An International Journal

Nature and Science

ISSN 1545-0740

Volume 7 - Number 9 (Cumulated No. 30), September 15, 2009

Marsland Press

P.O. Box 21126, Lansing, Michigan 48909, the United States

525 Rockaway PKWY, #B44, Brooklyn, New York 11212, the United States

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Nature and Science

ISSN: 1545-0740

Volume 7 - Number 9 (Cumulated No. 30), September 15, 2009

Cover Page, Introduction, Contents, Call for Papers, All in one file

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Emails: editor@sciencepub.net; sciencepub@gmail.com

Influence of explants type and plant growth regulators on *In vitro* multiple shoots regeneration of a Laurel from Himalaya

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Abstract: Micropropagation in a Laurel, Bay leaf tree (*Cinnamomum tamala* Nees. & Ebrm.) was studied with an aim to select best explant and effect of plant growth regulators (PGRs) for multiple shooting. Various explants were isolated from three year old seedlings. Multiple shoots were induced through callus culture from petiole with nodal segment, shoot tip, shoot with inter-nodal segments and leaf explants on woody plant medium (WPM) supplemented with various plant growth regulators (BA, IBA and kinetin) alone as well as in combinations with varying degree of success in the following order: petiole with nodal segment>apical shoot>shoot with internodes>leaf. Petiole with nodal segment explant and PGRs combination of BI was found best for callogenesis (compact greenish white), shooting (4 fold) and rooting (100%). The petiole with nodal segments from *in vitro* developed plantlets could be induced again to produce a large number of harvestable shoots. Harvested shoots were rooted *in vitro* in WPM supplemented with plant growth regulators (PGRs). Similarly BI combination of PGRs showed the significant results in callogenesis, multiple shooting and rooting regeneration in all four types of explants. The plantlets were transferred to theromocol cups after which they were replanted into poly bags and then to field. These plants survived with over 70% success under field conditions and exhibited vigorous growth. This system could be utilized for large-scale multiplication of *C. tamala* by tissue culture. Further observations will be continued. [Nature and Science. 2009; 7(9):1-7]. (ISSN: 1545-0740).

Key words: *Cinnamomum tamala*, petiole with nodal segment, explants, plant growth regulators, callogenesis, multiple shooting, acclimatization

Abbreviations: I= Indole butyric acid (IBA), B= 6-Benzyladenine (BA), K=Kinetin

1. Introduction

Cinnamomum is a large genus, many species of which yield a volatile oil on distillation. The genus *Cinnamomum* comprises several hundred species which occur naturally in Asia and Australia (Brandis, 1998). They are evergreen trees and shrubs and most species are aromatic. *Cinnamomum tamala* Nees and Eberm. (Family-Lauraceae) known as tejpatra in Sanskrit (Kirtikar and Basu, 1981), is a medium-sized tree, found in India along the North-Western Himalayas, in Sikkim, Assam, Mizoram and Meghalaya (Plate 1. A). It is also found in tropical and sub-tropical Asia, Australia, Pacific region and South Asia (Showkat et al., 2004). This evergreen species occurs as associated species in transitional evergreen broad leaf forest and is confined between sub–montane broad leaf ombrophilous forest (below 1000m) and mid montane broad leaf ombrophilous forest up to 3000m (Singh & Singh, 1992). Leaves of *C. tamala* (tejpat) are widely used as a spice and also yield an essential oil on distillation.

The essential oil of the leaves called tejpat oil is medicinally used as carminative, antiflatulent, diuretic, and in cardiac disorders (Showkat et al., 2004). "Ayurveda" describes the use of leaves of tejpatra in the treatment of ailments such as anorexia, bladder disorders, dryness of mouth, coryza, diarrhea, nausea and spermatorhea (Kapoor, 2000). It has hypoglyacemic and hypolipidemic properties (Kar et al., 2003). It is commonly used in food industry, because of its special aroma (Chang and Cheng, 2002). The main constituents of *C. tamala* leaves are a-pinene, camphene, myrcene, limonene, eugenol, p-cymene, methyl eugenol, eugenol acetate and methyl ether of eugenol (Smith et al., 2002; Saino et al., 2003). Eugenol (4-hydroxy-3-methoxy allylbenzene) is one of the main constituents of cinnamon oil (Fischer and Dengler, 1990; Dighe et al., 2005). It is used as a fragrance and flavoring agent, as analgesic in dental preparations and also as an insect repellant (Fischer and Dengler, 1990; Kermasha et al., 1994; Yuwono et al., 2002). It has antibacterial and antifungal activity (Smith et al., 2002) and strong antitermitic activity (Chang and Cheng, 2002). The oil's high eugenol content also makes it valuable as a source of this chemical for subsequent conversion into iso-eugenol, another flavoring agent. Cinnamon bark oil possesses the delicate aroma of the spice and a sweet

and pungent taste. Its major constituent is cinnamaldehyde but other, minor components impart the characteristic odor and flavor. It is employed mainly in the flavoring industry where it is used in meat and fast food seasonings, sauces and pickles, baked goods, confectionery, cola-type drinks, tobacco flavors and in dental and pharmaceutical preparations (FAO United Nations, 1995).

Owing to its high medicinal value and being an important ingredient of the spices the demand of *C. tamala* is increasing day by day and the species is being exploited from its natural pockets illegally (Ph.D observation of first author). The blatant exploitation of the species from the forest in the recent years has created serious concern about its long term health in the already diminishing natural populations. The habitat specific occurrence, poor regeneration status and short life span of seed results in the vulnerable status of the species in Uttarakhand (Ved et al., 2003; Sharma et al., 2009). Therefore there is a need to raise high quality individuals in large scale to fulfil the increasing demand on the one hand and help in conservation of the species on the other. Clonal propagation through tissue culture is an option but, no reports on *in vitro* propagation and vegetative propagation are available for this species. A step in this direction is present communication, which aims the establishment of *in vitro* micropropagation protocol of the species.

Tissue culture has tremendous potential in this context and could be useful in overcoming the above mentioned limitations. Micropropagation protocols have been used for some other species of this genus viz., *in vitro* shoot multiplication in *C. camphora* (Babu et al., 2003; Huang et al., 1997; Kalam et al., 2005) and *C. kanehirae* (Chang et al., 2002). In the present study an attempt has been made, for the first time, to develop an efficient *in vitro* micropropagation method of multiple shoot formation (and subsequent rooting) through callus culture, from various types of explants.

2. Materials and Methods

2.1 Plant material and explant preparation

Explants were collected from net house of HAPPRC nursery located at Srinagar in Pauri district of Uttarakhand state, India (560m amsl) where 2-3 year old seedlings of *C. tamala* were grown. Various explants viz. petiole with nodal segment, apical shoot tip, shoot with internode segment and leaf isolated from young shoots of healthy plants for culture initiation were selected. Explants were washed with tween-20 (5%, 5 min.) and successively surface disinfected with systemic fungicide, mercuric chloride ($HgCl_2$; 0.1% w/v, 2 min), Sodium hypochlorite with 4% available chlorine (2% v/v, 5 min) and ethanol (50% v/v; 2 min) solutions. The explants were thoroughly washed with sterilized double distilled water (X4) after each surface disinfection treatment under aseptic conditions.

2.2 Explant and culture preparation

Explants were placed vertically in conical flasks containing 30 ml of Lloyd and McCown (1980) woody plant medium (WPM) as basal medium containing agar (0.8% w/v) and sucrose (3%w/v). The medium was adjusted to pH 5.6 with 1 N NaOH or 1 N HCl in all the experiments, before autoclaving (1.05kg / cm^2, 121^0C; 20 min). The chemicals used were of analytical grade (Himedia, Qualigens, and Sigma) and the medium was dispensed into culture vessels (Borosil, India). All the cultures were incubated at 25 ± 1^0C in 16/8 hrs light/dark cycle on racks fitted with cool florescent tubes (Philips 40w; 24 and 60 µmol/m^2/s irradiance inside and outside the culture flasks, respectively). Sub culturing was carried out at 3-4 week intervals.

2.3 Callus induction and organogenesis

For this purpose different plant growth regulators (PGRs) such as Indole butyric acid (I, 5µM), 6-Benzyladenine (B, 2.5µM) and Kinetin (K, 2µM) alone as well as in combination were used as supplement with woody plant medium (WPM). WPM alone was considered as control treatment. The experimental design compared explant and PGRs combination interaction for callogenesis, multiple shooting as well as rooting of the species. Induced callus was transferred after 30 days in the same type of culture medium for growth and shoot regeneration. Regenerated shoots (~ 1cm long) were separated from the callus and counted. These shoots were than transferred to the above combinations of the culture medium for elongation and multiplication for another 30 days. After 4 weeks of incubation, shoots were separated from clumps and harvested for rooting also in the same medium.

2.4 Acclimatization and transfer of plantlets to soil

Plantlets with well-developed roots were removed from the culture medium and after washing the roots gently under running tap water, plantlets were transferred to plastic pots (10 cm diameter) containing autoclaved garden soil, farmyard manure and sand (2:1:1). All were irrigated with 1/8 MS basal salt solution devoid of sucrose and inositol every 4 days for two weeks. The potted plantlets were covered with porous polyethylene sheets for maintaining high humidity and were maintained under the culture room conditions. The relative humidity was reduced gradually and after 30 days the plantlets were transplanted to nursery and kept under shade in a net house for further growth and development.

2.5 Statistical analysis

The data were statistically analyzed using the

General Linear Models procedure for analysis of variance. One way ANOVA was applied to test the effect of the interaction of PGRs and explants separately. Analysis was applied for multiple shoot formation and rooting with least significant difference (LSD at 5% level of significance), which was calculated using ten replicates for every treatment (n=10).

3. Results

3.1 Influence of explant type

After 12 days of inoculation, callus induction was observed directly on the cut surface in all four types of the explants when cultured on WPM media supplemented with various combinations of plant growth regulators used with varying degree of success in the following order: petiole with nodal segment>apical shoot>shoot with internodes>leaf (Table 1). Petiole with nodal segment was found best explant for callusing and multiple shooting. Similarly apical shoot and shoot with internode were found relatively good for same in contrast to leaf explant that produced least callus and multiple shooting in all the six PGR combinations with control.

3.2 Influence of PGRs

The explant selection was the most critical feature in callus induction. The texture and type of callus depend on explant type as well as on the combination of growth regulators. The callus formed in media containing BI combination was compact and greenish white in all the four explants; IK combination formed compact and light green callus observed in petiole with nodal segment and apical shoot explant, while friable and white colored callus was obtained in shoot with internode and leaf explant; media supplemented with I showed compact light green colored callus in petiole with nodal segment, apical shoot and shoot with internode whereas in leaf explant friable and white colored callus was obtained; K supplemented media showed compact greenish white callus in petiole with nodal segment and apical shoot, compact light green callus in shoot with internode and friable light green callus in leaf explant; compact greenish white callus was found in petiole with nodal segment and apical shoot, compact light green in shoot with internode and friable light green callus in leaf explants when supplemented with B, in comparison to control in which friable, light green and white colored callus in all the explants was formed. Thus explant type had significant effect on callus induction (Table 1, Plate 1. B & C).

