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Studies on the Interaction of Natural Antifungals with Metal Ferrocyanides and Their Medicinal Applications

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Abstract: Manganese, Silver and Titanium ferrocyanides were synthesized and characterized by elemental and spectral studies. The stabilities of these metal ferrocyanides were investigated in the presence of acids, bases, organic solvents, tap and sea water at room and boiling temperature. The natural antifungal plants studied were azadirachta indica (Neem), ocimum sanctum (tulsi), cassia obtusifolia (money bush), cassia alata (canicro bush), tagetes patula (marigold). The natural antifungal plant extract with metal ferrocyanides complexes were found to be have more antifungal property in comparison to metal ferrocyanides and natural antifungals alone. Antifungal activity of natural antifungals, metal hexacyanoferrate(II) compound and natural antifungal metal ferrocyanide complexes were tested by well known cultured fungus (Aspergillus Niger). The titanium ferrocyanide with neem extract and manganese ferrocyanide with money bush extract complexes were found to have maximum and minimum antifungal property, respectively.
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Keywords: metal ferrocyanides, natural antifungals, aspergillus niger, medical value, skin infection.

1. INTRODUCTION

Antifungal extracts are to be obtained from various local plants in Guyana. These plants include azadirachta indica, cassia alata, cassia obtusifolia, oscimum sanctum and togetes patula. Studies on these plants have shown that they possess antifungal compounds mainly in their leaves, bark and fruits. The extract may show various activities depending on the method of extraction – such methods include wet, dry and steam distillation. In addition they are also used for various medicinal purposes such as laxative and antibacterial among others. Azadirachta indica is believed to be an answer to many incurable diseases. A mixture of the extracts from the leaves, bark, fruit and seeds is used efficiently to treat skin diseases[1]. Cassia alata leaves and flowers are used as remedy for asthma, bronchitis, diabetes, ulcers, scabies and skin diseases such as puritis eczema, etc.[2,3]. Cassia obtusifolia leaves extracts are known to be used to treat feet rashes, lotta, scabies, ringworm, and other skin infections. The oil extract of Ocimum sanctum consists of eugenol, eugenol methyl ether and carvacrol which are main contributors to the medicinal value of Tulsi. It helps to eradicate ringworm and other skin diseases when applied to such skin infections[4]. The whole herb of tagetes patula is used in coughs and dysentery, taken internally in the form of a decotion. Extracts of Marigold can be used as fungicides. In addition to antifungal properties plants extract also have various other medicinal uses[5-10]. To analyze the nature of metal ferrocyanides and the extracts, the best suited fungal spore is Aspergillus niger. A. niger is omnivorous and one of the most common easily identifiable species of the genus. A. niger may also be a common laboratory contaminant [11].

Primitive earth atmosphere was anoxygenic and reducing potential of atmosphere was not high enough hence metals like iron, chromium, molybdenum, manganese and tin etc. were in the form of their lower oxidation states. Considering the fact that cyanide was formed in all simulated

experiments of primitive earth conditions, cyanide could have combined with a large number of metal ions present in primeval sea. Consequently, several insoluble metal ferrocyanides of general formula $M_2[Fe(CN)_6] \cdot x\ H_2O$, where M = Fe, Cr, Mo, Zn, etc. could have been formed. It is well established that metal ferrocyanides acts as adsorbents[12], ion-exchangers[13] and photosensitizers[14].

Literature survey indicates that no report is available on medical value of natural antifungal – metal ferrocyanide complex. In view of this attempt were made to study medical application of these complexes. In addition present work describes synthesis, characterization and medical application of manganese, silver, titanium ferrocyanides – natural antifungal complexes.

2. EXPERIMENTAL SECTION

2.1 Chemicals

All chemicals used were of AnalaR grade and used as such without any further purification. Potassium ferrocyanide, manganese chloride, silver nitrate, titanium tetrachloride were obtained from BDH, Poole, England. Solutions were prepared in doubly distilled water.

2.2 Synthesis of metal ferrocyanides

Manganese and silver ferrocyanides were prepared by Kourim's method[15]. Whereas titanium ferrocyanide was prepared according to the procedure reported by Bastian et al. [16].

The manganese and silver ferrocyanides were prepared by adding potassium ferrocyanide (167 ml; 0.1 M) slowly to metal chloride/nitrate (500 ml; 0.1 M) with constant stirring. Reaction mixture was heated on water bath for 2-3 h and cured for 24 h. The precipitate was washed with distilled water and dried at 60 C. The dried product was ground and sieved to 125 µm BSS mesh size. In case of silver ferrocyanide all reactions were performed in the dark. Silver ferrocyanide was kept in the dark bottle.

The best condition for the preparation of titanium ferrocyanide involves variation in the mole ratio of titanium to hexacyanoferrate(II), which vary between 10 to 1 and 1 to 10, respectively. For this experiment we will use a 0.5 M solution of titanium tetrachloride in 2.0 M aqueous hydrochloric acid and 0.34 M solution of hexacyanoiron(II) acid. The solution of hexacyanoiron(II) acid is won by pouring a solution of potassium hexacyanoferrate(II) over a Dower-50-exchanger and then poured into the 2.0 M $HCl/TiCl_4$ solution. The filling material from the exchanger is centrifused out after 24 h and dried over phosphorous pentaoxide and potassium hydroxide in a vacuum desiccators. The dried product was washed with water free from chloride ions and then dried again in the vacuum desiccators. The dried product was ground and sieved to 125 µm BSS mesh size.

2.3 Characterization of metal ferrocyanides

Manganese, silver ferrocyanides are found to have light blue colour, while titanium ferrocyanide have forest green colour. All are amorphous solid and shows no X-ray pattern. The metal ferrocyanides were characterized on the basis of elemental and spectral studies.

The percentage composition of metals were determined by IL – 751 atomic absorption spectrophotometer. Carbon, hydrogen and nitrogen analysis were carried out by CEST – 118, CHN analyzer. Percentage composition of all three metal ferrocyanides are given in Table 1.

Infrared spectra of the metal ferrocyanides were recovered in KBr disc on Beckman IR – 20 spectrophotometer. All three metal ferrocyanides show a broad peak at 3800 cm^{-1} is characteristics of water molecules and OH groups. Also a peak at around 1600 cm^{-1} is due to H-O-H bending. A sharp band at 2000 $cm^{-1}$and a broad peak at 600 cm^{-1}were observed in all three metal ferrocyanides are characteristics of cyanide and Fe – C stretching, respectively. A band around 500 cm^{-1} is observed in all three metal ferrocyanides may be due to polymerization of metal – nitrogen bond.

2.4 *Stability of metal ferrocyanides*:

All three metal ferrocyanides were found to be stable in acids (HCl, H_2SO_4, HNO_3, CH_3COOH) bases (NaOH, KOH, NH_4OH) in concentration range 0.5 – 2.0 M at room and boiling temperature. Metal ferrocyanides unaffected by salt (LiCl, NaCl, KCl, NH_4Cl, RbCl, CsCl, $BaCl_2$ and $CaCl_2$) solutions at room temperature in concentration range of 0.5 – 2.0 M.

Metal ferrocyanides are also found to be stable in tap and atlantic ocean water at room and boiling temperature. The change in colour of metal ferrocyanides of various conditions are may be due to loss of water molecules from the compound.

2.5 Preparation of natural antifungal extracts

The extraction from cassia obtusifolia was done by wet method. The leaves of the plant were picked and soaked by covering with 95% ethanol solvent for 24 h. The ethanol – extract was filtered using glass wool. The filtrate was vaporized using a rotovapourizer at 45 C until all the ethanol is removed. The final extract was considered as the stock solutions from which further dilutions would be made for analysis cassia alata, cassia obtusifolia, ocimum sanctum and azadirachta indica extraction were done using dry method. The green leaves were dried for three days at 45 C, then grounded using an electric mill. The powdered leaves were then be soaked by covering with 95% ethanol solvent for 24 h. The soaking was repeated three times. The ethanol – extracts were then filtered by gravitation filtration using whatman filter paper. The ethanol was then be removed using rotovaporizer until the extract solidifies. The antifungal activity of plant extracts, metal ferrocyanides and plant extract – metal ferrocyanide complexes, were tested on a known cultured fungus, Aspergillus niger.

2.6 Test on antifungal activity

2.6.1 Testing the antifungal activity of metal ferrocyanides only Metal ferrocyanide (10 mg) was placed in a sterilized petri dish containing media.

The fungal spores were then sprayed on the entire bottom of the dish using an aspirator. The similar method was repeated using different metal ferrocyanides.

2.6.2 Testing the antifungal activity of extract only

The antifungal plant extract (10 mg) was placed by means of washing with 20 ml ethanol in a sterilized petri dish containing media. The fungal spores were sprayed on the entire bottom of petri dish using an aspirator. The same method was repeated using different plants extract.

2.6.3 Testing the antifungal activity of antifungal plant extract – metal ferrocyanide complexes

Metal ferrocyanide (10 mg) and antifungal plant extract (10 mg) were placed in sterilized petri dish containing media. The fungal spores were sprayed on the entire bottom of the petri dish using an aspirator. This method was repeated using different extract and metal ferrocyanide complexes.

2.6.4 Testing the antifungal activity of control (ethanol only) Ethanol (20 ml) was placed in sterilized petri dish containing media. The fungal spores were then sprayed on the entire bottom of the dish using an aspirator. The essay was left to stand in sealed container in an incubator at 28 C for 168 h.

3. RESULTS AND DISCUSSION

3.1 Antifungal activity of metal ferrocyanides only

Antifungal activities of manganese, silver and titanium ferrocyanides was studied. Titanium ferrocyanide and manganese ferrocyanide were found to have maximum and minimum antifungal property respectively. The following order of antifungal activity was observed in metal ferrocyanides:

Titanium ferrocyanide > silver ferrocyanide > manganese ferrocyanide

The observation of bioassay test of metal ferrocyanides with fungal spores are given in Table 3.

3.2 Antifungal activity of extract only

Antifungal activity of azadirachta indica, cassia alata, cassia obtusifolia, ocimum sanctum and targetes were studied. Azadirachta indica and cassia obtusifolia were found to have maximum

and minimum antifungal property respectively. The following order of antifungal property was

observed in plant extracts.

Azadirchta indica > tagetes patula > oscimum sanctum > cassia alata > cassia obtusifolia

The observations of bio assay test of natural antifungal extract only with cultured fungal spore are given in Table 4.

3.3 *Antifungal activity of metal ferrocyanides and metal antifungal complexes*

The following order of antifungal activity was observed in natural antifungal with metal ferrocyanide complexes.

(i) Manganese ferrocyanide
Azadirachta indica > tagetes patula > ocimum sanctum > cassia alata > cassia obtusifolia

(ii) Silver ferrocyanide
Azadirachta indica > tagetes patula > ocimum sanctum > cassia alata > cassia obtusifolia

(iii) Titanium ferrocyanide
Azadirachta indica > tagetes patula > ocimum sanctum > cassia alata > cassia obtusifolia

Titanium ferrocyanide – azadirachta indica and manganese ferrocyanide – cassia obtusifolia complexes were found to have maximum and minimum antifungal properties, respectively. The observation of bioassay test of metal ferrocyanide – natural antifungal complexes with cultured fungal spore are given in Table 5.

3.4 Antifungal activity of control (ethanol only)

It was observed that fungal spores were able to grow in the control. The growth of fungal spore was unaffected by ethanol.

Table 1. Elemental analysis of manganese, silver and titanium ferrocyanides

Metal Ferrocyanides*	Percentage found				
	Metal	Iron	Carbon	Hydrogen	Nitrogen
MnFc	26.90	13.12	16.30	2.80	18.60
AgFc	40.22	8.75	11.47	2.75	13.77
TiFc	25.35	11.95	15.62	3.17	18.25

*MnFc = Manganese ferrocyanide; AgFc = Silver ferrocyanide;

TiFc = Titanium ferrocyanide

Table 2. Infrared spectral data of manganese, silver and titanium ferrocyanides

Metal Ferrocyanides	Absorption frequencies (cm^{-1})				
	H_2O molecules/ OH groups	HOH bending	υ C ≡ N	υ Fe – C	Metal – N*
MnFc	3800	1600	2000	610	500
AgFc	3800	1600	2010	600	490
TiFc	3800	1615	2020	600	500

* metal – nitrogen band due to polymerization

Table 3. Observation of bioassay test of metal ferrocyanides only with cultured fungal spores

Manganese ferrocyanide	Silver ferrocyanide	Titanium ferrocyanide
Evidence of few fungal spores growth was seen	Few spores of fungus were seen in area where silver ferrocyanide was not present.	No evidence of fungal spores growth seen.

Bio assay: 10 mg metal ferrocyanide per petri dish

Room temperature: 30 ± 1 C

Time: 168 h

Cultured fungus: Aspergillus niger

Order of antifungal activity: TiFc > AgFc > MnFc

Table 4. Observations of bioassay of natural antifungal extract only with cultured fungal spore

Azadirachta indica	Cassia alata	Cassia obtusifolia	Ocimum sanctum	Tagetes patula
Evidence of small amount of spores was seen. It had least evidence of fungal spores.	Evidence of more fungal spores was seen but less than cassia obtusifolia extract	Evidences of wide spread fungal spores was seen in comparison to al other plant extract.	Evidences of fungal spores was seen but more than tagetes patula plant extract.	Evidences of small amount of spores was seen but more than azadirachta indica plant extract.

Bio assay: 10 mg plant extract per petri dish

Room temperature: 30 ± 1 C

Time: 168 h

Cultured fungus: Aspergillus niger

Order of antifungal activity: Azadirachta indica > targets patula > oscimum sanctum > cassia alata > cassia obtusifolia

Table 5. Observations of bioassay test of metal ferrocyanides and natural anti fungal extract with cultured fungal spore

MFc	Azadirachta indica	Cassia alata	Cassia obtusifolia	Ocimum sanctum	Tagetes patula
MnFc	Little sign of fungal growth seen	Some fungal spores seen growing but more than O. Sanctum	Clumps of fungus were seen in the petri dish maximum growth	Few fungal spores seen growing more than T. Patula	Evidence of small amount of fungal growth but more than A. Indica.
AgFc	Very little sign of fungal growth seen	More spores were seen growing than O. Sanctum	Some spores were seen growing in clumps.	Evidences of small amount of fungal growth but more than T. Patula	Evidences of small amount of fungal growth but more than A. Indica
TiFc	No evidence of fungal growth maximum inhibition	Evidences of small amount of fungal growth but more than O. Sanctum	Evidences of small amount of fungal growth but more than C. Alata	Few fungal spores seen growing but more than T. Patula	Little fungal spores seen growing

Bio assay: 10 mg metal ferrocyanide plus 10 mg antifungal plant extract per petri dish

Room temperature: 30 ± 1 C

Time: 168 h

Cultured fungus: Aspergillus niger

CONCLUDING REMARKS

The following conclusions can be drawn from the present studies

(a) Antifungal activity of secondary metabolities are enhanced through interaction with metal ferrocyanides.
(b) Titanium and manganese ferrocyanides were found to have maximum and minimum antifungal property, respectively.
(c) Azadirachta indica and cassia obtusifolia were found to have maximum and minimum antifungal properties, respectively.
(d) Azadirachta indica extract – titanium ferrocyanide complex and cassia obtusifolia extract – manganese ferrocyanide complex were found to have maximum and minimum antifungal property respectively.
(e) It may be also concluded from present studies that titanium ferrocyanide – azadiracha indica extract complex may be used as effective medicine for skin infection.

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Seasonal pattern of lichen fall from trees in an evergreen *Quercus Semecarpifolia* forest of Garhwal Himalaya, India

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ABSTRACT: The Himalaya is one of the richest sources with respect to the occurrence of lichen on oak species. These unique symbiotic organisms that contribute to biodiversity and are important as food and shelter for various wild animals are being lost because of unsystematic harvesting. We purpose that collection of fallen lichens would reduce lichen diversity loss. In the paper we have documented the seasonal pattern of lichen and twig fall, and frequency of fall of common genera in closed and open canopied forests of *Quercus semecarpifolia* (the brown oak) in a moist temperate forest of Garhwal Himalaya. The annual fall of marketable material was 6.4 kg/ha/yr in the open canopied forest. The lichen fall was maximum in the early summer seasons (April-May) at both sites. Lichen collection from the oak forests (*Quercus* species) is carried out without any consideration for sustainability. The branches are chopped and the bark scraped off using sickles and axes. [Nature and Science. 2009; 7(3):8-12]. (ISSN: 1545-0740).

Keywords: Lichen fall, *Quercus semecarpifolia*, Garhwal Himalaya

INTRODUCTION

According to the concept of basic adaptational strategy of plants (Grime 1977), lichens are stress-tolerant organisms. Such organisms are not expected to survive deficiency in resources (stress), as well as destruction of biomass (disturbance). In Uttarakhand and much of the other Himalayan regions people harvest lichens from forests, particularly from oak (*Quercus*) trees without any consideration for sustainability. The lichen collectors damage trees by chopping branches for collecting lichens and firewood from trees for cooking food while camping in/around forest sites. Poverty is so acute in some sections of the society that incomes of Rs.10, 000-35,000 per annum are enough economic incentives for them.

Depletion of lichen populations is a matter of concern from conservation standpoint because of several reasons; being unique symbiotic organisms they contribute to biodiversity; they are ecologically important as food, shelter and nesting material for a variety of wild animals and birds (Mc Cune and Geiser 1997). Among the animals, which use lichens as food, include the rare species, Himalayan musk deer, and others such as goats, sheep, pikas, mice and bats. Some birds use lichens as nesting material (Banfield 1974, Conner 1983). Studies of the Northwest Pacific forests indicate that lichens are important component of food chain, and they play a significant role in forest nutrient cycling (Pike 1978; Maser et al. 1985).

In this article we describe the seasonal pattern of lichen fall from trees in a brown oak forest (*Quercus semecarpifolia*). No data are available on lichen fall in this part of world. We understand that collection of fallen lichens would reduce the depletion of lichen diversity and forest degradation. In order to collect lichens from the ground it is important to know the period of year when lichen falls are high. *Quercus semecarpifolia* is possibly; the most widely distributed species in high altitude areas (above 2400m) of the Himalaya, and is in a serious problem because of poor regeneration and aging population.

MATERIALS AND METHODS

One plot each of 0.22ha. (110m X20m) was identified both within open canopied stand (located on a sun facing slope, having lower moisture and tree diversity) and closed canopied stand (slope having more in moisture, more forest cover, and more tree diversity of Kharsu oak) between 2750-2850m altitudes. 30 permanent plots of 1m² were placed within each the plot. The tree density in the area was estimated by placing 10, 10X10m² random quadrats (Saxena and Singh, 1982). Canopy cover was estimated using a densiometer.

The fallen lichen taxa from each permanent plot were collected at fortnightly interval (represented on monthly basis). Fallen twigs on ground, bearing lichens were collected carefully and were placed in poly

bags for further identification. The lichens were scratched of the twigs using a sharp knife. Fresh weight of the collected material (lichens & twigs) taken using an electronic balance which was oven dried at 60ºc for 48 hours till constant weight. Seasonally collected fallen lichen and twig samples were weighted separately and packed carefully in hard card board notes bearing proper information viz. date of collection, name of collector, plot number, forest site, condition of fallen lichen samples (as lichen found with or without twigs) which have been presented at the Centre for Ecological Studies, A.T. India, Ukhimath (Uttarakhand) India.

Fortnightly information on climatic conditions of the area was documented through interaction with local persons who live there for approximately seven or eight months every year from May to December. On and around collection dates records were made about the visit of troop of langurs (*Prestbytis entellus*), events of heavy snowfall, heavy rainfall, strong wind blowing, hails, human activities (such as harvesting of fodder, lopping of branches for fuel wood, timber and agriculture implements).

RESULTS

The tree density varied between 406 trees/ha at open canopied forest (OCF) and 712 trees/ha at closed canopied forest (CCF) and the forest cover between 42% and 58% (Table 1). The annual lichen fall from trees was 110.5 (±23) mg dry mass (DM)/m² and 158.5 (±28.6) mg DM/m² in OCF and CCF. CCF also had more twig fall than OCF 484.5(±136.5) mg vs. 378 (±129) mg DM/m² (Table 2 and 3).

Among the lichens *Everniastrum* was the largest major contributor to the mass of fallen lichen both in open and closed canopied sites. *Usnea* and *Parmotrema* are other regularly falling lichen species of the area. A total of ten fallen lichen taxa were recorded in the open canopied site of the study area, but in case of closed site it was nine. *Sulcaria* species of fallen lichens was not found in closed canopied site of the forest. *Parmelia, Leptogium* and *Sticta* rarely fall in the CCF, and in case of OCF fall of *Sticta* and *Sulcaria* species are rare, the frequency of these fallen lichens was below 1%.

The lichen fall peaked in April, and this month accounted for about 30% annual lichen fall. This was followed by May and July. Collecting lichens from ground in April and May is quit convenient because herbaceous cover was at the lowest point almost negligible.

Table 1: Representation of density and forest cover in OCF and CCF

Forest site	Forest strata	Species	Density (plants ha ha^{-1})	Forest cover (%)
OCF	Tree	*Quercus semecarpifolia*	280	42
		Rhododendron arboreum	100	
		Acer sp	26	
		Total	406	
CCF	Tree	*Quercus semecarpifolia*	293	58
		Rhododendron arboreum	380	
		Abies pindrow	13	
		Taxus baccata	13	
		Acer sp	13	
		Total	712	

Table 2: Seasonal pattern of lichen fall and twigs dry mass estimation in open canopied forest (OCF)

Year 2006-2007	Dry mass of fallen material (mg/m²)		No. of fallen lichen genera
	Lichens	Twigs	
May	14.5 (±2.0)	36.5 (±11.0)	8
June	10 (±1.5)	20 (±5.0)	7
July	14 (±4.0)	43.5 (±19.0)	6
August	6.5 (±1.5)	43 (±16.0)	6
September	13 (±4.0)	64 (±25.0)	6
October	5.5 (±2.0)	25 (±11.0)	9
November	6 (±1.0)	26 (±12.0)	7
December	4 (±1.0)	10 (±4.5)	8
January	4.5 (±1.0)	11 (±4.5)	8
February	*	*	*
March	*	*	*

April	32.5 (±5.0)	99(±23.0)	9
Total	110.5 (±23.0)	378 (±129.0)	

Table 3: Seasonal pattern of lichen fall and twigs dry mass estimation in closed canopied forest (CCF)

Year 2006-2007	Dry mass of fallen material (mg/m²)		No. of fallen lichen genera
	Lichens	Twigs	
May	32.5 (±7.5)	77 (±30.0)	9
June	7.5 (±1.0)	24.5 (±6.5)	6
July	21.5 (±5.5)	54 (±17.0)	6
August	10.5 (±2.0)	38.5 (±12.0)	6
September	10.5 (±2.5)	38.5 (±14.0)	6
October	1.5 (±o.1)	16.5 (±9.0)	4
November	6.5 (±2.0)	20.5 (±7.5)	6
December	4.5 (±1.0)	15 (±5.0)	5
January	8.5 (±1.5)	22.5 (±4.0)	7
February	*	*	*
March	*	*	*
April	55 (±5.5)	177.5 (±33.0)	7
Total	158.5 (±29.0)	484.5 (±137.0)	

*The lichen fall could not be counted during February and March because of the inaccessibility of sites due to heavy accumulation of snowfall.

Table 4: Frequency list of commonly fallen lichen genera in descending order in the study area:

S. No.	Fallen lichen genera	
	OCF	CCF
1.	*Everniastrum*	*Everniastrum*
2.	*Parmotrema*	*Usnea*
3.	*Usnea*	*Parmotrema*
4.	*Cetrariopsis*	*Heterodermia*
5.	*Heterodermia*	*Ramalina*
6.	*Ramalina*	*Cetrariopsis*
7.	*Leptogium*	*Leptogium*
8.	*Parmelia*	*Parmelia*
9.	*Sulcaria*	*Sticta*
10.	*Sticta*	*Sulcaria*

DISCUSSION

Seasonal pattern of lichen fall

The higher tree density and canopy cover contributed to larger total lichen fall mass in the CCF. The twig fall consisted of both twigs with attached lichens and twigs without lichens. The similarity in lichen fall patterns between the two study sites indicates that lichen fall has a definite seasonal pattern, the knowledge of which can help collectors to decide on strategy to collect them. Storms and movement of monkeys seem to hasten twig fall, as following such events lichens could be seen all over the place. Seasonal pattern of twig fall was similar to that of lichen fall, indicating that lichen growth would hasten twig fall. The lichen cover might hasten twig senescence, or lichens grow well on senescing twigs. The abscission of wood is promoted by higher temperatures in the annual cycle (summer and rainy seasons) although abscission continues, though irregularly, through out the year as a mechanism of canopy clearing by self-pruning (Singh and Singh, 1992). According to the concept of Stone (1989) allogenic factors caused by outward growth of oak canopy, including changes in microclimate and thickening and sloughing of bark, appear to be far more important to most species than changes brought on by the epiphytic species.

On the basis of hypothesis of Larson (1984), Lawrey (1981), and Topham (1977) epiphytes could be competing for light, branch surface space, and water. Fruticose lichens (*Usnea* spp) and foliose lichens

(*Everniastrum* spp) were found dominant on twigs, competition in *Usnea* species appeared to be mainly intrageneric and therefore *Usnea* species should not be affected by clearing other species from around them. The primary succession on oak branches is mostly influenced by the allogenic factors of microclimate change brought by outward canopy growth. However, within the framework of allogenic factors, autogenic factors of competition and facilitation are similar to those, which cause secondary succession (Stone, 1989). Stone (1989) reported that foliose and fruticose lichens developed fully in 9-12 years. The ten most frequent genera were the same in two forest sites, but differed in their order of importance (Table 4).

Doignon (1954) reported that lichens begin to colonize oak twigs in Europe at about five years of tree age. Foliose lichens began to colonize on oak at Fontainebleau, France at about 15-20years. Generally, lichens found on leaves of very long durability are not obligate folicolous but also belong to the corticolous flora, indicating that the obligately folicolous lichens are perhaps restricted to their habitats because they are relatively poor competitors in other habitats. Slightly higher moss coverage on the south side of trunks, (Rincon, 1993) suggested that the combination of abundant moisture and more sunlight may result in greater photosynthetic production which in bryophytes translates into greater volume and biomass growth.

Some experimental studies of Graham (1971), on corticolous lichen (bark inhabiting), showed that the lichen thallus is partly responsible for the modification of its own environment, by increasing its own water holding capacity, it would be possible to grow lichens over a period of years and determine their increase in size and dry weight. A concept given by Denison (1973), he studied on air quality monitoring with lichens in Willamette valley (Oregon), there are major differences in amounts of light and moisture on different sites of a tree trunk. Moisture varies because rainwater flows down channels in the bark of the trunk, living intervening areas well up in the tree receive similar amounts of light and moisture whether they are on the north or the south site of the tree. By examining the lichens on branches we can limit differences caused by variation in light and moisture.

Light affects growth by affecting the rate of photosynthesis and ultimately the amount of assimilate available to the fungus. Most lichens are as matter of fact photophils, and any light reduction would probably come about by gradual closing of the forest canopy over many years. Hakulinen (1966) reported reduction in lichen growth caused by less light might conceivably be offset by an increase in moisture in a shaded habitat.

The market lichens are sold along with twigs, therefore we need to consider both lichens and twigs to which they are attached. Thus the annual fall of marketable material is 6.4 kg/ha/yr in the CCF and 4.9kg/ha/yr in OCF. These lichens are sold at rates of approximately half a dollar/kg in the local markets (Upreti et al 2005). The price however doubles when these lichens reach the central market areas. A trained collector can easily collect 6-8kg of lichens with twigs from the ground (collecting lichens from attached twigs slow down the collection as the entire branches are cut or the lichens are scraped off along with the bark and portion of sapwood). A collector for the major part of the year can earn a reasonable income by collecting the fallen lichens without being destructive with some knowledge of the fall and seasonal pattern.

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Macrolichens Cover And Their Distribution Pattern On Two Common Phorophytes (*Quercus Semecarpifolia* And *Rhododendron Arboreum*) In A Temperate Forest Of Rudraprayag District Grahwal (Uttrarkhand), India

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ABSTRACT: Macrolichens cover and their distribution pattern on two common *Quercus semecarpifolia* and *Rhododendron arboreum* trees from the moist temperate forest (Chopta) of Garhwal Himalaya. Out of three d. b. h. classes trees (diameter at breast height), d. b. h. between 0.1-0.30 m, has found maximum cover of macro-lichens at southeast aspect. [Nature and Science. 2009;7(3):13-16]. (ISSN: 1545-0740).

Key Words- *Lichen cover, Macrlichens, Garhwal Himalaya*

INTRODUCTION

Lichens are most successful symbiotic organisms in nature, dominating 8% or more of the earth's terrestrial area (Ahmadjian 1995) and are amongst the most significant indicators of air pollution and ecosystem health (Richardson 1992, Wolseley et al 1995, Upreti 1995). They are very sensitive to microclimatic changes. Therefore any natural manmade disturbances are bound to affect lichen populations (Negi, 1996). Lichens are able to grow in diverse climatic conditions and on equally diverse substrata. They are widely distributed in almost all the phytogeographical regions of the world. Requisite moisture and light, unpolluted air and undisturbed substratum often favor optimum growth and abundance of lichens (Awasthi, 2000).

To assess the micro-lichen distribution pattern and cover on different tree species, the host tree species were identified through a survey and presence or absence of occurrence of macro-lichens on different tree species in an open canopied forest (ocf) and a closed canopied forest (ccf) were recorded. A total of eight tree species viz. *Rhododendron arboreum*, *R. anthopogon*, *Quercus semecarpifolia*, *Taxus baccata*, *Ilex dipyrena*, *Abies pindrow*, *Asculus indica*, and *Acer sp* were identified in both the forest between 2500m to 3500m altitudes. Due to variation of the aspects and vegetation type on the southeast aspect (ocf site) only two species viz. *Quercus semecarpifolia* and *Rhododendron arboreum* were present there. *Rhododendron arboreum* was less dominant than the *Quercus semecarpifolia*. Thus comparison of macro-lichen species distribution and cover on different parts viz. trunk, branch and twigs of these two tree species were performed.

The present paper, enumerates the cover of some macro-lichens of the Chopta area of the Garhwal Himalaya viz. *Usnea*, *Everniastrum*, *Parmotrema*, *Cetrariopsis* and *Ramalina.* Because these five lichen taxa are commercially exploiting in some high altitude area of Uttarakhand state.

METERIALS AND METHODS

Site

Chopta forest is situated between 2500m to 3500m altitudes along with Akashkamini valley of district Rudraprayag (Garhwal) India. A stratified random sampling method was employed. For the assessment of the macro-lichen species distribution pattern and cover on tree parts, the selected host tree species viz. *Quercus semecarpifolia* and *Rhododendron arboreum* were stratified into different parts or locations of tree parts, due to suitability of the work, on northwest aspect and south east aspect of the forest. The studied parts of the host tree are as trunk, branch, and twigs.

Sampling of host trees

The trees were categorized into three d. b. h. (diameter at breast height) classes viz. 0.1-0.30m, 0.31-0.60m and 0.61-0.90m. Five trees of each species on both the site of the forest of each d .b. h. class were selected randomly between 2500m to 3500m altitudes, and laid five quadrats (10cm²) randomly on each selected tree trunk and in each quadrat, number of individuals of small, medium and large macro-lichens were counted in each sample quadrat and noted properly. The d. b. h of the trunk was also recorded. Similarly three branches of each selected tree species randomly selected and placed randomly five quadrats

(5cm²) on each branch and count and noted the number of individuals of small, medium and thallus in each quadrat sample.

The summing of individuals of each small, medium and large macro-lichen taxa in total number of quadrats studied on total sample trees trunk (five trunks) and multiplied by calculated mean size of small, medium and large size of each selected macro-lichen and the calculated value is divided by total number of quadrats studied. The calculated mean cover represented by square centimeter size of the lichen on the tree part.

Formula (Kumar, 2008)-

$$C = T \times A / N$$

Where 'C' is the size wise cover (cm²) of a macro-lichen.
'T' is the total number of individuals of each small, medium & large size macro-lichen taxa in all quadrats studied on total number of sample trunk for each dbh class.
'A' is the calculated size of macro-lichen taxa.
'N' is the total number of quadrats studied on total sampled trunk of each dbh class.

Similarly for branch the lichen species cover (size wise) were calculated by the following formula (Kumar, 2008)-

$$C = B \times A / N$$

Where 'C' is the size wise cover (cm²) of macro-lichen taxa.
'B' is the total number of individuals of each small, medium & large size macro-lichen taxa in all quadrats studied on total number of branches for each dbh class tree.
'A' is the calculated mean size of that lichen taxa.
'N' is the total number of quadrats studied on total sampled branches of each d. b. h. class tree.

To estimate the lichen cover on twigs, a scale with ten centimeter marking at 1cm distance was used on five randomly selected twigs of each d. b. h. class tree, and the sum of total lichen cover on all sampled twigs of each d. b. h. class trees, was divided by total number of twigs sampled on that d. b. h. class trees (Table 1).

Table 1: Calculated mean size (cm²) of each major lichen taxa.

Major lichen species	Calculated mean size of major macro-lichen thallus (cm²)		
	Small	Medium	Large
Everniastrum	1.7	3.12	6.48
Parmotrema	1.74	3.08	5.0
Usnea	1.9	3.74	6.0
Ramalina	1.56	3.02	4.88
Cetrariopsis	1.74	3.1	5.94

RESULTS

The macro-lichen cover analysis on the tree parts at two different study sites are given in table 2, 3, and 4. In both the aspects young saplings of ***Quercus semecarpifolia*** (dbh between 0.01-0.30m) provides maximum lichen cover, and ***Rhododendron arboreum*** recorded minimum cover of macro-lichens.

Table 2: Lichen cover (cm²) on trunk of two phorophytes at southeast aspect (ocf).

Phorophyte (Trunk)	Trunk dbh (m)	Available mean surface area (m²) for covering/growth of lichens	Lichen cover (cm²/10cm²)
Q. semecarpifolia	0.1-0.30	0.33	7.46
	0.31-0.60	0.9	4.13
	0.61-0.90	8.4	3.2
R. arboreum	0.1-0.30	0.17	4.24
	0.31-0.60	1.31	2.31
	0.61-0.90	5.42	1.39

Table 3: Lichen cover (cm^2) on trunk of two phorophytes at northwest aspect (ccf).

Phorophyte (Trunk)	Trunk dbh (m)	Available mean surface area (m^2) for covering/growth of lichens	Lichen cover ($cm^2/10cm^2$)
Q. semecarpifolia	0.1-0.30	0.35	9.46
	0.31-0.60	0.95	1.43
	0.61-0.90	8.02	2.37
R. arboreum	0.1-0.30	0.12	0.61
	0.31-0.60	0.96	0.53
	0.61-0.90	4.36	0.85

Table 4: Lichen cover (cm^2) on branch of the phorophytes.

Forest	Branch	Lichen taxa	Lichen cover ($cm^2/5cm^2$)	Available mean surface area of the branch for growth of lichen taxa (m^2)
OCF	*Q. semecarpifolia*	*Everniastrum*	2.21	
		Parmotrema	0.69	
		Usnea	1.62	
		Ramalina	0.17	
		Cetrariopsis	0.24	
		Total cover	4.93	0.08
	R. arboreum	*Everniastrum*	0.92	
		Parmotrema	0.35	
		Usnea	0.42	
		Total cover	1.69	0.03
CCF	*Q. semecarpifolia*	*Everniastrum*	3.28	
		Parmotrema	0.34	
		Usnea	0.62	
		Ramalina	0.16	
		Cetrariopsis	0.07	
		Total cover	4.46	0.08
	R. arboreum	*Everniastrum*	0.08	
		Parmotrema	0.29	
		Total cover	0.37	0.03

DISCUSSION

The lichen cover indicates the tree growth function and also attributes for health and ecosystem function of the area. The corticolous lichens growth on tree bark is a useful indicator of young trees. The lichen cover on different parts of phorophytes at south east aspect (open canopied site) of the forest as young *Quercus semecarpifolia* tree diameter (dbh) between 0.1-0.30m, the lichen cover was $7.46cm^2/10cm^2$ and the available mean surface area of the trunk was recorded $0.33m^2$. The trunk diameter between 0.31-0.60m exhibit lichen cover $4.13cm^2/10cm^2$, when the available surface area of the trunk was recorded $0.901m^2$. Similarly the trunk diameter between 0.61-0.90m when the available surface area of the trunk was 8.40 m^2 and $3.20cm^2/10cm^2$ lichen cover was recorded.

In the northwest aspect (ccf) of the same forest, the *Q. semecarpifolia* young tree diameter (dbh) between 0.1-0.30m has $9.46cm^2/10cm^2$ lichen cover out of $0.35m^2$ available surface area of the trunk. The trunk diameter (dbh) 0.31-0.60m has $1.43cm^2/10cm^2$ of lichen cover in $0.95m^2$ surface area of the trunk. Similarly the trunk diameter 0.61-0.91m has lichen cover of $2.37cm^2/10cm^2$ out of $8.02m^2$ surface area of the trunk. Both the foliose lichen genera Everniastrum and *Parmotrema* covered about $1cm^2/10cm^2$ area of the *Q. semecarpifolia* while other lichen genera *Usnea*, *Ramalina* and *Cetrariopsis* exhibit lower lichen cover.

Lichen cover on branch of *Quercus semecarpifolia* was recorded as $4.93cm^2/5cm^2$ out of the available surface area of $0.08m^2$ at southeast aspect (ocf). In the north west aspect (ccf) it was recorded $4.46cm^2/5cm^2$

out of available area of 0.08m². In both the aspect the *Everniastrum* play a significant role providing as it constitutes the highest lichen cover (3cm²/5cm²). The other lichen taxa provide a poor representation (<1cm²/5cm² area) for lichen cover on *Quercus semecarpifolia* branch (Table 4).

The *Quercus semecarpifolia* tree at, northwest aspect exhibits the maximum lichen cover represented by more than 70% while southeast aspect has only 40% of lichen cover.

In the open canopied forest the *Rhododendron arboreum* trunk shows maximum lichen cover. The *Rhododendron arboreum* tree trunk in closed canopied site has poor lichen cover (< 1cm²/10cm²) as compare to the open canopied trees (Table 2 and 3).

The *Rhododendron arboreum* trunk dbh between 0.1-0.30m has 4cm²/10cm² of lichen cover. The trunk with 0.31-0.60m dbh has 2.31cm²/10cm² of lichen cover out of the available surface area of 1.31m². The trunk of dbh 0.61-0.91m recorded 1.39cm²/10cm² lichen cover out of 5.42m² available surface area of the trunk. It is interesting to note that the lichen cover was decreasing with increasing diameter of the *R. arboreum* trunk (Table 2 and 3).

Rhododendron arboreum branches in the open canopied forest has 0.92cm²/5cm cover of *Everniastrum* which is quite low in the closed canopied forest (0.08cm²/5cm), while the *Parmotrema* cover in both closed and open canopied forest was more or less similar. The fruticose lichen *Usnea* was not recorded only closed canopied *Rhododendron* forest (Fig. 4.12 and 4.13). The twigs and trunk of *Rhododendron arboreum* in northwest aspect bear 16% of lichen taxa than the southeast aspect, which has only 5% of lichens.

