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Effect of Heat Absorbed and Remitted by Copper Present In Molten Pb-Sb-Cu Alloy System on the Impact Strength and Hardness of the Solidified Alloy

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Abstract: The effect of heat absorbed and remitted by copper present in molten Pb-Sb-Cu alloy system on the impact strength, energy absorbance and hardness of the solidified alloy was studied following casting and cooling of the alloys in the furnace as well as computation of the heat absorbed and remitted by copper. The results of the investigation indicate that the impact strength, energy absorbance, and hardness of the sand cast Pb-Sb-Cu alloys increased with increase in the quantity of heat absorbed and remitted into molten Pb-Sb-Cu alloy system as result of increase in the weight (up to 45g) of copper added and distributed within the Pb-Sb alloy matrix. This was attributed to a wider temperature gradient believed to have been created (due to increased copper addition and distribution) during cooling of the alloy system as a result of the increased heat remittance into the system. This on one hand, increased the cooling time as desirably expected for increased energy absorbance and impact strength and on the other hand increased the temperature difference through which the cooling process desirably increased the hardness. It was found that increase in heat absorbance by the Pb-Sb-Cu alloy resulted from increased weight of copper added (up to 45g) and distributed within the Pb-Sb matrix. Furnace cooling conferred higher impact strength and energy absorbance on the Pb-Sb-Cu alloy compared with similar alloy cooled in water and air. Water cooling however, imparted greater hardness on Pb-Sb-Cu alloy compared with similar alloy cooled in air or furnace. [Nature and Science. 2009;7(7):1-7]. (ISSN: 1545-0740).

Keywords: Effect, Heat Absorbance and Remittance, Hardness, Impact Strength, Pb-Sb-Cu Alloy.

1. Introduction

The effect of tellurium on the mechanical properties of Pb-Sb alloy has been studied by Abrikosov [1]. The results of the investigation indicate that impact strength, tensile strength and hardness of the alloy is enhanced with addition of Te. He however, stated that the durability of the components made with this alloy cannot be guaranteed since Te is very radioactive. Several studies [2,3] have been carried out on lead-antimony alloy by addition of Sn to improve its mechanical properties and corrosion resistance. Results of the investigation indicate that addition of Sn to the Pb-Sb matrix increases both the tensile strength, hardness and corrosion resistance of the alloy. This makes Pb-Sb-Sn alloy suitable for coating tanks and pipes. Nwoye [4] reported that dispersion of Cu powder in Pb-Sb melt increases the impact strength and hardness of the alloy when cooled. He stated that the higher values of these mechanical properties (relative to those of Pb-Sb alloy) obtained is believed to be jointly as a result of Cu dispersion in the Pb-Sb matrix and the high level of purity (99.8%) of the copper powder used. This is in accordance with studies [5] which show that impurities in metals and alloys affect negatively their mechanical properties. It has been reported [5] that the effect of oxygen addition on Pb-Sb alloy is improvement in the corrosion resistance of the alloy due to the formation of transient oxide film as oxygen diffuses into the alloy. However, the alloy does not find wide industrial application due to the low mechanical properties attributed to it which includes tensile strength, impact strength and hardness. It has been reported [6] that addition of indium to Pb-Sb alloy increases the corrosion resistance of the alloy. Indium is added to the Pb-Sb alloy by ionic exchange through electrolytic process where indium is the anode and Pb-Sb, the cathode. Addition of 0.7% Al and 0.23% Bi to Pb-Sb alloy was found to increase the hardness, tensile strength, ductility and corrosion resistance of the alloy [7]. Arsenic addition to Pb-Sb-Sn alloy has been found to increase the corrosion resistance of the alloy due to its ability to reduce oxidation during service by formation of oxide film on the matrix [8]. However, this alloy has not found application in pipes and tanks because of its poisonous nature. Ackerrmann [9] reported, following characterization of Pb-Sb-Sn-Ni alloy, that addition of 0.25% Ni imparts good casting properties to Pb-Sb-Sn alloy. He also found that presence of Ni in the alloy increases the tensile and impact strength of Pb-Sb-Sn particularly at high temperature. He further stated that

the hardness and corrosion resistance of the alloy is tremendously improved with addition of 0.25% Ni. Several research works [4,10,11] have been carried out to improve the electrical conductivity of Pb-Sb alloy used as wet cell battery heads. Blumenthal [10] discovered that addition of cadmium enhances the electrical conductivity of Pb-Sb alloy tremendously. He however, stated that the alloy cannot find application in battery heads and plates because Cd is very radioactive and causes a volatile and explosive reaction when in contact with sulphuric acid for a long time. Rollason and Hysel [11] reported that addition of silver to Pb-Sb alloy increases very significantly the electrical conductivity of the alloy. He however, stated that this increase does not give a stable value due to impurities in the Ag. He stated that these impurities are Au, As, Sn, Cu and S. He further posited that these impurities create an unstable electrical field in the alloy of Pb-Sb-Ag. It is believed that this short coming has made the use of this alloy for battery heads and plates impossible since it obscures the precise electromotive force of the electrolyte in the battery. Nwoye [4] found that addition of copper powder by dispersion to Pb-Sb alloy improves the electrical conductivity of alloy greatly. It is believed that this breakthrough was possible because Cu used, had high purity level (99.8%). It is widely accepted that the mechanical properties of cast alloys and metals depend significantly on the chemical compositions of the material, casting temperature, casting technique, mould material, cooling medium and cooling rate. Studies [4,12,13] have shown that amongst cooling media such as water, air and furnace, water gives the highest cooling rate followed by air and then furnace. They posited that furnace cooling imparts better impact strength, ductility and tensile strength to cast metals and alloys followed by air cooling and then water cooling. They however, stated that water cooling imparts greater hardness to these materials followed by air cooling and then furnace cooling. Nwajagu [12] found that cooling an alloy from a higher temperature widens the temperature gradient and hence increases the hardness in the case of water cooling. It was therefore concluded that increased cooling time increases the tensile and impact strength.

It has been reported [14] that solid or liquid gains heat when introduced into a heated liquid or molten system, and then looses the heat to the system, to maintain temperature uniformity.

The aim of this research work is to study the effect of heat absorbed and remitted by copper present in molten Pb-Sb-Cu alloy system on the impact strength, energy absorbance and hardness of the solidified alloy. In this work, copper powder was added to the Pb-Sb melt by dispersion.

2. Materials and methods

ALLOY PREPARATION:

The materials used are antimonial lead scraps and electrolytic copper powder (200 mesh to dust type). They antimonial lead collected were melted together in order to obtain a fairly uniform composition of lead antimonial alloy, in case of any variation in antimony content. The melting operation was carried out at the forge, followed by casting of the alloys in sand mould and cutting to various sizes for use in the actual alloying. They melting crucible was of 260mm long, 200mm wide mild steel of about 100mm breadth with handle for carriage.

MOULD PREPARATION:

The preparation of the mould was done by first sieving the sand for aeration and mixing 6% moisture to give good green strength. The mould box of dimension 300mm wide, 100mm breadth and 500mm long was made from cast metal frame. A long hollow cylindrical pipe of 85mm long and 9mm diameter was used as the pattern for the cast. The mould was allowed to dry before use following its preparation.

CASTING TECHNIQUES:

A weighed quantity of lead antimony alloy (500g) was placed on the crucible and then placed inside the furnace. At 420^0C, the melt was slagged (since the whole constituent of the crucible have melted) and a weighed quantity of Cu added and the whole constituent stirred and returned to the furnace. After 5 minutes, the crucible was brought out of the furnace and poured into the mould. HEAT TREATMENT

The cast alloys were heat treated at a temperature of 180^0C to relieve stresses incurred during solidification of the alloys. The heat treatment was also carried out to homogenize the microstructure of the alloys prior to the testing of their mechanical properties.

IMPACT STRENGTH AND HARDNESS TEST

Following the heat treatment process, impact strength and hardness tests were carried out on the cast alloys (applying British standard procedures) using impact strength testing machine and Vickers hardness testing

machine respectively from the Mechanical Engineering Workshop of University of Nigeria, Nsukka. They energy absorbed by the alloy before fracture was calculated from the values of the impact strength by considering the cross-sectional area of the alloy sample. Heat absorbed by copper present in the molten Pb-Sb-Cu alloy was calculated considering the masses of copper added to the base alloy, the specific heat capacity of copper and the initial and final temperatures to which the copper was exposed.

CALCULATION OF IMPACT STRENGTH AND ENERGY ABSORBANCE OF Pb-Sb-Cu ALLOYS:
The striking energy of the impact strength testing machine is given by the equation [15];

$$S_E = M \times g \times H \quad (1)$$

Where

S_E = Striking energy of the impact strength machine (Kg/Fm)
M = Mass of hammer from the machine (g)
g = Acceleration due to gravity (m/s^2)
H = Height of hammer (rad.)

M = 3941Kg, g = $10m/s^2$, H = 90^0 ($\Pi/2$) (by conversion to radian) and $\Pi = 22/7$. Substituting these values into equation (1) gives;

S_E = 619300J (61930 KgFm)

Where 1Nm = 1J and 1KgF = 10N
Cross-sectional area, A (cm^2) of the alloy sample is given by the equation;

$$A = \Pi D^2/4 \quad (2)$$

Where D = 0.9cm; (Diameter of cross- section of the sample)
Substituting the of D into equation (2)

$$A = 0.6364cm^2$$

Energy absorbed at fracture, E_B (KgFm) is given by the equation (Mc Graw, 1982);

$$E_B = I_M \times A \quad (3)$$

Where

I_M = Impact strength of the alloy sample before fracture ($KgFm/cm^2$)

On heating the crucible containing antimonial lead and copper powder, it is believed that Cu present in the molten Pb-Sb-Cu alloy system gained or absorbed heat based on its own specific heat capacity and temperature exposed. Since the Cu added to the Pb-Sb alloy forms an alloy system; Pb-Sb-Cu, it is strongly conceived and believed that the heat absorbed or gained by the Cu is remitted into the alloy system formed, ensuring uniformity of temperature gradient within the alloy matix. This agrees with past reports [14]
Based on the foregoing,
Heat absorbed and remitted by Cu in the molten Pb-Sb-Cu alloy system is given by the equation[14];

$$Q = M C \Delta T \quad (4)$$

Where

Q = Quantity of heat absorbed and remitted by copper present in the Pb-Sb-Cu alloy (KJ)
M = Mass of copper powder added (g)
C = 0.385; Specific heat capacity of copper (J/g/K) [14]
ΔT = Change in temperature (between the initial and final temperature to which copper was exposed (K)

3. Results and discussion

Results of chemical analysis carried out on the materials used (as shown in Table 1) indicate that antimonial lead contains about 3.3% Cu in addition to Pb and Sb present. The percentage composition of the powdered Cu used is as received.

Table 1:Chemical composition of materials used

Material	Pb (%)	Sb (%)	Cu(%)
Antimonial lead	92	4.7	3.3
Copper powder	-	-	99.80

Effect of heat input resulting from copper addition (to Pb-Sb) on the impact strength of Pb-Sb-Cu alloy

The result of impact strength test (Fig.1) carried out on Pb-Sb-Cu alloys shows that irrespective of the cooling medium used the impact strength of the alloy increases with increase in the quantity of heat absorbed and remitted by Cu to the molten Pb-Sb-Cu alloy system.

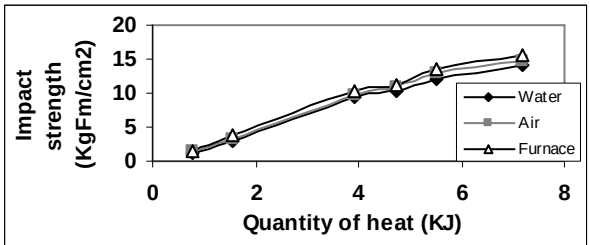

Fig. 1 Effect of heat absorbed and remitted by copper on the impact strength of Pb-Sb-Cu alloy

Fig. 4 indicates that increase in the quantity of Cu added to Pb-Sb matrix (up to 45g) increases the quantity of heat absorbed and remitted by it to the of Pb-Sb-Cu alloys formed. It is therefore believed that increased impact strength of the Pb-Sb-Cu alloys resulted from increased heat absorbed and remitted into the alloy system as a result of increased Cu addition and distribution within the Pb-Sb matrix (Fig. 4 and Table 3). This is true because increase in the heat absorbed and remitted by Cu into the Pb-Sb-Cu alloy system widens the temperature gradient prevailing during cooling of the system and increases the cooling time of the alloy which is considered ideal for better strength. This is in accordance with findings by Nwajagu [12]. This implies that increased heat absorbed by the Pb-Sb-Cu alloy is due to increased weight of Cu added and distributed within the Pb-Sb matrix.

Effect of heat input resulting from copper addition (to Pb-Sb) on the energy absorbance of Pb-Sb-Cu alloy

Energy absorbed by Pb-Sb-Cu alloys prior to fracture was calculated from the values of the impact strength using equation (3) following the calculation of the cross-sectional area of the alloy sample using equation (2). Fig. 2 shows that irrespective of the cooling medium used, energy absorbed by the alloy increases with increase in the quantity of heat absorbed and remitted by Cu present in the molten Pb-Sb-Cu alloy system.

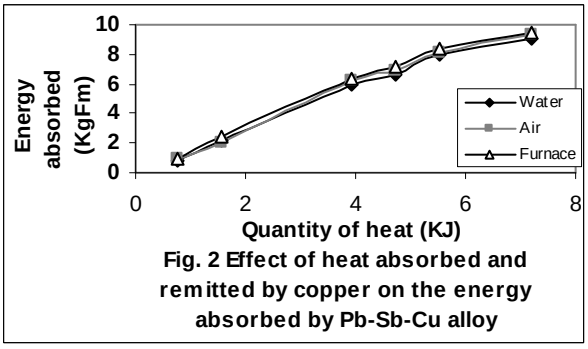

Fig. 2 Effect of heat absorbed and remitted by copper on the energy absorbed by Pb-Sb-Cu alloy

Comparing Fig. 4 and Table 3, it is strongly believed that since energy absorbed by the alloys is a derivative of the impact strength, increased energy absorbed by the Pb-Sb-Cu alloys also resulted from increase in the heat absorbed and remitted into the alloy system as a result of increased Cu addition and distribution (up to 45g) within the Pb-Sb matrix. This is also true because increase in the heat absorbed and remitted by Cu into the Pb-Sb-Cu alloy system widens the temperature gradient prevailing during cooling of the system and increases the cooling time of the alloy which is considered ideal for better strength

Effect of heat input resulting from copper addition (to Pb-Sb) on the hardness of Pb-Sb-Cu alloy

The hardness of Pb-Sb-Cu alloy was also found to increase with increase in the quantity of heat absorbed and remitted by Cu into the molten Pb-Sb-Cu alloy system cooling medium used. Comparison of Figs.3, Fig. 4 and Table 3 shows that increase in the hardness of the Pb-Sb-Cu alloys resulted from increase in the heat absorbed and remitted into the alloy system as a result of increased Cu addition and distribution (up to

45g) within the Pb-Sb matrix. This also implies that increase in heat absorbed and remitted by the Cu into the alloy system is due to increased weight of Cu added and distributed within the Pb-Sb matrix.

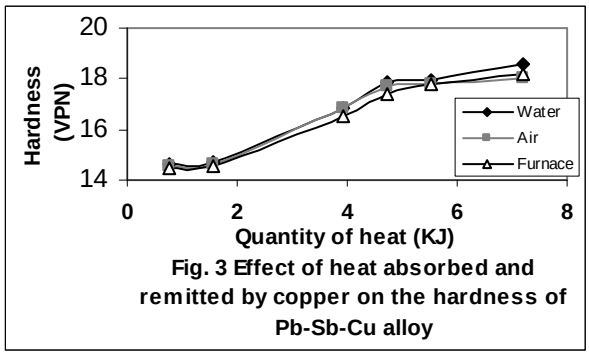

Fig. 3 Effect of heat absorbed and remitted by copper on the hardness of Pb-Sb-Cu alloy

This is believed to be so because increase in the heat absorbed and remitted into the Pb-Sb-Cu alloy system is likely to raise the temperature of the alloy system, widen the temperature gradient prevailing during cooling of the system and also increase the cooling time. The widening of the temperature gradient is believed to have increased the hardness the solidified Pb-Sb-Cu alloy especially when cooled in water. This is in agreement with past report [12]. However, increased cooling time imposed by increased heat absorption and remittance into the molten Pb-Sb-Cu alloy system imparted lower hardness on the alloys when they are cooled in the air and furnace. This is so because air and furnace cooling is associated with slower cooling rate which is not ideal for higher hardness. However, Fig. 3 indicates that the hardness of alloys cooled in air and furnace increases with increase in the quantity of heat absorbed and remitted into the molten alloy. This is a clear indication that the cooling rate increased with increase in the heat content of the molten alloy system. This is in accordance with the law guiding heat emission in solid bodies.

Effect of copper addition (to Pb-Sb alloy) on the quantity of heat absorbed and remitted into Pb-Sb-Cu alloy

Comparison of Figs.1-4, Tables 2 and 3 shows that increased addition of Cu (up to 45g) to the base alloy to form Pb-Sb-Cu alloy increased the quantity of heat absorbed and remitted into Pb-Sb-Cu alloy system. This invariable resulted to corresponding increase in the impact strength, energy absorbance and hardness of the Pb-Sb-Cu alloy due to increased cooling time and widened

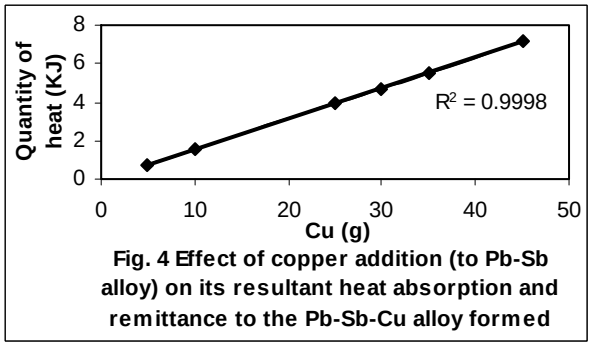

Fig. 4 Effect of copper addition (to Pb-Sb alloy) on its resultant heat absorption and remittance to the Pb-Sb-Cu alloy formed

temperature incurred. An evaluation of the correlation coefficient, R between the Cu added to the base alloy (Pb-Sb alloy) and heat absorbed and remitted to the formed Pb-Sb-Cu alloy (as in Fig.4) using the equation;

$$R = \sqrt{R} \qquad (5)$$

gives R = 0.9999 which is approximately unity. This represents a very ideal and desirable relationship between the experimental process parameters.

Table 2: Mechanical properties of Pb-Sb alloy (Alloy control of melting temperature 425^{0}C) cooled in water, air and furnace

Mech. Property	Water	Air	Furnace
Impact strength	1.01	1.18	1.26
Energy absorbed	0.64	0.75	0.80
Hardness	14.45	14.26	14.40

Table 3: Effect of copper addition (to Pb-Sb matrix) on the impact strength, energy absorbance and hardness of Pb-Sb-Cu alloy cooled in furnace

Cu (g)	Hardness (VPN)	Energy absorbance (KgFm)	Impact Strength (KgFm/cm^2)
5	14.49	0.96	1.50
10	14.56	2.40	3.80
25	16.53	6.35	10.20
30	17.40	7.20	11.30
35	17.80	8.40	13.40
45	18.20	9.45	15.50

Effect of cooling medium on the hardness, impact strength and energy absorbance of Pb-Sb-Cu alloy. Figs. 1-3 show that furnace cooling imparted better impact strength and energy absorbance to the alloy (compared with water and air cooling) in agreement with previous work [16,17]. This is suspected to be due to the formation of equiaxed structure in the microstructure of the alloys as a result of slower cooling rate imposed by furnace cooling. This agrees with past report [12]. This result implies that alloys cooled in the furnace can withstand greater stress or load (than water and air cooled alloys) before actually undergoing failure. This is in accordance with past reports [4, 12,13]. It was also found that water cooling the alloys imparted higher hardness to the alloy (compared with furnace and air cooling). This is suspected to be as a result of the formation of coarse grain within the alloy structure imposed by rapid cooling of water. Coarse grains achieved in this way have been found to give greater hardness [12,18].

Conclusion

Impact strength, energy absorbance and hardness of sand cast Pb-Sb-Cu alloys increased with increase in the quantity of heat absorbed and remitted into the molten Pb-Sb-Cu alloy system as a result of wider temperature gradient prevailing during cooling of the system and also increase in the cooling time. Furnace cooling confers higher impact strength and energy absorbance on cast Pb-Sb-Cu alloys compared with similar alloys cooled in water and air. Water cooling however, imparts greater hardness on Pb-Sb-Cu alloys compared with similar alloys cooled in air or furnace.

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It is the intention of the author to publish this work culled from his M. Eng. Thesis in honour of Prof. Sylvanus I. Okeke of Nnamdi Azikiwe University, Awka as a memorial research piece credited to his numerous publications in recognition and appreciation of his unequalled supervisory role during this research work.

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Model for Predicting the Upgraded Concentration of Iron during Solid-State Beneficiation of Iron Oxide Ore Pelletized with Powdered Potassium Chlorate

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Abstract: Model for predicting the upgraded concentration of iron during solid-state beneficiation of iron oxide ore has been derived. The model-predicted %Fe upgrades were found to agree with a direct relationship between %Fe values and weight input of $KClO_3$ as exhibited by %Fe upgrades obtained from the experiment. It was found that the model; $\%Fe = 1.57[(\ln(T/\gamma))^{2.58}]$ is dependent on the weight input of $KClO_3$ and the range of treatment temperature used (500-800^{0}C). It was found that the validity of the model is rooted in the expression $(\%Fe/\alpha)^N = \ln(T/\gamma)$ where both sides of the expression are correspondingly approximately equal to 4. The maximum deviation of the model-predicted values of %Fe from those of the corresponding experimental values was found to be less than 13% which is quite within the range of acceptable deviation limit of experimental results. Upgraded concentration of iron per unit increase in the treatment temperature evaluated from experimental and model-predicted results are 0.0545 and 0.0629%/^{0}C respectively, indicating proximate agreement. [Nature and Science. 2009;7(7):8-14]. (ISSN: 1545-0740).

Keywords: Model, Upgraded Iron, Pyrobeneficiation, Iron Oxide Ore.

1. Introduction

Nigeria`s largest known iron ore deposit estimated at 1250 metric tonnes have been found at Agbaja. It consists of oolitic and pisolitic structures rich in iron oxides, in a matrix that is predominantly clay [1]. Uwadiele [1] also found that the principal constituent of the ore is goethite, with minor hematite, maghemite, siderite, quartz, kaolinite pyrite and an average of 0.09%S.

It has been reported [2] that one of the most important factors influencing the desulphurization process during iron making is the state of oxidation of the bath.

Desulphurization of Agbaja iron oxide ore concentrate using solid potassium trioxochlorate (v) ($KClO_3$) as oxidant has been carried out [3]. The concentrate was treated at a temperature range 500–800^{0}C. The results of the investigation revealed that simultaneous increase in both the percentage of the oxidant added (up to 15g per 50g of ore) and treatment temperature (maximum 800^{0}C) used give the ideal conditions for increased desulphurization efficiency. This translates into high desulphurization efficiency when both oxidant concentration (up to 15g per 50g of ore) and treatment temperature (maximum 800^{0}C) are high.

The mechanism and process analysis of desulphurization of Agbaja iron ore concentrate using powdered potassium trioxochlorate (v) ($KClO_3$) as oxidant has been reported [4]. Concentrates were treated at a temperature range 500 – 800^{0}C. Results of the process analysis indicate that oxygen required for the desulphurization process was produced following decomposition of $KClO_3$ within a temperature range 375-502^{o}C. It was observed that this temperature range is the Gas Evolution Temperature Range (GETR) for sulphur present in Agbaja iron ore. Sulphur vapour and oxygen gas produced at this temperature range were believed to have reacted to form and liberate SO_2. The process analysis suggests that the mechanism of the desulphurization process involves gaseous state interaction between oxygen and sulphur through molecular combination. The results for the extent of desulphurization reveal that simultaneous increase in both the percentage of the oxidant added and treatment temperature used (up to 15g $KClO_3$ per 50g of ore and maximum of 800^{0}C respectively) are the ideal conditions for the best desulphurization efficiency.

Previous study [5] indicates that Agbaja oolitic iron ore, which has not been responsive to so many upgrading processes, has been upgraded to 73.4% Fe assay (starting from as-received concentrate assaying 56.2%Fe) by pyrometallurgical-oxidation method. Main parameters investigated were the effects of treatment temperature and oxidant ($KClO_3$) on the upgrading process. It was established that 800^{0}C is the optimum temperature for the upgrading step considering the range of temperature used (500-800^{0}C). It was observed from results of the investigation that both oxidant and temperature increase (up to 12g per 50g of

iron ore and maximum of 800^0C respectively) during the process are vital conditions for improving on the grade of the ore concentrate.

Following an intensive study [6] on selective oil agglomeration of Agbaja iron ore, the crude ore of Fe content (45.6%), was concentrated by oil agglomeration technique to 90% Fe recovery and 65% Fe assay. The researcher stated that the ore require grinding to minus 5µm to effect adequate liberation. These results were obtained at optimum pH 9. It was found [7] following studies on the effect of temperature on magnetizing reduction of Agbaja iron ore that the fine-grained oolitic Agbaja iron ore, which is not responsive to conventional processing techniques, can be upgraded by the magnetizing reduction method with an Fe recovery of 87.3% and Fe assay of 60% at 600^0C.

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

Model for predictive analysis of the concentration of sulphur removed by molecular-oxygen-induced desulphurization of Agbaja (Nigeria) iron oxide ore has been derived [9]. The model is expressed as;

$$\%S = \left(\frac{\text{LogT} - \text{Log}\,\alpha - \text{Log}k_n}{\mu\,\text{Log}\gamma} \right) \qquad (1)$$

Where

%S = Concentration of sulphur removed during the pyrometallurgical-oxidation process.

k_n= 9.75 (Decomposition coefficient of $KClO_3$ at the treatment temperature (600^0C)) determined in the experiment [9].

(µ) = 2.1739 (Oxidation coefficient of $KClO_3$ relative to the treatment temperature (600^0C)) determined in the experiment[9]

(α) = Weight of iron oxide ore added (g)

T = Treatment temperature used for the process (^{0}C)

(γ) = Weight of $KClO_3$ added (g)

D_e = 0.0415 (Assumed Desulphurization Enhancement Factor)

Substitution of these parameters into the model in equation (1) reduced it to ;

$$\%S = \left(\frac{0.0415}{\text{Log}\gamma} \right) \qquad (2)$$

The model was found to predict the concentration of sulphur removed, very close to the corresponding %S values obtained from the actual experimental process. It was found that the model is dependent on the values of the weight input of the oxidant ($KClO_3$) during the desulphurization process. The validity of the model was rooted in the expression $k_n[(\gamma)^{\mu\%S}] = T/\alpha$ where both sides of the expression were correspondingly almost equal.

The aim of this work is to derive a model for predicting the upgraded concentration of iron during solid-state beneficiation of Agbaja (Nigeria) iron oxide ore pelletized with powdered potassium chlorate. The powdered potassium chlorate was used in the preceding work [9] as oxidant.

2. Model

The solid phase (ore) is assumed to be stationary, contains some unreduced iron remaining in the ore. It was found [4] that oxygen gas from the decomposition of $KClO_3$ attacked the ore in a gas-solid reaction, hence removing (through oxidation) the sulphur present in the ore in the form of SO_2 .Equations (3) and (4) show this.

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

$$S_{(s)} \xrightarrow{\text{Heat}} S_{(g)} + O_{2\,(g)} \longrightarrow SO_{2\,(g)} \qquad (4)$$

Nwoye [4] posited that when sulphur inherent in the iron ore is removed in this stance, the concentration of iron present in the ore is upgraded since sulphur is an impurity element and leaves as SO_2.

2.1 Model Formulation

Experimental data obtained from research work [10] carried out at SynchroWell Research Laboratory, Enugu were used for this work.

Results of the experiment as presented in report [10] and used for the model formulation are as shown in Table 1. Computational analysis of the experimental data [10] shown in Table 1, gave rise to Table 2 which indicate that;

$$(\%Fe/\alpha)^N = \ln(T/\gamma) \quad \text{(approximately)} \qquad (5)$$

Introducing the value of N and γ into equation (5);

$$(\%Fe/1.57)^{0.3876} = \ln(T/\gamma) \qquad (6)$$

Since the inverse of 2.58 = 0.3876

$$(\%Fe/1.57)^{1/2.58} = \ln(T/\gamma) \qquad (7)$$

Multiplying the indices of both sides by 2.58;

$$\%Fe/1.57 = (\ln(T/\gamma))^{2.58} \qquad (8)$$

$$\%Fe = 1.57[(\ln(T/\gamma))^{2.58}] \qquad (9)$$

Where

%Fe = Upgraded concentration of iron during the beneficiation process

N = 0.3876 (Decomposition coefficient of $KClO_3$ during the beneficiation process at the treatment temperature range 500-800^{0}C) determined in the experiment [10].

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

(α) = 1.57(Oxidation coefficient of $KClO_3$ relative to its weight-input during the beneficiation process) determined in the experiment[10]

I_f = 2.58 (Assumed Temperature-Oxidant Interaction Factor)

T = Treatment temperature (^{0}C)

Equation (9) is the derived model

Table1: Variation of upgraded concentration of iron with treatment temperature.[10]

%Fe	T(^{0}C)	(γ) (g)
57.60	500	50
62.80	550	50
68.00	600	50
68.18	650	50
68.50	700	50
68.72	750	50
69.00	800	50

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

$(\%Fe/\alpha)^N$	ln(T/γ)
4.0403	3.9120
4.1779	4.0073
4.3089	4.0943
4.3132	4.1744
4.3210	4.2485
4.3264	4.3175
4.3332	4.3820

3. Boundary and Initial Condition

Iron oxide ore (in a furnace) pelletized with potassium chlorate (oxidant) is considered.The furnace atmosphere was not contaminated i.e (free of unwanted gases and dusts). Initially, atmospheric levels of oxygen were assumed just before the decomposition of $KClO_3$ (due to air in the furnace).Weight of iron oxide ore; (50g), and treatment temperature range; 500-800^{0}C were used. Treatment time; 360 secs.,

average ore grain size; 150μm, and weight-input of $KClO_3$ (oxidant); 10g were also used. These and other process conditions are as stated in the experimental technique [10].

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

4. Model Validation

Direct analysis and comparison of %Fe values predicted by the model and those obtained from the experiment were carried out to ascertain the validity of the formulated model.

Computational analysis and comparison between these %Fe values reveal deviations of model-predicted %Fe values from those of the experiment. This was attributed to the fact that the surface properties of the ore and the physiochemical interactions between the ore and the oxidant (under the influence of the treatment temperature) which were found to have played vital roles during the oxidation-beneficiation process [10] were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model-predicted %Fe values to those of the experimental %Fe values.

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

$$Dv = \left(\frac{M_D - E_D}{E_D} \right) \times 100 \qquad (10)$$

Where M_D = Model-predicted %Fe value

E_D = Experimental %Fe value

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

$$Cr = -Dv \qquad (11)$$

Therefore

$$Cr = -\left(\frac{M_D - E_D}{E_D} \right) \times 100 \qquad (12)$$

Introduction of the corresponding values of Cr from equation (12) into the model gives exactly the corresponding experimental %Fe values [10].

5. Results and Discussion

The derived model is equation (9).

Equations (8) and (9) show that since 2.58 is the index of the expression $\ln(T/\gamma)$, the values of the process parameters; T and γ as applied to the beneficiation process are simultaneously affected by the constant 2.58 towards iron upgrade. The constant is therefore assumed to be the interaction factor between treatment temperature and the oxidant (Temperature-Oxidant Interaction Factor) I_f since it is common to both T and γ mathematically. It was found that the model-predicted %Fe upgrades show a direct relationship with the treatment temperature, in agreement with %Fe upgrade from the experiment as in Table 1.

An ideal comparison of the quantity of water evaporated as obtained from experiment and as predicted by the model for the purpose of testing the validity of the model is achieved by considering the R^2 values. The values of the correlation coefficient, R calculated from the equation; using the r-squared values (coefficient of determination) from Figs.1 and 2 show a better correlation (0.9982) with model-predicted quantity of water evaporated than that obtained from experiment (0.69). This suggests that the model predicts more accurate, reliable and ideal quantity of evaporated water than the actual experiment despite its deviations from the experimental values.

$$R = \sqrt{R^2} \qquad (13)$$

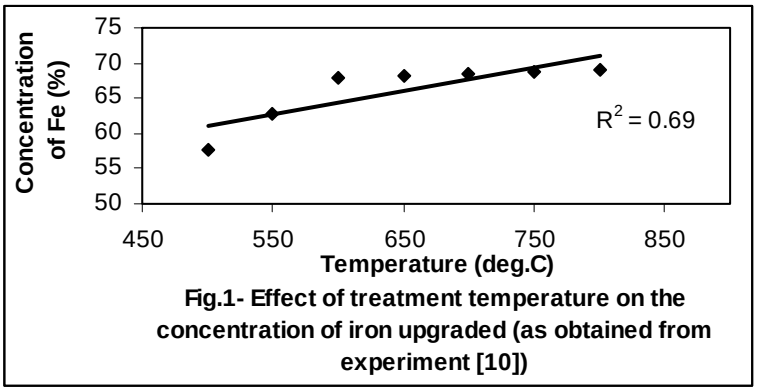

Fig.1- Effect of treatment temperature on the concentration of iron upgraded (as obtained from experiment [10])

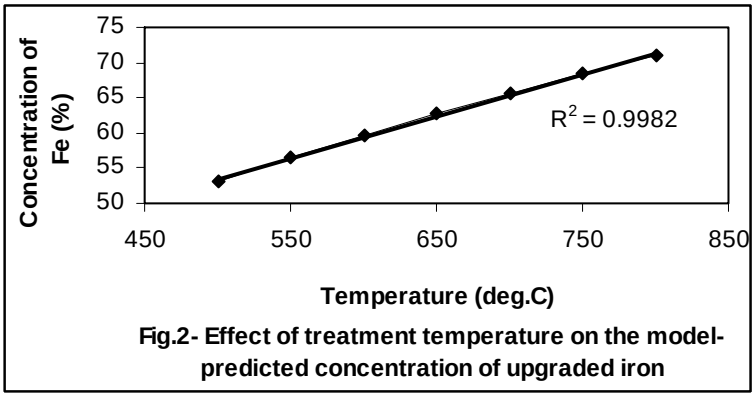

Fig.2- Effect of treatment temperature on the model-predicted concentration of upgraded iron

The concentration of iron upgraded per unit increase in the treatment temperature resulting from the reaction between the iron oxide ore and $KClO_3$ (oxidant) at a temperature range 500-800^{0}C was determined following comparison of the iron upgrade per unit increase in the treatment temperature obtained by calculations involving experimental results[10], and model-predicted results obtained directly from the model.

Iron upgrade per unit increase in treatment temperature, I_U (%/ ^{0}C) is calculated from the equation;

$$I_U = I/T \quad (14)$$

Therefore, a plot of concentration of iron upgraded I, against treatment temperature T, (as in Fig. 1) using experimental results gives a slope, S at points (57.6, 500) and (68.5, 700) following their substitution into the mathematical expression;

$$I_U = \Delta I/\Delta T \quad (15)$$

Eqn. (15) is detailed as

$$I_U = I_2 - I_1/ T_2 - T_1 \quad (16)$$

Where

ΔI = Change in the concentrations of upgraded I_2, I_1 at two treatment temperature values T_2, T_1. Considering the points (57.6, 500) and (68.5, 700) for (I_1, T_1) and (I_2, T_2) respectively, and substituting them into eqn. (16), gives the slope as 0.0545%/^{0}C which is the concentration of iron upgraded per unit increase in the treatment temperature during the actual experimental process. Also similar plot (as in Fig. 2) using model-predicted results gives a slope. Considering points (53, 500) and (65.58, 700) for (I_1, T_1) and (I_2, T_2) respectively and substituting them into eqn. (16) gives the value of slope, I_U as 0.0629%/^{0}C. This is the concentration of iron upgraded as predicted by the model. A comparison of these two concentrations of iron upgrade shows proximate agreement. Based on the foregoing, the model is believed to be very valid as a predictive tool.

A comparison of the values of %Fe from the experiment and those from the model shows very minimum positive and negative deviations less than 13% which is quite within the acceptable range of deviation limit of experimental results hence depicting the reliability and validity of the model. The validity of the model

is rooted in the expression $(\%Fe/\alpha)^N = \ln(T/\gamma)$ where both sides of the expression are correspondingly approximately equal to 4. Results in Table 2 show proximate agreement with equation (5) following the values $(\%Fe/\alpha)^N$ and $\ln(T/\gamma)$ evaluated from Table 1 as a result of corresponding computational analysis.

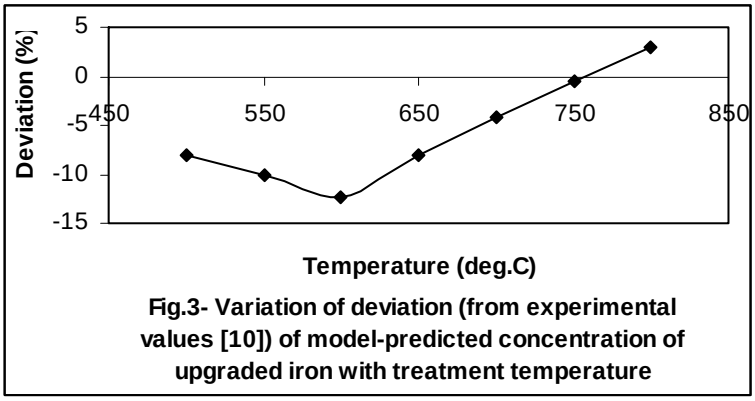

Fig.3- Variation of deviation (from experimental values [10]) of model-predicted concentration of upgraded iron with treatment temperature

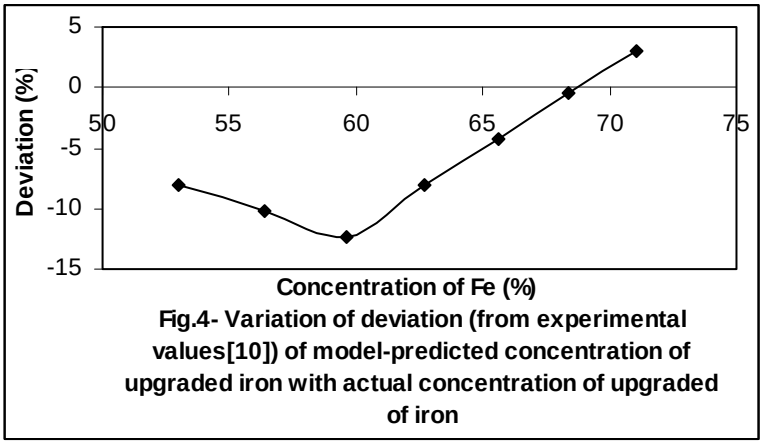

Fig.4- Variation of deviation (from experimental values[10]) of model-predicted concentration of upgraded iron with actual concentration of upgraded of iron

Comparison of Figs. 3 and 4 show that the positive and negative deviations (of the model-predicted concentration of iron upgraded) from actual experimental values show an undulating relationship with both the treatment temperature and concentration of iron upgraded. These curves indicate that the highest and least deviations; -12.34 and -0.52% are associated with treatment temperatures; 600-750^{0}C and iron upgrade; 59.61 and 68.36% respectively. This is so because the extent of deviation is a function of just the magnitude of the value whiles the sign (positive or negative) preceding the value indicates whether the deviation is deficit or surplus.

Correction factor to (in Fig. 5) also shows an undulating relationship with the concentration of iron upgraded. However, the orientation of the curve of correction factor against concentration of iron upgraded (Fig.5) is opposite that of the deviation against treatment temperature and concentration of iron upgraded (Figs. 3 and 4).

This is attributed to the fact that correction factor is the negative of the deviation as shown in eqns. (11) and (12). It is believed that the correction factor takes care of the effects of the surface properties of the iron oxide ore and the physiochemical interaction between the ore and the leaching solution which (affected experimental results) were not considered during the model formulation.

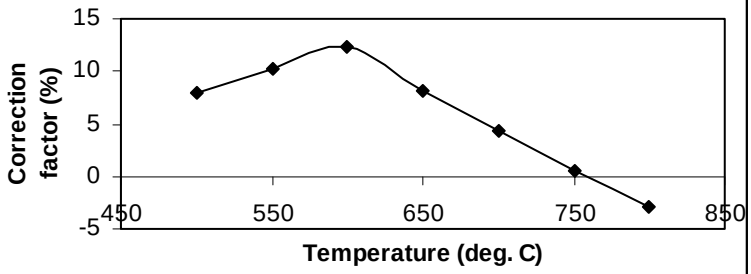

Fig.5- Variation of correction factor (to model-predicted concentration of upgraded iron) with treatment temperature

6. Conclusion

The model predicts the concentration of upgraded iron during solid-state beneficiation (at a temperature range 500-800^{0}C) of iron oxide ore pelletized with powdered potassium chlorate. The validity of the model is rooted in the expression $(\%Fe/\alpha)^N = \ln(T/\gamma)$ where both sides of the expression are correspondingly approximately equal to 4. The maximum deviation of the model-predicted concentration of iron upgraded from the corresponding experimental value is less than 13% which is quite within the acceptable deviation range of experimental results. Upgraded concentration of iron per unit increase in the treatment temperature evaluated from experimental and model-predicted results are 0.0545 and 0.0629%/^{0}C respectively, indicating proximate agreement.