Various combinations of PGRs had significant effects on callus, shoot and root regeneration. Explants cultured on PGRs-free basal medium (control) produced friable callus with no considerable shooting. Callus induction was markedly enhanced by BI combination which produced very good amount of compact and greenish white callus in all the explants except leaf explant. B, K and I alone were also found relatively far better than IK combination with respect to control. Compact callus type was obtained in almost all the explant and PGRs treatments except leaf explant and control, in which friable white and light green color callus was formed from which less number of shoot were obtained (Table 1).

Approximately over a period of 60 days initiation of shooting was observed in all type of explants from callus (Plate 1. D-F). A significant improvement was observed in all the explants by PGRs treatments. In case of petiole with nodal segment explant 4 fold multiple shooting (4.0±0.0 shoot/explant) with 100 % rooting was observed (Plate 1. G); in B, K, I and IK the same explant showed 2 to 3 fold multiple shooting (3.5±0.71, 3.1±0.72, 3±0.67 and 2.4±0.52 shoots/explant respectively) as well as rooting (3.5±0.52, 2.1±0.48, 2.6±0.52 and 1.8±0.63 rooting/explant respectively) in comparison to control (1.7±0.48 shooting and 1.4±0.52 rooting). The results of petiole explant on various PGRs are significantly different at $P<0.001$ level of significance. Similar results were also found in other explants- apical shoot, shoot with internode and leaf explants, that showed same trend of results in respect of the PGRs used over control and the differences are statistically significant at $P<0.001$ level significance for both multiple shooting as well as rooting (Table 2 & 3).

3.3 Acclimatization

Thus, observations from the present investigations clearly suggest that responses to organogenesis in *C. tamala* are influenced by explant type and specific hormonal combinations in the medium. After 30 days of acclimatization in culture condition 90% plantlets survived. Subsequently plantlets were transplanted to nursery and kept under shade in a net house for further growth and development. After 30 days these plants displayed 70% survival under field conditions and exhibited vigorous growth (Plate 1. H).

Table 1. Effect of different PGRs combination on callus induction, multiple shoot regeneration and rooting % on various explants

SI No.	Explants	Hormone combination	Callus formation	Kind of callus	Callus color	No. of shoots /explant	Rooting%
1	Petiole with nodal segment	Control	+	Friable	Light green	1.7	82
2		I	++	Compact	Light green	3	87
3		K	+++	Compact	Greenish white	3.1	87
4		B	+++	Compact	Greenish white	3.5	97
5		IK	++	Compact	Light green	2.4	75
6		BI	+++	Compact	Greenish white	4	100
7	Apical shoot	Control	+	Friable	White	1.5	67
8		I	++	Compact	Light green	2.4	88
9		K	++	Compact	Greenish white	2.3	87
10		B	+++	Compact	Greenish white	3.3	85
11		IK	++	Compact	Light green	2.2	68
12		BI	+++	Compact	Greenish white	3.6	89
13	Shoot with internode	Control	+	Friable	White	0.8	50
14		I	++	Compact	Light green	2.1	81
15		K	++	Compact	Light green	2.5	80
16		B	++	Compact	Light green	3	73
17		IK	+	Friable	White	1.3	77
18		BI	+++	Compact	Greenish white	3.4	74
19	Leaf	Control	+	Friable	White	0.4	0
20		I	+	Friable	White	1.2	67
21		K	+	Friable	Light green	1.3	77
22		B	+	Friable	Light green	1.5	73
23		IK	+	Friable	White	1	60
24		BI	++	Compact	Greenish white	2.2	77

I=Indole Butyric Acid (5µM), K=Kinetin (2µM), B=Benzyladenine (2.5µM), LSD-Least Significant Difference, + =Poor callus, ++ =Good callus, +++ =Very good callus

Table 2. Multiple shooting per explant on various explants using different combination of PGRs

	Plant growth regulators						
Explants	Control	I	K	B	IK	BI	P value (LSDat5%)
Petiole (nodal)	1.7±0.48	3±0.67	3.1±0.72	3.5±0.71	2.4±0.52	4.0±0.0	<0.001(0.50)
Apical tip	1.5±0.53	2.4±0.52	2.3±0.48	3.3±0.48	2.2±0.78	3.6±0.52	<0.001(0.49)
Shoot (Internode)	0.8±0.63	2.1±0.74	2.5±0.53	3±0.47	1.3±0.48	3.4±0.52	<0.001(0.50)
Leaf	0.4±0.52	1.2±0.63	1.3±0.67	1.5±0.71	1±0.82	2.2±0.63	<0.001(0.59)
	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
P value (LSDat5%)	(0.48)	(0.56)	(0.54)	(0.42)	(0.59)	(0.55)	

Table 3. Rooted shoots on various explants using different combination of PGRs

	Plant growth regulators						
Explants	Control	I	K	B	IK	BI	P value (LSDat5%)
Petiole (nodal)	1.4±0.52	2.6±0.52	2.7±0.48	3.4±0.52	1.8±0.63	4.0±0.0	<0.001(0.43)
Apical shoot	1.0±0.47	2.1±0.32	2.0±0.0	2.8±0.63	1.5±0.71	3.2±0.42	<0.001(0.42)
Shoot (Internode)	0.4±0.52	1.7±0.48	2.0±0.67	2.2±0.63	1.0±0.67	2.5±0.53	<0.001(0.51)
Leaf	0	0.8±0.79	1.0±0.67	1.1±0.32	0.6±0.69	1.7±0.48	<0.001(0.49)
	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001	
P value (LSDat5%)	(0.38)	(0.48)	(0.46)	(0.47)	(0.59)	(0.36)	

Table 4. Comparisons of shooting (Numbers/10 explant), rooted shoots and percent rooting response of four different explant type and six PGRs combinations in *C. tamala*

		Treatments					
Explants		Control	I	K	B	IK	BI
Petiole	Shoots	17	30	31	35	24	40
	Rooted shoots	14	26	27	34	18	40
	% Rooting	82	87	87	97	75	100
Apical shoot	Shoots	15	24	23	33	22	36
	Rooted shoots	10	21	20	28	15	32
	% Rooting	67	88	87	85	68	89
Shoot	Shoots	8	21	25	30	13	34
	Rooted shoots	4	17	20	22	10	25
	% Rooting	50	81	80	73	77	74
Leaf	Shoots	4	12	13	15	10	22
	Rooted shoots	0	8	10	11	6	17
	% Rooting	0	67	77	73	60	77

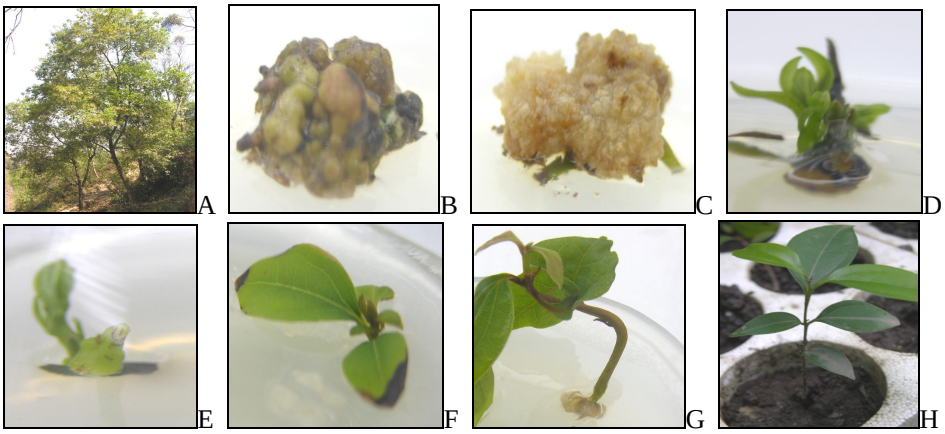

Plate 1. (A-G)
A. A mature tree of *Cinnamomum tamala*
B. Compact greenish white colored callus
C. Friable white colored callus
D. Multiple shooting from callus
E & F Separated shoots from callus
G. Rooting in shoots
H. Acclimatization of plantlets of *Cinnamomum tamala* in soil

4. Discussion

The procedure described here is the first successful plant regeneration system for *C. tamala* through indirect organogenesis using a range of explants and PGRs combinations. Thus, the results of the present investigation reflect the existence of a large inter-explant variability in callusing responses of the target taxa. Such variable responses for different explant types have also been reported in other species (Pereira et al., 2000; Dhar and Joshi, 2005). Such variations can be attributed to the physiological condition of the explant, which is determined by genetic factors (Baroncelli et al., 1978; Nagarathna et al., 1991).

The present investigation has shown that the response of *C. tamala* to micropropagation seems to be dependent more precisely on the explant type as well as on the PGRs treatments. The petiole with nodal segment was found the best source of explant both for callogenesis (Table 1) as well as multiple shooting with good rooting (Table 4) in all PGRs in comparison to other explants used. Similarly high regeneration capacity of petiole with nodal segment explant in comparison of other explants has also been reported in other species of this genus (for eg. *C. camphora* Babu et

al., 2003). Leaf explant performed poorly with all type of treatments in present experiment. Similar results were found in *C. camphora* and *C. verum* from leaf explant (Soulange et al., 2007). Therefore, explant type had significant effect on callus induction, shoot and root regeneration. Such types of variation in response to different explant type have been reported earlier (Luo and Jia, 1998; Rout et al., 1999; Manjkhola et al., 2005).

In *C. tamala*, a combination of BA and IBA was found to be suitable for induction of callus, multiple shooting and rooting in all type of explants in contrast to other treatments (Table 4). Efficacy of BA for shooting and IBA for rooting has also been found for plantlet regeneration in *C. kanehirae* (Chang et al., 2002), combination of BA and IBA for multiple shoot induction in *Arnebia euchroma* (Manjkhola et al., 2005) and for somatic embryogenesis in *Quercus semecarpifolia* (Tamta et al., 2009). Data from the current study provide strong evidence that growth regulator requirements for callus induction vary depending on the source of the explant. This possibly results from the variation in morphological and biochemical characters of different explant types, which affects cytokinin uptake and competence of cells to initiate callus or shoots.