The size of lichen cover may be affected by a number of climatic variations in the study area. The aspect variation, type of vegetation, darkness and disturbances, presence or absence of light, moisture and other climatic conditions play important role in growth and colonization of lichens. It also depends on the age and bark condition of the tree. The bark of the trees in closed canopied forest; provide excellent conditions for growth of other epiphytes viz. mosses, bryophytes, ferns, orchids, and angiosperms. Thus there remains little space for lichens to colonize.

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Food Habit of the Catfish *Chrysichthys auratus* (Geoffrey Saint – Hilaire, 1808) in Kainji Lake, Nigeria

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ABSTRACT

The food composition of *Chrysichthys auratus* in Kainji Lake was studied for a period of one year. One hundred and twenty specimens were used for the study. Frequency of occurrence and volumetric methods used to analyze the stomachs contents showed that the species fed on fish fry, insects, crustaceans, sand / mud, algae, vegetable matter, molluscs, detritus, nematode and other unidentified items. Seasonal variations of food items showed that stone particles, *Spirotaenia* spp, molluscs that included *Pila* spp, *Planorbis* spp, *Limnaea* spp, *Bulinus* spp were not ingested during rainy season while human food remains only was not ingested in dry season. There was no significant difference ($p>0.05$) in items ingested in both season. There was high degree of feeding intensity during the study period. *Chrysichthys auratus* could be said to be a bottom dwelling fish and omnivorous in Lake Kainji. [Nature and Science. 2009; 7(3): 17 - 22]. (ISSN: 1545 - 0740).

Key words: Food composition, habit, diversity, *Chrysichthys auratus*, Kainji Lake, Nigeria

INTRODUCTION

Lake Kainji (9^0 50' and 10^0 55'N; 4^0 25' - 4^0 45'E) is the largest man - made lake in Nigeria created after damming River Niger for electricity generation. The lake is blessed with a lot of fish species because of abundant food supply amongst other factors.

Fish just like any other animal require food in order to survive and differ considerably in what they consume as food. Abundance and availability of a potential food item according to Lagler *et al*. (1977) determine what fish consumed as food. Authors such as Arawomo and Fawole (1997), Abdullahi and Abolude (2001), Omoigberale and Aruoture (2002) worked on the food and feeding habits of *Hepsetus odeo*, *Sarotherodon galilaeus*, *Bagrus* species, and *Chromidotilapia guentheri* respectively in different water bodies.

Chrysichthys auratus is predominantly an inshore species, abundant in all shallow parts of the lake and commonly found in the commercial catches (Ajayi, 1987). Sturm (1984) reported that *Chrysichthys auratus* in Lake Tiga fed on larvae and pupae of chironomid, nymph and adult of hemipteran, adult of coleopteran and insects larvae as food in addition to ostracods, gastropods, molluscs, fish scales and prawn, higher plant tissues, plant seeds, organic detritus and inorganic matter. However, there is dearth of such information for the species in the lake.

This study tends to look at the food composition of this important fish species in the lake for better management.

MATERIAL AND METHODS

Fish of different sizes were procured from fishermen's catches. This was done monthly for a period of one year. Lengths (standard and total in cm) and weights (g) were taken using measuring board and weigh balance respectively. Fishes were cut opened and the stomachs carefully removed and preserved separately in 4% formalin solution at the landing sites in labelled sample bottles prior to analysis in the Laboratory.

Stomach contents analysis was assessed using a combination of Frequency of occurrence (Bowen, 1983) and volumetric method (Lima-Junior and Goitein, 2001). Stomach contents were identified using Mellanby (1979), Jeje and Fernando (1986).

Analysis of variance was used to test whether there is significant difference in food ingested based on seasons.

Prey Importance Index was obtained separately for each food item based on the method by Lima - Junior and Goitein (2001).

RESULTS AND DISCUSSION

Twenty - two distinct items (Table 1) were ingested by *Chrysichthys auratus*. These were re - classified into ten major groups. This shows that the food of this species is diversified containing both plant and animal materials. The species fed on fish fry, insects especially chironomid and choaborus larvae and pupae, crustacean, sand / mud, algae, vegetable matter, mollusc, detritus, nematode and other unidentified items, which were not significantly different ($p>0.05$). This is inline with the findings of Sturm (1984) and Risch (1986).

The species predominantly fed on detritus, plant tissues / remains and chironomid larvae and pupae. Sturm (1984) did report chironomid larvae and organic debris as most important constituents in the food of this species in Tiga Lake, which is in agreement with this study. The inclusion of sand / mud as food item is an indication that the species feed close or even at the bottom of the lake. Ajayi (1987) did report that *Chrysichthys auratus* fed both at the surface and bottom of the lake, and that the juveniles fed largely on detritus, insects larvae, crustaceans eggs, copepods and cladocerans while the adults preferred detritus, volvox, higher plants, bivalves and a variety of insect. Ikomi and Odum (1998) reported that the species fed predominantly on insects, fish and aquatic macrophytes with crustaceans and algae as minor part of the diet.

Welcomme (2001) reported that unspecialized feeders eat insects, zooplankton, detritus and plant matter according to their abundance, while Strum (1984) did report *Chrysichthys auratus* as a non - selective bottom feeder, which is inline with the findings of this study.

Prey importance index shows the importance of an item in the diet of fish. Detritus had the highest index (51%). This indicates that it is the most relevant item in the diet of the species during the period of study (Table 2). This was followed by plant tissues / remains (44.88%), chironomid larvae and pupae (31.3%), fish fry (26.40%), while the least was *Spirotaenia* spp (0.05%), which were positively correlated ($p<0.05$). The reliance on detritus could be due to its abundance, preference or the species being a bottom dweller as reported by Ogeibu and Ezeunara (2005). Ajayi (1987) did report that detritus was swallowed by *Chrysichthys auratus* at all the times.

Feeding intensity of fish can be determined based on degree of fullness of stomach. A total of one hundred and twenty stomachs were examined, where by eighty - seven contained food and thirty- three without food (Table 3). 43(35.83%) of the stomachs were full, 6 (5.0%) almost full, 27(22.5%) half full, 9 (7.5%) almost empty and 35 (29.17%) were empty. The relatively high percentage of full stomach suggests that food was abundant throughout the period of study. This indicates that there was high feeding intensity. Ogbeibu and Ezeunara (2005) reported that if percentage of full stomach was more than that of empty stomach there is high degree of feeding intensity, which is inline with the study.

Seasonal variation influences abundance and diversity in the diet of most tropical fishes. During rainy season *Bosmonia* spp, stone particles, S*pirotaenia* spp, *Pila* spp, *Planorbis* spp, *Limnaea* spp and *Bulinus* spp were not ingested, while human food remains was not ingested in dry season (Figure 1). This could be due to unavailability of such items during these seasons. Ogbeibu and Ezenuara (2005) did report that seasonal diversity of food items could influence food habits, diet and feeding intensity of fish.

Fish can broadly be classified into categories based on their predominant feeding habits (Welcomme, 1979). *Chrysichthys auratus* fed on wide range of items from plant to animal materials. Detritus is the predominant item ingested by the species in the lake. The species can be considered as omnivorous detrivore. This is in agreement with the findings of Welcomme (2001), Idodo – Umeh (2003) and Oronsaye and Nakpodia (2005).

Table1: Analysis of stomach contents of *Chrysichthys auratus auratus* by frequency of occurrence and volumetric methods.

	Items	**% Frequency of occurrences**	**% Volume**
FISH			
	Fish fry	17. 6	1.5
INSECTA			
	Chironomid larvae and pupae	20.6	1.76
	Choaborus larvae and pupae	4.6	0.39
	Insect fragments (limb, head, wing)	9.2	0.79
CRUSTACEAN			
	Diaptomus spp		
	Cyclops spp	3.8	0.33
	Daphnia spp	2.3	0.20
	Bosmonia spp	4.6	0.33
		2.3	0.02
Sand / Mud	Stone particles		
		3.8	0.33
Algae			
	Filamentous (green)		
	Spirotaenia spp	16.0	1.37
		2.3	0.02
Vegetable Matter	Plant tissues / remains		
	Seed / seed chaff	22.9	1.96
	Human food remains	10.7	0.92
		16.8	1.44
MOLLUSCA	*Pila* spp		
	Viviparous spp	3.1	0.26
	Planorbis spp	1.5	0.13
	Limnaea spp	3.1	0.26
	Bulinus spp	1.5	0.13
		0.8	0.07
Detritus		24.4	2.09
Nematode		9.9	0.85
Others	Unidentified items	13.6	0.25

Table 2: Measure of prey importance index of *Chrysichthys auratus*

Items		**% Importance Index of Prey**
FISH		
	Fish fry	26.40
INSECTA		
	Chironomid larvae and pupae	31.33
	Choaborus larvae and pupae	1.01
	Insect fragments (limb, head, wing)	5.52
CRUSTACEAN		
	Diaptomus spp	1.25
	Cyclops spp	0.46
	Daphnia spp	1.79
	Bosmonia spp	0.07
Sand / Mud		
	Stone particles	1.25
Algae		
	Filamentous (green)	21.92
	Spirotaenia spp	0.05
Vegetable Matter		
	Plant tissues / remains	44.88
	Seed / seed chaff	8.48
	Human food remains	24.19
MOLLUSCA		
	Pila spp	0.81
	Viviparous spp	0.20
	Planorbis spp	0.81
	Limnaea spp	0.20
	Bulinus spp	0.06
Detritus		51.00
Nematode		8.42
Others	Unidentified items	7.9

Table 3: Stomach contents classification of *Chrysichthys auratus* based on degree of fullness

No. of stomach examined	120
% stomach with food	87 (72.5)
% stomach without food	33 (27.5)
% Degree of Fullness	
Full (4/4)	43 (35.83)
Almost full (3/4)	6 (5.0)
Half (2/4)	27 (22.5)
Almost empty (1/4)	9 (7.5)
Empty (0/4)	35 (29.17)

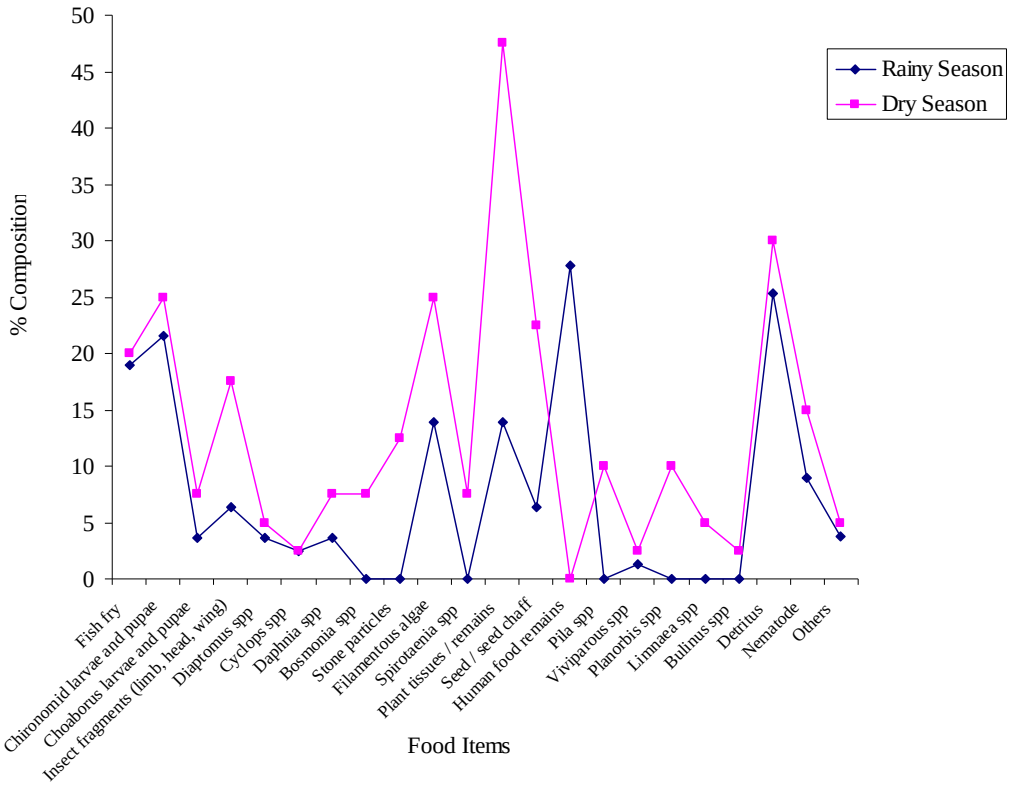

Figure 1: Seasonal variation of the percentage composition of items ingested by *Chrysichthys auratus*

CONCLUSION

Chrysichthys auratus fed on wide range of food items from plant to animal materials and can therefore be said to be omnivorous detrivore in the lake. Season had influence on items eaten by *Chrysichthys auratus* in the lake

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Relative Agronomic performance of different *Dioscorea* species found in different parts of Orissa.

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ABSTRACT: A study was under taken to quantify the relative agronomic performance of twelve *Dioscorea* species (11 wild and one cultivated species *D.esculenta*) found in different parts of Orissa .Various agro morphological character starting from plant height to yield per plant was evaluated among the twelve different *Dioscorea* species and presented in tabular form as per the standard agro metric method.The agronomic character analysis revealed that plant height was significantly superior in *D. hispida* (3.21 m) followed by the shortest height was noticed in *D. oppositifolia* (1.98 m). However at final stage of the crop highest number of leaves are found in *D. oppositifolia* (179) and *D.* wallichii (156). Tuber number per plant was the highest in *D. esculenta* (6.2) and there was only one tuber in *D. bulbifera.* The tuber: shoot ratio was significantly the highest in *D. wallichii* (2.43) and the lowest in *D. oppositifolie* (0.833). The ratio was very low in *D. bulbifera* and *D. hamiltonii* .The yield (kg/plant) was significantly highest in *D. bulbifera* (1.646 kg) and lowest yield was obtained with *D. belophylla* (0.654 kg) followed by *D. Pubera* (0.678 kg). From the study it is concluded that each species has their own identical agronomic character with certain similarities and dissimilarities among themselves. [Nature and Science. 2009;7(3):23-35]. (ISSN: 1545-0740).

Key words: Agro morphology, *Dioscorea*, Growth period, Orissa, Senescence, Tuber

INTRODUCTION

Yams (*Dioscorea* spp.) are tropical tuber crops grown as a source of carbohydrates, but also for use in ceremonial activities (Degras, 1993). They are cultivated world wide, but principally in West Africa, where approximately 95% of world annual production (37 million tones) were achieved in the yam growing belt (FAO, 2004). In East Africa, yams constitute an important subsistence food crop and component of the farming system (Wanyera et al., 1996). Out of six hundred species of *Dioscorea*, so far reported in the world only ten species are in commercial cultivation (Prain and Burkil ,1936). However, some species which are edible, yet, have not been domesticated because of several reasons like inferior quality of tuber, low yield, inaccessible deepest tubers and transformable poisonous forms of tuber. In India so far twenty-six species of *Dioscorea* have been reported (Abruna et al., 1981). At present thirteen *Dioscorea* species are available in Orissa. Out of them two species are cultivated and rest eleven are wild (Maharana ,1993). A study conducted by Niswass (1975) revealed that six *Dioscorea* species are edible in Tumudibandha area of Phulbani district. Arora and Singh (1978) reported that several wild yams are used as food items in the Eastern Ghat region. All the *Dioscorea* species available in the state of Orissa were used as food item as when required. (Martin and Ruberte , 1976).

Out of twelve *Dioscorea* species *D.belophylla, D. glabra, D. hamiltonii, D. oppositifolia, D. pubera, D. wallichii,* twine to right so placed under section *Enantiophyllum* and the rest six species are left twiner. Among them, the compound leave Dioscorea i.e. *D. hispida, D. kalkapershadii,D. pentaphylla* were under the section Lasiophyton . The air yam, *D. bulbifera* is under section opsophyton and *D. esculenta*, the cultivated species is under the section cambilium which produces a cluster of small tuber (Alvarez and Hahn, 1984 ; Coursey and Martin, 1970). In *Dioscorea*, the above ground vegetative mass includes stem, leaf and branch. The stem of yam is rope like structure, and of different shapes depending upon the species specificity. Species under the section *Enantiophyllum* twine to right whereas, others twine to left. Stems grow several meters before any branching occurs and appendages on the stem, like wing, spine, hairs etc., apparently prevent the stem from slipping from its support (Burkill ,1960 ; Coursey , 1967). The research being reported here was aimed at investing the agronomic parameters of different *Dioscorea* species found in different parts of Orissa as a guide to developing a more efficient agro technology and practical system

for yam cultivation.

MATERIALS AND METHODS

Tubers of twelve *Dioscorea* species were collected from different parts of Orissa and the species were grown in the experimental garden, P.G. Deptt. of Botany Utkal University during the year 2004-05 and 2005-06 as per the standard agronomic practices (Ferguson and Gumbs ,1976; Ferguson, 1980 ; Ferguson et al., 1984). Various agro morphological parameters were taken and evaluated as per the standard agro biometric method proposed by Panse and Sukhatme (1978). Harvesting of the tuber was done after all the vines dried and it was done around 300 days after planting. The various agronomic parameters taken in this study are as follows.

Aerial Agro morphological Parameters: Height of the plant was measured in the 1st week of December, when all most all species and cultivars attained the maximum linear growth or reached flowering. The plants were not given full privilege for attaining maximum height since the staking height was restricted to 1.5 meters. The height attained on this staking height was measured only. Average of four plants was taken and this was also followed in all other observations. Number of branches was calculated by counting the branches produced in the main stems and average was taken for computation of data. Number of stem was recorded by counting the main stems produced from the tuber piece planted. Thickness of main stem was measured during December when the branches attained the maximum growth. This was done with the help of dial micrometer and expressed in centimeters. Spread of the plant was measured by keeping a scale in the centre of the stake and average was taken. This relates to the width of entire plant mass attained on 1.5 meters staking system. Number of leaves in different species was counted in the 1st week of December i.e. a period when most of these plants attained maximum growth. Leaf areas of representative leaves were calculated at different stages of plant growth by placing the leaves on a graph paper. A factor was found out showing the relation with the multiplication of length and width. The factor was multiplied with length and width to get the leaf area. Eighth fully expanded leaf from top was only measured. Total leaf area was calculated by taking the average leaf area multiplied by number of leaves. It was calculated for the leaves present during December for both the years. Growth period was recorded in number of days from the date of sprouting of tubers till the vine started to decline. Senescence of first leaf in the vine was calculated by counting the days from the date of planting to the date when first leaf turned to yellow under natural condition but not by any disease.

Under Agronomic ground Parameters: Tuber initiation time was recorded in weeks by exposing the plants carefully and six weeks afterwards at week's interval in some extra observation plants. Root zone was measured by removing the soil carefully and tracing the root around the plant in four direction just before decline of the vine**.** Tuber to shoot ratio was calculated by harvesting the tuber just at the time of decline of the vine and by weighing the tuber and the vegetative growth. Number of tubers produced in each plant was calculated on the basis of number of tuber produced in the plants under observation. Length of tuber was measured in cm from the neck of the tuber to the basal tip of the tuber. Width of tuber was measured at three point's i.e. basal, middle and top portion and average was taken for tabulation of data. Tuber formation depth was assessed by measuring the soil depth from plain surface of soil to the depth where the tuber was formed. Immediately after harvest the tubers from observation plants were cleaned of soils adhered to it and weighed. Mean was taken for tabulation of data.

RESULT AND DISCUSSION

Result of the experiment: Data collected on various characters for the 11 wild and one cultivated *Dioscorea* species were analyzed for the respective years i.e. first year and second year. The pooled data was analyzed basing on six replications of two years. However, the I and II year data were given along with the pooled data for reference only (Lyonga and Ayuktaken , 1982; IJOYAH et al., 2006; Law-Ogbomo, 2007).

Result of the Aerial Agro morphological Parameters : Significant difference was observed for plant height in both the years and in pooled data also. Height was significantly superior in *D. hispida* (3.21 m) followed by *D. pubera* (3.21m) and *D. bulbifera* (3.05 m). However, significantly shortest height was

noticed in *D. oppositifolia* (1.98 m). *D. esculenta* (cultivated species) attained a height of 2.67 m only(Table-1). Out of 11 wild species only two species were having profuse branching habit i.e. *D. wallichii* (29.66) and *D. glabra* (21.58) and these two differed significantly. Among other species, *D. oppositifolia* was having the highest number of branches (10.50) and rest species were having less than 10 numbers of branches. Majority of the species were having 4-5 branches and no significant difference was observed between them (*D. pubera, D. belophylla, D. tomentosa, D. pentaphylla, D. kalkapershadii, D. hispida and D. bulbifera*). *D. esculenta* produced a good number of branches (8.9 / plant) (Table-1).Out of 12 species, 6 species produced a single stemmed plant. *D. oppositifolia* had significantly the highest number of stems (2.04/plant) followed by *D. esculenta* (1.05/ plant) and these two differed significantly (Table-1). Significant difference was observed for thickness of the main stem in both the years and pooled data. The stems were significantly of highest diameter in *D. hispida* (0.70cm) followed by *D. bulbifera* (0.69) and was no significant difference was observed between these two species. The stem diameter was shortest in *D. hamiltonii* (0.26 cm) followed by *D. oppositifolia* (0.27 cm) (Table-2). Number of leaves were counted at 2 month, 3 month, 4 month and at final growth phase. Significant difference was observed in I year, II year and pooled data. Significantly highest numbers of leaves were produced in *D.esculenta* at 2 months (56.79), 3 months (103.75), 4 months (162.06) and final stage (211.91). At all these stages, the next highest number of leaves was observed in *D. oppositifolia*. At 3 month stage all the species except the above two species produced less than 100 number of leaves per plant. At final stage, the leave number was more than 100 in all species of which *D. esculenta* only had more than 200 leaves per plant (211). However, *D. oppositifolia* (179) and *D. wallichii* (156) had good number of leaves at final stage (Table-2, 3). *D. wallichii* was the most spreading species (203.45 cm) which differed significantly with all other species. The least spreading species was *D. belophylla* (34.58 cm) which was at par with *D. hamiltonii* (35.41), *D. tomentosa* (44.50), *D. pentaphylla* (37.16cm), *D. kalkapershadii* (35.54cm), *D. hispida* (35.75cm) and *D. bulbifera* (41.45cm) (Table-4). Total leaf area (single side) was significantly highest in *D. wallichii* (30186 sq. cm) followed by *D. pubera* (21393 sq. cm) as compared to all the species but these two also differed significantly. The lowest total leaf area was recorded in *D. hamiltonii* (3280 sq. cm) followed of *D. oppositifolia* (3498 sq. cm) and these two were at par with *D. tomentoa* (8561sq.cm) and *D. pentaphylla* (7703 sq.cm) (Table-4). Significant difference was observed for the starting time of senescence of 1st leaf. The pooled data revealed that senescence time of 1st leaf was significantly earliest in *D. hispida* (94.91 days) followed by *D. bulbifera* (95.37 days) and *D. oppositifolia* (96.91 days) and all these were at par. The senescence was significantly delayed in *D. pentaphylla* (128.95 days) followed by *D. belophylla* (121.00 days) and significant difference was observed between these two (Table-6). Significant difference was observed for active growth period. Shortest period was observed with *D.bulbifera* (153.97days), followed by *D. kalkapershadii* (165.25 days) and *D. oppositifolia* (167.16 days).No significant difference was observed between *D.kalkapershadii*(165.25),*D.hispida* (169.08) and *D.oppositifolia* (167.16). Similarly no significant difference was observed among *D.hamiltonii* (193.00), *D.glabra*(192.08) and *D.pubera* (195.20) and also among *D. belophylla* (183.58), *D. tomentosa* (181.66) and *D. pentaphylla* (184.66) (Table-6).

Result of the Under ground Agronomic Parameters: Spread of root zone was significantly highest in *D. wallichii* (95.98 cm) followed by *D. glabra* (82.80 cm) and significant difference existed between them. The root zone was shortest in *D. habiltonii* (16.87 cm) followed by *D. belophylla* (21.66 cm) and significant difference was observed between them (Table-5).It was observed that the tuber initiation time was significantly different in different species under study and in both the years and in pooled data. It was earliest in *D. hispida* (5.79 weeks) followed by *D. bulbifera* (6.62 weeks) and there was significant difference between these two species. It was most delayed in *D. belophylla* (11.45 weeks) followed by *D. glabra* (10.45 weeks) and *D. esculenta* (10.41 weeks) and there was no significant difference between these two (Table-6).Tuber formation depth was significantly lowest in *D. pentaphylla* (11 cm) followed by *D. kalkapershadii* (11.66 cm) and *D. hispida* (16.87 cm). No significant difference was observed in the former two. Tuber formation depth was deepest in *D. belophylla* (43.62 cm) followed by *D. wallichii* (43.08 cm) and *D. hamiltonii* (41.70 cm), and all these species were at par. The depth was 24.6 cm and 22.5 cm respectively in *D. bulbifera* and *D. esculenta* (Table-7).Tuber length was significantly shortest (8.81 cm) in *D. esculenta* and the longest in *D. tomentosa* (60.48 cm).Tubers below 20 cm long were observed in *D. hispid* and *D. bulbifera* and beyond 40 cm was in *D. oppositifolia, D. glabra, D. wallichii* and *D. pentaphylla* (Table-7). Tuber width was significantly shortest in *D.oppositifolia* and *D.*

glabra (3.11 cm) followed by *D. pubera*, *D. tomentosa* (3.78 cm), *D. esculenta* (3.8 cm). Tuber width was significantly highest in *D. hispida* (16.86 cm) as compared to other species. However, *D. pentaphylla* (11.26 cm) and *D. bulbifera* (10.99 cm) significantly differed from each other (Table-8). Tuber number per plant was the highest in *D. esculenta* (6.20) followed by *D. glabra* (4.12) and these two differed significantly from each other. There was only one tuber in *D. bulbifera*, *D. hispida*, *D. kalkapershadii* and *D. pentaphylla* while it was one or two in *D. oppositifolia*, *D. hamiltonii* and *D. pubera* (Table-8). The tuber: shoot ratio was significantly the highest in *D. wallichii* (9.08) followed by *D. glabra*(7.89) and the lowest in *D. oppositifolie* (0.833). The ratio was very low in *D. bulbifera* (1.06) and *D. hamiltonii*(1.04) (Table-9).Significant difference was observed for yield in both the years and in pooled data. The yield was significantly highest in *D. bulbifera* (1.646 kg) followed by *D. glabra* (1.091 kg), *D. tomentosa* (1.074 kg), *D. pentaphylla* (1.060 kg) and *D. esculenta*(1.022kg).The lowest yield was obtained with *D. belophylla* (0.654 kg) followed by *D. Pubera* (0.678 kg) (Table-9).

Table.1.Plant height(m) ,Branch number, Number of main steams /plant and Thickness of main stem (cm) in different *Dioscorea* species

Sl. No.	Name of the species	Plant height (m)			Branch number /plant			Number of main steams /plant			Thickness of main stem (cm)		
		I	II	Pooled	I	II	Pooled	I	II	Pooled	I	II	Pooled
1	*D.belophylla*	2.403	2.303	2.353	4.583	4.250	4.417	1.000	1.000	1.000	0.423	0.427	0.425
2	*D.bulbifera*	3.217	2.883	3.050	4.167	5.667	4.917	1.083	1.083	1.083	0.703	0.693	0.698
3	*D. esculenta*	2.917	2.433	2.675	9.250	8.667	8.958	1.583	1.417	1.500	0.310	0.313	0.312
4	*D.glabra*	2.417	2.567	2.492	21.917	21.250	21.583	1.000	1.000	1.000	0.510	0.500	0.505
5	*D.hamiltonii*	2.797	2.833	2.815	4.833	4.750	4.792	1.083	1.250	1.167	0.273	0.257	0.265
6	*D.hispida*	3.450	2.983	3.217	4.667	4.500	4.583	1.000	1.000	1.000	0.723	0.690	0.707
7	*D.kalkapershadii*	3.237	2.517	2.877	4.417	4.833	4.625	1.000	1.000	1.000	0.337	0.337	0.337
8	*D.oppositifolia*	2.137	1.833	1.985	11.667	9.33	10.500	2.000	2.083	2.042	0.273	0.267	0.270
9	*D.pentaphylla*	2.823	2.900	2.862	4.500	4.917	4.708	1.000	1.000	1.000	0.337	0.337	0.337
10	*D.pubera*	3.237	3.183	3.210	8.167	6.833	7.500	1.083	1.333	1.208	0.377	0.367	0.372
11	*D.tomentosa*	2.197	1.823	2.010	5.667	4.250	4.958	1.000	1.000	1.000	0.327	0.320	0.323
12	*D.wallichii*	2.820	3.050	2.935	31.333	28.000	29.667	1.500	1.250	1.375	0.443	0.447	0.445
'F' test		Sig.**	Sig.**	Sig.**	Sig.**	Sig.**	Sig.**	Sig.**	Sig.**	Sig.**	Sig.**	Sig.**	Sig.**
C.D. (0.05)		0.262	0.3515	0.2494	0.2698	2.4162	1.324	0.3858	0.2845	0.2308	0.0141	0.0169	0.119

Table.2. Number of leaves at 2 month and 3 month stage in different *Dioscorea species*.

Sl. No	Name of the species	Number of leaves at 2 months			Number of leaves at 3 months		
		I	II	Pooled	I	II	Pooled
1	*D.belophylla*	43.833	44.083	43.958	86.033	90.000	88.017
2	*D.bulbifera*	26.833	24.500	25.667	54.667	54.933	54.800
3	*D. esculenta*	56.583	57.000	56.792	99.000	108.500	103.750
4	*D.glabra*	26.417	27.167	26.792	72.000	72.233	72.117
5	*D.hamiltonii*	37.337	38.750	38.042	84.833	96.000	90.417
6	*D.hispida*	24.167	29.167	26.667	64.333	72.167	68.250
7	*D.kalkapershadii*	20.333	21.833	21.083	51.000	48.167	49.583
8	*D.oppositifolia*	66.583	62.750	64.667	105.083	105.083	105.083
9	*D.pentaphylla*	31.500	32.500	32.000	57.167	56.167	56.667
10	*D.pubera*	20.750	21.833	21.292	68.417	66.167	67.292
11	*D.tomentosa*	26.833	25.000	25.917	54.000	60.333	57.167
12	*D.wallichii*	34.250	37.833	36.042	67.333	75.833	71.583
'F' test		Sig.**	Sig.**	Sig.**	Sig.**	Sig.**	Sig. **
C.D. (0.05)		5.354	4.4345	3.4916	5.198	8.162	5.424

Table.3. Number of leaves at 4 month and final crop growth stage in different *Dioscorea species*

Sl No.	Name of the species	Number of leaves at 4 months			Number of leaves at (25 weeks) final stage		
		I	II	Pooled	I	II	Pooled
1	*D.belophylla*	109.417	109.750	109.583	143.167	137.083	140.125
2	*D.bulbifera*	84.000	86.500	85.250	112.500	115.500	114.000
3	*D. esculenta*	162.217	161.917	162.067	213.833	210.000	211.917
4	*D.glabra*	86.833	88.833	87.833	111.083	116.250	113.667
5	*D.hamiltonii*	105.167	108.250	106.708	133.250	128.417	130.833
6	*D.hispida*	90.233	87.667	88.950	116.333	117.417	116.875
7	*D.kalkapershadii*	79.333	79.083	78.708	97.917	105.333	101.625
8	*D.oppositifolia*	135.583	135.167	135.375	188.333	170.083	179.208
9	*D.pentaphylla*	78.667	79.000	78.833	116.083	126.000	121.042
10	*D.pubera*	80.167	82.667	81.417	107.833	106.583	107.208
11	*D.tomentosa*	74.917	76.833	75.875	104.250	102.583	103.417
12	*D.wallichii*	104.583	100.500	102.542	157.917	154.167	156.042
'F' test		Sig. **	Sig.**	Sig.**	Sig.**	Sig.**	Sig. **
C.D. (0.05)		8.344	9.021	5.6831	10.215	15.414	9.588

Table.4. Spread of plant and leaf area (single side) in different *Dioscorea* species.

Sl. No.	Name of the species	Spread of plant (cm)			Leaf area/plant (sq.cm)		
		I	II	Pooled	I	II	Pooled
1	*D.belophylla*	36.167	33.000	34.583	18106.250	18799.000	18452.630
2	*D.bulbifera*	41.167	41.750	41.458	16123.170	16802.420	16462.790
3	*D. esculenta*	52.833	52.667	52.750	16350.170	11378.080	13864.130
4	*D.glabra*	173.583	190.250	181.917	5491.834	5485.500	5488.667
5	*D.hamiltonii*	35.250	35.583	35.417	3308.667	3251.667	3280.167
6	*D.hispida*	35.667	35.833	35.750	18665.170	19446.670	19055.920
7	*D.kalkapershadii*	35.750	35.333	35.542	13448.500	14026.170	13737.330
8	*D.oppositifolia*	56.917	61.500	59.208	3533.083	3463.500	3498.292
9	*D.pentaphylla*	39.500	34.833	37.167	7055.584	8351.000	7703.292
10	*D.pubera*	44.000	55.167	49.583	22489.170	20297.580	21393.380
11	*D.tomentosa*	45.333	43.667	44.500	8659.000	8463.750	8561.375
12	*D.wallichii*	218.333	188.583	203.458	29991.920	30380.670	30186.290
'F' test		Sig. **	Sig.**	Sig.**	Sig.**	Sig.**	Sig. **
C.D. (0.05)		16.287	13.642	11.944	1767.9	1658.1	2719.5

Table.5. Starting time of senescence of 1^{st} leaf and diameter of root zone in different *Dioscorea species*.

Sl. No.	Name of the species	Start of senescence in 1^{st} leaf (Days)			Diameter of root zone (cm)		
		I	II	Pooled	I	II	Pooled
1	*D.belophylla*	120.833	121.167	121.000	18.400	24.933	21.667
2	*D.bulbifera*	96.000	94.750	95.375	33.267	37.433	35.350
3	*D. esculenta*	110.667	109.917	110.292	24.000	31.967	27.983
4	*D.glabra*	119.000	120.667	119.833	75.300	90.300	82.800
5	*D.hamiltonii*	112.500	113.833	113.167	16.700	17.033	16.867
6	*D.hispida*	96.667	93.167	94.917	23.567	27.400	25.483
7	*D.kalkapershadii*	103.333	121.333	112.333	34.300	35.367	34.833
8	*D.oppositifolia*	96.167	97.667	96.917	21.700	29.567	25.633
9	*D.pentaphylla*	133.167	124.733	128.950	35.700	40.433	38.667
10	*D.pubera*	114..667	113.667	114.167	28.800	30.500	29.650
11	*D.tomentosa*	117.833	107.417	112.625	58.467	72.467	65.467
12	*D.wallichii*	113.667	110.917	112.292	91.300	100.667	95.983
'F' test		Sig. **	Sig.**	Sig.**	Sig.**	Sig.**	Sig. **
C.D. (0.05)		7.373	12.5159	7.935	0.2806	3.8086	3.544

Table.6.Tuber initiation time and growth period in different *Dioscorea* species.

Sl. No.	Name of the species	Tuber initiation time (weeks)			Growth period (Days)		
		I	II	Pooled	I	II	Pooled
1	*D.belophylla*	11.417	11.500	11.458	184.583	182.583	183.583
2	*D.bulbifera*	6.335	6.917	6.625	155.083	152.867	153.975
3	*D. esculenta*	10.083	10.750	10.417	177.750	171.000	174.375
4	*D.glabra*	10.500	10.417	10458	191.167	193.000	192.083
5	*D.hamiltonii*	9.500	10.250	9.875	192.000	190.000	193.000
6	*D.hispida*	5.417	6.167	5.792	166.583	171.583	169.083
7	*D.kalkapershadii*	8.167	8.167	8.167	161.333	169.167	165.250
8	*D.oppositifolia*	8.250	8.917	8.583	163.083	171.250	167.167
9	*D.pentaphylla*	7.333	7.417	7.375	184.583	180.750	184.667
10	*D.pubera*	7.167	7.583	7.375	194.583	195.833	195.208
11	*D.tomentosa*	6.333	7.417	6.875	182.917	180.417	181.667
12	*D.wallichii*	10.417	10.083	10.250	181.8333	174.750	178.292
'F' test		Sig.**	Sig. **	Sig.**	Sig.**	Sig.**	Sig. **
C.D. (0.05)		0.3204	1.0379	0.5611	1.0601	7.848	4.851

Table.7.Tuberformation depth and length of tuber in different *Dioscorea* species.

Sl. No.	Name of the species	Tuber formation depth (cm)			Length of tuber (cm)		
		I	II	Pooled	I	II	Pooled
1	*D.belophylla*	46.500	40.750	44.625	20.167	20.100	20.133
2	*D.bulbifera*	24.167	25.167	24.667	15.700	15.533	15.617
3	*D. esculenta*	22.667	22.333	22.500	8.700	8.933	8.817
4	*D.glabra*	34.583	35.500	35.042	45.267	34.067	44.667
5	*D.hamiltonii*	40.917	42.500	41.708	33.167	30.933	32.080
6	*D.hispida*	16.667	17.083	16.875	19.250	20.700	19.975
7	*D.kalkapershadii*	11.917	11.417	11.667	26.533	26.100	26.317
8	*D.oppositifolia*	39.417	35.500	37.458	43.500	37.083	40.292
9	*D.pentaphylla*	11.333	10.667	11.000	44.067	44.500	44.283
10	*D.pubera*	31.500	32.667	32.583	38.900	36.533	37.717
11	*D.tomentosa*	19.833	42.000	20.917	61.400	59.567	60.483
12	*D.wallichii*	41.833	44.333	43.083	46.867	43.600	45.233
'F' test		Sig. **	Sig.**	Sig.**	Sig.**	Sig. **	Sig. **
C.D. (0.05)		3.853	5.675	3.481	7.453	4.905	4.229

Table.8.Width of tuber and number of tubers in different *Dioscorea* species.