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

Acknowledgement

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Extinction of Species from Establishment of Large Water Projects: The Case of *Aloe polyphylla* in Lesotho

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Abstract: Water is one resource that can be used in different ways. It is sometimes shared between countries as some countries have too little while others have more. When this sharing of water happens normally biodiversity is negatively affected from constructions involved. Some of these species have extinct while others are left highly endangered. Spiral aloe (*Aloe polyphylla*), endemic to Lesotho only but valuable worldwide is a good example. The aim of this study was to learn about the life of *Aloe polyphylla* and then assess how far did Lesotho Highlands Water Project, as a large water projects endanger this life of Aloe polyphylla in Lesotho. The research also involved finding out which measures were used to either avoid or remediate this problem. The research was done between May and July 2008. Interviews were conducted in Katse Botanical Garden, 'Muela Hydropower station, Ts'ehlanyane National Park, Ts'ehlanyane village and Ministry of Environment in Lesotho. Both closed-ended and open-ended interviews were used to gather information. Purposive methods were used to sample the respondents in all areas of study. The results showed that *Aloe polyphylla* has been highly affected that it has extinct in Ts'ehalnyane area. No natural Aloe polyphylla was found. The results also showed that propagation has been used as a remediation method. [Nature and Science. 2009;7(7):15-20]. (ISSN: 1545-0740).

Keywords: Extinction, Large Water Projects, *Aloe polyphylla*, Ts'ehlanyane National Park

1. Introduction

Lesotho is a mountainous country whose largest resource is its abundance of water, which results from an annual precipitation of approximately 1 000 mm in the highland areas. Most of this precipitation occurs during the summer months from October to April, although snow does fall periodically throughout the year. The approximate absolute maximum and minimum temperatures for the project area are 29 ℃ and 7 ℃, with a mean monthly temperature of 12 ℃ and with the project area lying between 1 700 and 2 100 m above sea level, evaporation losses are minimal (Bell and Haskins, 1997). It is because of this characteristic of Lesotho that the two Governments of both Lesotho and Republic of South Africa (RSA) had an agreement that Lesotho has to sell water to RSA. As a result of this agreement, Lesotho Highlands Water Project (LHWP) was born. It is been run by Lesotho Highlands Development Authority (LHDA).

LHWP was established in 1978 by the Governments of Lesotho and RSA, and a joint feasibility study was undertaken. The study recommended that the project to transfer water, under gravity, from the upper reaches of the Orange River to RSA should be constructed in four phases over period of some 30 years. The water supplied to RSA will meet the increasing demand and enable industrial growth to continue (De Bruiyn et al, 2000).

LHWP is situated in the Kingdom of Lesotho and the adjoining north-eastern part of the Orange Free State Province of the RSA in an area underlain by Triassic and Jurassic basalts of the Lesotho Formation and Triassic sandstones and mud rocks of the Karoo Sequence. It consists of a series of dams and tunnels to convey water from the Lesotho Highlands to the industrial centre of the RSA. LHWP just like any other project of its nature and size posed some problems on the land where it has been implemented. It has negatively affected biodiversity found in the areas where it has been implemented both directly and indirectly.

Spiral aloe (*Aloe polyphylla*, which means "many leaves") is a rare perennial succulent and beautiful aloe native to the basaltic Maluti Mountains in Lesotho, Africa, a small country entirely surrounded by RSA. It

is from the Liliaceae family, Aloe genus and *Aloe polyphylla* Schönland ex Pillans. It has leaves spine-tipped with the distinctive spiral arrangement, flowers pale red to salmon. It has fleshy leaves, bright green in colour with yellow-white teeth along the edges. The most striking feature of this aloe is the perfect spiral in which its leaves are arranged. This may be clockwise or anti-clockwise. This species is now considered highly endangered. This species and others were part of biodiversity found in the location chosen for LHWP implementation. As a result, the implementation of LHWP highly contributed to its present status. LHWP has negatively affected Aloe polyphylla both directly and indirectly. The most highly affected location was Ts'ehlanyane area. One of the Adits of LHWP was constructed in this area. *Aloe polyphylla* was last seen in Ts'ehlanyane during that the time of that construction.

This study tried to find out what happened and what measures were taken to either reverse or correct the situation in Ts'ehlanyane in relation to extinction of *Aloe polyphylla*.

2. Description of the Study Area

Phase 1A of this project involved construction of two big dams and a hydropower station. Several tunnels were constructed to move water from the main dam, Katse to 'Muela (location of hydropower). When that happened, a tunnel was constructed at Ts'ehlanyane area. This area lies at the interface between the Eastern Mountain Province of Lesotho and the lowlands, which are surrounded by the relatively dry interior of RSA and also has plenty of freshwater resources. It is deep in the front range of the Maluti Mountains at the foot of the Holomo Pass. It has an altitude ranging from 1 940 to 3 112 m and is considered mostly sub-alpine. This is one of the richest places of biodiversity of Lesotho. It is at this village where Ts'ehlanyane National Park (TNP) is located.

It lies at the junction between Ts'ehlanyane and the Holomo Rivers. Over 5 600 hm^2 of extremely rugged mountain terrain are protected within this park, which includes one of the very few remaining indigenous woodlands in Lesotho. These amongst others include Che-che *(Leucosidea sericea*) woodland with a number of rare undergrowth plants that are unique to this woodland habitat. On the banks of the rivers and streams are stands of Berg Bamboo *(Thamnocalamus tessellatus)* which are of cultural significance to Basotho. The reserve also encompasses a reasonable proportion of very rare mountain "fynbos" that do not occur anywhere else in the world and also recorded to be in excess of 220 flowering plant species. The diversity of this habitat types is exceptionally high and derived from the large altitudinal range that the park has. Almost all species found in this park, with the exception of the clawless otter *(Aonyx capensis)*, grey rhebuck *(Pelea capreolus)* and rock hyrax *(*Procavia capensis*)* are considered to be endangered in the park area.

It comprises principally of the Sub-alpine Belt, but includes a limited area of Alpine Tundra, up to 3 112 m. Its lowest point (c. 1 940 m) is just above the interface between the Sub-alpine and Montane Belts. This consists of rolling upland plateau, which extends westwards of the Drakensberg escarpment. This area is characterized by sharp convex break of slope and is entirely underlined by basaltic rocks. It also has large straight simple basaltic slopes falling from about 3 200 to 2 600 m. TNP also consists principally of the cliff faces, and precipitous scarp and valley slopes of the Sub-alpine Belt. This kind of characteristic leads to very active mass wastage processes and as a result only few areas have deep soils in the park. A high proportion of the park consists of thin skeletal soils lying directly over rock. The soils are relatively young and shallow, derived from the underlying basalt or dolerites. Soils of the summit areas and valley sides are generally shallow (< 600 mm) and of medium texture (loams to clay loams). This is one reason why it had *Aloe polyphylla* in abundance.

These Alpine and Sub-alpine Belts are sensitive environments, which respond rapidly to disturbances and poor land-use methods. This is one way of showing why it had to be protected after the construction of the LHDA's Adit. While the soils are inherently stable by virtue of their high organic matter content and favourable state of aggregation, they are nevertheless susceptible to erosion from high intensity rain storm, particularly if the vegetation cover on steep slopes is decreased. Erosion gullies form rapidly following poor siting of roads, paths or other forms of development. Recovery rates are extremely slow. Bared or eroded areas may not recover in many decades. The unique attributes of the alpine flora and ecosystem clearly indicate the need for priority to be given to sustainable land uses and appropriate conservation measures for the vegetation.

Many of the high altitude vegetation formations present in this park are found nowhere else, and constitute two of the seven floristic regions of Africa South of the Sahara, namely the Afro-alpine and the

Afromontane Regions. Considering the scale of this park, at least 90% of this area lies between 2 100 and 2 900 m. There are several major vegetation types in each belt or bioclimatic zone found in this park and are reported to be poorly distinguished on the ground as a result of anthropogenic factors.

Ts'ehlanyane people depend on subsistence agriculture and harvesting natural resources for a variety of needs, mainly firewood, handcrafts, medicine, food, construction, and socio-cultural amenities. They have done so since time immemorial and are singularly responsible for the good conservation value that the area represents. The area has the longest history of conservation championed by a local traditional authority in Lesotho (Letšela et al, 2003). Figure 1 shows the location of Ts'ehlanyane National Park.

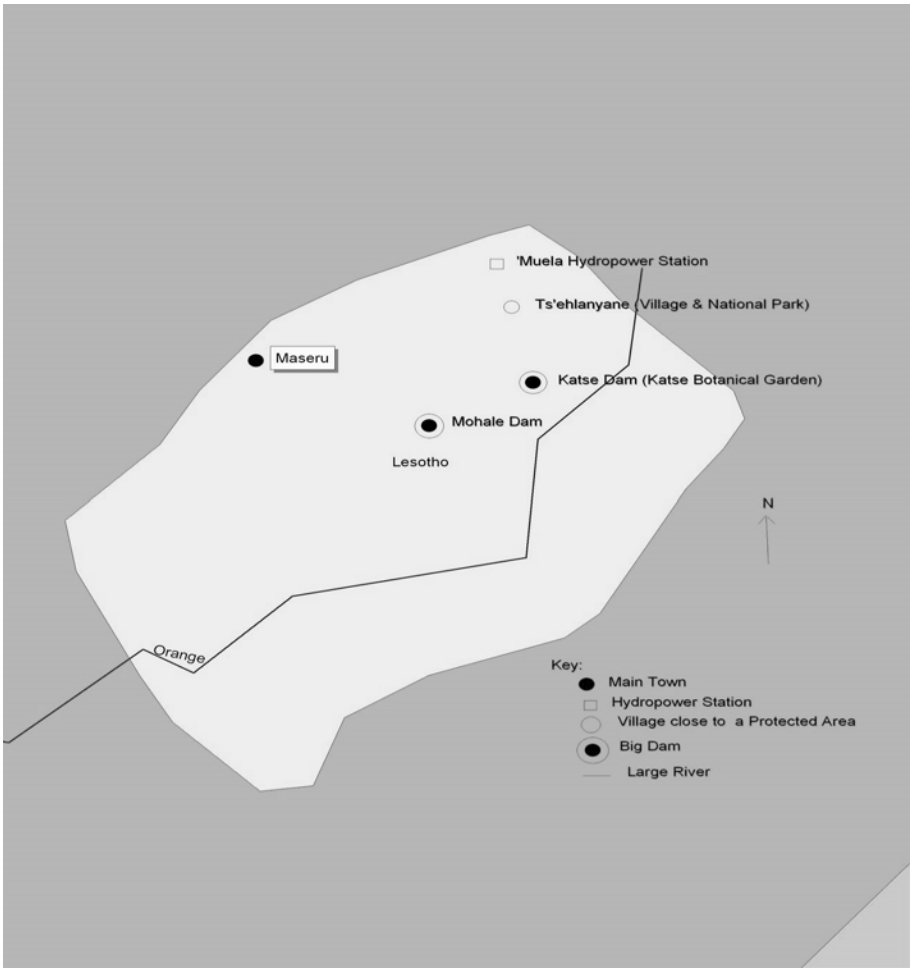

Figure 1 The location of Ts'ehlanyane National Park (June/July 2008, Research)

3. Materials and Methods

The research was carried out from May to July 2008. Both open-ended and closed-ended interviews were conducted in the three protected areas. The questionnaires used in the closed interviews were developed by the researcher with the guidance from previous studies and other related agencies. The other interviews (mostly open-ended) were conducted in Ts'ehlanyane village. The respondents were workers from TNP, the botanists, villagers (traditional doctors, farmers, herd boys & shepherds) from Ts'ehlanyane village. Experts from the Ministry of Environment were also interviewed about their involvement and consent about the protection of spiral aloe.

The qualitative methods were used to construct strata in the village. A stratified sampling method was used to divide the village into four strata. The aim of this research was to obtain more rich information and that means the respondents have to be people who really have the rich information about the subject of the study.

In cases like this, Babbie (1992) indicates that it is important that the sample is chosen on the basis of the researcher's knowledge of the population in which the study is to be conducted. Polit and Beck (2003) therefore say that a purposive or judgmental sampling which is based on the belief about the researcher's knowledge about the population can be used to hand-pick sample members. In this way, the sample members will be people with the necessary information for the study. Respondents were then chosen using purposive sampling in all areas of the study as to make sure that they all have good knowledge about *Aloe polyphylla.*

The researcher adopted a phenomenological (qualitative) approach, using semi-structured interviews and observations as data-collection techniques (Polit and Beck, 2003). During the interviews narrative-description data was collected (Polit and Beck, 2003). The semi-structured, in-depth interviews with the individual respondents were the primary data-collection tool. The advantages of semi-structured interviews were that more complex issues could be triggered, answers could be clarified and more in-depth as well as sensitive information could be obtained (Bowling, 1997). To further enrich and support the data, the researcher' observations, field notes as well as taking pictures of *Aloe polyphylla* formed part of the study. Tables and standard method were used to analyse data. Results from all protected areas were them compared to make a conclusion of the results.

4. Results

4.1 Findings about the nature and life of *Aloe polyphylla*

The characteristics of *Aloe polyphylla*: The results show that Aloe polyphylla is a stem-less high altitude succulent. It prefers basaltic soils. Its leaves have a distractive spiral arrangement (clockwise & anticlockwise). This arrangement of its leaves is unique. It has also been discovered that these leaves are thorny at the edges and have apple green colour.

The results about uses of *Aloe polyphylla*: It has been found that most people use *Aloe polyphylla* for ornamental purposes. It is also used for medicinal purposes as well as in manufacturing of toiletries.

The species living in harmony with *Aloe polyphylla*: *Aloe polyphylla* can be found together with amongst others various *Crassula species, Erica species, Themeda triandra, Urginea capitata, Bulbine narcissfolia, Cotyledon orbiculata and Festuca caprina.*

The results of relationship between *Aloe polyphylla* and the species it lives together with: It was mentioned that all species are high altitude species and are unified by the moisture. They are all looking for moisture. Another relationship though not yet fully proven was the other species seem to be acting as protection to *Aloe polyphylla* as where these species are found, *Aloe polyphylla* is always hiding under them.

4.2 Findings about the availability of *Aloe polyphylla* in Ts'ehlanyane area

The results show that presently natural Aloe polyphylla is not available at this area but it used to be present. It was last seen before the construction of LHDA Adit that transfers water from Katse dam to 'Muela dam.

4.3 Findings about the causes of extinction of *Aloe polyphylla*

It has been found that a lot of people came to this place in search for work in the construction and while waiting to be hired, they started illegally harvesting *Aloe polyphylla* in large quantities to sell it. The other cause is easy access to the area resulting from the road constructed as part of the Adit construction. The area became more accessible to everyone so most people came to harvest this species illegally. Other causes of extinction of *Aloe polyphylla* mentioned are illegal collection for illegal sales and overgrazing.

4.4 Results on the protection and remediation measures for *Aloe polyphylla*

Before the implementation of the project, *Aloe polyphylla* was collected together plants with other species that were to be affected by the project activities and propagated in Katse botanical garden. In places where it was not collected prior to the construction, in Ts'ehlanyane area in particular, a nature reserve was then established to reintroduce *Aloe polyphylla* to this area as it has extinct after the construction. LHDA also established a National Park in Ts'ehlanyane area to protect this species together with some other ones affected by the project too. Another measure taken was to encourage people living around the affected

areas to establish their own household botanical gardens in order to reduce pressure on the little ones left in the wild.

5. Discussions

5.1 The nature and life of *Aloe polyphylla*

Aloe polyphylla is a succulent so it has some other general characteristics of all succulent but it has its own distinguishing features. The unique arrangement of its leaves with both clockwise and anticlockwise spirals is one of the main unique features of this species. It falls in the group of stem-less succulents with thorny leaves and has leaves with green apple colour. It is these unique features it has that make it too attractive that most people have decided to use it more as an ornamental plant. This is the most popular use of it. It can also been used for medicinal purposes too.

As it has been mentioned that this species is a succulent, it is clear that it leaves under harsh conditions. It can be found in places with little moisture living in harmony with amongst others species like: *Crassula species, Erica species, Themeda triandra, Urginea capitata, Bulbine narcissfolia, Cotyledon orbiculata* and *Festuca caprina*. *Aloe polyphylla* and all these other species are highlands species and they serve as a protection for it. In most cases it can be found somehow under these species.

5.2 The availability of *Aloe polyphylla* in Ts'ehlanyane area

Ts'ehlanyane area is one of the few places in Lesotho that are natural niches of this aloe. But unfortunately, it cannot be found there anymore. This result from illegal overharvesting that happened during the construction of the Adit of the giant project in Lesotho, LHDA/LHWP. As in any other large project, most people waited everyday to be hired. It is during that time that *Aloe polyphylla* extinct from Ts'ehlanyane. Most people harvested it in large quantities that it had no time to recover.

One of the features of big projects is road construction. LHDA also constructed roads. These roads made some places accessible which were formerly not accessible. It is in this places where *Aloe polyphylla* is found, rocky inaccessible places. Even the little species which were left after the illegal overharvesting were harvested as its habitats became accessible to almost everyone.

5.3 The protection and remediation measures for *Aloe polyphylla*

After all the destruction it caused. LHDA took some measures to reestablish the harmed land. One measure used was a nature reserve. Ts'ehlanyane National Park was established as a way to conserve the biodiversity found in the area of its construction. But unfortunately, at the time when the reserve was established, *Aloe polyphylla* has already extinct. Instead, it has been propagated here in the reserve too. There are also plans to establish colonies for this species and try to plant it in larger quantities. This is from the fact it is been suspected that *Aloe polyphylla* has still chances of naturally appearing in this area. This result from suspicion that there are some few species that might have grown from the seeds that fell from the last big species to be harvested illegally.

Besides this nature reserve, there are botanical gardens established with the same purpose. One belongs to LHDA while others belong to the people living around the project areas. They are all under the supervision of LHDA.

6. Conclusions

LHWP has highly affected *Aloe polyphylla* in Lesotho. Some of the measures taken to correct this situation were late in some places like Ts'ehlanyane as it has already extinct when measures were implemented. In cases where it was protected prior to the implementation of the project, it has been fully saved. This clearly shows that, it is better and safer to protect biodiversity prior to establishments than correcting the harm caused. In short, prevention is better than cure, so it will be wiser for all project managers to see to it that they avoid or prevent problems than correcting them. That is not only safer for biodiversity but also reduces costs of implementation of corrective measures which are in most cases expensive and as a result become unsustainable.

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Prunus Cerasoides D. Don (Himalayan Wild Cherry): A Boon To Hill- Beekeepers In Garhwal Himalaya

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Abstract: This article describes Prunus Cerasoides D. Don (Himalayan Wild Cherry): A Boon To Hill-Beekeepers In Garhwal Himalaya. [Nature and Science. 2009;7(7):21-23]. (ISSN: 1545-0740).

INTRODUCTION

Prunus cerasoides (Family Rosaceae) the Himalayan Wild Cherry is a sacred plant in Hindu mythology. It is found in Sikkim, Nepal, Bhutan, Myanmar, West China and India (Collett, 1921; Gaur, 1999 and Polunin & Stainton, 1984). In India the plant is restricted to sub-montane and montane Himalaya ranging from 1500-2400 m asl. In Garhwal Hills it is distributed abundantly in temperate zones of Pauri, Tehri, Chamoli and Uttarkashi districts. Locally it is known as 'Panyyan'. It is worshipped in all auspicious occasions by the inhabitants. People never cut the whole tree and use only its twigs in rituals as the wood are forbidden to be used as fuel. Thus it is common to observe quite old trees of *Prunus cerasoides* in the area. But the potential of the plant as rich source of pollen and nectar for honey bees is not tapped adequately.

As the winter starts, restricted patches in the hilly regions impart a spring look due to this plant. It blooms in October and lasts up to mid -December. Its pinkish- white flowers are the rich source of nectar and pollen for bees (Sharma, 1970; Crane *et. al.*, 1984; Gaur and Nawani, 1989; Pratap, 1997 and Gaur and Tiwari, 2001). In this period the swarms of honey bees can be observed gathering nectar and pollen heavily from these trees. In Garhwal Himalaya, November and December is a period when flowering is minimum, only few wild and ornamental herbs bloom which hardly fulfill the need of honey-bees. Thus beekeepers are compelled to use artificial feeding to bees. Hence *Prunus cerasoides* can serve as a boon for beekeepers. Its plantation is the need of people, bees and plant itself. The apicultural value of the plant is non-comparable as it blooms in hills during the dearth period and is visited by the three native Asian honey-bees (*Apis cerana indica*, *A. dorsata* and *A. florae)* and also by introduced European species *Apis mellifera*. In this way artificial feeding is not necessary for those beekeepers whose colonies are in the surroundings of *Prunus cerasoides*.

The botanical description of the plant, features of pollen and properties of honey are as follows.

Botanical Description: D. Don, *Prodr. Fl. Nepal* 239, 1825; Naithani, *Fl Chamoli* 1:201, 1984; Gaur, *Fl Garhwal*, 226, 1999; Collett *Fl. Simlensis* 156, 1921; Osmaston, *A Forest Flora for Kumaun*, 202, 1927.
Deciduous trees, up to 10 m high; bark reddish brown, exfoliating in thin circular strips. Leaves conduplicate in bud, elliptic or ovate-lanceolate, 3.5-8.5 cm, apex acuminate, both surfaces glabrous, dark glossy, shining above, finely simple or double serrate, with gland tip teeth; petioles 1.2-2 cm long; stipules long, subulate. Flowers pinkish white 1.2-2.5 cm across, appearing before the leaves in umbellate fascicles (figure 3); pedicels 0.5-2cm long. Calyx bell shaped, 5-lobed; lobes ovate-acute. Petals 5, obovate. Fruits red or yellow, ovoid, 1.2-1.5 cm long, glabrous, shining, supported by base of calyx tube; 1- seeded. Flowering period October to December and fruiting, Febuary to November.

Pollen: Grains 3 – zonicolporate, colpus broad, lip pointed, endocolpium indistinct. Exine surface finely straite, straie thick. Exine 2.5 µm thick, ecto exine as thick as endoexine; columella indistinct; AMB circular, triangulate 39x28 µm. Shape sub-prolate. Figures (4-6).

Honey:

Physical Properties: Colour, reddish brown; appearance shining; aroma strong and pleasant; flavor slightly bitter.

Chemical Constituents : Water-8.80%, Nitrogen 0.022%, Ash 0.08%, Minerals , Na-14 ppm, K 195 ppm, Ca-47 ppm, reducing sugars-63.97 %.

Apicultural Value: All the four species of *Apis* present in India namely *Apis cerana indica, A. dorsata, A. florae* and *A. mellifera* visit the flowers of *Prunus cerasoides* for its rich nectar (N1) and pollen (P1). The honey is slightly bitter in taste but medicinal in properties. Inhabitants of this region use *Prunus cerasoides* honey to treat eye ailments.

Other Uses: Although the plant is conserved for religious purposes, used in rituals by the local inhabitants, yet have some other uses such as for making walking sticks, leaves for fodder, and fruits for making sauces. A gum exuding from trunk and branches is used by honeybees as honeydew. The bark paste is applied on contusions (Gaur, 1999).

Propagation: Natural regeneration of the plant is by seeds and it prefers temperate climate. Regeneration can be achieved by direct sowing or by transplanting nursery raised seedlings.
The plant is strongly recommended for plantation as rich source of pollen and nectar to honeybees besides its religious value. It needs to be brought under afforestation programmes in gardens, avenues and in dry waste lands.

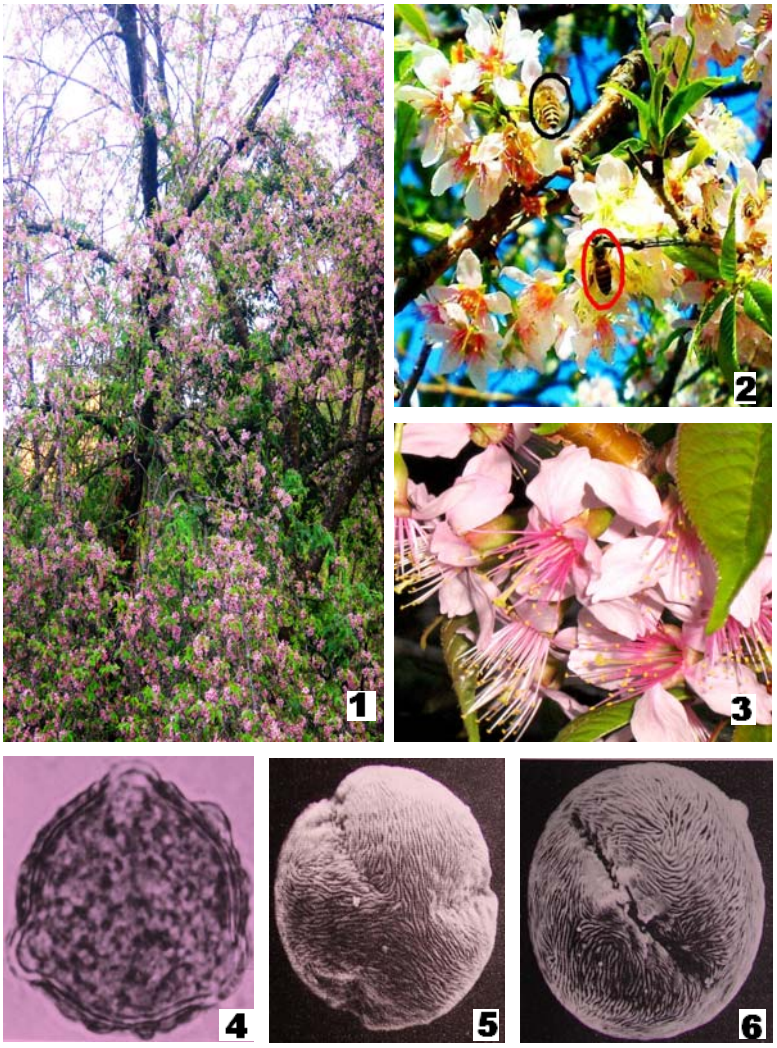

PLATE 1: Figure 1-6. 1: The tree in full bloom. 2: Inflorescence with *Apis dorsata* (red circled) and *A. cerana indica* (black circled) worker bees gathering nectar. 3: Flowers. 4: Light microphotograph of pollen (polar view). 5: SEM microphotograph in polar view. 6: SEM microphotograph in equatorial view.

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Biochemical Quantification of protein , Fat, Starch, Crude fibre, Ash and Dry matter content in different Collection of Greater Yam (*Dioscorea alata* L.) found in Orissa.

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Abstract

A study was under taken to quantify the biochemical composition of protein, fat, starch , crude fibre, ash and dry matter in different collections (C1-C22) of *Dioscorea alata* fresh tubers. In the present study C-18 had the highest dry matter (33.33%) and lowest was in C-3 (24.91%). The Average dry matter was highest in intermediate shape groups and collections with white flesh tubers. Starch percentage is highest in C-20 (82.51%) followed by C-1, C-7, C-3, C-22 and lowest is estimated in C-15 (78.36%). The highest protein content in dry matter of tuber was observed with C-1 (9.67%) and the lowest protein content was observed with C-13 (7.31%). The lowest fat content, however, was observed in C-11 (0.67%) and highest value was with C-2 (1.24%) . C-1 had the lowest ash content of 1.89 % whereas C-15 had the highest value of 7.08 %. However, the ash content was towards the higher side in the intermediate group (5.85). The crude fiber range was higher in the intermediate group i.e. out of five collections four were having more than 2% crude fiber. From the present investigation it is concluded that different collections of *D.alata* vary greatly for their dry matter , starch, protein , fat ,ash and crude fiber content depending on different collection groups and the geographical regions. [Nature and Science. 2009;7(7):24-32]. (ISSN: 1545-0740).

Keywords: Greater Yam, Dry matter, Biochemical estimation, Different collections

Introduction

Roots and tubers are the most important food crops of very ancient origin in the tropics and subtropics. Theses crops are associated with the human existence, survival, and their socio-economic history. The Indo-Burma region is the centre of origin of taro and Asiatic edible yams. The two hot spots of global biodiversity viz. North Eastern Himalayas and Western Ghats are particularly rich in wild relatives of tropical root and tuber crops Burkill, (1960). Root crops occupy nearly 50 million hectares of arable land and account for a global production of 560 million tones. Nigeria alone accounts for 70% of the total yam production. In terms of the productivity and gross return, yam ranks second among all the root and grain producing food crops (FAO, 2004). It also ranks second in dry matter and energy production per hectare (IITA, 2004).Yams belong to the genus *Dioscorea* of family *Dioscoreaceae* an important members of the oldest monocot. More than 600 species have been reported under this genus Coursey,1976).Out of so many species of *Dioscorea* only ten species have been domesticated and commercially cultivated. In India so far 26 species of *Dioscorea* have been reported (Panneerselvam , 2007). Among the *Dioscoreas D.alata* is the leading species grown globally as well as all over the state of Orissa. It is highly polymorphic in relation to shape and colour of the tuber. Basing on the shape of the tuber and colour of the cortex or flesh , some selections were made from the collections of *D.alata* on different parts of the state .Out of the collections only 22 cultivars were included in the present study. Detail information of these 22 cultivars of *D.alata* is presented in Table-1&2.

Yams are a valuable source of carbohydrates, fibers, and low level fats, which makes them a good dietary source and could be eaten as boiled yam , fufu or fried in oil (Osman ,1990). Several species of Yams also have medicinal properties and the tuber is said to contain some pharmacologically active substances including dioscorine, saponin and sapogenin [20]. The objective of the present study therefore, was to examine the biochemical composition of dry matter, starch, protein, ash, crudefiber and fat content of different collection of *D.alata* found in different parts of Orissa and the selection of the cultivated variety for higher crop production for the yam cultivars of the state (Niswass, 1985).

Materials and Methods

Highly polymorphic *D.alata* tubers were collected from different parts of Orissa during the year 2005-06 . Basing on the shape of the tuber and colour of the cortex or flesh, some selections were made .Out of the collections only 22 cultivars were included in the present study . All the 22 collections were grouped under three shape types namely (1) Pyramidal (2) Cylindrical (3)Intermediate (those in between pyramidal and cylindrical types) and four flesh colours namely (1) White, (2) Cream, (3) Yellowish pink (4) Violet. Detail information of these 22 cultivars of *D.alata* are presented in Table-1&2 [23].

Table-1. *D.alata* L., Collections based on shape of the tuber

Sl. No	Shape of tuber	Total collection	Code number of different *D.alata* collections
1	Pyramidal shape	9	C1, C7, C18, C19, C11, C12, C13, C3, C20
2	Intermediate shape	5	C5, C6, C2, C15, C21
3	Cylindrical shape	8	C4, C14, C8, C16, C17,C 9, C10, C22

Table-2 *D.alata* L., Collections based on colour of the tuber

Sl .No	Colour of flesh	Total collection	Code number of different *D.alata* collections
1	White	11	C1, C7, C18, C19, C5, C6, C4, C14, C8, C16, C17.
2	Cream	4	C11, C2, C9, C10
3	Yellowish pink	3	C12, C13, C15.
4	Violet or pink	4	C3, C20, C21, C22

These 22 collections (C1-C22) were grown in the experimental garden, P.G. Deptt. of Botany Utkal university as per the standard agronomic practices with stacking and non stacking system and harvesting of the tuber was done after all the vines dried and it was done around 300 days after planting. There were three replications and in each replication 22 treatments were randomly distributed. In each treatment 16 plants were grown besides border rows. Observations were recorded in four randomly selected plants (Bradbury and Holloway,1988; AOAC,1984).

Drymatter: Drymatter in tuber was calculated by taking 100 gms of freshly harvested tuber from a representative sample of tuber and drying the sample at 40^0C till a constant weight was obtained and the value was expressed in percentage [Cozzolino and Labandera,2002; Egesi et al.,2003; Ferguson, et al.,1980).

Starch: Starch was calculated from a representative 2.5 gms of powered dry tuber following the standard method and titrating with Fehling's solution A + B. The percent of starch was calculated by the AOAC,(1990) method. (Greenwood-Barton, 1961 ; Macrae , et al.,1974). , Osisiogu and Uzo,1973; , Prain and Burkill,1936;).

Protein: Protein content of the yam tuber was estimated on the basis of nitrogen content of the tubers and on dry matter basis. The micro Kjeldhals distillation method as per Jackson (1967) was used for such estimation. The protein content was estimated by 'N' percent x 6.25 considering that the protein contains 16 percent nitrogen (Balogun and Fetuga**,** 1986; Bressani,1994; Gary, 1986; Amoo 1998; Adeyeye,1995).

Fat: Fat content was estimated as per the standard procedure indicated in methods of analysis of AOAC (1990) and the value was expressed in percentage (Panneerselvam , 2007; Vogel, 1980).

Crude fiber: Crude fiber was estimated as per standard procedure stated in methods of analysis AOAC (2001) and value was expressed in percentage (Martin and Rhodes, 1977; Osisiogu and Uzo,1973).

Ash: Ash was calculated on dry matter basis of tuber as per the standard procedure specified in methods of analysis of AOAC (1984).The value was expressed in percentage.

Result and Discussion

Dry matter in tuber: During the studies significant difference was observed for the dry matter content in tubers of different collections of *D. alata* in both the years of observations and also in the pooled analysis. The dry matter was the highest in the tubers of C-18 (33.33%) followed by C-13 (32.75%) and no significant difference was observed in these two cultivars. The lowest dry matter content was observed in C-3 (24.91%) which was significantly the lowest as compared to the rest of he collection. The mean value was 29.19%, 30.43% and 28.09% respectively in pyramidal, intermediate and cylindrical type and 29.70%, 28.56%, 30.47% and 26.81% respectively in white, cream, yellowish pink and violet flesh colour group of tuber (Table-3) (Martin ,1974 ; Onwueme and Charles,1994).

Starch content of tuber : As regard to the starch content, significant differences were recorded . Analysis of angular transformed values revealed that starch content was highest in C-20 (82.51%) but this was at par with C-1, C-7, C-3 and C-22. The lowest starch content was however, observed with C-15 (78.36%). The mean was 81.44%, 79.55% and 80.20% respectively in the pyramidal, intermediate and cylindrical types and 80.52%, 79.77% 80.07% and 81.82% respectively in the white, cream, yellowish pink and violet coloured flesh groups (Table-4) (Maynard,1970).

Protein (% in dry matter of tuber): Protein content in the tuber was significantly differed among the collections. The highest protein content in dry matter of tuber was observed with C-1 (9.67%) and it was however, at par with C-4, C-14, C-16, C-17 and C-9. The lowest protein content was observed in C-13 (7.31%). The mean was 8.55, 8.16% and 7.75% respectively in pyramidal intermediate and cylindrical types and 8.03%, 7.8 %, 8.34 % and 8.35% respectively in white, cream, yellowish pink and violet cololured flesh tuber groups (Table-5) (Ogungbenle,1998).

Fat content: Fat content was assessed from the dried material of the tuber of different collections of *D. alata* in both the years. There was significant difference among the collections. In the first year the range was 0.64% to 1.30 % and in the second year it was from 0.73 to 1.20 % whereas in the pooled data it was 0.67 to 1.24%. The lowest fat content, however, was observed in C-11 (0.67%) and highest value was with C-2 (1.24%). The white fleshed types including the cylindrical types had less than 1% fat as indicated from the observations. The means value was 0.88 %, 0.92 % and 0.81% respectively in pyramidal, intermediate and cylindrical types. However, the white, cream, yellowish pink and violet flesh colour tuber groups had 0.84, 0.91, 0.75 and 0.92 per cent respectively (Table-5) (Onwueme and Charles,1994).

Ash content (Dry matter basis) : Significant difference was observed for the ash content in the tubers. C-1 had the lowest ash content of 1.89 % whereas C-15 had the highest value of 7.08 %. However, the ash content was towards the higher side in the intermediate group (5.85). The mean value was 3.82 %, 5.85 % and 4.37 % respectively for pyramidal, intermediate and cylindrical group, whereas, it was 4.25%, 5.1% , 5.16 % and 3.48 % respectively in white, cream, yellowish pink and violet coloured tuber groups (Table-6) [Pucher et al., 1948; Vogel, 1980).

Crude fiber: The crude fiber content of the tuber was in a range of 1.39 to 2.60 % in the first year of the study while, it ws 1.46 to 2.53% in the second year. There was significant difference among the collection of *D. alata* as regard to crude fiber content. The range was higher in the intermediate group i.e. out of five collections four were having more than 2% crude fiber. The mean was 1.95 %, 2.27 % and 1.96 % respectively in the pyramidal, intermediate and cylindrical groups whereas it was 1.94 %, 2.25 %, 2.32 % and 1.81 % respectively in the white, cream, yellowish pink and violet colour flesh tuber groups (Table-6) [Sadasivam, and Balasubramanian,1985; SPYN ,2003).

Table .3. Drymatter content (%) in different collections of *D. alata* L.

Collection	Dry matter content in tuber (%)		
Pyramidal	I	II	Pooled
C-1	32.48 (28.83)	31.82 (27.80)	32.15 (28.31)

C-7	34.03 (31.33)	34.30 (31.76)	34.17 (31.54)
C-18	35.71 (34.06)	34.00 (32.60)	35.26 (33.33)
C-19	31.04 (26.60)	30.70 (26.08)	30.82 (26.34)
C-11	32.43 (28.76)	32.26 (28.50)	32.35 (28.63)
C-12	31.94 (28.00)	32.47 (28.83)	32.21 (28.41)
C-13	34.69 (32.41)	35.12 (33.10)	34.90 (32.75)
C-3	29.66 (24.5)	30.22 (25.33)	29.94 (24.91)
C-20	32.24 (28.66)	32.22 (28.43)	32.23 (28.54)
Intermediate			
C-5	33.82 (31.00)	34.03 (32.41)	33.92 (31.71)
C-6	34.69 (32.33)	34.75 (32.50)	34.67 (32.41)
C-2	33.67 (30.73)	33.56 (30.58)	33.61 (30.65)
C-15	33.33 (20.20)	33.62 (30.33)	33.47 (30.26)
C-21	31.52 (27.33)	31.30 (27.00)	31.41 (27.16)
Cylindrical			
C-4	32.41 (28.75)	32.37 (28.66)	32.39 (28.70)
C-14	32.62 (29.08)	32.58 (29.00)	32.60 (29.04)
C-8	32.41 (28.73)	32.26 (28.50)	32.34 (28.61)
C-16	31.73 (27.66)	32.16 (28.33)	31.94 (27.99)
C-17	32.79 (29.33)	32.15 (28.33)	32.47 (28.80)
C-9	31.16 (26.76)	31.09 (26.66)	31.12 (26.71)
C-10	31.89 (26.16)	33.43 (30.38)	32.66 (28.27)
C-22	31.11 (26.70)	31.17 (26.58)	31.14 (26.64)
'F' test	Sig.**	Sig.**	Sig.**
S.E (m) ±	0.485	0.413	0.326
C.D (0.05)	0.980	0.835	0.640

N.B.: Data in parenthesis are actual value and analyzed data are angular value

Table .4. Starch content (in dry matter) in different collections of *D. alata* L.

Collection	Starch content in tuber (%)		
Pyramidal	I	II	Pooled
C-1	64.95 (82.06)	65.14 (82.33)	65.04 (82.19)
C-7	65.49 (82.80)	64.68 (81.80)	65.08 (82.30)
C-18	64.15 (81.00)	64.40 (81.33)	64.28 (81.17)
C-19	63.79 (80.50)	63.91 (80.66)	63.85 (80.58)
C-11	63.60 (80.23)	63.56 (80.16)	63.58 (80.20)
C-12	64.64 (81.66)	64.23 (81.40)	64.43 (81.53)
C-13	63.67 (80.33)	64.13 (81.30)	63.90 (80.32)
C-3	64.89 (82.00)	65.14 (82.33)	65.02 (82.16)
C-20	65.75 (83.13)	64.82 (81.90)	65.28 (82.51)
Intermediate			
C-5	63.48 (80.06)	63.60(80.23)	63.54 (80.14)
C-6	63.24 (79.73)	63.26 (79.73)	63.24 (79.73)
C-2	62.84 (79.16)	62.49 (78.66)	62.66 (78.91)
C-15	62.60 (78.83)	61.96 (77.90)	62.28 (78.36)
C-21	64.20 (81.06)	63.55 (80.16)	63.88 (80.61)
Cylindrical			
C-4	63.93 (80.70)	63.96 (80.73)	63.95 (80.71)
C-14	63.41 (79.96)	62.84 (79.16)	63.12 (79.56)
C-8	63.79 (80.50)	63.08 (79.50)	63.43 (80.00)
C-16	62.02 (81.00)	62.24 (78.30)	63.30 (79.65)
C-17	63.43 (80.00)	63.05 (79.46)	63.24 (79.73)

C-9	63.01 (79.40)	63.79 (80.50)	63.40 (79.95)
C-10	63.41 (79.96)	63.53 (80.13)	63.47 (80.04)
C-22	65.65 (88.00)	64.22 (81.03)	64.93 (82.01)
'F' test	Sig.**	Sig.**	Sig.**
S.E (m) ±	0.285	0.479	0.301
C.D (0.05)	0.575	0.967	0.591

N.B.:- Data in parenthesis are actual value and analyzed data are angular value

Table .5. Protein and fat percent in dry matter basis in different collections of *D. alata* L.