5. Conclusion

In conclusion, the present investigation reports an efficient and easy-to-handle protocol for organogenesis through callus for Cinnamomum tamala. Petiole with nodal segment is the best explant source for the species and very good callus can be induced in WPM medium supplemented with Benzyladenine (2.5µM) and Indole butyric acid (5µM) combination. In the context of best explant selection, the maximum 4 fold shooting and 100 % rooting was found in petiole with nodal segment explant. The sequentially decreasing order was found in apical shoot, shoot with internode and leaf explant and the differences were statistically highly significant in all the PGRs treatments. Similarly in the best PGRs selection experiment, BI combination was found superior in all the explants. The successively decreasing order was found in Benzyladenine, Kinetin, Indole butyric acid alone and Indole butyric acid+ Kinetin combination and control treatments in all the explants with highly significant results.

Following the procedure described here, approximately 4 well rooted plantlets can be developed from a single explant, within 4-5 months without harvesting or damaging the source material. This protocol could be useful in multiplying an elite stock of the species within a limited time. The importance of developing the callus line has increased over the years because of active compound production, and the possibility of genetic transformation in the pharmaceutical sector. The present callus regeneration system may also be important for advance studies on genetic improvement and, in the future, also has considerable potential as an alternative means for production of known and new secondary metabolites.

Acknowledgements:

This study was financed by the National Medicinal Plants Board, Government of India.

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7/2/2009

DNA Extraction from Different Preserved Tissue of *Cassidula aurisfelis* for PCR study

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Abstract: Preserving DNA within tissue make the ability to collect and stabilize samples in the field or operating room, also making it easier to use the sample for histology and DNA isolation. Two preservative methods, Ethanol 95% and TNES-Urea Buffer were used. The best method makes DNA quality clear and sharp on gel electrophoresis. TNES-Urea buffer make clear pattern. Two extraction methods were using for yield, quality and suitability of genomic DNA for RAPD marker amplification in *Cassidula auresfellis.* Phenol chloroform method makes clear and sharp DNA quality for preserved samples (Ethanol 95% and TNES-Urea Buffer) and the purity between 1.0847 - 1.6715. Screening of RAPD marker produced 90% in TNES-Urea Buffer samples and 70% in Ethanol 95%.
[Nature and Science. 2009;7(9):8-14]. (ISSN: 1545-0740).

Key words: Preservation, DNA extraction, PCR, RAPD, snail.

1. Introduction

Cassidula aurisfelis is known as Angulated Shoulder Ear Snail, Angulate Vassidula or Cat's Ear Cassidula in English (Smith, 1992). It belongs to the great division or phylum called the Mollusca and from class of gastropoda. The shell is one piece (univalve) and may be coiled and uncoiled. Body bilaterally symmetrical and unsegmented, usually with definite head. Feeding habits of snails are as varied as their shape and habitats, but all include the use of some adaptation of the radula.

Owing to recent innovation in molecular biological techniques, such as polymerase chain reaction (PCR) and DNA automated sequencing, nucleic acid data are becoming more and more important in biology (Hillis *et al.,* 1996). One of the modern marker techniques for studying genetic variability is Random Amplified Polymorphic DNA, RAPD (Williams *et al.,* 1990). The technique requires no prior knowledge of the genome and it needs only a small amount of DNA (Hadrys *et al.,* 1992). Using this technique polymorphism can be detected in closely related organism.

Preservation is really important procedure to make samples keep on original quality. According to Dessaure *et al*., (1996), preservation of tissues for DNA extraction is important because it can protect these potentially valuable resources. In this study, there are two preservative were used to examine and determined the effects to DNA of the samples (TNES-Urea buffer and ethanol 95% solution). Usually TNES-Urea buffer were used in fish preservation. These study were detect the effected of TNES-Urea buffer to the DNA of mollusk. Ethanol solutions are one of the methods for tissue preservation for DNA analysis. Ethanol is suitable to the storage of vertebrate tissue and has been used successfully in DNA hybridisation and sequencing (Dessaur *et al*., 1996). 95 - 100% ethanol at ambient temperature were used to tissue samples from invertebrate for molecular studies (Winsor, 1998).

2. Materials and Methods

2.1 Sample Collections.

The samples of *Cassidula aurisfelis* were collected randomly from the area in Setiu Wetland, Setiu, Terengganu. 15 individuals were collected randomly around this area by hand packing. All the samples were collected during the low tide of water. The length, width, thickness and weight from each sample were measured.

2.2 Preservation

There are two preservative for preservation, Ethanol 95% and TNES-Urea Buffer. Ethanol 95% were prepared by dilutes 100% Ethanol to 95% Ethanol. The solution of TNES-Urea Buffer (Tris ; for 200 ml : 2 ml of 1 M pH 7.5 ; final conc. : 10 mM, NaCl ; for 200 ml : 5 ml of 5 M ; final conc. : 125 mM, EDTA-2Na ; for 200 ml : 2 ml of 0.5 M pH 7.5 ; final conc. : 10 mM,

SDS ; for 200 ml : 10 ml of 10 % ; final conc. : 0.5 %, Urea ; for 200 ml : 48.05 g ; final conc. : 4 M) were mixed (Asahida *et al*., 1996). This samples preservation was saved for three and four month.

2.3 Kit Wizard™ Genomic DNA Purification (Promega)

DNA from all snail body tissue was extracted from the samples by using Kit Wizard™ Genomic DNA Purification (Promega). About 70 mg of all body tissue were used for the extraction of the DNA. 600 µl of nuclei lysis were added to the all body tissue into 1.5 ml micro centrifuge tube. The mixtures then were homogenized to get the lysat. Then the sample was incubated in the water bath at 65°C for about 15 to 20 minutes. After that, it was treated with 3.0 µl of RNase. The sample then was incubated again in water bath at 37°C for 15 to 20 minutes. Next is the sample was left at room temperature for 5 minutes.

About 200 µl Protein Precipitation were added in the sample and then the sample were vortex at highest maximum speed for about 20 seconds. Then the sample was centrifuged at 14,000 rpm at room temperature for 3 minutes. The supernatant that contain DNA will be removed to put into a new micro centrifuge which contains 600 µl of isopropanol. The sample was centrifuged once again at 14,000 rpm at room temperature for 2 minutes. Next step is 600 µl of ethanol (70 %) were added to the pellet to wash the DNA. Once again the sample was centrifuged at 14,000 rpm at room temperature for 1 minute. Then the DNA was dried at room temperature for 10 to 15 minutes. Then the DNA was resuspended with 100 µl of DNA rehydration for 1 hour. The DNA extraction sample was keep at -20°C to avoid DNA from degradation.

2.4 Phenol-Chloroform Method

DNA was extracted based on the Phenol-chloroform method described by Brown *et al.* (1991) with some modifications. Digestion buffer at volume of 500 µl containing (1 % (w/v) Sodium Dodecyl Sulphate 0.8 %, Triton X-100, 0.5 M NaCl, 0.1 M Tris-Hcl at pH 9, 0.01 M EDTA) were added into 1.5 ml microcentrifuge tube which containing 70 mg all snail body tissue and then the 40 µl of 10 % (w/v) SDS and Proteinase K (20 mg/ml solution) were added. The tube was shaken gently and was incubated at 55^0C for 1 to 2 hours. The sample was treated with 25 µl of RNase. Then, the mixture was left at room temperature for 15 to 30 minutes. The sample were treated with 500µl of phenol:chloroform:isoamyl alcohol (25:24:1) and gently the tube were vortexed to homogenize.

The sample was left at room temperature for 10 minutes before doing centrifugation at 13,000 rpm for 5 minutes. The top later is aqueous and were remove and dispersed into the new microcentrifuge tube. The step of adding phenol:chloroform:isoamyl alcohol were repeated twice. The samples were treated with 500 µl of chloroform:isoamyl alcohol (24:1) and were centrifuged at 13,000 rpm for 5 minutes. The upper aqueous layer was mixed with 1 ml of ice-cold absolute ethanol by rapid inversion of the tubes several times. Then, centrifuge at 6,000 rpm for 30 minutes and after that the precipitated DNA were collected at the bottom tubes as a white pellet. The pellet was washed with 500 µl of 70 % of ethanol and was centrifuge at 6,000 rpm for 15 minutes. The DNA was allowed to dry at room temperature. Then resuspended with 100 µl TE buffer (10 mM Tris and 1 mM EDTA, pH 8) for at least 24 hours at room temperature to fully dissolved before proceeding to the next step. This DNA extraction samples will be kept in – 20^0C to avoid DNA degradation.

2.5 Measurement of DNA Purity and Quality

The samples were separated by agarose gel electrophoresis through 1.0 % of agarose gel in 1.0 X TBE. Electrophoresis was run at 55 volts for 1 to 2 hours. Then, the gel was stained with ehidium bromide for 20 to 30 minutes and washed with distilled water for 5 to 10 minutes. The gel was photographing with Image Master VDS (American Pharmacia, Biotech).

The genomic DNA was quantified using UV-spectrophotometer. The quantity of DNA were measured by obtaining the absorbance reading at 260 nm and the purity of DNA were estimated by calculating the ratio of absorbance reading at 260nm and 280nm. The quantification can be determined base on ratio ($OD_{260}/_{280}$). An OD of 1 corresponds to approximately 50 µg/ml for double-stranded DNA (Sambrook *et al.,* 1989). The DNA concentration was determined by the formula:

DNA concentration = OD_{260} × 50 µg/ml × dilution factor (Linacero *et al.,* 1998).

2.6 Screening of RAPD Primers

10 RAPD primers (Table 1) from Operon Technology were screened from a single individual. Primers that have the basic of sharpness, clarity of the profile and the existence of polymorphism usually were chosen for further study. (D'Amato and Corach, 1997).

The total reaction volume of 25 µl were used with the final concentration containing 1.0 × of reaction buffer included the concentration of genomic DNA 50 ng, Fermentas Magnesium Chloride 4.0 mM, Fermentas Taq DNA Polymerase 2 units, Fermentas dNTP-mixture 0.4 mM and primer 10 pM.

The DNA was amplified by using a Master Cycles

Gradient (Eppendorf). The amplification were programmed at 45 cycles for 30 seconds of denaturation at 94°C, 30 seconds of annealing temperature at 36°C, 1 minutes of primers extension at 72°C and final extension of 2 minutes at 72°C.