Sl. No.	Name of the species	Width of tuber (cm)			No. of tubers / plant		
		I	II	Pooled	I	II	Pooled
1	*D.belophylla*	4.033	3.933	3.983	2.000	2.167	2.083
2	*D.bulbifera*	10.650	11.333	10.992	1.000	1.000	1.000
3	*D. esculenta*	3.867	3.733	3.800	6.417	6.000	6.208
4	*D.glabra*	2.967	3.267	3.117	4.417	3.833	4.125
5	*D.hamiltonii*	7.800	7.633	7.767	1.083	1.250	1.167
6	*D.hispida*	16.667	16.467	16.867	1.000	1.000	1.000
7	*D.kalkapershadii*	5.500	5.700	5.600	1.000	1.000	1.000
8	*D.oppositifollia*	3.100	3.133	3.117	1.250	1.167	1.208
9	*D.pentaphylla*	11.200	11.333	11.267	1.000	1.000	1.000
10	*D.pubera*	3.833	3.733	3.783	1.667	2.000	1.833
11	*D.tomentosa*	3.633	3.933	3.783	3.417	3.250	3.333
12	*D.wallichi*	4.667	4.333	4.500	2.500	2.333	2.417
'F' test		Sig. **	Sig.**	Sig.**	Sig.**	Sig.**	Sig. **
CD (0.05)		1.0785	0.644	0.188	0.658	1.099	0.5964

Table.9. Shoot: Tuber ratio and plant yield in different *Dioscorea* species. [*Including bulbuil]

Sl. No.	Name of the species	Shoot tuber ratio			Yield (kg/plant)		
		I	II	Pooled	I	II	Pooled
1	*D.belophylla*	1.393	1.417	1.400	0.703	0.605	0.654
2	*D.bulbifera**	1.047	1.083	1.065	1.560	1.732	1.646
3	*D. esculenta*	1.267	1.203	1.235	1.026	1.018	1.022
4	*D.glabra*	7.950	7.833	7.892	1.057	1.124	1.091
5	*D.hamiltonii*	1.040	1.050	1.045	0.759	0.780	0.770
6	*D.hispida*	3.357	3.700	3.528	0.934	0.936	0.935
7	*D.kalkapershadii*	2.157	2.300	2.228	0.937	0.901	0.919
8	*D.oppositifolia*	0.783	0.833	0.808	1.056	0.844	0.950
9	*D.pentaphylla*	2.450	2.420	2.435	1.182	0.937	1.060
10	*D.pubera*	1.633	1.483	1.558	0.703	0.653	0.678
11	*D.tomentosa*	1.243	1.267	1.255	1.084	1.064	1.074
12	*D.wallichi*	9.150	9.010	9.080	1.041	0.867	0.954
'F' test		Sig.**	Sig.**	Sig.**	Sig.**	Sig.**	Sig. **
CD(0.05)		0.783	0.667	1.8372	0.232	0.124	0.141

DISCUSSION

From the various agronomic aspect of the study it is concluded that right twining species are normally shorter than left twining species. Prain and Burkill (1936), Singh and Arora (1978) reported that out of several wild species growing in the jungles of Orissa *D. bulbifera* and *D. hispida* climbs up to 15 meters. But in this investigation, all the species are forced to grow with limited staking. Therefore the plant height is reduced to 20-50 percent of the height observed in nature. *D. oppositifolia* and *D. tomentosa* produced the shortest vine length respectively 1.9 meter (m) and 2.01 m whereas *D. hispida* (3.21m) and *D. pubera* (3.21 m) attained highest linear growth. *D. wallichii* produced highest number of branches (29.66 branches) followed by *D. glabra* (21.58 branches). *D. esculenta* and *D. oppositifolia* produced 8.9 and 10.5 number of branches respectively. Other species produced less than 8 branches per vine. It is clear from the study that a yam plant should produce around 10 numbers of branches for proper growth of 8000-10000 vines in a hectare to get ample sunlight (Okpara and Omaliko, 1995). Staking plants of *P.tetragonolobus* affects vegetative and reproductive growth but tuberous roots (number and size) were not clearly affected, although in other species such as *Dioscorea* species, this treatment strongly favors tuber number and size of individual tubers (Enyi,1972a ; Igwilo, 1989; Ndegwe et al, 1990).

Thick stems of a yam help to cling the stake properly. In the present study, *D. bulbifera* (0.69 cm), *D. hispida* (0.7 cm) and *D. glabra* (0.5 cm) produce stems more than 0.5 cm thick. The thin and wiry branches of few species trails on ground unless staking is provided at early stage. The canopy of vines in left twining species is low, nearly 50% of the right twining species. *D. glabra* (181 cm) and *D. wallichii* (208 cm) spread up to 2 m. where as, all other species restricted their canopy within 60 cm. A crowding leaf mass reduce the rate of photosynthesis due to flat leaves, restricted sunlight and low stomatal opening. As many of the *Dioscoreas* are not having erect leaves for which a low canopy may allow penetration of more sunlight for higher photosynthesis (Ramirez and Rodriguez, 1975).The leaf production ability of right twining species is higher than left twining species. At final count (25 weeks after planting) the average leave number was 137 and 128 respectively in right and left twining species (Table -3, 4). But leaf number was highest in *D. esculenta* (211) followed by *D. oppositifolia* (179).

In this trial, senescence process is quicker in left twining species (109.08 days) than right twining species (112.89 days, Table 37). Surprisingly species like *D. hispida, D. bulbifera and D. oppositifolia* start senescence before 100 days of crop growth. Leaf production was lowest in *D. kalkapershadii* (101) followed by *D. tomentosa* (103). All *Dioscorea* having compound leaves produced less number of leaves (103-121). In *D. glabra* and *D. pubera* respectively produced 117 and 107 leaves per plant. However, the total leaf area was highest in *D. wallichii* (30186 sq. cm) and *D. pubera* (21393 sq. cm) because of their large leaf. Leaf area index (LAI), photosynthesis ability, short prebulking period are important in all *Dioscorea*. Flach (1979) reported four phases in yam development namely (1) establishment, (2) development of leaf area, (3) starch accumulation, (4) ripening i.e. diminishing of leaf area accompanying starch accumulation and these phases are in 6 weeks, 11 weeks, 8 weeks and 14 weeks duration respectively. Therefore, it is important to select a cultivar which completes the first and second phase in a shorter period following a rapid bulking and maturation period. However, Degras (1976) proved that bulking is initiated in first phase itself. Quamina et al (1982) also reported same finding. Haynes et al (1967) believed that in *D. alata,* leaf area decreases as tuberization begins. In the present investigation tuber initiation time was earliest in *D. hispidda* (5.79) weeks) followed by *D. bulbifera* (6.62 weeks) and *D. tomentosa* (6.8 weeks). Species having earlier tuber initiation yielded higher because of a longer bulking period.

Data recorded on diameter of root zone revealed (Table -5) that root zone is wider in right twining species (45.43 cm) than left twining species (37.86 cm). It is highest in *D. glabra* (82 cm) followed by *D. wallichii* (95.98 cm) and *D. tomentosa* (65.46 cm). Lowest was recorded in *D. hamiltonii* (16.87 cm) followed by *D. belophylla* (21.66 cm). In right twining species the yield was reduced proportionately with

reduced root zone and same trend was also observed in left twining species, but in a lesser degree. Ferguson et al., (1976) reported that compaction in root area reduced the yield of tuber in *D. alata* by reduced leaf growth. Formation of tuber in a yam is the most peculiar feature. In the present study, the tuber initiation was quickest in *D. hispida* (5.79 weeks) followed by *D. bulbifera* (6.62 weeks). On an average, most of the left twining species initiated tuber formation earlier (7.54 weeks) when compared to the right twining species (9.66 weeks). However *D. belophylla* (11.45 weeks), *D. wallichii* (10.25 weeks) and *D. esculenta* (10.41 weeks) are very late to initiate the tuber formation.

As observed, the initiation of senescence process is significantly earliest in *D. oppositifolia* (96.91 dys) followed by *D. bulbifera* (95.37 days) and *D. hispida* (94.91 days). Earlier senescence is observed in a yam where tubers form close to soil moisture surface and produce a good number of leaves or easily prone to moisture stress. *D. bulbifera* produces a good amount of bulbils and *D. hispida* tubers are produced very close to soil surface (16.87 cm) where as *D. pentaphylla* and *D. belophylla* tubers are deep seated. As a result, they are late to start the senescence phase. Above all, the start of senescence and growth period are highly variable among the species ranging 94-128 days and 153-195 days respectively. Several good characters required for an ideal yam farming as observed in *D. hispida*. Since it is a poisonous one search may be made to locate non poisonous types. *D. dumetorum* an African species akeen to *D. hispida* has several non poisonous forms.

All yams under the section *Enantiophyllum* produce tubers at an average depth of 38.9 cm as compared to 17.93 cm in left twining species. Shallow seated tubers are easy to harvest and they effectively utilize the nutrients and soil moisture. However, all functional roots are clustered around the neck. All species under section *Lasiophyton* are shallow seated. Tubers of *D. pentaphylla* are formed very close to soil surface (11 cm) so also *D. kalkapershadii* (11.41 cm). *D. belophylla* produced tubers at highest depth (43.62 cm) so also in *D. wallichii* (43.08 cm) and *D. hamiltonii* (41.70 cm). Tubers of all yams under section *Enantiophyllum* measure in between 20.13 cm to 45.23 cm whereas in left twining species the length is in between 8.81 cm to 60. 48 cm. *D. esculenta* tubers measure 10 cm in length, whereas, tubers of *D. tomentosa* are the longest (60.48 cm). *D. hispida* and *D. bulbifera* are ideal type yams as regard to length and width of tuber. *D. esculenta* also possess a good shape in this regard. Most of the yams grown and marketed in Africa and Latin America are of medium length which facilitate for their proper storage and marketing. Such tubers are ideal for transportation. Martin et al (1974) reported that ideal yam cultivars should bear in pair or threes and spherical or cylindrical in shape, not often branched and have smooth but thickened skin that resists abrasion. Number of tuber per plant is highest (6.2) in *D. esculenta*, a member of *Cambilium*. Also tubers are more in *D. glabra* (4.12). In yams, shape of tuber in a particular variety is important as the number of tuber. A tuber should be of within 30 cm length and weighs up to 1-2 kg. None of the species produce tubers suitable for marketing except *D. hispida*, *D. pentaphylla* and *D. kalkapershadii*. The tuber of *D. oppositifolia* are very long for which they break into small pieces during harvesting. *D. pubea* produces less leaves (107.2) with comparison to other species. This favors for longer vine life and late to start senescence (114 days after sprouting). *D. oppositifolia* during its growth period produces 179.2 leaves. The *Disocorea* with more leaves maintains a longer growth period. Species with shorter growth period yield more as observed in *D. bulbifera* and *D. hispida*. A short prebulking period with early tuber initiation are observed in them. Growth and developmental process in yams have studied by Campbell (1962), Enyi (1972b), Sobul (1972), Sadik and Okereke (1975) Degras et al. (1977) and Oyolu (1982), and it was opined that a yam plant should be of a short duration with high yield. All the 12 species were observed for crop duration over 2 years and it is revealed that left twining species are shorter duration (171.5 days) than right twining species (184.88 days). Among the right twining species *D. oppositifolia* and except *D. tomentosa* and *D. pentaphylla* other left twining species exhibited less than 180 days duration. The shortest growth period was observed in *D. bulbifera* (153.9 days) followed by *D. Kalkapershadii* (165.25 days) and *D. oppositifolia* (167.16 days). Species having more than six months growth period are *D. pubera* (195.2 days), *D hamiltonii* (193 days) and *D. glabra* (192.0 days).

Many wild *Dioscorea* could not be domesticated primarily due to their poor yield and tubers of inferior quality. Among the wild species, highest yield was recorded in *D. bulbifera*(1.646 kg) where 70 % yield contributed by bulbils. The study reveals that yield is higher in species with shallow seated tubers than deep seated tubers. Tubers with wiry shape are poor yielder. The average yield per plant is 850 gm in

right twining species (Table -9) and 1.11 kg in left twining species. Therefore, many species under *Enantiophyllum* remained in obscurity due to poor yield. Similarly, many left twining species with good yield could not be domesticated owing to tubers of poor quality. *Dioscorea* species yielded more than one kg tuber per plant are *D. glabra, D. tomentosa, D. pentaphylla, D. bulbifera* and *D. esculenta*. Very little information is available on the yield potential of different wild species found in Orissa. The yield is very low in *D. belophylla* (0.654 kg) because of deep seated tuber i.e. 43.62 cm, highest among all. Species with deepest tubers invariably suffer from moisture stress. Further, a moderate to low shoot: root ratio is favorable for yield (Sivan, 1980). The yield in *D. wallichii* is only 0.954 kg/plant with the highest shoot root ratio of 1: 9.080 on the other hand *D. oppositifolia* yielded 0.950 kg/plant with a shoot root ratio of 1: 0.808. More vegetative growth in a tuber crop requires more photosynthates for maintenance of vegetative part hence produce a poor tuber yield. *D. tomentosa* and *D. pentaphylla* yielded more than 1 kg/ plant of tuber. However, yield of tuber is not the only criteria for selection of species; rather the quality of the tuber plays an important role for domestication. Tubers of *D. oppositifolia*, *D. hamiltonii*, *D. belophylla*, *D. wallichii* and *D. tomentosa* are of very good quality. *D. pentaphylla* and *D. Kalkapershadii* tubers are inferior and fibrous in nature (Leon , 1976; Lyonga and Ayuktaken, 1982). The ability to form tubers is, in the first instance, dependent on the genetics of the variety (Martin, 1978) and is affected by environmental factors such as day length, temperature and some cultivation practices including species specific character (King & Risimeri, 1992).

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Model for Predictive Analysis of the Concentration of Sulphur Removed by Molecular- Oxygen-Induced Desulphurization of Iron Oxide Ore

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Abstract: Model for predictive analysis of the concentration of sulphur removed by molecular-oxygen-induced desulphurization of iron oxide ore (powdered potassium chlorate being the oxidant) has been derived. The model; %S = 0.0415/Logγ 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 is believed to be rooted in the expression $k_n[(\gamma)^{\mu\%S}] = T/\alpha$ where both sides of the expression are correspondingly almost equal. The positive or negative deviation of each of the model-predicted values of %S from those of the corresponding experimental values was found to be less than 33% which is quite within the range of acceptable deviation limit of experimental results. [Nature and Science. 2009;7(3):36-40]. (ISSN: 1545-0740).

Keywords: Model, Predictive Analysis, Sulphur Removed, Iron Oxide Ore, Molecular Oxygen.

1. Introduction

Agbaja iron ore has been found to consist principally of goethite, with minor hematite, maghemite, siderite, quartz, kaolinite pyrite and an average of 0.09%S [1]. It was also found to have oolitic and pisolitic structures rich in iron oxides, in a matrix that is predominantly clay. It has been reported [2] that sulphur transfer from metal to slag or slag to gas during desulphurization involves oxygen transfer in the opposite direction. They found [2] that the mechanism of such desulphurization involves oxidation of sulphur resident in the metal or slag by oxygen from the slag through ionic exchange between the oxygen and sulphur, since the whole system is made up of liquid/molten condition during this process. They maintained that oxygen in the slag comes from CaO, which is one of the products of decomposition of $CaCO_3$ being deposited into the slag as a slag forming agent.

Studies [3] carried out on gas-slag system during iron making, indicates that at oxygen partial pressure below about 10^{-5} atm., sulphur dissolves in the melt as sulphide ions; at oxygen partial pressure higher than 10^{-3} atm., sulphur enters the melt as sulphate ions. In both cases, it was reported [3] that both the sulphide and sulphate ions leave the furnace through the slag. It was therefore concluded [3] that the mechanism of such desulphurization process is oxidation of sulphur by oxygen from the slag through ionic exchange between the two participating elements.

Turkdogan and Darken [4] found that at a temperature well below about 1600^0C, the pyrosulphate reaction also occurs. They found that this reaction was an enhancement to the desulphurization process actually taking place in the furnace. Also oxygen for this process was found [4] to come from the slag, engaging sulphur in ionic exchange; being the mechanism of such process.

It was discovered that one of the most important factors influencing the desulphurization process during iron making is the state of oxidation of the bath [5].

Nwoye [6] found that Agbaja 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 [6] that 800^0C is the optimum temperature for the upgrading step considering the range of temperature used (500-800^0C). It was also 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.

Desulphurization of Agbaja iron oxide ore concentrate using solid potassium trioxochlorate (V) ($KCI0_3$) as oxidant has been carried out [7]. The concentrate was treated at a temperature range 500 - 800^0C. 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 800^0C) 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 800^0C) are high.

The mechanism and process analysis of desulphurization of Agbaja iron ore concentrate using powdered potassium trioxochlorate (v) ($KCIO_3$) as oxidant has been reported [8]. Concentrates were treated at a temperature range 500 - 800^0C. 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 [8] 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 [8].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 800^0C respectively) are the ideal conditions for the best desulphurization efficiency [8].

The aim of this work is to derive a model for predictive analysis of the concentration of sulphur removed by molecular-oxygen-induced desulphurization of Agbaja (Nigeria) iron oxide ore. In the actual experimental work [9] preceding the present, powdered potassium chlorate was used as oxidant.

2. Model

The solid phase (ore) is assumed to be stationary, contains some unreduced iron remaining in the ore. It was found [9] that oxygen gas from the decomposition of $KClO_3$ attacked the ore in a gas-solid reaction, hence removing (through oxidation) the sulphur present in the ore in the form of SO_2 .Equations (1) and (2) show this.

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

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

2.1 Model Formulation

Experimental data obtained from research work [9] carried out at SynchroWell Research Laboratory, Enugu were used for this work. Results of the experiment as presented in report [9] and used for the model formulation are as shown in Table 1.

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

$$k_n[(\gamma)^{\mu\%S}] = T/\alpha \quad \text{(approximately)} \qquad (3)$$

Taking logarithm of both sides

$$\text{Log}\,(k_n[(\gamma)^{\mu\%S}]) = \text{Log}\,(T/\alpha) \qquad (4)$$

$$\text{Log}k_n + \text{Log}[(\gamma)^{\mu\%S}] = \text{Log}T - \text{Log}\,\alpha \qquad (5)$$

$$\text{Log}k_n + \mu\%S\text{Log}\gamma = \text{Log}T - \text{Log}\,\alpha \qquad (6)$$

$$\mu\%S\text{Log}\gamma = \text{Log}T - \text{Log}\,\alpha - \text{Log}k_n \qquad (7)$$

$$\%S = \frac{\text{Log}T - \text{Log}\,\alpha - \text{Log}k_n}{\mu\,\text{Log}\gamma} \qquad (8)$$

Introducing the values of μ, T, k_n and α into equation (8) (since they are constants) and evaluating further, reduces it to;

$$\%S = \frac{0.0415}{\text{Log}\gamma} \qquad (9)$$

$$\%S = \frac{D_e}{\text{Log}\gamma} \qquad (10)$$

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)

Table 1: Variation of concentration of sulphur removed with weight input of $KClO_3$.[9]

(γ)	(α)	%S
8	50	0.0346
9	50	0.0360
10	50	0.0400
11	50	0.0470
12	50	0.0500

Table 2: Variation of T/α with $k_n[(\gamma)^{\mu\%S}]$

T/α	$k_n[(\gamma)^{\mu\%S}]$
12	11.4003
12	11.5804
12	11.9125
12	11.9973
12	11.9982

3. Boundary and Initial Condition

Consider iron ore (in a furnace) mixed with potassium chlorate (oxidant). The furnace atmosphere is not contaminated i.e (free of unwanted gases and dusts). Initially, atmospheric levels of oxygen are assumed just before the decomposition of $KClO_3$ (due to air in the furnace).Weight of iron oxide ore used; (50g), and treatment time; 360secs. were used. Treatment temperature; 600^{o}C, ore grain size; 150µm, and weight of $KClO_3$ (oxidant); (8-12g) were also used. These and other process conditions are as stated in the experimental technique [9].

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

4. Model Validation

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

Analysis and comparison between these %S values reveal deviations of model-predicted %S values from those of the experiment. This is attributed to the fact that the surface properties of the ore and the physiochemical interactions between the ore and the oxidant (under the influence of the treatment temperature) which were found to have played vital roles during the oxidation process [9] were not

considered during the model formulation. This necessitated the introduction of correction factor; to bring the model-predicted %S values to those of the experimental %S values.

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

$$Dv = \frac{Sp - Se}{Se} \times 100 \quad (11)$$

Where Sp = Predicted %S values from model

Se = Experimental %S values

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

$$Cf = -Dv \quad (12)$$

Therefore

$$Cf = - \left(\frac{Sp - Se}{Se} \right) \times 100 \quad (13)$$

Introduction of the corresponding values of Cf from equation (13) into the model gives exactly the corresponding experimental %S values [9].

5. Results and Discussion

The derived model is equation (9). A comparison of the values of %S from the experiment and those from the model shows positive and negative deviations less than 33% which is quite within the acceptable deviation limit of experimental results hence depicting the reliability and validity of the model. This is shown in Table 3. The validity of the model is believed to be rooted in equation (3) where both sides of the equation are correspondingly almost equal. Table 2 also agrees with equation (3) following the values of $k_n[(\gamma)^{\mu\%S}]$ and T/α evaluated from Table 1 as a result of corresponding computational analysis. The value 0.0415 has a direct relationship with the value of %S as shown in equation (9). This indicates that the constant contributes directly (as a multiplying factor) to the predicted concentration of sulphur removed from the ore. Based on the foregoing, the constant is denoted as Desulphurization Enhancement Factor D_e.

Table 3: Comparison between %S removed as predicted by model and as obtained from experiment [9].

$\%S_{exp}$	$\%S_M$	Dv (%)	Cf (%)
0.0346	0.0460	+32.95	-32.95
0.0360	0.0435	+20.83	-20.83
0.0400	0.0415	+3.75	-3.75
0.0470	0.0399	-15.11	+15.11
0.0500	0.0385	-23.00	+23.00

Where $\%S_{exp}$ = %S values from experiment [9].

$\%S_M$ = %S values predicted by model

6. Conclusion

The model predicts the concentration of sulphur removed by molecular-oxygen-induced desulphurization of the iron oxide ore. The validity of the model is rooted in equation (3) where both sides of the equation are correspondingly almost equal. The deviation of the model-predicted %S values from those of the experiment is less than 33% which is quite within the acceptable deviation limit of experimental results.

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

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Model for Predictive Analysis of the Concentration of Dissolved Iron Relative to the Weight Input of Iron Oxide Ore and Leaching Temperature during Sulphuric Acid Leaching

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Abstract: Model for predictive analysis of the concentration of dissolved iron during leaching of iron oxide ore in sulphuric acid solution has been derived. The model %Fe = 0.987(μ/T) was found to predict %Fe dissolved with high degree of precision being dependent on the values of the leaching temperature and weight of iron oxide ore added. It was observed that the validity of the model is rooted in the expression %Fe = N(μ/T) where both sides of the relationship are correspondingly approximately equal. The positive or negative deviation of each of the model-predicted values of %Fe (dissolved) from those of the experimental values was found to be less than 19% which is quite within the acceptable range of deviation limit for experimental results, hence depicting the usefulness of the model as a tool for predictive analysis of the dissolved iron during the process. [Nature and Science. 2009;7(3):41-47]. (ISSN: 1545-0740).

Keywords: Model, Predictive Analysis, Dissolved Iron, Sulphuric Acid, Iron Oxide Ore, Leaching Temperature.

1. Introduction

Several studies have been carried out to evaluate the use of different organic and inorganic acids for dissolution of iron. Dissolution of iron oxides and oxyhydroxides in hydrochloric and perchloric acids has been evaluated [1]. Lim-Nunez and Gilkes [2] evaluated the use of synthetic metal-containing goethite and haematite for similar work while Borghi et al. [3] studied the effect of EDTA and Fe(II) during the dissolution of magnetite. It has been reported [4] that goethite can be dissolved in several inorganic acids belonging to the families of the carboxylic and diphosphoric acids in the presence of reducing agents. The effectiveness of several organic acids (such as acetic, formic, citric, ascorbic acids etc.) used for dissolving iron from iron compounds has also been evaluated [5]. Oxalic acid was found to be the most promising because of its acid strength, good comlexing characteristics and high reducing power, compared to other organic acids. Using oxalic acid, the dissolved iron can be precipitated from the leach solution as ferrous oxalate, which can be re-processed to form pure haematite by calcinations [6]. Many researchers have studied the use of oxalic acid to dissolve iron oxide on a laboratory scale [7-13]. Lee et al [14] used 0.19-0.48M oxalic acid to dissolve hydrated iron oxide. Iron dissolution was found [14] to reach 90% for a 20% slurry within 60mins. using 0.19M oxalic for the finer fraction (< 150μm) containing 0.56% Fe_2O_3. The coarser fraction (>150μm) containing 1.06% Fe_2O_3 achieved a lower iron removal, reaching a steady state of only 78% after 1 h of leaching. Although the pH was not measured or controlled, it was expected that the liquor pH is < pH1 at the oxalic acid concentration range studied (0.19-0.48). Taxiarchou et al.[6] found that the maximum iron dissolution of only 40% is within 3 h at temperatures in the range 90-100^{0}C. At 0.5M oxalate and all temperatures (25, 60 and 80^{0}C) the dissolution of iron was faster at a lower pH in the range pH 1-5 studied. Biological processes for iron dissolution have been evaluated by several researchers based on the use of several micro organisms that were easily sourced and isolated. Mandal and Banerjee [15] recently presented their findings on the study of the use of Aspergillus niger and their cultural filtrates for dissolving iron present in iron compounds. It has been found that dissolution of iron oxide is via a photo-electro chemical reduction process, and involves a complicated mechanism of charge transfer between the predominant oxalate species, namely ferric oxalate $Fe(C_2O_4)_3^{3-}$, ferrous oxalate $Fe(C_2O_4)_2^{2-}$ acting also as an auto catalyst, and the oxalate ligand on the iron oxide surface [16].

Iron oxide dissolution in oxalic acid has been found to be very slow at temperatures within the range 25-60^{o}C, however, its rate increases rapidly above 90^{o}C [17]. The rate of the dissolution process also increases with increasing oxalate concentration at the constant pH values set within the optimum range of

pH 2.5-3.0. The dissolution of fine pure haematite (Fe_2O_3) (105-140μm) was discovered to follow a diffusion-controlled shrinking core model at this optimum pH [17].

Taxiarchour et al [18] reported that it took close to 40h to dissolve 80% of pure haematite slurry (97% purity, 0.022% w/v or 0.21% g/L Fe_2O_3) at pH 1. He stated that even at 90^0C, it required close to 10h to achieve 95% dissolution of iron of the slurry at pH 1. They also dissolved iron using 0.1-0.5M oxalic acid (pH1-5) to dissolve iron from a 20% w/v slurry (83% of particle size in the range 0.18-0.35mm, containing 0.029% Fe_2O_3). The iron oxide concentration in the leach is equivalent to 0.058g/L Fe_2O_3.

The speciation of Fe(III) oxalate and Fe(II) oxalate has been found [19] to be governed by pH and total oxalate concentration. For a having pH › 2.5 and an oxalate concentration higher than 0.1M, the most predominant Fe(III) complex ion existing is $Fe(C_2O_4)_3^{3-}$. At these conditions, (pH › 2.5 and an oxalate concentration higher than 0.1M) the predominant Fe(II) complex species is $Fe(C_2O_4)_2^{2-}$.

Nwoye [20] derived a model for quantitative analysis of dissolved iron in oxalic acid solution in relation to the final pH of the solution during leaching of iron oxide ore;

$$\gamma = 0.5\left(\frac{K_1[\%Fe_2O_3]+K_2[\%Fe]}{[\%Fe][\%Fe_2O_3]}\right) \quad (1)$$

where

K_1 and K_2 = Dissolution constants of Fe and Fe_2O_3 respectively.

γ = pH of leaching solution after time t (mins.).

The values of the dissolution constants compared with those of % Fe and % Fe_2O_3 from the experiment [20] indicate clearly that the constants K_1 and K_2 are numerical equivalence of the chemical resistance to the dissolution of Fe and Fe_2O_3 (respectively) in oxalic acid solution. It was found that $K_1 \approx 2K_2$ indicating twice chemical resistance to the dissolution Fe compare to that of Fe_2O_3. This expression agreed with the higher percentage of Fe_2O_3 dissolved compared to that of the corresponding Fe.The model also predicted the final pH of the leaching solution when the concentrations of Fe and Fe_2O_3 dissolved (at a temperature of 30^0C and average ore grain size; 150μm) are known.

Models for computational analysis of the concentration of dissolved haematite and heat absorbed by oxalic acid solution during leaching of iron oxide ore have been derived [21]. These models are:

$$\%Fe_2O_3 = K(\gamma/\mu) \quad (2)$$

$$Q = K_C\mu \quad (3)$$

Where

$\%Fe_2O_3$ = Concentration of dissolved haematite in oxalic acid solution.

γ = Final pH of the leaching solution at time t at which $\%Fe_2O_3$ was obtained.

μ = Weight of iron oxide added into the oxalic acid leaching solution (g)

K = Constant of proportionality associated with haematite dissolution

K_C = Constant of proportionality associated with heat absorption

Q = Quantity of heat absorbed by oxalic acid solution during the leaching process (J)

Nwoye [21] found that optimization of the weight input of iron oxide ore could be achieved using the model; ($\%Fe_2O_3 = K(\gamma/\mu)$) by comparing the concentrations of dissolved haematite at different weights input of the iron oxide ore, with the view to identifying the optimum weight input of iron oxide ore that gives the maximum dissolution of Fe_2O_3. The model also indicates that the concentration of haematite dissolved during the leaching process is directly proportional to the final pH of the leaching solution and inversely proportional to the weight input of the iron oxide ore.

It was also found [21] that values of Q obtained from both the experiment and model ($Q = K_C\mu$) agree to the fact that leaching of iron oxide ore using oxalic acid solution is an endothermic process, hence the absorbed positive heat energy by the leaching solution. The quantity of heat energy absorbed by the oxalic acid solution during the leaching process (as calculated from the model; $Q = K_C\mu$) was found to be directly proportional to the weight input of the iron oxide ore. These results were obtained at initial pH 6.9, average grain size of 150μm and leaching temperature of 30^0C. The constants of proportionality K and K_C associated with the respective derived models were evaluated to be 0.0683 and 66.88 respectively [21].

Model for predictive analysis of the quantity of heat absorbed by oxalic acid solution during leaching of iron oxide ore has been derived [22]. It was observed that the validity of the model is rooted in the expression $(\ln Q)/N = \sqrt{T}$ where both sides of the relationship are correspondingly almost equal. The model was found to depend on the value of the final solution temperature measured during the experiment. The respective deviation of the model-predicted Q values from the corresponding experimental values was found to be less than 21% which is quite within the acceptable range of deviation limit of experimental

results. The positive values of heat absorbed as obtained from experiment and model were found to agree and indicate that the leaching process is endothermic in nature.

Nwoye [23] derived a model for predicting the time for dissolution of pre-quantified concentration of phosphorus during leaching of iron oxide ore in oxalic acid solution as:

$$\tau = \left(\frac{\mathrm{Log}\left(\frac{P^{1/4}}{1.8} \right)}{\mathrm{Log}\, T} \right) \qquad (4)$$

Where

T= Leaching temperature (^{0}C) in the experiment [23], taken as specified leaching temperature (^{0}C) aiding the expected dissolution of phosphorus .

N= 1.8 (Dissolution coefficient of phosphorus in oxalic acid solution during leaching of iron oxide ore) determined in the experiment [23].

P = Concentration of dissolved phosphorus (mg/Kg) in the experiment [23], taken as pre-quantified concentration of phosphorus expected to dissolve after a leaching time t (mg/Kg) in the model.

τ = Leaching time (sec.) in the experiment [23], taken as time for dissolution of the pre-quantified concentration of phosphorus (hrs) in the model.

The model was found to depend on a range of specified leaching temperatures (45-70^{0}C) for its validity. It was found [23] that the time for dissolution of any given concentration of phosphorus decreases with increase in the leaching temperature (up to 70^{0}C), at initial pH 5.5 and average grain size of 150µm. The model (formulated at conditions; leaching temperature of 25^{0}C, initial solution pH 5.0 and average grain size; 150µm) is dependent of the final pH and temperature of the leaching solution. The model shows that the concentration of iron dissolved during the leaching process is directly proportional to the third power of the ratio of final leaching and temperature.

It has been found [24, 25] that the final pH of the leaching solution depend on the leaching time, initial pH for the leaching solution and the leaching temperature.

Model has been derived [26] for predicting the concentration of phosphorus removed during leaching of iron oxide ore in oxalic acid solution. The model is expressed as;

$$P = \left(\frac{150.5}{\mu\alpha} \right) \qquad (5)$$

Where

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

(μ) = Weight input of iron oxide ore (g)

(α) = Final pH of the leaching solution at the time t when P is evaluated

150.5 = (pH coefficient for phosphorus dissolution in oxalic acid solution during the process) determined in the experiment [26].

The model [26] predicted the concentration of phosphorus removed, with high degree of precision being dependent on the final pH of the leaching solution and weight input of the iron oxide ore. It also shows that the concentration of phosphorus removed (at a temperature of 25^{0}C, average ore grain size; 150µm and initial leaching solution pH 5.5) is inversely proportional to the product of the final pH of the leaching solution and the weight input of the iron oxide ore.

Nwoye [27] derived a model for predictive analysis of the concentration of phosphorus removed (relative to the initial and final pH of the leaching solution) during leaching of iron oxide ore in sulphuric acid solution. It was observed that the validity of the model is rooted in the mathematical expression; $(P/N)^{1/3} = (e^{\gamma/\alpha})$ where both sides of the relationship are almost equal. The model; $P = 4.25(e^{\gamma/\alpha})^3$ shows that the concentration of phosphorus removed is dependent on the values of the initial and final pH of the leaching solution. In all, the positive or negative deviation of the model-predicted phosphorus concentration from its corresponding value obtained from the experiment was found to be less than 29%.

Nwoye et al. [28] derived a model for predicting the concentration of dissolved iron during leaching of iron oxide ore in sulphuric acid solution. The model is stated as;

$$\%Fe = 0.35(\alpha/T)^3 \qquad (6)$$

Where T = Solution temperature at the time t, when the concentration of dissolved iron is evaluated. (^{0}C)

0.35= (pH coefficient for iron dissolution in sulphuric acid solution during the leaching process) determined in the experiment [28].

α = Final pH of the leaching solution at the time t, when the concentration of dissolved iron is evaluated.

The aim of this work is to derive a model for predictive analysis of the concentration of dissolved iron relative to the ore weight input and leaching temperature during leaching of Agbaja (Nigeria) iron oxide ore in sulphuric acid solution.

2. Model

The solid phase (ore) is assumed to be stationary, contains the un-leached iron remaining in the ore. Hydrogen ions from the sulphuric acid attack the ore within the liquid phase in the presence of oxygen.

2.1 Model Formulation

Experimental data obtained from research work [29] carried out at SynchroWell Research Laboratory, Enugu were used for this work.

Results of the experiment as presented in report [29] and used for the model formulation are as shown in Table 1.

Computational analysis of the experimental data [29] shown in Table 1, gave rise to Table 2 which indicate that;

$$\%Fe = N(\mu/T) \quad \text{(approximately)} \qquad (7)$$

Introducing the value of N into equation (7)

$$\%Fe = 0.987(\mu/T) \qquad (8)$$

where

T= Leaching temperature at the time t when the concentration of dissolved iron is evaluated.(^{0}C)

N=0.987(Temperature coefficient for iron dissolution in sulphuric acid solution during leaching of iron oxide ore) determined in the experiment [29].

μ= Weight of iron oxide ore added during the leaching process. (g)

Equation (2) is the derived model**.**

Table1: Variation of leaching temperature with dissolved iron.[29]

T (^{0}C)	%Fe
55	0.0734
57	0.0725
60	0.0556
65	0.0554
68	0.0552
70	0.0551

Table 2: Variation of %Fe with N(μ/T)

%Fe	N(μ/T)
0.0734	0.0718
0.0725	0.0693
0.0556	0.0658
0.0554	0.0607
0.0552	0.0580
0.0551	0.0564

3. Boundary Conditions

Consider iron ore in cylindrical flask 30cm high containing leaching solution of sulphuric acid. The leaching solution is stationary i.e (non-flowing). The flask is assumed to be initially free of attached bacteria. Initially, atmospheric levels of oxygen are assumed. Weight of iron oxide ore used; 4g, initial pH of leaching solution; 6.7 and leaching time; 30 minutes were used. Range of leaching temperatures used; 55-70^{o}C. Ore grain size; 150µm, volume of leaching solution; 0.1 litre and sulphuric acid concentration; 0.1mol/litre was used. These and other process conditions are as stated in the experimental technique [29].

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

4. Model Validation

The formulated model was validated by direct analysis and comparism of %Fe values from model data and those from the experimental data for equality or near equality.

Analysis and comparison between these data reveal deviations of model data from experimental data. This is believed to be due to the fact that the surface properties of the ore and the physiochemical interactions between the ore and leaching solution which were found to have played vital roles during the leaching process [29] were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model data to that of the experimental values.

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

$$Dv = \frac{Dp - DE}{DE} \times 100 \qquad (10)$$

Where

Dp = Predicted %Fe values from model

DE = Experimental %Fe values

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

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

Therefore

$$Cf = -\left(\frac{Dp - DE}{DE}\right) \times 100 \qquad (12)$$

Introduction of the corresponding values of Cf from equation (12) into the model gives exactly the corresponding experimental %Fe values [29].

5. Results and Discussion

The derived model is equation (2). A comparison of the values of %Fe from the experimental data and those from the model shows very minimum positive and negative deviations less than 19% which is quite within the acceptable deviation limit of experimental results, hence depicting the reliability and validity of the model. This is shown in Table 3. The validity of the model is believed to be rooted on equation (1) where both sides of the equation are correspondingly approximately equal. Table 2 also agrees with equation (1) following the values of %Fe and N (µ/T) evaluated from Table 1 as a result of corresponding computational analysis.

Table3: Comparison between %Fe dissolved as predicted by model and as obtained from experiment [29]

$\%Fe_{exp}$	$\%Fe_{M}$	Dv (%)	Cf (%)
0.0734	0.0718	-2.18	+2.18
0.0725	0.0693	-4.41	+4.41
0.0556	0.0658	+18.35	-18.35
0.0554	0.0607	+9.57	-9.57
0.0552	0.0580	+5.07	-5.07
0.0551	0.0564	+2.36	-2.36

Where

$\%Fe_{exp}$ = %Fe values from experiment [29]

$\%Fe_M$ = %Fe values predicted by model

6. Conclusion

The model predicts quantitatively the concentration of dissolved iron (relative to the weight input of ore and leaching temperature used) during leaching of Agbaja iron oxide ore. The validity of the model is believed to be rooted on equation (1) where both sides of the equation are correspondingly approximately. The deviation of the model-predicted %Fe values from those of the experiment is less than 19% which is quite within the acceptable deviation limit of experimental results. 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.

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Model for Calculating the Solution pH during Hydrogen Peroxide Leaching of Iron Oxide Ore

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Abstract: Model for calculating the solution pH during hydrogen peroxide leaching of iron oxide ore has been derived. It was observed that the validity of the model is rooted in equation (1) where both sides of the equation are correspondingly approximately equal to 2. The model; $\gamma = \exp(K_C(\%Fe_2O_3/\%Fe)^N)$ was found to depend on the values of the % concentrations of dissolved iron and haematite from experiment. The respective deviation of the model-predicted pH values from the corresponding experimental values was found to be less than 20% which is quite within the acceptable range of deviation limit of experimental results. [Nature and Science. 2009;7(3):48-54]. (ISSN: 1545-0740).

Keywords: Model, Calculation, Solution pH, Hydrogen Peroxide, Iron Oxide Ore. Leaching.

1. Introduction

Studies [1-4] have been carried out involving the use of different acids for the leaching of iron oxide ore. Also attempts have been made to leach pyrite using strong oxidizing agent like hydrogen peroxide because of the inert nature of the pyrite.

It has been found [5] that oxalic acid (0.05-0.15M) is the best extractant for removing iron from iron compounds. The dissolution was found to increase with acid concentration within the range (0.05-0.15M) studied. In this case, both hydrogen ions and oxalate were increased. Using 0.15M oxalic acid approximately, 70% of the iron could be extracted from a slurry (20%w/v) containing 0.93% iron oxide (of goethite and haematite phases) at 100^0C within 90mins. The iron oxide concentration in the leach is equivalent to 1.86g/L Fe_2O_3.It was found [6], following studies carried out on the electrochemical dissolution of haematite (α-Fe_2O_3), maghetite, (γ-Fe_2O_3), goethite, (α-FeOOH) and lepidochrocite, (γ-FeOOH) in hydrochloric and oxalic acid using voltammetry that the hydroxyl-oxides of FeOOH can be reduced also via soluble Fe(III) species at 0.6-0.8 V (vs Ag-AgCl), where as haematite and maghetite dissolve only via direct reduction of the solid at -0.55 to -0.60V (vs AG-AgCl). These potentials were determined as peaks on voltammograms conductedwith stationary electrodes made from these iron oxides and hydroxyl-oxides. This study [6] on the electrchemical behaviour of different types of iron oxides confirms the electrochemical nature of haematite reductive dissolution. This further explains why it is easier to dissolve hydroxyl-oxides such as goethite where dissolution can take place via both reduction (solid and aqueous species) and complexation [7] whereas haematite dissolves mainly via solid reduction [8]. Oxalate can easily be reductant for such aprocess, as shown in its Eh-pH diagram [9].