Collection	Protein % content			Fat % content		
Pyramidal	I	II	Pooled	I	II	Pooled
C-1	3.20(10.25)	3.01(9.10)	3.10(9.67)	0.92(086)	0.90(0.82)	0.84
C-7	2.89(8.50)	2.89(8.37)	2.89(8.43)	0.92(0.86)	0.92(0.96)	0.86
C-18	2.75(7.60)	2.69(7.26)	2.72(7.43)	1.00(1.00)	1.06(1.13)	1.06
C-19	2.91(8.65)	2.89(8.39)	2.90(8.52)	0.92(0.85)	0.89(0.79)	0.82
C-11	2.78(8.20)	2.73(7.55)	2.75(8.40)	0.80(0.64)	0.84(0.70)	0.67
C-12	2.56(8.27)	2.58(8.27)	2.56(8.72)	0.86(0.75)	0.85(0.73)	0.74
C-13	2.57(7.22)	2.59(7.40)	2.58(7.31)	0.86(0.74)	0.86(0.74)	0.74
C-3	2.95(7.74)	2.92(7.80)	2.93(7.77)	1.11(1.24)	1.09(1.20)	1.22
C-20	2.72(9.41)	2.78(9.38)	2.75(9.36)	0.95(0.92)	1.01(1.02)	0.97
Intermediate						
C-5	2.87(6.63)	2.74(6.73)	2.80(6.68)	0.97(0.95)	0.90(0.82)	0.88
C-6	287(6.60)	2.93(6.54)	2.90(6.57)	0.92(0.85)	0.89(0.80)	0.82
C-2	2.54(8.72)	2.58(8.54)	2.56(8.63)	1.13(1.30)	1.08(1.18)	1.24
C-15	2.68(9.59)	2.72(9.39)	2.70(9.46)	0.96(0.93)	0.93(0.86)	0.89
C-21	2.68(9.38)	2.79(9.60)	2.74(9.94)	0.85(0.73)	0.90(0.81)	0.77
Cylindrical						
C-4	3.09(8.38)	3.06(8.37)	3.08(8.37)	0.87(0.77)	0.89(0.80)	0.78
C-14	3.06(7.21)	3.09(7.83)	3.08(7.53)	0.90(0.82)	0.93(0.88)	0.85
C-8	2.68(7.60)	2.93(7.26)	2.91(7.43)	0.95(0.91)	0.98(0.84)	0.87
C-16	3.06(9.12)	3.06(9.50)	3.06(9.31)	0.90(0.82)	0.87(0.75)	0.79
C-17	30.02(8.30)	3.08(6.81)	3.05(8.45)	0.84)0.72)	0.86(0.74)	0.73
C-9	3.08(7.45)	3.06(7.73)	3.07(7.59)	0.96(0.94)	0.94(0.89)	0.91
C-10	2.94(6.49)	2.89(6.67)	2.91(6.58)	0.91(0.85)	0.91(0.83)	0.84
C-22	2.58(6.69)	2.62(6.87)	2.60(6.78)	0.85(0.72)	0.87(0.75)	0.74
'F' test	Sig.**	Sig.**	Sig.**	Sig.**	Sig.**	
C.D (0.05)	0.052	0.052	0.050	0.025	0.045	

Data in parenthesis are actual value and analyzed data are ⌐ value

Table. 6. Ash and crude fiber content in the dry matter of tuber in different collections of *D. alata* L.

Collection	Ash (%)			Crude fiber (%)		
Pyramidal	I	II	Pooled	I	II	Pooled
C-1	(1.74)1.31	(2.05)1.43	(1.89)	(1.42)1.19	(1.46)1.21	(1.44)
C-7	(2.34)1.52	(2.94)1.71	(2.64)	(1.80)1.34	(1.90)1.38	(1.55)
C-18	(4.90)2.20	(4.62)2.14	(4.76)	(1.31)1.34	(1.36)1.36	(1.35)
C-19	(3.92)1.97	(4.34)2.03	(4.13)	(2.14)1.46	(2.23)1.49	(2.23)
C-11	(4.93)2.22	(5.37)2.31	(5.15)	(2.46)1.66	(2.40)1.54	(2.43)

C-12	(4.67)2.15	(4.66)2.15	(4.66)	(2.28)1.50	(2.41)1.55	(2.34)
C-13	(5.59)2.36	(6.09)2.46	(5.34)	(2.10)1.44	(2.14)1.46	(2.12)
C-3	(2.20)1.43	(2.40)1.55	(2.34)	(1.60)1.26	(1.51)1.22	(1.55)
C-20	(2.74)1.63	(3.30)1.31	(3.02)	(1.69)1.30	(1.66)1.29	(1.57)
Intermediate						
C-5	(5.45)2.33	(5.72)2.39	(5.53)	(1.90)1.37	(2.00)1.41	(1.95)
C-6	(5.00)2.23	(4.78)2.18	(4.89)	(2.21)1.48	(2.26)1.50	(2.23)
C-2	(6.72)2.59	(6.76)2.60	(6.74)	(2.26)1.56	(2.44)1.55	(2.35)
C-15	(5.89)2.62	(7.27)2.69	(7.06)	(2.50)1.53	(2.41)1.55	(2.45)
C-21	(4.58)2.13	(5.36)2.31	(4.97)	(2.08)1.44	(2.26)1.50	(2.17)
Cylindrical						
C-4	(3.60)1.89	(3.70)1.92	(3.65)	(1.39)1.17	(1.50)1.22	(1.44)
C-14	(4.58)2.14	(4.65)2.15	(4.61)	(1.71)1.30	(1.78)1.33	(1.74)
C-8	(3.95)1.98	(4.46)2.11	(4.20)	(2.60)1.61	(2.53)1.59	(2.56)
C-16	(5.98)2.44	(5.60)2.36	(5.79)	(2.24)1.49	(2.23)1.49	(2.23)
C-17	(4.66)2.15	(4.60)2.14	(4.63)	(1.64)1.27	(1.63)1.27	(1.63)
C-9	(3.56)1.38	(3.77)1.94	(3.66)	(2.10)1.44	(2.10)1.44	(2.10)
C-10	(4.98)2.23	(4.73)2.17	(4.85)	(2.15)1.46	(2.09)1.44	(2.12)
C-22	(3.53)1.87	(3.76)1.94	(3.64)	(1.33)1.35	(1.83)1.35	(1.33)
'F' test	Sig.**	Sig.**		Sig.**	Sig.**	
C.D (0.05)	0.109	0.148		0.057	0.057	

Data in parenthesis are actual value and analyzed data are ⌐ value

CONCLUSION

D. alata cultivars are used as staple food in many communities of tropical world. As per Egbe and Treche (1984). *D. alata* cultivars in average contain 24.47% dry matter and 72.6% starch, 8.24% protein and 0.24% fat in dry matter. In the present study C-18 had the highest dry matter (33.33%) and lowest was in C-3 (24.91%). Average dry matter was highest in intermediate shape and collections with white flesh. Starch percentage is highest in C-20 (82.51%) followed by C-1, C-7, C-3 and C-22 and lowest is estimated in C-15 (78.36%). The highest protein content in dry matter of tuber was observed with C-1 (9.67%) and the lowest protein content was observed with C-13 (7.31%). The lowest fat content, however, was observed in C-11 and (0.67%) highest value was with C-2 (1.24%). C-1 had the lowest ash content of 1.89 % whereas C-15 had the highest value of 7.08 %. However, the ash content was towards the higher side in the intermediate group (5.85). The crude fiber range was higher in the intermediate group i.e. out of five collections four were having more than 2% crude fiber (Vogel, 1980). From the present investigation it is concluded that different collections of *D.alata* vary greatly for their dry matter, starch protein, fat, ash, and crude fiber content with respect to their agro climatic and wild genetic stock (Brown, 1995).

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In the search for a new field of mathematics

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Abstract: Needless to say, mathematics is the mother of all of science. Algebra and geometry were the first two branch of mathematics. Primitive man started to pronounce 1, 2, 3, …. etc. nearly 30000 years ago. Today, there are more than 50 branches of mathematics. In this submission, the authors propose a new idea for the origin of a new field of mathematics. A preliminary result and an open problem are discussed. [Nature and Science. 2009;7(7):33-40]. (ISSN: 1545-0740).

Key Words: : number theory, algebra, geometry, Euclidean postulates, non-Euclidean geometries and physical applications to geometry.

PACS:02.40.Dr

Journey through Euclid

Euclid's 5th Postulate states that lines will always intersect at some point unless they are parallel. However, this is an *axiom*, not a *theorem*. In other words, Euclid just assumed this to be a geometric truth, without proof. Many subsequent mathematicians believed this Postulate was independent of the other 4 Postulates; one could prove it as a Theorem using only the other Postulates. However, nobody was ever able to complete such a proof, and in 1868, the mathematician Beltrami formally proved that the 'Axiom of Parallels' was completely independent of the other Postulates.

What does this mean? Apart from Euclid's Postulate, there is no guarantee that parallel lines cannot meet. Thus the several varieties of 'non-Euclidean' Geometry (where parallel lines can meet) can be entirely consistent.

Why did mathematicians feel the need to deduce the parallel postulate from the other axioms of geometry? After all, if you are going to start from some axioms, it doesn't much matter how many there are. Nobody seemed to mind that the other axioms were independent of each other.

Suspicion of the parallel postulate goes back to Euclid, who was the first person to notice (in writing at any rate) that it was needed for some arguments. Whenever he could, he avoided using it, even if that meant producing longer proofs. Did people have some inkling of non-Euclidean geometry, some premonition that the parallel postulate might be false?

No, they certainly did not. However, they felt uneasy about the parallel postulate because it was more complicated to state than the other axioms, and not quite as obviously true. If you have a line L and a point x not on it and claim that there is a line M through x that does not meet L, then you are making a statement about the whole, infinite line M and are therefore on dodgier ground than you are with the other axioms. It seems strange to have to deduce that the angles of a triangle add to 180 by appealing to what goes on unboundedly far away.

Incidentally, there were some serious attempts at proofs of the parallel postulate, but they all turned out to depend on hidden assumptions that were themselves equivalent to the parallel postulate (as is obvious if one bears hyperbolic geometry in

mind). For example, one proof used the fact that for every triangle there is a similar triangle of any given size - which is false in the hyperbolic plane.

The development of non-Euclidean geometry caused a profound revolution, not just in mathematics, but in science and philosophy as well.

The philosophical importance of non-Euclidean geometry was that it greatly clarified the relationship between mathematics, science and observation. Before hyperbolic geometry was discovered, it was thought to be completely obvious that Euclidean geometry correctly described physical space, and attempts were even made, by Kant and others, to show that this was necessarily true. Gauss was one of the first to understand that the truth or otherwise of Euclidean geometry was a matter to be determined by experiment, and he even went so far as to measure the angles of the triangle formed by three mountain peaks to see whether they added to 180. (Because of experimental error, the result was inconclusive.) Our present-day understanding of models of axioms, relative consistency and so on can all be traced back to this development, as can the separation of mathematics from science.

The scientific importance is that it paved the way for Riemannian geometry, which in turn paved the way for Einstein's General Theory of Relativity. After Gauss, it was still reasonable to think that, although Euclidean geometry was not *necessarily* true (in the logical sense) it was still *empirically* true: after all, draw a triangle, cut it up and put the angles together and they will form a straight line. After Einstein, even this belief had to be abandoned, and it is now known that Euclidean geometry is only an approximation to the geometry of actual, physical space. This approximation is pretty good for everyday purposes, but would give bad answers if you happened to be near a black hole, for example.

Even before Beltrami proved the independence of the Parallel Postulate, mathematicians were still able to work on Projective Geometry. In the early 17th Century, Kepler suggested the notion of 'points at infinity' where parallel lines would intersect; meanwhile Desargues and Pascal began to study Geometry using only intersections. Once Kepler's idea was taken seriously, Geometers saw that the Geometry of intersections (incidence relations) could be made into a wholly consistent theory. As suggested above, if all lines are guaranteed to meet at one point, the study of intersections does not have to make any exceptions (a flaw of Euclidean Geometry). Finally, in 1871, Klein proved that the entire theory of Projective Geometry is independent of the Parallel Postulate.

Hyperbolic geometry

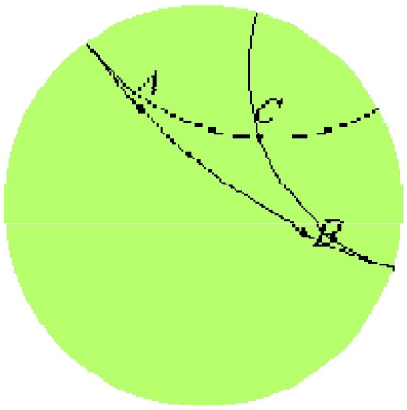
Two important geometries alternative to Euclidean geometry are elliptic geometry and hyperbolic geometry..

These three geometries can be distinguished by the number of lines parallel to a given line passing through a given point. For elliptic geometry, there is no such parallel line; for Euclidean geometry (which may be called parabolic geometry), there is exactly one; and for hyperbolic geometry, there are infinitely many.

It is not possible to illustrate hyperbolic geometry with correct distances on a flat surface since a flat surface is Euclidean. Poincaré, however, described a useful model of hyperbolic geometry where the "points" in a hyperbolic plane are taken to be points inside a fixed circle (but not the points on the circumference). The "lines" in the hyperbolic plane are the parts of circles orthogonal, that is, at right angles to the fixed circle. And in this model, "angles" in the hyperbolic plane are angles between

these arcs, or, more precisely, angles between the tangents to the arcs at the point of intersection. Since "angles" are just angles, this model is called a *conformal* model. Distances in the hyperbolic plane, however, are not measured by distances along the arcs. There is a more complicated relation between distances so that near the edge of the fixed circle a very short arc models a very long "line."

Once this model is accepted, it is easy to see why there are infinitely many "lines" parallel to a given "line" through a given "point." That is just that there are infinitely many circles orthogonal to the fixed circle which don't intersect the given circle orthogonal to the fixed circle but do pass through the given point.



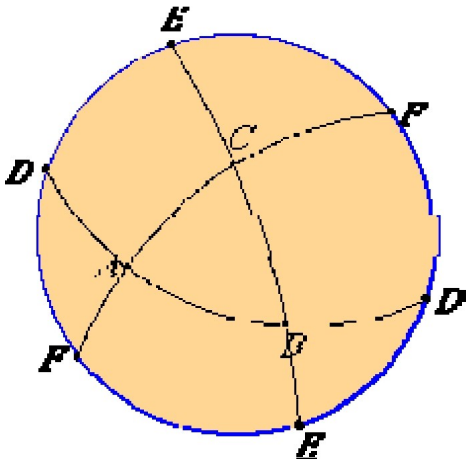
In the diagram, *AB* is a "line" in the hyperbolic plane, that is, a circle orthogonal to the circumference of the shaded disk which represents the hyperbolic plane. A "point" *C* lies in that plane. Two "lines" are shown passing through *C,* one gets close to the line *AB* in the direction of *A,* the other gets close in the direction of *B.* But these two "lines" don't intersect *AB* since the arcs representing them only intersect on the circumference of the disk, and points on the circumference don't represent "points" in the hyperbolic plane.

These two parallel "lines" are called the *asymptotic* parallels of *AB* since they approach *AB* at one end or the other. There are infinitely many parallels between them. (In much of the literature on hyperbolic geometry, the word "parallels" is used for what are called "asymptotic parallels" here, while "nonintersecting lines" is used for what are called "parallels" here.)

Elliptic geometry

Plane elliptic geometry is closely related to spherical geometry, but it differs in that antipodal points on the sphere are identified. Thus, a "point" in an elliptic plane is a pair of antipodal points on the sphere. A "straight line" in an elliptic plane is an arc of great circle on the sphere. When a "straight line" is extended, its ends eventually meet so that, topologically, it becomes a circle. This is very different from Euclidean geometry since here the ends of a line never meet when extended.

The illustration on the right shows the stereographic projection of one hemisphere. Since only one hemisphere is displayed, each "point" is represented by one point except those "points" such as *D, E,* and *F* on the blue bounding great circle which appear twice.



A "triangle" in elliptic geometry, such as *ABC,* is a spherical triangle (or, more precisely, a pair of antipodal spherical triangles). The internal angle sum of a spherical triangle is always greater than 180°, but less than 540°, whereas in Euclidean geometry, the internal angle sum of a triangle is 180° as shown in Proposition I.32.

Elliptic geometry satisfies some of the postulates of Euclidean geometry, but not all of them under all interpretations. Usually, Post.1, to draw a straight line from any point to any point, is interpreted to include the uniqueness of that line. But in elliptic geometry a completed "straight line" is topologically a circle so that any pair of points on it divide it into two arcs. Therefore, in elliptic geometry exactly two "straight lines" join any two given "points."

Also, Post.2, to produce a finite straight line continuously in a straight line, is sometimes interpreted to include the condition that its ends don't meet when extended. Under that interpretation, elliptic geometry fails Postulate 2.

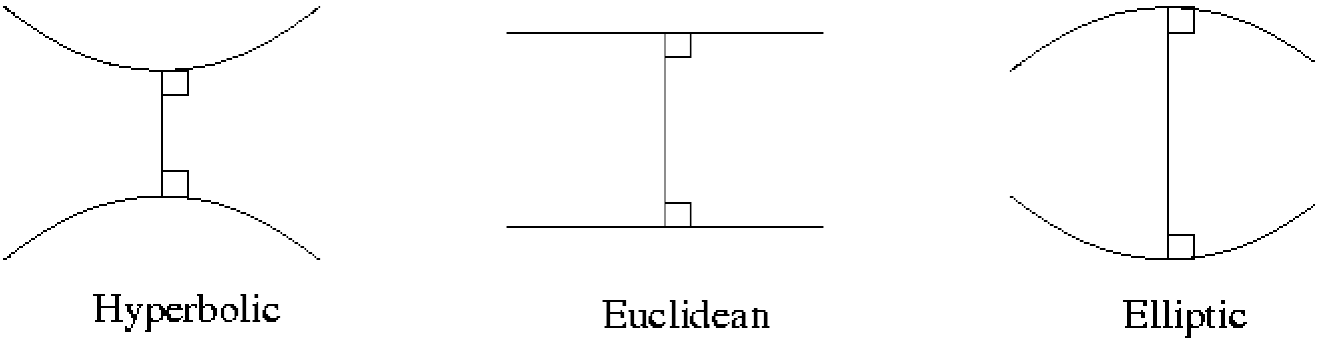
Elliptic geometry fails Post.5, the parallel postulate, as well, since any two "straight lines" in an elliptic plane meet. That is, any two great circles on the sphere meet at a pair of antipodal points.

Finally, a completed "straight line" in the elliptic plane does not divide the plane into two parts as infinite straight lines do in the Euclidean plane. A completed "straight line" in the elliptic plane is a great circle on the sphere. Any two "points" not on that "straight line" include two points in the same hemisphere, and they can be joined by an arc that doesn't meet the great circle. Therefore two "points" lie on the same side of the completed "straight line."

The proof of this particular proposition fails for elliptic geometry, and the statement of the proposition is false for elliptic geometry. In particular, the statement "the angle *ECD* is greater than the angle *ECF*" is not true of all triangles in elliptic geometry. The line *CF* need not be contained in the angle *ACD.* All the previous propositions do hold in elliptic geometry and some of the later propositions, too, but some need different proofs.

Another way to describe the differences between these geometries is as follows: consider two lines in a plane that are both perpendicular to a third line. In Euclidean and hyperbolic geometry, the two lines are then parallel. In Euclidean geometry, however, the lines remain at a constant distance, while in hyperbolic geometry they "curve away" from each other, increasing their distance as one moves farther from the point of intersection with the common perpendicular. In elliptic geometry, the

lines "curve toward" each other, and eventually intersect; therefore no parallel lines exist in elliptic geometry.



Behavior of lines with a common perpendicular in each of the three types of geometry

In a nutshell:

Euclid (circa 300 BC) produced the definitive treatment of Greek geometry and number theory in the 13 volume Elements.

Ptolemy (circa 130 AD) assumed that there was at least one line parallel to a line through a given point which is equivalent to Euclid's postulate-circular reasoning

Proclus (410-485) assumed parallel lines are always equidistance which is an added assumption about parallel lines.

Wallis (1616-1703) proved the Parallel Postulate assuming a postulate about Similar Triangles which is equivalent to Euclid's postulate-circular reasoning.

Saccheri (1667-1733) worked with quadrilaterals, now called Saccheri quadrilaterals, where the base angles are rights angles and the sides adjacent to the base are congruent.

The question is: what can be proven about the summit angles, <D and <C? Without assuming the Parallel Postulate, it can be proven that the two summit angles are congruent. Then, there are three distinct possibilities:

The summit angles are acute angles.
the summit angles are right angles.
the summit angles are obtuse angles.

What Saccheri Finally Wrote Was: "The hypothesis of the acute angle is absolutely false, because [it is] repugnant to the nature of the straight line!" (Greenberg, p.155)

Clairaut (1713-1765) proved the Parallel Postulate assuming a postulate about the Existence ofRectangles which is equivalent to Euclid's postulate-circular reasoning.

Legendre (1752-1833) worked with the Parallel Postulate assuming a postulate about angle sum of a triangle being equal to 180o which is equivalent to Euclid's postulate-circular reasoning.

\Lambert (1728-1777) worked with quadrilaterals, now called Lambert quadrilaterals, which have three right angles. The question is what can be said about the fourth angle?

Since so many mathematicians had tried to prove Euclid's Parallel Postulate, Klügel did his doctoral thesis in 1763 finding the flaws in 28 different proofs of this postulate. The thesis led d'Alembert to call Euclid's Parallel Postulate "the scandal of geometry." (Greenberg, p.161)
The Hungarian Farkas Bolyai wrote to his son János:

You must not attempt this approach to parallels. I know this way to its very end. I have traversed this bottomless night, which extinguished all light and joy in my life. I entreat you, leave the science of parallels alone. I thought I would sacrifice myself for the sake of truth. I was ready to become a martyr who would remove the flaw from geometry and return it purified to mankind. I turned back when I saw that no man can reach the bottom of the night. I turned back unconsoled, pitying myself and all mankind.

I have traveled past all reefs of this infernal Dead Sea and have always come back with broken mast and torn sail. The ruin of my disposition and my fall date back to this time. I thoughtlessly risked my life and happiness. (Greenberg, pp: 161-162) The son János Bolyai (1802-1860)wrote back:

It is now my definite plan to publish a work on parallels as soon as I can complete and arrange the material. When you, my dear Father, see them, you will understand; at present I can only say nothing except this: that out of nothing I have created a strange new universe. All that I have sent you previously is like a house of cards in comparison to a tower.(Greenberg, p. 163)

When János's father send his work to Gauss (1777-1855), Gauss wrote back that he, in essence, had done this work but would never publish it since: Most people have not the insight to understand our conclusions and I have encountered only a few who received with any particular interest what I comcated to them.

(Greenberg, p. 178)

- **C Lobachesky (1792-1656) was the mathematician first to publish an account of non-Euclidean geometry in 1829. However, the original was published in Russian. It was not until 1840 that the work was published in German and received some recognition. Since his work openly challenged Kant's view of space as "a priori" knowledge, he was fired 1846 from his university post.**
- C **In 1868, Beltrami settled the question about Euclid's Parallel Postulate by proving that no proof was possible.**
- C **Riemann (1826-1866) developed elliptic geometry starting in 1854.**
- C **Klein, Beltrami and Poincaré worked in the last half of the 19th century in developing models for hyperbolic geometry.**

- **In 1882, Pasch developed one of the first modern set of axioms for Euclidean geometry.**
- **In 1902, Hilbert, a great champion of the axiomatic method, published a set of axioms which filled the gaps for Euclidean geometry.**
- **In 1932, Birkhoff developed a new set of axioms for geometry, based totally on the connections between geometry and real numbers and include distance and angle as undefined terms.**
- **Gödel, in 1940, proved that no mathematical system can be complete.**

Equivalent Statements for Hyperbolic Geometry:

- **Given a line and a point P not on, there are at least two distinct lines through P parallel to.**
- **Every triangle has angle sum less than 180o**
- **If two triangles are similar, then the triangles are congruent.**
- **There exist an infinite number of lines through a given point P parallel to a given line.**
- **In the Saccheri quadrilateral, the summit angles are congruent and less than 90o**
- **In the Lambert quadrilateral, the fourth angle is less than 90o.**
- **Rectangles do not exist.**

Geometry is the second field of mathematics. It is the extension of number theory. There is no exact period for the origin of classical geometry. Euclid of Alexandria was the first mathematician who compiled Elements which contains propositions and constructions. In Elements, Euclid assumed five postulates. Euclid could not prove the parallel postulate. After Euclid almost all the mathematician attempted to deduce the fifth postulate from the first four postulates. But unfortunately all of them failed. The studies on this famous historical problem gave birth to two consistent models of non-Euclidean geometries. These affine geometries are widely used in quantum physics and relativistic mechanics. Also, the surveys and research led to a number of propositions equivalent to the fifth postulate. One among them is Saccheri's similar triangle proposition. In this work the authors derive the preliminary result and sincerely propose the open problem by using a physical phenomena.

Preliminary Result

In classical and Riemannian geometries we can construct similar triangles. But it is impossible to draw a triangle similar to the given triangle in Lobachevskian geometry. Let ABC be the given Lobachevskian triangle. By using computer technology and software magnify this triangle. And let $A'B'C'$ be the magnified triangle of the given Lobachevskian triangle ABC. It is well known that in magnification the angles are preserved. So, the Lobachevskian triangles ABC and $A'B'C'$ are similar. Without assuming Euclid's fifth postulate, we have derived this preliminary result. This establishes Saccheri's above said theorem [1,2,3,4]. But it has been shown once and for all that the fifth postulate is a specialcase. The author has proved this impossibility and published his paper [6]. This computer-cum-mathematical work has no equations at all.

Conclusion

Magnification is a Universal phenomenon. This technique is applied in physics, astronomy, biology, medicine, architecture, particle physics, genetics, microbiology and in chemistry. Without magnification deep studies and research in the above said fields are impossible. For the first time in the history of mathematics, the authors applied magnification technology and obtained a solution for a nearly 4300 year old parallel postulate problem. To put it in a layman's language, an impossible has been shown to be possible. This is a problematic problem. Further studies will give birth to a new branch of mathematical science.

ACKNOWLEDGEMENTS

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Dicsussion:

Since we have derived (21) without assuming the parallel postulate. (21) establishes the fifth Euclidean postulate.[2 - 7] Our construction,i.e figure 1 can be extended to both hyperbolic and elliptic spaces also.Through out this work, we have applied only the fundamental operations of number theory and algebra.So, (21) is consistent.If it is inconsistent, immediately it implies that one plus two is NOT equal to three.This is absurd.Similarly to brand that (21) is incorrect is also absurd.Only God is the Number One expert.The almighty reveals some message through (21).We have to probe into (21) which will definitely give birth to a new field of science.

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Molecular Characterization of *Valeriana* Species with PCR, RAPD and SDS PAGE

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Abstract

Characterization of plant germplasm using molecular techniques is playing an important role in the management and utilization of plant genetic resources. The present study deals with the standardization of the protocol for isolation of DNA from *Valeriana wallichi* DC. and *Valeriana officinalis* L., to standardize the protocol for isolation of total soluble protein and to standardize the PCR condition for RAPD analysis and SDS-PAGE analysis for total soluble protein. The DNA quality was detected by UV spectrophotometer and Agarose gel electrophoresis. The DNA of leaf tissues from two biotypes of *Valeriana* was analyzed and the amount of DNA in mg per gm was calculated by taking absorbance at 260 nm/290 nm. The ratio of absorbance 260 nm/280 nm was found to be in the range of 1.6 to 1.8 and the DNA yield ranged from 0.59 µg/ml to 0.90 µg/ml. This work shows that the DNA, which was isolated by some modifications in the CTAB method, was of high quality containing very low contamination of terpenoids and polysaccharides. The chemicals, which were used during isolation of DNA by CTAB method, increase DNA purity by removing all impurities. Long-term chloroform isoamylalcohol treatment removes chlorophyll, pigments and dyes. Overnight treatment of RNase degrades RNA. Other precipitates (detergents, protein, polysaccharides etc.) were removed by additional step of phenol:chloroform:isoamylalcohol (25:24:1,v/v/v) and phenol:chloroform (24:1). DNA isolated by this method yielded strong and reliable amplification products showing it's compatibility for RAPD-PCR using AG2 primer. Reproducible amplifiable products were observed in all PCR reactions. Thus the results indicate that the optimized protocol for DNA isolation and PCR was amenable to plant species belonging to different genera which is suitable for further work on diversity analysis. [Nature and Science. 2009;7(7):41-49]. (ISSN: 1545-0740).

Key words: *Valeriana,* DNA, SDS-PAGE, RAPD

Introduction

India, with its tropical climate and varied ecological features, is rich in biodiversity of plants. There are many families, which comprise so many medicinal plants for e.g. Ranunculaceae, Valerinaceae etc. Valerinaceae is a well-known medicinal family in India and contain only 10 genera and about 370 species, mostly distributed in North Temperate region. Some species are reported from high altitude region of tropical zone only. The members of family Valerianaceae have long been used as sedatives in Europe and Asia. Valerianaceae occurs naturally throughout the world except Australia and New Zealand. Among 250 known species, three have commercial importance: *Valeriana wallichi* DC (Indian Valerian), *Valeriana edulis* Nutt., ssp. *procera* F.G. Mayer (the Mexican Valerian) and *Valeriana officinalis* L. The plants have diploid, tetraploid and octaploid forms and therefore display considerable morphological diversity. Indian valerian root yields a volatile oil (0.5-2.12%) and root rhizomes are reported to contain upto 3.82% monoterpene derivatives called 'Valepotriates' used in preparation of medicines (Bajaj, 1999). The dried rhizome and roots of *V. officinalis* L. are used medicinally in certain cardiac ailments. The fresh juice of the rhizomes and roots containing a volatile oil is used against nervous disorders and certain cardiac disease. The efficiency of the drug is lost on drying. To an evolutionary biologist or a breeder, variation among plants species has always fascinated a mind of enquiry and helped to select desirable variants or breed a new from of greater agronomic value.

Taxonomic studies and molecular characterization of medicinal plants play an important role in generating new crop varieties with the high yield potential and resistance to environmental stresses. DNA based markers provide powerful and reliable tools for discerning variation within germplasm and to study evolutionary relationships (Gepts, 1993, Joshi et al., 2009). PCR based techniques have been used successfully in DNA fingerprinting of plant genomes and in genetic diversity studies. These techniques include RAPD (Randomly amplified polymorphic DNA),

RFLP (Restriction Fragment Length Polymorphism), SSR (Simple Sequence Repeat) and AFLP (Amplified fragment Length Polymorphism). So far, the most used techniques seems to be PCR and RAPD (Chong et aI., 1994; Lowe et aI., 1996).These procedures, due to their great sensitivity, constitute a powerful technique widely used for enzymatic amplification of stretches from small amounts of DNA and provide an alternative approach to distinguish genotypic variants. In RAPD technique short oligonucleotides of arbitrary sequences are used singly to support the amplification of the plant genome and amplification products are separated by gel electrophoresis. RAPD technique has also been used in plants for the construction of genetic maps (Reitar *et al*., 1992) and molecular characterization (Mehmood et al., 2008). Very little information exists on the molecular aspects of *Valeriana species, which* requires high quality DNA. Therefore, the present study was planned with following objectives

1. Taxonomic consideration and protocol standardization for isolation of DNA from *Valeriana* species (i.e. *V. officinalis* L. and *Valeriana wallichi DC.*).
2. To standardize protocol for isolation of total soluble protein, PCR conditions for RAPD analysis and SDS-PAGE analysis for total soluble protein.

Material and methods

Plant material

Two species, *V. wallichii* DC., *and V. officinalis L.* were taken for experiment. *V .officinalis L.* was collected from G.B. Pant Institute of Himalayan Environment & Development, Kosi – Katarmal, Almora (Uttarakhand) and grown at glass house of Deptt. of Botany D.S.B. campus Nainital(Kumaun University Nainital). *V. wallichii* DC. was collected from Ayarpata Nainital .

DNA Extraction

Total genomic DNA was extracted using CTAB method (Doyle & Doyle, 1987) with some modification. 1gm freshly harvested leaf whose gel was removed was ground to fine pulp using liquid nitrogen along with 0.1 g PVP. Extraction buffer (pH-8) preheated to 65ºC containing 2% CTAB (w/v), 5.0 M NaCl, 0.5 M EDTA and 0.5 m tris HCl were added to the pulp in a centrifuge tube, shacked and incubated for 1 hour at 65 ºC in a water bath with intermittent shacking and swirling in every half an hour. To this equal volume of Chloroform:Isoamylalohol (24:1) was added and mixed by inversion for 30 min and centrifuged at 12,000 rpm for 15 min. Supernatant was transferred to a new tube and was precipitated with equal volumes of cold Isopropanoal, and gently mixed to produce fibrous DNA and incubated at -20ºC for 30 min. Samples were centrifuged at 12,000 rpm for 15 min. The pellete was washed with 70% ethanol and kept for drying. After drying, the pellete was dissolved in 3 µl of TE buffer (1 mM EDTA and 10 mM Tris HCL pH-8). To remove contaminating RNA 5 µl of RNAs (10 mg/ml) was added. The tubes were incubated over night at 37ºC. Dissolved DNA was extracted with equal amount of Phenol:Choloroform: Isoamylalcohol (25:24:1.v/v/v) and centrifuged at 8000 rpm for 15 min. then aqueous layer was transferred to a fresh 15 ml tube and equal volumes of chloroform:isoamyalcohol (24:1) was add and centrifuged at 12,000 rpm for 15 min. Finally supernatant was transferred to a fresh tube, equal volume of absolute alcohol and 1/10 volume of sodium acetate were added and incubated at -20ºC for 30 min and centrifuged at 12,000 rpm for 15 min. The final pellet was dried and resuspended in TE buffer.

Analysis of *Valeriana* sp.through RAPD

For RAPD analysis, the standards given in **Table 1** are used. In RADP analysis both the species were analyzed and compare simultaneously. For RAPD analysis, a large number of universal primers (decamers) (1,2,3,4,5,6,7 and 8) are used. For the optimization of RAPD reaction from *Valeriana* species, arbitrary oligonucleotide primers were used for amplification to standardize the PCR condition. The reaction was carried out in a DNA Thermocycler (Biometra). Reaction without DNA were used as a negative controls. Protein was extracted from the leaves of *V. wallichii* DC. and V. *officinalis* L. both (1 g each) using Borate Buffer (pH 9.0) and centrifuged at 12000rpm for 30 min.

Table 1 Standards for RAPD amplification

Steps	Temp(°C)	Duration
Lid temp	105	
T1	94	2min
T1	94	30sec
T2	55 (gradient of 10 °C)	45sec
T3	72	30sec

DNA amplification

For polymerase chain reaction 02 oligonucleotide primers (17-30 nucleotide) were used. The analysis conditions and quantification of product are given in **Table 2** and **Table 3**.

Table 2 Analysis of *Valeriana sp.*Through PCR

Contents	Amount (µl)	Conc.
Water	21.9	-
Assay buffer	0.5	10x
dNTPs	1.0	1mM
Primers	0.4	1µM
Taq	0.2	1U
Template (sample DNA)	1.0	25.0ng

Note- Primer used is AG21.

Table 3 Thermocycler programme

Steps	Temp(°C)	duration
Lid temp	105	-
T1	94	2min
T1	94	30sec
T2	55	45sec
T3	72	30sec

Agarose gel electrophoresis

PCR products were electrophorased on 1.5 %(w/v) agarose gels, in 1x TAE Buffer at 50 V for 3 h and than stained with ethidium bromide (0.5 µi/ml). Gels with amplification fragments were visualized and photographed under UV light (260 and 280 nm).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS – PAGE)

Glass slabs, spacers and spacers were washed with water and wiped with tissue paper. Plates are set in the gel caster and sealed with the help of sealing agar (1% agarose). Constituents of the resolving gel were mixed in the given ratio as per given in table. Resolving gel was poured down from one edge of the spacer, leaving a gap of two to three cm. on top. Water saturated n-butanol was pipetted above it. The gel was allowed to polymerize for 45 – 90 min. After polymerization the gel surface is rinsed several times with triple distilled water and drained well. Comb was placed keeping a distance of 1 - 1.5 cm. from separating gel, and the constituents of stacking gel were mixed and were poured from one side of comb. It was allowed to polymerize for 45 – 60 minutes. After polymerization the comb was removed carefully and the plates were fixed on to the electrophoresis apparatus. 10 – 20 µg samples were loaded in each well. The gel was run at 15µA constant current until the dye front reached the bottom of the gel. The protein bands were detected by silver staining.

Data analysis

Concentration of protein were determined by Lawry's method (1951) and comparing the concentration of the samples with standard curve value of BSA. Bands with same mobility were

treated as identical fragments. The positions of PCR bands were compared with molecular weight standards.

Results and Discussion

Taxonomic consideration

Most taxonomists place the family Valerinaceae along with the Caprifoliaceae in the order Dipsacales or Rubiales. Hutchinson treats it as a member of Valerinales. From the studies undertaken and data collected its correct position is the Dipsacales among with the Caprifoliaceae and other families.

Valeriana species

Valerian is a common name given to the genus *Valeriana*. The genus *Valeriana* (family – Valerinaceae) comprises large number of species, around 350, which are throughout the world (Bantly et al., 1983). Out of these 20% have been recorded as used for medicinal purposes. This herb may have been named Valerian after the Roman Emperor Valerian (Pubhus Licinius Valerianus, 253-260 A.D.), who first used it in medicine. The other sources mention the name derived from Latin word "Valere" means to be strong and healthy.

About 12 species have been reported to occur in India. Three species viz. *V. wallichii, V. hardwickii, and V. pyrolaefolia* are found in the temperate Himalayas from Kashmir to Bhutan and Khasia hills at 1200-1800m. *V. officinalis* is native to Europe and north and south – west Asian countries. *Valeriana wallichi* (commonly known as tagar, samao) is a perennial herb. The roots and rhizome with or without stolons are used in unani and ayurvedic system of medicine.

Valeriana officinalis L. (Common Valierin or Garden Heliotrope) (Plate 1)

A common glabrous herb; attain a height up to 1 m. Stem solitary, erect. Furrowed and hollow. Leaves opposite, pinnate, lower leaves with long petioles and upper small. Flower appears in panicle, corymb, and white or dull white in colour, fragrant, small calyx many toothed and corolla 5-lobed. Fruit oblong, ovate, small, smooth, without hairs, one seeded. Rootstock is thicker, short with many fasciculate rootlets. This species is native to Europe and Kashmir in India at 2500 m altitude. Now a day it is cultivated in many parts of India and world. The plant is propagated by seeds or through rootstocks. It grows in temperate climate and in India cultivated in Kashmir, H.P., U.P. and Uttarakhand. Medium fertile soil, rich in humus is suitable for this crop. Seeds are sown in well-drained raised beds in the nursery during April-May or July- August.

The plant consists of rhizome, stolons and roots, which constitute the drug. Roots are harvested after two years during their dormancy in November. Roots are washed thoroughly, washed properly and dried. The root contains epoxy-iridoid esters called valepotriares, which include valtrate didrovaltrate, acevaltrate, isovaltrate and isovaleroxyhydroxy didrovaltrate. It also contains chatinine and valerine alkaloids and volatile oil.

The drug is said to be sedative, hypertension, antispasmodic, and stomachic. The juice of fresh rhizome is used in treatment of hysterical fits and other nervous disorders and flatulence. The root preparation is also known as tonic and stimulant. The fresh juice is administered in case of insomnia and in certain cardiac preparations. The essential oil of root is used as a flavour in food products and beaverages.

Plate 1 *Valeriana officinalis* L.

***Valeriana wallichi* DC. *(syn V. jatamansi jones)* (Plate 2)**

It is another species of similar properties as that of *V. officinalis* L. it is a perennial herb upto 45 cm tall and stems tuffed. Flowers are white tinged with pink. Rootstock thick and horizontal, aromatic and nodular. This species is native to Himalayan region of India, Nepal and South Western China. Roots of this plant are useful in diseases of eye, blood, liver and spleen enlargement. They are useful in clearing of voice. The crushed leaves are rubbed in extreme forehead. The root preparations are used as cosmetic and hair oil.

Plate 2 *Valeriana wallichi DC*.

Extraction of Genomoic DNA

The extracted protein of *Valeriana* sp. was of high quality as it showed a reading between 1.6 and 1.8 after calculating the ratio 260/290 nm absorbances. The DNA yield obtained ranged from 0.59µl/ml to 0.90µl/ml.

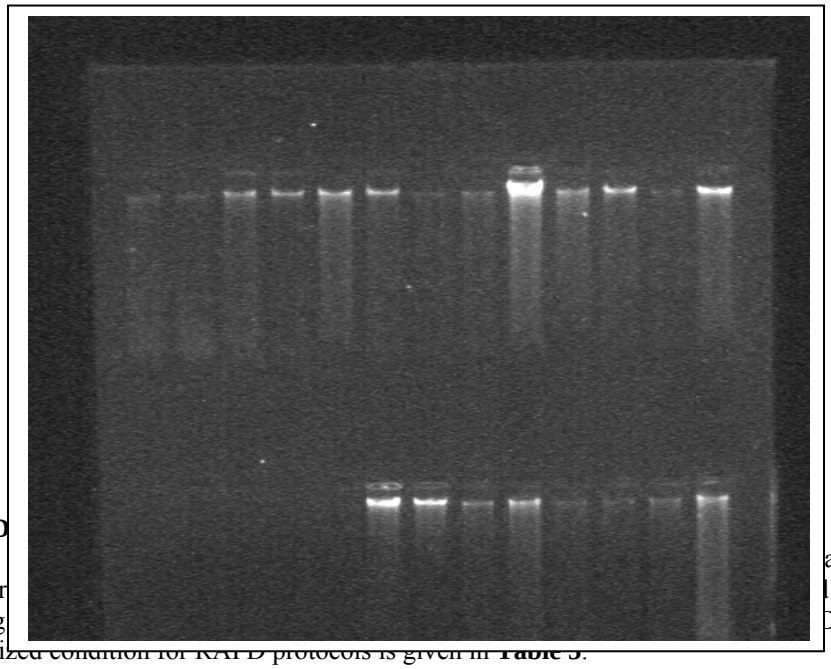
Total genomic DNA

The quality and quantity of DNA isolated was checked in UV spectrophotometer and agarose gel electrophoresis (**Fig. 1.**). The DNA bands extracted with modified CTAB method did not show any smearing or RNA contamination. The concentration of genomic DNA ranged between 1 to 2µl/ml. DNA isolated by this method yielded strong and reliable amplification products showing it's compatibility for RAPD-PCR using AG2 primer. DNA isolated by this method was of high quality and there was very low contamination of terpenoids and polysaccharides. The presence of terpenoids reduces the yield and purity of extracted DNA since it is a strong oxidizing agent and binds to DNA covalently, making it useless for research applications (Perterson et al. 1997; Porebaski et al. 1977). Tannins, terpenes and resins are considered as secondary metabolites, are also difficult to separate from DNA (Ziegenhagen and Scholz, 1998). Certain polysaccharides are known to inhibit RAPD reactions. They distort results in many analytical applications and therefore, lead to wrong interpretations (Kotchoni et al. 2003).