Table 1 Code, sequence, nucleotide length and G+C content of primers used in RAPD analysis

No.	Primer Code	P Primer sequence 5' to 3'	Nucleotide length	G+C content (%)
1	OPA 01	CAGGCCCTTC	10-mers	70
2	OPA 02	TGCCGAGCTG	10-mers	70
3	OPA 03	AGTCAGCCAC	10-mers	60
4	OPA 04	AATCGGGCTG	10-mers	60
5	OPA 05	AGGGGTCTTG	10-mers	60
6	OPA 06	GGTCCCTGAC	10-mers	70
7	OPA 07	GAAACGGGTG	10-mers	60
8	OPA 08	GTGACGTAGG	10-mers	60
9	OPA 09	GGGTAACGCC	10-mers	70
10	OPA 10	GTGATCGCAG	10-mers	60

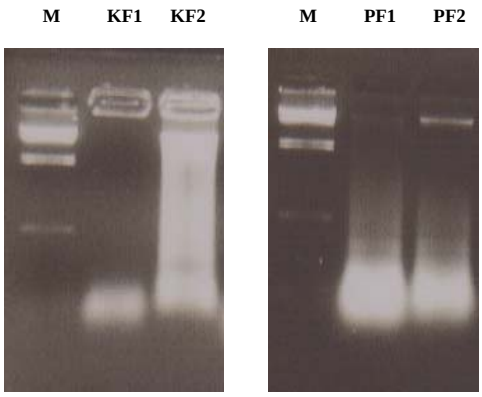

Figure 1 Genomic DNA extracted for fresh tissues, λ DNA/Hind III marker (lane M), (K - Wizard Genomic DNA Purification Kit (Promega) protocol, P – Phenol Chloroform Method, F – Fresh tissue).

3. Result and Discussion

3.1Extraction of DNA

The DNA extraction method was obtained from Kit Wizard™ Genomic DNA Purification and Phenol-chloroform Method. The Genomic DNA was successfully extracted and observed to have impurity and purity. Using Phenol-chloroform method, the clear band and high purity of DNA was obtained. According to Zhang and Hewitt (1998), the samples collected from remote areas have to be preserved before DNA analysis is carried out. The fresh tissue, muscles or blood sample provides the best source of DNA biological analysis (Parenrengi, 2001).

Fresh samples extraction had shown (Figure 1) no DNA and degraded band for Kit Wizard™ Genomic DNA Purification. For Phenol-chloroform method, the samples also degraded. The degraded for fresh samples usually causes by contamination of fresh samples by contain other particles when doing extraction.

Extraction using Kit Wizard™ Genomic DNA Purification for preserved sample in TNES-Urea Buffer produced degraded band for third month preserved and no DNA for fourth month preserved on the electrophoresis gel. However, sample preserved in 95% ethanol show no DNA and degrade band for third and fourth month preserved (Figure 2).

Phenol-chloroform had a good result, the DNA clear banding pattern was obtained in third and fourth month in TNES-Urea buffer and Ethanol 95% preserved samples. Clear banding pattern are shown in Figure 3.

Samples preserved in TNES-Urea buffer yielded proper and nice band fragments on the electrophoresis agarose gel. TNES-Urea buffer consist a few chemicals such as Tris-HCl, Natrium Chloride, EDTA, Sodium Dedocyl Sulphate, which had an agent that assimilate the whole tissue samples and DNA. All the samples that been preserved in TNES-Urea buffer were fully digested in the solution. These make easy to lyses the muscle of samples.

For samples preserved in ethanol 95% were producing improper result due to degradation. All the tissue samples in ethanol 95% became hard and maintain condition as long as it had been preserved but the ethanol solution sometimes is evaporated and dried. Tissue samples in ethanol not easy to be lyses and homogenized.

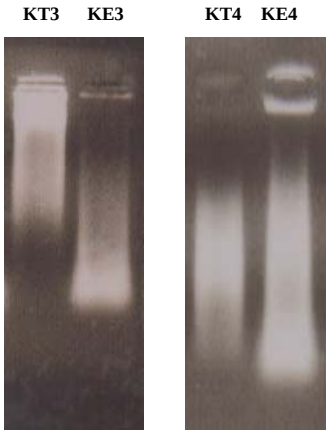

Figure 2 Genomic DNA extracted for Kit Wizard™ Genomic DNA Purification, λ DNA/Hind III marker (lane M), (T – TNES-Urea Buffer, E – Ethanol 95%, 3 – Third month, 4 – Fourth month).

During organic extraction, protein contaminants are denatured and partition either with the organic phase or at the interface between organic and aqueous phases, while nucleic acids remain in the aqueous phase. Phenol used in this protocol is buffered to prevent oxidized products in the phenol from damaging the nucleic acids (University of Regina, 1998).

3.2 Purity and Quantity of DNA

The DNA purity using Wizard Genomic DNA Purification Kit (Promega) was ranged from 1.1870 to 1.3554, while DNA purity with Phenol-chloroform Method was from 1.0847 to 1.6715. The range was estimated quantitatively from the ratio between the reading of absorbancy at 260nm and 280nm ($OD_{260}/_{280}$) in UV-Biophotometer. Quantity of DNA with Wizard Genomic DNA Purification Kit (Promega) calculated ranged from 337.50 to 906.00µg/mL while quantity of DNA with Phenol-chloroform Method ranged from 160.00 to 1005µg/mL. Through the result obtained, *Cassidula aurisfelis* contains high concentration of

DNA using Phenol Chloroform Method. This high concentration of DNA will banding pattern of DNA amplification. The values of the DNA purity and quantity of Kit Wizard™ Genomic DNA Purification and Phenol-chloroform Method are shown in Table 2.

Previous studies suggested the use of genomic DNA ranging from 1.8 to 2.0 in purity for PCR requirement in amplification of DNA. Purity of genomic DNA lower than 1.8 was contaminated with protein, while more than 2.0 purity of genomic DNA was suspected to be contaminated with organic matters residue, derived from the DNA extraction (Sambrook *et al.*, 1989).

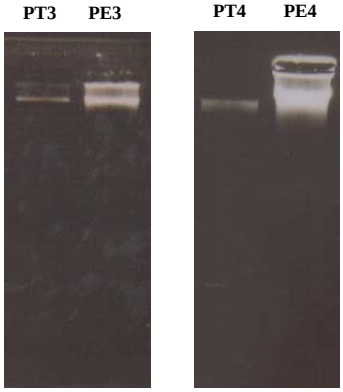

Figure 3 Genomic DNA extracted for Phenol-Chloroform method, λ DNA/Hind III marker (lane M), (T – TNES-Urea Buffer, E – Ethanol 95%, 3 – Third month, 4 – Fourth month).

In this case, the causes for obtain high quality DNA such as food or feces remaining inside the abdomen, which could promote the degradation of the DNA and contribute to contamination (Zhang and Hewitt, 1998). For the poor quality, according to Pearson and Sterling (2003), some tissues contain large amount of connective tissue and are difficult to digest, these can be ground in a mortar and pestle before being digest with nuclei lysis.

3.3 Screening of RAPD Primers

Ten primers from the Operon 10 mers (Operon Kit A) (OPA 01 to OPA 10) with 60% – 70% GC content were used during the screening of the RAPD primers. Screening using TNES-Urea Buffer produced more amplication band on gel electrophoresis (Figure 4). The site of band shown between 150 – 1200bp. The degrade DNA from preserved samples in Ethanol 95% (Figure 5), shown poor result which only OPA 02, OPA 03, OPA 04, OPA 07, OPA 08, OPA 09 and OPA 10 produced band on gel electrophoresis. The site of band shown between 250 – 1200bp.

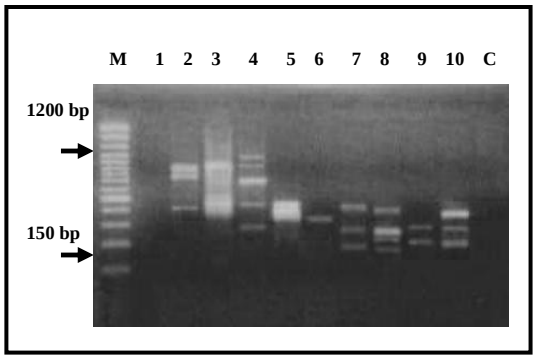

Figure 4 RAPD (TNES-Urea buffer) banding patterns for screening of 1st base primers, OPA 01 to OPA 10 (lane 1 to 10). (Lane M is a marker 100bp ladder plus, C - Control).

Lower number of amplicons in extracted DNA of preserved tissue in TNES-Urea buffer and Ethanol 95% suggests presence of contaminants like polysaccharide and polyphenols as well as RNA, which inhibits *Taq* polymerase (Scott and Playford, 1996). DNA quality is a major factor in genetic analysis using molecular markers in earlier reports on plants (Weeden et al., 1992; Staub et al., 1996) and it same with animals.

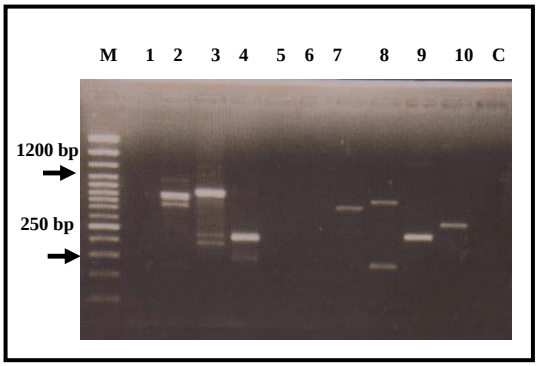

Figure 5 RAPD (Ethanol 95%) banding patterns for screening of 1st base primers, OPA 01 to OPA 10 (lane 1 to 10). (Lane M is a marker 100bp ladder plus, C - Control).

Some polymorphisms were easy to score whereas other bands appeared to produce ambiguous fragments (William *et al*., 1990). The best primers will produce more than three fragments and clear. The number of fragments generated is dependent on the primer sequence rather than to the nucleotide length.

Table 2 Observed density (OD) of purity and quantity of DNA for Genomic DNA extracted by Kit Wizard™ Genomic DNA Purification and Phenol-chloroform Method.

Sample	Average OD_{260}	Average OD_{280}	Ratio OD_{260}/OD_{280}	Quantity DNA(µg/mL)
KF1	0.1365	0.1090	1.2523	341.25
KF2	0.1880	0.1490	1.2617	470.00
KT3	0.1350	0.1030	1.3107	337.50
KE3	0.3205	0.2700	1.1870	801.25
KT4	0.1640	0.1210	1.3554	410.00
KE4	0.3600	0.2820	1.2766	906.00
PF1	0.1970	0.1390	1.4173	492.50
PF2	0.1230	0.0950	1.2947	307.50
PT3	0.0640	0.0590	1.0847	160.00
PE3	0.0935	0.0820	1.1402	233.75
PT4	0.1270	0.0970	1.3093	317.50
PE4	0.4020	0.2405	1.6715	1005.00

Table 3 Number of bands for *Cassidula aurisfelis* generated from OPA 01 - OPA 10 in differences preservation extracted using Phenol-Chloroform Method.