It has been reported [10] that dissolution of haematite in oxalic acid is via a reductive mechanism which made Fe(III) non-existent in the solution. The overall reaction was therefore found [10] to be a redox reaction, forming two half cells: Oxidation of oxalate to form carbonic acid or carbon dioxide:

$$HC_2O_4^- = H^+2CO_2 + 2e^- \quad (1)$$

Reduction of haematite forming Fe(II) oxalate:

$$2H^+ + Fe_2O_3 + 4HC_2O_4^- + 2e^- = 2Fe(C_2O_4)_2^{2-} + 3H_2O \quad (2)$$

The dissolution reaction is therefore:

$$H^+ + Fe_2O_3 + 5\ HC_2O_4^- = 2Fe(C_2O_4)_2^{2-} + 3H_2O + 2CO_2 \quad (3)$$

The overall reaction indicates that species involved in the leaching would be hydrogen ions, oxalate and iron oxide (haematite particles).

The presence of Fe^{2+} was found to significantly enhance the leaching of iron extraction from silica sand at a temperature even as low as 25°C [11]. Ferrous oxalate however is oxidized quickly by air during the dissolution and in general an induction period of a few hours was observed to exist unless a strong acidic environment (<pH 1) or an inert atmosphere is maintained. Maintaining the high level of ferrous oxalate in the leach liquor using inert gas, was found to enhance the reaction kinetics.

The dissolution of iron oxide is believed to take place via a photo-electro chemical reduction process, involving a complicated mechanism of charge transfer between the predominant oxalate species, namely ferric oxalate $Fe(C_2O_4)_3^{3-}$, ferrous oxalate $Fe(C_2O_4)_2^{2-}$ acting also as an auto catalyst, and the oxalate ligand on the iron oxide surface [12]. The dissolution of iron oxides in oxalic acid was found to be very slow at temperatures within the range 25-60°C, but its rate increases rapidly above 90°C [13]. The dissolution rate also increases with increasing oxalate concentration at the constant pH values set within the optimum range of pH 2.5-3.0. At this optimum pH, the dissolution of fine pure haematite (Fe_2O_3) (105-140μm) follows a diffusion-controlled shrinking core model.

It has been reported [14] that the leaching of 3g/L pure haematite (98.2% purity, 105-140μm size range) using 0.048-0.48M oxalic acid at 80-100^{0}C passed through a maximum peak at pH 2.5. Dissolution of haematite was found to be slower than magnetite ($FeO.Fe_2O_3$) and other hydrated iron oxide such as goethite (α-FeOOH), lapidochrosite (γ-FeOOH) and iron hydroxide ($Fe(OH)_3$)
[14].

It has been found [15] that increasing the concentration of HCl results in a significant reduction in the rate of pyrite dissolution. The report also states that hydrochloric acid has an inhibiting effect on the oxidation of pyrite. This was attributed to chloride ions which are known to have a high tendency for adsorption. Also the formation of chloride complexes of iron (III) was found [15] to be possible, resulting in a decreasing concentration of the free iron (III) ions. However, no work has been reported on the effect of chloride ions and HCl concentration on the dissolution of iron during leaching of iron oxide ore.

Nwoye [16] derived a model for computational analysis of the concentration of dissolved haematite and heat absorbed by oxalic acid solution during leaching of iron oxide ore.
The model also indicates that the concentration of haematite dissolved during the leaching process is directly proportional to the final pH of the leaching solution and inversely proportional to the weight input of the iron oxide ore. The model [16] indicated that values of Q obtained from both the experiment and model agree to the fact that leaching of iron oxide ore using oxalic acid solution is an endothermic process, hence the absorbed positive heat energy by the leaching solution. The quantity of heat energy absorbed by the oxalic acid solution during the leaching process was found to be directly proportional to the weight input of the iron oxide ore. These results were obtained at initial pH 6.9, average grain size of 150μm and leaching temperature of 30^{0}C. The constants of proportionality K and K_C associated with the derived model were evaluated to be 0.0683 and 66.88 respectively.

Nwoye [17] derived a model for the computational analysis of the solution temperature during leaching of iron oxide ore in hydrochloric acid solution. The model is expressed as:

$$T = e^{(8.9055/\gamma)} \quad (4)$$

where

T= Solution temperature during leaching of iron oxide ore using hydrochloric acid.(^{0}C)
N= 8.9055(pH coefficient for hydrochloric acid solution during leaching of iron oxide ore) determined in the experiment [17].
γ = Final pH of the leaching solution at the time t when the solution temperature is evaluated.

The model is dependent on the value of the final pH of the leaching solution which was found to also depend on the concentration of iron dissolved in the acid. The prevailed process conditions on which the validity of the model depended on include: initial pH 2.5, leaching time; 30 minutes, leaching temperature; 25°C, average ore grain size; 150μm and hydrochloric acid concentration at 0.1mol/litre.

The mixed potential model of leaching assumes that the charge transfer processes occurring at the mineral surface are those that control the rate of dissolution [18].

The dissolution of iron ore has been investigated in the presence of oxygen at elevated temperatures and pressures [19-23]. The result of the works indicates that presence of oxygen enhances dissolution of iron. The use of hydrogen peroxide as the oxidizing agent for hydrometallurgical processes has been increasingly studied. McKibben [19] studied the kinetics of aqueous oxidation of pyrite by hydrogen peroxide at pH 1-4 and 293-313K.

Model for computational analysis of heat absorbed by hydrogen peroxide solution (relative to the weight of iron oxide ore added) has been derived [24]. The values of the heat absorbed Q as predicted by

the model were found to agree with those obtained from the experiment that the leaching process is endothermic in nature hence the positive values of Q and the absorbed heat. The deviations of the predicted Q values from the experimental values were found to be within the acceptable range. The model was found to be dependent on the weight of iron oxide ore added to solution in the course of leaching.
The model is stated as:

$$Q = e^{1.04(\sqrt{W})} \quad (5)$$

where

Q= Quantity of heat energy absorbed by hydrogen peroxide solution during the leaching process (J)

N= 1.04 (Weight-input coefficient) determined in the experiment[24].

W = Weight of iron oxide ore used (g)

Model for the calculation of the concentration of dissolved haematite during hydrogen peroxide leaching of iron oxide ore has been derived [25]. The model; $\%Fe_2O_3/\%Fe = (\mu)^{1/6}$ was found to depend on both the % concentration of dissolved iron and weight input of iron oxide ore from experiment. The validity of the model was found to be rooted on the expression $\%Fe_2O_3 \approx \%Fe\sqrt{(\mu)^{1/3}}$ where both sides of the relationship are correspondingly almost equal. The deviation of the model-predicted concentration of dissolved haematite from the corresponding experimental values is less than 30% which is quite within the acceptable range of deviation limit of experimental results. The model indicates that the dissolved % ratio of extreme oxidation stage of iron to that of its extreme reduction stage is approximately equal to one-sixth (1/6th) power of the weight input of iron oxide ore during the leaching process.

It has been discovered [26,27] that the initial pH of a leaching solution plays vital role in determining the extent metal dissolution from their respective mineral ores. Other factors were said to include leaching temperature, grain size of ore, concentration of chemical used as leachant as well as oxygen content of the leaching solution.

The aim of this work is to derive a model for calculating the solution pH (concentrations of dissolved Fe and Fe_2O_3 being known) during leaching of Itakpe (Nigerian) iron oxide ore in hydrogen peroxide solution.

2. Model

The solid phase (ore) is assumed to be stationary, contains the un-leached iron remaining in the ore. Hydrogen ions from the hydrogen peroxide attack the ore within the liquid phase in the presence of oxygen.

2.1 Model Formulation

Experimental data obtained from research work [28] carried out at SynchroWell Research Laboratory, Enugu were used for this work. Results of the experiment as presented in report [28] and used for the model formulation are as shown in Table 1. Computational analysis of the experimental data [28] shown in Table 1, gave rise to Table 2 which indicate that;

$$\ln\gamma = K_C\left(\frac{\%Fe_2O_3}{\%Fe}\right)_N \quad \text{(approximately)} \quad (6)$$

Introducing the values of the constants K_C and N into equation (6)

$$\ln\gamma = 1.2111\left(\frac{\%Fe_2O_3}{\%Fe}\right)^{1.33} \quad (7)$$

$$\gamma = \exp\left[1.2111\left(\frac{\%Fe_2O_3}{\%Fe}\right)^{1.33}\right] \quad (8)$$

Where $\%Fe_2O_3$ = Concentration of dissolved haematite in hydrogen peroxide solution during leaching (%) %Fe = Concentration of dissolved iron in hydrogen peroxide solution during leaching (%)

N = 1.33 (Iron oxidation-reduction endpoint ratio) determined in the experiment [28].

(γ) = pH of the leaching solution after time t (30 minutes)

K_C = 1.2111(Fe_2O_3-Fe leachibility coefficient) determined in the experiment [28].

Equation (8) is the derived model .

Table 1: Variation of the concentrations of dissolved haematite and iron with pH of leaching solution.[28]

$\%Fe_2O_3$	%Fe	(γ)
0.0017	0.0012	6.78
0.0020	0.0014	6.82
0.0501	0.0350	6.89
0.0013	0.0009	7.40
0.0042	0.0029	7.57
0.0022	0.0016	7.83
0.0079	0.0054	9.25
0.0081	0.0053	9.26

Table 2: Variation of $K_C(\%Fe_2O_3/\%\ Fe)^{1.33}$ with Inγ

$K_C(\%Fe_2O_3/\%\ Fe)^{1.33}$	Inγ
1.9247	1.9140
1.9464	1.9199
1.9513	1.9301
1.9749	2.0015
1.9821	2.0242
1.8498	2.0580
2.0089	2.2246
2.1290	2.2257

3. Boundary and Initial Condition

Consider iron ore in cylindrical flask 30cm high containing leaching solution of hydrogen peroxide. The leaching solution is stationary i.e (non-flowing). The flask is assumed to be initially free of attached bacteria. Initially, atmospheric levels of oxygen are assumed. Weight of iron oxide ore used; (10g). The initial pH of leaching solution; 6.5 and leaching time; 30 minutes were used. A constant leaching temperature of 25^{o}C was used. Ore grain size; 150μm, volume of leaching solution; 0.1 litre and hydrogen peroxide concentration; 0.28mol/litre were also used.. These and other process conditions are as stated in the experimental technique [28].

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

4. Model Validation

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

Analysis and comparison between these γ values reveal deviations of model-predicted values of γ from the corresponding experimental values. This is believed to be due to the fact that the surface properties of the ore and the physiochemical interactions between the ore and leaching solution which were found to have played vital roles during the leaching process [28] were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model-predicted γ values to those obtained from the experiment.

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

$$Dv = \frac{Dp - DE}{DE} \times 100 \quad (9)$$

Where Dp = Predicted γ values from model
DE = Experimental γ values
Correction factor (Cf) is the negative of the deviation i.e

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

Therefore

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

Introduction of the values of Cf from equation (11) into the model gives exactly the corresponding experimental γ values. [28]

5. Results and Discussion

The derived model is equation (8). A comparison of the values of γ from the experiment and those from the model shows minimum positive and negative deviation hence depicting the reliability and validity of the model. This is shown in Table 3. The respective positive and negative deviation of the model-predicted γ values from the corresponding experimental γ values is less than 20% which is quite within the acceptable range of deviation limit of experimental results. Table 2 also agrees with equation (6) following the values of $K_C[(\%Fe_2O_3/\%Fe)^{1.33}]$ and Inγ evaluated from Table 1. The validity of the model is believed to be rooted on equation (6) where both sides of the equation are correspondingly approximately equal to 2.

Table 3: Comparison between γ as predicted by model and as obtained from experiment [28].

γ_{exp}	γ_M	Dv (%)	Cf (%)
6.78	6.85	+1.03	-1.03
6.82	7.00	+2.64	-2.64
6.89	7.04	+2.18	-2.18
7.40	7.21	-2.57	+2.57
7.57	7.26	-4.10	+4.10
7.83	6.36	-18.77	+18.77
9.25	7.45	-19.46	+19.46
9.26	8.41	-9.18	+9.18

Where γ_{exp} = γ values from experiment [28]
γ_M = γ values predicted by model

6. Conclusion

The model calculates the pH of the leaching solution relative to known concentrations of dissolved iron and haematite during hydrogen peroxide leaching of Itakpe iron oxide ore. The validity of the model is rooted on equation (6) where both sides of the equation are correspondingly approximately equal to 2. The deviation of the model-predicted γ values from the corresponding experimental γ values is less than 20% which is quite within the acceptable range of deviation limit of experimental results.

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

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6. Conclusion

The model calculates the pH of the leaching solution relative to known concentrations of dissolved iron and haematite during hydrogen peroxide leaching of Itakpe iron oxide ore. The validity of the model is rooted on equation (6) where both sides of the equation are correspondingly approximately equal to 2. The deviation of the model-predicted γ values from the corresponding experimental γ values is less than 20% which is quite within the acceptable range of deviation limit of experimental results.

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Model for the Evaluation of the Quantity of Water Evaporated during Oven Drying of Clay

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Abstract

Model for the evaluation of the quantity of water evaporated during oven drying of clay has been derived. The model; $\mu = \exp[(\ln\tau/2.9206)^{1.26}]$ indicates that the quantity of evaporated water during the drying process is dependent on the drying time, the evaporating surface being constant. It was found that the validity of the model is rooted in the expression $In\mu = (\ln\tau/Log\gamma)^{N}$ where both sides of the expression are correspondingly almost equal. The respective deviation of the model-predicted quantity of evaporated water from the corresponding experimental value is less than 30% which is quite within the acceptable deviation range of experimental results, hence depicting the usefulness of the model. [Nature and Science. 2009;7(3):55-58]. (ISSN: 1545-0740).

Keywords: Model, Water, Evaporation, Oven Drying, Clay.

1. Introduction

Studies [1] have shown that the contents of the basic clay materials are divided into three groups. The first group involves clays containing mainly the mineral kaolinite. The second groups are clays containing mineral rnontmorillorite, while the third group are clays which are intermediate product of disintegration of mica into kaolin. Unal [2] reported that the structure of sinters and pellets may be divided into two parts viz, the mineral and the pores. He stated that the properties of pellets and sinters are closely related to the mineral constituents.

Furnass [3] reported that voids volume in packed dispersed powder depend on the ratio of smallest size (Ss) to largest size (Ls) particle as well as the percentage of constituent monosized particles. He maintained that the smaller the (Ss/Ls) ratio, the more continuous the distribution and the lower the void volume of the system. Singer and Singer [4] found that on heating dried clays, water is given off. With time, a hard but porous piece forms. A swollen appearance might occur during the release of some gases, but overall shrinkage must occur when verifications set in leading to a strong dense piece.

Nwoye [5] reported that chemical composition of the pellet, pelletisation parameters and firing conditions affect the shrinkage of clay pellets. He posited that the rate of chemical reaction is very much dependent on the gas-solid contact area, which is mostly governed by the porosity of the pellet. He stated that shrinkage of clay is probably due to volume change resulting from evacuation of water from the voids, reduction of the size of the pores as well as decrease in the interparticle separation.

It has been reported [6] that fine particles shrink more, are denser and exhibit excellent mechanical properties. Studies [6] carried out to investigate the relationship between particle size and size distribution with linear drying shrinkage, firing shrinkage and apparent porosity shows that no visible relationship exists between particle size and linear drying shrinkage. In this work [6], finer particles were found tend to shrink more. They concluded that the finer the particle size, the lesser the apparent porosity and greater the bulk density.

The behaviour of ceramic products has been found [7] to be very dependent on their composition, grain size, grain distribution, structure of grain and pores. Nwoye [8] also posited that the grain size and grain distribution of the clays have significant effect on their physical and technological properties (binding ability, shrinkage and plasticity).

It has been reported [9] that pores are deleterious to the strength of ceramics not only because they reduce cross-sectioned area over which the load is applied but more importantly act as stress concentrators. Reed [10] described firing as having three stages through which it proceeds; preliminary reactions which include binder burnout, elimination of gaseous product of decomposition and oxidation, sintering as well as cooling which may include thermal and chemical annealing.

Several works [1, 6, 10, 11] have been carried out on shrinkage of clay during drying. In all these works, porosity has been shown to influence the swelling and shrinkage behaviour of clay products of different geometry. It has been reported [10] that drying occurs in three stages; increasing rate, constant and decreasing rate. He pointed out that during the increasing rate; evaporation rate is higher than evaporating surface hence more water is lost. At constant rate, the evaporation rate and evaporation surface are constant. He posited that shrinkage occurs at this stage. Keey [11] also in a similar study suggested that at this stage, free water is removed between the particles and the interparticle separation decreases, resulting in shrinkage. During the decreasing rate, particles make contacts as water is removed, which causes shrinkage to cease.

The present work is to derive a model for evaluating the quantity of water evaporated during oven drying of Ukpor (Nigeria) clay at 90^0C.

2. Model formulation

Experimental data obtained from research work [12] carried out at SynchroWell Research Laboratory, Enugu were used for this work. Results of the experiment used for the model formulation are as shown in Table1.Computational analysis of the experimental data [12] shown in Table 1, gave rise to Table 2 which indicate that;

$$\ln\mu = (\ln\tau/\text{Log}\gamma)^N \quad \text{(approximately)} \qquad (1)$$

$$\mu = \exp[(\ln\tau/\text{Log}\gamma)^N] \qquad (2)$$

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

$$\mu = \exp[(\ln\tau/2.9206)^{1.26}] \qquad (3)$$

Where

(μ) = Weight of water lost by evaporation during the drying process (g)

(γ) = Area of evaporating surface (mm^2)

N = 1.26; (Collapsibility coefficient of binder-clay particle boundary at the drying temperature of 90^0C) determined in the experiment [12].

(τ) = Drying time (mins.).

Table 1: Variation of quantity of evaporated water with drying time.[12]

(τ)	(γ)	(μ)
30	833	2.60
50	833	3.90
70	833	5.00
90	833	5.48
110	833	6.10
130	833	6.50

Table 2: Variation of Inμ with $(\ln\tau/\text{Log}\gamma)^N$

$\ln\tau$	Logγ	Inμ	$(\ln\tau/\text{Log}\gamma)^N$
3.4012	2.9206	0.9555	1.2116
3.9120	2.9206	1.3610	1.4452
4.2485	2.9206	1.6094	1.6036
4.4998	2.9206	1.7011	1.9862
4.7005	2.9206	1.8083	1.8214
4.8675	2.9206	1.8718	1.9033

3. Boundary and Initial Conditions

Consider a rectangular shaped clay product of length 49mm, width 17mm, and breadth 9mm exposed to drying in the furnace while it was in wet condition. Initially, atmospheric levels of oxygen are assumed. Atmospheric pressure was assumed to be acting on the clay samples during the drying process (since the furnace is not air-tight). The grain size of clay particles used is 425μm, weight of clay and binder (bentonite) used (for each rectangular product); 100g and 10g respectively, quantity of water used for mixing; 2% (of total weight), drying temperature used; 90^0C, area of evaporating surface;833mm^2 and range of drying time used; (30-130 mins.).

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

4. Model Validation

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

Analysis and comparison between these μ values reveal deviations of model-predicted μ from those of the experimental values. This is believed to be due to the fact that the surface properties of the clay and the physiochemical interactions between the clay and binder, which were found to have played vital role during the evaporation process [12] were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model-predicted μ value to that of the corresponding experimental value (Table 3).

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

$$Dv = \frac{Pw - Ew}{Ew} \times 100 \quad (4)$$

Where Pw = Quantity of water evaporated as predicted by model (g)

Ew= Quantity of water evaporated as obtained from experiment (g) [12]

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

$$Cf = -Dv \quad (5)$$

Therefore

$$Cf = -100 \left(\frac{Pw - Ew}{Ew}\right) \quad (6)$$

Introduction of the value of Cf from equation (6) into the model gives exactly the corresponding experimental value of μ [12].

5. Results and Discussion

The derived model is equation (3). A comparison of the values of μ obtained from the experiment and those from the model shows little deviations hence depicting the reliability and validity of the model (Table 3). The respective deviation of the model-predicted quantity of evaporated water from the corresponding experimental value is less than 30% which is quite within the acceptable deviation range of experimental results, hence depicting the usefulness of the model. It was found that the validity of the model is rooted in equation (1) where both sides of the equation are correspondingly almost equal. Table 2 also agrees with equation(1) following the values of Inμ and $(\ln\tau/\text{Log}\gamma)^N$ evaluated from Table1 as a result of corresponding computational analysis.

Table 3: Comparison between quantities of evaporated water as predicted by model and as obtained from experiment [12].

μ_{exp}	μ_M	Dv (%)	Cf (%)
2.60	3.3589	+29.19	-29.19
3.90	4.2427	+8.79	-8.79
5.00	4.9706	-0.59	+0.59
5.48	5.6067	+2.31	-2.31
6.10	6.1805	+1.32	-1.32
6.50	6.7081	+3.20	-3.20

where μ_{exp} = μ values obtained from experiment[12]
μ_M = μ values predicted by model.

6. Conclusion

The model evaluates the quantity of water lost by evaporation during drying of Ukpor (Nigeria) clay at 90^0C. It was found that the validity of the model is rooted in equation (1) where both sides of the equation are correspondingly almost equal. The respective deviation of the model-predicted quantity of evaporated water from the corresponding experimental value is less than 30% which is quite within the acceptable deviation range of experimental results, hence depicting the usefulness of the model.

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

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Effects of growth promoter Boldenone undecylenate on weaned male lambs

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Abstract

This study aimed to observe the effects of an anabolic androgenic synthetic commercial steroid (Boldenone, BOL) on the growth performance of prepubertal male lambs. Lambs were divided into three equal groups (n=4), the first group injected 5mg boldenone, the second group injected 2.5 mg and the third one injected olive oil served as control. All treated groups received 5 injections at three week interval. Blood samples and body weight were taken until the seventh week after last injection. Blood serum total proteins, albumin, urea, total cholesterol and high density lipoproteins (HDL), ALT and AST and creatinine were recorded in addition to some whole blood haemogram parameters. Testosterone, T_3 and T_4 were assayed. The results indicated a significant increase in body gain in treated groups, total proteins and haemoglonbin with a decrease in urea. An insignificant increase in testosterone was recorded in both treated groups. The study proved that boldenone improved the performance of male lambs and treated lambs reached puberty earlier than control. [Nature and Science. 2009;7(3):61-69]. (ISSN: 1545-0740).

Introduction

Anabolic androgenic steroids (AAS) is an official definition for all male sex steroid hormones, their synthetic derivatives and their active metabolites are synthetic derivatives of the male testosterone originally designed for therapeutic uses to provide enhanced anabolic potency with negligible androgenic effects (**Clark and Henderson, 2003)**. They are also used to enhance strength and endurance in canine, equine and human athletes through increasing muscle protein production **(Teale and Houghton, 1991; Schänzer & Donike, 1992; Schänzer, 1996).**

Boldenone (1,4-androstadiene-17beta-ol-3-one; BOL) and its precursor boldione (1,4-androstadiene-3-17dione; ADD) are used as anabolic steroids in livestock **(Cannizzo et al., 2007)**. This drug has been developed for veterinary use: with a low androgenic potency and a very long half-life and trace amounts can easily be detected for months after discontinued use (http://en.wikipedia.org/wiki/Boldenone). BOL increases muscle size due to promotion of positive nitrogen balance by stimulating protein production and reducing protein destruction, moreover it produces a retention of body water, nitrogen, sodium, potassium and calcium ions (**Forbes,1985 & Mooradian et al., 1987**). BOL improves growth and feed conversion in veal calves and therefore might be used illegally to achieve more efficient meat production (**Schilt et al., 1996; Arts et al., 1996; De Brabander et al., 2004 & Vanoosthuyze et al., 1994**). ADD is used by bodybuilders as a product with an even greater anabolic potency than BOL itself (**De Brabander et al., 2004&Geyer et al., 1987**). BOL is used as a growth promoter for beef cattle in the United States (**Sone et al. 2005).** BOL is used also for treatment of debilitation in cats **(Boebel and Ehrenford,1978)**

AAS have potent anabolic activity, increase muscle mass and aggression in animals **(Williams et al., 2000)**. In fillies, the age of first ovulation and the second breeding season was significantly delayed in those treated with the high dose (**Skelton et al., 1991)**. However, female rats treated with nandrolone decanoate showed estral acyclicity and there was destruction of follicular units and an absence of corpus luteum in the ovaries. In the uterus, the drug promoted morphological alterations, characterized by vacuolated epithelium and endometrial stroma fibrosis (**Gerez et al., 2005**) and have inhibitory effects on female hamster reproduction **(Triemstra and Wood (2004).**

Boldenone sulphate has provided direct evidence for the endogenous nature of boldenone in entire male horses (**Ho et al., 2004**). The abuse of boldenone has been reported in human, equine and greyhound dog sports (**Schänzer and Donike, 1992)**. In addition to the growth promoting effects, anabolic steroids have been shown to adversely affect the cardiovascular, hepatic, and endocrine systems **(Yesalis et al., 1993**). AAS administration will disturb the regular endogenous production of testosterone and gonadotrophins that may persist for months after drug withdrawal. Many other adverse effects associated with AAS misuse include disturbance of endocrine and immune function, alterations of sebaceous system and skin, changes of haemostatic system and urogenital tract (**Hartgens and Kuipers 2004).**

Plasma levels of testosterone do not permit detection of illegal treatments because plasma androgens always remained within the physiological range. Illegal treatment could be detected in blood samples when they were collected at least every 20 days (**Simontacchi et al., 2004).**

The objectives of the this study were to determine the effects of boldenone-17-undecylenate administration on body gain performance, whole blood and blood serum protein and lipid metabolites, liver and kidney function, testosterone and thyroid hormones of prepubertal weaned male lambs.

Materials and Methods

Animals: Twelve weaned male lambs belonging to the research farm of Animal Reproduction Research Institute were divided into three equal groups. Animals in full dose group (n=4) received an intramuscular injection of 5 mg boldenone undecylenate while those of half dose group received 2.5 mg of boldenone undecylenate. Animals in control group (n=4) injected olive oil and served as control. Dosages were chosen according to literature (**Rosa Gastaldo et al., 2006**). Five injections were given at 3-week intervals for 15 weeks. All animals kept in the same yard under natural day light and temperature and fed the same nutrition .Water and blocks of salts were fed ad libitum

Blood sampling: Blood samples with and without anticoagulant were collected at each injection every 21 days and at the 7^{th} week after the fifth injection via jugular veinipuncture and serum was harvested then sera were stored at -20C° for clinical chemistry and hormonal assays.

Whole blood analysis: Differential leucocytic count was read by using Lishman's stain and the white blood cells was determined by method described by **Schalm (1986),** Determination of haemoglobin content was performed using method described by **Drabkin(1982).**

Clinical Chemistry: Total protein (g/dL), albumin(g/dL), total cholesterol(mg/dL), high density lipoproteins**(HDL)** and urea (mg/dl) were measured using diagnostic kit according to **Henery,(1968); Drupt,(1974); Watson,(1960)**; **Stein (1986)** and **Fawcett(1960)** respectively. The serum globulin was calculated by subtracting the value of albumin from the value of total protein according to **Doumas and Biggs (1972).** Serum creatinine was determined according to (**Bartles et al., 1972**), AST(GOT), and ALT (GPT) were determined according to **Reitman and Frankle (1957)**

Hormone profiles:

Total T_3 and T_4 assays were performed by radioimmunoassay RIA using Coat-A-Count kits (total $T_4$1 1081 and $T_3$1 501 for T_4 and T_3, respectively ; Diagnostic products Corp. Los Angles, CA) according to **Milter and Albyl, l985; Wrutniak et al., 1985**. Sensitivity of the assay was 0.25µg /dl and 7ng/dl for T_4 and T_3. Mean T_4 and T_3 intra-assay and inter-assay CVs were 3.9, 6.3 and 7.3,14.95 , respectively. Testosterone was assayed using the same RIA kit according to **Tietz (1994).** Sensitivity, intra and inter-assay coefficients of variation were 0.05ng/ml, 10 and 8.4, respectively.

Statistical analysis:

Data were subjected to statistical analysis using Statistical Package for Social Science **(SPSS 16, 2007).** The effect of treatments and injections were studied using split simple one-way ANOVA. The Duncan's multiple range tests was used in separating differences between significant means.

Results and discussion

Body gain: Lambs of both treated groups significantly ($P<0.05$) gained more body weight than control (Table 1). As well as, **Sinnett-Smith et al. (1983)** reported a significant increase in weight gain of lambs implanted with 80 mg of trenbolone acetate (TBA) which they attributed in part to a decrease in muscle protein degradation. Also, **Henricks et al. (1982)** reported an increase in growth rate of heifers administered 300 mg of TBA as an ear implant for 62 d. In ewe lambs, **DeHaan et al. (1987)** reported an increase in average daily gain (ADG) for ewes treated prenatally with testosterone propionate and rams treated prenatally with testosterone propionate grew at similar rates compared with controls rams but at a higher rate than prenatally treated ewes. In contrast, **Cannizzo et al., (2007**) detected no statistically relevant difference between different groups of veal calves treated with boldenone. Similarly, neither dosage of anabolic steroid nor duration of treatment had a significant effect on weight gain when compared to controls **(Howe and Morello, 1985)**. Moreover, average daily gain was not affected by trenbolone acetate (TBA) in rams or ewes (**Lough et al., 1993)**. Rams implanted with TBA gain BW faster than the control rams during the first 56 d **(Sillence et al. 1987**).

Total Proteins, albumin, globulin and urea: In this study, levels of total proteins in both treated groups were significantly higher when compared to those of controls (table 2) within all injections. Levels of total proteins significantly returned to pretreatment levels after last injection in only

full dose group but still high in half dose group till the end of the experiment. Albumin level was significantly low in both treated groups (table 2) at second injection but was high at fifth injection in both treated groups compared to control. In contrast to the present findings, pretreatment and post-treatment measurements of plasma albumin concentration did not indicate any beneficial effect of 0.5 mg/kg, 1.0 mg/kg, and 1.5 mg/kg of an anabolic steroid **(Finco et al., 1984).**

Globulin levels were high in both treated groups compared to control lambs (table 2) within all treatments. Within both treated groups, globulin levels were significantly higher than pretreatment levels even after last injection in only half dose group (Table 2). Similarly, **Jockenhovel et al., (1999)** found that serum total globulins were significantly increased in all treatments whereas the parenteral treatment modes showed a lower increase of total globulins. Urea levels decreased significantly in full dose group after first injection and half dose group after second injection of male lambs compared to control lambs indicating the decrease in protein breakdown (Table 2).In agreement with our results, estradiol 17β increased nitrogen retention and decreased blood urea nitrogen concentrations (**Cecava and Hancock,1974&Istasse et al.,1988)**.Similar to the presented results, animals in the treatment groups converted food to live-weight gain more efficiently faster and had lower levels of blood urea and to a lesser extent serum albumin than untreated controls (**Galbraith and Watson, 1978)**. It was clear from the present results that the increase in body gain was attributed to the increase in serum total proteins and globulin which indicated improvement in lambs health and immunity and a decrease in protein breakdown. Since anabolism is defined as any state in which nitrogen is differentially retained in lean body mass, either through stimulation of protein synthesis and/or decreased breakdown of protein anywhere in the body **(Kuhn, 2002)**. From the other point of view, plasma concentrations of amino acids and serum urea were similar in both conditions **(Christiansen et al.,2005)**.

Total cholesterol and HDL: Total cholesterol levels showed only significant difference between groups of animals during second injection (table 2). In control lambs, cholesterol levels were lower than those of treated groups during all injections except at the fifth injection. As well as, a significant increase of total cholesterol was observed in all treatment groups **(Jockenhovel et al., 1999)**. Plasma cholesterol concentrations in Suffolk males implanted with trenbolone acetate and oestradiol-17β exhibit a biphasic response to implantation and the magnitude of the response was directly related to the dose level of the ear implant (**Scaife et al., 1982)**. On the other hand, **Berg et al., (2002**) stated that total cholesterol did not increase. The high density lipoproteins were declined significantly after first injection of boldenone in only the half dose group (Table 2) but its levels increased more than those of first injection in full dose group at the second injection and in control group at the third one. In agreement with the present results, plasma concentrations of total cholesterol and high-density lipoprotein cholesterol, were not affected by trenbolone acetate (TBA) in either rams or ewes **(Lough et al., 1993)**, for rams, but high-density lipoprotein cholesterol showed a significant decrease **(Jockenhovel et al., 1999)** .In addition, **Hartgens and Kuipers (2004)** recorded a depression of serum high-density lipoprotein -cholesterol levels and significant time effects were noted for HDL cholesterol. Storage of frozen plasma might affect concentrations of plasma lipid metabolites, especially HDL cholesterol **(Bachorik et al., 1980).** Total and LDL-cholesterol were similar, HDL-cholesterol was distinctly lower in athletes were still abuse AAS (**Urhausen et al., 2003**).

Creatinine, Aspartate aminotransferase(AST) and Alanine aminotransferase(ALT).

Creatinine were increased significantly in both treated groups of lambs at the third and fourth injections compared to other injections but declined again at the fifth and after last injections to levels still higher than those of first and second injections. Although plasma creatinine concentrations after androgen administration were significantly higher than those before androgen administration but changes were not observed in plasma urea values (**van Miert et al., 1988**). ALT levels (table 2) decreased significantly in all groups after first injection but a significant increase was observed in only full dose group at the third injection. AST levels were low at the second injection in both treated groups compared to control but were higher in both treated groups at the fifth one than control lambs (table 3). AST levels were higher in control lambs at the 2nd and 4th injections compared to both treated groups. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were higher (**Urhausen et al., 2003**) in athletes abusing AAS.

Testosterone (T): In the present experiment (Table 3), testosterone concentrations were insignificantly higher than 1ng/ml in both treated groups increase after first injection but control lambs reached this level at the time of the fifth injection indicating the delayed puberty in control lambs. In agreement with our findings, serum testosterone levels in treated groups with AAS were significantly higher than that in control group **(Urhausen et al., 2003**;**Takahashi et al., 2004&Gabr and Shaker, 2006**). In contrast, the administration of T alone did not induce any variation in plasma testosterone **(Simontacchi, et al.,**

2004). Also, **Shimomura et al., (2005)** showed that the treatment of rats with ethinylestradiol alone significantly decreased testosterone levels in serum and the testis. Men treated with testosterone enanthate intramuscularly every 21 days had normal testosterone **(Jockenhovel et al., 1999)**. **Demisch and Nickelsen (1983)** referred the high testosterone levels to the disturbance in the distribution of the androgen between the plasma proteins.

Triiodothrnine(T_3)and Tetraiodothyronine (T_4): Levels of T_3 were higher at the fifth injection (Table 3) in the treated groups of lambs compared to pretreatment levels but during other injection T_3 levels were lower than at first pretreatment injection. A significant increase in T_4 was observed in control lambs (Table 3) at the third injection compared to both treated groups but a non significant increase was also observed at the last and after last injections. There were no significant treatment effects on mean basal plasma concentrations of thyroxine (T_4) or triiodothyronine (T_3) among horses treated with boldenone undecylenate, given twice weekly for 12 days but resulted in a significant time effect on overall mean basal plasma T_4 and T_3 concentrations **(Morris and Garcia, 1985).** In rams and ewes subcutaneously implanted with TBA, **Kahl et al. (1992)** found a decrease in plasma thyroxine and hepatic 5'-deiodinase activity. The enzyme, 5'-deiodinase, converts thyroxine to 3,5,3'-triiodothyronine (T_3), which was the metabolically active thyroid hormone. **Donaldson et al. (1981)** also noted a decrease in plasma thyroxine of growing wethers implanted with 140 mg of TBA. This might suggest a decrease in lipid metabolism and (or)turnover of lipid in the lambs implanted with TBA. At physiological concentrations, T3 was involved with lipogenesis in the liver (**Blennemann et al., 1992**).

Blood haemogram: The haemoglobin concentration in blood of both treated groups was decreased after the second injection (Table 4) while its concentration decreased in control lambs after the third one. The neutrophils count was higher in full dose lambs at the third injection and was high in half dose group in the second injection compared to other injections. Conversely, lymphocyte count was lower in full dose lambs at the third injection and was low in half dose group in the second injection compared to other injections. In contrast to the presented data of lambs, hemoglobin,leucocytes and platelets were significantly higher in athletes were still abusing AAS **(Urhausen et al., 2003**). Large doses of androgens have been employed in the treatment of refractory anemias and have resulted in some increase in reticulcytosis and haemoglobin levels **(Katzung, 1989)**.Anabolic steroids could also stimulate erythropoiesis the mechanism for this effect may occur by stimulating erythropoietic stimulating factor **(Donald, 1989**).

Conclusion

The use of anabolic androgenic steroids in animals could be recommended in breeding animals to enhance puberty and increase body gain in animals of low body gain and during nutritional stress but its use in fattening animals must be done under control and the recommended withdrawal must not be less than three months before slaughter to avoid its side effect on humans consumers. There is a necessity for further research to distinguish between naturally occurring and illegally used boldenone forms.