Co precipitation of polysaccharides is avoided by adding a selective precipitant of nucleic acid i.e.CTAB. Additions of PVP along with CTAB may help in removal of impurities because it forms a complex with polyphenols through hydrogen bonds. Long – term chloroform isoamyl alcohol treatment removes chlorophyll and other coloring substances such as pigments and dyes.

Many DNA isolation producers also yield large amounts of RNA (Doyle and Doyle, 1987). RNA in samples can chelate Mg^{+2} and can reduce the yield of PCR. An overnight treatment of RNAse degraded RNA and yielded RNA free pure DNA. Other precipitates (detergents, proteins, polysaccharides) were removed by additional precipitation step of phenol; chloroform; isoamyl alcohol (25:24:1,v/v/v) and phenol:chloroform(24:1) (Khanka et al., 2009).

W W W W W W M O O O O O O



RAPD ation of $MgCl_2$, primer time intervals during D protocol. The optimized condition for RAPD protocols is given in **Table 5**.

Table 5 Optimization of RADP-PCR reaction parameters for analysis of *Valeriana* sp.

PCR parameters	Tested range
$MgCl_2$ (mM)	1,2,3,4
DNTPs (mM)	0.1, 0.2, 0.3, 0.4
Primer concentration (µM)	0.2, 0.3, 0.4
Taq polymerase	0.2, 0.3, 0.4
Initial denaturation time interval at 94ºC (min)	2,3,4,5
Annealing temperature (ºC)	30, 35, 38, 40, 42
Time interval (sec)	20, 30, 40
Reaction volume (µl)	5, 10, 15
Number of cycles	30, 35, 40, 45, 50

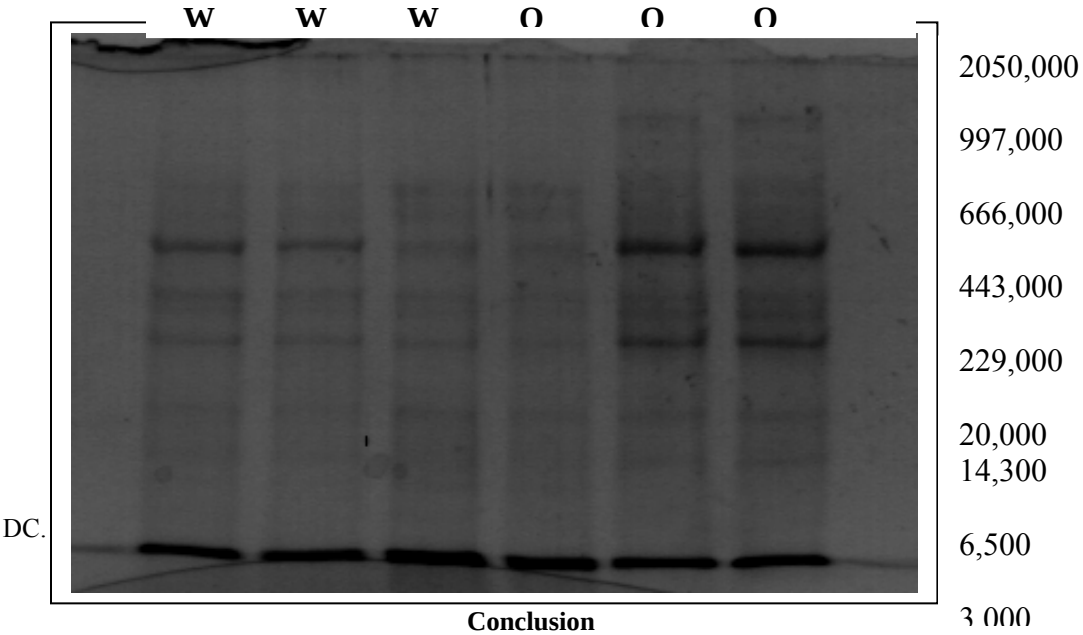
PCR amplification of DNA extracted from two species of *Valeriana* i.e. *V.* wa*llichi* DC. and *V. officinalis* L. Out of these, not any random decamer showed any positive result (**Table 6**). PCR product was run on 1.5 % agarose gel.

Table 6 Sequence of primers which gave negative results during RAPD analysis of DNA extracted from *V.* wa*llichi* DC. and *V. officinalis* L.

Sl. No.	Primers	*V.* wa*llichi* DC.	*V. officinalis* L.
1.	5'-GCAGGGATAGC-3'	-	-
2.	5'-GTCCTCAAACG-3'	-	-
3.	5'-GTCCTACTCG-3'	-	-
4.	5'CTACTACCGC-3'	-	-
5.	5'CTACACAGGC-3'	-	-
6.	5'-CCTGATGACC-3'	-	-
7.	5'-GTCCTTAGCG-3'	-	-
8.	5'-TGCCGAGCTC-3'	-	-

Protein profiling

SDS-PAGE (**Fig. 2**) analysis revealed 9 and 8 bands in *V.* wa*llichi* DC. and *V. officinalis* L. respectively, where molecular weight ranged from 3kDa to 43kDa. Silver stained revealed 12, 11 bands in *V.* wa*llichi* DC. and *V. officinalis* L. respectively, where molecular weight ranged from 3kDa to 205 kDa. This dissimilarity shows than composition of the gel differs and some proteins are present in very low concentration in one species, some are absent while distinct proteins are present in each species.



Conclusion

The study include optimize a protocol for isolation of total genomic DNA and PCR condition for RAPD analysis of *Valeriana spp*. which have high level of terpenoids, poyphenols and secondary metabolites. DNA extraction was done by modifying some steps of CTAB method originally develop for other plants (Doyle and Doyle, 1987). The DNA quality and quantity were detected by UV spectrophotometer and agarose gel electrophoresis. The DNA yield obtained ranged from 0.59µl/ml to 0.90µl/ml. The concentration of genomic DNA ranged between 1µl/ml to 2 µl/ml. This work shows that the DNA, which was isolated by some modifications in the CTAB method, was of high quality containing very low contamination of terpenoids and polysaccharides. The chemicals, which were used during isolation of DNA by CTAB method, increase DNA purity by removing all impurities.

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OPTIMIZATION OF 2, 4 DICHLOROPHENOL DEGRADABLE CRUDE EXTRACTS PRODUCED BY *Pseudomonas aeruginosa* USING BOX BEHNKEN DESIGN

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ABSTRACT: *Pseudomonas aeruginosa* was grown on mineral medium containing 2, 4 dichlorophenol as a sole source of carbon and energy. Process optimization was carried out by developing 17 combinations using Box Behnken design to identify the best combinations of the parameters which involved in the production biomass to obtain high yield of crude extract. The highest protein concentration in biomass from 17 combinations obtained from the experiment is 4.99 mg/ml (35 ml of medium, 6 ml of inducer and 6 ml of inoculum). The point prediction from the analysis of variance for response surface cubic model for the production of protein concentration (4.88 mg /ml) is 35 ml of medium, 4.5 ml of inducer and 4 ml of inoculum. [Nature and Science. 2009;7(7):50-60]. (ISSN: 1545-0740).

Keywords: 2, 4 Dichlorophenol, Crude extract, *Pseudomonas aeruginosa,* Optimization , ANOVA and Box Behnken design

1. INTRODUCTION

Tabak *et. al.*, (1964) described microbial metabolism of aromatic carbon compounds. The purpose of this investigation was to determine the ability of specifically adapted bacteria to degrade phenol and substituted phenols, and to study the relationship between the chemical structure of phenol derivatives and cyclic hydrocarbons and their susceptibility to decomposition by organisms adapted to related aromatic compounds.

Walter Reinke *et. al.*, (1984) isolated 2,4 dichlorophenol-degrading bacterium (, strain WR1306) by continuous enrichment from a mixture of soil and sewage sample & grown in a chemostat on a mineral medium with 2,4 dichlorophenol. Respiration data and enzyme activities in cell extracts as well as the isolation of 3-chlorocatechol from the culture fluid are consistent with the degradation of 2,4 dichlorophenol. Michel Rutgers *et. al.*, (1993) used nutristat to grow pentachlorophenol (PCP)-degrading microorganisms. Rebecca M Goldstein *et. al.*, (1985) explained the reasons for possible failure of inoculation to enhance biodegradation.

Ayami Nakagawa *et. al.*, (2006) found 32% of DCP was degraded within 1h.He is the first one to prove dechlorination pathway by Zygomycetes. Khadar valli *et. al.*, (1991) examined the degradative pathway of 2,4-dichlorophenol by P. chrysosporium. They showed that this pathway involves several cycles of oxidation and subsequent quinone reduction and hydroquinone methylation.

Mohammad Edrissi and Nima Razzaghi asl (2007) discussed the application of RSM method in optimizing complexation of iron with piroxicam. A response surface methodology (RSM) based on a Box-Behnken design was applied for study on ferrous ions binding ability to piroxicam in aqueous solution as a function of three numerical factors (extraction time, pH, piroxicam concentration) and extractant type as a categorical variable each in three levels.

Experimental designs nowadays have been regarded as one of the most favorable techniques in covering a large area of practical statistics and obtain unambiguous results with the least expense. Response surface

method (RSM) designs help to quantify the relationships between one or more measured responses and the vital input factors. The most popular response surface methodologies are Central Composite, Box-Behnken designs.

Box-Behnken design is an efficient and creative three-level composite design for fitting second-order response surfaces. It is an independent quadratic design. The methodology is based on the construction of balance designs which are rotatable and enable each factor level to be tested several times. Each factor or independent variable can be placed at one of three equally spaced values (coded as –1, 0, and +1). In this design the treatment combinations are at the midpoints of edges of the cubical design region and at the center.. Box-Behnken designs provide excellent predictability within the spherical design space and require fewer experiments compared to the full factorial designs or central composite designs. The number of required experiments for Box-Behnken design can be calculated according to $N = k^2 + k + c_p$, where k is the factor number and c_p is the replicate number of the central point.

In the present investigation, crude cell extracts from the enriched strain *P. aeruginosa* on 2,4 dichlorophenol was immobilized on sodium alginate beads and the beads were packed in a glass column to study the degradation. Seventeen sets of combinations of process parameters were developed to produce crude extracts. The experiment was carried out in different concentrations 2,4 dichlorophenol in the immobilized beads which contains crude extracts of *P. aeruginosa*.

2. MATERIALS AND METHODS

2.1 Maintenance and cultivation of microorganism

The strain *P. aeruginosa* was obtained from NCIM, Pune, India. The strain was sub cultured in nutrient broth. The broth was incubated in the shaker with 175 rpm and at 37ºC overnight. Sterile plates containing nutrient agar of specified composition were streak plated with the overnight cultures. The culture on the plates was used as the source for the entire experiment. The mineral medium with specified composition (Table 1) of chemical substances was prepared to conduct the experiment. The pH of the mineral medium was adjusted to 7.0 by using 2 NH_2SO4 or 2N NaOH solution. 50 ml of the medium was taken in each of 250 ml Erlenmeyer flasks and were sterilized at 1.5 kg/cm^2 (gauge) for 20 min. After cooling to room temperature, the medium was added with 2, 4 dichlorophenol and inoculated in a laminar flow chamber. The flasks were then incubated on a rotary shaker for 48 h at 30ºC and 175 rpm, for full growth of the strain. The sub cultured strains were stored at 5ºC.

Table 1 Composition of Medium

Ingredients	**Concentration (g/l)**
NH_4NO_3	1.0
$(NH_4)_2SO_4$	0.5
NaCl	0.5
K_2HPO_4	1.5
KH_2PO_4	0.5
$Mgso_4.7H_2O$	0.5
$CaCl_2$	0.01
Double distilled Water	1 l

2.2 Suspension of washed cells and cell extracts

Cells grown on 2,4 dichlorophenol as the sole carbon source, were harvested in the mid-exponential growth phase by centrifugation (8,000 rpm for 10 min at 4°C), washed with sodium phosphate buffer (pH 7.0, 50 mM), and suspended in the same buffer. The cell extracts were prepared by disrupting the cells by ultrasonic disintegration (Labsonic-P of Labsonic-Germany). The resulting cell lysate was centrifuged at 8,000 rpm for 10 min at 4°C, and the supernatant, containing approximately 10 to 20 mg of protein ml^{-1}, was the crude cell extract (containing 2,4 dichlorophenol degrading enzyme). The concentrations of protein content in the crude extracts were measured using UV Visible Spectrophotometer (Hitachi UV 2800).

2.3 Optimization of the process parameters

Process optimization was carried out by conducting 17 experiments (Table 2) to identify the best combinations of the parameters which involved in the production biomass to obtain high yield of crude extract. The parameters, the volume of mineral medium (20,35 and 50 ml), inducer (3 -6 ml) and inoculum (2 - 6 ml) were selected. The mineral medium, Inducer (2, 4 dichlorophenol) and inoculum were processed as mentioned different cultures were obtained by varying the three parameters and processed to obtain its crude extract. The concentration of the crude extract was measured at 280 nm. The data obtained from 17 experiments, were used to find out the optimum point of the process parameters by using Box Behnken Design in Response surface methodology. All the data were treated with the aid of Design Expert from Stat-Ease.

3. RESULTS AND DISCUSSION

3.1 Analysis of variance

Based on design of experiment, 17 combination were developed (Table 2) and processed to obtain crude extracts as mentioned in this paper. The data obtained from the experiments were used to the analysis of variance (Table 3 and 4). The Model F-value of 6.366E+007 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, BC, A^2, B^2, C^2, A^2B, A^2C, $AB^2$are significant model.

Table 2 Combination of process variables

Run	A:Medium (ml)	B:Inoculum (ml)	C:Inducer (ml)	Crude extract (mg/ml)
13	35	6	6	4.99
6	35	2	6	4.88
7	35	4	4.5	4.74
8	35	4	4.5	4.74
9	35	4	4.5	4.74
10	35	4	4.5	4.74

11	35	4	4.5	4.74
4	20	6	4.5	3.57
1	20	2	4.5	2.97
5	35	2	3	2.05
2	20	4	3	1.98
3	20	4	6	1.34
15	50	4	3	0.96
17	50	6	4.5	0.56
16	50	4	6	0.31
12	35	6	3	0.23
14	50	2	4.5	0.15

Table 3 Analysis of variance (ANOVA):

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	60.13575	12	5.011312	6.366E+007	< 0.0001	significant
A-Medium(ml)	1.05401	1	1.05401	6.366E+007	< 0.0001	
B-Inoculum(ml)	0.727097	1	0.727097	6.366E+007	< 0.0001	
C-Inducer(ml)	14.42253	1	14.42253	6.366E+007	< 0.0001	
AB	0.008603	1	0.008603	6.366E+007	< 0.0001	
AC	2.72E-06	1	2.72E-06	6.366E+007	< 0.0001	
BC	0.920256	1	0.920256	6.366E+007	< 0.0001	
A^2	24.39735	1	24.39735	6.366E+007	< 0.0001	
B^2	1.120969	1	1.120969	6.366E+007	< 0.0001	
C^2	5.894329	1	5.894329	6.366E+007	< 0.0001	
ABC	0	0				
A^2B	0.925412	1	0.925412	6.366E+007	< 0.0001	
A^2C	9.87168	1	9.87168	6.366E+007	< 0.0001	

AB^2	1.790021	1	1.790021	6.366E+007	< 0.0001	
AC^2	0	0				
B^2C	0	0				
BC^2	0	0				
A^3	0	0				
B^3	0	0				
C^3	0	0				
Pure Error	0	4	0			
Cor Total	60.13575	16				

Table 4 Regression Analysis

Std. Dev.	0	**R-Squared**	1
Mean	2.804	**Adj R-Squared**	1
C.V. %	0	**Pred R-Squared**	N/A
PRESS	N/A	**Adeq Precision**	3.1E-308

Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The application of response surface methodology (Kenneth et.al, 1995, Khuri, A.I.,) yielded the following regression equation, which is an empirical relationship between the logarithmic values of protein yields and test variables in coded unit.

Final equation in terms of coded factors with coefficients values (Table 5):

Y (Crude extract mg/ml) = + 4.74 - (0.51 * A) - (0.43 * B) + (1.90 * C) - (0.046* A * B) –(8.250E-004 * A * C) + (0.48 * B * C) - (2.41 * $A^{2)}$ - (0.52 * $B^{2)}$ - (1.18 * $C^{2)}$ + (0.68 * A^2 * B) – (2.22 * A^2 * C) – (0.95 * A * B^2)

Where Y is response that is the protein concentration is expressed in logarithmic values and A, B, and C are the coded values of the test variable medium, inducer and inoculum respectively.

Table 5 Coefficients obtained from regression analysis

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	4.737	1				
A-Medium(ml)	-0.513	1				2
B-Inoculum(ml)	-0.426	1				2
C-Inducer(ml)	1.899	1				2
AB	-0.046	1				1
AC	-0.001	1				1
BC	0.480	1				1
A^2	-2.407	1				1.0058
B^2	-0.516	1				1.0058
C^2	-1.183	1				1.0058
A^2B	0.680	1				2
A^2C	-2.222	1				2
AB^2	-0.946	1				2
AC^2 ALIASED A, AB^2						
B^2C ALIASED C, A^2C						
BC^2 ALIASED B, A^2B						
A^3 ALIASED A						
B^3 ALIASED B						
C^3 ALIASED C						

3.2 Analysis of process variables by response surface plots

The optimum values of the selected variables were obtained by solving their regression equation and analyzing response surface contour plots. Response Surface plots as a function of two factor at a time maintaining all other factors at a fixed level (zero for instance) are more helpful in understanding both the main and interaction effects of the two factors. The plots can be easily obtained by calculating the data from the model. The values were taken by one factor, where the second varies with constant of a given Y - values. The yield values of the different concentrations of the variable can also be predicted from respective response surface plots. Figures 1 to 6 shows the relative effects of the two variables with protein concentration level. The coordinates of the central point within the highest contour levels in each of these figures corresponded to the optimum concentrations of the respective components.

Figure 1 and 2 show their contour and response surface plot obtained as a function of volume of inoculum vs. medium with protein concentration, while all other variables are maintained at zero level (coded units). Figure 3 and 4 show their contour and response surface plot obtained as a function of volume of inducer vs. medium with protein concentration. Figure 5 and 6 show their contour and response surface plot obtained as a function of volume of inducer vs. inoculum with protein concentration.

3.3 Optimum Values

The protein production was predominantly influenced by medium and inducer concentration. From the Contour plots the red color shows the region of the desirability for the production of protein. The point prediction from the analysis of variable for response surface cubic model for the production of protein concentration (4.88 mg /ml) is 35 ml of medium, 2 ml of inducer and 6 ml of inoculum.

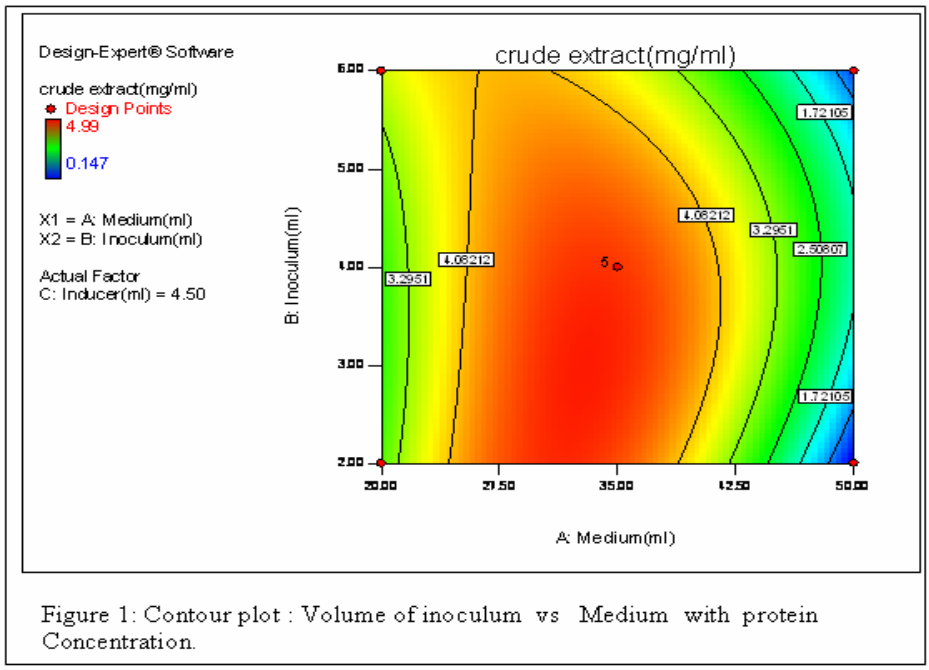

Figure 1: Contour plot : Volume of inoculum vs Medium with protein Concentration.

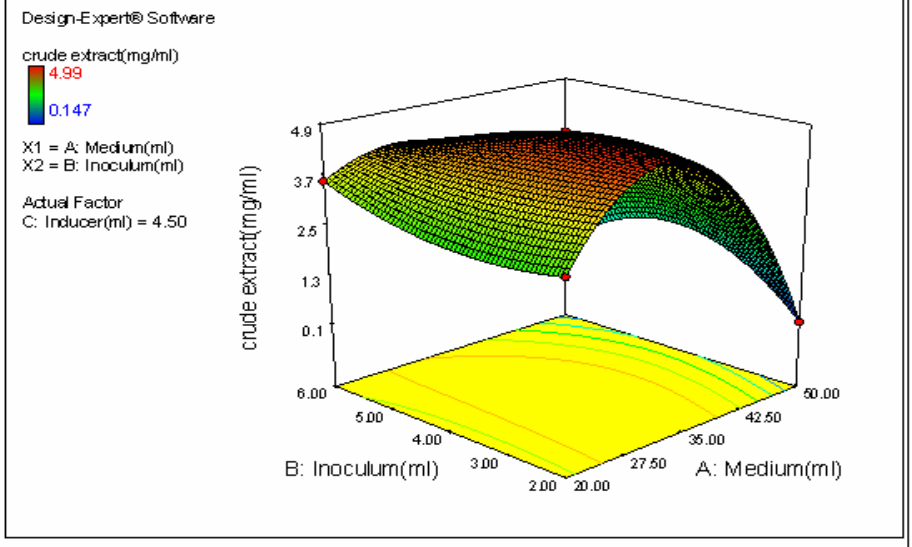

Figure 2: Response surface plot : Volume of inoculum Vs. Medium with protein Concentration.

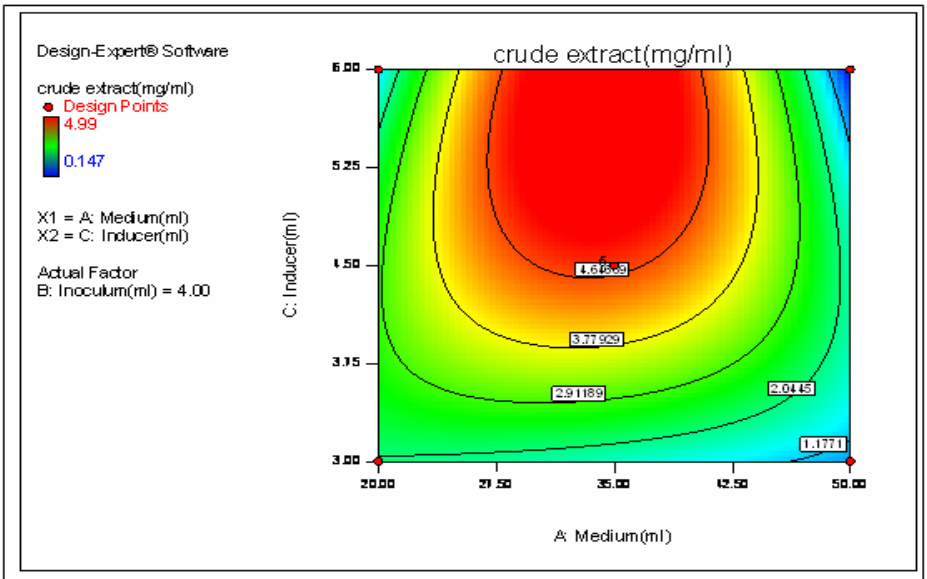

Figure 3: Contour plot : Volume of inducer Vs. Medium with Protein concentration.

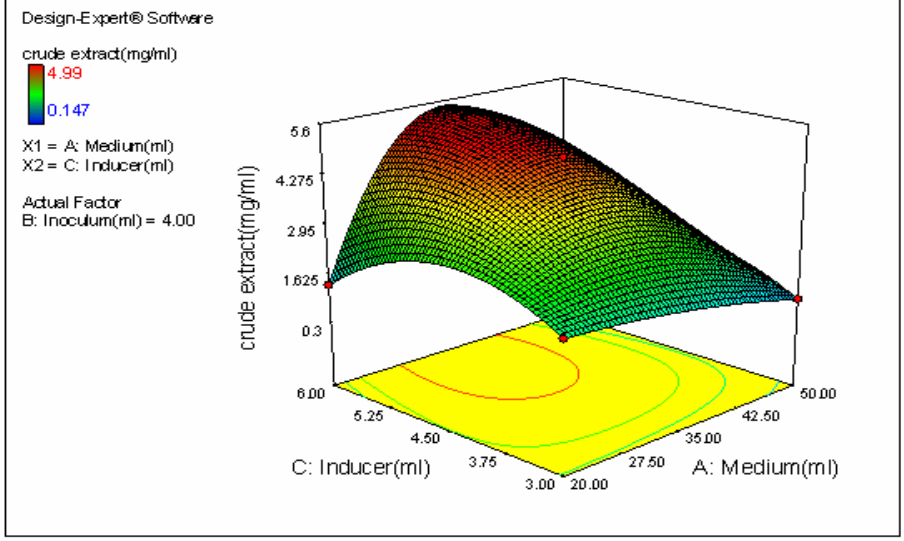

Figure 4: Response surface plot : Volume of inducer Vs. Medium with protein concentration.

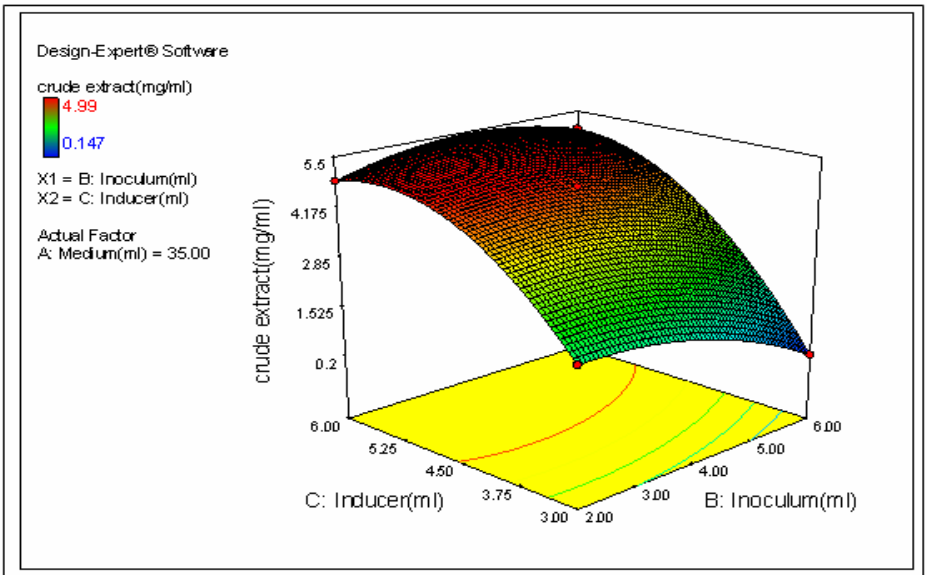

Figure 6: Response suface plot : Volume of inducer Vs. Inoculum with protein concentration.

Table 6 Predicted value from Box -Behnken design

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding	
A	Medium(ml)	35	20	50	0	Actual	
B	Inoculum(ml)	2	2	6	0	Actual	
C	Inducer (ml)	6	3	6	0	Actual	
Response	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
Crude extract(mg/ml)	4.88	0	4.88	4.88	0	4.88	4.88

PI - Prediction interval

CI - Confidence interval

SE Mean – Standard error of the mean.

SE Pred – Standard error of prediction

4 Conclusion

2,4 Dichlorophenol can induce the synthesis of enzymes in *Pseudomonas aeruginosa* that are able to break down hydrocarbons including 2,4 dichlorophenol. In this work the process conditions were optimized to produce crude extracts. Immobilization of the crude extracts obtained from *Pseudomonas aeruginosa* increases the efficiency of the extract and they have been used to study the degradation of 2,4 dichlorophenol in a packed bed column. Thus it has been concluded that this study will yield good results if extended to large-scale applications.

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A Case Study: Nainital High Altitude Zoo, Ecotourism and People Participation

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Abstract: The main aim of present study in Pandit Govind Bullabh Pant, High altitude Zoo, Nainital was to assess the role of local community in conservation and management of wildlife. There were 19 wild animals and 22 different kinds of pheasants. 18 different organizations and people adopted 16 different type of Carnivorous, Omnivorous & Pheasants. The local community and local organizations of Nainital were so keen to conserve and manage the wildlife of high altitude's Zoo, so they are much interested to adoption of wildlife. This is a new step to conservation and management of wildlife in high altitude Nainital Zoo. [Nature and Science. 2009;7(7):61-66]. (ISSN: 1545-0740).

Key words: Conservation, management, participation, ecotourism

Introduction

Conservation and management of wildlife not only preserving the remaining flora and fauna but also helping in promoting economic activities that brings money through tourism. It also contributes towards maintenance of biodiversity of landscape. Different wildlife's such as colorful birds, animals and other form of life in the forest are the important maintaining the ecosystems. Distraction of forests or its reduction will cause disappearance of much wildlife. As far as the concern of Indian region it has total area of 32 million hectare with rich in biological diversity. India is one of the 12-mega biodiversity centers in the world. It is estimated that about 45,000 species of plants comprises 15,000 species of which several hundred species are endemic to India. Besides this the region is also rich in fauna and containing about 65,000 species of animals. This richness in biological diversity is due to immense variety of climatic and altitudinal condition coupled with varied ecological habitats. These vary from the humid tropical Western Ghats to the hot desert of Rajasthan from the cold desert of Ladakh and the icy mountain Himalayas to the warm costs of Peninsular India. The following two conservation strategies are most famous for flora and fauna. In-situ conservation is the conservation of genetic resources through their maintenance with in natural or even human made ecosystem in which they occur. This is an ideal system for genetic resources conservation. This type includes a system of protected areas of different categories such as National Parks, sanctuaries, Natural Reserves, Natural Monuments, Cultural Landscapes, Biosphere Reserves, sacred Groves etc.

The origin of Zoo may be said to have commended with the opining of the London Zoo in 1828. Most of the older Zoos in North America and Europe were founded in the later part of the 19th century after 1870. During that period animal species were being regularly discovered and the various zoos were keen to collect as many different kinds of animals as possible for public display. (Sharma, 2000).

The Zoo movement in India is also one of the oldest in world. The setup Zoo was in Madras in the year of 1855, which is seen followed by Trivandrum (1857), Bombay (1863), Hyderabad (1959) and Guwahati (1960).

Ecotourism is viewed as a means of protecting natural areas through the generation of revenues, environmental education and the involvement of local people. In such ways both conservation and development are being promoted in sustainable forms. Local economic benefits from Zoo ecotourism have been documented both in the form of increased employment opportunities and incomes. The introduction of Zoo ecotourism can encourage socio economic development as desired by the community contribution, which ecotourism can make to biodiversity and integrity of natural areas are as important as the potentially positive effects on adjacent communities. The provision of environmental education through enhancement

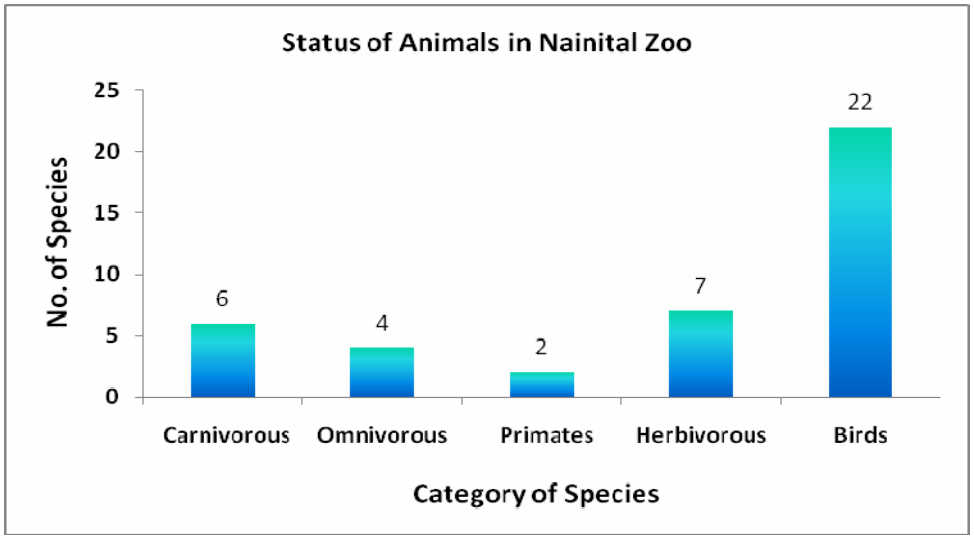
of opportunities to appreciate nature is fundamental to the success of ecotourism. Biodiversity and natural areas can provide this service in return for economic revenues, which can contribute to protected area conservation.

Zoo ecotourism is regarded as being more than tourism to natural areas and should be viewed as a means of combining the goals of resource conservation and local development through tourism in a synergistic fashion. This means that are should be taken to ensure that goal of tourism development do not interfere with the goals of protecting natural areas and biodiversity, local population may become advocates for protection of their natural resources and take pride in the unique surrounding which attract outsiders (Kandari & Chandra, 2004).

Description of Study Area

The Nainital high altitude Zoo is situated at 2100 m above sea level on the hill of Sher Ka Danda in Uttarakhand. The Zoo was established in 1964 on the name of Pandit Govind Bullabh Pant. The zoo is spread over 4.69 ha. The main objectives of Zoo are to conserve the high altitude Himalayan birds and animals which all endemic and endangered and to create awareness about our rich Himalayan fauna among the general people.

On the basis of animal kingdom there are five categories animals found in the High Altitude Nainital Zoo.



Methodology

The present study was carried out in April 2004 to May 2005. According to our study plan, we visited zoo weekly and collected the respected data based our objectives in relation to ecotourism. There after we developed a list of peoples and organization they were interested to adoption of zoo animals for conservation point of view.

Ecotourism with People Participation

Zoo is an important ex- situ conservation procedure. It is for not only the conservation and management of endangered wild animals and birds but also play a very crucial role in the ecotourism,

education and public awareness about the wildlife. Therefore the zoo authority has invited the people and organizations are interested in the wild life conservation and management for the adoption of wild species kept in zoo for their annual expenditure. 18 different organizations and peoples adopted 16 different type of Carnivorous, Omnivorous & Pheasants. The total annual donated amount was Rs. 123950 by different organizations and individuals. The total annual expenditure of different animals was Rs. 107600. So this is a very good sign for the management and conservation of different animal species.

Table- List of different organization and people who are adopting the wildlife in Nainital Zoo

[Sl. No.	Species	Adopted by	Donated annual amount (Rs)	Annual expenditure (Rs.)
1	Leopard	Nainital Bank	10,000	
		U. K. Photographer, Hotel & Restaurants Nainital	11,000	31,300
2	Leopard Cat	Boat House Club, Nainital	7000	7,000
3	Hill Fox	CHEA, Nainital	6,800	4,700
		Rana Conustraction Co., Almora	800	
4	Himalayan Black Bear	State Bank Of India, Nainital	15,000	
		Kumaun Mandal Vikas Nigam	24,100	24,100
5	Palm Civet Cat	Hotel Prashant, Tallital, Nainital		
6	Himalayan Civet Cat	Hotel Wecome Resort, Tallital, Nainital	3,800	3,800
7	Serow	CHEA, Nainital	11,200	11,200
8	Lady Amherst Pheasant	Consul Printers, nainital	3,750	1,500
9	Silver Pheasant	Miss. Pryas Priyanka C/O Prema Gosh, Mallital, Nainital	3,000	1,500
10	Reeves Pheasant	Mr. B. K. Bisht & Mrs. Renu Bisht, Senior Advocate, Nainital	1500	1,500
11	Golden Pheasant	Mrs. Sunita & Mr. Manoj Sah		
12	Edward Pheasant	Sajjanlal, Gopichand, Nainital	3,000	1,500
13	White Pea Fowl	Mrs. Alka, Mr. Sunil Nigam, Long view		
		Mrs. and Mr. Deep Chandra Pandey, Executive Engineer, Irrigation Department, Nainital	6,000	3,000

14	Steppe Eagle	Alok Sah & Rakhi Sah, Heritage Restaurant	3,000	2,500
15	Indian Great Horned Owl	Fair Havens, Nainital	7,000	7,000
16	Spot Bellied Eagle Owl	CHEA, Nainital	7,000	7,000

Ecotourism Activities

Among the tourists visited the Zoo, children (5-12 years age) and adult (>12 years) were 20.75 % and 79.3% respectively (Table 1 & 2). Total number of tourists visited in the Zoo during 2004 – 2005 was 94,884. Maximum tourists (26.6%) were visited in the month of June 2005 and followed by the month of May 2005 (15.9%). However; very small number of tourists came in the month of February 2006 (Table 3).

As far as economy concerned, Zoo authority earned total rupees seventeen lakhs and one thousand forty only (Rs. 1701040. Of this amount received from adult and children tourists was 88.4 and 11.6% respectively (Table- 3). Of the total amount, about 26.3% were earned during June 2005 (Table- 3). Of this amount, 29.8% came from children and adult tourists, respectively (Table 2 & 3).

Table 1. Ecotourism Activities of 5- 12 years Visitors during 2004- 2005

Sl. No.	Months	Visitor (5-12 Years)	Amount (Rs.)	% Contribution
1	April	1258	12580	6.4
2	May	3405	34050	17.3
3	June	5862	58620	29.8
4	July	1082	10820	5.5
5	August	375	3750	1.9
6	September	849	6490	4.3
7	October	1318	13180	6.7
8	November	1995	19950	10.1
9	December	1041	10410	5.3
10	January	743	7430	3.8
11	February	464	4640	2.4
12	May	1272	12720	6.5

Table 2. Ecotourism Activities of > 12 years Visitors during 2004- 2005

Sl. No.	Months	Visitor (>12 Years)	Amount (Rs.)	% Contribution
1.	April	5332	106640	7.1
2.	May	11643	232860	15.5
3.	June	19390	387800	25.8
4.	July	6770	135400	9.0
5.	August	3022	60440	4.0
6.	September	3518	70360	4.7
7.	October	3306	106120	7.1
8.	November	4738	94760	6.3
9.	December	4690	93800	6.2
10.	January	3264	65280	4.3
11.	February	2789	55780	3.7
12.	May	4758	95160	6.3

Conclusion

The nature-based tourism involves education and interpretation of sustainable natural environment. Ecotourism is viewed as a means of protecting natural areas through the generation of revenues and the participation of local people. In such a way both conservation and management would be promoted in sustainable forms. Nainital high altitude Zoo is one of the single Zoo established in high altitude region. There were 19 wild animals and 22 different kinds of pheasants. 18 different organizations and peoples adopted 16 different type of Carnivorous, Omnivorous & Pheasants. The local community and local organizations of Nainital were so keen to conserve and manage the wildlife of high altitude's Zoo, so they are much interested to adoption of wildlife. This is a new step to conservation and management of wildlife in high altitude Nainital Zoo. As far as economy concerned, Zoo authority earned total rupees seventeen lakh and one thousand forty only (17, 01040=00). Of this amount received from adult and children tourists was 88.4 and 11.6% respectively. Of the total amount, about 26.3% were earned during June 2005 (Table- 3). Of this amount, 29.8% came from children and adult tourists, respectively.

Table 3. Ecotourism Activities of total visitors years Visitors during 2004- 2005

S. No.	Months	Total Visitors	Amount (Rs.)	% Contribution
1.	April	6590	119220	7.0
2.	May	15048	266910	15.7
3.	June	25252	446420	26.3
4.	July	7852	146220	8.6
5.	August	3397	64190	3.7
6.	September	4367	78850	4.6
7.	October	6624	119300	7.0
8.	November	6733	114710	6.7
9.	December	5731	114210	6.2
10.	January	4007	72710	4.3
11.	February	3253	60420	3.6
12.	May	6030	107880	6.3

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Evaluation of Disease Intensity of Some Rust Fungi at Nainital Hills

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Abstract: Eight common disease causing rust fungi of the study area were selected for the evaluation of disease Intensity study. These were *Aecidium deutziae* on *Deutzia aecidium, Coleosporium clematidis* on *Clematis buchananiana, Melampsora ciliata* on *Populus nigra, Phragmidium incompletum* on *Rubus nivens, Puccinia nepalensis* on *Rumex nepalensis, Puccinia oxalidis* on *Oxalis dehradunensis, Puccinia padwickii* on *Cyathula tomentosa* and Raveneli*amitteri* on *Indigofera heterantha* in three selected localities in different attitudinal range i.e. Tallital (1800-2100), Ayarpatta (2100-2300 m) and Mallital (2300-2500 m) during 2006 & 2007. Results of Analysis of Variance indicated that both locality and year had the significant impact to influence the disease development by the rust fungi when considered together. *Melampsora ciliata* showed maximum disease development (38.72%) followed by *Puccinia padwickii* (37.20), *Puccinia oxalidis* (34.92%), *Puccinia nepalensis* (34.55), *Phragmidium incompletum* (28.87%), *Coleosporium clematidis* (17.97) and *Aecidium deutziae* (17.20%) and *Ravenelia mitteri* (15.88%). The data recorded at Mallital and Ayarpata localities did not differ significantly compared to Tallital locality. The average disease intensity during 2006 & 07 did not differ significantly and it was 28.47% in 2006 and 27.85 in 2007. [Nature and Science. 2009;7(7):67-72]. (ISSN: 1545-0740).