Number of band	OPA 1	OPA 2	OPA 3	OPA 4	OPA 5	OPA 6	OPA 7	OPA 8	OPA 9	OPA 10
TNES	0	3	2	4	2	1	3	3	2	3
Ethanol 95%	0	3	3	2	0	0	1	2	2	1

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7/7/2009

Model for Evaluating Essential Volume Parameters During Drying of Wet Clay

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Abstract: Model for evaluating essential volume parameters during drying of wet clays has been derived. The clays were prepared using three different grain sizes; <100μm, 100-300μm and 300-1000 μm and fired to a temperature of 1200^0C for 18hrs. The derived model;

$$V_o = \left(\frac{V}{e^{(x+\gamma)}}\right)$$

is dependent on the total volume of clay body before drying, the expansion factor and the volume fraction of water removed following the drying process. The expansion factor which forms part the model was found to depend significantly on the grain size of clay materials, clay mineralogy and the water expansion coefficient.
[Nature and Science 2009;7(9):15-21]. (ISSN: 1545-0740).

Keywords: Model, Evaluation, Volume Parameters, Drying, Wet Clays.

1. Introduction

Reed (1988) 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 (Barsoum,1997;Viewey and Larrly,1978;Keey, 1978) 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 (Reed,1988) 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 (1978) also in a similar study suggested that at this stage, free water is removed between the particles and the inter-particle separation decreases, resulting in shrinkage. During the decreasing rate, particles make contacts as water is removed, which causes shrinkage to cease.

Model for calculating the volume shrinkage resulting from the initial air-drying of wet clay has been derived (Nwoye, 2008). The model;

$$\theta = \gamma^3 - 3\gamma^2 + 3\gamma \qquad (1)$$

calculates the volume shrinkage when the value of dried shrinkage γ, experienced during air-drying of wet clays is known. The model was found to be third-order polynomial in nature. Olokoro clay was found to have the highest shrinkage during the air drying condition, followed by Ukpor clay while Otamiri clay has the lowest shrinkage. Volume shrinkage was discovered to increase with increase in dried shrinkage until maximum volume shrinkage was reached, hence a direct relationship.

Nwoye et al. (2008) derived a model for the evaluation of overall volume shrinkage in molded clay products (from initial air-drying stage to completion of firing at a temperature of 1200^0C). It was observed that the overall volume shrinkage values predicted by the model were in agreement with those calculated using conventional equations. The model;

$$S_T = \alpha^3+\gamma^3-3(\alpha^2+\gamma^2)+3(\alpha+\gamma) \qquad (2)$$

depends on direct values of the dried γ and fired shrinkage α for its precision. Overall volume shrinkage was found to increase with increase in dried and fired shrinkages until overall volume shrinkage reaches maximum.

Nwoye (2009a) derived a model for calculating the quantity of water lost by evaporation during oven drying of clay at 90^0C. The model;

$$\gamma = \exp[(\ln t)^{1.0638} - 2.9206] \qquad (3)$$

indicated that the quantity of evaporated water, γ during the drying process is dependent on the drying time t, the evaporating surface being constant. The validity of the model was found to be rooted in the expression $(\text{Log}\beta + \ln\gamma)^N = \ln t$.

Model for predictive analysis of the quantity of water evaporated during the primary-stage processing of a bioceramic material sourced from kaolin has been derived by Nwoye (2009b). The model;

$$\alpha = e^{(\ln t/2.1992)} \quad (4)$$

shows that the quantity of water α, evaporated at 110^0C, during the drying process is also dependent on the drying time t, where the evaporating surface is constant. It was found that the validity of the model is rooted on the expression $(\ln t/\ln\alpha)^N = \text{Log}\beta$ where both sides of the expression are correspondingly approximately equal to 3. The respective deviation of the model-predicted quantity of evaporated water from the corresponding experimental value was found to be less than 22% which is quite within the acceptable deviation range of experimental results.

Model for quantifying the extent and magnitude of water evaporated during time dependent drying of clay has been derived (Nwoye et al.,2009). The model;

$$\gamma = \exp((\ln t/2.9206)^{1.4}) \quad (5)$$

indicates that the quantity of evaporated water γ during the drying process (at 90^0C) is dependent on the drying time, t the evaporating surface being constant. It was found that the validity of the model is rooted in the expression $\ln\gamma = (\ln t/\text{Log}\beta)^N$ where both sides of the expression are correspondingly almost equal.

The present work is to derive a model for evaluating essential volume parameter during drying of wet clays.

2. Materials and Methods

2.1 Clay preparation

All clays (Olokoro, Ukpor, and Otamiri) used were collected in lumps from deposits. These clays were allowed to dry in air for 96 hours. Each of those clay samples were crushed and sieved to <100μm, 100-300μm and 300-1000 μm particle sizes using assembly of sieves and sieve shaker. Each sample was manually homogenized using 2% starch as binder. Samples were mixed with water (6% of the total weight of dry materials). The plastic clays from the three clay samples were kneaded using hand to expel any trapped air from the clays. The samples were moulded in a rectangular wooden mould of dimension 70mm x 17mm x 9mm for each of the clay sample.

2.2 Firing process and evaluation of essential volume parameters

These samples were dried under the laboratory temperature condition (25^0C) for 18hrs after which they were carefully packed in saw-dust to prevent them from cracking and absorbing moisture from the surrounding. These samples were then fired using electric kiln. The samples were charged at lower temperature (125^0C) after which the temperature was increased to 1200^0C. These samples were fired for 18hrs and then cooled in furnace for the same time limit.

After firing, new volumes, V_o, V_R were calculated using the new dimensions of the drying sample. The initial volume of the sample V, which is the total volume, was also calculated using the initial dimensions of the sample before drying. The values of the volume shrinkage, water removed (x) and expansion factor (γ) were also calculated. The values of the correlations between $\ln(V/V_o)$ and x were calculated using regression analysis method where $\ln(V/V_o)$ is Y axis and x, X axis.

Following experiment carried out,

V = Total volume of clay body before drying (mm^3)
V_o = Dry volume of clay body after drying (mm^3)
x = Volume fraction of water removed after drying
V_R = Volume of water removed after drying
(γ) = Expansion factor

Past report (Cooke,1988) has shown that volume shrinkage can be calculated using the formular;

$$\%\ Vs = \left(1-\left(1-\left(\frac{L_1 - L_2}{L_1}\right)^3\right)\right) \times 100 \quad (6)$$

Where

L_1 = Dried length of sample after air-drying (mm)
L_2 = Fired Length (mm)
Vs = Volume shrinkage (%)

3. Model Formulation

Results of the experiment carried out in this work as shown in Tables 2-12 indicate that;

$$\ln\left(\frac{V}{V_o}\right) - x = \gamma \quad (7)$$

Where x, γ and $\ln(V/V_o)$ from equation (7) are fractional values.

$$x = \left(\frac{V - Vo}{V}\right) \quad (8)$$

$$V_R = V - Vo \quad (9)$$

Evaluating equation (7), reduces it to;

$$\ln\left(\frac{V}{V_o}\right) = x + \gamma \quad (10)$$

Taking the exponential of both sides of equation (10)

$$\left(\frac{V}{V_o}\right) = e^{(x+\gamma)} \quad (11)$$

$$V_o = \left(\frac{V}{e^{(x+\gamma)}}\right) \quad (12)$$

Equation (12) is the derived model

4. Boundary and Initial Conditions

Consider a rectangular shaped clay product of length 70mm, 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 sizes for the clay materials used are, <100μm, 100-300μm and 300-1000μm. Drying temperature used; 1200^0C, and drying time used; 18hrs. 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. All the water in the clay body was assumed to have been removed during the drying process.

5. Model Validation

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

Analysis and comparison between these V_o values reveal deviations of model-predicted V_o 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 expected to have played vital role during the evaporation of water were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model-predicted V_o value to that of the corresponding experimental value.

Deviation (Dn) (%) of model-predicted values from the experimental V_o values is given by

$$Dn = \left(\frac{P_D - E_D}{E_D} \right) \times 100 \qquad (13)$$

Where

P_D = Dry volume of clay (after drying) as predicted by model (mm^3)

E_D = Dry volume of clay (after drying) as obtained from experiment (mm^3)

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

$$Cf = -Dn \qquad (14)$$

Therefore

$$Cf = -100 \left(\frac{P_D - E_D}{E_D} \right) \qquad (15)$$

Introduction of the value of Cf from equation (15) into the model gives exactly the corresponding experimental value of V_o.

6. Results and discussions

Comparison of Tables 1-10 shows that the magnitude of the various essential volumes and other related parameters vary with the clay mineralogy, clay particle size, coefficient of expansion and the expansion factor. Figs. 1-9 show close alignment between curves (V_o(exp) and V_o(mod)) of the dry volume of clay as obtained from experiment and derived model respectively. The degree of this alignment is a clear indication of the validity and precision of the derived model. The model shows similarities with Cooper`s equation (Cooper,1978); $\ln(V/V_s) = SC$, where V= Total volume of clay the body before drying, V_s = dry volume of clay body after drying, C = volume fraction of water in the clay body at any point in time and S = slope. In the case of the past related work (Cooper,1978), a plot of $\ln(V/V_s)$ against C gives the slope S as the coefficient of expansion α_c. It was found from the present work that since all the water in the clay body was removed, a plot of $\ln(V/V_o)$ against x also gives the slope as the coefficient of expansion α_c. Based on the foregoing, $V_s = V_o$ and C = x.