Table (1): Effect of different doses of BOL on body weight of weaned male lambs (Mean ±SE)

Treatment	Full dose**	Half dose*	control
Initial body weight/kg	27.25±1.18 [a]	25.50±2.25a	26.25±1.75
At second injection	26.75±1.38a	24.5±2.06a	25.75±1.49
At third injection	26.75±1.38a	25.0±2.04a	26.25±1.65
2 weeks post last injection	32.25±1.32b	30.00±2.27ab	28.25±1.97
4 weeks post last injection	35.0±1.78bc	32.50±2.10b	30.75±2.09
7 weeks post last injection (Final)	37.75±2.25c	35.50±2.10 b	33.25±1.44
Final gain/kg*	10.5±1.19[y]	10.5±0.50[y]	7.0±0.71[x]
Final body gain%(Final/InitialX100)	38.5 [y]	41.2[y]	26.7 [x]

Means with different superscripts a, b, c within column and x, y, z within row are significantly different at p<0.05,*P<0.001, ** P<0.0001

Table (2): Effect of different injections and doses of BOl on total proteins, albumin, globulin, urea total cholesterol, HDL, creatinine, ALT and AST(Mean ±SE)

Traits	Treatments	Injections					
		At first	At second	At third	At forth	At fifth	Post last
Total protein*** (g/dL)	Full **	6.74±0.26^{*abcy}	7.62±0.52^{**abcy}	7.78±0.44^{cdy}	7.60±0.21^{**bcdy}	7.29±0.41^{*abcy}	6.60±0.26^{ab}
	Half *	6.08 ± 0.46^{axy}	7.823±0.32^{by}	7.16±0.52^{abcxy}	7.05±0.37^{abcy}	7.71±0.42^{by}	7.02±0.18^{ab}
	control	5.29±0.16^{x}	5.94±0.10^{abx}	6.29±0.23^{bx}	5.71±0.32^{ax}	5.89±0.27^{x}	6.18±0.19^{b}
Albumin (g/dL)	Full	2.47±0.14	2.38±0.17^{*x}	2.61±0.47	2.41±0.37	2.41±0.37^{*xy}	2.57±0.11
	Half	2.42 ±0.11	2.27±0.07^{x}	2.45±0.42	2.42±0.22	2.86±0.29^{y}	2.72±0.13
	Control*	2.14±0.13^{b}	2.54±0.13^{by}	2.50±0.27^{b}	2.37±0.19^{b}	1.50±0.19^{x}	2.48±0.14^{b}
Globulin*** (g/dL)	Full **	4.27±0.31^{aby}	5.22±0.48^{**aby}	5.17±0 .37^{bc}	5.26± 0.14^{**bcy}	4.88± 0.71^{abc}	4.04±0.34^{ab}
	Half	3.66±0.47^{axy}	5.55±0. 30^{by}	4.70±0.74^{ab}	4.63±0.38^{aby}	4.85±0.69^{ab}	4.30±0.25^{ab}
	control	3.14±0.13^{x}	3.28±0.23^{x}	3.79±0.30	3.34±0.29^{x}	4.39±0.27	3.07±0.23
Albumin/ Globulin	Full	0.59± 0.07	0.48±0.09^{**x}	0.52±0.11^{x}	0.45 ±0.10^{*x}	0.56 ±0.15	0.86±0.31
	Half	0.70 ±0.11	0.42 ±0.06^{x}	0.59±0.17^{xy}	0.53±0.19^{xy}	068±0. 21	0.49±0.13
	control	0.67±0.12	0.76±0.27^{y}	0.68±0.11^{y}	0.73±0.17^{y}	035±0.10	0.65±0.22
Urea** (mg/dL)	Full ***	37.59 ± 1.63^{b}	32.71±1.24^{b*}	20.66±2.25^{*ax}	23.62±1.32^{**ay}	22.05±0.77^{*ax}	25.99±1.24^{*ax}
	Half ***	34.94±1.62^{c}	37.79±1.31^{c}	22.31±3.77^{abx}	17.68±1.46^{ax}	23.56±1.33^{bxy}	24. 78±0.82^{bx}
	Control*	36.06±1.6^{b}	35.23±2.48^{b}	31.50±2.07^{y}	30.99±2.11^{z}	26.96±1.3^{y}	30.94±1.2^{y}
Total ** cholesterol (mg/dL)	Full **	39.96±5.32^{ab}	55.68±3.62**^{by}	55.13±6.72^{b}	40.06±3.74^{ab}	29.67±2.89^{a}	45.14±4.15^{ab}
	Half	38.08±6.13^{ab}	40.96±3.24^{abx}	44.11±6.34^{ab}	32.09±2.95^{a}	30.67±2.69^{a}	44.61±2.62^{ab}
	control	35.22±4.92	37.02±2.96 ^{x}	40.99±2.81	29.74±2.79	29.86±3.42	41.56±1.3
HDL (mg/dL)	Full **	19.96±3.26^{ab}	23.39±2.43^{bxy}	15.47±1.09^{a}	17.85±2.39^{ab}	16.06±0.99^{a}	14.76±0.47^{a}
	Half **	23.18±1.89^{c}	21.01±1.10^{bcx}	19.98±2.78^{bc}	13.76±1.28^{a}	15.00±0.37^{ab}	15.91±1.07^{ab}
	control	22.46±2.55	22.29±1.66^{y}	23.25±3.77	18.34±1.82	16.19±2.22	14.96±0.4
Creatinine (mg/dL)	Full **	0.67±0.12^{a}	0.70±0.09^{a}	1.14±0.15^{b}	1.22±0.07^{b}	0.73±0.11^{**ay}	0.94±0.06^{*abx}
	Half ***	0.51±0.14^{a}	0.82±0. 80^{ab}	1.16±0.15^{b}	1.13±0.11^{b}	0.85±0.02^{abx}	0.92±0.10^{aby}
	control	0.60±0.09	0.75±0.08	1. 38±0.08	0. 79±0.21	0.88±0. 08^{y}	0.99±0.06^{x}
ALT(GPT) Units/ml	Full ***	51.50±4.79^{b}	37.13±6.39^{a}	59.50±2.36^{***by}	23.75±2.25^{a}	24.75±2.84^{a}	25.19±1.67^{a}
	Half *	47.25±3.33^{b}	33.75±3.29^{ab}	31.00±4.89^{ax}	20.75±0.75^{a}	32.25±1.03^{ab}	29.94±2.91^{a}
	control	41.00±4.55	35.25±3. 21	26.00±3.00^{x}	21.50±0.87	28.75±4.40	27.75±2.82
AST(GOT) Units/ml	Full *	76.75±3.66^{a}	76.13±3.64^{**acx}	80.25±3.82^{a}	62.50±3.75^{**ax}	138±24.47^{**by}	88.31±1.012^{a}
	Half **	62.75±6.75^{a}	64.13±2.38^{ax}	86.75±9.04^{ab}	59.25±3.25^{ax}	131±11.45^{cy}	109.3±14.3^{bc}
	control	66.75±6.60	92.38±8.29^{y}	91.50±17.3	97.3±1.01^{y}	55.0±5.93^{x}	84.94±6.63

Mean with different superscripts a, b, c within row and x, y, z within column are significantly different at P <0.05, *,**,** in the treatments indicate significance within groups and in other cells indicate significance between treatments. * P < 0.05,** P<0.001, *** P<0.0001

Table (3): Effect of different doses and injections of BOL on testosterone(T), T_3 and T_4 hrmones

Traits	groups	At first	At second	At third	At forth	At fifth	Post last
Testo-sterone ng/ml	Full	0.59±0.43	1.79±0.62	1.79±0.42	1.34±0.23	2.38±0.64	6.07±2.76
	Half	0.49±0.37	1.06±0.19	1.27±0.32	1.35±0.41	4.47±2.42	2.74±0.49
	control	0.55±0.25	0.60±0.09	0.73±0.21	0.45±0.13	2.40±1.56	1.61±0.49
T_3 ng/dl	Full***	74.31±4.26^{bc}	58.86±6.57^{ab}	45.79±5.79^{a}	49.73±4.35^{a}	81.36±6.70^{c}	74.97±2.87^{bcy}
	Half***	79.45±4.29^{c}	60.17±4.26^{ab}	52.28±5.18^{a}	61.90±4.63^{ab}	90.62±5.95^{c}	76.62±4.12^{bcxy}
	control	77.23±3.56^{c}	60.94±2.29^{ab}	46.75±4.39^{a}	56.39±2.27^{ab}	85.31±7.66^{d}	60.83±3.21^{bx}
T_4 µg/dl	Full***	6.10±0.28^{c}	3.66±0.24^{b}	1.90±0.31^{**ax}	3.09±0.30^{**b}	3.17±0.35^{b}	2.72±0.23^{ab}
	Half***	5.17±0.44^{d}	4.05±0.32^{c}	1.65±0.15^{ax}	2.37±0.30^{ab}	2.95±0.37^{b}	2.64±0.20^{ab}
	control	5.45±0.28^{c}	3.36±0.12^{ab}	3.61±0.46^{aby}	3.02±0.24^{ab}	3.59±0.21^{ab}	2.92±0.32^{a}

Mean with different superscripts a,b,c,d within row , x,y,z within column are significantly different at P < 0.05, *P<0.05**P<0.001 , *** P<0.0001

Table (4) Effect of different doses and injections of BOL on White blood cell count, neutrophil , lymphocyte count and ahemoglobin g %

Traits	groups	At first	At second	At third	At forth	At fifth	Post last
Haemo-Globin g%	Full **	13.6±0.53^{b}	13.7±0.4^{b}	12.8±0.5^{b}	11.7±0.3^{ab}	9.9±0.7^{a}	11.6±0.7^{ab}
	Half **	13.4±0.29^{c}	13.3±0.4^{bc}	12.6±0.8^{abc}	11.5±0.5^{ab}	11.1±0.3^{a}	11.9±0.4^{abc}
	Control***	13.5±0.28	13.5±0.3	13.2±0.6	9.9±0.2	9.1±0.2	11.8±0.4
White blood cells/10^3	Full	12.8±1.49	12.2±2.1	11.4±1.2	10.7±.5	12.18±0.57	12.6±0.6
	Half	13.07±1.83	13.9±1.0	13.2±1.8	13.9±2.5	12.75±0.55	11.6±0.7
	control	12.95±1.09	13.1±1.2	12.7±1.5	10.7±1.0	11.32±0.63	11.1±0.9
Neutrophils %	Full*	32.00±2.55^{ab}	28.1±3.9^{a}	43.3±3.1^{b}	32.5±3.4^{ab}	34.7±5.2ab	39.0±1.9^{ab}
	Half **	25.25±1.55^{a}	42.1±3.6^{b}	28.3±1.9^{a}	31.5±3.1^{ab}	38.0±1.0^{ab}	27.2±4.4^{a}
	Control**	28.62±1.89	35.1±3.2	36.5±3.4	22.0±2.0	36.0±3.0	41.3±3.4
Lympho-Cytes %	Full **	64.25±2.25^{b}	64.3±1.6^{b}	52.5±4.5^{a}	64.8±3.2^{b}	62.7±5.8^{b}	55.8±2.7^{ab}
	Half **	67.25±1.65^{ab}	53.3±3.8^{a}	66.5±2.9^{ab}	62.5±3.3^{ab}	59.0±1.0^{ab}	68.8±4.7^{b}
	Control**	65.75±1.41	58.8±2.4	60.3±3.3	74.5±4.5	61.2±3.3	55.1±3.5
	Control**	28.62±1.89	35.1±3.2	36.5±3.4	22.0±2.0	36.0±3.0	41.3±3.4
Lympho-Cytes %	Full **	64.25±2.25^{b}	64.3±1.6^{b}	52.5±4.5^{a}	64.8±3.2^{b}	62.7±5.8^{b}	55.8±2.7^{ab}
	Half **	67.25±1.65^{ab}	53.3±3.8^{a}	66.5±2.9^{ab}	62.5±3.3^{ab}	59.0±1.0^{ab}	68.8±4.7^{b}
	Control**	65.75±1.41	58.8±2.4	60.3±3.3	74.5±4.5	61.2±3.3	55.1±3.5

Mean with different superscripts a,b,c,d within row , x,y,z within column are significantly different at P < 0.05, *P<0.05**P<0.001 , *** P<0.0001

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Distribution of Aquatic Fungi in Relation to Physicochemical Factors of Kosi River in Kumaun Himalaya (India)

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Abstract: Watermolds- the members of Chytridiomycetes and Oomycetes fungi, and possess ability of colonizing a variety of substances. Kosi river water was assessed with reference to watermolds diversity, pH, water temperature and total organic matter at 3 study sites viz., Kosi, Kwarab and Khairna, from November 2000 to October 2001. During study, a total of 16 fungal species belonging to 7 genera of watermolds were isolated. Maximum number of fungal species was recorded during spring and rainy season, while minimum number of watermolds was observed during winter season. During present study, sterile species of watermolds showed dominance followed by eccentric species. The interaction of physicochemical factors greatly influence to the diversity of watermolds. [Nature and Science. 2009;7(3):70-74]. (ISSN: 1545-0740).

Key words: *watermolds, Kosi river, physicochemical factors.*

1. Introduction

Aquatic fungi contribute significantly in aquatic ecosystem and are seriously concerned with the utilization and degradation of animal and plant remains (Johnson, 1956; Sparrow, 1968). The members of Chytridiomycetes and Oomycetes are mostly aquatic and commonly known as watermolds. They are widely distributed and ubiquitous in occurrence (Liu and Volz, 1976; Nelson and Scott, 1962; Cummins et al, 1966). These fungi are mostly aquatic but several of them reported from other habitat too. Majority of them play a vital role in degradation of complex organic matter in simple one and recycling of nutrients. The watermolds are primarily saprophytic in nature but they have ability to colonize a variety of substances, as primary invaders. A few of them show parasitic association with living hosts, i.e. plants and animals and showed remarkable influence on biological productivity (Scott, 1961; Sati, 1981; Sati et al., 1992; Khulbe and Verma, 1983).

These fungi are mostly aquatic and commonly known as watermolds, but several of them reported from other habitat too. The study of watermolds have been carried out in all over the world by Coker (1923), Middleton (1943), Johnson (1956), Scott (1961), Seymour (1970), Robertson (1980) and Dick (1990). The studies of watermolds from various Indian water habitats were made by different investigators including Bhargava (1946), Dayal and Thakurji (1968), Khulbe (1977), Mer et al. (1980), Manoharachary (1991), Mer and Khulbe (1984), Mishra and Dwivedi (1987) and Sati (1997). The present paper deals with the study of distribution and seasonality of watermolds in Kosi river. In the present investigation, Kosi river water has been analyzed for watermolds diversity.

2. Material and Methods

Kumaun Himalaya - a north west region of India, is located between 28^0 44' - 30^0 49' N latitude and 78^0 45'- $81^0$1' E longitude in the western part of Central Himalaya. Climatically, major parts of this area represents a temperate zone and monsoon pattern of rain fall with dry summer and winter. The Kosi – a perennial river of Uttarakhand (India) was selected for the present study which originates from southern slope of the Bhatkot - Kausani range (2517 m) near Kausani and enters the Bhabar near Ramnagar (346 m). It drains 150 Km area in the mountains of Almora, Nainital and Udham Singh Nagar districts. For the present investigation three sites were selected on Kosi river viz., Kosi (1110 m), Kwarab (1000 m) and Khairna (790 m).

The water samplings were made seasonally for a period of one year during November, 2000 to October, 2001 from Kosi river in sterilized plastic bottles. 50 ml of composite sample of each water sample was pored into sterilized petridishes, baited with different sterilized animal and plant baits. Colonized baits

were washed with sterilized water and placed in different sterilized petridishes containing sterile water. The isolates were purified by single hypha culture method and identified with the help of various standard monographs (Coker, 1923; Johnson, 1956; Scott, 1961 and Dick, 1990).The physico-chemical properties (pH, water temperature, and total organic matter) of water analyzed by following standard methods of APHA (1989).

3. Result and Discussion

16 species belonging to seven genera of Blastocladiales, Saprolegniales and Peronosporales have also been recovered from Kosi river water (Table 1). Of these, one species belong to Blastocladiales of the class Chytridiomycetes, while twelve species belongs to Saprolegniales and 3 species belongs to Peronosporales of the class Oomycetes.

The Kosi river water at Kosi yielded 12 species of watermolds, whereas 8 and 10 fungal species were isolated at station Kwarab and Khairna stations of Kosi river, respectively (Table 1). On genera identification, *Achlya* showed higher diversity with 4 species followed by *Saprolegnia* with 3 species, while *Aphanomyces, Dictyuchus and Pythium* represent 2 species each. During present study, 4 species of watermolds viz., *Achlya* sps., *Dictyuchus sterile, Saprolegnia* sps. and *Pythium debaryanum* were observed most frequent occurring species as they recovered from all the stations. On the other hand, 6 fungal species viz., *Allomyces anomalous, Achlya klebsiana, Dictyuchus monosporous, Thraustotheca clavata, Pythium undulatum* and *P. afertile* were isolated only once and showed restricted distribution.

It is evident from the results presented in table 2 that the frequency of watermolds and concentration of different nutrients show variation considerably in different seasons in all the three stations. Kosi river water were found alkaline throughout the study period and ranged from 7.4-8.75 at Kosi, 7.56-8.66 at Kwarab and 7.86-8.66 at Khairna (Table 2). Water temperature of Kosi river varied from site to site due to altitudinal, topographical and various environmental factors, such as vegetation cover, human activities etc. Water temperature ranged between 11.5-24.0 ^{0}C at Kosi, 11.5-25.0 ^{0}C at Kwarab and 11.0-25.4 ^{0}C at Khairna. The maximum amount of total organic matter were recorded at Khairna (190-225 mg/l), while minimum at station Kosi (104-180 mg/l).

Watermolds are of ephemeral nature and consequently exhibits seasonality in aquatic system. During the present study, Kosi river at station Kosi showed highest diversity of watermolds (12 sps.). It might be due to wide range of pH (7.4-8.75) with moderate water temperature (11.5-24.0 ^{0}C). The mixing of fungal inoculums or spores through surface water runoff of catchment and nearby forest area alongwith rain water into river, might be responsible for higher diversity of watermolds during rainy season.

The maximum number of fungal species recovered in Kosi river at station Kosi (5 sps.) and Kwarab (4 sps.) in rainy season, and at Khairna (5 sps.) during spring season. The higher number of watermolds during spring and rainy season might be due to high amount of organic matter alongwith moderate water temperature. The results are similar to Klick and Tiffany (1985). Water temperature showed a positive significant correlation with total number of fungal species at station Kosi ($r = - 0.8266$, $P \leq 0.05$), and Khairna. ($r = - 0.9942$, $P \leq 0.01$). Higher temperature during summer and low temperature (below 15 ^{0}C) during winter has been found unfavorable for most of the aquatic fungi (Dayal and Tendon, 1962; Khulbe, 1991). On the basis of relative contribution, rainy season contribute highest occurrence of watermolds (62.5%), whereas both the spring and summer season contribute 43.75 % occurrence of watermolds. The minimum number of watermolds was observed during winter season, showed only 25% relative contribution. Lower temperature during winter might account for least number of species (Khulbe and Bhargava, 1977). It is interesting to note that 8 species of watermolds were failed to induce their antheridia and oogonia (sterile species) and showed their dominance followed by 6 eccentric species.

Table 1: Seasonal variation of watermolds in a freshwater river Kosi during November, 2000 to October, 2001.

S. No.	Fungal Species	Seasons				
		Winter 2000	Spring 2001	Summer 2001	Rainy 2001	Autumn 2001
1.	*Allomyces anomalous*	-	C	-	-	-
2.	*Achlya klebsiana*	-	-	-	B	-
3.	*A. americana*	-	-	C	B	-
4.	*A. prolifera*	-	-	A	B	-
5.	*Achlya sps.*	-	C	A,B	A,C	-
6.	*Aphanomyces leavis*	-	A	C	A	C
7.	*Aphanomyces sps.*	-	-	A,C	A	A
8.	*D. monosporous*	-	-	-	A	-
9.	*Dictyuchus sterile*	A,B,C	-	-	C	-
10.	*Saprolegnia ferax*	A,C	A	-	-	-
11.	*S. parasitica*	-	A,C	A	-	-
12.	*Saprolegnia sps.*	A	B,C	B	-	-
13.	*Thraustotheca clavata*	-	-	-	-	A
14.	*Pythium debaryanum*	C	C	-	B	A,B,C
15.	*P. undulatum*	-	-	-	-	B
16.	*P. afertile*		-	-	A	-
	TOTAL	**4**	**7**	**7**	**10**	**5**

A= Kosi; B= Kwarab; C= Khairna; - = species absent.

Table 2: Some Physicochemical parameters of river Kosi during November, 2000 to October, 2001.

Months	Physicochemical parameters								
	KOSI			KWARAB			KHAIRNA		
	pH	Water Temp. (^{0}C)	Total Org. Matter (mg/l)	pH	Water Temp. (^{0}C)	Total Org. Matter (mg/l)	pH	Water Temp. (^{0}C)	Total Org. Matter (mg/l)
Winter, 2000	7.97	11.5	104	8.59	11.5	113.5	8.36	11.0	190
Spring ,2001	8.75	14.5	140	8.66	14.0	191	8.66	16.5	205
Summer, 2001	7.4	22.5	175	7.56	21.5	155	7.93	23.0	225
Rainy, 2001	8.1	24.0	153	8.05	25.0	139.5	7.98	25.0	198
Autumn, 2001	8.09	19.0	186	7.99	17.5	140	7.86	18.0	220

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HYDROGEOLOGICAL CHARACTERIZATION AND WATER SUPPLY POTENTIAL OF BASEMENT AQUIFERS IN TARABA STATE, N.E. NIGERIA

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ABSTRACT

The basement complex area of Taraba State lies within Latitude $6^0 30^I$N to $9^0 30^I$N and Longtitude $9^0 00^I$E to $12^0 00^I$E, and is about 41,200km^2. The objective of this study is to characterize the hydrological nature and water-supply potential of the basement aquifers of the study area. Borehole logs were studied and on the basis of results of lithologic logs, weathered overburden and fractured-rock aquifer units were delineated. Pumping test results analysed from 39 hand pump boreholes reveal that transmissivity values range from 0.3m^2/day to 19.7m^2/day, thus indicating aquifer of negligible to high potentials. The hydraulic conductivity value vary from 3.3×10^{-2} m/day to 7.0×10^1 m/day which correspond to moderate specific capacity values as revealed by most boreholes. The basement aquifers have a total groundwater reserve of 281.8×10^6 m^3 with the recoverable reserve of 201.3m^2 per annum. The recoverable reserve per capita based on the present projected population in the next 25 years is 108.4m^3. The basement aquifers, therefore, when fully developed can sustain domestic, agricultural and industrial activities. On the basis of the aquifer types and characteristics identified, it is recommended that borehole field be designed in densely populated areas and infiltration galleries constructed along perennial streams. [Nature and Science. 2009;7(3):75-83]. (ISSN: 1545-0740).

Keywords: basement complex, groundwater, aquifer, borehole, wells, Nigeria.

INTRODUCTION

The area of study covers the basement complex area of Taraba State. It lies between latitude $6^0 30^I$N to $9^0 30^I$N and longitude $9^0 00^I$ to $12^0 00^I$E and has an areal extent of about 41,200 km^2. The area is generally hilly and is largely drained by River Benue, Katsina, Ala and Donga (Fig. 1). The people of Taraba State are predominantly farmers and most of them are found in the rural areas. Groundwater is an important source of water supply and plays an important role in industry, agriculture and domestic use (Achyara, 2004, Foster et al, 2008, MacDonald et al, 2005 (a), Srinivasa et al., 2000). Water supply to the communities of the area under consideration is grossly inadequate. Recent surveys carried out in 1997 and 1998 by the Petroleum (Special) Trust Fund (PTF) under the National Rural Water Supply Programme revealed that more than 90% of the people in Taraba State lack adequate and potable water supply. This is expressly manifested by the drying-up of hand dug wells and streams which constitute the dominant source of water supply to the people. Eighty hand pump boreholes were supposed to be drilled and eighty rehabilitated under the National Rural Water Supply Programme to ease the water supply problem in the State. However, not all the boreholes were drilled or rehabilitated due to accessibility

problem and subsequent winding up of the PTF. The problem however still remains due to inadequacy of the facilities and over usage. The national standard of 500 people to one hand pump borehole has not been met due to over population in the rural areas of the State. Some communities especially those in the basement complex terrain have no access to hand pump boreholes due to poor geologic environment.
The traditional sources of water supply from streams, hand dug wells and seepages are seasonal and hence unreliable (Adelana & MacDonald, 2008). This study therefore intends to evaluate the groundwater resource potentials of the basement complex terrain of the State. The study will also suggest appropriate scheme that would ensure continuous and sustainable water supply to the people.

METHOD OF STUDY

The study was carried out by conducting base line surveys in different localities covering the entire State. The baseline surveys involved identification of the different sources of water supply, rock types, accessibility and population of the communities visited. Measurement of depth to water level in hand dug wells were carried out. The aforementioned activities led to the choice of probable sites for the drilling of the proposed hand pump boreholes. Geophysical surveys consisting of the Electromagnetic (EM) and Vertical Electrical sounding (VES) techniques using EM34-3 Geonics and ABEM Terrameter SAS300C were employed to confirm the actual sites for the drilling of the boreholes. The present report involves the use of data from 43 hand pump boreholes located in the basement complex terrain. The boreholes were each pump tested for a period of 120 minutes. The results of the pumping test were analysed using Jacob and Cooper (1946) method for the purpose of evaluating the hydraulic properties of the aquifers. Storage coefficient for the aquifers could not be determined due to the short duration of the pumping test.

GEOLOGY OF THE STUDY AREA

The study area is underlain by the undifferentiated Basement Complex rocks which consist mainly of the migmatites, gneisses and the Older Granites. Tertiary to Recent basalts also occur in the area (Fig. 1). The undifferentiated Basement Complex particularly the migmatites, generally vary from coarsely mixed gneisses to diffused textured rocks of variable grain size and are frequently porphyroblastic (Macleod, et al 1971). This rock unit constitutes principally the undifferentiated igneous and metamorphic rocks of Precambrain age (Grant, 1971.)

The Pan African Older Granites are equally widespread in the area. They occur either as basic or intermediate intrusives (Turner, 1964). Different kinds of textures ranging from fine to medium to coarse grains can be noticed on the Older Granites (McCurry, 1976). Other localized occurrences of minor rock types include some doleritic and pegmatitic rocks mostly occurring as intrusive dykes and vein bodies. These occurrences are common to both the undifferentiated Basement Complex and the Older Granite rocks (Carter et al., 1963, McCurry, 1976). The Tertiary basalts on the other hand are found in the

Mambila Plateau mostly formed by trachytic lavas and extensive basalts which occur around Nguroje (du Preez, 1965).

RESULTS

Aquifer types

In basement terrain, groundwater development is met with difficulties due to lack of primary porosity in the bedrock. The secondary porosities such as joints, fault and weathered zones are the sources of groundwater occurrence and movement (Chiton and Foster, 1995, Foster et al 2008, Srinivasa, 2000, Wright and Burgess, 1992). Hence, the secondary porosity and weathered zones constitute the different aquifer systems. Borehole lithologic logs revealed two water bearing zones in the area; namely the weathered zone and the fractured-rock zone (Fig. 2). The basement complex rocks of the study area were subjected to different degrees of weathering which led to the formation of thick weathered materials in some places. The weathered materials range in thickness from 3m to 37.73m with an average of 17.8m, and consist of sandstone, clays and silts. The fractured-rock zone is overlain by the weathered zone and acts as conduct for groundwater movement. The zone is recharged by infiltration through the upper weathered zone.

Aquifer Properties

Available data on thirty-nine (39) hand pump boreholes were analysed for the determination of Transmissivity (T) and hydraulic conductivity (K). Attempts were not made to isolate the aquifer properties of the weathered overburden aquifers and that of the fractured-rock aquifers. This is because in the course of drilling these boreholes, both aquifers were merged and screened. Results from Table 1 reveal that transmissicity values (MacDonald et al, 2005 (b)) vary from $0.3m^2$/day to $19.7m^2$/day with an average of $2.9m^2$/day. Most values are below $5.0m^2$/day.

The hydraulic conductivity is simply computed from the relationship T = Kb where b represents the aquifer thickness. This was obtained by subtracting static water level from overburden and fractured-rock zone. The hydraulic conductivity values range from 3.3×10^{-2}m/day to 7.0×10^{-1}m/day with an average of 1.78×10^{-1}m/day. The specific capacity values computed for the boreholes vary from $0.60m^3$/day/m to $31.30m^3$/day/m. Higher values occur in BH7, BH8, BH9, BH24, BH37 and BH43 with low drawndown values (Table 1).

The specific capacity can be related directly to aquifer properties K and T (Dike, 1994). Though, specific capacity data do not show correspondence with transmissivity, hydraulic conductivity and borehole yield, but here two boreholes (BH6 and BH19) exhibit such a relationship (Table 1). The performances of the boreholes have been classified into 4 groups based on the range of specific capacity values (Table 2). Seventy-nine per cent (79%) of the boreholes have moderate performance. This also corresponds to

moderate hydraulic conductivity values (3.3. x 10^{-2}m/day to 7.0 x 10^{-1}m/day) and negligible to high transmissivity values (0.3m^3/day to 19.7m^2/day). Borehole yields range from 6.77m^3/day to 21.6m^3/day with an average of 14.41m^3/day.

DISCUSSION

The basement aquifer properties evaluated reveal that the transmissivity values range from 0.3m^2/day to 19.7m^2/day with an average of 2.90m^2/day. According to Offodile (2002), a transmissivity range of 5 to 50m^2/day could be regarded as high potential in crystalline rock situations. By the above standard, the basement aquifers in the area are classified as aquifers of negligible to high potentials. The hydraulic conductivity values vary from 3.0 x 10^{-2}m/day and 7.0 x 10^{-1}m/day with an average of 1.90 x 10^{-1}m/day. The range of values reveals moderate hydraulic conductivity (Todd, 1980). The specific capacity values for the boreholes do not show correspondence with transmissivity, hydraulic conductivity and borehole yields. This could be attributed to differences in the degree of weathering, presence or absence of fractures in some places and method of construction of the wells. Based on specific capacity values, most of the boreholes have moderate performance which corresponds to moderate hydraulic conductivity and negligible to high transmissivity values. Borehole yields range from 6.77m^3/day to 21.6m^3/day with an average of 14.41m^3/day. The total yield of the boreholes is about 620.04m^3/day which can sustain a population of 24, 802 based on water supply standard of 25 litres per day for rural communities (Babatola, 1997).

Water supply situation is grossly inadequate in the area covered by this study. Water supply from the hand pump boreholes is supplemented by water from streams, ponds and hand dug wells. The existing hand pump boreholes are over-stretched due to over population in the rural area. Frequent breakdown of the boreholes occur thereby creating acute water shortages. This situation compels the villagers to resort to the traditional system of water supply through streams, ponds and hand dug wells. Where water cannot be obtained due to the seasonal nature of these sources, water supply is obtained through groundwater mining few metres below the stream beds. Where such streams do not exist, people have to trek several kilometers in search of water.

Table 1: HYDRAULIC CHARACTERISTICS OF SOME HANDPUMP BOREHOLES IN THE STUDY AREA

BH Na	LOCATION	DEPTH (m)	YIELD (m^3/day	SWL (m)	DRAWDON (m)	SC (m^3/day/m)	AQUIFER THICKNESS (m)	TRANSMISSITY (m^3/day)	HYDRAULIC CONDUCTIVITY (M/day
1.	Mararaba Baissa	19.00	6.77	6.97	11.25	0.60	ND	0.39	ND
2.	Nasarawa	12.90	14.40	5.90	2.00	7.20	ND	ND	ND
3.	Bunduwa	48.18	12.96	5.86	7.21	1.80	ND	0.51	ND

4.	Chanchanji	96.10	17.28	6.80	4.60	3.76	ND	2.57	ND
5.	Kwambai 2	24.80	11.52	8.51	3.30	3.49	ND	1.75	ND
6.	Muji 2	4.00	12.10	7.00	4.51	2.68	8.00	1.29	1.60×10^{-1}
7.	Jenuwarikya	50.76	12.67	6.71	0.63	20.11	22.29	5.80	2.6×10^{-1}
8.	Basang	48.00	17.28	4.50	0.91	18.99	19.50	5.00	2.6×10^{-1}
9.	Kpakye	45.00	17.28	3.20	1.71	10.11	14.80	18.10	1.22×10^{-1}
10.	Kpambo Yirom	54.20	12.67	5.00	7.26	1.75	14.00	0.61	4.4×10^{-2}
11.	Sabongida	59.00	11.23	6.30	11.30	0.99	12.40	0.43	3.4×10^{-2}
12.	Nzurkwem	80.00	12.96	13.55	ND	ND	34.50	0.97	2.8×10^{-2}
13.	Lissam	51.60	11.23	5.20	13.40	0.91	8.50	0.38	4.5 4.4×10^{-2}
14.	Yelwa	49.18	12.96	4.36	9.50	1.36	19.64	0.64	3.3 4.4×10^{-2}
15.	Kwaitab	50.67	15.84	8.46	3.70	4.28	14.54	0.88	6.1 4.4×10^{-2}
16.	Karamti	41.97	14.40	4.37	3.75	3.84	30.63	1.46	4.8 4.4×10^{-2}
17.	Mayoselbe	44.90	14.40	8.42	3.34	4.31	10.58	1.01	9.5 4.4×10^{-2}
18.	Saukakahuta	48.00	ND	5.90	ND	ND	9.10	ND	ND
19.	Tudunwada	26.70	17.28	8.80	3.30	5.24	6.20	2.10	3.4 4.4×10^{-1}
20.	Gangpenton	55.20	17.28	7.33	9.16	1.89	10.67	0.57	5.3 4.4×10^{-2}
21.	Hamdallahi	51.46	17.28	6.63	3.96	4.36	6.37	1.44	$2.3 \times 4.4 \times 10^{-2}$
22.	Garbachede	91.30	13.25	2.55	1.04	12.74	ND	4.50	ND
23.	Garin Yusufu	55.80	14.40	7.80	3.40	4.24	22.20	1.91	8.6 4.4×10^{-2}
24.	Sansani	46.40	18.70	4.32	6.22	3.01	10.68	1.87	1.8 4.4×10^{-1}
25.	Gangdole	42.54	17.28	7.68	2.02	8.55	28.32	19.7	7.0 4.4×10^{-1}
26.	Dakka	103.20	14.40	5.85	4.77	3.02	ND	2.53	ND
27.	Tudunwada	59.20	10.51	6.20	6.88	1.53	ND	0.54	ND
28.	Bitako	55.70	14.40	2.97	2.59	5.56	32.10	2.93	9.1×10^{-2}
29.	Lakwati	61.67	11.23	2.60	4.81	2.33	ND	0.87	ND
30.	Monkin	37.36	11.23	9.50	9.66	1.16	ND	0.76	ND
31.	Yakoko	34.35	14.40	9.44	4.79	3.01	ND	0.59	ND
32.	Gov. Lodg. Zing	16.90	14.40	8.00	1.53	9.41	ND	3.20	ND
33.	Yonko	27.96	15.84	4.21	9.37	1.67	ND	0.56	ND
34.	Mararaba Kunini	54.00	14.40	5.28	ND	Nd	3.00	1.51	5.0×10^{-1}
35.	Tsoro Dangung	45.83	17.57	8.60	1.47	11.95	13.40	2.98	2.2×10^{-1}
36.	Jekadafari	42.00	14.40	3.00	6.80	2.12	ND	0.57	ND
37.	NTA Vill. Jalingo	42.00	20.16	6.56	7.93	2.54	28.44	1.23	4.3×10^{-2}
38.	Wuro Sambe	37.00	11.52	26.00	ND	ND	ND	ND	ND
39.	Sabongari Jalingo	49.00	14.40	5.27	4.67	3.08	37.73	1.14	3.0×10^{-2}
40.	Kantiyel	31.00	21.6	10.05	ND	ND	ND	ND	ND
41.	Tutare	30.00	21.6	4.92	0.69	31.30	26.58	15.7	5.9×10^{-1}
42.	Jauro Manu	45.87	10.08	6.87	1.33	7.58	26.13	3.6	1.4×10^{-1}
43.	Garin Shuaibu	23.86	11.52	8.26	2.04	5.65	ND	1.92	ND

BH: Borehole

ND: No data

Table 2: CLASSIFICATION OF SPECIFIC CAPACITIES IN THE STUDY AREA

Group	Specific Capacities	Borehole performance
	Range (m^3/day/m)	Excellent performance
1	<31.30	BH 41
		Good performance
2	11.95-31.30	BH7, BH8, BH22, BH35
		Moderate performance
3	1.16-11.95	BH2, BH3, BH4, BH5, BH6, BH9, BH10, BH14, BH15, BH16, BH17, BH19, BH20, BH21, BH23, BH24, BH25, BH26, BH27, BH28, BH29, BH30, BH31, BH32, BH33, BH36, BH37, BH39, BH42, BH43,
		Poor performance
4	0.60-1.16	BH1, BH11, BH13

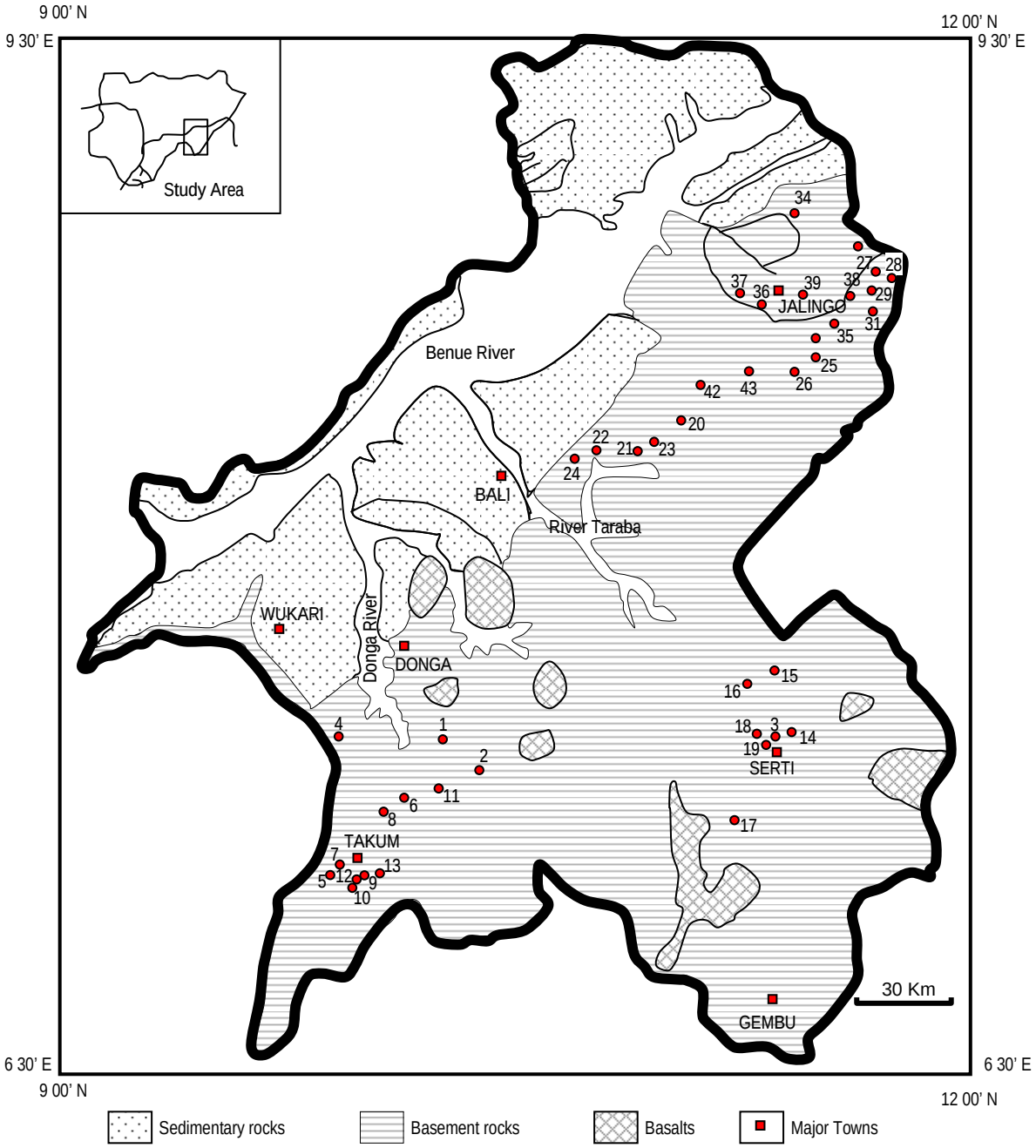

FIG. 1: BOREHOLE LOCATION MAP AND DRAINAGES OF THE STUDY AREA. MODIFIED FROM BADAFASH CONSULTING ENGINEEERS (1991)

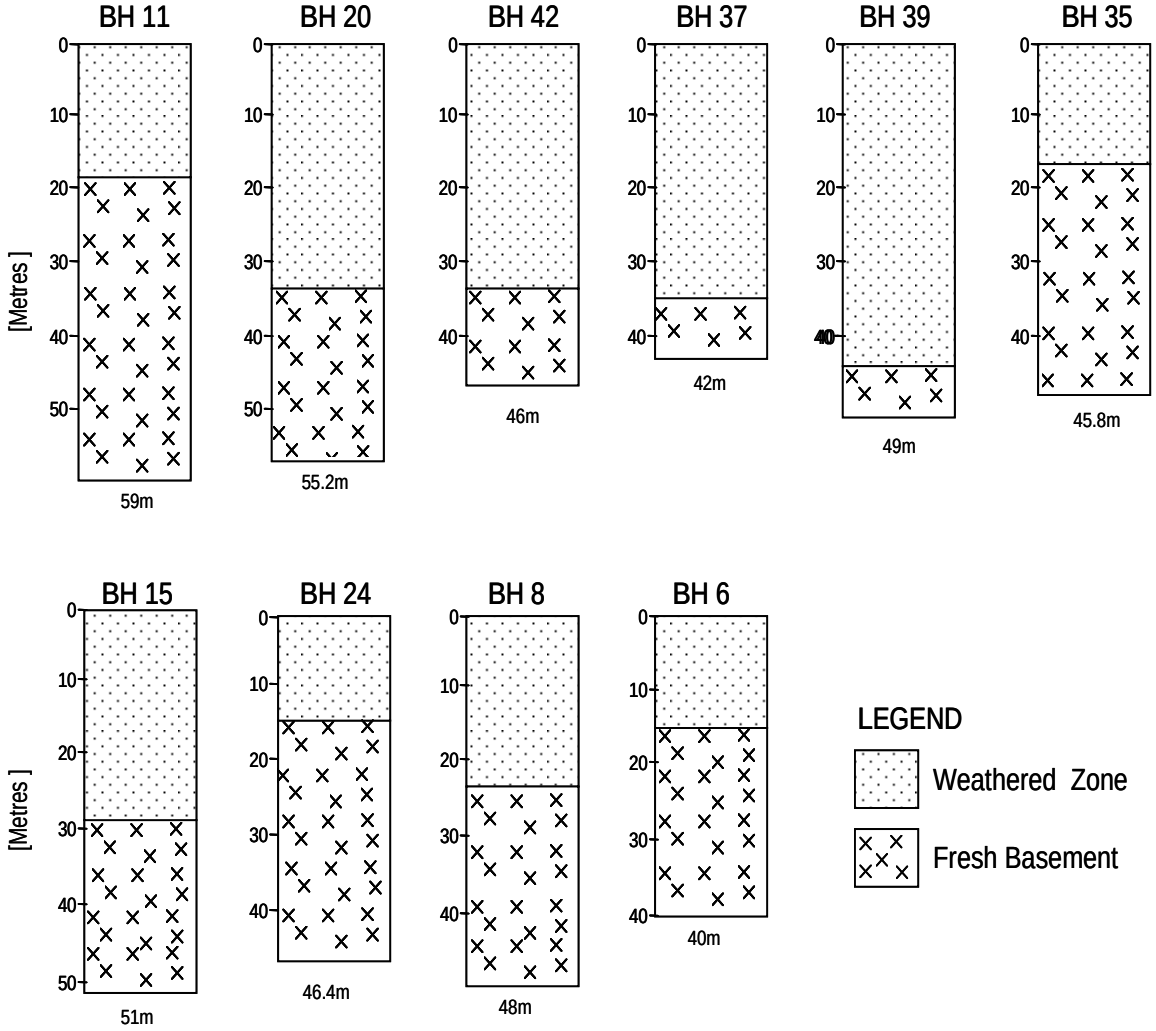

FIG. 2: LITHOLOGIC SECTION OF SOME HAND PUMP BOREHOLES IN THE BASEMENT AREAS

CONCLUSION

On the basis of borehole lithologic logs, weathered and fractured aquifer units were delineated in the area. The hydraulic properties of the basement aquifers indicate negligible to high transmissivity and moderate specific capacity values as revealed by most boreholes. This suggests boreholes of good performance for rural water supply. The aquifers have a total reserve of 281.8 x $10^6 m^3$ which is at present under exploited. The present population of the area is 1.4 x 10^6 and groundwater reserve is 281.8 x $10^6 m^3$. The recoverable reserve per capita amounts to $201.3 m^3$ per annum. With the projected population of 2.6 x 10^6 in the next 25 years at the growth rate of 2.5%, the recoverable reserve per capita is about $108.4 m^3$. The basement aquifers therefore when fully developed can sustain domestic, agricultural and industrial needs of the ever increasing population of the area. It is therefore recommended that borehole field should be designed in densely populated areas and infiltration galleries be constructed along perennial streams in order to meet the urgent water demand of the people.