Key words – Rust fungi, Disease Intensity, Uredinales, Basidiomycetes, Nainital

INTRODUCTION

The rust fungi are a unique and interesting group of fungi traditionally classified in the order Uredinales of the class Basidiomycetes, which are characterized by the presence of basidia and basidiospores (Cummins & Hiratsuka, 2003). The word rust fungi originates from the fact that rust sori are often reddish-orange and can be identified easily to family and generic levels. (Hiratsuka and Sato,1982).In the natural habitat, rust fungi are obligate parasites on living plants, although a few species have now been cultured successfully on artificial media (axenic culture). Rust fungi cause diseases in a wide range of host plants ranging from Pteridophytes (mainly ferns), Gymnosperms (particularly conifers) and flowering plants (both monocotyledons and dicotyledons).

Since there are very few reports dealing with rust fungi of Nainital hills, the present study was undertaken to evaluate the rust disease intensity of most common rust fungi of this area.

Materials and Methods

STUDY AREA

Collections of rust fungi were made from 2006 to 2007 between the altitudes ranging from 1800 to 2611m throughout the year the entire study area, but for more detailed study, collections were made from the selected localities during March to December. Since September to November is the peak season for the growth and development of the rust fungi, both uredinial and telial stages were collected during this period. However, in many cases, uredinial stages associated with aecial stages usually predominate during pre-peak season, while telial stages in the post-peak season. The disease intensity was studied in three selected localities in different altitudinal ranges i.e. Tallital (1800-2100m), Ayarpatta (2100-2300m) and Mallital (2300-2500m).

FIELD SURVEY

Detailed field surveys were undertaken to select out the most common disease causing rust fungi throughout the study area in different altitudinal ranges and finally eight common disease causing rust fungi were selected for the disease intensity study. They are: *Aecidium deutziae* Dietel, *Coleosporium clematidis*

Barclay, *Melampsora ciliata* Barclay, *Phragmidium incompletum* Barclay, *Puccinia nepalensis* Barclay & Dietel, *P. oxalidis* Dietel & Ellis, *P. padwickii* Cummins and *Ravenelia mitteri* H. Sydow in three selected localities in the study area (Mitter & Tondon,1932, 1938). The assessment of disease rating was done during September to October when the maximum disease development was exhibited by these selected rust fungi during the years 2006 and 2007.

For assessing disease intensity, the scale used is based on the method of Elliott and Jenkins (1946) in the disease appraisal of leaf blight of corn caused by *Helminthosporium turcicum* Pass., Ahmed (1976) of til disease caused by *Synchytrium sesamicola*, Lacy.and Pangtey (1979) of *Dolichos biflorus* L. caused by *Collectotrichum capsicum* (Sydow) Butler & Bibsy, *Phoma medicaginea* Malbr. ex Roum. and *Pyrenochaeta dolichi* Mohanty.

The diseased leaves were classified into five categories on the basis of total precent area covered by the spots i.e. (i) 10 (ii) 25 (iii) 50(iv) 75 and (v) 100. Five random samples were taken from each site and in each sample 100 leaves were observed. In case of *Indigofera heterantha* and *Oxalis dehradunensis*, each leaflet was considered as one leaf. Thus 500 leaves were picked up from one locality. All the leaves were examined and classified according to their grade of infection by comparing with the diagrammatic scale prepared and illustrated in Fig. 1.

The disease intensity was calculated by the formula given below following Naumov (1924), Ahmed (1976) and Pangtey (1979).

Degree of disease intensity per infected leaf = d

Number of infected leaves studied per site = x

Average disease intensity per infected leaf (F) = $\frac{d_1 + d_2 + d_3 + d_4 + d_5 \ldots . d_x}{x}$

Total number of leaves studied per site =m

Average disease intensity per site (P) = F. x /m

No. of site examined in the locality = y

Average disease intensity in the locality (I) = $\frac{P_1 + P_2 + P_3 + P_4 + P_5 \ldots\ldots P_x}{Y}$

RESULTS

The results of disease intensity of rust fungi recorded during 2006 and 2007 are presented in Tables 1, 2, 3, 4 and 5.

The Results of analysis of variance (**Table1)** indicated that both locality and year had the significant impact to influence the disease development by the rust fungi in the study area when considered together.

The comparative disease intensity of two years due to the various rust fungi in three localities has been given in (Table 2).The average per cent diseases intensity in 2006 and 2007 due to Aecidium deutziae was recorded to be 12.13 and 12.28 respectively at Tallital, 14.55 and 16.19 at Ayarpatta and 25.02 and 23.03 at Mallital locality. It was found that Mallital locality had maximum disease intensity (25.02 in 2006 and 23.03 in 2007) and Tallital showed the minimum (12.13) in 2006, while Ayarpatta (12.28) in 2007.

The data of Table 3 showed that the disease intensity of *Aecidium deutziae* ranged from 12.20% to 24.03% in three localities. *Coleosporium clematidis*, *Puccinia nepalensis* and *Ravenelia mitteri* did not differ significantly in three localities. *Melampsora ciliata* had maximum disease intensity at Mallital locality and minimum at Ayarpatta locality. Similarly *Phragmidium incompletum* exhibited maximum disease intensity at Mallital locality, followed by Ayarpatta and Tallital localities. *Puccinia oxalidis* showed

more or less same disease intensity at Ayarpatta and Mallital localities than the Tallital locality. *Puccinia padwickii* had the highest disease intensity at Ayarpatta locality, followed by Mallital locality and lowest at Tallital locality.

The data of Table 4 showed that the mean disease intensity was slightly higher in 2007 than in 2006 as shown by *Coleosporium clematidis*, *Melampsora ciliata*, *Puccinia oxalidis* and *Ravenelia mitteri*, while *Aecidium deutziae*, *Phragmidium incompletum*, *Puccina nepalensis* and *Puccinia padwickii* accounted for slightly higher in 2006 than in 2007. However, the average higher disease intensity in 2006 and 2007 was recorded in *Melampsora ciliata* (38.59% and 38.86%), followed by *Puccinia padwickii* (41.09% and 33.31%), *Puccinia nepalensis* (35.39% and 33.71%), *Puccinia oxalidis* (32.99% and 36.82%) and *Phragmidium incompletum* (29.09% and 28.64%), *Aecidium deutziae* (17.22% & 17.17%), The remaining two rust fungi viz *Coleosporium clematidis*. and *Ravenelia mitteri*, showed lower average disease intensity in 2006 and 2007 varied respectively (17.90% &18.03%) , (15.83% and 16.21%).

The data of Table 5 revealed that the average disease intensity during 2006 and 2007 did not differ significantly and it was 28.47% in 2006 and 27.85% in 2007. The mean disease intensity recorded at Tallital, Ayarpatta and Mallital localities was found, to be 24.43%, 29.36% and 30.68%.respectively; However, Mallital and Ayarpatta localities appeared to be more prone to the disease infection than the Tallital locality.

Similarly, three localities when considered together, the average disease intensity exhibited by individual rust fungi found to be highest in *Melampsora ciliata* (38.72%) followed by *Puccina padwickii* (37.20%), *Puccina oxalidis* (34.92%), *Puccinia nepalensis* (34.55%), *Phragmidium incompletum* (28.87%), *Coleosporium clematidis* (17.97%), *Aecidium deutziae* (17.20%), *Ravenelia mitteri* (15.88%).These results indicated that in the same prevailing environment conditions of the study area, *Melampsora ciliata*, *Puccinia padwickii, Puccinia oxalidis and Puccinia nepalensis* had stronger potential in causing higher disease intensity as compared to other rust fungi.

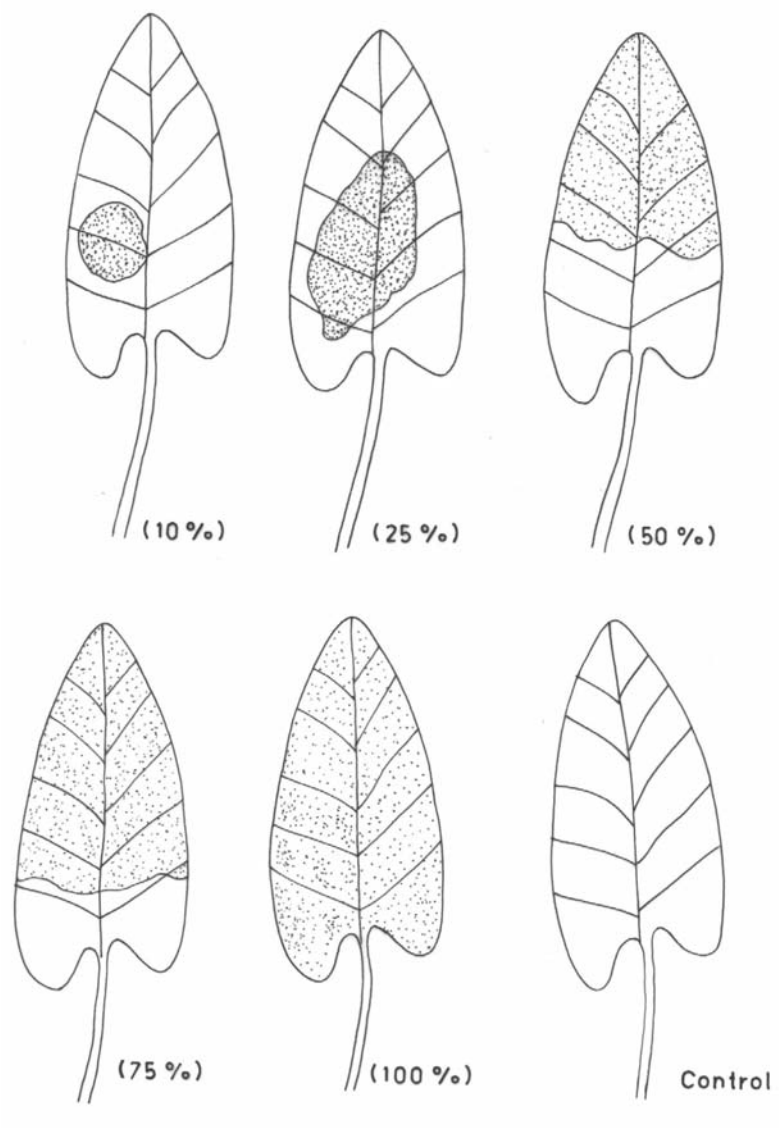

Figure1. Diagramatic representation of disease intensity due to the various rust fungi

Table 1. Analysis of variance on data for disease causing rust fungi on different host species

Source of variation	Sum of squares (ss)	Degree of freedom (df)	Mean Square (Mss)	F	Significance level
Fungus	19684.035	7	2812.01	103.27	P < 0.001
Locality	1737.48	2	868.74	31.9	P < 0.001
Year	23.23	1	23.23	0.85	NS
Fungus × Locality	6206.48	14	443.32	16.28	P < 0.001
Fungus × Year	568.54	7	81.22	2.98	P < 0.01
Locality × Year	458.45	2	229.23	8.42	P < 0.001
Fungus × Locality ×Year	908.42	14	64.89	2.38	P < 0.01
Error	5228.06	192	27.23		

Table 2. Disease rating of different disease causing rust fungi at Tallital, Ayarpatta & Mallital localities during 2006 and 2007.

S. N.	Disease causing fungi	Tallital (1800-2100m)		Ayarpatta (2100-2300m)		Mallital (2300-2500m)		Mean
		2006	2007	2006	2007	2006	2007	
1	*Aecidium deutziae*	12.13	12.28	14.55	16.19	25.02	23.03	17.21
2	*Coleosporium clematidis*	18.95	17.99	15.55	15.75	19.25	20.35	17.97
3	*Melampsora ciliata*	43.95	42.61	35.91	38.84	35.91	35.12	38.72
4	*Phragmidium incompletum*	15.75	15.92	29.6	27.88	41.92	42.13	28.87
5	*Puccinia nepalensis*	28.39	35.35	37.33	38.57	40.44	27.22	34.55
6	*Puccinia oxalidis*	20.66	27.01	37.53	43.27	40.82	40.23	34.92
7	*Puccinia padwickii*	38.84	38.25	43.95	42.61	40.47	19.07	37.2
8	*Ravenelia mitteri*	11.09	11.74	15.52	16.71	19.74	20.19	15.83
	Average	**23.72**	**25.14**	**28.74**	**29.98**	**32.95**	**28.42**	**28.16**
	Average of two years	*24.43*		*29.36*		*30.68*		

NOTE: Figures are expressed in percentage and mean of five replicates.

Table 3.Average Disease rating of different disease causing rust fungi at three different localities of two year

S. N.	Disease causing fungi	Tallital (1800-2100m)	Ayarpatta (2100-2300m)	Mallital (2300-2500m)	Mean
1	*Aecidium deutziae*	12.20	15.37	24.03	17.20
2	*Coleosporium clematidis*	18.47	15.65	19.80	17.97
3	*Melampsora ciliate*	43.28	37.38	35.52	38.72
4	*Phragmidium incompletum*	15.84	28.74	42.03	28.87
5	*Puccinia nepalensis*	31.87	37.95	33.83	34.55
6	*Puccinia oxalidis*	23.82	40.40	40.53	34.92
7	*Puccinia padwickii*	38.55	43.28	29.77	37.20
8	*Ravenelia mitteri*	11.42	16.12	19.97	15.88
	Average	**24.43**	**29.36**	**30.69**	**28.16**

Standard error of Fungus species (S) 0.953
Standard error of Locality (L) 0.583

Standard error of Species × Locality (S × L) 1.650

NOTE: Figures are expressed in percentage and mean of five replicates.

Table 4.Average Disease rating of different disease causing rust fungi at three localities in each year

S. N.	Disease causing fungi	2006	2007	Mean
1	*Aecidium deutziae*	17.22	17.17	17.20
2	*Coleosporium clematidis*	17.92	18.03	17.98
3	*Melampsora ciliata*	38.59	38.86	38.73
4	*Phragmidium incompletum*	29.09	28.64	28.87
5	*Puccinia nepalensis*	35.39	33.71	34.55
6	*Puccinia oxalidis*	32.99	36.84	34.92
7	*Puccinia padwickii*	41.09	33.31	37.20
8	*Ravenelia mitteri*	15.45	16.21	15.83
	Average	**28.47**	**27.85**	**28.16**

Standard error of Fungus species (S) 0.953, **Standard error of Year (Y)** 0.476, **Standard error of Species x Year (S x Y)** 1.347
NOTE: Figures are expressed in percentage and mean of five replicates

Table 5 Disease rating of different disease causing rust fungi at three different localities, *viz*. Tallital, Ayarpatta & Mallital during years 2006 and 2007

Agroclimatic localities	2006	2007	Mean
Tallital	23.72	25.14	24.43
Ayarpatta	28.74	29.98	29.36
Mallital	32.95	28.42	30.69
Average	**28.47**	**27.85**	**28.16**

Standard error of Locality (L) 0.583, **Standard error of Year (Y)** 0.476, **Standard error of Locality × Year (L × Y)** 0.825
NOTE: Figures are expressed in percentage and mean of five replicates

DISCUSSION

Results of the study conducted indicated that both locality and year had significant impact in influencing the disease development by the rust fungi in the study area. The average disease intensity during 2006 and 2007 did not differ significantly and it was 28.47% and in 2006 and 27.85 in 2007. The data recorded at Mallital and Ayarpatta localities did not differ significantly compared to Tallital locality.

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Thermal decomposition kinetics of peanut shell

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Abstract: A great importance was attached to harness of plant tissue because of resource and energy crisis. Through biologic and thermal chemistry technique, nature plant tissue as peanut shell shoot could be transferred to gaseous, liquid fuel used as fuel or chemical raw material. The thermal decomposition of peanut shell was composed of three step TG weight loss. Effects of temperature scanning rate, grain size and purge- gas flow rate on peanut shell decomposition character have been studied. Thermodynamic activation parameters were computed from the thermal data using Coats and Redfern methods, which confirm first order kinetics. This result could be used in peanut shell gasification process or thermal decomposition optimization. [Nature and Science. 2009;7(7):73-78]. (ISSN: 1545-0740).

Keywords: Coats & Redfern Method, Kinetics, Peanut Shell, Thermal Analysis, Thermal Decomposition,

1. Introduction

Today, great importance was attached to harness of plants tissue because of resource and energy crisis. Plant tissue was yielded through plant photosynthesis,it is a kind of transformed solidified,dposited solar energy.It is a kind of steady energy and it could be regenerated by plant. Compare to wind energy,solar energy,plant tissue energy do not confined by weather.It is of high energy density and could be transferred,stored,,transported just like normal mineral fuel.Through biologic or thermal chemistry technique nature plant tissue such as cane,peanut shell,short could be transferred to gaseous,liquid fuel used as fuel or chemical raw material.

Thermogravimetric technique has been an important research method on thermal stablisation & thermal decomposition.TG could provide theory assistance for material heating treatment and applications [1,2]. TG is an easy,quick,precise measure method. By mathematical analyzing TG data, thermal decomposition activation parameters can be obtained [3,4].This paper discusses thermal decomposition of peanut shell & its thermal decomposition effectors were studied kinetic functions was obtained and kinetic parameters were calculated. The result can be used in peanut shell thermal decomposition optimization.

2.Experimental

Peanut shell washed, dried & porphyrised to 120 μm - 380 μm before using thermal decomposion. Rigaku model 8150 thermoanalyser(Thermaflex) was used for simultaneous recording of TG-DTA curves at a heating rate of 5-10 min^{-1} .For TG, the instrument was calibrated using calcium oxalate while for DTA, calibration was done using indium metal, both of which were supplied along with the instrument. A flat bed type aluminum crucible was used with α – alumina (99% pure) as the reference material for DTA. Purge gas was used as N_2 with varying rate 15-70 min^{-1}. The number of decomposition steps was identified using TG curves. The activation energy and Arrhenius constant of the degradation process was obtained by Costs and Redfern method [5].

3. Result & Discussion

3.1 Effects of thermal gravity analysis condition on peanut shell decomposition

3.1.1. *Peanut shell thermal decomposition*

The peanut shell was thermally decomposition in three decomposition steps (Fig.1 is the curve of peanut shell thermal decomposition). The first decomposition step of estimated mass loss 6% occurred at 8^0-120^{o}C due to dehydration process. The DTA curve also confirms endothermic peak at peak temp. 94^{o}C (Maximum peak temperature The second decomposition step of estimated mass loss of 54% and occurred at 200-380^{o}C.Major mass loss shows this step was the Main thermal decomposition process. The DTA curve again shows endothermic peak at 330^{o}C. The third decomposition step of estimating mass loss mass 16% occurred at 380-700^{o}C,it was the thermal decomposition of residual.

3.1.2 *Effects of Grain Size on Peanut Shell Thermal Decomposition*

The curves of three grain size thermal decomposition were almost the same trends in fig. 2 the same purge gas flow rate and temperature scanning rate, the smaller the grain size was the lower the thermal decomposition beginning temperature was the bigger the grain size was, the higher the thermal decomposition beginning temperature was, it was explain on the basis of the heat transmission. The bigger grain size was, the heat transmission was slower, so the beginning thermal decomposition was delayed.

3.1.3 *Effect of temperature scanning rate on Peanut shell thermal decomposition*

Effect of temperature scanning rate on peanut shell thermal decomposition was similar to that of grain size. The thermal decomposition curves of three temperature rising rate were almost shows the same trend. At the same grain size and purge gas flow rate, the faster the temperature rising rat was the higher the beginning thermal decomposition temperature was. This was because of heat transmission. The samples temperature was always lower than the heating temperature. So the beginning of thermal decomposition was delayed.

3.1.4 Effect of Purge- gas rate on peanut shell thermal decomposition

The thermal decomposition curves (fig.3) of three purge-gas flow rate have been indicated. At the same grain size & temperature scanning rate, the faster purge-gas flow rate was, the lower the residual gravity was when the purge-gas flow rate was fast, gaseous product of thermal decomposition could be departed quickly, this promote the thermal decomposition process undergoing thoroughly, so the residual gravity was low.

3.2. Kinetics of thermal decomposition

In recent years there has been increasing interest in determining the rate – dependent parameters of solid-state non-isothermal decomposition reactions by analysis of TG curves. Several equations [5-11] have been proposed as means of analyzing a TG curve and obtaining values for kinetic parameters. Many authors [5-10] have the advantage of this method over the conventional isothermal method. The rate of a decomposition process can be described as the product of two separate functions of temperature and conversion [7], using

$$d\alpha/dt=k(T)f(\alpha) \qquad (1)$$

Where α is the fraction decomposed at time t, k (T) is the temperature –dependent function and f(α) is the conversion function dependent on the mechanism of decomposition. It has been established that the temperature – dependent function k(T) is of the Arrhenius type and can be considered as the rate constant k.

$$k=Ae^{E^*/RT} \qquad (2)$$

Where R is the gas constant in (kJ deg^{-1}mol^{-1}). Substituting Eq. (2) into Eq.(1), we get

$$d\alpha/dt= A/\theta e^{E^*/RT}\ f(\alpha) \qquad (3)$$

where θ is the linear rate dT/dt. On integration and approximation, this equation can be obtained in the following form

$$\log g(\alpha) = -2.303E^*/RT + \log[AR/\theta E^*] \quad (4)$$

Where g(α) is a function of α dependent on the mechanism of the reaction. The integral on the right hand side is known as temperature integral and has no closed for solution. So, several techniques have been used for the evaluation of temperature integral. Most commonly used methods for this purpose are the differential method of Freeman and Carroll [6], integral method of Coats and Redfern [5], the approximation method of Horowitz and Metzger [10]. The kinetic parameters calculated by the Horowitz-Metzger method revealed no significant difference with that evaluated by the Coats-Redfern method. So integral method of Coats-Redfern and using this method various kinetic parameters calculated.

The kinetic analysis parameters such as activation energy (ΔE*), enthalpy of activation (ΔH*), entropy of activation (ΔS*), free energy change of decomposition (ΔG*) were evaluated graphically by employing the Coats –Redfern relation (5):

$$\text{Log}\,[-\log(1-\alpha)/T^2] = \log[AR/\theta E^*(1-2RT/E^*)] - E^*/2.303RT \quad (5)$$

Where α is the mass loss up to the temperature T, R the gas constant, E* is the activation energy in J mol^{-1}, θ the linear heating rate and (1-2RT/E*) = 1. A plot of left hand side of Eq. (5) against 1/T gives a slope from which E* was calculated and A (Arrhenius constant) was determined from the intercept. From relevant data, linearization plots have been drawn in fig. 4 confirms first order kinetics.

The entropy of activation (S*) and the free energy change of activation (G*) were calculated using Eqs. (6) & (7):

$$\Delta S^* \ (JK^{-1}mol^{-1}) = 2.303\ R\ [\log(Ah/kT) \quad (6)$$

$$\Delta G^* \ (Jmol^{-1}) = \Delta H^* - T\Delta S^* \quad (7)$$

Where k and h are the Boltzmann and Planks constant, respectively. The values of E*,A, ΔS*,ΔH*and ΔG*for the decomposition steps of the peanut shell has been calculated(Table 1). The negative values of the entropies of activation are compensated by the values of the enthalpies of activation, leading to almost the same values for the free energies of activation [2]. This result could be used in peanut shell gasification process or thermal decomposition optimization.

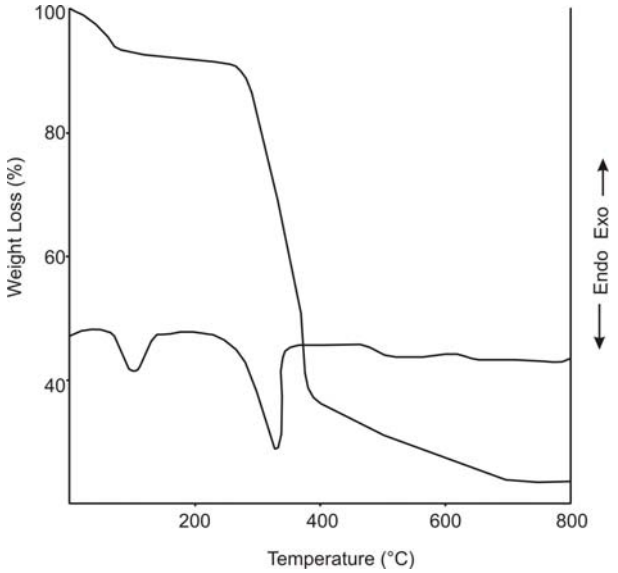

Fig 1: TG/ DTA curves of peanut shell thermal decomposition

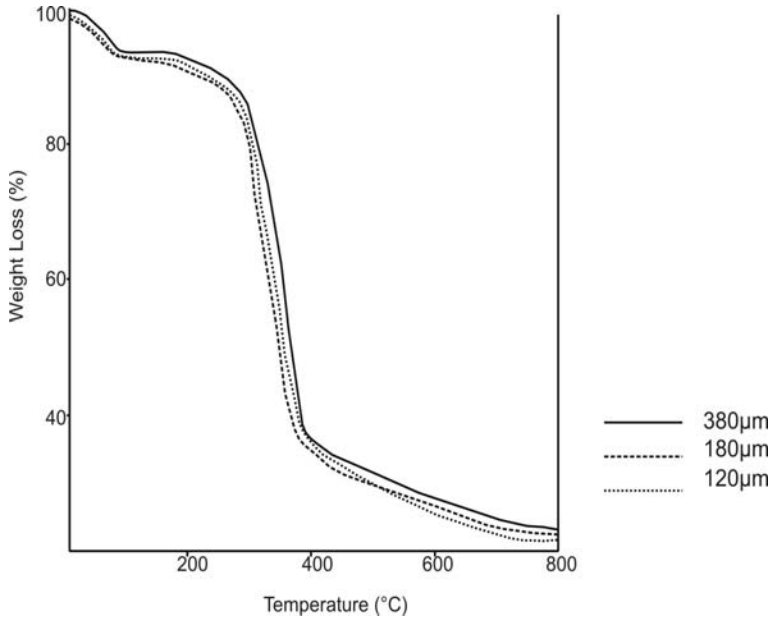

Fig 2: Effect of grain size on peanut shell decomposition

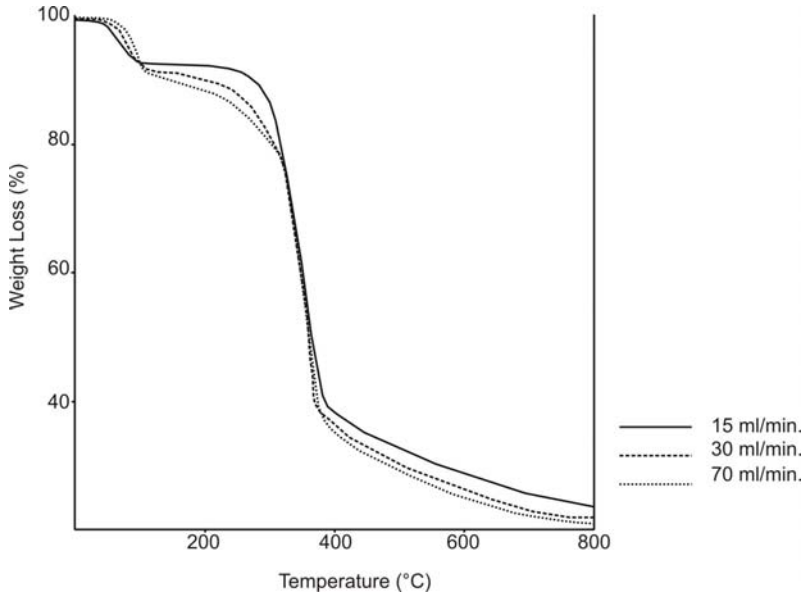

Fig 3: Effect of purge-gas flow rate on peanut shell decomposition

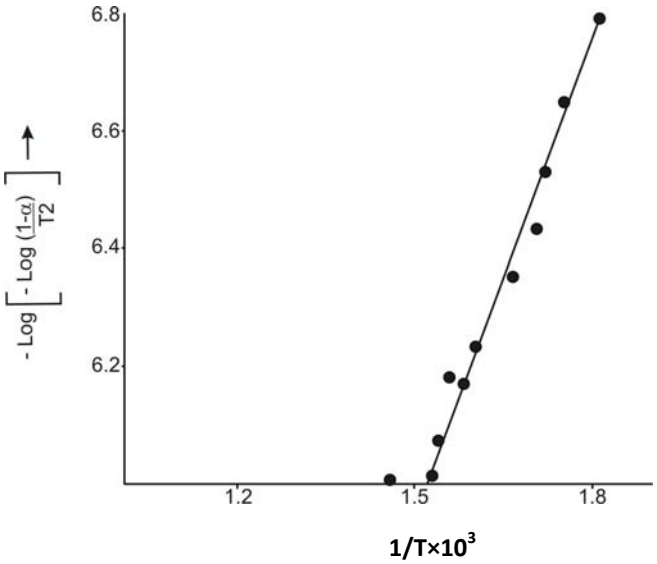

Fig 4: Coats and Redfern Linearization plot of peanut shell thermal decomposition

Table 1: Thermodynamic activation parameters of the peanut shell

S.No	Step	E*(Jmol^{-1})	A(×10^2 s^{-1})	ΔH*(Jmol^{-1})	ΔS*(JK^{-1}mol^{-1})	ΔG*(kJmol^{-1})
1	I	59.86	5.18	21.32	-197.76	110.17
2	II	50.97	4.24	48.75	-199.93	117.40
3	III	54.95	4.57	19.25	-199.91	126.36

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Staphylococcus aureus - A Cause of Fatal Toxic Shock Syndrome In Egyptian Horses (First record)

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Abstract

Our study investigated the cause of an outbreak in Arabian and foreign breed equine farm with mortality rate 18.82%, the animals showed acute watery diarrhea and colic followed by death. However the animals were treated with multiple broad spectrum antibiotics. Postmortem and histopathological findings indicate generalized toxaemia in the form of severe congestion in all vital organs, pneumonia, endocarditis, gastroenteritis and nephritis. Bacteriological examination showed isolation of *S. aureus* from all cases which were tested for their sensitivity toward different antibiotics. Results reveals that all *S. aureus* isolated from infected and dead animals were 100% resistant to all tested antibiotics with an exception for vancomycin which was used to control the progress of cases in the farm. The excessive nonspecific antibiotics treatment leads to propagation of opportunistic multiple drug resistant *S. aureus* which release enterotoxins leading to toxic shock syndrome that end fatally after development of signs of toxemia and septicemia leading to increased morbidity and mortality rates. In Egypt this study was the first record for multiple drug resistant *S. aureus* toxic shock syndrome as a cause of an outbreak in equine stable subjected to multiple stressful conditions. In conclusion, Staphylococcus isolates were biochemically identified and their sensitivity against different antibiotics as well as their pathological lesions indicated that this type of *S. aureus* may be MRSA and the strains need further detection of the toxic genes by using molecular biology techniques. [Nature and Science. 2009; 7(7):79-87]. (ISSN: 1545-0740).

Keywords: *S. aureus* / Toxic Shock Syndrome / Equine / Pathology.

Introduction

S. aureus is a bacterium, frequently living on the skin or in the nose of a healthy human and animals that can cause illnesses ranging from minor skin infections and abscesses, to life-threatening diseases such as pneumonia, meningitis, endocarditis, toxic shock syndrome (TSS) and septicemia which may be rapidly fatal **[1, 2].** They tend to cause different types of infections and differ in their typical antibiotic resistance profiles. The importance of methicillin resistant *S.aureus* (MRSA) in veterinary medicine is not well established **[3]**. However, MRSA outbreaks in horses suggest that this organism might be an emerging problem in the equine population **[4, 5].** MRSA infection has been reported in different animal species; sheep, goat and cows **[2]**, dogs **[6]** and hospitalized horses **[7]** and their transmission between infected horses and veterinary personnel has been documented.

In this investigation *S. aureus* multiple drug resistant was isolated form all cases in infected equine farm. The strains were identified by bacterial isolation, identification, antibiotic resistance test and pathological examination indicating an outbreak of toxic shock syndrome caused by multiple drug resistant *S. aureus* (MRSA) .To our knowledge this study considered the first record of toxic shock syndrome (TSS) in Egyptian equine.

Materials and method

Animals and clinical sampling:

Total number 17 cases out of 93 cases of horses (3cases pure Arabian and 14 cases mixed breed) at the private stable in Cairo, Egypt were dead after suffering from acute severe watery diarrhea and colic, stiffness in gate, congestion of external mucous membrane, loss of appetite with slight transient fever (39˚C

- 40˚C), severe sweating and sudden death shortly 1-2 days after the onset of clinical symptoms. The sick animals showed no response for treatment using multiple broad spectrum antibiotics (Oxytetracycline, Sulphaguanidine, Streptomycin and Cephadrine). Full clinical examination of the animals were carried out, 11 blood samples were collected for virological examination, 12 fecal samples for parasitic infection, 5 vaginal, 10 nasal and 12 fecal swabs were collected for bacteriological examination. Food samples from infected farm were collected for mycotoxin evaluation and total bacterial count, also drinking water samples were collected for examining water quality.

Post mortem examination:

Post mortem and clinical examination of internal organs were carried out after death directly. Specimens were taken form different internal organs including liver, kidney, spleen, heart, lung, ceacum, intestine for bacteriological examination and other samples from the same organs were fixed in 10% neutral buffer formalin for pathological examination, processed routinely and sectioned at 4-5 micron thick, then stained with haematoxyline and eosin for microscopic examination **[8].**

Bacteriological sampling and monitoring bacterial profile:

Bacterial swabs were collected under aseptic conditions, including nasal swabs **[3]** vaginal swabs and rectal swabs **[9].** Cultivation of samples, isolation and purification of the isolates were carried out using media purchased from (Oxoid); swabs were inoculated into a tube containing 10 ml Tryptic soy broth. The broth was incubated at 37ºC for 24 hrs then streaked from the enriched broth onto Nutrient, Mannitol, Blood and MacConkey agar plates. The swabs were also inoculated into Selenite-F-broth for 16 hrs then sub cultured onto Salmonella- Shigella agar medium then plates were incubated at 37°C for 16-18 hrs according to **[10, 11]**. Identification of isolates includes morphological examination by Gram's Method **[12].** Biochemical identification carried out according to **[13,14]** including catalase, oxidase, indole, methyl red, Voges Proskauer, Simmon's citrate, urease test, hydrogen sulphide production on triple sugar iron agar medium, sugar fermentation test using different sugars, arginine hydrolysis test, hippurate hydrolysis test, nitrate reduction test, coagulase test were carried out.

***S.aureus* identification and characterization**:

Staphylococcus isolates were streaked onto Mannitol salt agar with 2 µg/mL oxacillin and incubated aerobically at 35°C for 48 hrs. Colonies identified as *S. aureus* were diagnosed according to **[14, 15]** as Gram positive, non-spore forming cocci, arranged in form of single, pairs, short chains or in irregular clusters. The colonies are circular, smooth and glistening. On blood agar, they are beta-hemolytic. Colonies are colorless to yellow. Biochemically, they are coagulase positive and are maltose fermenter to differentiate *S. aureus* from other Staphylococci. Confirmation of strains was carried out using Staphytect plus dry spot **(Oxoid)** as latex identification for *S. aureus*. Agar diffusion antibiotic sensitivity test was carried out for all isolated strains during the outbreak according to **[16, 17, 18, 19],** Antibiotic discs were obtained from Oxoid including B-lactams [penicillin-G (10 units), amoxicillin/clavulinic acid (20/10 µg/ml), cefotaxime (30 µg/ml)] , macrolides [erythromycin (15 µg/ml)], aminoglcosides [gentamicin (10 µg/ml)], fluoroquinolones [ciprofloxacin (5 µg/ml), ofloxacin (5 µg/ml)] cefadroxil (30 µg/ml), cefoperazone(75 µg/ml), tetracycline (30 µg/ml), tobramycin (10 µg/ml), sulpha/ trimetho (23.75+1.25 µg/ml), amikacin(30 µg/ml) , amoxy/fluclox (25 µg/ml) and vancomycin (30 µg/ml).

Results

Water samples were free from pathogenic bacteria. Food samples were free from mycotic infection and mycotoxins contamination; aflatoxins, ochratoxins and fumonisin. Virological as well as parasitological examinations showed negative results.

Clinical findings of infected animals showed dullness, dehydration and depression of a horse just before death **Fig. [1]**.Horse suffering from severe watery diarrhea, colic, stiffness in gate, slight fever (39-40˚C) , congestion of mucous membranes, loss of appetite followed by a short period of severe sweating ending with tremors and death **Fig. [2]**. Postmortem examination was carried out showing severe congestion and hemorrhages in intestine and caecum **Fig. [3]**. Severe congestion and hemorrhages in the heart and lung as shown in **Fig.[4].** Histopathological examination showed signs of generalized toxemia in the animal tissue.

The lung showed alveolar emphysema, edema and interstitial lymphocytic infiltration in the lung tissue as shown in **Fig. [5]** and hemorrhages as in **Fig .[6]**, kidney tissue showed severe degenerations and interstitial hemorrhages as shown in **Fig.[7**] as well as hyaline cast in the renal tubules as shown in **Fig. [8].** Severe gastritis with mononuclear cellular infiltration and congestion of blood capillaries was shown in **Fig. [9]**. Caecum showed congestion and hemorrhages of the blood capillaries in the caecal mucosa **Fig. [10**]. Lesions of the heart showed degeneration and severe oedema between the cardiac muscle bundles **Fig. [11**].These clinical and pathological changes indicate signs of toxemia.

Bacteriological studies revealed the presence of *S. aureus* isolates completely identified in all tested samples as Gram-positive cocci, grape-like, large, round, golden-yellow colonies, β-hemolysis on blood agar plates. Biochemical identification revealed; catalase positive, coagulase positive test *S. aureus*, isolates were subspecies: *S. aureus aureus*. The incidence of isolation of *S. aureus* was reached 100% from examined samples; nasal, vaginal and rectal swabs as well as tissue samples; liver, kidney, spleen, heart, lung, ceacum, intestine. Other isolates recovered from examined samples with lower incidence as streptococcus spp. (20%) from nasal swabs only, salmonella (17.65%) and (20.00%) from rectal and nasal swabs respectively. *E. coli* (11.76%) from rectal swabs only. **Table [I]** showed highest rate of isolation was from the rectal swabs followed by vaginal swabs then nasal swabs and finally internal organs of dead case. The total number of isolates showed that the highest incidence was *S.aureus* followed by salmonella then streptococcus and *E.coli*. All isolated strains were tested for their sensitivity toward different antibiotics. Results reveals that all *S.aureus* isolated from infected and dead animals were 100% resistant to all tested antibiotics as shown in **Table [II**] and **Fig. [12, 13].** The previous multiple drug resistant *S. aureus* isolates were then tested against vancomycin showed high sensitivity.

Discussion

Our study investigated the cause of an outbreak in equine farm with mortality rate 18.82% showing severe watery diarrhea, colic, loss of appetite with slight transient fever (39˚C - 40˚C), severe sweating and sudden death. However the animals were treated with multiple broad spectrum antibiotics. These results agree with **[20]** who stated that several problems in which diarrhea is one of the symptoms can be quickly fatal in equine, diarrhea caused by bacteria will usually elevate the horse's temperature a degree or two for a short time during invasion of the intestinal lining, after that temperature may drop back to normal.

Postmortem examination was carried out showing severe congestion in all vital organs, these findings indicate generalized toxaemia. Histopathological examination showed signs of generalized toxemia in the animal tissue in the form of pneumonia, endocarditis, gastroenteritis and nephritis. These results agree with bacteriological findings which indicate multiple drug resistant *S. aureus* from all examined samples which was accused of causing toxic shock syndrome in equine. These findings agree with **[21]** who found that clinical MRSA infection in horses ranges from simple skin and soft tissue infections to bacteriaemia/ septicemia, pneumonia, septic arthritis, endocarditis and osteomyelitis. Also, Results agree with **[22]** which reported that some strains of *S. aureus* carry exotoxins; toxic shock syndrome toxin 1 (TSST-1) which are superantigen cause toxic shock syndrome if they are released systemically. They added that, *S. aureus* can produce several enterotoxins which cause staphylococcal gastroenteritis (food poisoning) causing symptoms including nausea, vomiting, diarrhea, abdominal cramps and muscle cramps.

The incidence of isolation of *S.aureus* reached 100%. All isolated strains were tested for their sensitivity toward different antibiotics. Results reveals that all *S.aureus* isolated from infected and dead animals were 100% resistant to all tested antibiotics. This agree with **[23]** who reported that the majority of MRSA isolates were multidrug resistant. Also, it agree with **[24]** who mentioned that Fluoroquinolone-resistant *S. aureus* strains should be suspected of being MRSA. Also, **[24, 25, 26]** proved that antibiotic susceptibility tests can also be used to identify MRSA. Also, these results agree with **[11]** who proved that MRSA either produce potent toxins or resist a wide range of antibiotics. Also, results agree with **[21]** who reported that Methicillin resistance in *S. aureus* are resistant to all penicillins, cephalosporins and members of their classes. They added that, resistance to methicillin represents resistance to all B-lactam antimicrobials. Results also agree with **[27]** who proved that the antimicrobial therapy is not required for eradication and control of MRSA colonization in horse's farm.

Our study proved that the previous multiple drug resistant *S. aureus* isolates showed sensitivity toward vancomycin. Results agree with **[28]** who mentioned that MRSA is multiple drug resistant to different

antibiotics as well as Beta lactams and are only susceptible to vancomycin. Results agree with **[29, 30]** who mentioned that *S. aureus* is an opportunistic pathogen which can cause diseases ranging from superficial soft-tissue infections to life-threatening bacteremia and toxic shock syndrome. Our investigation proved that the horses highly affected in the outbreak were a mixture of imported horses from different localities and Arabian breed, Case history revealed that horses were completely exhausted due to massive training program for race, high environmental temperature (40-43˚C), excessive antibiotics treatment and high mortality (18.82%) without proper identification and antimicrobial sensitivity test, such treatment can result in prolonged delay in the administration of effective therapy and subsequent propagation of opportunistic multiple drug resistant *S. aureus* which release enterotoxins leading to toxic shock syndrome end fatally after developing signs of toxemia and septicemia leading to increased morbidity and mortality rates. These findings agree with **[3, 5, 31]** who proved that MRSA infection may be an emerging disease in horses, its infection become endemic on horse farms because of the extensive movement of horses, especially thoroughbreds and standard breeds. Also results agree with **[11]** who abuse MRSA of being a critical pathogen responsible for a great morbidity and mortality especially among immunosuppressed cases. Also, results agree with **[21]** mentioned that animals at high-risk of MRSA infection are the immunosuppressed, antimicrobial-treated, and surgically incised animals. They added that the most significant problems associated with the emergence of MRSA is treatment failure caused by empirical treatment of presumed *S. aureus* infections with B-lactam antimicrobials and added that without proper identification of the MRSA isolate by culture and antimicrobial-sensitivity testing, such treatment can result in a prolonged delay in administration of effective therapy and subsequent increase in morbidity and mortality.