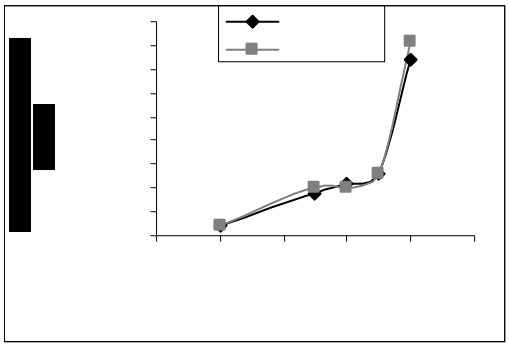

Fig.1 Comparison of the dry volumes of Olokoro clay as obtained from experiment and as predicted by model. (for particle size; <100μm)

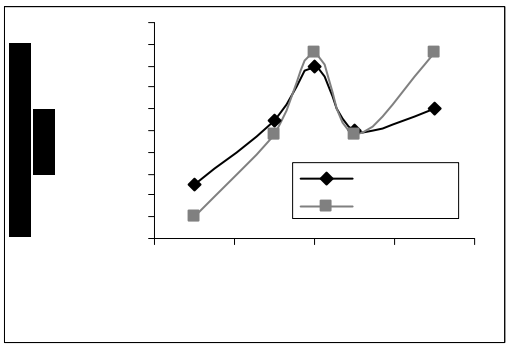

Fig.2 Comparison of the dry volumes of Olokoro clay as obtained from experiment and as predicted by model. (for particle size;100-300μm)

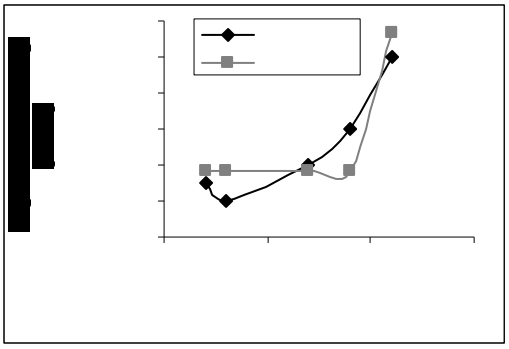

Fig.3 Comparison of the dry volumes of Olokoro clay as obtained from experiment and as predicted by model. (for particle size;300-1000μm)

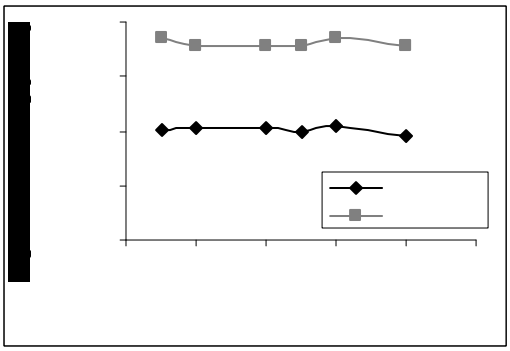

Fig.4 Comparison of the dry volumes of Ukpor clay as obtained from experiment and as predicted by model. (for particle size; <100μm)

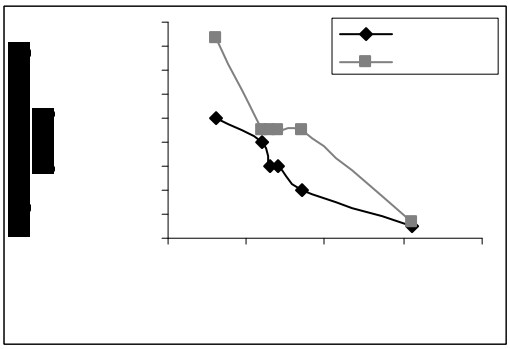

Fig.5 Comparison of the dry volumes of Ukpor clay as obtained from experiment and as predicted by model. (for particle size;100-300μm)

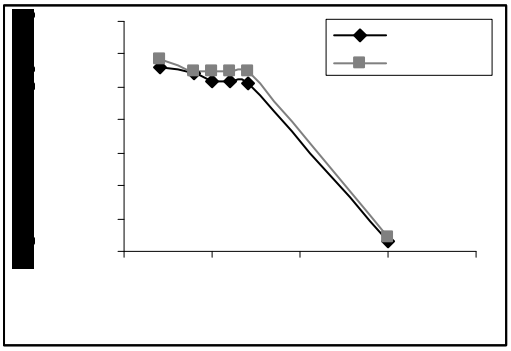

Fig.6 Comparison of the dry volumes of Ukpor clay as obtained from experiment and as predicted by model. (for particle size;300-1000μm)

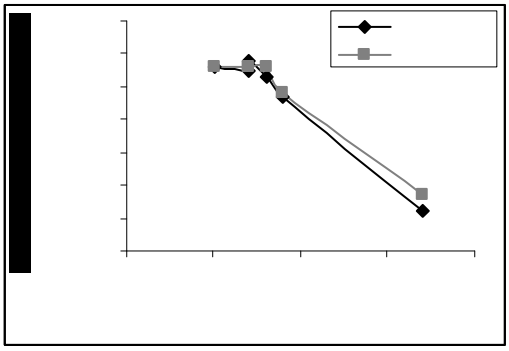

Fig.7 Comparison of the dry volumes of Otamiri clay as obtained from experiment and as predicted by model. (for particle size; <100μm)

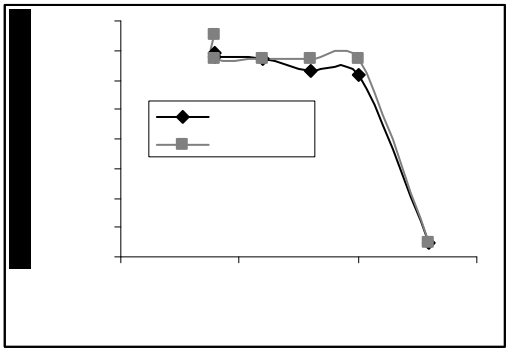

Fig.8 Comparison of the dry volumes of Otamirir clay as obtained from experiment and as predicted by model. (for particle size;100-300μm)

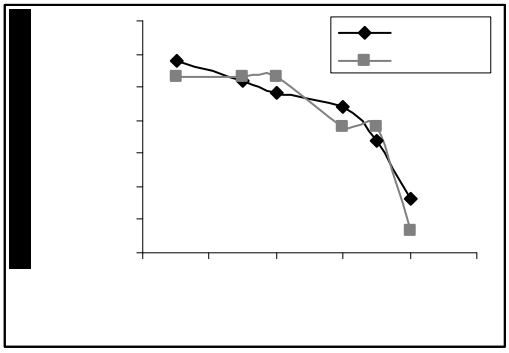

Fig.9 Comparison of the dry volumes of Otamiri clay as obtained from experiment and as predicted by model. (for particle size;300-1000μm)

Table 1: Chemical composition of clays used

Source	Al_2O_3	Fe_2O_3	TiO_2	MgO	CaO	SiO_2	N_2O	K_2O	Loss of ignition
Otamiri	15.56	0.05	1.09	-	0.29	69.45	0.01	0.21	13.10
Olokoro	20.10	7.05	-	0.75	1.26	45.31	0.05	0.09	11.00
Ukpor	31.34	0.63	2.43	0.14	0.06	51.43	0.04	0.10	12.04

Table2: Relationship between volume shrinkage and essential volume parameters resulting from drying of Olokoro clay (for particle size; <100µm), $\alpha_c = 0.7$

Vs	V_o(exp)	V	V_R	x	V/V_o	ln (V/V_o)	γ (10^{-3})
25.63	7610.4	8400	789.6	0.0940	1.1038	0.0987	4.7
25.66	7612.2	8400	787.8	0.0938	1.1035	0.0985	4.7
25.60	7609.7	8400	790.3	0.0941	1.1039	0.0988	4.7
25.64	7610.6	8400	789.4	0.0940	1.1037	0.0987	4.7
25.66	7613.2	8400	786.8	0.0937	1.1033	0.0983	4.7
25.65	7610.8	8400	789.2	0.0940	1.1037	0.0987	4.7

Table3: Relationship between volume shrinkage and essential volume parameters resulting from drying of Olokoro clay (for particle size; 100-300µm), $\alpha_c = 0.57$

Vs	V_o(exp)	V	V_R	x	V/V_o	ln (V/V_o)	γ (10^{-3})
25.07	7629.6	8400	770.4	0.0917	1.1010	0.0962	4.5
25.09	7629.8	8400	770.2	0.0917	1.1009	0.0961	4.4
25.03	7629.1	8400	770.9	0.0918	1.1010	0.0963	4.5
25.03	7630.4	8400	770.6	0.0917	1.1009	0.0961	4.4
25.06	7630.2	8400	769.8	0.0916	1.1009	0.0961	4.5
25.05	7629.7	8400	770.3	0.0917	1.1010	0.0962	4.5

Table4: Relationship between volume shrinkage and essential volume parameters resulting from drying of Olokoro clay (for particle size; 300-1000µm), $\alpha_c = 0.43$

Vs	V_o(exp)	V	V_R	x	V/V_o	ln (V/V_o)	γ (10^{-3})
24.82	7638.0	8400	762.0	0.0907	1.0907	0.0951	4.4
24.84	7638.2	8400	761.8	0.0907	1.0997	0.0951	4.4
24.86	7638.6	8400	761.4	0.0906	1.0997	0.0950	4.4
24.77	7637.9	8400	762.1	0.0907	1.0998	0.0951	4.4
24.78	7637.8	8400	762.2	0.0907	1.0998	0.0951	4.4
25.85	7634.4	8400	761.6	0.0907	1.0997	0.0950	4.3

Table5: Relationship between volume shrinkage and essential volume parameters resulting from drying of Ukpor clay (for particle size; <100µm), $\alpha_c = 0.45$

Vs	V_o(exp)	V	V_R	x	V/V_o	ln (V/V_o)	γ (10^{-3})
23.57	7680.0	8400	720.0	0.0857	1.0938	0.0897	2.9
23.56	7680.2	8400	719.8	0.0857	1.0937	0.0896	2.9
23.58	7680.4	8400	719.6	0.0857	1.0937	0.0896	2.8
23.54	7680.2	8400	719.8	0.0857	1.0937	0.0896	2.9
23.53	7680.1	8400	719.9	0.0857	1.0937	0.0896	2.8
23.60	7679.6	8400	720.4	0.0858	1.0938	0.0897	2.8

Table6: Relationship between volume shrinkage and essential volume parameters resulting from drying of Ukpor clay (for particle size; 100-300µm), $\alpha_c = 0.33$

Vs	V_o(exp)	V	V_R	x	V/V_o	ln (V/V_o)	γ (10^{-3})
23.14	7694.4	8400	705.6	0.0840	1.0917	0.0877	3.7
23.12	7694.6	8400	705.4	0.0840	1.0917	0.0877	3.7
23.17	7694.2	8400	705.8	0.0840	1.0917	0.0877	3.7
23.13	7694.4	8400	705.6	0.0840	1.0917	0.0877	3.7
23.06	7694.8	8400	705.2	0.0840	1.0916	0.0876	3.6
23.31	7693.9	8400	706.1	0.0841	1.0918	0.0878	3.7

Table7: Relationship between volume shrinkage and essential volume parameters resulting from drying of Ukpor clay (for particle size;300-1000µm), $\alpha_c = 0.3$

Vs	V_o(exp)	V	V_R	x	V/V_o	ln (V/V_o)	γ (10^{-3})
22.96	7700.4	8400	699.6	0.0833	1.0909	0.0870	3.7
22.94	7700.8	8400	699.2	0.0832	1.0909	0.0870	3.8
23.05	7690.6	8400	709.4	0.0845	1.0922	0.0882	3.7
22.97	7700.2	8400	699.8	0.0833	1.0909	0.0870	3.7
22.95	7700.3	8400	699.7	0.0833	1.0909	0.0870	3.7
22.92	7701.2	8400	698.8	0.0832	1.0907	0.0868	3.6

Table8: Relationship between volume shrinkage and essential volume parameters resulting from drying of Otamiri clay (for particle size; <100µm), $\alpha_c = 0.17$