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Electro- reduction of 4-nitrobenzaldehyde in basic medium at Different Electrodes

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Abstract: Electro-organic synthesis of 4,4'-bis(hydroxymethyl)hydazobenzene has been carried out by the electroreduction of 4-nitrobenzaldehyde at copper and amalgamated forms of copper and zinc electrodes under alkaline conditions using galvanostatic techniques. The electro reduction of 4-nitrobenzaldehyde has been also studied by cyclic voltammetry at glassy carbon electrode under alkaline conditions, which indicate the reducible behaviour from its cyclic voltammogram. Isolated product was characterized by T.L.C., usual laboratory qualitative tests and IR, NMR spectral analysis to be 4,4'-bis(hydroxymethyl)hydazobenzene. Effect of different parameters like current density, temperature, depolarizer concentration and nature of cathode material on yield percentage and current efficiency have been investigated. Number of electrons have also been calculated to confirm the isolated product to be 4,4'-bis(hydroxymethyl)hydazobenzene. [Nature and Science. 2009;7(3):84-98]. (ISSN: 1545-0740).

Keywords: 4-nitrobenzaldehyde, cyclic voltammetry galvanostatic technique.

Introduction

Electroreduction of aromatic compounds having nitro and aldehyde groups may be controlled to give aniline, azo, azoxy, hydrazo and alcohol respectively [1-6]. Cathodic reduction of nitro and aldehyde groups containing aromatic compounds and other pharmacologically important compound [7-11] have also been studied at the different electrodes but the formation of 4,4'-bis(hydroxymethyl)hydazobenzene from the reduction of 4-nitrobenzaldehyde has not been reported in literature. The Voltammetric techniques showed themselves to be an excellent alternative for the study of the reaction mechanism [12-13] and analytical determination of organic compounds . Compared with other methods , this new procedure (Cyclic Voltammetry) possesses following advantages, such as a low detection limit, a rapid response, excellent reproducibility , simplicity and low cost[14] . typical Cyclic Voltammograms were recorded within the wide range (200mV to -1600 mV)of the potential at pH 11.0and at different scan rates(50,100,200,500 $mVsec^{-1}$)fig.1. The 4-nitrobenzaldehyde reduction is irreversible as evident by cyclic voltammogrames at higher scan rates and anodic waves did not appear under any circumstances. The current function ($I_{pc/v}{}^{1/2}$) can be scan rate independent (fig:2) while E_{pc} (cathodic peak potential) varies negatively as the scan rate increases.

It is evident form the literature that the reduction 4-nitrobenzaldehyde has not been studied in detail. Reduction of 4-nitrobenzaldehyde has, therefore, been studied at different metal such as Copper, amalgamated copper (Cu-Hg) and amalgamated zinc (Zn-Hg) electrodes galvanostatically. Effect of various experimental parameters such as current density, temperature and concentration of depolarizer on yield percentage has been investigated for the reduction of 4-nitrobenzaldehyde in order to obtain optimum conditions.

Material and Methods

4-nitrobenzaldehyde used was of laboratory grade and other chemicals like sodium hydroxide, sulphuric acid, diethylether etc. were of analytical grade. All the solutions were prepared in conductivity water. Electrolytic cell used was a 250 ml beaker (tall form) with the provision of porous pot, magnetic stirrer, magnet bar, thermometer, cathode and agar-agar gel glass tube (bridge) to measure the cathodic potential (vs Saturated Calomel Electrode, SCE). Other components of the cell assembly were as follows [15-19].

Cathode : different metal strips

Catholyte : 5% (w/v) aqueous sodium hydroxide + 4-nitrobenzaldehyde

Total volume of catholyte : 100ml

Anode : lead strip (PbO_2)

Anolyte : aqueous sodium hydroxide solution

Distance between cathode & anode : 2.5 cm (approximately)

Catholyte prepared was of different concentration from 0.05M to 0.2M. The temperature was controlled to the desired value by having ice cold water in outer Jacket of Electrolysis cell. A magnetic stirrer was used for agitation.

The desired current according to current density range was applied from the current regulated power supply (galvanostat) developed by CDPE, Department of Physics, University of Rajasthan, Jaipur. The cathodic potential measured by digital multimeter RISH Multi 14S via saturated calomel electrode (SCE) through agar-agar gel bridge in each case i.e. at all electrode under investigation in the presence and absence of depolarizer to get suitable current density range for reduction under the experimental conditions imposed.

In all cases a theoretical quantity of current was passed depending upon the amount of depolarizer taken.

After electrolysis, the catholyte was neutralized with 5% (w/v) sulphuric acid solution and cooled to 278 – 283 K in ice. This solution was then treated with diethylether to extract the product and the same was washed with ice cold water to remove the base or salt (if any) and allowed to evaporate the ether. After evaporation, a yellowish-orange solid was obtained, which was subject to the usual physiochemical methods of analysis. The following observations were made.

1. Solid was soluble in ether, acetone, chloroform and partially in water, and burns with sooty flames shows aromatic nature of compound.
2. The product gave highly pungent and displeasing smell indicating the formation of dimerisation product.
3. A single clear spot on silica gel-G plate was obtained in iodine chamber. (80% C_6H_6+ 20% ethyl acetate medium) confirming that the product was a single compound and not a mixture.
4. The melting point of the product was found to be 453K.
5. The percentage of Carbon ,Hydrogen and Nitrogen in the product was determined by PERKIN ELMER elemental analyzer.
6. IR spectra were recorded in KBr on a SHIMADZU 400-50 infrared spectrophotometer (ν_{max} in cm^1).
7. ^1H NMR spectra were recorded on JEOL AL 300 ^1H NMR spectrophotometer using $CDCl_3$ as solvent and TMS as an internal standard (chemical shift in δppm).
8. All the characteristic data are collectively given in Table 1.

C, H, N, estimation value

The observed values of the carbon, Hydrogen and Nitrogen, in the product, were 68.34%, 5.96%, 11.78% respectively, as compared to the their theoretical values, which are 68.85%, 6.5%, 11.47% respectively, thus, confirming the product.

Results and Discussions

Polarization curves

The polarization data are given in Table 2(a), 2(b) and 2(c). From which polarization curves have been drawn for 4- nitrobenzaldehyde and are given in figs. 3, 4 and 5 for all the electrodes investigated. Comparison of these polarization curves in presence and in the absence of depolarizer shows suitable current density ranges for reduction. The data for potential vs. time curves are also given in Table 2(d) and curves have been drawn in Fig. 6.

Polarization curves for the reduction of 4-nitrobenzaldehyde at copper, zinc and their amalgamated forms (Cu-Hg and Zn-Hg) show that virtually polarization takes place and hence reaction can be expected.

The proper current density ranges to be 0.057 to 0.067, 0.091 to 0.105 and 0.080 to 0.091 Amp. $cm.^{-2}$ for copper, zinc and their amalgamated forms (Cu-Hg and Zn-Hg) respectively . (Table 3).

After conducting experiments at various current densities optimum current densities have been determined for all three electrodes (Cu, Cu-Hg and Zn-Hg) at 0.062, 0.098 and 0.085 Amp. $cm.^{-2}$ respectively. (Table 4).

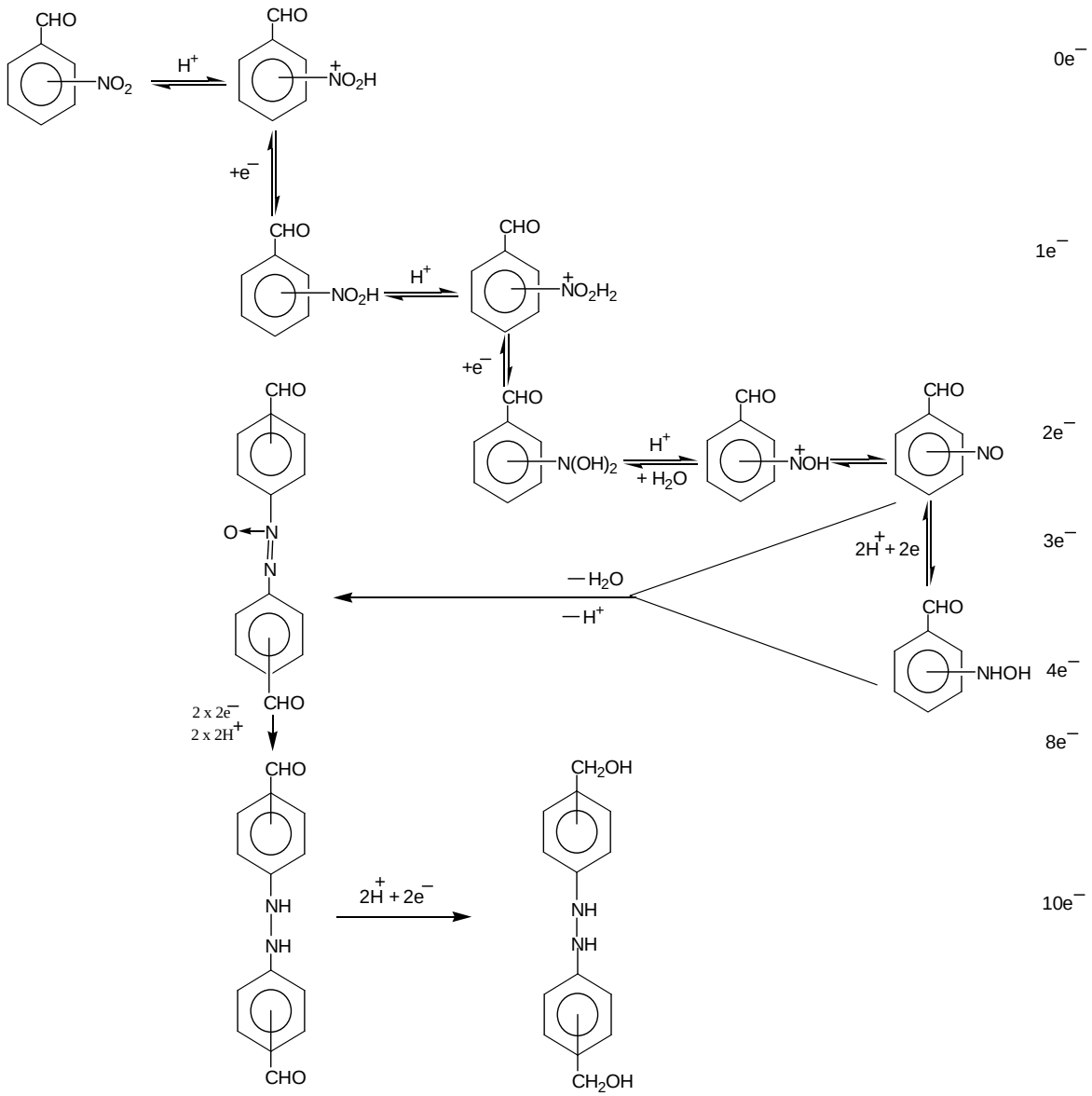
Conclusions

The formation of 4,4'-bis(hydroxymethyl)hydazobenzene by the electroreduction of 4-nitrobenzaldehyde in alkaline medium involves ten electrons process, mechanism is given in scheme 1. This is confirmed by working electrode potential Vs time curves (Fig. 6). It is seen from the curves that the cathodic potential becomes constant after theoretical time during electrolysis approximately required for ten electron process according to Faraday's law.

An electrolytic method for its preparation will be of industrial importance. The literature reviewed so far does not propose any method for the preparation of this compound on any scale. Thus , 4,4'-bis(hydroxymethyl)hydazobenzene can be prepared by the cathodic reduction of 4- nitrobenzaldehyde at any of the electrodes investigated.

Acknowledgement

The author (Sadhana Sharma) is thankful to coordinator of CAS-UGC (Centre of Advanced Studies) for JRF and Prof. and Head, Department of Chemistry, University of Rajasthan, Jaipur for providing laboratory facilities.



4,4'-bis(hydroxymethyl)hydazobenzene

Figure

Table 1: I.R. and N.M.R. data for the product.

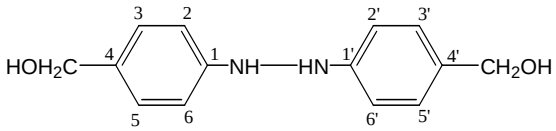
Crystal / Amorphous state of compound	M.P.	IR data ($c.m^{-1}$)	NMR data (δ ppm)	Product compound confirmed
Yellow Needle Shaped Crystal	453K	3330 sharp one spike of –NH 3100 – OH 3030 = CH (Aromatic hydrogen) 1450-1550 (Aromatic system) 830– para substituted aromatic compound peak	δ (7.3) aromatic protons (merged) integrated for 8 protons δ (3.9) Two alcoholic protons δ (7.9) for –NH integrated Two protons δ (3.52) Four protons of benzyl group	 4,4'-bis(hydroxymethyl) hydrazobenzene

Table 2(a): Cathodic Polarization data of 4- nitrobenzaldehyde at copper (Cu) electrode in aqueous 5 %(w/v) sodium hydroxide and methanol solution.

Cathode area = 19.10 cm^2, Temperature = 25±1°C

S. No.	Current (Amp.)	Current Density (c.d., Amp.cm^{-2})	-log c.d.	Potential (- V) vs. SCE			
				Without depolarizer	With depolarizer		
					0.05M	0.10M	0.20M
1	0.10	0.005	2.30	0.573	0.511	0.404	0.374
2	0.20	0.010	2.00	0.573	0.511	0.404	0.374
3	0.30	0.015	1.82	0.573	0.511	0.404	0.374
4	0.40	0.021	1.67	0.573	0.511	0.404	0.374
5	0.50	0.026	1.58	0.573	0.511	0.404	0.374
6	0.60	0.031	1.51	0.610	0.523	0.504	0.374
7	0.70	0.036	1.44	1.498	0.637	0.611	0.550
8	0.80	0.041	1.38	1.585	0.741	0.700	0.696
9	0.90	0.047	1.33	1.649	0.844	0.800	0.810
10	1.00	0.052	1.28	1.720	0.938	0.940	0.928
11	1.10	0.057	1.24	1.739	1.040	1.023	1.080
12	1.20	0.062	1.20	1.776	1.175	10116	1.315
13	1.30	0.067	1.17	1.815	1.380	1.190	1.620
14	1.40	0.073	1.13	-	1.660	1.315	2.070

Table 2(b): Cathodic Polarization data of 4- nitrobenzaldehyde at amalgamated copper (Cu-Hg) electrode in aqueous 5 %(w/v) sodium hydroxide and methanol solution. Cathode area= 14.18cm^2, Temperature = 25±1°C

S. No.	Current (Amp.)	Current Density (c.d., Amp.cm^{-2})	-log c.d.	Potential (- V) vs. SCE			
				Without depolarizer	With depolarizer		
					0.05M	0.10M	0.20M

1	0.10	0.007	2.15	0.609	0.542	0.522	0.500
2	0.20	0.014	1.85	0.609	0.542	0.522	0.500
3	0.30	0.021	1.67	0.609	0.542	0.522	0.500
4	0.40	0.028	1.55	0.609	0.542	0.522	0.500
5	0.50	0.035	1.45	0.609	0.542	0.522	0.500
6	0.60	0.042	1.37	1.215	0.550	0.531	0.500
7	0.70	0.049	1.31	2.191	0.656	0.650	0.550
8	0.80	0.056	1.25	2.223	0.763	0.747	0.630
9	0.90	0.063	1.20	-	0.882	0.830	0.724
10	1.00	0.070	1.15	-	1.007	0.906	0.785
11	1.10	0.077	1.11	-	1.214	0.984	0.850
12	1.20	0.084	1.075	-	1.459	1.085	0.900
13	1.30	0.091	1.040	-	1.736	1.220	0.960
14	1.40	0.098	1.008	-	2.530	1.380	1.100
15	1.50	0.105	0.98	-	-	1.550	1.150

Table 2 (c): Cathodic Polarization data of 4-nitrobenzaldehyde at amalgamated zinc (Zn-Hg) electrode in aqueous 5% (w/v) sodium hydroxide and methanol solution. Cathode area=18.62cm^2, Temperature= 25±1^{o}C

S. No.	Current (Amp.)	Current Density (c.d., Amp.cm^{-2})	-log c.d.	Potential (- V) vs. SCE			
				Without depolarizer	With depolarizer		
					0.05M	0.10M	0.20M
1	0.10	0.005	2.30	1.543	1.406	1.340	0.713
2	0.20	0.011	1.95	1.543	1.406	1.340	0.713
3	0.30	0.016	1.79	1.543	1.406	1.340	0.713

4	0.40	0.021	1.67	1.543	1.406	1.340	0.713
5	0.50	0.026	1.58	1.543	1.406	1.340	0.713
6	0.60	0.032	1.49	1.548	1.406	1.356	0.713
7	0.70	0.037	1.43	2.143	1.420	1.385	0.730
8	0.80	0.042	1.37	2.216	1.456	1.440	0.753
9	0.90	0.048	1.31	2.234	1.513	1.470	0.789
10	1.00	0.053	1.27	2.283	1.573	1.510	0.820
11	1.10	0.058	1.23	2.321	1.656	1.610	0.863
12	1.20	0.064	1.19	2.358	1.779	1.730	0.916
13	1.30	0.069	1.16	2.393	1.920	1.845	0.979
14	1.40	0.075	1.12	2.426	2.220	1.925	1.038
15	1.50	0.080	1.10	2.457	2.325	2.020	1.097
16	1.60	0.085	1.07	2.490	2.434	2.121	1.065

Table 2(d): Polarization data for long term electrolysis (Potential vs. Time) of 4- nitrobenzaldehyde (0.1M) at copper (Cu) amalgamated copper (Cu-Hg) amalgamated zinc (Zn-Hg) electrodes in aqueous 5 %(w/v) sodium hydroxide and methanol solution. Temperature = 25 ± 1°C

S. No.	Time (min.)	Potential (-V) Vs SCE at copper electrode	Potential (-V) Vs SCE at amalgamated copper (Cu-Hg) electrode	Potential (- V) Vs SCE at amalgamated zinc (Zn-Hg) electrode
1	0	1.085	1.340	1.900
2	20	1.145	1.400	1.960
3	40	1.256	1.700	2.250
4	60	1.386	2.156	2.340
5	80	1.595	2.235	2.396
6	100	1.630	2.250	2.405

7	120	1.650	2.270	2.423
8	140	1.650	2.270	2.428
9	160	1.650	2.270	2.428
10	180	1.650	2.270	2.428

Table 3: Effect of current density on yield at different cathodes [Temperature 298 ± 1K, catholyte 100 ml (aqueous 5 %(w/v) sodium hydroxide + methanol solution containing 4-nitrobenzaldehyde (0.1M))]

S.No.	Cathode	Current density (Amp.Cm^{-2})	Yield of the product (%)
1.	Cu	0.057	62
		0.062	70
		0.067	63
2.	Cu-Hg	0.091	71
		0.098	76
		0.105	69
3.	Zn-Hg	0.080	69
		0.085	77
		0.091	72

Table 4: Optimum conditions for electroreduction of 4-nitrobenzaldehyde (medium: aqueous 5% (w/v) sodium hydroxide + methanol solution) with 0.1 M concentration, Temperature 298 ±1

Cathode	Temperature (±1K)	Depolarizer concentration	Current density (Amp.Cm^{-2})	Current efficiency (%)
Cu	298	0.1 M	0.062	88
Cu-Hg	298	0.1 M	0.098	92
Zn-Hg	298	0.1 M	0.085	92

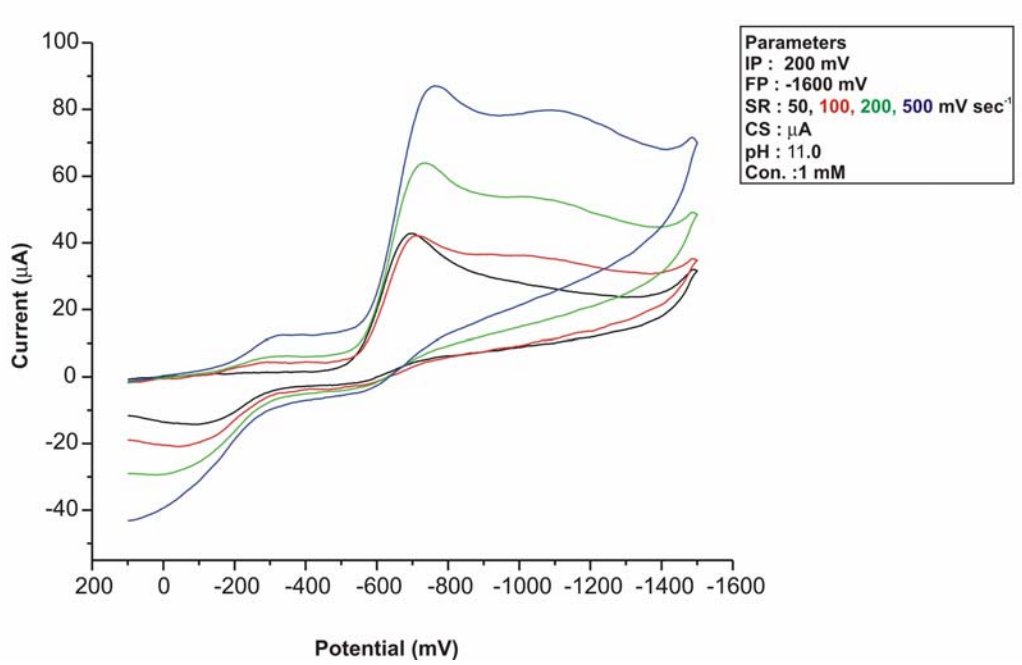


Fig 1: Effect of Scan rate on Cyclic Voltammetric behavior of 4- nitrobenzaldehyde at pH: 11.0

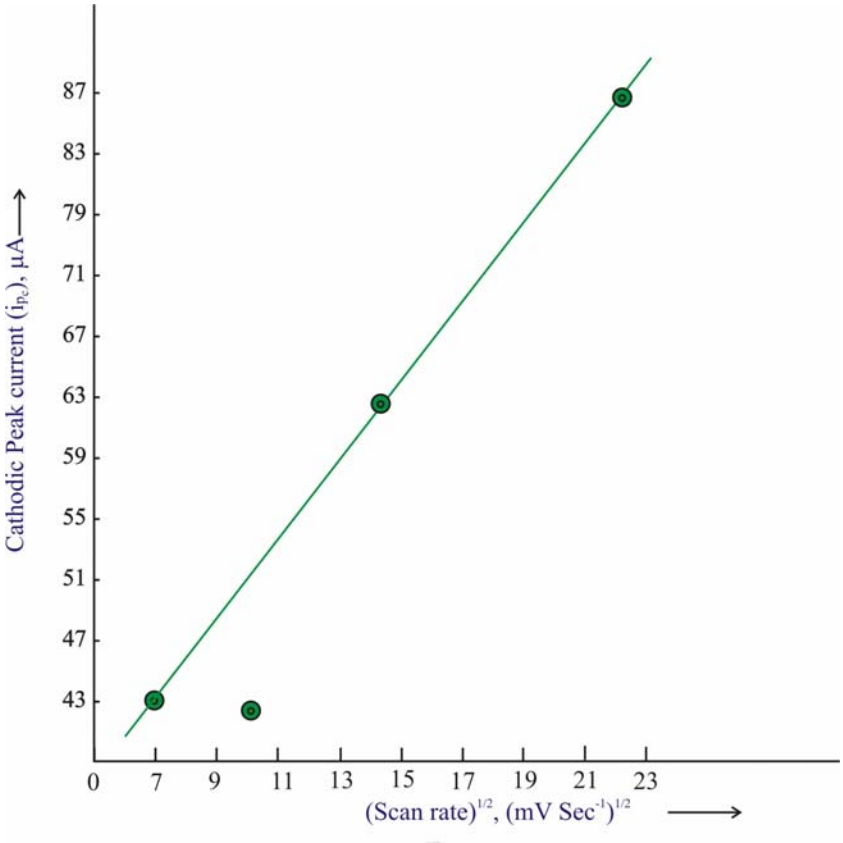


Fig. 2. Plot of I_{pc} versus $\nu^{1/2}$ from votammogram in fig. 1 for 4-nitrobenzaldehyde(1Mm) at pH: 11.0.

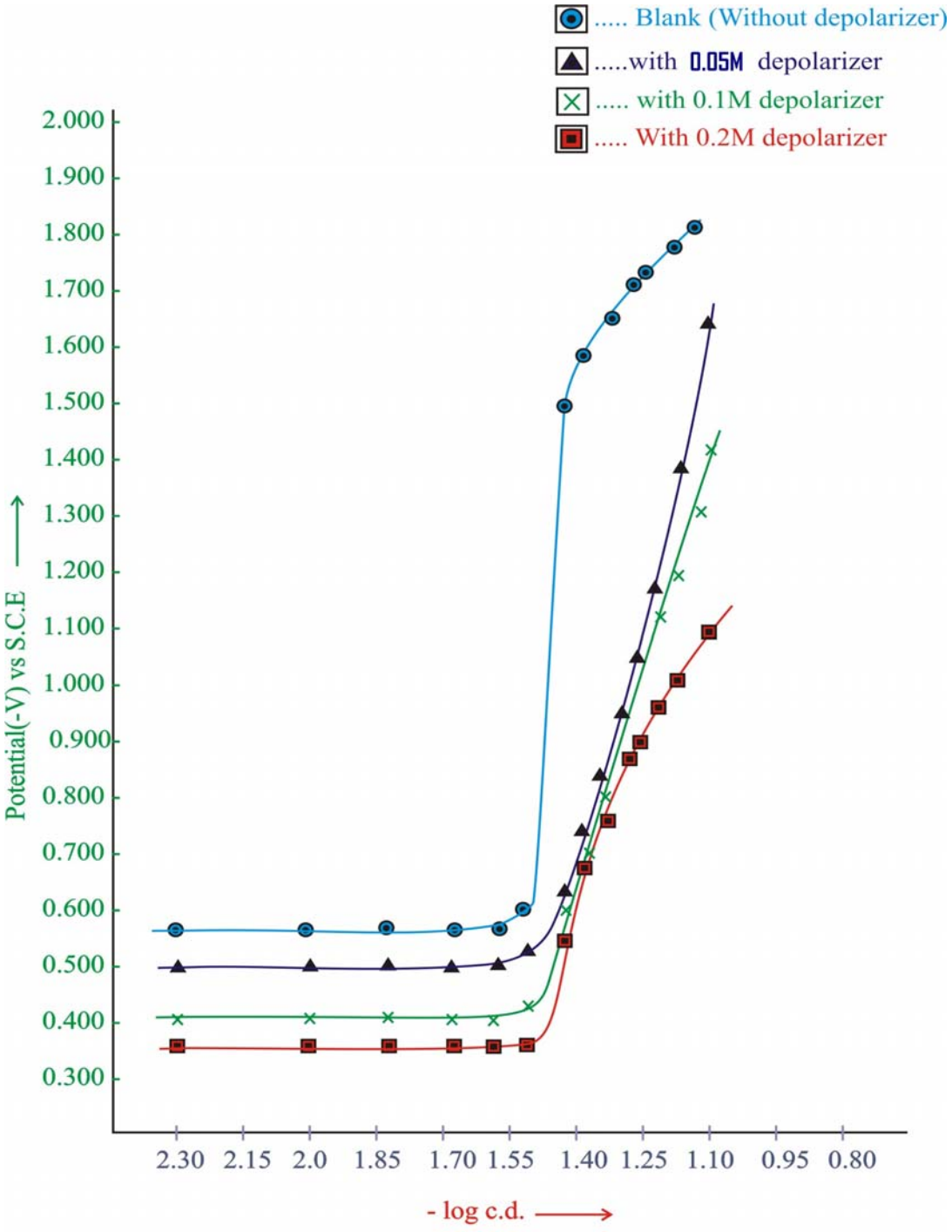


Fig 3: Cathodic Polarization Curves of 4- nitrobenzaldehyde in aqueous 5% (w/v) NaOH + methanol medium, temperature [298+1K] at Copper (Cu) electrode

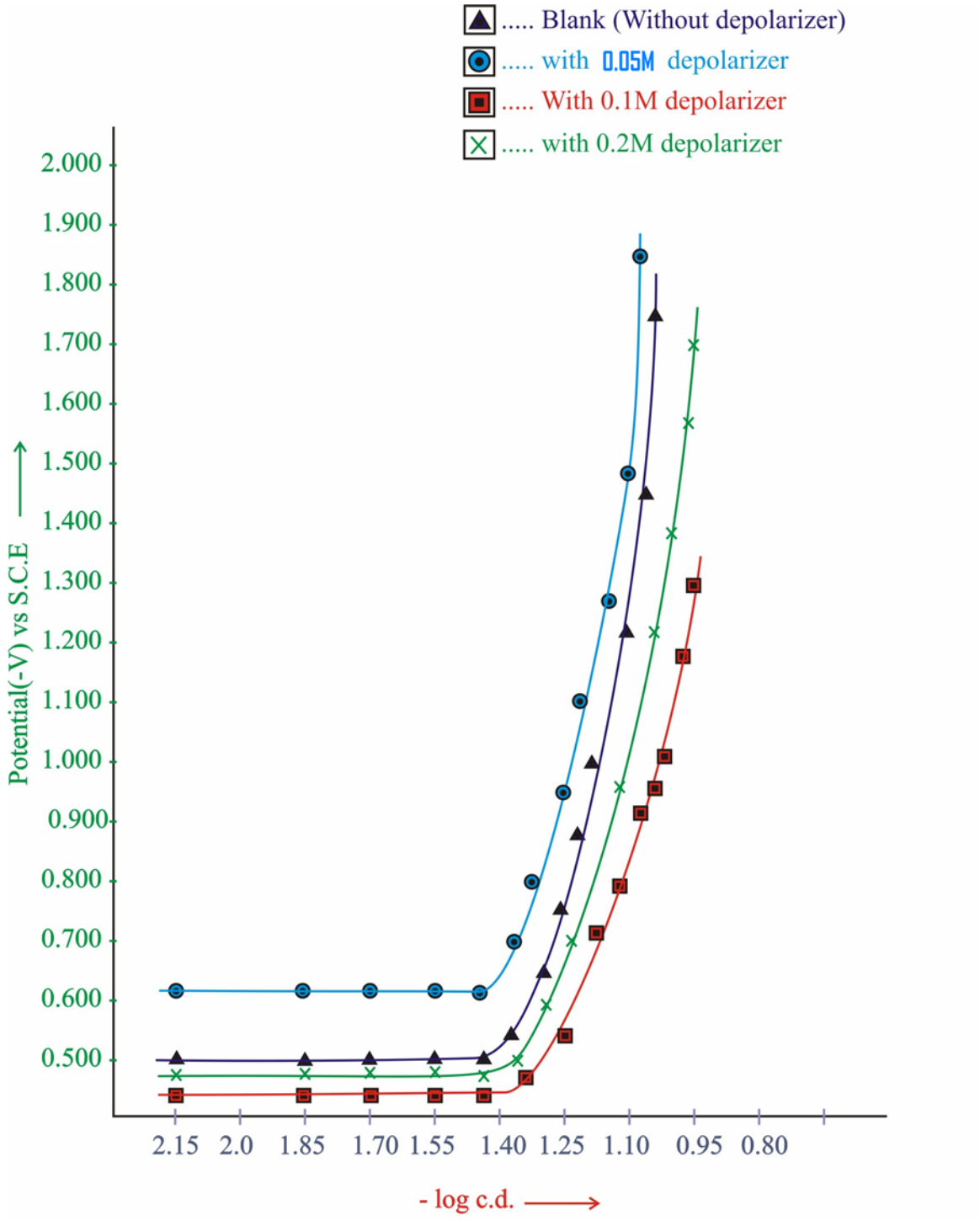


Fig 4: Cathodic Polarization Curves of 4- nitrobenzaldehyde in aqueous 5% (w/v) NaOH + methanol medium, temperature [298+1K] at amalgamated Copper (Cu-Hg) electrode

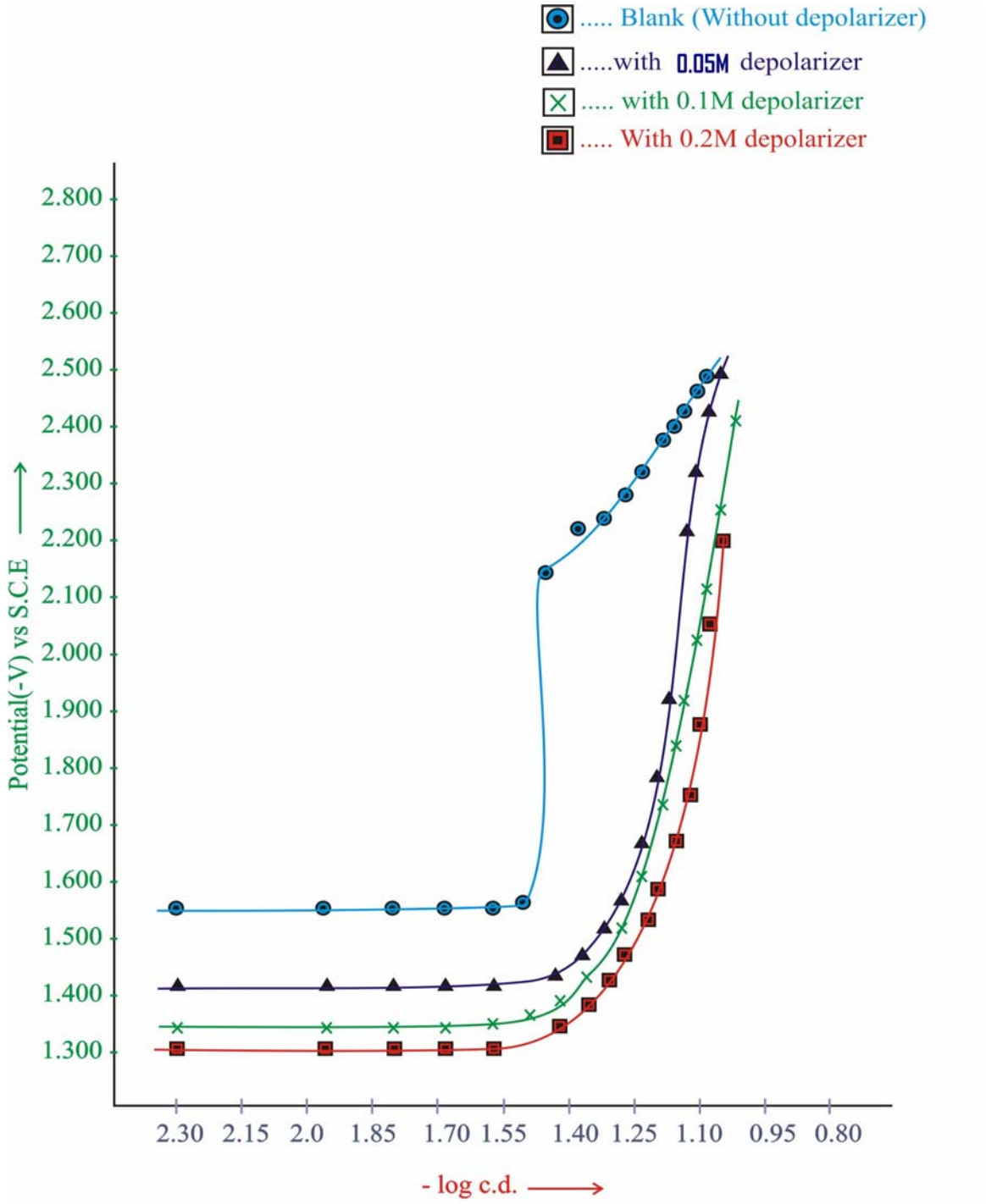


Fig 5: Cathodic Polarization Curves of 4- nitrobenzaldehyde in aqueous 5% (w/v) NaOH + methanol medium, temperature [298+1K] at amalgamated Zinc (Zn-Hg) electrode

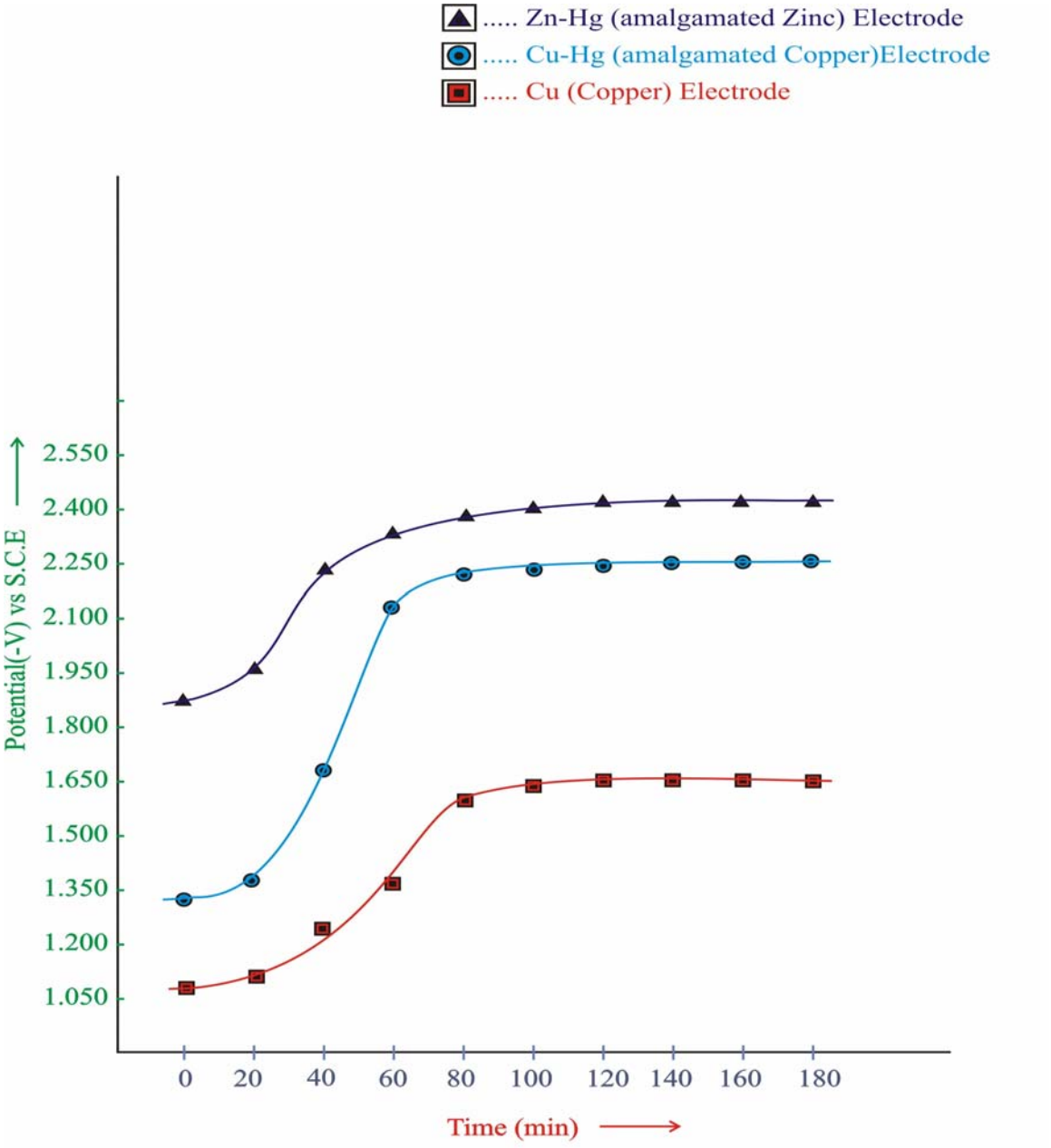


Fig 6: Time vs Potential Curves of 4- nitrobenzaldehyde (0.1M) in aqueous 5% (w/v)NaOH + methanol medium, temperature [298+1K]

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Single Node Callus Culture: Improvement for Micropropagation of *Solanum tuberosum (cv. Kufri Himalini)*

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Abstract:

In the present study MS media supplemented with different growth regulators such as BAP and Kn was used for callus induction and shoot regeneration. Shoots regenerated from callus were shifted to MS media supplemented with different combinations of NAA and Kinetin for shoot elongation and root formation. Percentage response to callus induction was best (60%) on medium with the combination of 0.6 mg/l BAP + 0.6 mg/l Kn. Shoot height (6.4 ± 0.6), node number (5.0 ± 0.7), root length (8.2 ± 0.5) and rooting percentage (80%), was reported highest on medium with 0.1 mg/l NAA + 0.01 mg/l Kn. After rooting on shoots the plantlets were shifted to sterile soil field pots for acclimatization. . The plantlets were survived well as about 70-80%. [Nature and Science. 2009;7(3):99-103]. (ISSN: 1545-0740).