In our study MRSA was isolated from the nares of healthy animals after the end of outbreak. These finding agree with **[3]** who proved that Animals can be colonized with MRSA for variable periods of time without developing clinical disease and added that there are no proven options to eradicate MRSA from horse's nares.

The horse stable where the outbreak occurred was closely situated near a large dog farm and as the dogs are asymptomatic carriers for MRSA therefore they might be accused of being the source of infection for the nearest horses stable. This agrees with **[32]** who mentioned that *S. aureus* recovered from less than 10% of dogs and cats in most studies, although carriage rates are as high as 90%. **[5, 33]** had evidence that some MRSA strains may be spreading in equine populations, most canine and feline. They added that these strains might be particularly well-adapted to transmission in horses. **[6]** Isolated MRSA from 133 animal cases, 131 were isolated from equine and 2 from canine. These results agree with **[34]** who isolated MRSA from 69 dogs and one horse. Also, **[3]** reported that MRSA was found in 13% of horses on one farm in the province and in 5% of horses on another farm. **[33]** Found that MRSA infections become more common in horses. Results also agree with **[35]** who isolated MRSA from 16% of horses tested at a university equine clinic in the U.K.

In Egypt this study was the first record for multiple drug resistant *S.aureus* toxic shock syndrome as a cause of an outbreak in equine stable suffering from multiple stressful conditions. This study needs further investigation of bacterial toxin by molecular biology as an accurate tool of bacterial toxin identification. This agree with **[36]** who mentioned that diagnosis of MRSA in horses depend on laboratory identification of *S. aureus* from clinical specimen but identification of MRSA required additional testing to identify phenotypic resistance or the presence of *mec-A* gene using molecular technique.

Table [I]: Incidence of bacterial isolation from different sites of infected living and dead equine cases.

Isolated strains	Rectal swabs (17)		Vaginal swabs (5)		Nasal swabs (10)		Internal organs of dead case (8)		Positive samples (40)	
	No	%	No	%	No	%	No	%	No	%
S.aureus	17	100.00	5	100.00	10	100.00	8	100.00	40	100.00
streptococcus	0	0.00	0	0.00	2	20.00	0	0.00	2	5.00
salmonella	3	17.65	1	20.00	0	0.00	0	0.00	4	10.00
E.coli	2	11.76	0	0.00	0	0.00	0	0.00	2	5.00
Mean ±SE	**1.29 ±0.31**		**1.20 ±0.54**		**1.20 ±0.38**		**1.00 ±0.35**		**1.20 ± 0.19**	

Table [II] Antibiotic sensitivity test of *S.aureus* isolates, salmonella and *E.coli* recovered from rectal swabs of healthy and infected groups.

Isolated strains	***S.aureus***		**Salmonella spp.**	***E.coli***	
Animal group **Antibiotics**	**Infected group (40)**	**Healthy group (6)**	**Infected group (3)**	**Infected group (2)**	**Healthy group (8)**
Amikacin (30)	100.00% R	66.67%S 33.33% R	66.67%I 33.33% R	100.00% S	100.00% S
Amoxicillin/ clavulinic acid (20/10)	100.00% R	66.67%S 33.33% R	100.00% S	100.00% S	100.00% S
Amoxy/fluclox (25)	100.00% R	50.00%S 16.67% I 33.33% R	100.00% S	100.00% S	100.00% S
Cefadroxil (30)	100.00% R	66.67%S 33.33% R	100.00% S	100.00% S	100.00% S
Cefoperazone (75)	100.00% R	66.67%S 33.33% R	100.00% S	100.00% S	100.00% S
Cefotaxime (30)	100.00% R	66.67%S 33.33% R	100.00% S	100.00% S	100.00% S
Ciprofloxacin (5)	100.00% R	66.67%S 33.33% R	100.00% S	100.00% S	100.00% S
Erythromycin (15)	100.00% R	66.67%S 33.33% R	66.67%S 33.33% I	100.00% S	100.00% S
Gentamicin (10)	100.00% R	50.00%S 16.67% I 33.00%R	100.00% S	100.00% S	100.00% S
Ofloxacin (5)	100.00% R	66.67%S 33.33% R	100.00% S	100.00% S	100.00% S
Oxytetracycline (30)	100.00% R	66.67%S 33.33% R	100.00% S	100.00% S	100.00% S
Penicillin-G (10 units)	100.00% R	33.33% R 33.33% I 33.33% S	66.67%I 33.33% R	50.00% S 50.00% I	100.00% S
Sulpha/trimetho (23.75+1.25)	100.00% R	50.00%S 16.67% I 33.00%R	100.00% S	100.00% S	100.00% S
Tobramycin (10)	100.00% R	66.66%S 33.33% R	100.00% S	100.00% S	100.00% S

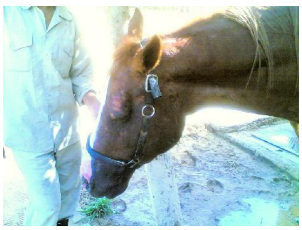


Figure [1] sick horse, showing dullness, dehydration and depression just before death.

Figure [2] the same horse after death.

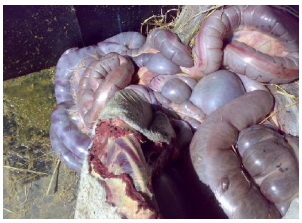


Figure [3] severe congestion and hemorrhages in intestine and ceacum.

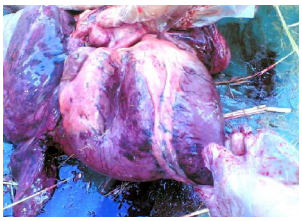


Figure [4] severe hemorrhages in the heart.

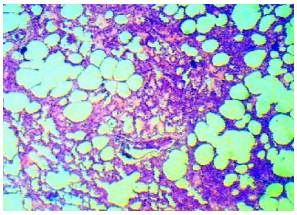


Figure [5] alveolar emphysema and lymphocytic infiltration. H&E (x 100)

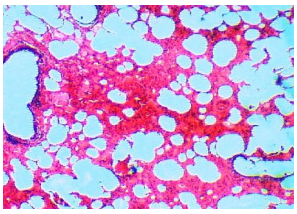


Figure [6] alveolar emphysema and interstitial edema and hemorrhage. H&E (x 100)

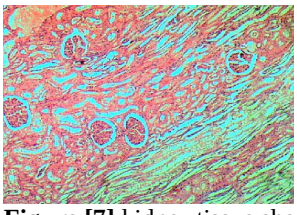


Figure [7] kidney tissue showed severe degenerations and interstitial hemorrhage. H&E (x 100)

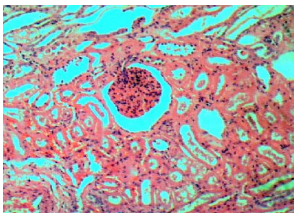


Figure [8] kidney tissue showed severe degenerations and hyaline cast. H&E (x 100)

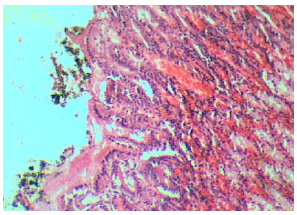


Figure [9] severe gastritis with mononuclear cells infiltration and congestion of blood capillaries. H&E (x 100)

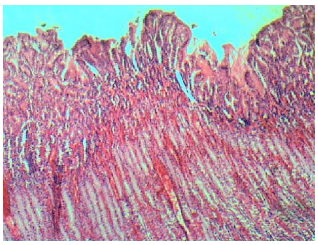


Figure [10] severe hemorrhages in the caecal mucosa. H&E (x 100)

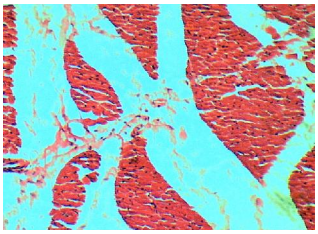
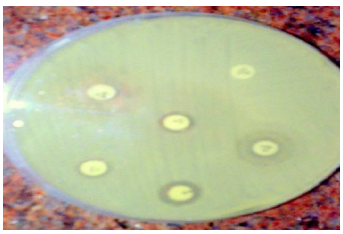


Figure [11] severe edema in the heart tissue. H&E (x 100)



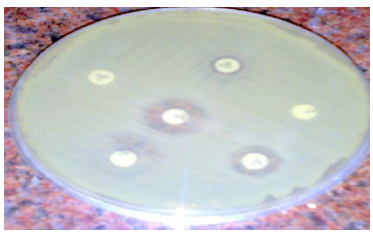


Figure [12] and figure [13] Agar diffusion antibiotic sensitivity test showing multiple drug resistant *S. aureus*.

Conclusion and Recommendations

-Misuse of antibiotics must be forbidden as it might be the real cause of outbreaks due to their immunosuppressive effect on infected animals due to prolonged nonspecific treatment. Rapid diagnosis in outbreaks should be carried accurately and should include screening of unusual causes and not only for

suspected diseases. Researchers recommended that veterinary hospitals initiate surveillance programs for MRSA infections including rapid screening using PCR or Real time PCR, particularly in horses to clarify the role of MRSA in equine outbreaks.

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5/13/2009

Beautiful Geometry

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Abstract: In this work, without assuming the fifth Euclidean postulate, the following theorem was proved: There exist a number of spherical quadrilaterals whose interior angle sum is equal to 360 degrees. [Nature and Science. 2009;7(7):88-89]. (ISSN: 1545-0740).

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Key Words: Euclid, elements, postulates ,non-Euclidean geometries, spherical triangles

MSC: 51 M04
PACS: 02.40.Dr, 02.40Ky

1. Construction

Let NA', NB', NC' be the segments of a sphere S whose north pole is N as shown in fig.1 Choose a ponit A on NA'. With center N, radius NA, describe an arc cutting AB' at B and AC' at C.

2. Result

So, NA = NB = NC (1)
Take a point F on NA. With center N, radius NF draw an arc meeting NB at E
And NC at D. So, NF = NE = ND (2)
From (1) in triangle NAC, angle NAB = angle NCB (3)
And in triangle, NBC, angle NBC = angle NCB (4)
From (3) and (4) we obtain that angles NAB=NBC=NCB = 90 degrees (5)
Similarly from (2) we can show that angles, NFE= NEF= NED=NDE = 90 degrees (6)
From (5) and (6) we get that the sum of the interior angles of spherical
quadrilateral BCDE is equal to 360 degrees (7)

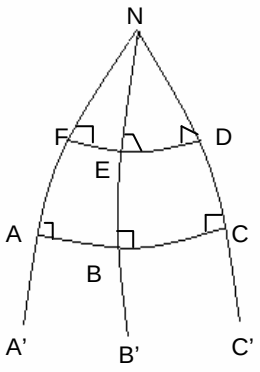

Figure 1 [Spherical]

Discussion Needless to say, (7) is controversial but consistent.Construction of a number of quadrilaterals such as BCDE is very easy. How is it possible? What is the mystery? There is something hidden treasure of physical geometry. The classical geometry is widely used in mechanics. The principles of non - Euclidean geometries are applied in quantum mechanics and general theory of relativity. A turning point in geometry always influenced theoretical physics. There are many burning problems in physics. Further investigations to be devoted on this mystery will unlock this problematic problem and give rise to a new field of physical geometry.

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Wikipedia [Parallel Postulate] Section2,Proposition 7

Rare metal (Ta-Sn-Li-Be) distribution in Precambrian pegmatites of Keffi area, Central Nigeria

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Abstract: Rare metal bearing pegmatite occurring in two major structural forms; as vertically dipping and low lying pegmatites around keffi area, central Nigeria have been studied with a view to elucidate their petrographic features and rare metal (Ta-Sn-Li-Be) distribution. Geological mapping, first on a scale of 1:50,000, then on a larger scale of 1:5000 reveal that these pegmatite bodies which intrude older lithologies of Schist and Older Granite have varied and distinct mineralogical zones. The vertically dipping type exhibit more mineralogical zonations with five distinct zones identified. These are Muscovite-Quartz- Microcline- Albite- Tourmaline (MQMAT), Lepidolite-Muscovite-Tourmaline (LMT), Microcline-Albite (MIA), Quartz-Muscovite-Albite (QMA) and Quartz–Beryl-Muscovite-Albite-Tourmaline (QBMAT) zones respectively. However, the low lying bodies exhibit less zonal complexity with only two distinct mineralogical zones, viz the Quartz- Microcline- Tourmaline zone (QMIT) and the Quartz- Albite- Microcline (QAM) zones. Chemical analysis for rare metal Ta-Nb –Sn –Li-Be content of the pegmatite bodies using the inductively coupled plasma (ICP AES) Instrumentation technique show that these rare metals have preference for specific mineralogical zones. For example, Ta has affinity for the albite and tourmaline rich zones while Li concentration is greater in zones of Lepidolite and microcline enrichment. Sn, which has the highest values generally, shows preference mainly for the LMT zone. Generally however, the pegmatite bodies are more of a Sn-Li prospect with subordinate Ta-Nb enrichment. The distinct compositional preference of rare metal content and mineralogical zones is thus a veritable exploration guide for rare metal exploration in the pegmatite of the study area. [Nature and Science. 2009;7(7):90-99]. (ISSN: 1545-0740).

Key words: Pegmatite , raremetal, mineralogical, analysis, zonations

Introduction

Pegmatite bodies of Nigeria, intrude generally discordantly, older lithologies of the Schist belt, the Gneiss migmatite complex and the Older granite suites. These pegmatite bodies were hitherto thought to be restricted to a 400 Km NE-SW trending belt from the southwestern part around Abeokuta to the north central parts around Jos (Jacobson and Webbs, 1946; Kainnard, 1984). (Fig.1) Recently however, Garba (2002), Okunlola and King (2003), Okunlola, (2005), Okunlola and Somorin (2006) have shown evidence that they may not be restricted to only this confine. Also, in a recent study, Okunlola (2005) revealed at least 3000 sizeable bodies ranging between 10-1500m in length and sometimes up to 50m in width distributed across and beyond the earlier defined belt with diverse structural orientation, and varied morphological composition. The increase in global demand for these rare metals notably Ta, Nb, and Sn has led to renewed interest in the search for viable deposits especially in Nigeria (Okunlola, 1998). Consequently, this study aims at ascertaining the mineralogical characteristics and rare metals (Ta-Nb-Sn-Li) distribution in the pegmatite bodies around Keffi area with a view to elucidating their possible economic potentials and serve as an exploration guide for raremetal mineralization in the pegmatites of the area.

Method of study:

The study involved systematic geological mapping initially on a scale of 1: 50,000 covering a total area of about 475Km^2 extending from Lat.8^0 40"N and 8^0 50"N, and Long. 7^0 51"E and 8^0 05"E. This was followed by a large scale deposit mapping of two identified pegmatite bodies at Angwan Doka and Angwan Mallam area of the study area respectively on a scale of 1: 5000. The reason for the latter large scale mapping is to study closely the pegmatite bodies and possibly use the identified petrographic and chemical features as basis for evaluating the rare metal potentials of the other pegmatite bodies in the area. During the mapping on a scale of 1:50.000, lithological relationships with other rock units were established. However, during the large scale mapping, identified mineralogical zones was sampled and the representative samples were studied petrographically and composite samples also analyzed for rare metals Ta, Sn, Li, and Be using Inductively Coupled Plasma Atomic Spectro-Photometry (ICP-AES) method at the Activation laboratories Canada.

Field relationship and Petrography

Rocks of the Nigerian Basement Complex of Precambrian age underlie the project area. The Nigerian Basement Complex lies east of the West African Craton and North West of the Congo Craton in a Mobile Belt affected by mainly the Pan African Orogeny. (Rahaman,1976, 1992). Three main groups are identified namely: The Migmatite gneiss complex, the Schist belts and the Pan African Older Granites (Okunlola, 2001)(Fig.2)

The pegmatite which are believed to be late intrusive members of the Pan African Older Granite suite in this study area intrude the rocks of the Toto-Gadabuike Schist belt earlier described by Onyeagocha (1984).

In this study area, (Fig. 3) the schistose rocks are pelitic to semi-pelitic schists. Calc-gneiss, amphibolite and dolerite are the minor rocks present. The schists are fine to medium-grained, strongly foliated rocks. Some of them contain numerous porphyroblasts of staurolite, garnet, kyanite, and andalusite. The dominant foliation, which is defined by a mineralogical and lithological banding is essentially flat lying and it is axial planar to flat –lying fold. It also defines the major structure of the area. Field and petrographic studies showed that they were affected by an early episode of medium pressure metamorphism that at its peak attained the kyanite grade. This was followed by an episode of low pressure metamorphism during which there was widespread development of andalusite and. /or cordierite. Areas underlain by these rocks are characterized by low relief and form extensive pediplain broken by occasssional low lying hills and inselberg of Older granites.

Members of the Older granite suites are intrusive into the schist and vary in composition from tonalite to granodiorite to true granite. They are coarse grained, structurally isotropic rock except where they have been affected by a late episode of shearing. In these shear zones the rocks have augen to mylonitic texture.

Pegmatite veins varying in sizes from only a few centimeters to 0.5km in length and about 30-60m wide are abundant in the area (Fig 3). These are coarse grained rocks rich in quartz, feldspar and micas. The main accessory minerals are tourmaline, beryl columbotantalite, cassiterite and garnet. Mineralogical zoning is common in larger bodies with two types of pegmatite distinguished on the basis of their structural inclination. The first and most abundant type occur as concordant intrusions in areas underlain by schist thus forming near flat lying bodies (Fig. 4) while the second type occurs as steeply dipping to nearly vertical bodies intruding mainly members of the Older granite suites. At Angwan Doka for example, excavations of these pegmatite veins (Fig. 5) up to a depth of 15 m in most cases by informal miners have exposed the structural attitude and mineralogical zonations of these pegmatite bodies. The initial mapping on a scale of 1: 50,000 reveal about 12 pegmatite bodies in the study area. Two out of these bodies located at Angwan Mallam and Angwa Doka were then closely studied on a scale of 1:5000 (Figs 4 and 5) because of their relatively large size and because they represent the flat lying bodies in the case of Angwan Mallam vein and the steeply dipping type in the case of the Angwan Doka vein respectively

The Angwan Mallam pegmatite exhibits some form of crude mineralogical zoning. Located between Latitudes 7^0 56.8' E and 7^0 57.2'E, and Longitudes 8^0 45.0N and 8^0 45.3N, it is about 400m long and 300m wide (Fig). Its contact with the schist is well exposed. The Angwan Doka Pegmatite vein on the other hand is mineralogically more complex than the Angwan Mallam vein and occurs between Latitudes $8^0$0.5″N and $8^0$1.58″N, and Longitudes $8^0$45″E and $8^0$45.66″E of the study area. It is a 1.5Km long and 400m wide steeply dipping pegmatite dyke with a strike of 120^0. It is intrusive into the granodiorite and forms a low-lying ridge.

Two mineralogical zones can be recognized in the Angwan Mallam vein. These are (i) the quartz-muscovite-microcline-tourmaline (QMAT) and (ii) quartz-albite-microcline (QMA) zones (Fig 4). However, excavations of the body show that the zoning is irregular (Fig8)

The quartz-muscovite-microcline (QMAT) zone consists of very coarse aggregate of light green muscovite, microcline and quartz. The books of muscovite can be as thick as 10cm sometimes, while tourmaline occurs usually in minor amounts as small crystals usually intergrown with muscovite but coarser crystals are found in the portions rich in quartz and microcline. The quartz-albite-microcline (QMA) zone is composed of large crystals of microcline, and quartz with abundant pockets of fine-grained albite and muscovite. (Fig.6) This zone is more susceptible to weathering than the former with pockets of fine-grained aggregates of albite and quartz being common.

The Agwan Doka pegmatite body on the other hand is generally coarse grained. However, pockets fine-grained albite-rich portions with saccharroidal texture with small flakes of muscovite and/or lepidolite are also present. They are generally milky white to buff coloured except for the lepidolite-rich portions that are purplish in colour.

Five mineralogical zones were recognized within the vein (Fig 5, 10). These are:

i. Muscovite- Quartz-Microcline-Albite-Tourmaline (MQMIAT) zone
ii. Lepidolite-Muscovite-Tourmaline (LMT) zone

iii. Microcline-Albite (MIA) zone
iv. Quartz-Muscovite-Albite (QMA) and
v. Quartz-Beryl-Muscovite-Albite-Tourmaline (QBMAT) zone.

The MQMIAT zone which is the most extensive forms the outer border zone of the intrusion. It is composed of large books of muscovite measuring up to 8 x 5cm, milky-white and sometimes smoky cobbly quartz, and an aggregation of albite and microcline which is found in the lower parts. (Fig. 7) Pockets rich in fine-grained albite and muscovite are also present in the zone. Black tourmaline associated with quartz is common in this zone and indeed some portions are composed almost entirely of an aggregate of black tourmaline and quartz.
The LMT zone extends for about 100m along the strike and has a maximum width of about 40m. Lepidolite in this zone occurs as small, purple flakes intergrown with albite. Cobbles composed of coarse-grained aggregate of quartz; muscovite, tourmaline, and occasionally beryl are present as inclusions in this zone. This zone weathers very easily and fresh rocks are rare even in deep pits.
The MIA zone comprises mainly of microcline and albite. Microcline occurs as large milky white to slightly brownish grains intergrown with finer-grained quartz, albite and minor muscovite. Pockets of white fine-grained albite are also present. In the case of the Quartz-Muscovite-Albite (QMA) zone, it is composed of a fine-grained aggregate of quartz, muscovite and albite. The quartz is often clear and colourless while the muscovite occurs as small flakes. The QBMAT zone on the other hand is limited to the south eastern portion of the intrusion. Beryl occurs together with quartz and minor amounts of muscovite, albite and tourmaline. The Beryl grains are euhedral, light bluish green crystals varying in size from small (2 x 3cm) to large (10-20cm) or more .

Rare metal distribution

Channel samples collected from pits sunk into the Angwan Mallam and Doka pegmatite veins during the second phase of the exploration work were analysed for their rare metal Ta, Nb, Sn, Li and Be content. The summaries of the analytical results of composite whole rock samples in ranges and means are presented in Tables 1 and 2. Table 1 shows that generally, the analysed samples of the Angwan Mallam pegmatite vein are enriched in Sn and to some extent Ta and Nb, but are poor in Li and Be. Mineralogically, they are deficient in Lepidolitic mica and Beryl minerals. Average Sn concentration is 760 ppm but some samples have values as high as 6000 ppm, while Ta values range from 5 – 418 ppm with an average of 65 ppm. More than 66% of the samples have Ta values higher than 33ppm and about 20% of the samples have values > 200ppm. Nb values are generally lower than those of Ta (5-376 ppm) with highest concentration also in zones of Ta enrichment. Ta/Nb ratio range from 1 - 1.5 which are low compared to the Ta/Nb ratios in pegmatite bodies from adjacent Nassarawa (Udegi) area where average Ta/Nb ratio is about 3.5 but are as high as 10 in places. (Okunlola and King, 2003). Average Li and Be values are 27 ppm and 13 ppm respectively.

The Angwan Doka vein on the other hand is enriched in Li and to some extent Sn, relative to the Angwan Mallam body (Table 2). Li values are particularly high (range 17 – 11,000 ppm, average 731 ppm). This value reflects the enriched presence of lepidolite in the Angwan Doka pegmatite. Average Sn content is 870 ppm, therefore the Angwan Doka pegmatite seem to be a better Li-Sn prospect.
The vein also has pockets of Ta enrichment with values as high as 328 ppm. Only 7% of the samples have Ta values > 200 ppm. The areas that are considered as having high values of Ta are highlighted in Figures. Ta/Nb ratio range from 1 – 1.5 which shows that relative to Nb, there is marginal Ta enrichment. The average Be concentration (11 ppm) is low generally in the vein.

In terms of specific distribution of the rare metals relative to the mineralogical zones (Tables 3 and 4), in the Angwan Mallam pegmatite, Ta is noticeably enriched in the Quartz- Muscovite-Albite (QMA) zone while Sn concentration is highest in the (QMAT). Nb values also are highest in the QMA zone.

In the Angwan Doka pegmatite body, Ta concentration is highest in the Microcline, Albite, (MIA) zone (235 ppm) and Quartz- Beryl- Muscovite- Albite- Tourmaline (QBMAT) zone (174 ppm). Sn on the other hand is more in the Lepidolite- Muscovite- Tourmaline, (LMT) zone with an average concentration of 1056 ppm and also the MIA (100 ppm). Highest Li concentration is also recorded in the LMT zone (3580 ppm) followed bythe Muscovite- Quartz-Microcline-Albite-Tourmaline (MQMAT) zone. Nb is generally low with highest values (15 ppm) in the MIA zone.
From the mineralogical and rare metal compositional features of the Angwan Doka pegmatite therefore, it could be classified as a complex type, lepidolite sub type pegmatite. (Cerny, 1986). It is also compositionally similar to the Broen Derby pegmatite, Colorado, the Phanga pegmatite, Thailand

and Wodgina pegmatite. The Agwan Mallam pegmatite on the other hand is an Albite type with compositional similarities to the Heng Shen pegmatite Grand gong, China. (Pollard, 1989).

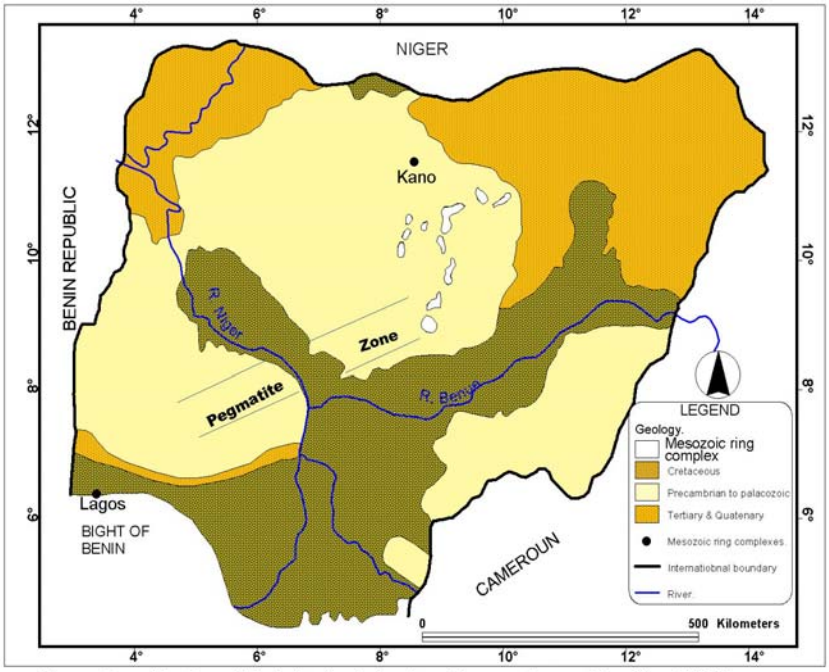

Fig. : General Geology of Nigeria showing the location of the pegmatite zone (after Kinnaird 1984).

Fig1 General geological map of Nigeria showing location of pegmatite zone

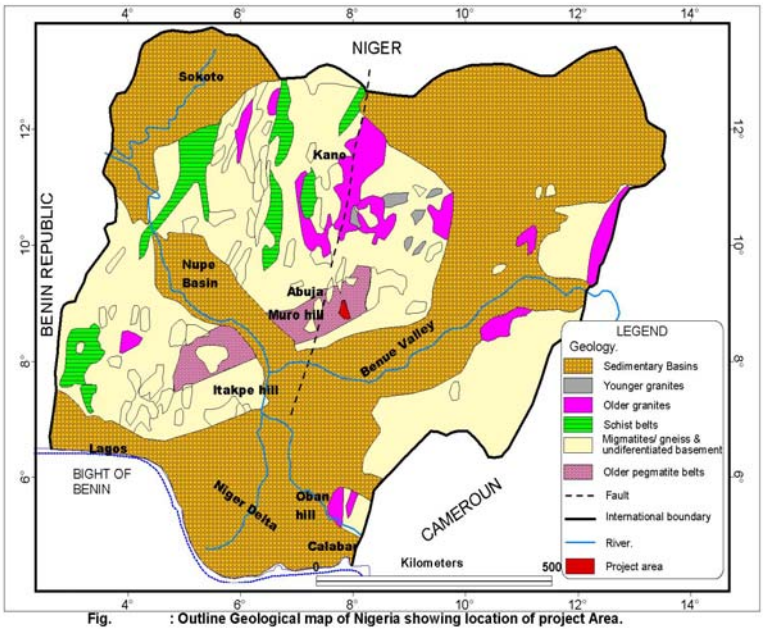

Fig. : Outline Geological map of Nigeria showing location of project Area.

Fig 2 Outline Geological map of Nigeria showing location of project area

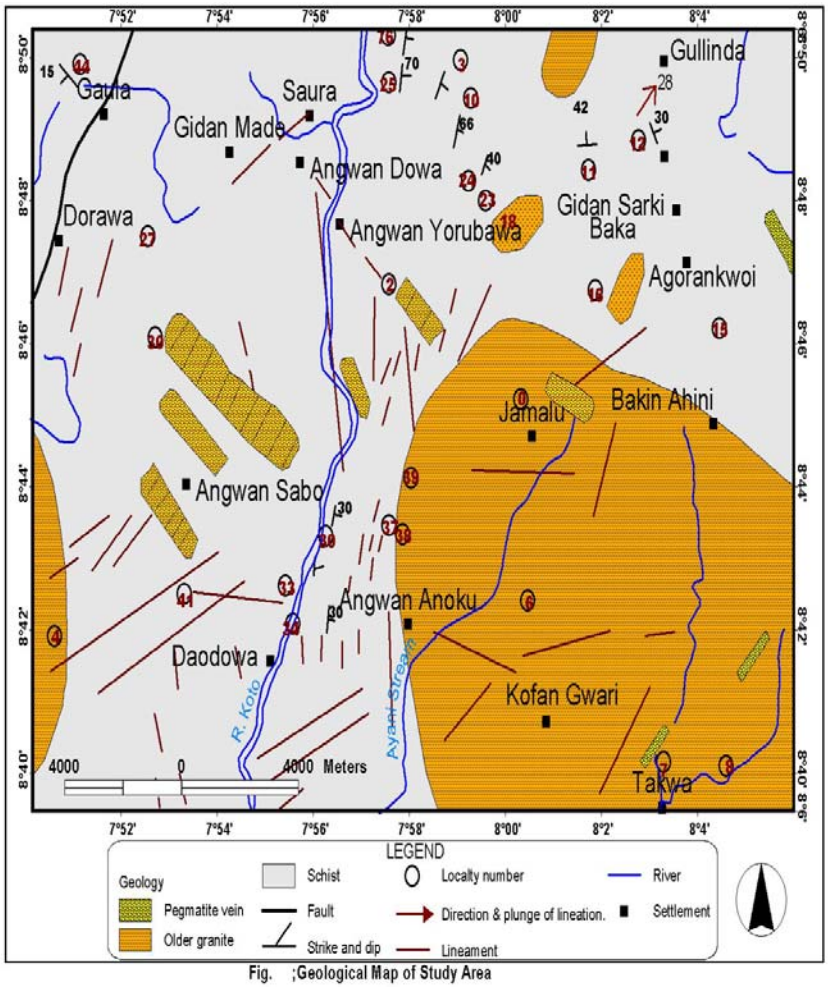

Fig. ;Geological Map of Study Area

Fig 3 geological map of study area

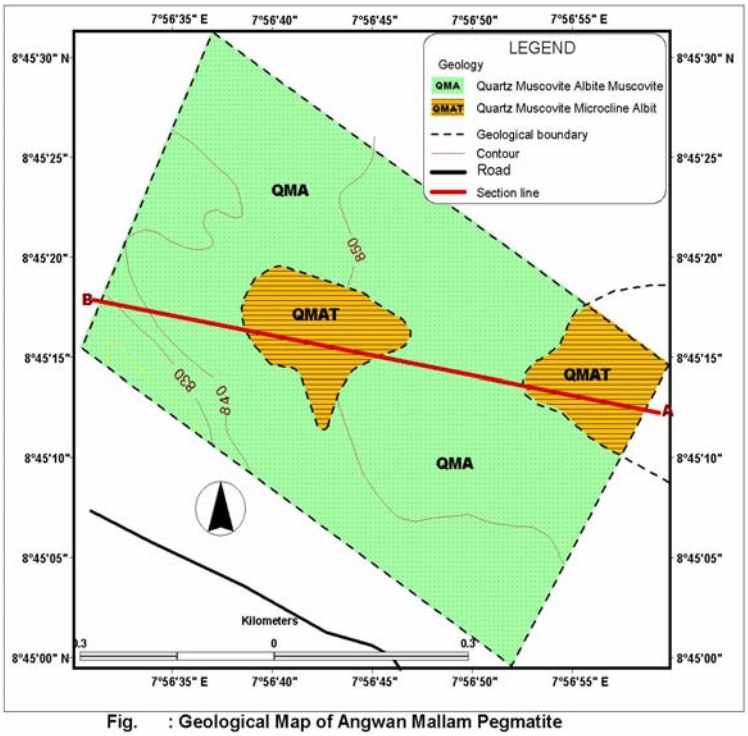

Fig. : Geological Map of Angwan Mallam Pegmatite

Fig 4 geological map of Agwan mallam pegmatite

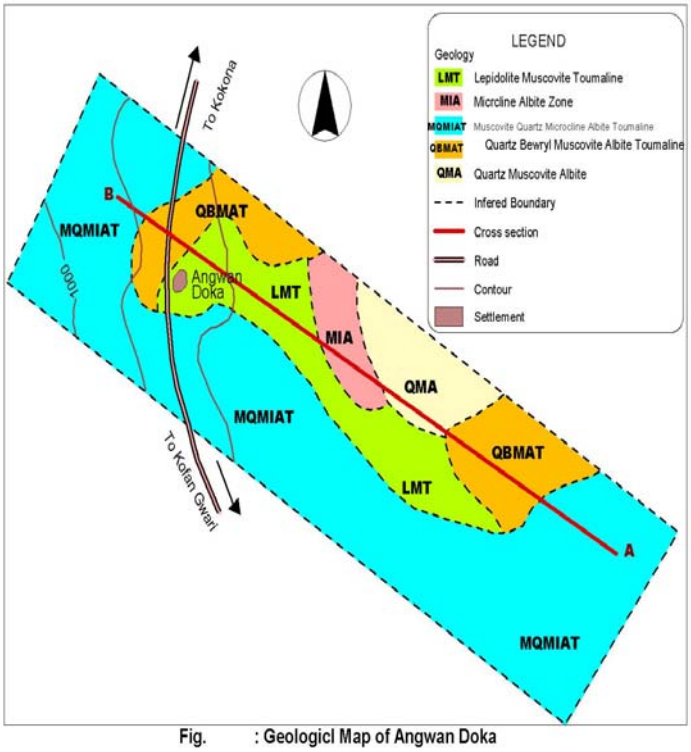

Fig. : Geologicl Map of Angwan Doka

Fig 5 Geological map of Angwan Doka area

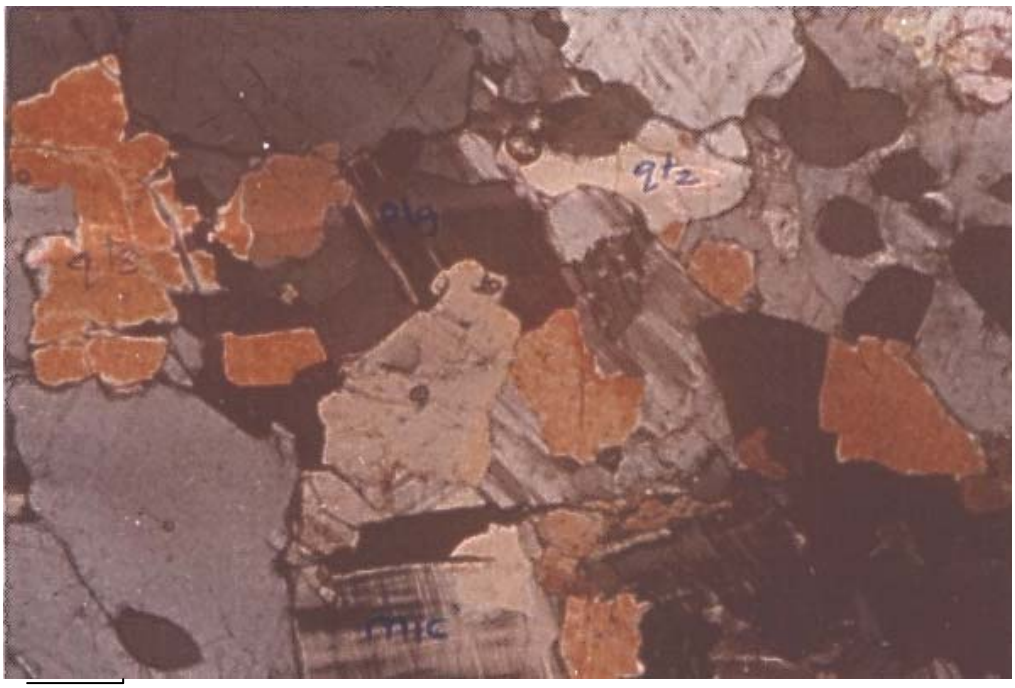

Fig 6 Photomicrograph of Quartz-Microcline Albite zone in transmitted light of the Agwan Mallam pegmatite body
Bar scale :2mm

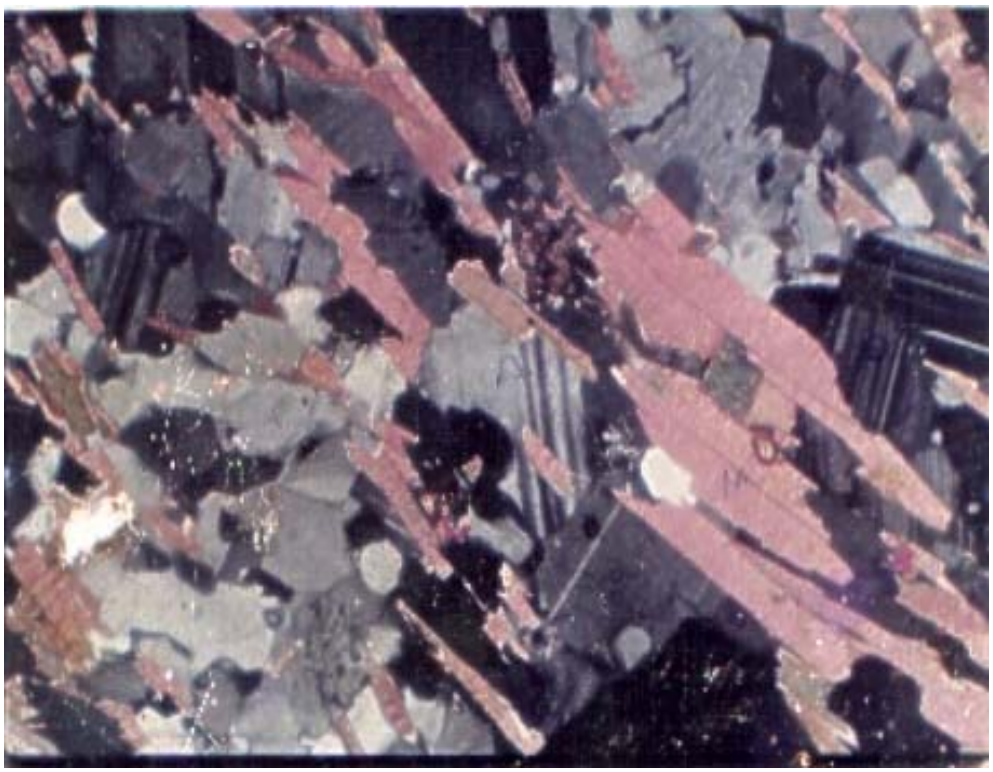

Fig 7 Photomicrograph of Quartz-Muscovite –Albite -Tourmaline zone of the Agwa Mallam pegmatite body

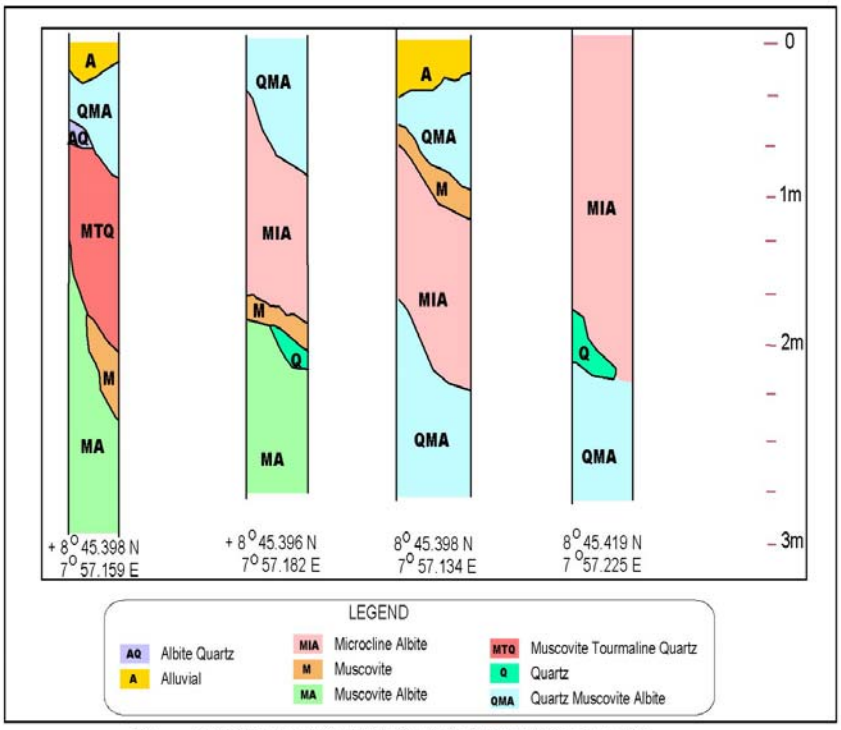

Fig. Typical Representative Pit Profiles in the Angwan Mallam Pegmatite.