Vs	V_o(exp)	V	V_R	x	V/V_o	ln (V/V_o)	γ (10^{-3})
20.52	7780.8	8400	619.2	0.0737	1.0796	0.0766	2.9
20.50	7780.6	8400	619.4	0.0737	1.0796	0.0766	2.9
20.52	7780.5	8400	619.5	0.0738	1.0796	0.0766	2.8
20.54	7779.7	8400	620.3	0.0738	1.0797	0.0767	2.9
20.62	7776.2	8400	623.8	0.0743	1.0802	0.0771	2.8
20.53	7780.3	8400	619.7	0.0738	1.0796	0.0766	2.8

Table9: Relationship between volume shrinkage and essential volume parameters resulting from drying of Otamiri clay (for particle size; 100-300µm), $\alpha_c = 0.25$

Vs	V_o(exp)	V	V_R	x	V/V_o	ln (V/V_o)	γ (10^{-3})
19.93	7804.3	8400	595.7	0.0709	1.0763	0.0735	2.6
19.91	7804.7	8400	595.3	0.0709	1.0763	0.0735	2.6
19.89	7804.9	8400	595.1	0.0708	1.0762	0.0734	2.6
19.89	7804.8	8400	595.2	0.0709	1.0763	0.0735	2.6
19.95	7804.2	8400	595.8	0.0709	1.0763	0.0735	2.6
19.98	7798.5	8400	601.5	0.0716	1.0771	0.0743	2.7

Table10: Relationship between volume shrinkage and essential volume parameters resulting from drying of Otamiri clay (for particle size;300-1000µm), $\alpha_c = 0.26$

Vs	V_o(exp)	V	V_R	x	V/V_o	ln (V/V_o)	γ (10^{-3})
19.63	7833.6	8400	566.4	0.0674	1.0723	0.0698	2.4
19.61	7833.9	8400	566.1	0.0674	1.0723	0.0698	2.4
19.64	7833.4	8400	566.6	0.0675	1.0723	0.0698	2.3
19.66	7833.2	8400	566.8	0.0675	1.0724	0.0699	2.4
19.68	7831.8	8400	568.2	0.0676	1.0726	0.0701	2.5
19.67	7832.7	8400	567.3	0.0675	1.0724	0.0699	2.4

Conclusion

The model evaluates essential volume parameters during drying of wet clay. The model is dependent on the total volume of clay body before drying, the expansion factor and the volume fraction of water removed following the drying process. The expansion factor which forms part the model was found to depend significantly on the grain size of clay materials, clay mineralogy and the water expansion coefficient.

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7/9/2009

Model for Computational Analysis of Water Absorption In Clay Materials Exposed To Hot-Humid Environment

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Abstract: Model for computational analysis of water absorption in clay materials exposed to hot-humid environment has been derived. These clay materials were prepared using different grain sizes; <100μm, 100-300μm, 300-1000 μm and their respective mixtures. The derived model;

$$\beta = \left(\frac{\gamma}{\alpha\left[(S)^{1.0753}\right]} \right)$$

was found to be dependent on the bulk density, apparent porosity and the shrinkage sustained on the clay body at any point in time under the hot-humid condition. The validity of the model is rooted on the expression; $S = (\gamma/\alpha\beta)^{0.93}$ where both sides of the expression are correspondingly almost equal. The maximum deviation of the model-predicted quantity of absorbed water from the corresponding experimental values is 7.5% which is within the acceptable range of deviation limit for experimental results. [Nature and Science 2009;7(9):22-25]. (ISSN: 1545-0740).

Keywords: Model, Water Absorption, Clay Materials, Hot-Humid Environment.

1. Introduction

Past report (Reed,1988) has 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. Barsoum (1997), Viewey and Larrly(1978) and Keey (1978) have studied the 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. Reed (1988) reported 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. The researcher posited that shrinkage occurs at this stage. In a similar study, Keey (1978) suggested that at this stage, free water is removed between the particles and the inter-particle separation decreases, resulting in shrinkage. During the decreasing rate, particles make contacts as water is removed, which causes shrinkage to cease.

Nwoye (2008) derived a model for calculating the volume shrinkage resulting from the initial air-drying of wet clay. The model;

$$\theta = \gamma^3 - 3\gamma^2 + 3\gamma \qquad (1)$$

calculates the volume shrinkage when the value of dried shrinkage γ, experienced during air-drying of wet clays is known. The model was found to be third-order polynomial in nature. Olokoro clay was found to have the highest shrinkage during the air drying condition, followed by Ukpor clay while Otamiri clay has the lowest shrinkage. Volume shrinkage was discovered to increase with increase in dried shrinkage until maximum volume shrinkage was reached, hence a direct relationship.

Model for the evaluation of overall volume shrinkage in molded clay products (from initial air-drying stage to completion of firing at a temperature of 1200^0C) has been derived by Nwoye et al. (2008). It was observed that the overall volume shrinkage values predicted by the model were in agreement with those calculated using conventional equations. The model;

$$S_T = \alpha^3+\gamma^3-3(\alpha^2+\gamma^2)+3(\alpha+\gamma) \qquad (2)$$

depends on direct values of the dried γ and fired shrinkage α for its precision. Overall volume shrinkage was found to increase with increase in dried and fired shrinkages until overall volume shrinkage reaches maximum.

Nwoye (2009a) derived a model for calculating the quantity of water lost by evaporation during oven drying of clay at 90^0C. The model;

$$\gamma = \exp[(\ln t)^{1.0638} - 2.9206] \quad (3)$$

indicated that the quantity of evaporated water, γ during the drying process is dependent on the drying time t, the evaporating surface being constant. The validity of the model was found to be rooted in the expression $(\text{Log}\beta + \ln\gamma)^N = \ln t$.

Nwoye (2009b) derived a model for predictive analysis of the quantity of water evaporated during the primary-stage processing of a bioceramic material sourced from kaolin .The model;

$$\alpha = e^{(\ln t/2.1992)} \quad (4)$$

indicates that the quantity of water α, evaporated at 110^0C, during the drying process is also dependent on the drying time t, where the evaporating surface is constant. It was found that the validity of the model is rooted on the expression $(\ln t/\ln\alpha)^N = \text{Log}\beta$ where both sides of the expression are correspondingly approximately equal to 3. The respective deviation of the model-predicted quantity of evaporated water from the corresponding experimental value was found to be less than 22% which is quite within the acceptable deviation range of experimental results.

Model for quantifying the extent and magnitude of water evaporated during time dependent drying of clay has been derived (Nwoye et al.,2009). The model;

$$\gamma = \exp((\ln t/2.9206)^{1.4}) \quad (5)$$

indicates that the quantity of evaporated water γ during the drying process (at 90^0C) is dependent on the drying time, t the evaporating surface being constant. It was found that the validity of the model is rooted in the expression $\ln\gamma = (\ln t/\text{Log}\beta)^N$ where both sides of the expression are correspondingly almost equal.

The present work is to derive a model for computational analysis of water absorption in Ukpor clay materials exposed to hot-humid environment.

The present work is to derive a model for computational analysis of water absorption in Ukpor clay materials exposed to hot-humid environment.

2. Materials and Methods

2.1. Model Formulation

Results of the experiment previously carried out (Nwoye,2006) were used for the model derivation. These results as shown in Table 1 indicate that;

$$S = \left(\frac{\gamma}{\alpha\beta}\right)^N \quad \text{(approximately)} \quad (6)$$

Introduction of the value of N to equation (6) reduced it to;

$$S = \left(\frac{\gamma}{\alpha\beta}\right)^{0.93} \quad (7)$$

Dividing the indices of both sides of equation (7) by 0.93 redu

$$\left(\gamma\right) \quad (8)$$

$$S^{1/0.93} = \alpha\beta$$

$$(S)^{1.0753} = \left(\frac{\gamma}{\alpha\beta}\right) \quad (9)$$

$$\beta = \left(\frac{\gamma}{\alpha\left(S\right)^{1.0753}}\right) \quad (10)$$

Where

N = 0.93; Coefficient of shrinkage for Ukpor clay at 1200^0C (determined in the experiment (Nwoye,2006)

(γ) = Bulk density of the clay body in the hot-humid environment (g/cm^2)

(α) = Fractional value of apparent porosity of the clay body in the hot-humid environment

(β)= Fractional value of water absorbed by the clay body under the hot-humid environment

Equation (10) is the derived model

3. Boundary and Initial Conditions

Consider a rectangular shaped clay product of length 70mm, 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 sizes for the clay materials used are, <100μm, 100-300μm, 300-1000μm and their respective mixtures. The hot-humid environment was at a temperature; 1200^0C, and the resident time of clay bodies under the environment; 18hrs. The boundary conditions are: atmospheric levels of oxygen at the top and bottom of the clay samples since they are dried under the atmospheric condition. No external force due to compression or tension was applied to the drying clays. The sides of the particles and the rectangular shaped clay products are taken to be symmetries.

4. Model Validation

The formulated model was validated by direct analysis and comparison of the model-predicted β values and those from the experiment (Nwoye,2006) for equality or near equality.

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

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

$$Dv = \left(\frac{\beta_M - \beta_{exp}}{\beta_{exp}} \right) \times 100 \quad (11)$$

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

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

Therefore

$$Cf = -100 \left(\frac{\beta_M - \beta_{exp}}{\beta_{exp}} \right) \quad (13)$$

Introduction of the value of Cf from equation (13) into the model gives exactly the corresponding experimental value β_{exp}.

5. Results and discussions

The model is equation (10). It was found that the model is dependent on the bulk density, apparent porosity and the shrinkage sustained on the clay body at any point in time under the hot-humid condition. The validity of the model was found to be rooted on the expression; $S = (\gamma/\alpha\beta)^{0.93}$ where both sides of the expression are correspondingly almost equal. Table 2 also agrees with equation (6) following comparison of the value S and that of $(\gamma/\alpha\beta)^{0.93}$ evaluated from Table 1 as a result of corresponding computational analysis. Table 3 shows that the maximum deviation of the model-predicted quantity of absorbed water from the corresponding experimental values is less than 7.5% which is within the acceptable range of deviation limit for experimental results.

The model can be useful to engineers for carrying out failure or survival analysis of clay materials (exposed to hot-humid environment while in service) relative to the varied clay porosity, water absorption, bulk density and shrinkage sustained in the clay bodies under this service environment. This is because swelling of clay materials is likely when water absorbed by the materials becomes excessive. Nwoye (in press) found that swelling process weakens the grain boundaries and also loosen the clay-binder interface leading to collapse of the microstructure of the clay material. This implies failure.

Conclusion

The model computes the quantity of absorbed water in clay materials exposed to hot-humid environment. The model is dependent on the bulk density, apparent porosity and the shrinkage sustained on the clay body at any point in time under the hot-humid condition. The validity of the model is rooted on the expression; $S = (\gamma/\alpha\beta)^{0.93}$ where both sides of the expression are correspondingly almost equal. The maximum deviation of the model-predicted quantity of absorbed water from the corresponding experimental values is less than 7.5% which is within the acceptable range of deviation limit for experimental results.