Key words: *Kufri himalini*, growth regulators, callus induction, shoot regeneration, rooting and acclimatization

Introduction:

As a crop of high biological value for its protein and a substantial amount of vitamins, minerals and trace elements, it is undoubtedly a very important crop in many countries (Gebre and Sathyanarayana 2001). Potato is a semi-perishable crop susceptible to many diseases and insect pests. Production of quality planting material is essential not only for improving domestic potato productivity but also to ensure minimum commercial quality. Shortage of good quality seed has been recognized as the single most important factor limiting potato productivity in the developing countries. The availability of tissue culture technology for rapid multiplication of disease-free planting material has facilitated potato seed production to a great extent (Dodds 1988). The recent advancement in tissue culture and the flexibility of organ development in potato allows alternative methods of propagation through *in vitro* techniques.

Much work has been carried out on callus induction and growth in potatoes. This has resulted in a range of protocols and procedures being established by researchers since tissue culture gained an importance in plant propagation, conservation and breeding (Ahloowalia 1982; Wareh et al., 1989). Callus is used for most of these transformation methods such as particle gun (McCabe et al., 1998) and *Agrobacterium tumefaciens*-mediated transformation (Stiekema et al., 1988) as well as initiation of cell culture. A callus from an explant tissue occurs as a result of dramatic changes in the appearance and metabolism of the cells (Aitchison et al., 1978). Induction of callus, physical disorganization of cultured cells, is thought as result of the breakdown of intercellular physical and chemical communication (Lindsey and Jones, 1992). It has been already an established fact from the earlier findings in which the callus culture showed higher multiplication rate in comparison to other methods of *in vitro* culture, as in nodal culture the major factors limiting the rates of multiplication, short height of the plantlets and the low number of nodes on the plantlets (Gebre and Sathyanarayana 2001). Improvements have been made possible by callus culture and addition of growth regulators to the medium. Keep these points on view the present study was done using nodal explants for callus culture to reduce the losses due to conventional propagation methods of potato.

Material and Methods:

The nodal segments as explants were taken from the plants of *Kufri Himalini* and washed thoroughly in running tap water and surface sterilized with Tween-20 for 10 minutes. Sterilized explants were rinsed with sterile double distilled water for 3-4 times. These explants were treated with 0.5% (4%

concentrated sodium hypochloride, qualigence) for 5 minutes and finally rinsed with sterilized double distilled water for 3-4 times to remove the traces of sterilants.

For callus induction, surface sterilized nodal segments were transferred to full strength MS media (Murashige and Skoog, 1962) supplemented with different growth regulators such as BAP and Kn and incubated at 25 ^{0}C up to 16 hr photoperiod. These hormones were used separately and with combinations of each other at different concentrations (0.2, 0.4, 0.6, and 0.8 mg/l). After 35 days of culturing, calli induced were analyzed and scored depending on growth, texture and colour.

Shoots regenerated from callus were shifted to MS media supplemented with different combinations of NAA (0.1 mg/l) and Kinetin (0.01 mg/l, 0.001 mg/l, 0.1 mg/l) for shoot elongation and root formation. After rooting the complete plantlets were transferred to sterilized soil field pots for acclimatization. The mixture of soil, sand and vermi compost was used for hardening in the ratio of 2:1:1.

Results:

Callus induction: All the concentrations of growth regulators (BAP and Kn) induced callus on nodal explants when cultured on MS media with varying degrees of success (Table-1). Direct regeneration of shoots from callus (Plate.1-a and b) was observed with 60% response on medium containing the combination of BAP and Kn (0.6 + 0.6 mg/l). Concentrations of 0.6 mg/l BAP induced good amount of compact, light green callus with 40% shoot regeneration. Kn with 0.4 mg/l concentration was also sowed very good compact callus but poor shoot regeneration was occurred in this concentration.

Shoot elongation, Rooting of shoots and Hardening: The results for shoot elongation and rooting indicate (Table-2 and 3; Plate1-c and d) that in MS media with 0.1 mg/l NAA + 0.01 mg/l Kn showed higher growth of shoots and rooting percentage. Shoot height reached 6.4 cm. (±0.6) with 5.0 (±0.7) nods in MS+0.1 mg/l NAA + 0.01 mg/l Kinetin media and 5.3 cm. (±1.2) with 4.2 (±0.8) nods and 4.0 cm. (±0.6) with 2.7 (±0.7) nods in other media. The MSKN2 (0.1 mg/l NAA + 0.001 mg/l Kn) having low concentration of Kinetin and NAA and MSKN3 (0.1 mg/l NAA + 0.1 mg/l Kn) combinations having higher concentration of Kinetin and low concentration of NAA, responded the least mean Shoot height and number of nodes. Low concentration of Auxin (0.1 mg/l NAA) plus moderate concentration of Cytokinine (0.01 mg/l Kinetin) showed good development of complete plantlets from nodal segments. Hardening of the well rooted plantlets was done in the potting mixtures of soil, sand and vermi compost (2:1:1) and kept under poly house condition for survival and growth. The plantlets were survived well as about 70-80%.

Table-1 Effect of BAP and Kn on callus induction and shoot regeneration using nodal explants

Hormones concentrations with MS media (mg/l)		Callus formation	Kind of callus	Callus color	Shoot regeneration percentage
BAP	Kn				
Control		-	-	-	0
0.2	-	+	Friable	White	0
0.4	-	+	Friable	White	0
0.6	-	+++	Compact	Light Green	40
0.8	-	++	Compact	Light Green	20
-	0.2	-	-	-	0
-	0.4	+++	Compact	Greenish White	20
-	0.6	++	Compact	Light Green	10
-	0.8	+	Friable	White	0
0.2	0.2	+	Friable	White	0
0.4	0.4	+	Friable	White	0
0.6	0.6	+++	Compact	Greenish White	60
0.8	0.8	++	Compact	Greenish Brown	20

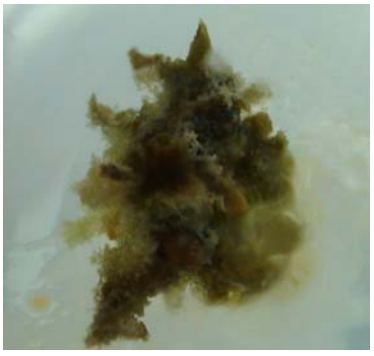
Observations were recorded after 35 days of culture: (-, No callus production; +, poor callus; ++ good callus; +++ very good callus)

Table-2: Effect of different hormonal combinations with MS media on shoot height, node number, and root length after 35-40 days of culture:

Growth regulators (mg/l)			Shoot height (cm)	Node number	Rooting Percentage	Root length (cm)
NAA	Kn	Symbol used				
0.1	0.01	MSKN 1	6.4 ± 0.6	5.0 ± 0.7	80	8.2 ± 0.5
0.1	0.001	MSKN 2	5.3 ± 1.2	4.2 ± 0.8	60	6.8 ± 0.8
0.1	0.1	MSKN 3	4.0 ± 0.6	2.7 ± 0.7	50	5.3 ± 0.9

Table-3: Effect of different hormonal combinations with MS media on shoot and root fresh weight and root: shoot ratio after 35-40 days of culture:

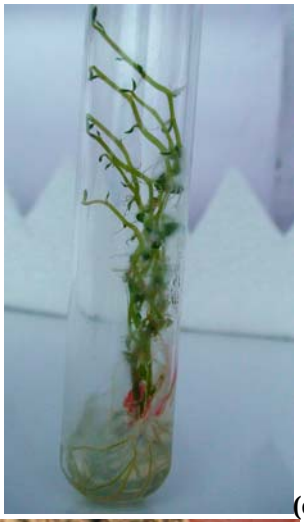
Hormonal Combination	Shoot fresh weight	Root fresh weight	Root: Shoot ratio
MSKN 1	0.226 ± 0.01	0.144 ± 0.01	1.57 ± 0.1
MSKN 2	0.171 ± 0.03	0.120 ± 0.07	1.42 ± 0.2
MSKN 3	0.141 ± 0.05	0.112 ± 0.08	1.27 ± 0.5



(a)

(b)


(c)


(d)


(e)

Plate 1 (a-e): Callus induction, shoot regeneration and rooting in potato cv. *Kufri Himalini*
(a) Callus formation on 0.6 mg/l BAP+0.6 mg/l Kn MS media **(b)** Shoot regeneration **(c-d)** Shoot Multiplication, elongation and rooting on 0.1 mg/l NAA + 0.01 mg/l Kinetin MS media **(e)** Hardening of plantlet to the mixture of sterile soil, sand and vermi compost

Discussion:

The present study was carried out with an aim to *in vitro* culture of potato cv. *Kufri Himalini* through callus culture to get disease free, uniform plantlets and for mass multiplication of cultures. This was undertaken to standardize the micropropagation technique of potato cv. *Kufri Himalini* using callus culture. *Kufri Himalini* is a medium maturing late blight resistance variety released for hills by ICAR (Anonymous, 2005). Potato, like other Solanaceous crops, shows considerable regenerative ability in culture, producing adventitious shoots both directly from organ tissue and from callus under appropriate conditions (Wang and Huang, 1975; Skirvin *et al.* 1975; Roest and Bokelmann, 1976). Through *in vitro* propagation of potato by serial culture of axillaries shoots has been reported by a number of workers and has become established as an effective means of rapidly multiplying new or existing cultivars in diseases-free conditions (Nozeran *et al.*, 1977; Goodwin *et al.*, 1980; Hussey and Stacey, 1981).

The explants selection was the most critical feature in callus induction. The texture and type of callus depend on the concentrations of growth regulators. The callus formed in media containing 0.2 and 0.4 mg/l BAP and 0.8 mg/l Kn showed friable and white callus while the callus produce in media containing 0.6 mg/l BAP and the combination of BAP (0.6 mg/l) and KN (0.6 mg/l) were compact and greenish white in colour. Higher shoot regeneration was observed in the media containing 0.6 mg/l BAP + 0.6 mg/l Kn. For shoot regeneration several workers have used various hormonal combinations of different

hormones with MS salts. Similarly as the observations of the present study Morozova *et al.* (1977) obtained largest number of regenerated plants from the early variety Izobilie using low concentration of kinetin (0.001 mg/l) while midlate variety Istrinskii gave best results under higher concentration (0.01 mg/l). This could attribute the fact that the regeneration of shoots is not totally dependent on hormonal combinations but the variety is also responsible for *in vitro* shoot proliferation. The combined effect of NAA and kinetin was reported to be better in cvs. Bintje, Desiree, Gracia and Ostara that developed the largest number of plantlets on a medium containing 0.5 mg/l NAA, 0.4 mg/l kinetinand 0.7 mg/l thiamine (Maroti *et al.*, 1982). The result is also supported by the findings of Ruzic *et al.*, (1997), in which they reported 0.01 mg/l NAA, 0.1 mg/l GA and 1 mg/l kinetin proved in production of prolific and healthy shoots and good root development.

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Chromosomal Abnormalities Arising Under The Action Of Antibiotics In *Pisum Sativum*

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Abstract

Studies on genetic as well as cytological effects of antibiotics in higher plants are scanty at present. Therefore, in this study we tried to investigate chromosomal and gene mutations arising under the action of antibiotics. *Pisum sativum* (2n=14) which belongs to sub family *papilionaceae* of family *leguminosae* and has comparatively small number of good size chromosome and is a very important legume crop of India, has been chosen as test system for evaluating mutagenic potency of some of the antibiotics. The effect of antibiotics (viz., amoxicillin, streptomycin and tetracycline) treatment on biological damage and genetic changes in M1 and subsequent generation (M2) of its progeny were evaluated in relation to the following parameters; germination, seedling injury, cytological observations, pollen sterility. [Nature and Science. 2009;7(3):104-112]. (ISSN: 1545-0740).

Introduction

Antibiotics are the substances which are produced by microorganism and act against microorganism (e.g., *Pencillium notatum*). Most antibiotics known till date are products of actinomycetes and some are from fungi and bacteria (e.g., *Streptomyces* spp.). Most antibiotics have been tried for plant disease control. The commonly used antibiotics are streptomycin, tetracycline, griseofulvin, cycloheximide and aurofungin. Although antibiotics are used for disease control, it has many side effects, one of them being gene mutations. These antibiotics enter in the tissues/organs as chemical compounds and penetrate membrane system. Chemical reaction may occur before a pharmacodynamic action and metabolites formed may cause unwanted toxic effects before being excreted. If these chemical compounds react with genetic material (DNA) heritable changes (beneficial/harmful) may be induced. EMS (Svetleva *et. al*., 2005), DES and some antibiotics are being used extensively for inducing mutation in the microbes, lower plants (Takano *et. al*., 2003) and higher plants. Most of the antibiotics affect DNA, RNA and protein synthesis. Antibiotics induce chromosomal abnormalities such as erosions, diplo chromatids, pycnosis, micronuclei, bridges with or without fragments etc. Induction of genetic damage by mitomycin-c has also been reported (Vig, 1997). Mutagenic potency of antibiotic has been demonstrated in *Phaseolus vulgaris* L (Prasad *et. al*., 1981). All these observations necessitate the screening of antibiotics for the harmful short term and overall insidious long term effect on human health and of noxious effects on plants, bacteria and animals. The mutagenic action of antibiotics as well as chemicals has been extensively evaluated with various tests on plants like wheat, barley (Ehrenberg, 1971), maize (Nilan *et. al*., 1976), tradescentia (Taro, 1982), soybean (Carroll *et. al*., 1985), but *Pisum sativum* (Duc, 1989) being one of the pillars of the classical genetics has not been commonly used in mutagenic studies. *Pisum sativum* L (2n=14) has 7 pairs of recognizable chromosomes (3 large, 3 medium and 1 small chromosome) out of which 3 pairs are metacentric, 4 are submetacentric or subterminal. The average size of the chromosomes falls within the range of 4.29µ to 7.12µ.

Materials and Methods

Seeds (variety ps-49) were obtained from seed production center of G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand).

Seed treatment:

Dry and dormant seeds of *P. sativum* were treated separately with 0.1, 0.2, 0.3, 0.4 and 0.5% freshly prepared solutions of amoxicillin, streptomycin and tetracycline for 4 hrs with constant shaking under laboratory conditions (24±2°c). Control seeds were also soaked in distilled water for the same period for which treatment was given. All treated seeds were thoroughly washed to remove the trace of

chemicals. There after 50% of seeds in single layer were allowed to germinate on moist filter paper and 50% were directly sown in the field.

Field preparation:

Land was prepared by using well decayed cow dung manure. Total 100 seeds for each dose were sown in 4 different plots of size 2.25 m^2 each with row to row distance of 30 cm, plant to plant distance 30 cm and 25 seeds were sown per plot. Control seeds were sown adjacent to the treated seeds in the same manner as that of treated seeds. M_1 seeds were harvested plant wise and were sown directly in the next season to raise M_2 progenies. M2 seeds were also collected plant wise. Different observations were made for both M1 and M2 generations. Data collected for different characters in M1 and M2 generations were statistically analyzed.

Results

Germination:

Germinated seeds of both control as well as treated were counted every 24 hrs from the time of treatment till 10^{th} day. Total number of seeds germinated till 10^{th} day after treatment were used to study the effects of antibiotics on germination, both in M1 and M2 generations.

It was observed that tetracycline is more toxic than amoxicillin and streptomycin with regards to effect on percent germination. The reduction in germination frequency at its lowest dose 0.1% (53.3% that of control) is much lower than the percent germination seen at the highest concentration that is 0.5% of either amoxicillin (81.35% that of control) or streptomycin (83% that of control) (Table 1).

Seedling injury:

Ten germinated treated and untreated seeds were randomly selected for measurement of root and shoot length. The root and shoot length were measured every 24 hrs after drawing their outline on centimeter graph paper. The lengths of root and shoot were read from the graph paper every day up to 10^{th} day, from the day of emergence of root and shoot. Data on mean root and shoot length on 10^{th} day after their emergence, at M1 and M2 are summarized in Table 2 and 3, respectively.

A dose dependent decrease in both root and shoot length in M1 and M2 was noticed, although both root and shoot length at M2 were found to be longer than M1 at all concentrations of antibiotics. Tetracycline was found to be most effective, exhibiting 53% and 78% reduction in root and shoot length, respectively, at 5% concentration, where as, the corresponding reductions were 34% and 39% in streptomycin treatment and 28% and 31%, respectively, in case of amoxicillin treatment.

Correlation coefficient between root and shoot length at M1 generation for amoxicillin, streptomycin and tetracycline and at M2 for amoxicillin and streptomycin indicates highly significant ($p>0.001$) positive co-relation between root and shoot length for both amoxicillin and streptomycin, and significant negative co-relation for shoot length in case of tetracycline treatment in M1 generation. However, positive correlation at lower doses, 0.1-0.2% of amoxicillin and 0.1-0.3% and 0.4-0.5% streptomycin was observed in M2 generation. The results further indicate that shoot is more sensitive to antibiotics treatment than root (Table 4).

Cytological observations:

Root tip mitosis and PMCs were studied at M1 and M2 generations. Chromosomal abnormalities were scored at different phases of both mitosis and meiosis and chromosomal clumping was the most common effect observed at all concentration of all antibiotics. Maximum number of such type of chromosomes (28.35%) was recorded in tetracycline treatment at M1 generation (Table 7). Occurrence of micronuclei was observed only at higher concentrations of both amoxicillin and streptomycin (Table 5, 6). It was evident from the data that the frequency of a particular type of dose and the nature of chromosomal aberration is both under the influence of dose and chemical composition of antibiotic.

Similarly, in case of meiosis, chromosomal abnormalities were observed at different stages of meiosis until the formation of pollen grain. All the treated chromosomes showed bridges, fragments, laggards and bridges with or without fragments. The frequency of occurrence of such abnormalities increased with the increasing concentration of the antibiotic.

Pollen sterility:

One of the commonest features of mutagenic treatment is pollen sterility which is associated with the induction of chromosomal changes involving translocation, inversion and deletion. Higher numbers of sterile pollens were observed after amoxicillin treatment as compared to streptomycin

treatment in both the generations. Comparing the mean and variance in M1 and M2 generations, four different relationship have been discerned and are listed below (a) increased mean and decreased variance (0.5% amoxicillin at M1, 0.1% at M2, 0.5% streptomycin at M1 and 0.1% streptomycin at M2) (b) decreased mean and decreased variance (0.1% amoxicillin and 0.1% streptomycin at M2) (c) decreased mean with increased variance (0.5% amoxicillin at M1 and 0.1% amoxicillin at M2, 0.1% and 0.4% streptomycin at M2) (d) increased mean and equal variance (0.5% amoxicillin and streptomycin at M1 and 0.4% streptomycin at M1 and M2). Increased CV (%) then control except 0.1% amoxicillin at M2 was noticed at almost all the concentration of amoxicillin and streptomycin.

Table 1: Effect of antibiotics on the 10th day of germination following (% that of control) treatment

Treatment (Percent)	Generation	Amoxicillin	Streptomycin	Tetracycline
0	M1	100.00	100.00	100.00
	M2	100.00	100.00	100.00
0.1	M1	94.00	96.00	53.30
	M2	89.33	94.66	0.00
0.2	M1	90.66	93.33	50.60
	M2	82.66	88.00	0.00
0.3	M1	88.00	90.00	46.60
	M2	74.66	83.00	0.00
0.4	M1	86.60	85.00	45.30
	M2	70.66	80.00	0.00
0.5	M1	81.30	83.00	40.00
	M2	70.00	78.00	0.00

Table 2: Effect of antibiotics on mean length (cm) of roots and shoots on 10th day of emergence on M_1 generations

Treatment (Percent)	Root/Shoot	Amoxicillin	Streptomycin	Tetracycline
		Mean±S.E.	Mean±S.E.	Mean±S.E.
Control	R	6.42±0.168	6.42±0.168	6.42±0.168
	S	10.4±0.724	10.4±0.724	10.4±0.724
0.1	R	5.86±0.437	5.0±0.160	3.06±0.248
	S	9.10±0.367	9..1±0.303	2.12±6.231
0.2	R	5.54±0.36	5.18±0.147	2.72±0.258
	S	9.0±0.416	8.3±0.520	1.34±0.180
0.3	R	5.50±0.063	4.92±0.120	2.64±0.309
	S	8.74±0.223	7.66±0.460	1.24±0.121
0.4	R	4.16±0.153	4.88±0.245	2.60±0.459
	S	7.72±0.708	6.98±0.75	1.28±0.263
0.5	R	4.28±0.306	4.56±0.30	2.52±0.314
	S	7.2±0.599	6.4±0.92	1.18±0.174

Table 3: Effect of antibiotics on mean length (cm) of roots and shoots on 10th day of emergence on M2 generations

Treatment (Percent)	Root/Shoot	Amoxicillin	Streptomycin
		Mean±S.E.	Mean±S.E.
Control	R	10.74±0.480	10.74±0.480
	S	14.26±0.180	14.26±0.180
0.1	R	9.1±0.303	8.42±0.260
	S	10.12±0.591	13.14±0.291
0.2	R	7.58±0.222	8.20±0.268
	S	9.30±0.258	11.64±0.098
0.3	R	7.12±0.290	7.46±0.447
	S	8.94±0.401	10.8±0.243
0.4	R	7.16±0.476	7.18±0.136
	S	8.2±0.260	10.3±0.243
0.5	R	6.98±0.359	7.04±0.093
	S	7.72±0.708	9.0±0.310

Table 4: Correlation coefficient between root and shoot following treatment with antibiotics at M1 and M2 generation

Treatment (Percent)	Generation	Amoxicillin	Streptomycin	Tetracycline
Control	M1	0.360***	0.360***	0.360***
	M2	0.291**	0.291**	0.291**
0.1	M1	0.382***	0.714***	–0.0500***
	M2	0.412***	0.006	0.00
0.2	M1	0.188	0.332***	–0.0551
	M2	0.686***	0.780***	0.00
0.3	M1	0.825***	0.458***	0.285**
	M2	–1.00	0.086	0.00
0.4	M1	0.583***	0.451***	–0.246*
	M2	0.165	0.507***	0.00

0.5	M1	0.746***	0.447***	–0.263**
	M2	0.398***	–0.036	0.000

***Significant at 0.1% level
**Significant at 1% level
*Significant at 5% level

Table 5: Chromosomal abnormalities induced by different doses of amoxicillin in *Pisum sativum* L.

Treatment (Percent)	Generation	Total number of cells scored	Total number of dividing cells	Metaphase		Anaphase			Telophase	
				Fragment (%)	Clumped cells (%)	Bridge (%)	Bridge with F (%)	Erosion (%)	Bridge (%)	Micro nuclei (%)
0.1	M1	2576	193	–	6.40	4.48	–	–	–	–
	M2	2511	254	–	4.00	–	–	–	–	–
0.2	M1	2264	134	–	8.17	5.61	3.00	–	–	2.45
	M2	2060	190	–	7.52	1.05	2.00	–	–	1.29
0.3	M1	2564	145	5.44	8.98	5.75	2.34	2.93	–	3.52
	M2	2096	179	–	6.58	2.15	2.00	–	–	2.00
0.4	M1	2694	139	7.61	10.51	6.07	3.28	2.18	1.0	4.78
	M2	2664	165	1.26	3.24	3.29	1.77	–	–	2.56
0.5	M1	2500	124	9.76	11.01	10.41	4.18	1.5	1.5	6.56
	M2	2550	146	2.74	4.29	2.70	2.45	–	–	3.98

Table 6: Chromosomal abnormalities induced by different doses of streptomycin at M_1 and their progenies M_2

Treatment (Percent)	Generation	Total number of cells scored	Total number of dividing cells	Metaphase		Anaphase			Telophase	
				Fragment (%)	Clumped cells (%)	Bridge (%)	Bridge with F (%)	Erosion (%)	Bridge (%)	Micro nuclei (%)
0.1	M_1	2615	216	–	7.42	5.99	2.91	–	–	–
	M_2	2264	232	–	3.52	–	–	–	–	–
0.2	M_1	2540	194	5.3	8.56	7.03	4.96	1.0	–	3.0
	M_2	2079	164	2.3	3.96	2.2	–	–	–	2.0
0.3	M_1	2028	140	6.46	8.95	7.33	4.99	2.0	2.4	5.95
	M_2	2138	185	3.96	5.30	3.0	1.0	–	–	2.0
0.4	M_1	2928	169	8.98	11.56	7.44	5.0	2.76	2.0	6.29
	M_2	2305	173	4.20	6.50	3.56	2.5	2.4	–	3.28
0.5	M_1	2324	122	9.0	13.00	8.62	5.5	1.92	3.4	6.56
	M_2	2756	175	5.2	6.95	4.0	3.8	1.0	–	3.98

Table 7: Chromosomal abnormalities induced by different doses of tetracycline at M1 generation

Treatment (Percent)	Total number of cells scored	Total number of dividing cells	Metaphase % clumped cells
0.1	2984	165	11.2
0.2	2806	139	18.15
0.3	2629	112	20.85
0.4	2820	135	25.76
0.5	2694	112	28.35

Table 8: Effect of Amoxicillin on pollen sterility in *Pisum sativum* at M1 and their progeny M2

Treatment (Percent)	Generation	Number of pollen grains scored	Sterility (%) ± S.E.*	Variance	C.V. (%)
Control	M1	722	8.03±1.20	7.29	23.27
	M2	692	7.28±1.09	5.99	18.84
0.1	M1	898	13.47±3.0	45.19	27.76
	M2	596	8.53±0.368	0.676	8.48
0.2	M1	762	16.92±4.66	108.69	40.38
	M2	659	9.76±2.19	24.05	38.07
0.3	M1	694	19.74±4.13	85.29	33.68
	M2	589	10.37±2.36	27.87	43.12
0.4	M1	629	23.52±4.70	110.79	35.54
	M2	856	12.61±3.07	47.29	31.80
0.5	M1	598	26.42±5.81	169.29	41.17
	M2	739	15.96±3.38	57.29	32.0

(* S.E. of mean)

Table 9: Effect of streptomycin on pollen sterility in *Pisum sativum* at M1 and their progeny M2

Treatment (Percent)	Generation	Number of Pollen grains scored	Sterility (%) ± S.E.*	Variance	C.V. (%)
0.1	M1	728	15.65±2.72	37.19	26.71
	M2	538	11.15±1.84	16.99	34.34
0.2	M1	694	18.87±4.75	113.19	40.57
	M2	738	13.77±4.40	96.79	47.75
0.3	M1	605	25.28±4.76	113.29	34.77
	M2	698	17.19±3.98	79.49	37.12
0.4	M1	525	28.0±5.42	147.29	41.25
	M2	629	23.05±5.44	148.49	42.0
0.5	M1	495	31.51±4.50	101.69	32.30
	M2	548	25.36±3.96	78.69	31.90

(* S.E. of mean)

Discussion

The reduction in germination percentage observed in the present case may be due to any one of the following effects of antibiotic treatment (a) disruption of mechanism of gene action controlling protein synthesis (b) inhibition of several enzyme systems responsible for germination (c) chromosomal damages (Sinha *et. al*., 1972) (e) dissimilar mode of its action. Growth depression had been also affected in almost equal proportion in amoxicillin and streptomycin treatment. However, the shoot depression was relatively very much pronounced at tetracycline treatment. Here, in this case decrease in seedling height may be ascribed to injury caused at cellular level either to gene controlled biochemical or physiological process or acute chromosomal aberration or both, whereas inhibitory effect at lower doses may be either due to mitotic arrest or mitotic delay resulting in growth retardation.

The injury of seedling height is one of the commonest parameter used in evaluating the effectiveness, efficiency and specificity of mutagens in the study of mutagenesis. Studies already made and reported in relation to differential and combined treatment of various groups of mutagens (a) dose dependent reduction (Shaikh *et. al*., 1983) and (b) stimulation in seedling growth at lower doses. Reduction in the seedling height may be due to inhibition of protein synthesis or disruption of mechanisms of gene action controlling its synthesis in the embryonic cell and preventing the entry of cells at G1 to further phases of cell cycles. This may result into either inhibition of emergence of root or shoot or growth retardation.

Chromosomal damage is also supposed to be one of the most common factors for retardation in growth. Root and shoot showed different degree of sensitivity may be due to the facts that the growth of root depends on the cell division whereas that of shoot on cell elongation. More growth retardation in

root and shoot at M1 generation and less effect at M2 is due to the effect of antibiotics which damage more in M1 but persists up to M2 in very dilute states.

The effect of physical and chemical mutagens on plant height has extensively been studied (Shaikh *et. al*., 1983) in different plants and in all the cases on adverse effect of these has been reported. In garlic (Choudhary *et. al*., 1980), in Anethum (Deshmukh, 1981)**,** in *Plantago ovate* (Dube *et. al*., 1981). It has been reported that the magnitude in reduction of height is a function of disc i.e. higher the dose greater is the reduction. Reduction in the plant height with the use of different physical and chemical mutagens is supposed to be the consequence of a number of factors i.e. induced destruction of plant, production of diffusible growth reducing substances, changes in specific activity of enzymes, inhibition of DNA synthesis, chromosomal aberration and reduction in the number of internodes which either may be due to the reduction in cell length without any alteration in cell breadth or reduction in cell number (Gottschalk *et. al*., 1983).

Yield is one of the most common factors for the selection of desired genotypes towards positive and negative direction. Increased, decreased and unchanged variance with mean for number of pod/plant, number of seeds/plant and seed weight/plant as a result of different treatment with mutagens in leguminous crop has been extensively studied (Shaikh *et. al*., 1983; Pathirana *et. al*., 1983). The increased mean at M2 than M1 but still below the control could be assigned to the recovery effect which is a consequence of an elimination of bad genes after selfing. The increase in both mean and variance has suggested unidirectional occurrence of mutations in quantitative traits. The increase in variance reveals the existence of increase in genotypic variability which offers an opportunity of artificial selection following the treatments and also suggests the occurrence of higher frequency of micro-mutations.

Antibiotics which functions like other physical and chemical mutagens induce chromosomal damage, disturb mitotic index and effect the relative frequency of prophase, metaphase, anaphase and telophase. Cell division involves a number of biochemically different processes alongside the chromosome duplication and segregation and thus the chemical which interferes with any of the cytological or biochemical events may induce cytological abnormalities i.e. clumped metaphase, fragments, bridges with or without fragment in anaphase, erosions, telophase bridges and micronuclei.

Decline in prophase is an indication of prevention of entry of cells into division after G_2, antimitotic activity and induction of chromosome aberrations. The increase in metaphase and anaphase and decrease in telophase illustrates the prevention of cell cycle at the end of anaphase and embryonic cell at different stages of interphase at the time of treatment (Amer *et. al*., 1974). Higher frequency of abnormalities at M1 and less at M2 is suggestive of the fact that the chromosomal abnormalities produced at M1 due to direct action of chemicals which go on decreasing in subsequent generation and shows elimination. From the observed different rates of elimination of the various aberrations it can be concluded that the rate of elimination depends on the type of aberration, dose and ploidy. All the antibiotics used were noticed to produce a large number of abnormalities but variability in the degree of damage were different. This may be either due to variation in permeability of the solution or its degree of penetration or dissimilar mode of action.

With the increase of dose there is a corresponding increase in the frequency of aberrations. A single dominant gene is capable of causing meiotic irregularities resulting in partial or complete failure of chromosome pairing (Amer *et. al*., 1974; Grover *et. al*., 1980).

One of the commonest features of the mutagenic treatments is pollen sterility which is associated with the induction of chromosomal changes involving translocation, inversion and deletion. These abnormalities are eliminated during succeeding cell division. Some of the abnormalities remained undetected during mitosis and persist up to gamete formation till M1, M2 or subsequent generation and consequently, cause pollen sterility. Pollen sterility may not only be as a result of the effect of mutagen of polygenic system but also on the genes controlling the meiotic behaviors.

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Biodiversity in Uttrakhand Himalaya region

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Abstract: Himalaya means Abode of Snow. The Himalaya have been the supreme benefactor and protector of our country in many ways from million of years. Though youngest of the mountain chains in the world, the Himalaya have attracted tourists, philosophers, scientists and saints alike. The Uttrakhand Himalaya region provides a matchless wealth of medicinal and aromatic plants & is known to be a natural reservoir. Uttrakhand region is well known for its biodiversity. This paper aims to evaluate the present conditions of resources as a form of natural vegetation, agricultural crops, horticultural farming, herbs, tea garden practices and economic development of the Uttaranchal Himalaya. [Nature and Science. 2009;7(3):113-125]. (ISSN: 1545-0740).

Introduction

The Himalayas present a storehouse of bio-diversity, where flora and fauna vary extensively with climate diversity from one region to the other. Initially, if an attempt is made to divide the forests types based on the standard classification of tropical, sub-tropical, temperate and alpine, it becomes difficult to describe the rich diversity of the Himalayan forests. However, extensive commercial felling of forest has been reported in this region, especially in the last few decades when urban centers began to grow in areas near the forests. While the forests, farmlands and grasslands are of extreme importance for the agripastoral economy within the Himalayan region, the other renewable resource, water, has always been crucial for thickly populated plains further down in the south and east. The Himalayas has one of the highest hydropower potentials of the world, which includes three of the mightiest rivers of the world, i.e. Indus, Ganga and Brahmaputra. Besides, water resources, vast extended grasslands and dense forests in some highland parts and in the foothills of the Himalaya have certain specific advantages such as a tremendous potential for tourism. However, extreme climate variations and inaccessible terrain make it difficult to exploit and utilize all the diversified natural resources of the great Himalaya.

Forests constitute one of the most important natural resource. They play a vital role in social, cultural, historical, economic and industrial development of any country and in maintaining its ecological balance. Forests are considered not only the resource base for sustenance of the population but also as the storehouses of the biodiversity. Forests are solely responsible to maintain and improve the moisture regime and provide clear air. They moderate floods and make the streams perennial. They also produce humus and thereby maintain soil fertility. The diversity in climatic and physical setting produces a markedly diverse flora and fauna. With increasing catastrophes (mass wasting, cloud bursts, avalanches) forests have been severely fragmented at many places degraded, causing threat to local extinction to many wild species of plants.