Fig 8 Typical representative pit profile of the Agwan Mallam pegmatite

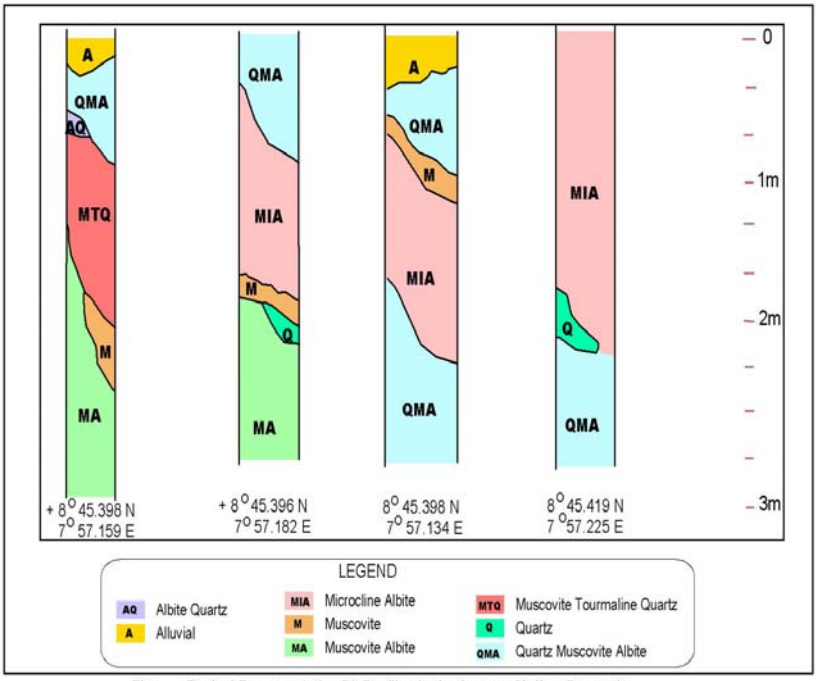

Fig. Typical Representative Pit Profiles in the Angwan Mallam Pegmatite.

Fig 9

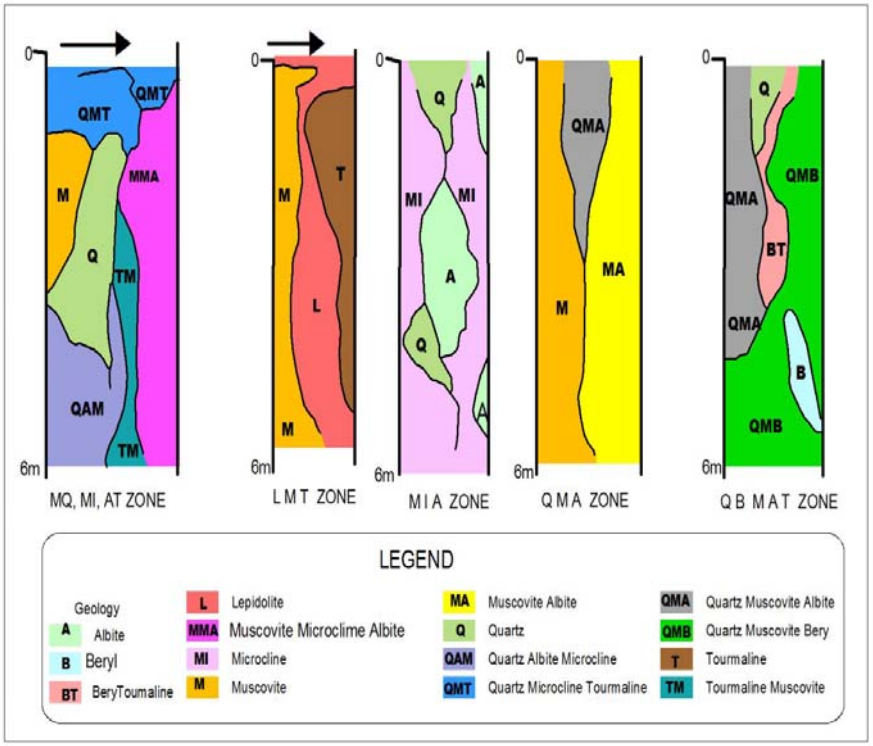

Fig10 Typical representative pit profile of the Agwan Doka pegmatite body

TABLE 1: Summary of Raremetal distribution of the Angwan Mallam and Agwan Doka pegmatite veins (ppm) (n=10)

	Agwan Mallam		AgwanDoka	
Element	**Range**	**Average**	**Range**	**Average**
Ta	5 – 418	65	5-328	39
Nb	5 – 376	51	6-355	40
Sn	5 – 6000	760	5-3373	638
Li	5 – 111	27	7-6000	731
Be	5 - 58	13	4-212	11

TABLE 2: Average rare metal distribution in relation to mineralogical zones in the pegmatite bodies

Mineralogical zones (Agwan Doka)	**Ta**	**Sn**	**Li**	**Nb**
MQMIAT	5	15	850	5
LMT	32	1056	3580	5
MIA	235	900	282	15
QMA	174	158	421	6
QBMAT	15	76	185	5

Mineralogical zones (Agwan mallam)	**Ta**	**Sn**	**Li**	**Nb**
QMAT	35	1522	28	18
QMA	320	150	32	105

Conclusions

The study area which comprises mainly schist and older granite rocks have been intruded by vertical and low lying horizontally dipping pegmatites. These Pegmatite bodies which vary in sizes have been shown to be complex with varied mineralogical zonations. Attempts have been made to study on a larger scale these two types. These have revealed different levels of mineralogical compositional complexities.

The flat lying Agwan Mallam Pegmatite is the less complex with two main mineralogical zones while the vertically dipping pegmatite type as typified by the Agwan Doka outcrop is more complex with five distinct mineralogical zones. Results of the chemical analysis of the samples of pegmatites for rare metals-Ta-Nb-Li-Be content show enrichment of these metals in preferred zones. For instance, Ta is associated more with zones rich in albite and muscovite in both pegmatite types specifically, QMA zone in the Agwan mallam pegmatite, and MIA zone in the Angwa Doka pegmatite. Li, on the other hand is associated with lepidolite, muscovite and tourmaline rich zones especially in the Agwan Doka body where the LMT and the QMIAT zones are preffered prospects.

Generally, the two pegmatite types are Sn-Li rich pegmatite with subordinate Ta- Nb concentration. The Sn enrichment is noticeably associated with lepidolite, muscovite, and albite rich zones. The flat lying pegmatites have also been shown to be poorer Li prospects compared to the vertical dipping type. The knowledge of the distribution of the rare metals and their enrichment in known mineralogical zones as shown in this study will thus be a veritable tool in exploring preferentially,these rare metals in the pegmatite bodies of this study area

Acknowledgements

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Prediction Of Productivity Of Spent Lubricant Oil Uncontaminated And Contaminated Soil Amended With Organic Wastes Using Modified Productivity Index In Abakaliki, Nigeria

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Abstract: A research was carried at the Teaching and Research farm of the Faculty of Agriculture and Natural resources Management, Ebonyi State University, Abakaliki to predict the productivity of spent lubricant oil contaminated and uncontaminated soil amended with organic wastes. Maize (Oba super II hybrid) variety was used as a test crop. The productivity index was modified by excluding sufficiencies for aeration and electrical conductivity. Soil parameters namely available water capacity, bulk density, rooting depths, soil horizons and soil P^H were used to compute productivity index. The results indicated that modified productivity indices for uncontaminated soil amended with organic wastes were highest when compared with contaminated soil. Similarly, Maize grain yields increased with increase in productivity index and decreased with decrease in productivity index. Relationship between productivity index and maize grain yield showed highly significant correlation (r= 0.96 at p >0.01) in uncontaminated soil but was reduced to r= 0.79 at p <0. 01 in contaminated soil. This represented 18% increase in correlation coefficient of uncontaminated soil over contaminated soil. Spent lubricant oil contamination of soil could not probably be a limiting factor to soil productivity. [Nature and Science. 2009;7(7):100-112]. (ISSN: 1545-0740).

Keywords: Modified, Prediction, Productivity, Spent Lubricant oil, Soil.

INTRODUCTION

Soil is a universal recipient of myriad of organic wastes and chemicals (Brady and Weil, 2002) produced in modern industrial society. Modern industrial society has manufactured among other things plastic and plasticizers, paints, refrigerants, fuels and lubricants. These products when they enter into the soil become contaminants and capable of reducing its productivity. Soil contaminants are any chemical, physical, biological or radiological materials introduced into the soil. Oil products and hydrocarbons are the most common sources of soil contamination. Contamination of soils could also result in most cases from careless use of chemicals such as fertilizer or simply out of ignorance (Lauhanen ***et al.,*** 2004). Soil contamination occurs when contaminants reach any level that is harmful (Adesodun, 2004).

Lubricant oil contaminated soil has serious fertility problems (Vuoto ***et al.,*** 2005). Soil properties such as bulk density, soil porosity, infiltration rate, hydraulic conductivity, Soil p^H, moisture content, soil type and amount of major nutrients are affected as well as microbial and mineral concentrations of soil. These attributes of soil could be used to evaluate soil productivity. Soil productivity according to Soil Science Society of America (1975) is the capacity of a soil in its normal environment to produce a particular plant or sequence of plants under a specified management system. This is expressed when soil is able to produce a good crop yield with a minimal input at a given set of practices.

Spent lubricant oil contamination had been widely reported by (Atuanya, 1987) and most common in Nigerian cities. Abakaliki is affected by oil drained from machines by mechanics" which is often discarded indiscriminately on the soil. Incidentally, researchers do not seem to take particular interest in this direction which has resulted to paucity of information. The objective of this study was to predict the productivity of a spent lubricant contaminated and uncontaminated soil amended with organic wastes using modified productivity index of Pierce ***et al.*** (1983).

Materials and Methods

Location and Site Description

The experiment was conducted at the Teaching and Research farm of the Faculty of Agriculture and Natural Resources Management of Ebonyi State University, Abakaliki. The area is located by latitude 06^0 4' N and longitude 08^0 65' E of the derived savannah zone of Nigeria.

The rainfall pattern is bimodal. The mean annual minimum rainfall is 1800 mm while the mean annual maximum rainfall is 2000 mm spread between April to early November. There is short spell in August popularly referred to as "August break". At onset of rainfall, it is violent and often torrential lasting for 1-2 hours. The minimum temperature is 27 ^oC while maximum is 31 ^oC. The relative humidity is highest during rainy season (80%) and declines to 60% in dry season especially at harmattan period. The bedrock geology is shale residuum due to successive marine deposit. The soil belongs to the order ultisol classified as Typic Haplustult (FDALR, 1985).

Field Methods

A total land area of 0.032 hectare was cleared and used for the experiment. The land was demarcated into blocks and plots using a 2 x 4 x 4 split plot in randomized complete block design. Plots measured 2 x 2 m and were separated by 0.5 m while blocks were set apart by 1m giving a total of 32 plots. Spent lubricant oil was sourced from mechanic village, Abakaliki. It was spread uniformly using spraying machine on plots receiving it and allowed one week before applying organic wastes treatment. Organic wastes of burnt rice husk dust, un-burnt rice husk dust and saw dust were applied at 20 t ha^{-1} equivalent to 8 kg $plot^{-1}$ on both contaminated and uncontaminated soils.

Maize Oba super II hybrid variety which was used as a test crop was planted two seeds per hole after two weeks of organic wastes treatment. The plants were spaced 25 x 75 cm. They were thinned to one per hole after two weeks of germination leaving 53,000 stands per hectare. Harvest was taken at maturity of crops and yield data adjusted to 14% moisture content.

Soil samples were collected with auger and core. The samples were collected at 0-15, 15-30, 30-45 and 45-60 depths. Core samples were used to determine physical properties of the soil after passing it through 2 mm sieve.

Laboratory Methods

Bulk density was determined by the method described by Blake and Hartge (1986). Available water capacity determination was by Stolte (1997) method using 1000 kpa (wilting point) and 1500 KPa (permanent wilting point). Soil p^H was by 1:2.5 soil / water ratio and values read in p^H meter.

Productivity Index Model and its Modification.

Pierce ***et al.*** (1983) productivity index is stated as follows:

$$PI = \sum_{i=1}^{r} (A_i \times B_i \times C_i \times D_i \times E_i \times wf_i) \quad \ldots\ldots 1$$

Where

PI = Productivity index

A_i = sufficiency for available water capacity for the ith soil layer

Bi = sufficiency for aeration for the ith soil layer

C_i = sufficiency for p^H for the ith soil layer

D_i = sufficiency for bulk density for the ith soil layer

E_i = sufficiency for electrical conductivity for the ith soil layer

Wf_i = Root weighting factor

r = Number of horizons in the rooting zone

Pierce ***et al***. (1983) productivity index was modified to exclude sufficiencies for aeration and electrical conductivity.

Modified productivity index (PIM).

$$PIM = \sum_{i=1}^{r} (A_{ii} \times C_i \times D_i \times Wfi) \quad \ldots\ldots 2$$

Where

PIM = Modified productivity index

A_i = sufficiency for available water capacity for the ith soil layer

C_i = sufficiency for p^H for the ith soil

Di= sufficiency for bulk density for the ith soil layer.

Wfi= root weighting factor.

r= Number of horizons in the rooting zone.

Data Analysis

Data generated after soil analysis were used to compute productivity index and also used to determine relationship between soil properties and grain yield using correlation analysis (Steel and Torrie, 1980).

Results and Discussion

Productivity Index Parameters and Ascribed Sufficiency Values for Contaminated Soil.

Tables 1-4 show soil properties, ascribed sufficiency values and predicted productivity indices for contaminated soils. The soil properties and their individual sufficiency values were used in the computation of productivity index. The result indicated highest productivity indices of 0.57, 0.77, 0.34 and 0.31, respectively for UBRHD, BRHD, BRHD and UBRHD in Tables 1-4.

The control plots contaminated with spent lubricant oil but unamended with organic wastes recorded least productivity indices. These represent 83, 81, 82 and 81%, respectively relative to control productivity indices. This further indicates that organic wastes could be used in ameliorating spent lubricant oil contamination of soil. This result suggest that unburnt and burnt rice husk dust that predicted higher productivity index could be preferred in treatment of spent lubricant oil contaminated soil (Tables 1-4).

Soil Productivity Index Parameter and Ascribed Sufficiency Values for Uncontaminated Soil.

The results on Table 5-8 show soil productivity index parameters and ascribed sufficiency values for uncontaminated soil. Results followed the same trend as in contaminated soil by PI being highest in organic wastes amended soil while they were reduced in control plots un-amended. Productivity indices were 0.87, 0.54, 0.49 and 0.41, respectively for BRHD, SD, BRHD and UBRHD treatments. These results accounted for 44, 82, 14 and 44%, respectively when compared with control plots un-amended with organic wastes.

High productivity index suggest soil with improved soil properties that could boost crop yield. The organic wastes enhanced soil properties such as available water capacity, bulk density, rooting zone and P^H of the soil. Several researchers (Opara-Nadi 1990; Nnabude and Mbagwu, 2000; Agbim, 1985) had earlier reported positive effects of organic wastes in increasing soil productivity.

Soil Productivity Index and Grain Yield of Maize of Contaminated and Uncontaminated Soil Amended with Organic Wastes

Table 9 Shows soil productivity index values and maize grain yields for contaminated and uncontaminated soil amended with organic wastes. The table indicated that mean productivity index value for uncontaminated soil is 0.28 with a corresponding mean maize grain yield value of 1.0 t ha^{-1}. Productivity index recorded highest (0.77) value in burnt rice husk dust amended organic waste in uncontaminated soil, which also had highest maize grain yield of 2.3 tha^{-1}. However, control plots un-amended with organic wastes recorded least value of productivity index and a corresponding least value of maize grain yield (Table1). The table further showed a general and consistent decline in maize grain yield. This suggests that productivity index increase and / or decrease with maize grain yield. This finding is consistent with the report of Nwite (2002) and Anikwe (1999) that maize grain yield increased with productivity index and declined with fall in productivity index.

Similarly, results of contaminated soil show that the mean value for productivity index is 0.34 with a corresponding maize grain yield of 1.06t ha^{-1}. Burnt rice husk dust amended organic waste recorded the highest productivity of 0.58 and a corresponding maize grain yield of 2.2. tha^{-1}. Furthermore, control plot un-amended with organic waste recorded least productivity index of 0.10 and also maize grain yield of 0.3 tha^{-1}. Productivity index had been described by Pierce ***et al.*** (1983), Anikwe (1999) and Nwite (2002) as a veritable tool for predicting soil productivity.

Relationship between Productivity Index and Grain Yield of Maize

Table 10 shows the relationship between productivity indices and maize grain yields of contaminated and uncontaminated soils amended with organic wastes. The results show positive correlation on the two soils. There was a highly significant relationship ($r=0.96$; $P>0.01$) between productivity index and maize grain yield in uncontaminated soil. However, relationship between productivity index and maize grain yield showed significant correlation coefficient of $r = 0.79$ at $P<0.01$ in contaminated soil. The uncontaminated soil increased by 18% in correlation coefficient relative to contaminated soil. The results suggest that although spent lubricant oil could reduce soil productivity, it was not totally limiting. This could be possible since spent lubricant oil contamination has little or no effect on physical properties of soil such as available water capacity, bulk density and rooting depth that are used as parameters for assessing the productivity in this study. Furthermore, Ogbohodo ***et al.*** (2001) had noted that oil contamination had little or no effect on physical properties of soil.

Conclusion

The results of this study indicate that spent lubricant oil contaminated and uncontaminated soil amended with organic wastes could be predicted using modified productivity index. The predicted productivity indices of uncontaminated soils were generally higher relative to contaminated soils. Similarly, productivity indices corresponded with maize grain yields. This showed that spent lubricant oil contamination of soil is probably not limiting to soil productivity. Furthermore, organic wastes could be used to ameliorate spent lubricant oil contamination of soil.

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Table1. Soil Productivity and Ascribed Sufficiency values for uncontaminated soil

	CONTROL							
Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^3)	1.57	1.72	1.72	1.76	0.79	0.29	0.24	0.19
AWC (cm/cm)	0.36	0.46	0.49	0.49	1.00	1.00	1.00	1.00
p^H in KCL	4.8	5.5	5.4	5.1	0.09	0.00	0.00	0.00
Depth of rooting								

Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI								0.10

BRHD

Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^{-3})	1.09	1.21	1.44	1.64	1.00	1.00	1.00	0.59
AWC (cm/am)	0.31	0.42	0.46	0.46	1.00	1.00	1.00	1.00
p^H in KCL	5.6	5.0	4.6	4.2	0.00	0.00	0.19	0.39
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI								0.87

UBRHD

Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^{-3})	1.14	1.57	1.67	1.74	1.00	0.79	0.49	0.20
AWC (cm/cm)	0.32	0.38	0.55	0.69	1.00	1.00	1.00	1.00
p^H in KCL	5.6	5.4	5.4	4.4	0.00	0.00	0.00	0.29
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI								0.14

SD

Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^{-3})	1.18	1.45	1.59	1.61	1.00	1.00	0.70	0.69
AWC (cm/cm)	0.56	0.62	0.68	0.93	1.00	1.00	1.00	1.00
p^H in KCL	5.3	5.2	5.2	4.0	0.00	0.00	0.00	0.49
Depth of rooting Zone	60	60	60	60	1.00	1.00	1.00	1.00
PI								0.49

Table 2. Soil Productivity and Ascribed Sufficiency values for uncontaminated soil.

CONTROL

Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^{-3})	1.72	1.78	1.86	1.88	0.29	0.09	0.00	0.00
AWC (cm/cm)	0.26	0.40	0.37	0.67	1.00	1.00	1.00	1.00
p^H in KCl	5.1	5.3	4.3	5.5	0.00	0.00	0.00	0.00
Depth of rooting Zone	60	60	60	60	1.00	1.00	1.00	1.00
PI								0.10

BRHD

Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^{-30})	1.14	1.49	1.49	1.65	1.00	1.00	1.00	1.00
AWC (cm/cm)	0.36	0.47	0.44	0.53	1.00	1.00	1.00	1.00
p^H in KCl	5.3	5.4	5.4	4.4	0.00	0.00	0.00	0.00
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI								0.29

UBRHD

Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	45-60,	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^{-3})	1.49	1.55	1.57	1.79	1.00	0.79	0.88	0.09
AWC (cm/cm)	0.39	0.43	0.70	0.45	1.00	1.00	1.00	1.00
p^H in KCl	5.4	5.4	5.4	4.6	0.00	0.00	0.00	0.00
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI	0.17							

SD

Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^{-3})	1.54	1.61	1.67	1.69	0.89	0.69	0.49	0.39
AWC (cm/cm)	0.47	0.60	0.67	0.75	1.00	1.00	1.00	1.00
p^H in KCl	4.0	4.2	4.9	5.0	0.48	0.39	0.09	0.00
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI	0.45							

Table 3. Productivity index and Ascribed sufficiency values for uncontaminated soil

CONTROL

Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	15-60	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^{-3})	1.57	1.59	1.68	1.75	0.79	0.70	0.40	0.19
AWC (cm/cm)	0.47	0.52	0.56	0.58	1.00	1.00	1.00	1.00
p^H in KCl	5.9	4.4	4.3	4.3	0.29	0.00	0.30	0.30
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI	0.42							

BRHD

Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	15-60	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^{-3})	1.50	1.52	1.70	1.83	1.00	1.00	0.39	0.00
AWC (cm/cm)	0.25	0.36	0.54	0.65	1.00	1.00	1.00	1.00
p^H in KCl	5.3	4.5	4.5	4.4	0.00	0.20	0.20	0.29
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI	0.49							

UBRHD

Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^{-3})	1.51	1.58	1.61	1.77	0.99	0.78	0.69	0.10
AWC (cm/cm)	0.40	0.54	0.67	0.74	1.00	1.00	1.00	1.00
p^H in KCl	5.3	4.5	4.5	4.4	0.00	0.20	0.20	0.29
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI	0.45							

SD

Soil Properties	Soil Depth (cm)	Ascribed Sufficiency

	0-15, 15- 30, 30-45, 45-60				0-15, 15-30, 30-45, 45-60			
Bulk density (gcm^{-3})	1.52	1.67	1.68	1.89	0.98	0.49	0.40	0.00
AWC (cm/cm)	0.39	0.59	0.68	0.93	1.00	1.00	1.00	1.00
p^H in KCL	5.4	4.9	4.4	4.1	0.00	0.09	0.29	0.40
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI	0.43							

Table 4. Productivity index and Ascribed sufficiency values for uncontaminated soil.

CONTROL

Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15, 15- 30, 30-45, 45-60				0-15, 15-30, 30-45, 45-60			
Bulk density (gcm^{-3})	1.51	1.5	1.69	1.70	0.99	0.79	0.39	0.39
AWC (cm/cm)	0.42	0.49	0.56	0.60	1.00	1.00	1.00	1.00
p^H in KCl	5.5	5.2	4.4	4.3	0.00	0.00	0.29	0.30
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI	0.23							

BRHD

Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15, 15- 30, 30-45, 45-60				0-15, 15-30, 30-45, 45-60			
Bulk density (gcm^{-3})	1.68	1.71	1.73	1.74	0.40	0.30	0.29	0.20
AWC (cm/cm)	0.37	0.37	0.44	0.63	1.00	1.00	1.00	1.00
p^H in KCl	4.5	4.4	4.4	4.3	0.20	0.29	0.29	0.30
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI	0.32							

UBRHD

Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15, 15- 30, 30-45, 45-60				0-15, 15-30, 30-45, 45-60			
Bulk density (gcm^{-3})	1.57	1.64	1.66	1 .70	0.79	0.59	0.49	0.39
AWC (cm/cm)	0.53	0.60	0.60	0.63	00	1.00	1.00	1.00
p^H in KCl	5.7	5.3	4.6	4.5	0.00	0.30	0.19	0.20
Dept of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI	0.41							

SD

Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15, 15- 30, 30-45, 45-60				0-15, 15-30, 30-45, 45-60			
Bulk density (gcm^{-3})	1.48	1.79	1.80	1.81	1.00	0.09	0.00	0.00
AWC (cm/cm)	0.42	0.42	0.56	0.60	1.00	1.00	1.00	1.00
p^H in Kcl	5.5	5.2	4.4	4.3	0.00	0.00	0.29	0.30
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI	0.29							

Table 5. Productivity index and Ascribed sufficiency values for contaminated soil

CONTROL								
Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^{-3})	1.56	1.76	1.77	1.86	0.80	0.19	0.10	0.00
AWC (cm/cm)	0.44	0.52	0.55	0.56	1.00	1.00	1.00	1.00
p^H in KCl	5.5	5.5	5.4	4.3	0.00	0.00	0.00	0.30
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI							0.10	

BRHD								
Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60
Bulk desity (gcm^{-3})	1.60	1.61	1.71	1.91	0.69	0.69	0.39	0.00
AWC (cm/cm)	0.31	0.47	0.57	0.64	1.00	1.00	1.00	1.00
p^H in KCl	5.5	5.3	4.5	4.3	0.00	0.00	0.20	0.30
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI								0.27

UBRHD								
Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^{-3})	1.30	1.56	1.62	1.65	1.00	0.80	0.60	0.50
AWC (cm/cm)	0.49	0.50	0.56	0.58	1.00	1.00	1.00	1.00
p^H in KCl	5.4	5.1	4.4	4.1	0.00	0.00	0.29	0.40
Depth or rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI								0.57

SD								
Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^{-3})	1.36	1.49	1.70	1.82	1.00	1.00	0.39	0.00
AWC (cm/cm)	0.44	0.49	0.60	0.87	1.00	1.00	1.00	1.00
p^H in KCl	5.5	5.1	4.3	4.3	0.00	0.00	0.30	0.30
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00
PI							0.30	

Table 6. Productivity index and Ascribed Sufficiency values for contaminated soil

CONTROL								
Soil Properties	Soil Depth (cm)				Ascribed Sufficiency			
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60
Bulk density (gcm^{-3})	1.36	1.71	1.73	1.95	1.00	0.30	0.29	0.00
AWC (cm/cm)	0.35	0.38	0.44	0.45	1.00	1.00	1.00	1.00
p^H in KCl	5.6	5.1	4.4	4.0	0.00	0.00	0.29	0.49
Depth or rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00

PI									0.15
			BRHD						
Soil Properties			Soil Depth (cm)			Ascribed Sufficiency			
			0-15, 15- 30, 30-45, 45-60			0-15, 15-30, 30-45, 45-60			
Bulk density (gcm^{-3})	1.45	1.46	1.55	1.63	1.00	1.00	0.88	0.59	
AWC (cm/cm)	0.35	0.38	0.40	0.43	1.00	1.00	1.00	1.00	
p^H in KCl	5.5	4.5	4.5	4.2	0.00	0.20	0.20	0.39	
Depth or rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00	
PI									0.77
			UBRHD						
Soil Properties			Soil Depth (cm)			Ascribed Sufficiency			
			0-15, 15- 30, 30-45, 45-60			0-15, 15-30, 30-45, 45-60			
Bulk density (gcm^{-3})	1.67	1.70	1.78	1.85	0.49	0.30	0.09	0.00	
AWC (cm/cm)	0.44	0.50	0.58	0.65	1.00	1.00	1.00	1.00	
p^H in KCl	5.6	5.4	4.3	4.3	0.00	0.00	0.30	0.30	
Depth or rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00	
PI									0.15
			SD						
Soil Properties			Soil Depth (cm)			Ascribed Sufficiency			
			0-15, 15- 30, 30-45, 45-60			0-15, 15-30, 30-45, 45-60			
Bulk density (gcm^{-3})	1.48	1.58	1.78	1.84	1.00	0.78	0.09	0.00	
AWC (cm/cm)	0.35	0.47	0.50	0.65	1.00	1.00	1.00	1.00	
p^H in KCl	5.5	4.5	0.45	4.2	0.00	0.20	0.20	0.39	
Depth or rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00	
PI									0.39

Table 7. Productivity index and Ascribed sufficiency values for contaminated soil

			CONTROL						
Soil Properties			Soil Depth (cm)			Ascribed Sufficiency			
			0-15, 15- 30, 30-45, 45-60			0-15, 15-30, 30-45, 45-60			
Bulk density (gcm^{-3})	1.64	1.74	1.79	1.91	0.59	0.20	0.20	0.00	
AWC (cm/cm)	0.36	0.38	0.41	0.50	1.00	1.00	1.00	1.00	
p^H in KCl	5.4	5.2	4.5	4.3	0.00	0.00	0.30	0.20	
Depth or rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00	
PI									0.60
			BRHD						
Soil Properties			Soil Depth (cm)			Ascribed Sufficiency			
			0-15, 15- 30, 30-45, 45-60			0-15, 15-30, 30-45, 45-60			
Bulk density (gcm^{-3})	1.64	1.74	1.79	1.91	0.59	0.20	0.20	0.00	
AWC (cm/cm)	0.36	0.38	0.41	0.50	1.00	1.00	1.00	1.00	
p^H in KCl	5.4	5.2	4.5	4.3	0.00	0.00	0.30	0.20	
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00	

PI									0.34
	UBRH								
Soil Properties	Soil Depth (cm)				Ascribed Sufficiency				
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60	
Bulk density (gcm^{-3})	1.42	1.68	1.77	1.78	1.00	0.40	0.10	0.09	
AWC (cm/cm)	0.35	0.38	0.51	0.76	1.00	1.00	1.00	1.00	
p^H in KCl	5.6	5.2	4.6	4.3	0.00	0.09	0.09	0.30	
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00	
PI									0.32
	SD								
Soil Properties	Soil Depth (cm)				Ascribed Sufficiency				
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60	
Bulk density (gcm^{-3})	1.34	1.48	1.81	1.82	1.00	1.00	0.09	0.00	
AWC (cm/cm)	0.42	0.43	0.45	0.57	1.00	1.00	1.00	1.00	
p^H in KCl	4.7	4.5	4.4	3.9	0.10	0.20	0.29	0.52	
Depth or rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00	
PI									0.30

Table 8. Productivity index and Ascribed sufficiency values for contaminated soil

	CONTROL								
Soil Properties	Soil Depth (cm)				Ascribed Sufficiency				
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60	
Bulk density (gcm^{-3})	1.45	1.76	1.77	1.79	1.00	0.19	0.10	0.09	
AWC (cm/cm)	0.32	0.46	0.47	0.50	1.00	1.00	1.00	1.00	
p^H in KCl	5.6	5.1	4.3	4.3	0.00	0.00	0.30	0.30	
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00	
PI									0.06
	BRHD								
Soil Properties	Soil Depth (cm)				Ascribed Sufficiency				
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60	
Bulk density (gcm^{-3})	1.59	1.59	1.63	1.66	0.70	0.70	0.59	0.49	
AWC (cm/cm)	0.41	0.50	0.59	0.59	1.00	1.00	1.00	1.00	
p^H in KCl	5.4	5.3	4.9	4.3	0.00	0.00	0.09	0.70	
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00	
PI									0.26
	UBRHD								
Soil Properties	Soil Depth (cm)				Ascribed Sufficiency				
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60	
Bulk density (gcm^{-3})	1.67	1.67	1.68	1.90	0.49	0.49	0.40	0.00	
AWC (cm/cm)	0.40	0.50	0.55	0.58	1.00	1.00	1.00	1.00	
p^H in KCl	5.6	5.1	4.4	4.4	0.00	0.00	0.29	0.29	
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00	

PI									0.31
				SD					
Soil Properties	Soil Depth (cm)				Ascribed Sufficiency				
	0-15,	15- 30,	30-45,	45-60	0-15,	15-30,	30-45,	45-60	
Bulk density (gcm^{-3})	1.76	1.77	1.79	1.82	0.19	0.10	0.09	0.00	
AWC (cm/cm)	0.34	0.43	0.44	0.47	1.00	1.00	1.00	1.00	
p^H in KCL	5.3	4.4	4.1	4.1	0.00	0.29	0.40	0.40	
Depth of rooting Zone (cm)	60	60	60	60	1.00	1.00	1.00	1.00	
PI									0.11

Table 9. Soil productivity index and maize grain yield of uncontaminated and contaminated soil

	Uncontaminated soil			Contaminated		
Treatments	PI	Maize grain yield (t ha^{-1})		TreatmentsPI	maize grain yield (t ha^{-1})	
C	0.10	0.4		C	0.10	0.30
C	0.15	0.6		C	0.10	0.30
C	0.06	0.2		C	0.42	1.3
C	0.06	0.2		C	0.23	0.6
B	0.27	1.8		B	0.58	2.2
B	0.77	2.6		B	0.29	0.8
B	0.24	1.3		B	0.49	1.5
B	0.26	1.7		B	0.32	0.9
U	0.57	2.4		U	0.14	0.4
U	0.15	0.6		U	0.17	0.7
U	0.32	1.2		U	0.45	1.4
U	0.31	1.0		U	0.14	1.1
S	0.30	0.9		S	0.49	1.5
S	0.39	1.8		S	0.54	2.0
S	0.30	0.9		S	0.43	1.2
S	0.11	0.5		S	0.29	0.8
Total	4.46	16.1		Total	5.45	17.0
Mean	0.28	1.01		Mean	0.34	1.06

C – Control
B – Burnt
U – Unburnt
S – Sawdust

Table 10. Relationship between productivity index and maize grain yield

Dependent parameters	regression model	correlation (r) Coefficient	Soil type
PI VS maize grain yield (tha^{-1})	Y = 3.47 x – 0.12	0.96 **	uncontaminated
PI VS maize grain yield (tha^{-1})	Y = 2.99 x + 0.17	0.79 *	contaminated

AWC - Available water capacity
BRHD - Burnt rice husk dust
Control - control plot un-amended with organic wastes
PI - Productivity index
SD - Saw dust
UBRHD - Un-burnt rice husk dust
VS - Versus
* - Significant
** - Highly significant

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Pedology of Oak and Pine Forests in Indian Central Himalaya

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Abstract: For the analysis of physicochemical properties of soil it was taken from two different forest sites (*P. roxburghii* Sarg. and *Q. leucotrichophora* A. Camus) that are degraded and non-degraded forests at Lamgarha block of Almora district in Kumaun Central Himalaya. Soil texture varied from loamy to sandy loam. Soil moisture ranged from 6.6-18.1% and showed fixed seasonal pattern and maximum in rainy season (Mid-September) followed by winter and summer. Water holding capacity was more or less similar in all the sites. The soil was acidic with pH ranging from 5.5-6.5 across the sites. Soil nutrient concentration also varied across the study sites. [Nature and Science. 2009;7(7):113-115]. (ISSN: 1545-0740).

Key words: Physicochemical analysis, degraded forest, non-degraded forest.

Introduction

Nature and composition of soil vary considerably with geological formations, aspect, and degree of slope, climate and vegetation (Saxena and Srivastava, 1973). In the Himalayan state of Uttaranchal (India) the rich alluvial soil of Tarai is quite different from poor bare soil of mountain and almost desert like soil of trans-Himalayan zone (Negi, 1990).

This paper is an attempt to study physicochemical properties of soil in different status of forest viz., degraded and non-degraded forest sites or natural sites in Guna Van Panchayat (VP) at Lamgarha block in Almora District. It varies from 79°43.2 E longitude, and 29°33.04 latitude and altitudinal variation from 1800 m to 1900 m. It is located about 29 km far from main Almora town. In these study sites, various forage development and carbon sequestration related forestry based projects were implemented. It is also a research site for a NGO i.e. Central Himalayan Environmental Association (CHEA), Nainital. The site being a hilly region comprises of numerous hills and forests. Some of the important forests were locally named as chir pine (*P. roxburghii* Sarg.) forests and banj oak (*Q. leucotrichophora* A. Camus) forest. They are categorized as Guna banj oak degraded and non-degraded forest sites and Guna chir-pine degraded and non-degraded forest sites.

Climate is warm temperate with moderate summers and severe winters with annual rainfall in 2004 was 832.0 mm and in 2005 was 921.9 mm (Lamgarha block office, 2005). Three seasons: warm and wet rainy season (June-September), cool and dry winter season (October-February) and hot and dry summer season (March-May) is quite distinct here.

Materials and Methods

For the present study fieldworks were done in peak rainy/ growth season (September) in 2004, in winters 2004 (December) and in summers 2005 (April). Depending upon vegetational conditions and extent of degradation the whole study area was distributed in the following degraded and non-degraded sites as-

Guna non-degraded oak forest (site 1), Guna degrade oak forest (site 2), Guna non-degraded pine forest (site 3) and (site 4) as a non-degraded pine forest.

In order to collect soil samples (0-30 cm depth) grasses, mosses, litter and other plant residues were removed from soil surface. Thereafter 100 g of soil was collected in a plastic bag which was sealed and labelled properly. Soil texture was determined by using sieves of various size classes, whereas soil moisture content on dry weight basis, soil pH by potentiometer method and soil nitrogen, phosphorus, potassium, soil carbon and organic matter were analyzed at Niglat soil testing laboratory of ICAR (Indian Council of

Agriculture and Research, New Delhi) at Bhowali of Nainital district of Uttaranchal (India). In all three seasons the soil moisture and water holding capacity were measured by Zobel et al. 1987. Besides soil moisture content remaining soil parameters were measured only during December.

Results and Discussion

Soil moisture showed fixed seasonal trend, i.e. maximum in rainy season followed by winter and summer showing direct relationships with precipitation. In the study site it ranged form 6.56±0.16 to 18.07±0.44 (Table 1). Almost sites facing north aspect, soil moisture was comparatively higher favouring the growth of mosses.

Amount of sand, silt and clay were estimated to be 43.74±2.41%, 52.08±2.49% and 4.17±0.28% respectively at Guna non-degraded oak forest site, 41.04±2.25%, 53.34±2.94% and 5.61±1.20% respectively at Guna degraded oak forest site (Table 2). It was estimated as 45.88±2.01%, 49.56±2.09% and 4.55±0.24% for sand, silt and clay respectively at Guna non-degraded pine forest site, 83.29±1.39%, 8.35±0.69% and 8.35±0.69% respectively for sand, silt and clay at Guna degraded pine forest site (Table 2). Hence, soil texture was loam at degraded sites and sandy loam at non-degraded sites. Water holding capacity was more or less similar irrespective of seasons but slightly higher in winters followed by rainy and summer seasons. It was found in the range of 43.34±1.26% to 45.27±0.72% (Table 1), which was more or less similar to the reported value of Rikhari et al. (1991) i.e. 52% to 67% found in Kumaun Himalaya. It was found to be the highest at degraded or regenerating sites.

Soil porosity was 38.93±2.95% to 54.39±1.46%, while the bulk density was ranged between 1.24±0.12% to 1.94±0.24% g/cm^3. It was found to be the highest at non-degraded forest sites. Soil pH was 5.5±0.0 - 6.5±0.0 at degraded forest sites (Table 2). This clearly indicates the acidic nature of the soil. The pH value at non-degraded sites was within the reported range of Rikhari et al. (1991) which was from pH 4.7 to 6.8 in Kumaun Himalaya and near to Teare (1986) that was from pH 4.62- 5.9 at different sites of Lalitpur district. Organic matter varied from 2.84±0.26% at degraded pine forest site to 5.05±0.17% at non-degraded oak forest site (Table 2). It was within the reported range of Shrestha (1979) which was from 1.68% to 17.0% at Godawari. Nitrogen ranged from 0.13±0.07% at degraded pine forest site to 0.33±0.12% at non-degraded oak forest site (Table 2). It was also found to be in close findings of Baral (1983) that was from 0.137% to 0.385 at Phulchoki (Nepal) but higher then that of Shrestha (1979) who reported it to be in a range of 0.07% to 0.26% and Sah & Ram (1989)valued it from 0.16% to 0.22%. The higher value may be due to the fact that these forest sites possessed sand stone soil which was acidic in nature. This type of soil as stated by Rathore (1971) is usually rich in organic matter and nitrogen. Theses two soil nutrients were also highly related to each other as shown by Shrestha (1979) and Gupta et al. (1989). High values of both soil nutrients at relatively moist soils of non-degraded sites were due to high decomposition rate because of the high soil moisture, the decomposition rate is accelerated, as also shown by Rastvorova & Tereshenkova (1978). Available phosphorus at these sites varied from 42.12±9.07 kg/ha at degraded pine forest site to 56.00±10.85 kg/ha at non-degraded oak forest site (Table 2). It was within the reported range of Shrestha (1979) who found it to be from 1.03 to 71.15 kg/ha but lower than that of Baral (1983) that was from 44.66 to 90.66 kg/ha. Potassium ranged from 226.19±31.08 kg/ha at degraded pine forest site to 388.57±33.07 kg/ha at non-degraded oak forest site (Table 2). Both these soil nutrients were maximum at non-degraded oak forest site followed by degraded oak forest site and non-degraded pine forest site. The value of soil carbon ranged from 1.14±.003% at degraded oak forest site to 1.40±.007% at non-degraded pine forest site. It was found 1.17±.005% at non-degraded oak forest site while, 1.15±.003 at non-degraded pine forest site (Table 2).

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Table 1 Seasonal Soil Moisture, Water Holding Capacity (W.H.C), Porosity and Bulk Density at different studied sites.

Sites types	**Soil moisture (%)**			**W.H.C. (%)**	**Porosity (%)**	**Bulk density (g/cm^3)**
	September, 2004	**December, 2004**	**April, 2005**			
Site 1	15.65±0.92	11.54±0.16	6.82±0.28	43.34±1.26	38.93±2.95	1.62±0.11
Site 2	14.62±0.59	11.76±0.17	6.56±0.16	43.97±1.98	54.39±1.46	1.24±0.12
Site 3	14.97±0.88	11.37±0.20	7.25±0.25	43.44±0.56	41.94±1.48	1.94±0.24
Site 4	18.07±0.44	11.34±0.24	7.76±0.23	45.27±0.72	42.17±2.81	1.66±0.14

Table 2 Physicochemical Characteristics of soil at different sites

Parameters	**Site 1**	**Site 2**	**Site 3**	**Site 4**
pH	5.50±0.00	6.20±0.00	6.50±0.00	6.50±0.00
N (%)	0.33±0.12	0.20±0.08	0.19±0.11	0.13±0.07
P (kg/ha)	56.00±10.85	50.00±10.38	48.36±8.42	42.12±9.07
K (kg/ha)	388.57±33.07	267.40±36.17	263.67±27.14	226.19±31.08
C (%)	2.93±0.021	1.85±0.017	2.76±0.023	1.65±0.013
O.M (%)	5.05±0.17	3.18±0.63	4.75±0.23	2.84±0.26
Sand (%)	43.74±2.41	41.04±2.25	45.88±2.01	83.29±1.39
Silt (%)	52.08±2.49	53.34±2.94	49.56±2.09	8.35±0.69
Clay (%)	4.17±0.28	5.61±1.20	4.55±0.24	8.35±0.69

(O.M. = Organic matter, N = Nitrogen, P = Phosphorus, K = Potassium, C = Carbon)

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Optimization Of 2, 4 Dichlorophenol Degradable Crude Extracts Produced by *Pseudomonas aeruginosa* Using Box Behnken Design

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ABSTRACT: *Pseudomonas aeruginosa* was grown on mineral medium containing 2, 4 dichlorophenol as a sole source of carbon and energy. Process optimization was carried out by developing 17 combinations using Box Behnken design to identify the best combinations of the parameters which involved in the production biomass to obtain high yield of crude extract. The highest protein concentration in biomass from 17 combinations obtained from the experiment is 4.99 mg/ml (35 ml of medium, 6 ml of inducer and 6 ml of inoculum). The point prediction from the analysis of variance for response surface cubic model for the production of protein concentration (4.88 mg /ml) is 35 ml of medium, 4.5 ml of inducer and 4 ml of inoculum. [Nature and Science. 2009;7(7):117-128]. (ISSN: 1545-0740).

Keywords: 2, 4 Dichlorophenol, Crude extract, *Pseudomonas aeruginosa*, Optimization, ANOVA and Box Behnken design

1. INTRODUCTION

Tabak *et. al.*, (1964) described microbial metabolism of aromatic carbon compounds. The purpose of this investigation was to determine the ability of specifically adapted bacteria to degrade phenol and substituted phenols, and to study the relationship between the chemical structure of phenol derivatives and cyclic hydrocarbons and their susceptibility to decomposition by organisms adapted to related aromatic compounds.

Walter Reinke *et. al.*, (1984) isolated 2,4 dichlorophenol-degrading bacterium (, strain WR1306) by continuous enrichment from a mixture of soil and sewage sample & grown in a chemostat on a mineral medium with 2,4 dichlorophenol. Respiration data and enzyme activities in cell extracts as well as the isolation of 3-chlorocatechol from the culture fluid are consistent with the degradation of 2,4 dichlorophenol. Michel Rutgers *et. al.*, (1993) used nutristat to grow pentachlorophenol (PCP)-degrading microorganisms. Rebecca M Goldstein *et. al.*, (1985) explained the reasons for possible failure of inoculation to enhance biodegradation.

Ayami Nakagawa *et. al.*, (2006) found 32% of DCP was degraded within 1h.He is the first one to prove dechlorination pathway by Zygomycetes. Khadar valli *et. al.*, (1991) examined the degradative pathway of 2,4-dichlorophenol by P. chrysosporium. They showed that this pathway involves several cycles of oxidation and subsequent quinone reduction and hydroquinone methylation.

Mohammad Edrissi and Nima Razzaghi asl (2007) discussed the application of RSM method in optimizing complexation of iron with piroxicam. A response surface methodology (RSM) based on a Box-Behnken design was applied for study on ferrous ions binding ability to piroxicam in aqueous solution as a function of three numerical factors (extraction time, pH, piroxicam concentration) and extractant type as a categorical variable each in three levels.

Experimental designs nowadays have been regarded as one of the most favorable techniques in covering a large area of practical statistics and obtain unambiguous results with the least expense. Response surface method (RSM) designs help to quantify the relationships between one or more measured responses and the vital input factors. The most popular response surface methodologies are Central Composite, Box-Behnken designs.

Box-Behnken design is an efficient and creative three-level composite design for fitting second-order response surfaces. It is an independent quadratic design. The methodology is based on the construction of balance designs which are rotatable and enable each factor level to be tested several times. Each factor or independent variable can be placed at one of three equally spaced values (coded as –1, 0, and +1). In this design the treatment combinations are at the midpoints of edges of the cubical design region and at the center.. Box-Behnken designs provide excellent predictability within the spherical design space and require fewer experiments compared to the full factorial designs or central composite designs. The number of required experiments for Box-Behnken design can be calculated according to $N = k^2 + k + c_p$, where k is the factor number and c_p is the replicate number of the central point.

In the present investigation, crude cell extracts from the enriched strain *P. aeruginosa* on 2,4 dichlorophenol was immobilized on sodium alginate beads and the beads were packed in a glass column to study the degradation. Seventeen sets of combinations of process parameters were developed to produce crude extracts. The experiment was carried out in different concentrations 2,4 dichlorophenol in the immobilized beads which contains crude extracts of *P. aeruginosa*.

2. MATERIALS AND METHODS

2.1 Maintenance and cultivation of microorganism

The strain *P. aeruginosa* was obtained from NCIM, Pune, India. The strain was sub cultured in nutrient broth. The broth was incubated in the shaker with 175 rpm and at 37ºC overnight. Sterile plates containing nutrient agar of specified composition were streak plated with the overnight cultures. The culture on the plates was used as the source for the entire experiment. The mineral medium with specified composition (Table 1) of chemical substances was prepared to conduct the experiment. The pH of the mineral medium was adjusted to 7.0 by using 2 NH_2SO4 or 2N NaOH solution. 50 ml of the medium was taken in each of 250 ml Erlenmeyer flasks and were sterilized at 1.5 kg/cm^2 (gauge) for 20 min. After cooling to room temperature, the medium was added with 2, 4 dichlorophenol and inoculated in a laminar flow chamber. The flasks were then incubated on a rotary shaker for 48 h at 30ºC and 175 rpm, for full growth of the strain. The sub cultured strains were stored at 5ºC.

Table 1 Composition of Medium

Ingredients	Concentration (g/l)
NH_4NO_3	1.0
$(NH_4)_2SO_4$	0.5
NaCl	0.5
K_2HPO_4	1.5
KH_2PO_4	0.5
$Mgso_4.7H_2O$	0.5
$CaCl_2$	0.01
Double distilled Water	1 l

2.2 Suspension of washed cells and cell extracts

Cells grown on 2,4 dichlorophenol as the sole carbon source, were harvested in the mid-exponential growth phase by centrifugation (8,000 rpm for 10 min at 4°C), washed with sodium phosphate buffer (pH 7.0, 50 mM), and suspended in the same buffer. The cell extracts were prepared by disrupting the cells by ultrasonic disintegration (Labsonic-P of Labsonic-Germany). The resulting cell lysate was centrifuged at 8,000 rpm for 10 min at 4°C, and the supernatant, containing approximately 10 to 20 mg of protein ml^{-1}, was the crude cell extract (containing 2,4 dichlorophenol degrading enzyme). The concentrations of protein content in the crude extracts were measured using UV Visible Spectrophotometer (Hitachi UV 2800).

2.3 Optimization of the process parameters

Process optimization was carried out by conducting 17 experiments (Table 2) to identify the best combinations of the parameters which involved in the production biomass to obtain high yield of crude extract. The parameters, the volume of mineral medium (20,35 and 50 ml), inducer (3 -6 ml) and inoculum (2 - 6 ml) were selected. The mineral medium, Inducer (2, 4 dichlorophenol) and inoculum were processed as mentioned different cultures were obtained by varying the three parameters and processed to obtain its crude extract. The concentration of the crude extract was measured at 280 nm. The data obtained from 17 experiments, were used to find out the optimum point of the process parameters by using Box Behnken Design in Response surface methodology. All the data were treated with the aid of Design Expert from Stat-Ease.

3. RESULTS AND DISCUSSION

3.1 Analysis of variance

Based on design of experiment, 17 combination were developed (Table 2) and processed to obtain crude extracts as mentioned in this paper. The data obtained from the experiments were used to the analysis of variance (Table 3 and 4). The Model F-value of 6.366E+007 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, BC, A^2, B^2, C^2, A^2B, A^2C, $AB^2$are significant model.

Table 2 Combination of process variables

Run	A:Medium (ml)	B:Inoculum (ml)	C:Inducer (ml)	Crude extract (mg/ml)
13	35	6	6	4.99
6	35	2	6	4.88
7	35	4	4.5	4.74
8	35	4	4.5	4.74
9	35	4	4.5	4.74
10	35	4	4.5	4.74
11	35	4	4.5	4.74
4	20	6	4.5	3.57
1	20	2	4.5	2.97
5	35	2	3	2.05
2	20	4	3	1.98
3	20	4	6	1.34
15	50	4	3	0.96
17	50	6	4.5	0.56
16	50	4	6	0.31
12	35	6	3	0.23
14	50	2	4.5	0.15

Table 3 Analysis of variance (ANOVA):

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	60.13575	12	5.011312	6.366E+007	< 0.0001	significant
A-Medium(ml)	1.05401	1	1.05401	6.366E+007	< 0.0001	
B-Inoculum(ml)	0.727097	1	0.727097	6.366E+007	< 0.0001	
C-Inducer(ml)	14.42253	1	14.42253	6.366E+007	< 0.0001	
AB	0.008603	1	0.008603	6.366E+007	< 0.0001	
AC	2.72E-06	1	2.72E-06	6.366E+007	< 0.0001	
BC	0.920256	1	0.920256	6.366E+007	< 0.0001	
A^2	24.39735	1	24.39735	6.366E+007	< 0.0001	
B^2	1.120969	1	1.120969	6.366E+007	< 0.0001	
C^2	5.894329	1	5.894329	6.366E+007	< 0.0001	
ABC	0	0				
A^2B	0.925412	1	0.925412	6.366E+007	< 0.0001	
A^2C	9.87168	1	9.87168	6.366E+007	< 0.0001	
AB^2	1.790021	1	1.790021	6.366E+007	< 0.0001	
AC^2	0	0				
B^2C	0	0				
BC^2	0	0				
A^3	0	0				
B^3	0	0				
C^3	0	0				
Pure Error	0	4	0			
Cor Total	60.13575	16				

Table 4 Regression Analysis

Std. Dev.	0	**R-Squared**	1
Mean	2.804	**Adj R-Squared**	1
C.V. %	0	**Pred R-Squared**	N/A
PRESS	N/A	**Adeq Precision**	3.1E-308

Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The application of response surface methodology (Kenneth et.al, 1995, Khuri, A.I.,) yielded the following regression equation, which is an empirical relationship between the logarithmic values of protein yields and test variables in coded unit.

Final equation in terms of coded factors with coefficients values (Table 5):

Y (Crude extract mg/ml) = + 4.74 - (0.51 * A) - (0.43 * B) + (1.90 * C) - (0.046* A * B) –(8.250E-004 * A * C) + (0.48 * B * C) - (2.41 * $A^{2)}$ - (0.52 * $B^{2)}$ - (1.18 * $C^{2)}$ + (0.68 * A^2 * B) – (2.22 * A^2 * C) – (0.95 * A * B^2)

Where Y is response that is the protein concentration is expressed in logarithmic values and A, B, and C are the coded values of the test variable medium, inducer and inoculum respectively.

Table 5 Coefficients obtained from regression analysis

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	4.737	1				
A-Medium(ml)	-0.513	1				2
B-Inoculum(ml)	-0.426	1				2
C-Inducer(ml)	1.899	1				2
AB	-0.046	1				1

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
AC	-0.001	1				1
BC	0.480	1				1
A^2	-2.407	1				1.0058
B^2	-0.516	1				1.0058
C^2	-1.183	1				1.0058
A^2B	0.680	1				2
A^2C	-2.222	1				2
AB^2	-0.946	1				2
AC^2 ALIASED A, AB^2						
B^2C ALIASED C, A^2C						
BC^2 ALIASED B, A^2B						
A^3 ALIASED A						
B^3 ALIASED B						
C^3 ALIASED C						

3.2 Analysis of process variables by response surface plots

The optimum values of the selected variables were obtained by solving their regression equation and analyzing response surface contour plots. Response Surface plots as a function of two factor at a time maintaining all other factors at a fixed level (zero for instance) are more helpful in understanding both the main and interaction effects of the two factors. The plots can be easily obtained by calculating the data from the model. The values were taken by one factor, where the second varies with constant of a given Y - values. The yield values of the different concentrations of the variable can also be predicted from respective response surface plots. Figures 1 to 6 shows the relative effects of the two variables with protein concentration level. The coordinates of the central point within the highest contour levels in each of these figures corresponded to the optimum concentrations of the respective components.

Figure 1 and 2 show their contour and response surface plot obtained as a function of volume of inoculum vs. medium with protein concentration, while all other variables are maintained at zero level (coded units). Figure 3 and 4 show their contour and response surface plot obtained as a function of volume of inducer vs. medium with protein concentration. Figure 5 and 6 show their contour and response surface plot obtained as a function of volume of inducer vs. inoculum with protein concentration.

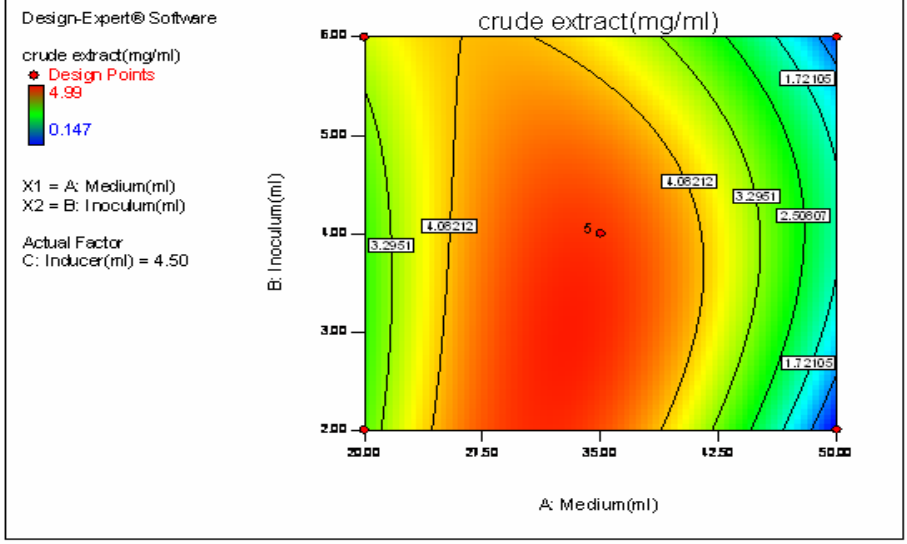

Figure 1: Contour plot : Volume of inoculum vs Medium with protein Concentration.

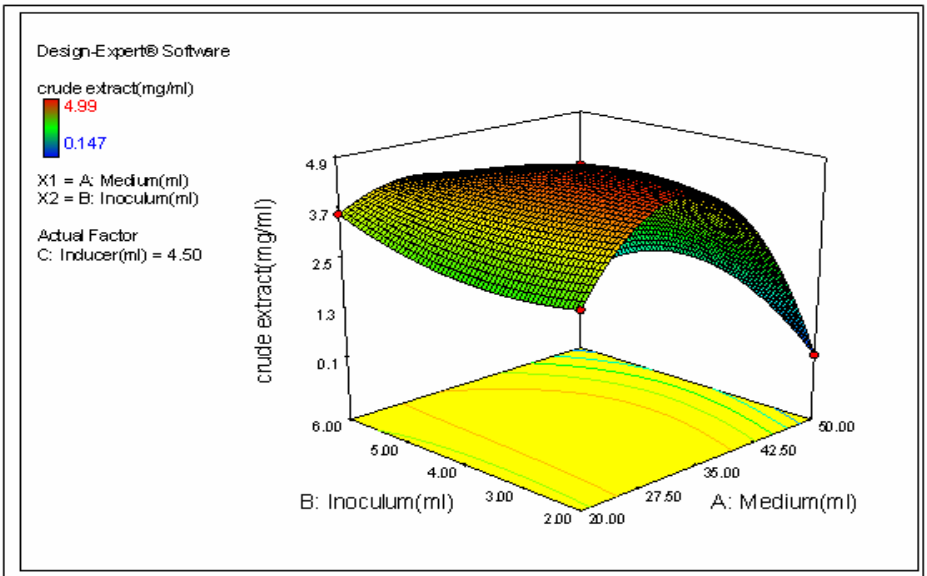

Figure 2: Response surface plot : Volume of inoculum Vs. Medium with protein Concentration.

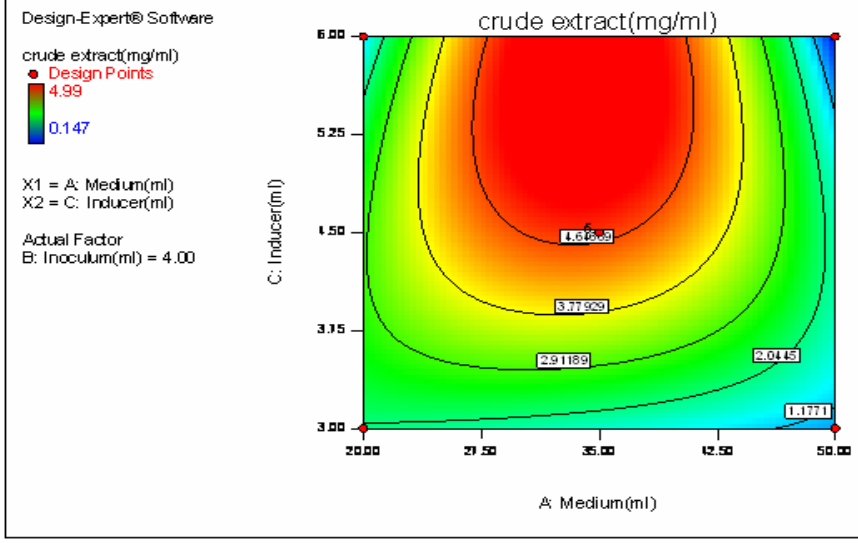

Figure 3: Contour plot : Volume of inducer Vs. Medium with Protein concentration.

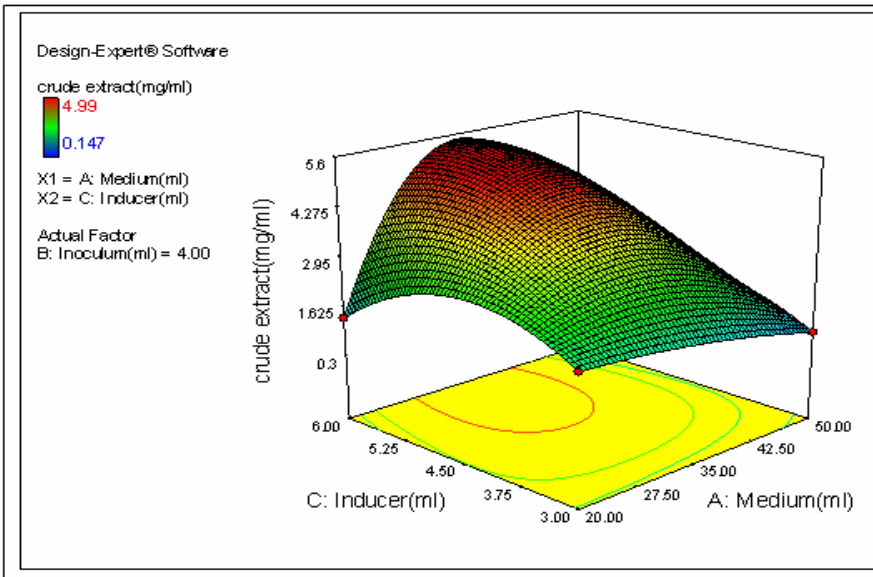

Figure 4: Response surface plot : Volume of inducer Vs. Medium with protein concentration.

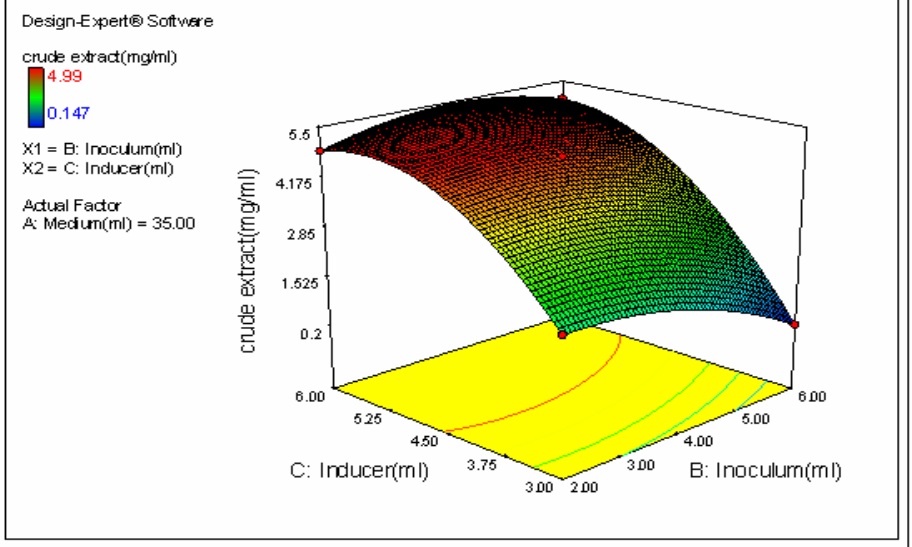

Figure 6: Response suface plot : Volume of inducer Vs. Inoculum with protein concentration.

3.3 Optimum Values

The protein production was predominantly influenced by medium and inducer concentration. From the Contour plots the red color shows the region of the desirability for the production of protein. The point prediction from the analysis of variable for response surface cubic model for the production of protein concentration (4.88 mg /ml) is 35 ml of medium, 2 ml of inducer and 6 ml of inoculum.

Table 6 Predicted value from Box -Behnken design

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding	
A	Medium(ml)	35	20	50	0	Actual	
B	Inoculum(ml)	2	2	6	0	Actual	
C	Inducer (ml)	6	3	6	0	Actual	
Response	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
Crude extract(mg/ml)	4.88	0	4.88	4.88	0	4.88	4.88

PI - Prediction interval

CI - Confidence interval

SE Mean – Standard error of the mean.

SE Pred – Standard error of prediction

4 Conclusion

2,4 Dichlorophenol can induce the synthesis of enzymes in *Pseudomonas aeruginosa* that are able to break down hydrocarbons including 2,4 dichlorophenol. In this work the process conditions were optimized to produce crude extracts. Immobilization of the crude extracts obtained from *Pseudomonas aeruginosa* increases the efficiency of the extract and they have been used to study the degradation of 2,4 dichlorophenol in a packed bed column. Thus it has been concluded that this study will yield good results if extended to large-scale applications.

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Efficient *in vitro* multiplication of Syrian Rue (*Peganum harmala* L.) using 6-benzylaminopurine pre-conditioned seedling explants

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Abstract
The frequency of multiple shoot regeneration of two seedlings explants i.e. shoot apex and cotyledonary node with both cotyledons of *Peganum harmala* was affected by the concentration of benzylaminopurine (1.11 - 15µM) in the regeneration medium as well as significantly influenced by the preconditioning of seeds with 0.5 - 11.1µM benzylaminopurine prior to excision of explants. Among the untreated explants, average number of 4 shoots per explant was observed on MS medium supplemented with 4.44µM BAP in shoot apex and cotyledonary node explants with 100% and 92% frequency of regeneration respectively. While among the explants excised from the seedlings raised on MS medium containing 11.1µM BAP, the average number of shoot formation per explant was increased up to 6 in case of shoot apex and 6 - 7 in cotyledonary node explants cultured on MS medium supplemented with 4.44µM BAP, with in four weeks of culture. Regenerated shoots were rooted on MS medium containing half strength salts, 3% sucrose and 5µM IBA with 80% efficiency. The plantlets were successfully established in soil where 90% of them survived into morphological normal plants. [Nature and Science. 2009;7(7):129-134]. (ISSN: 1545-0740).

Abbreviations: BAP- 6-Benzylaminopurine, IBA- Indole-3-Butyric Acid, MS- Murashige and Skoog

Key words: Cotyledonary node; *In vitro* regeneration; Micropropagation; *Peganum harmala*; Shoot apex; Syrian Rue.

1. Introduction

Peganum harmala L. (Syrian Rue), a medicinally important perennial herb of family Zygophyllaceae (Ananymous, 1986; Stewart, 1972). But recently it has been placed in Nitrariaceae family (Sheahan and Chase, 1996). *Peganum* is distributed over semi arid areas of North-West India, North-Africa and central Asia.

The plant is having biochemical, pharmaceutical (Baytop, 1999) and ornamental importance and used as abortifacient, aphrodisiac, emmenagogue, galactogogue and diuretic. It enriches the blood and is useful in weakness of muscles and brain (Chatterjee, 1997; Kiritikar, 1995; Sharma, 1988). Carboline alkaloids like harmine, harmaline, harmalol, peganine, vasicine, vasicinon, deoxyvasicione, peganone-1 (3-6-dihydroxy-8-methoxy-2methyl anthraquinone), peganone-2 (8- hydroxy – 7 methoxy – 2 methyl anthraquinone) obtained from various parts of this plant are used against a number of diseases (Aarons, 1977; Sobhani et al 2002).

Medicinally the fruits and seeds are digestive, diuretic, hallucinogenic, hypnotic, antipyretic, antispasmodic, nauseant, emetic, narcotic and uterine stimulant (Chatterjee, 1997; Kiritikar, 1995; Sharma, 1988). A red dye obtained from seeds is widely used in Turkey and Iran for colouring carpets (Baytop, 1999). Leaves are useful in asthma, colic, dysmenorrhea, hiccup, hysteria, neuralgia and rheumatism (Chatterjee, 1997; Kiritikar, 1995; Sharma, 1988). The plant has also been used as antimicrobial (Adday et al 1989; Alkofahti et al 1990; Prashanth et al 1999), antitumoral (Prashanth et al 1999), in curing malaria (Kiritikar et al 1995) and has insecticidal potential (Ahmed et al 1981).

Peganum harmala is propagated by seeds (Saini and Jaiwal, 2000). One of the constraints of this conventional propagation is of very short span of seed viability. No reliable data are available about seed germination, growth and fruiting of the plant in domesticated or natural settings (Khawar et al 2005). Because of increasing exploitation of the natural population for its wide use in traditional medicine and since the plant grows as wild and not cultivated; it is facing the problem of extinction (Saini and Jaiwal, 2000). There is no alternative mode of multiplication to propagate and to conserve the genetic stock of this medicinal plant. Some preliminary work on micropropagation of this species has been done by a few workers (Saini and Jaiwal, 2000; Khawar et al 2005). But the frequency of regeneration as well as number

of shoots per explants was low with lesser efficiency of establishment of plants in soil. Hence the present study was designed to develop efficient *in vitro* procedure for multiple shoot formation using shoot apex and cotyledonary node explants so that such explants could be used as storage tissue for cryo-preservation and rapid propagation of this plant stock. Besides it, the effect of different dose of 6-benzylaminopurine growth regulator preconditioning of seeds before the harvest of seedling explants was also studied.

2. Materials and Methods

The seeds of *Peganum harmala* were collected from the plants growing wild in Haryana, state of India. Uniform and healthy seeds were selected and surface sterilized first by washing with liquid detergent (Tween-20) and rinsing gently under tap water this is followed by sterilization with 0.2% mercuric chloride ($HgCl_2$) solution for 5 - 6 minutes under the sterile conditions and finally were thoroughly washed seven to eight times with sterilized distilled water to remove traces of mercuric chloride.

2.1 Inoculation of seeds without pretreatment

The surface sterilized seeds were germinated aseptically on MS basal medium containing 3% (w/v) sucrose and 0.8% (w/v) agar. Explants like shoot apex (0.5-1.0 cm) and cotyledonary node with cotyledons (1.0-1.5cm) were excised from 10-15 days old seedlings with the help of sharp sterilized blade and cultured in vertically upright position on MS medium containing 3% (w/v) sucrose, 0.8% (w/v) agar and supplemented with different concentrations of BAP (1.11µM, 2.22µM, 4.44µM, 11.1µM).

2.2 Inoculation of seeds with pretreatment

The surface sterilized seeds were germinated aseptically on MS medium supplemented with different concentration of BAP (0.5 µM, 2.0 µM, 11.1 µM and 15.0 µM). The shoot apex (0.5-1.0 cm) and cotyledonary node with cotyledons (1.0-1.5cm) explants were excised from 10-15 days old pre-cultured thickened seedlings with the help of sharp sterilized blade and cultured on MS medium supplemented with 4.44µM BAP.

In all the cases, the pH of medium was adjusted to 5.8 by adding 0.1 N HCl or 0.1 N NaOH before adding agar-agar and autoclaving. The media was poured and equally distributed in the cultural tubes (25x150 mm). These cultural test tubes were plugged with non-absorbent cotton plugs (cotton wrapped in muslin cloth) and sterilized in an autoclave at 1.05 atmospheric pressure at 121^{o}C for 15-20 minutes. At least 24 cultures for each treatment and all experiments were repeated three times. All the cultures were maintained under a 16hr/day photoperiod (80µE $m^{-1}s^{-1}$) of cool- white fluorescent light at $25^{0}\pm2^{0}$C.

The elongated multiple shoots (2-3 cm) formed in various media were excised individually and implanted in culture tubes containing MS medium (half strength salts and 3% sucrose) with 2.5µM, and 5.0µM IBA under aseptic conditions for rooting. The cultures were maintained under the similar physical culture conditions as above for shoot regeneration.

The plantlets with well developed roots were thoroughly washed in tap water to remove agar medium from roots and transferred to earthen pots containing sterilized soil and sand mixture in the ratio of 1:1. Each pot was covered with a polythene bag containing small holes, to maintain high humidity around the plants and was kept in culture room. The pots were irrigated with 1/4 MS salt solution on alternate days. The polythene bags were removed after about two weeks for 3-4h daily to expose the plants to the conditions of natural humidity. After further one week, the plants were transferred to bigger pots containing sand and garden soil in 1:3 ratios, and were maintained under natural conditions of day length and temperature in the green house.

3. Results and Discussion

The seeds collected freshly from the fields showed 60% germination on MS basal medium with in 8 -10 days while 2 year old stock showed only 25% germination on MS medium. The concentrations of cytokinins are known to be critical in shoot regeneration under *in vitro* conditions and among the various cytokinins BAP is the most widely used and most effective cytokinin for *in vitro* regeneration in various plant species such as *Plumbago rosea* (Harikrishan and Hariharan, 1996), *Alinia galangal* (Anand and Hariharan, 1997), *Vigna mungo* (Saini and Jaiwal, 2002), *Vigna radiata* (Sonia et al 2007), *Vigna unguiculata* (Chaudhary et al 2007). Therefore, in the present study the effect of different concentrations of BAP supplied at the time of regeneration either alone or in combination of different dose of BAP during germination was assessed using two seedlings explants viz: shoot apex and cotyledonary node with cotyledons. The shoot apices cultured on MS basal medium directly elongated into single shoots without

callus formation at the cut ends, and the shoots gradually developed roots at the basal end, resulting into complete plantlets in 100% of the cultures. The cotyledonary node explants when cultured on MS basal medium produced an average of 1.5 shoots per explant from the axils of the cotyledons in 98% of the cultures without much callus formation at the basal cut end. It was found that MS medium without growth regulator failed to induce callus and multiple shoots from the explants, probably due to the insufficient level of endogenous growth regulators in explants and therefore required an exogenous supply of growth regulators for the response.

When the explants shoot apex and cotyledonary node with cotyledons excised from the aseptically raised seedlings germinated on MS basal medium were inoculated on MS medium fortified with different concentration of BAP (1.11µM - 11.1µM) induced a variable amount of callus at the base of explants with in 8-10 days and followed by multiple shoot differentiation within 15 days of culture (Table-1). Initiation of the callus was perhaps due to the exogenous supply of growth regulators which disturbed the established polarity and induced the callus formation. Similar observations have been made in hypocotyls and cotyledon explants of *Sesbania grandiflora* (Khattar and Mohan Ram, 1983) and *Leucaena leucocephala* (Singh and Lal, 2007). Shoot apex and cotyledonary node with cotyledon showed maximum number of shoots 4.00±0.40 and 4.05±0.40 per explant with 100 % and 92% regeneration respectively on MS medium fortified with 4.44µM BAP (Table-1, Figure1a, 1b). At this concentration of BAP, the *in vitro* regenerated shoots showed an average shoot length of 1.1 cm. The length of the shoots showed an inverse relationship with the concentration of BAP (Table 1).

When the explants harvested from the aseptically raised seedlings germinated on MS medium fortified with various concentration of BAP (0.5µM - 15.0 µM) were inoculated on MS medium supplemented with 4.44µM BAP showed the significant enhancement in shoot regeneration efficiency (Table-2). Preconditioning of shoot apex and cotyledonary node with cotyledons explants at 11.1µM BAP was the most effective in increasing the number of shoots with an average of 6.04±0.61 and 7.01±0.70 number of shoots per explant with 98% and 90% regeneration frequency, respectively than those without preconditioning treatment (an average of 4 shoots / explant) (Table 2, Figure1c, 1d). However, no significant difference in the shoot length was observed due to preconditioning of explants. Similar work of BAP pretreatment has been reported by different workers on different plants such as *Vigna mungo* (Saini and Jaiwal, 2002, 2005, 2007), *Linnum usitaissium* (Yildiz and Ozgen, 2004; Burbulis et al 2005).

The *in vitro* regenerated shoots (2 – 3 cm long) implanted on half strength MS basal medium without growth regulator showed very less profuse rooting in only 1%. While the shoots implanted on MS medium containing half strength salts, 3%sucrose and fortified with the IBA (5.0µM) was found effective in regeneration of the profuse roots with 80% efficiency (Table-3, Figure1e)

Rooted shoots were planted in pots having sterilized soil and sand mixture in the ratio of 1:1. These plantlets irrigated with 1/4 strength MS salt solution. High humidity was maintained for initial 15 days with the help of polythene bags. After this, pots were regularly exposed to natural conditions for 3-4 hours daily for acclimatization of plantlets. After about a week these acclimatized plants were shifted to the greenhouse where they grew normally with 90% survival rate (Figure1f).

Form the present study we can conclude that after giving the preconditioning to the explants, there was an efficient increase in the multiple shoot formation rather than without preconditioning. Therefore, the protocol could be used for the mass multiplication of this highly important medicinal plant species in a short duration as well as the phenomenon of preconditioning of seedlings with cytokinin like BAP prior to excision of explants may be exploited for the efficient multiplication of other plant species.

Table-1 Effect of different concentration of BAP on multiple shoot formation from shoot apex and Cotyledonary node with cotyledons explants of *Peganum harmala* cultured on MS medium.**

Explant	Plant growth regulator(µM)	% regeneration	No. of shoots per explant (mean±SE)*	Mean length of shoot (cm)
Shoot apex	0.0	100	1.00±0.10[a]	2.95
	1.11	100	1.75±0.17[a]	2.11
	2.22	100	1.21±0.12[a]	1.52
	4.44	100	4.00±0.40[b]	1.14
	11.1	85	1.77±0.17[a]	0.82
Cotyledonary node	0.0	98	1.50±0.15[a]	2.41

with cotyledons	1.11	96	1.75±0.17[a]	2.22
	2.22	96	2.36±0.23[a]	1.30
	4.44	92	4.05±0.40[b]	1.12
	11.1	92	2.09±0.20[a]	0.75

*For different explants separately, mean values followed by same letter are not significantly different according to Newman-Keul's multiple range test (p=0.05)
**Data based on 72 explants per treatment and taken after 28 d of culture

Table-2 Effect of BAP pretreatment of shoot apex and cotyledonary node with cotyledons explants of *Peganum harmala* on MS medium supplemented with different concentration of BAP before cultured on MS medium containing 4.44µM BAP.**

Explant	BAP(µM) Pretreatment	% regeneration	No. of shoots per explant (mean±SE)*	Mean length of shoot (cm)
Shoot apex	0.0	100	4.00±0.40[a]	1.14
	0.5	100	4.22±0.46[a]	1.16
	2.0	100	4.55±0.47[a]	1.12
	11.1	98	6.04±0.61[b]	1.10
	15.0	90	5.67±0.57[b]	1.00
Cotyledonary node with cotyledons	0.0	98	4.05±0.40[a]	1.12
	0.5	96	4.30±0.46[ab]	1.12
	2.0	96	5.07±0.52[b]	1.09
	11.1	90	7.01±0.70[c]	0.98
	15.0	80	6.92±0.75[c]	0.98

*For different explants separately, mean values followed by same letter are not significantly different according to Newman-Keul's multiple range test (p=0.05)
**Data based on 72 explants per treatment and taken after 28 d of culture

Table-3 Effect of different concentration of IBA on rooting of *in vitro* regenerated shoots cultured on MS medium half strength salts and 3% sucrose.**

IBA concentration (µM)	% of shoot rooted
0.0	1.0[a]
2.5	45.0[b]
5.0	80.0[c]

* Values followed by same letter are not significantly different according to Newman-Keul's multiple range test (p=0.05)
** Data recorded after 20 days of culture.

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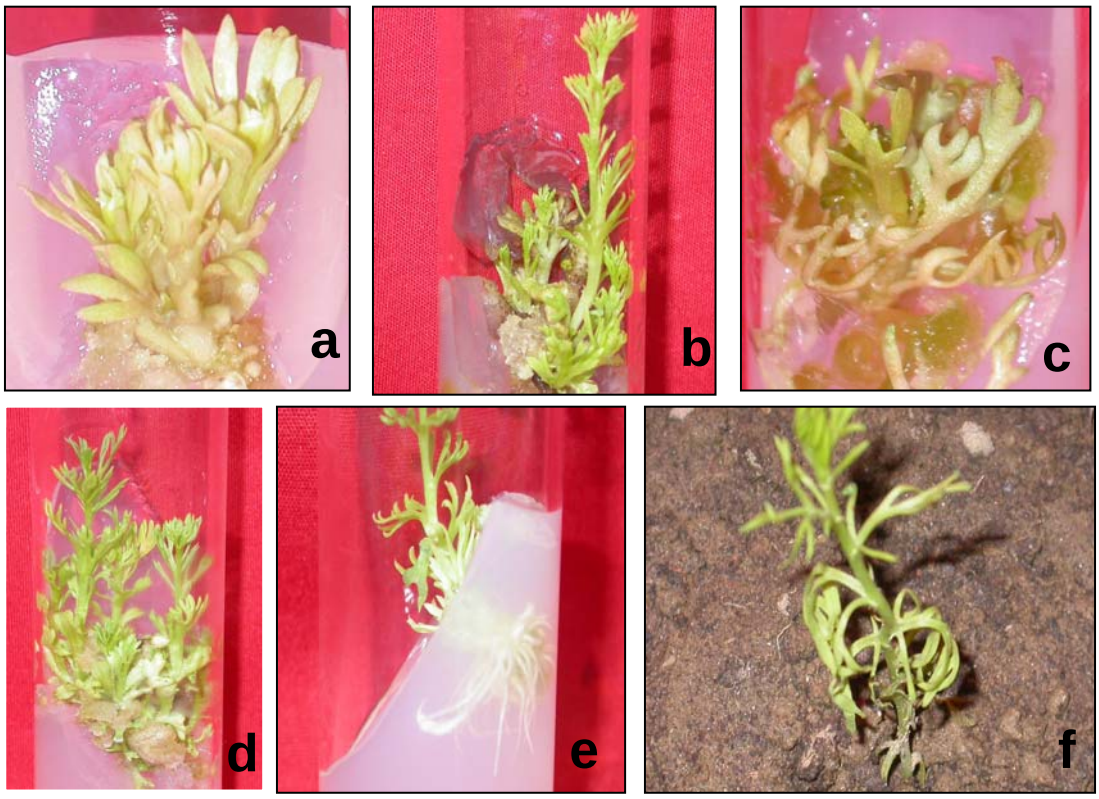

Figure1 (a-f): *In vitro* regeneration of *Peganum hamrala.* a, Regeneration of multiple shoots from shoot apex on MS+4.44µM BAP; b, Shoots developed from cotyledonary node with cotyledons on MS+4.44µM BAP; c, Regeneration of multiple shoots from preconditioned shoot apex at 11.1µM on MS+4.44µM BAP; d, Regeneration of multiple shoots from preconditioned cotyledonary node with cotyledons at 11.1µM on MS+4.44µM BAP; e, Regeneration of roots from shoot on ½ MS+5.0µM IBA; f, Transplanted plantlets in pots containing garden soil.

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(6) Cover Page: Put detail information of authors and a short title in the cover page.
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(8) Figures and Tables: Use full word of figure and table, e.g. **"Figure 1.** Annul Income of Different Groups**", Table 1. Annual Increase of Investment".**
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Reference Examples:

Journal Article: Hacker J, Hentschel U, Dobrindt U. Prokaryotic chromosomes and disease. Science 2003;301(34):790-3.
Book: Berkowitz BA, Katzung BG. Basic and clinical evaluation of new drugs. In: Katzung BG, ed. Basic and clinical pharmacology. Appleton & Lance Publisher. Norwalk, Connecticut, USA. 1995:60-9.

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(2) Abstract: including Background, Materials and Methods, Results, and Discussions.
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