Sustainability of forest ecosystem is an essential component of the environmental conservation efforts and any degradation of forests will have an adverse impact on various systems, such as water resources, agriculture bio diversity, environment, climate and human health, besides its impact on the subsistence living of tribal and other communities living in around forest areas. Therefore, the function with respect to conservation of soil, water, forests and biodiversity are vital for the welfare of present and future generations.

Climatic extremes like cloudbursts, hailstorms and earthquakes play a critical role as an environmental constraint. Global warming is affecting ice and glacier cover in the region. Furthermore, due to inaccessibility of the region, it is difficult to quantify and assess the damage caused by these natural events. The environmental constraints against the exploitation of natural resource also include other natural

phenomena like mass wasting, high seismic activity, landslides, glacial lake outburst floods, erosion and sedimentation.

Soil erosion is one of the major environmental constraints, which results in frequent flooding in the plains downstream and damage to agriculture, life and infrastructure. The livestock population in the region has also increased during the recent past and problem of grazing in the high altitudes has acquired serious dimension, as a result the vegetative cover is decreasing which has resulted in the loss of topsoil due to excessive erosion. The relatively soft rocks and favorable climatic conditions are the factors behind quick weathering followed Workshop on Himalayan Ecology by mass wasting and landslides. Intense monsoon rainfall also accelerates soil erosion in the region. The Himalayan rivers also carry a very heavy sediment load especially during summer and rainy season, which provides conditions for river shifting.

Cloudburst is a natural phenomenon in Himalayas and it has become frequent in the recent years. Geomorphic features like cirques, hanging valleys with broad valley, erosional glacial features with open valley on one side and dense forests especially of Oak in another side are present with average height of 5000-6500 feet. It always occurs in areas where the southern face has agriculture land. These artificial deposits are less compact, loosely laid on the surface of various types of pre-existing landforms and are prone to erosion lands transportation in bed load due to mixture of finer sand silt and coarse gravel even boulders and get a lot of direct sunlight and northern face lie behind the area and have thick forests. Cloud always move south to north in the Himalayas and when they come up against cirque like features, where the valley is closed, they become stagnant and get piled up and after some time cloud precipitate suddenly by heavy discharge in rivers and usually cause massive damage to forests and other property.

Biodiversity

Natural resources as a form of minerals and petroleum products are lagged behind in the region. Therefore, the mainland of Uttarakhand is industrially backward. Furthermore, whatever the minerals are found they are not properly mined because the fragility of terrain does not permit to do it. Heavy investment and lack of technological development, on the other hand, are making slow the process of utilization of these resources. But the availability of forests resources, wild lives, agricultural crops, extensive grasslands, varieties of herbs, flowers, fruits, vegetables, water, rearing of animals, goats and sheep is high. The feasible climatic conditions boost up the suitability for growing horticultural crops on the one hand and on the other they play a vital role for sustainability of the populace. Cultivation of fruit crops, herbs, flowers, off-season vegetables, high yield variety (HYV) of animals and tea garden practices can be improved with giving scientific inputs. Tourism can also be practiced to improve the economy and the augmentation of employment. But owing to fragility of mountain terrain and poor infra-structural facilities, these given resources of the region could not be utilized properly, particularly the forest and water resources. Now we are discussing biodiversity in Uttrakhand Himalaya in details:

Forest diversity

Among the natural resources of Uttarakhand, forests are the most important, both economically The and environmentally. The forest area is reported to be 3,466 thousand hectares and accounts for around 62.27 % of the area of Uttarakhand. The alpine and tropical rainforests that cover most parts of the state make natural habitats of some of the best-known wildlife creatures. The Jim Corbett National Park is home to Royal Bengal Tigers. Another rainforest in the region is Rajaji National Park famous for its large number of pachyderms. Alpine forests in the region include the Valley of Flowers National Park (known for its amazing variety of flowers), Nanda Devi National Park, Govind National Park and Gangotri National Park.

Uttaranchal Himalaya is very rich in forest resources and diversity. The plant diversity is found extremely rich from the valley regions to the highly elevated alpine meadows, locally known as *kharaks* or *bugyals*

The main forest types of the state according to altitude are described as follows:

(1) Deodar forests (*Cedrus deodara*)

They are found in Chakrata region, Tons Valley & Uttarkashi, and the highly elevated regions of water parting mainly Alaknanda, Nandakini, Pindar and Mandakini (1,650~2,300 m). The Deodar is a tall coniferous tree used for house construction and paneling.

(2) Blue Pine forests (*Pinus wallichiana*)

The Blue Pine is also known as *Kail*. It is found in Chakrata, Tons Valley, Uttarkashi and Joshimath areas. These are found mostly mixed with Deodar forests (1,650~2,300 m).

(3) Chir forests (*Pinus roxburghii*)
The Chir pine is found in the whole of Uttaranchal Hills. The forests exist mostly in the places of 1,000 to 1,650 m. This pine is used for making packing cases and paneling in interior decoration and also used as firewood.
(4) Teak forests (*Tectona grandis*)
The forests have been artificially planted in the *Terai* belt of the Kumaon region. The plant is a broad-leaved tree and flourishes in the plains. It is the best timber, with great demand for construction work as well as for furniture making.
(5) Bamboo forests (*Dendrocalamus spp.*)
Bamboo forests are found abundant in the Lansdowne and Kalagarh areas of Garhwal region (325~1,000 m). Bamboo is known as the poor man's timber. Its versatility of use makes it a force of livelihood for a large number of people. It is a broad-leaved species and helpful for reducing soil erosion.
(6) Oak forests (*Quercus sp.*)
Oak forests are found in the whole area of Uttaranchal between 1,325 m and 1,925 m. It is used for firewood and charcoal manufacturing. It is a broad-leaved tree.
(7) Fir (*Abies pindrow*) & spruce (*Picea smithiana*) forests
The two species of trees are found mostly between 2,300 m and 2,950 m. The forests exist in Chakrata, the Tons Valley, Uttarkashi, Pauri and Pithoragarh.
(8) Sal forests (*Shorea robusta*)
The Sal is found in the foothills of the Himalaya in *Terai* and *Bhabar* areas (500 m~1,000 m). The forests are rich in species, such as Rohini, Amultas, Kanju and Amla. Table 1 shows forest diversity based on altitude (below 1,000 m to snow line) in the State.

In the present study area, the forests are depleting at a large scale. The depletion of forests is causing severe impediments for the stability of landscape. Most of the forest patches are clearing for agricultural fields due to mounting population pressure on the hilly slopes of the state. Therefore, the rate of deforestation is high. As it is reported that the highest rate of deforestation in any biome is in tropical upland forest, i.e., 1.1% per year (FAO 1993), the similar situation is applied with the state. The people that depend on the forest for firewood are high resulting degradation of forest and deterioration of environment. A case study of the five villages is done on the bases of their elevation and distance from the road head. Per day firewood consumption (in kg) both in summers and winters is calculated as an average in the following table: Table 2 reveals that the elevation and distance form the road play a crucial role in determining per day firewood consumption in the villages. The author collected the data related with per day fire consumption and forest types after an extensive field visit and household survey with sampling method. The highly elevated villages consume more firewood than the villages in low-lying areas. Firewood consumption also depends on the availability of forestland. In the areas, with dense vegetation, more firewood is collected than that in the other areas.

Degradation of natural forests is a global problem (Guppy 1984, Sayer and Whitmore 1991). People have been destroying forests for millennia ever since agriculture was started (William 1989). In the Himalaya, deforestation is argued to be not a recent phenomenon too. It has a long history, being well established in late eighteenth century at least (Mahat *et al.* 1986). The heavy pressure of population on the agricultural land due to high growth rate of human population compelled the inhabitants of the region to solve the problem through merging forestland either for settlement or for agricultural practices. However, the extent of impairment of various processes attributed to vegetal degradation depends upon the range of other factors including past histories, intensity of removal of natural vegetation, patterns of natural regeneration and /or other human interferences (Valdiya and Bartarya 1989 & 1991, Gilmour *et al.* 1989, Ramakrishnan *et al.* 1992, Alford 1992).

The management of the forest resource in the mainland of Uttaranchal is getting setback due to two reasons: first, an inaccessibility of the temperate forestland and second, the depletion of forest in surrounding of settlements. The instability of the land due to natural phenomena (terrestrial and atmospheric), such as slope failure, landslides, mass movements and rock fall, further accentuate the problem of depletion of forest. There may be two solutions for sustainable management of forest resources. (i) The proper utilization of the waste woods, which are found in the inaccessible areas of the region through small-scale village based forest industries. That will help the economic development of the region as well as the sustainable livelihood of the populace there. (ii) Afforestation, around the settlements and the areas, where soil erosion is more prone, should be done to stabilize hill slopes.

Agricultural diversity

Agriculture is the main stay of the people of Uttrakhand. Of the total population, more than 75% people are engaged either with the main occupation of agriculture or its allied practices.The study on agriculture in Uttarakhand State demands better planning separately for the mountainous mainland and foothill plains. The prospects of agriculture development in the hill region of uttrakhand appear very limited due to a number of constraints imposed by the natural environment. In this region, which is predominantly agricultural, population pressure has far exceeded the carrying capacity of the land and it, therefore appears imperative that the economy be supplemented by activities allied to agriculture such as vegetable growing, horticulture, bee-keeping, poultary farming, dairy farming etc.Which should, in a phased manner, become vital components of the rural economic structure- as per environmental conditions and needs of the village people. In the mainland of Uttaranchal, traditional subsistence agriculture is dominant in farming system. But their viability in terms of sustainable livelihood is insufficient. Therefore, the rate of out-migration from the region is high. Among the principal crops rice, wheat, millets, barley, pulses and oil-seeds are grown in the entire state. The ratio of pulses and oil seeds is comparatively low. Fortunately, the state has high diversity in food grains, vegetables, fruits, oil-seeds and pulses in all the altitudinal climatic zones, such as tropical, temperate, and cold. The feasibility of climatic conditions and diversity in crops may help for sustainable farming. Wheat occupies highest percent (33.54%) in the total sown area, followed by rice (23.51%) and millets (12.76%). Cultivation of vegetables is extending and now accounts for 12.45% of the total cropped area of the state. The economic viability of off-season vegetables, pulses and oil-seeds in the farming system of the state is noteworthy while the cropped area with these crops is very small. The area with off-season vegetables can be raised for sustainable livelihood because the environmental conditions are very suitable for this production.

Biodiversity of medicinal and aromatic plants

Ayurveda has prospered in the laps of Himalaya and as such herbs growing here find high place in the Ayurvedic texts. It is an accomplished fact besides medicinal plants, Himalaya are the home of many species of aromatic nature.

Medicinal Plants

In India, there are about 3,000 medium to large scale drug manufacturing units which utilize several drug plants, both from cultivated as well as wild sources, as raw materials. Whereas bulk of this raw material is obtained from forest, only a few plants are under systematic cultivation. Despite the close relationship between the forest and pharmacy, very little efforts have been made to maintain, manage and develop the drug plant resources of the Himalayan forests.

At present there is neither any organization to estimate productivity of even selected forests at regular intervals nor has there been quantitative assessment of the growing stock of any of the crude drugs/medicinal plants. Obviously, it would be hazardous to make estimates of even more valuable medicinal plants growing wild in the forest or annually collected in the Himalayan states. It is known that about a thousand of plant species distributed in various climatic zones in India are found to have medicinal virtues but only a handful of these are commercially collected for use within the country and for exports.

Table 1 describes the important/major chemical constituents/active principles of some commercially important medicinal plants, traditionally collected from the Himalaya, which would provide a glimpse of a wide variety of the medicinal plant components of the Himalayan forests.

Table 1: Commercially important medicinal plants from forests

Plants	**Chemical constituents**
	Active principles
Bantalnag	Aconiting, chasmacontine
Aconitum chasmanthum	Chasmonthine

Aconite/Atis	Atisine, heteratisin &
Aconitum heterophyllum	hetisine
Bach	Asargone
Acorus calamus	
Vasaka	Vasicine & Vasicinone
Adhatoda vasica	
Kirmala	Santonin
Artemisia maritima	
Safed Musli	Asparagin
Asparagus adscendens	
Nim	Nimbin, nimbinin, nimbidin,
Azadirachta indica	Azadirachtin, salannin
Rasaut	Berberine
Berberies aristata	
Pashanbed	Sitosterol, bergenin
Bergenia ciliata	
Amaltas	Fosticacodom, rhein,
Cassia fistula	Sennoside A & B
Brahma manduki	Brahmoside, brahminoside
Centella asiatica	Asiatic acid
Hirantutiya	Colchicine
Colchicum luteum	
Satpura	Daphnin
Daphne papyracea	
Dhatura	Scopolamine, hyoscyamine
Datura metel	Atropine
Dhattura	Atropine, hycocine
Datura stramonium	Hyoscyamine
Kins	Diosgenin
Dioscorea deltoidea	

Ephedra	Ephedrine
Ephedra nebrodenis	
Sankhapushpi	Betaine, evolvine
Evolvulus alsinoides	
Kotu	Rutin
Fagopyrum esculentum	
Kutki	
Gentiana kurroo	
Kalihari	Chelidonic acid, colchicine,
Gloriosa superba	lumicolchicine
Kurchi	Conessine, holarrhimine
Holarrhena antidysenterica	Kurchine, conarrhimine
Jatamansi	Jatamansone & jatamansic
Nardostachys jatamansi	Acid
Nandru	Hyoscyamine & hyoscine
Physochlaina praealta	
Kuru	Picrorhizin, kutkin
Picrorhiza kurroa	
Papra	Podophyllin, podophyllotoxin,
Podophyllum hexandrum	Podophyllic acid
Rhubarb	Emodin, physcione, chrysophanol
Rheum emodi	
Chiraita	Ophelic acid, chiratin,
Swertia chirata	Amarogentin
Jangli Piyaz	Scillarens A & B
Urginea indica	
Banafsha	Rutin, cyanin, methyl
Viola odorata	Salicylate, odoratine
Ashwagandha	Withasomine, withaferin
Withania somnifera	A & withanolide

The trade in crude drug in largely unorganized and statistics on their production and utilization are generally not available. Medicinal plants/crude drugs are good foreign exchange earners. To boost indigenous production and to avoid imports of crude drugs, the cultivation and exploitation of indigenous and exotic medicinal plants should be encouraged in the national afforestation programmes, such as Social Forestry, Farm Forestry, etc.

Aromatic Plants

Himalaya are repository of many aromatic or essential oil bearing plants and it is difficult to enumerate their entire wealth. However, the present review gives the status of some important aromatic plants which need the attention to explore different fields, so that the scientists may be able to deal with the species judiciously and the entrepreneurs may exploit them according to the need of the trade and industry.

Table 2 highlights the commercially important aromatic plants for their essential oil value in our country. The essential oil contents of these plants compare well with those plants grown in other parts of the world and commercial exploitation of these plants is feasible which can meet the requirement of various industries, viz, perfumery, cosmetics, soap, toilet, hair oil, pharmaceuticals, food, alcoholic beverages, etc.

Table 3 includes such aromatic plants which occur locally in the Himalaya. These do not either occur elsewhere or have not so far been exploited commercially. In nature, yield of essential oils from these species is however, low but their production can be increased by suitable cultivation techniques and proper methods of exploitation.

Table 4 consists of some lesser known aromatic plants of Himalaya and recent investigations have explored the possibility of their commercial exploitation while a few species though known need proper attention.

The value of essential oils obtained from various aromatic plants (Tables 2-4) lies in the presence of certain commercially important components or chemical constituents which suggest their industrial utility. It has been assessed that the aggregate value of essential oils used in processed food, toilets, cosmetics and pharmaceutical preparations is around Rs. 300 crores. Their demand is going to increase four times by the end of this century. Essential oils are good foreign exchange earners too involving low transportation cost. Attempts should, therefore, be made to explore the new possible species having essential oils and to grow them in suitable areas so as to meet national demands, boost exports and avoid imports.

Table 2: Commercially exploited aromatic plants of Himalayas

	Plants	**Chemical constituents**
1.	*Bach* Acorus calamus	Asarone, calamenol, calamene
2.	*Bel* Aegle marmelos	L-phellandrene, citronellal, para-cymene, citral
3.	*Angelica* Archangelica	L-pinene, phellandrene, osthole, terpenes & sesquiterpenes, L-phellandrene, para-cymene
4.	*Chura* Angelica glauca	L-pinene, L-phellandrene, Selinene, L- cadinene
5.	*Zira* Carum carvi	Carvone, a terpene & traces of carvacrol

6.	*Tej Pat* Cinnamomum tamala	Cinnamic aldehyde, linapool
7.	*Ferula* Jaeschkeana	Azulenes, pinene, cadinene
8.	*Aaraar* Jumiperus communis	Friots, pinenes
9.	*Dunp* Juniperus macropoda	Cedrol, Iimonene, 4-terpineol
10.	*Jatamansi* Nardostachys jatamans	Jatamansone & Jatamansic acid
11.	*Kuth* Saussurea Lappa	Dihydro costus lactone, costunolids,costol
12.	*Ner* Skimmia laureola	Linalyl acetate, linalool
13.	*Stinking Roger* Tagetes minuta	Aromadendrene, tagetone
14.	*Banajwain* Thymus serpyllum	Carvacrol, para-cymene, gama-terpinene
15.	*Tejbal* Zanthoxylum alatum	L-phellandrene, traces of linalool, a sesquiterpene
16.	*Indian lichens chrilla* Parmelia nepalensis, P. nilgherrensis, Ramalina sub- complanta & Usnea lucea	Resinoids

Table 3:Commercially unexploited aromatic plants of Himalayas

	Plants	**Chemical constituents**
1.	Artemisia dracunculus	Methyl-chavicol & paramethoxy cinnamaldehyde
2.	Chaerophyllum villosum	L-pinene, car-3-ene
3.	Elsholtzia pilosa	1-8 cineole, terpinyl acetate limonene
4.	Heracleum canescens	Pinenes, L-terpinene, phenylethyl acetate

5.	Heracleum candicans	Pinenes, dihydro-carveol, carveolacetate
6.	Pushkar Inula racemosa	Alantolactone, isolantolactone & sesquiterpenes
7.	Zufa yabis Nepeta ciliaris	Tricyclic sesquiterpene, pinocarvone, L-Pinene
8.	Billilotan Nepeta hindostana	Limonene, citronellal, geranyl acetate, citronellol
9.	Komal Prangos pabularia	Myrcenae, camphene, L-pinene, osthole, 4-Camphene, borneol

Table 4: Lesser known aromatic plants of Himalaya

	Plants	**Chemical constituents**
1.	Caucalis anthriscus	Benzyl alcohol
2.	Rangchari Elsholtzia polystachya	1,8-cineole & 2- subsituted furan derivatives
3.	Sitruti Hedychium spicatum	Ethyl ester of p-methoxy cinnamic acid
4.	Ligusticum elatum	Pinenes, sabinene, car-3-ene
5.	Lavangalata Luvunga scandens	Cineole, methyl cinnamatem & sesquiterpenes
6.	Sathra Origanum vulgare	Pinenes, dipentene, linalool, sesquiterpenes
7.	Kheshavo Selinum tenuifolium	Limonene, elemol, terpineol, geraniol
8.	Moor Selinum vaginatum	Pinenes & limonene
9.	Vanayamani Seseli indicum	Limonene, selinene
10.	Bhootakeshi Seseli sibiricum	Pinenes, myrcene, p- cymene, β-pheldandrene

Considering the economic value of the components of aromatic plants in each group, it may be necessary to grow them according to their industrial demand. Since methods of cultivation and exploitation of most of the aromatic plants have not been perfected, it may be useful to conduct agronomical trials before establishing a particular crop. Determination of optimum stage and season of harvesting is also essential to obtain good quality product with maximum yield. Cultivation, recovery of essential oils and isolation of their components from aromatic plants is labour intensive and generates lot of employment also. Therefore, introduction and growing of aromatic plants in forest areas, or in crop rotation with agriculture crops in Agro-Forestry Programmes, or as rehabilitation crops in Social Forestry Programmes is in the interest of national development and is likely to fetch very good returns. Based on these considerations, significant advantage can be obtained from the aromatic plants. With this in view, a list of species which should receive attention in Himalayan region are highlighted in three groups. The presence of various constituents are also indicated against each species which are suggestive of the scope of utilization by different industries for flavouring foods, drinks and alcoholic beverages, perfumery, cosmetics soaps etc.

Uttarakhand is a storehouse of a rich variety herbs and medicinal and aromatic plant species. The Government intends to exploit this advantage. Special emphasis is on R&D.

• An integrated action plan has been drawn up for this purpose in coordination with the Government of India and other concerned agencies in the State and elsewhere in the country.

• R&D in the area of Medicinal Plants and commercial production of applications and formulations will be developed in conjunction with Research Institutions and reputed companies.

• A Medicinal and Aromatic Plants Export Zone has been set up covering seven districts of Uttarakhand and Specialized Herbal Parks are in the offing.

Medical and health care

The salubrious climate, pollution free environment and the availability of a wide range of flora and fauna in the mountainous terrain, make Uttarakhand an ideal location for developing centres for alternative medicine and health care facilities. Possibilities of establishing gyms, sauna and related facilities, which would motivate health care enthusiasts exist in plenty.

Forest product- Herbs and spices

A significant portion of Uttarakhand is under forest cover (almost 70 percent). There is, thus, excellent potential for the development of forest resources based Industries in the State. In addition, there is ample scope to develop industries based on forest and agro-wastes such as lantana, pineneedles, plant and vegetative fibers such as Rambans, etc.

Horticulture and flouriculture

Uttarakhand has almost all the different Agro-geo climatic zones making it particularly conducive to commercial horticulture and floriculture. Horticulture schemes are being promoted in a big way through adequate incentives and facilities. Given the constraints on the productivity of field crops, a shift from the cultivation of low value field crops to high value crops, such as fruits and vegetables appears to be the most obvious option in the state. The Uttarakhand region has proved to be suitable for growing different types of temperate, sub-tropical and tropical fruits. Also, the region has wide scope for growing different kinds of vegetables, flowers, ornamental plants, mushrooms, and medicinal plants in its different climatic zones. Temperate fruits, such as apple, pear, peach, plum, apricot, cherry and walnut, are grown in the places of 1,000 m~3,000 m (Sati 2004). The places from 300 m to 1,400 m are planted with citrus, mango, litchi, banana, guava, papaya, strawberry and different vegetables and crops. Market forces and institutional set-up created for gearing horticultural development led to economic growth but at the cost of equity. Prosperous farmers benefited more than small and marginal farmers (Swarup and Sikka 1987).

The land under fruit cultivation is 190,192 ha and under vegetable 80,332 ha, accounting for 29% and 15% of the total area sown, respectively. The total production of fruits and vegetables in Uttarakhand is estimated to be 345,339 metric tones and 492,785 metric tones, respectively according to data available in 2003. Apple is the most important among the various fruits grown in the region and its cultivated land is 54,000 ha.

Among vegetables, potato is the most importance crop. In the state fruits are grown in the different altitudinal zones, and among them apple, peach, citrus, mango, plum, apricot, walnut, litchi and other fruits are important. The land used for production of various vegetables in the region was almost twice increased

and their production was increased by 2.5 times during the past two decades. This clearly indicates that growing of fruits and vegetables has spread over larger areas of land. However, lack of proper marketing facilities, and absence of post-harvest technologies and storage are serious constraints to a more rapid and systematic development of fruit and vegetable cultivation and marketing in the region. Cultivation of off-season vegetables in different altitudinal zones of Uttarakhand in summer offers extensive market (Mehta, G. S. 1990).

Herbs are naturally grown in the meadows *(kharaks*). Fortunately, the state has extensive meadows along the Great Himalayan Ranges. These herbs are locally utilized for medicinal purposes with the positive results. Simultaneously, a tea garden practice is centuries old, but presently its extension is available in very limited areas. Following the creation of Uttarakhand State, the government has launched the scheme for establishing tea gardens in different geographically suitable areas.

Floriculture is being developed in a big way in order to supply the domestic market and penetrate foreign markets. Floriculture can help generate employment opportunities as well as augment incomes of farmers. The climate being ideal for growing flowers nearly round the year it is proposed to establish Floriculture Parks with common infrastructure facilities for sorting, pre-cooling, cold chain, processing, grading and packing/marketing facilities.

Agro and food processing industries

The Uttrakhand Government will assist in establishing small & medium size Agro Parks, Food Parks etc., which will provide common infrastructure facilities for storage, processing, grading and marketing, thus ensuring that surplus fruits and vegetables do not go waste as at present. Four Agri Export Zones have already been declared under the AEZ scheme of the Government of India for Leechi, Horticulture, Herbs, Medicinal Plants and Basmati Rice(Maikhuri, R.K, et.al 1995). Further, efforts will continue to promote production for export and provide access to domestic and export markets for products from the State. Uttarakhand has been included in difficult area category by the Ministry of Food Processing Industry (MFPI), Government of India and hence units being set up in Uttarakhand will be eligible for higher incentives under the schemes of MFPI. The State Government is also providing matching subsidy for projects under various schemes of Agricultural & Processed Food Products Export Development Authority (APEDA), National Horticulture Board (NHB), Ministry of Food Processing Industry (MFPI) and the Natural Medicinal Plant Board (NMPB) subject to a maximum limit of Rs. 20 lakhs.

Biotechnology

Biotechnology (BT) is poised to make significant contributions in agriculture, human and animal health care, environment management and process industries. Rare species of plants and animals found in the Uttrakhand state, add to its natural advantage in this sector. In this context, a MOU has already been signed between Rabo India Finance Company, Infrastructure Development Finance Company and the G.B.Pant University of Agriculture and Technology in order to forge strategic cooperation to jointly pursue initiatives in the sphere of research in food and agriculture sectors. A high level Biotechnology Board is also being setup under the Chairmanship of the Hon'ble former Chief Minister N. D.Tewari.

Eco-tourism

The Uttrakhand region, with its lofty mountain peaks and glaciers is the source region of many important rivers of North India. The region also abounds in a vast range of natural phenomena including glaciers, waterfalls, lakes, winding river valleys and mountain slopes showing a wide variety of flora and fauna. The entire region, therefore, presents an endless variety of scenic beauty and it may not be an exaggeration to say that the Himalaya is one of the most picturesque mountain systems of the world (Joshi S.C,2004). The hills of Uttaranchal have all the ingredients for adventure packed with excitement and thrills - an unexplored valley, towering peaks, flowing rivers, snow-capped mountains, a splendid combination of flora & fauna and vast tracts of virgin snow. Mountaineering, trekking, skiing, river rafting, canoeing, kayaking, fishing, angling, aero sports - there lies a whole world of activities to satiate the wildest of sprits. Bhagirathai, Chowkhamba, Nanda Devi, Kamet, Pindari, Har ki Doon, Dayara, Kafni, Auli, Munsyari, Pauri, Pithoragarh, Kodiyala are some of the hotspots where adventure seekers can have an experience of a lifetime. Uttaranchal also treasures some of the most breathtaking destinations for Wildlife Tourism - presenting a perfect retreat for picnics and excursions.The snow-capped mountains, Rolling Meadows, high-altitude lakes and dense forests in the hills of Uttaranchal support exotic wildlife, bird life and plant

life. These wonderful creations of nature add a dash of splendour to nature's abundance that exists in the environs of the Uttaranchal. Some National Parks and Sanctuaries are created to preserve this gift of wildlife and enable visitors to have a privileged view of the same – Corbett National Park, Rajaji National Park, Govind Wildlife Sanctuary, Nanda Devi National Park and Kedarnath Sanctuary. In the wilderness of these Parks and Sanctuaries animals like tiger, elephant, leopard, leopard cats, jungle cats, fishing cats, snow leopard, panther, snow cock, tahr, musk deer, chitals, barking deers, sambar, Himalayan black bears, brown bears, bharals, monals, crocodiles, gharials (descendants of the prehistoric reptiles) can be seen along with many species of birds, butterflies and snakes. Uttaranchal, a land resplendent in awesome natural splendour is a jewel of the glittering Himalayan necklace. In its range of natural beauty, Uttaranchal is known for White Mountain peaks, blue ribbons of meandering rivers, eye-catching pink and red rhododendrons and birds of vivid plumage. From the most modern facilities at Mussoorie, Narendranagar and Nainital to the untouched and pristine beauty of its snow-clad peaks, rivers and forests, Uttaranchal is indeed a paradise for tourists.

It can be *concluded*, the future prospects of the available resources for sustainable development of the state are as follows:

(1) Horticultural Development, in this context, offers significant scope and could become a lead sector in many parts of Uttarakhand, particularly in the low hills and middle mountains. As indicated earlier, a variety of fruits are grown in the region during different crop seasons.

(2) A large variety of minor forest produce, medicinal plants, and natural fiber plants found across the region, and these could be harnessed advantageously to produce useful and high-value items.

(3) Promotion of ringal (bamboo) plantation in larger uncultivable areas and its use to make useful marketable products could thus be both economically and environmentally profitable. In some relatively high altitude areas, the production of ringal-based goods could become an important economic activity. There is ample scope for developing this activity for larger markets, both for creating gainful employment opportunities for the local population and also for the protection of the environment, as it has also been recognized as a plant that binds the soil, thus preventing soil erosion and landslides.

(4) Rearing sheep and goats have special advantages in the hills and mountains at altitudes of 700m and above. It has been a traditional activity of the Bhotia households in high altitude areas of Pithoragarh, Almora, Chamoli, Uttarkashi and Dehradun districts. The Animal Husbandry Department of the State Government is involved in improving the breads of sheep in order to increase the production of better quality wool in the region.

(5) Woolen industries have been given significant importance during the recent past. A greater segment of the population, particularly women and relatively low-income groups, is participating increasingly in the production of woolen items as a part-time or full-time activity.

(6) Tea garden practice is centuries old but presently its existence is very less. This practice will definitely help for economic development of the region.

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Tree Layer Characterstic and Regenration Pattern of Central Himalayan Forest in Relation to Catchment Area

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ABSTRACT: The present study was carried out in the forest located in the catchments of Nainital lake located in Uttarakhand. To study various qualitative and quantitative parameters, the study site was divided into North- East aspect and South – West aspect. In North East aspect two forests, Oak forest (hill base), and Oak-conifer forest (hill top and mid hill) were studied. Along the South-West aspect also three forests were studied: Oak forest (hill base), mixed forest (mid hill) and mixed forest (hill top). A total of 8 tree species were reported across all the study sites. These were: *Quercus leucotrichophora, Quercus floribunda, Cupress torulosa, Cedruss deodara, Rhododendron arboreum, Acer pictum, Aesculus indica and Cornus macrophylla.* The density within each forest ranged from 10 – 250, 30- 220, 120 – 380, 10 – 150, 20- 130 and 20 – 180 trees/ ha. Total density was found ranging from 220-560 trees / ha across the study sites. Abundance/frequency (A/F) ratio was found ranging from 0.01- 0.08 among the forest sites indicating that the species were distributed in regular, random and random – contagious pattern. The total basal area ranged from 16.7-69.9 m^2/ ha and the total IVI (269.93 – 299.9) across the forest studied. The moisture in the northern aspect ranged from 45.14% to 32.29%, where as the soil moisture in southern aspect ranged from 39.68% to 30.12%. The soil carbon in the northern aspect is found to be higher than the southern aspect. The soil carbon in the northern aspect ranged between 2.9% (0-10cm) to 1.3%(30-60cm),where as in southern aspect it varies from 2.3%to0.8%.Bulk density ranged between 0.88gm g cc^{-1} to 0.91gm gcc^{-1}in northern aspect while in southern aspect it ranged 0.79g $cc^{-1}$to 0.82g cc^{-1}. Based on the study, it is concluded that the forests which were close to the human habitation has suffered much damage than the sites far from the human settlements. The poor occurrence of seedlings and saplings in the studied forests indicate that immediate efforts are needed to boost the regeneration and employing suitable silvicultural and management practices for preserving the catchment protection value of the forest of Nainital lake catchment. [Nature and Science. 2009;7(3):126-130]. (ISSN: 1545-0740).

KEY WORDS: Vegetation analysis, composition, catchments, regeneration

INTRODUCTION

The Indian subcontinent is a region of moderate to very high biodiversity including two of the global hot spot of vascular plant endemism in the Western Ghats and the Eastern Himalaya (Myers et al, 2000). In the central Himalayan region forest is potential vegetation above up to 3500 to 4000 m elevation. (Singh and Singh,1987).The Himalayan forest vegetation ranges from tropical dry deciduous forest in the foothills to alpine meadow above timberline (Singh and Singh,1992), however as data collected from land's imaginaries indicate, right now only 29% of the reported area (51,000sq km^2) is forested and good forests (with more than 60%forest cover) occur only in 4.4% of the area (Singh and Singh, 1987). Concentration of human settlements in the forest area, lopping and felling and occasional fire spreading from pine forest have reduced the area under forest (Champion and Seth,1968).The plant diversity is found extremely rich from the valley regions to the highly elevated alpine meadows (Sati, 2005). Forest diversity is the main source of live hoods of people living in Uttrakhand central Himalaya (Ram et al, 2004). Among the natural resource of Uttarakhand, forest are the most important both economically and environmentally. The forest area is reported to be 3,466 thousand ha and accounts for around 62.27%0f the area of the Uttarakhand. Through out the state, serious environmental problems have already emerged. There environmental problems are particularly noticeable in main land of Uttarakhand as a form of degradation and depletion of forest resources (Sati, 2005). In Uttrakhand, composition of forest is diverse varies from place to place because of varying topography such as plains, foothills and upper mountains (Singh, 2006). Strategies are being suggested to receive the forest cover in the region, including the replacement of agriculture by forestry (Singh and Singh, 1987), because of the human onslaught on the forest, the ecosystem succession of communities and regeneration affects. To study the forest it is necessary to know the phytosociology of the forest. According to (Sexana and Singh, 1982), Himalaya offers and array of forest types below the timber line, and is cradle of major river of India harboring a network of catchments areas. As the catchments efficiency depends upon the type quality and stratification of forest vegetation a quantitative evaluation of its vegetation is pre-requisite. Some scientist have made few studies (Puri, 1960), (Champion and Seth, 1968 et al) found variation in the humid tropical forest and forest in the South East Asia respectively. Regeneration of the structure of a forest community can be communicated by constituting a profile diagram. The present study deals with tree layer compositions of forest and regeneration pattern of central Himalayan forest in relation to catchments area.

MATERIAL AND METHODS

The study area Nainital is located between 29º 21'- 28º 24' and north latitude between 1938 m to 2292 m elevation. For the detail study of tree layer composition, characteristic of forest and aspect along the elevational gradient i.e. hill base (1938-2000m) hill slope (2000-2110m) and hill top (2110-2292m).In the past both the aspect are subjected to landslides (based on personal inventory with the locals), on aspects between located 1938 to 2110m, the characteristic features anthropogenic disturbances and absence of regeneration. Altitudinaly the study area was located in a temperate environment but latitudnally it exists with in the subtropical belt. The basic climate pattern is governed by monsoon. The annual rainfall was 200-300mm of which three fourth occurred in rainy season (Mid June to September). The mean monthly temperature ranged between 17˙0˚ c (January) and 33˙0˚ c (April), and mean minimum temperature ranged between 0˙0˚ c (January) and 14.5˚ c (August). The study was conducted during the year 2004-2005 from each site; the composite soil samples were collected from 0-10cm, 10-30cm, 30-60cm.depths packed in polythene bags and brought to the laboratory for

analysis. Moisture content was determined on dry weight basis. Soil bulk density was determined by (Mishra1968). Soil carbon percentage was determined by (Walkey and Blacks's methods, 1958).Vegetation analysis made for all the sites. Tree layer was analyzed sampling 10 quadrates 10x10 size in each site. The size and number of sample was determined following (Saxana and Singh, 1982). The vegetational data were quantitatively analyzed for abundance, density, and frequency (Curtis and Mcintosh, 1950). The importance value index (IVI) for the tree layer was determined as the some of the relative frequency, relative dominance (Curtis, 1950).The distribution pattern of different species using the ratio of abundance to frequency.

RESULTS AND CONCLUSION

SOIL

The moisture in the northern aspect ranged from 45.14% to 32.29%, where as the soil moisture in southern aspect ranged from 39.68% to 30.12%. The soil carbon in the northern aspect is found to be higher than the southern aspect. The soil carbon in the northern aspect ranged between 2.9%(0-10cm)to 1.3%(30-60cm),where as in southern aspect it varies from 2.3%to0.8%.Bulk density ranged between 0.88gm g cc^{-1} to 0.91gm gcc^{-1}in northern aspect while in southern aspect it ranged 0.79g $cc^{-1}$to 0.82g cc^{-1}.

TREE LAYER

On the northern aspect at the hill base the lowest tree density was reported for *Acer pictum* (10ind-ha^{-1}) and the highest for *Quercus leucotrichophora* (150ind-ha^{-1}). At the hill slope the lowest density was reported for *Aesculus indica* and *Cupress torulosa* (20ind –ha $^{-1}$each) and highest for (Quercus leuchotrichophora130ind-ha^{-1}), Where as at hill top the lowest density was reported for *Rhododendron arboreum* (20ind ha^{-1}) and highest for *Quercus leucotrichophora* (130ind-ha^{-1}), where as at hill top the lowest density was reported for *Aesculus indica* and *Cupress torulosa* (20ind-ha^{-1}each) and highest and highest for Quercus leuchotrichophora (130ind-ha^{-1}), where as hill top the lowest density was reported for *Rhododendron arboretum* (20ind ha $^{-1}$) and highest for *Quercus leucotrichophora* was (180ind-ha^{-1}). *Quercus leucotrichophora* was dominant all the sites (IVI=76.33) and hill top (IVI=74.59) and *Cedruss deodara* at hill slope (IVI=102.64).

In the southern aspect at the hill base the lowest density was reported for *Cornus macrophylla* and *Cupress torulosa* (10ind ha^{-1}) and highest for *Quercus floribunda* (250ind^{-1}). Density at hill slope ranged between (30ind-ha^{-1}*). Rhododendron* arboretum and *Cupress torulosa* (220ind ha^{-1}) for *Quercus floribunda*, where as it ranged between 120ind ha^{-1} *Quercus leuchotrichophora* and 380ind –ha $^{-1}$for Quercus floribunda. The maximum (IVI=120.26) followed by *Quercus leuchotrichophora*(IVI= 82.04) and *Cupress torulosa*(IVI=78.1). At hill slope *Quercus leucotrichophora* was the dominant species (IVI= 85.73) followed by *Cupress torulosa* (IVI= 82.04) and Cupress *torulosa(*IVI=78.1). At the hill slope *Quercus leucotrichophora* was the dominant species (IVI=85.73) followed by *Cupress torulosa*(IVI=78.55) and *Quercus floribunda* (Quercus *leucotrichophor*(IVI=77.98).At the hill top IVI of *Quercus leuchotrichophora* respectively.

REGENRATION PATTERN

The study sites were to close human habitation, this indicates that lack of regeneration in each forest site there were identified no seedlings and saplings because the sites were also constructed by the builders and encroached by the local residents.

Conclusion

Loss of forest in central Himalayan region results in severe, ecological and economic cost lost watershed protection, regional climate change, reduced supply of timber, fuel wood, fruits etc, and also affects peoples lives (Jagdish et al,1997). As far as the species concerned in study area, total 08 species were reported in all the forest sites; however the range was 2-5 species, which were much less than the value 28 reported for central Himalayan forests (Upetri et al,1985) and 17 species for Oak Reserve forest (Bisht and Lodhiyal, 2005).This indicates that the most of the species has been disappeared from the study forest site because of human pressure and illegal tree cuttings this tempo need to be checked.

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