Table 1: Chemical composition of clays used

Source	Al_2O_3	Fe_2O_3	TiO_2	MgO	CaO	SiO_2	N_2O	K_2O	Loss of ignition
Otamiri	15.56	0.05	1.09	-	0.29	69.45	0.01	0.21	13.10
Olokoro	20.10	7.05	-	0.75	1.26	45.31	0.05	0.09	11.00
Ukpor	31.34	0.63	2.43	0.14	0.06	51.43	0.04	0.10	12.04

Table1: Variation of apparent porosity, water absorption, bulk density and volume shrinkage with grain size of Ukpor clay body exposed to hot-humid environment. (Nwoye,2006)

Grain size (μm)	α	β	γ	S (%)
(A) <100	0.2367	0.1869	1.29	23.57
(B) 100-300	0.2446	0.1872	1.27	23.14
(C) 300-1000	0.2486	0.1874	1.26	22.96
A + B	0.2391	0.1869	1.28	23.36
A + C	0.2403	0.1872	1.27	23.29
B +C	0.2448	0.1899	1.26	23.07
A + B + C	0.2407	0.1871	1.29	23.21

Table 2: Variation of $(\gamma/\alpha\beta)^{0.93}$ with S

$(\gamma/\alpha\beta)$	$(\gamma/\alpha\beta)^{0.93}$	S (%)
29.1596	23.0275	23.57
27.7358	21.9800	23.14
27.0458	21.4710	22.96
28.6432	22.6480	23.36
28.2322	22.3456	23.29
27.1040	21.5140	23.07
28.6444	22.6489	23.21

Table3: Comparison of the quantities of water absorbed by the clay body under the hot-humid environment as obtained from experiment (Nwoye, 2006) and derived model.

β_{exp}	β_M	Dv (%) 9%)	Cf (%)
0.1869	0.1823	-2.46	+2.46
0.1872	0.1771	-5.40	+5.40
0.1874	0.1743	-6.99	+6.99
0.1869	0.1808	-3.26	+3.26
0.1872	0.1790	-4.38	+4.38
0.1899	0.1761	-7.27	+7.27
0.1871	0.1822	-2.62	+2.62

Where

β_M = Absorbed water by clay body under hot-humid environment as predicted by model

β_{exp} = Absorbed water by clay body under hot-humid environment as obtained from experiment (Nwoye,2006)

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7/9/2009

Bacterial Agent of Respiratory Manifestation in Cattle and The Associated Biochemical Alterations in Menoufiea Governorate

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ABSTRACT This study was carried out on a feedlot cattle farm at Menoufiea governorate suffering from respiratory distress, fever, with mild to continuous coughing, loss of appetite, nasal discharge, depression and reduction in fertility. The morbidity rate was 25 % and the mortality rate reached 4%. Mycoplasma was suspected to be incriminated as causative agent. Nasopharyngeal swabs and blood samples were taken from the diseased animals. Bacteriological examination of recovered isolates revealed that *Mycoplasma bovis* (20%) and this was confirmed by Polymerase Chain Reaction (PCR).The studied biochemical parameters revealed a significant decreased in serum total protein, albumin, total globulin and consequently in A/G ratio. Liver enzyme alanine aminotrasferase (ALT) was significantly decreased while aspartate aminotransferase (AST) did not change. [Nature and Science 2009;7(9):26-30]. (ISSN: 1545-0740).

KEY WORDS: Mycoplasma, PCR, Serum protein, AST, ALT

1-INTRODUCTION

Bovine respiratory disease (BRD) complex is the most important cause of mortality and culling of weaned calves and young animals after arrival at a feedlot (Kelly and Janzen ,1986). Infectious agents implicated in bovine respiratory disease include viruses, bacteria, mycoplasma and chlamydia. Many viruses have been associated with respiratory disease in cattle, many acting in conjunction with bacteria to produce severe pneumonia (Radostits, et al., 1994). Mycoplasmas are prokaryotes which lack a true cell wall and are known to cause chronic diseases in man and animals. Mycoplasma bovis is associated with a variety of bovine diseases, including pneumonia, polyarthritis, tenosynovitis and mastitis, which cause considerable economic losses (Henderson and Ball, 1999).

The infection is usually introduced to M. bovis-free herds by clinically healthy calves or young cattle shedding the mycoplasma and once established on multi-age sites it becomes very difficult to eradicate. Its appearance on some farms suffering low grade respiratory disease can lead to increased morbidity and mortality (Gourlay et al., 1989). Infected cattle shed the mycoplasma via the respiratory tract for many months and even years where they act as reservoirs of infection (Pfutzner, 1990). Contact animals become infected via the respiratory tract, the teat canal or genital tract; artificial insemination with infected semen is another common route (Pfutzner, 1990). The male genital tract can become infected with M. bovis through contact with other animals or, possibly, via a heavily contaminated environment.

This study aimed to diagnose the cause of respiratory affection in a farm of feedlot cattle, together with monitoring the biochemical changes associated with the causative agent.

MATERIAL AND METHODS

A feedlot farm at Menofia governorate suffering from respiratory distress, mild to continuous coughing, loss of appetite, nasal discharge and depression with morbidity rate 25 % and mortality rate 4%. Vaccination program against viral diseases was maintained together with strict internal and external parasite control. Nasal swabs were taken from 15 of both diseased and apparently healthy animals for identification of the causative microorganism, also blood samples were taken for biochemical investigation.

1-Isolation of Mycoplasma: The samples were cultured on Modified HayFlicks medium (Rosendal, 1994) and B.H.S.L medium (Carmicheal et al., 1972) as described by (Sabry and Ahmed 1975). Digitonin sensitivity test as described by (Freundt et al., 1973) was done to differentiate between genus Mycoplasma and Acholeplasma, where genus Mycoplasma was digitonin sensitive and Acholeplasma was digitonin resistant, (Thurmond et al., 1989). Biochemical characterization was carried to differentiate the purified Mycoplasma isolates using different biochemical tests as glucose fermentation test, arginine test (Sabry, 1968) , and

film and spots formation (Cottew, 1983). Serotyping was carried by growth Inhibition test (GI), according to (Clyde 1964) with reference antisera.

2-Polymerase chain reaction (PCR):

a) Preparation of samples for DNA extraction (Yleana et al., 1995): 5ml of a 24 hour broth cultures of isolates were centrifuged for 10 minutes at 12000 r.p.m. The pellet was washed twice in 1 ml of PBS pH 7.2 and suspended in 50 µl PBS. The cell suspension was heated directly at 100oC for 10 min. in a heat block to break the cell membranes, and then cooled on ice for 5 min. Finally, the cell suspension was centrifuged for 5 min. and the supernatant containing chromosomal DNA was collected and stored at -20^{o}C until used.

b) Primer selection (Yleana et al., 1995): Two oligonucleotide primers were selected for the detection of M. bovis. The sequence of the primers was (prepared by Sigma):

Forward:5-CCTTTTAGATTGGGATAGCGGATG-3

Reverse:5-CCGTCAAGGTAGCATCATTTCCTAT-3

Procedure for DNA amplification: PCR amplification was performed in 50 µl reaction mixture consisting of 5 µl of 50 ng M. agalactiae 90 min. at 100 volts, DNA Ladders: 100 bp (Pharmacia), Cat. No. 27-4001- 01, USA was added then stained with ethidium bromide. After electrophoresis, the gel was visualized by UV transillumination and photographed. Arthritis and reduced weight gain in calves, mastitis in cows and reproductive problems in both cows and bulls (Romváry et al., 1977; Kreusel et al., 1989; ter Laak et al., 1992; Pfützner and Sachse, 1996; Nicholas and Ayling, 2003). It can occasionally be involved in other diseases such as meningitis (Stipkovits et al., 1993), otitis media (Walz et al., 1997) and abortion (Byrne et al., 1999).

This study was made on a feedlot farm at Menofia governorate suffering from respiratory distress with mild to continuous coughing, loss of appetite, nasal discharge and depression. The morbidity rate was 25 % and the mortality rate reached 4%. The nasal swabs (n=20) taken were examined for bovine respiratory viruses, bacteria but DNA, 10 µl of 10 x Taq buffer (10mM tris- HCl [pH 8.8], 50 mM KCl), 1 µl of 50 pM of each primer, 1.5 mM MgCl2, 1 µl of 2U of Taq thermostable DNA polymerase, 1 µl of 50 uM of each dNTP, and 31µl of DNase- RNase- free, deionized water. The thermal profiles were as follows: Denaturation at 94 oC for 45 seconds, primer annealing at 60oC for 1 min., and extension at 72oC for 2 min.the amplifications were performed for 30 or 35 cycles with a final extention step at 72oC for 3 min. After the reaction, the amplified DNA was electrophoresed on 1.5% agarose gel for

3-Biochemical tests**:** serum total protein concentration was detrmined according to Sannenwirth and Jarett (1980), albumin concentration was made according to the methods described by Drupt (1974) and serum globulin detected by subtraction. Serum Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activities were made as recorded by Reitman and Frankel (1957).

Results and Discussion

M. bovis is an important but sometimes overlooked pathogen in cattle. It causes major economic losses mainly by causing pneumonia,

Table (1) showed the recovery rate of mycoplasma from nasal swabs as 20% (4 out of 20) and Acholeplasma spp was found to be 5% one out of 20. In other studies the recovery rate was13% (El-Shater and Eissa, 2001); 63 out of 432 calves (15%) via culture (Wiggins et al., 2007) and 31.58% (Eissa et al., 2007).

The obtained isolates were subjected to the polymerase chain reaction (PCR) for confirmation of the results using the 16S rRNA gene for M. bovis. It was found that the obtained 4 Mycoplasma isolates were positive to the used gene giving positive band at 360 bp, so the isolates confirmed as *M. bovis* (image 1). A similar results were obtained by Hotzel et al. (1996); Ghadersohi et al. (1997); El-shater and Eissa (2001); Susan (2006) and Eissa et al. (2007).

Table (1) recovery rate of mycoplasma isolated

Sample	Primary isolation				Expected type
	No Examined	No. +ve recovered	Digitonin	%+ve	
Nasal swabs	20	4	+ve	20%	Mycoplasma Spp
		1	-ve	5%	Acholeplasma spp

M. bovis infection represents a major disease burden for cattle producers worldwide, emphasizing the need for a reliable method of molecular typing for outbreak investigation and epidemiological surveillance (McAuliffe et al, 2004). Chavez et al., (1995) reported that the use of PCR makes the identification of *M. bovis* much shorter comparing to the conventional culture methods.

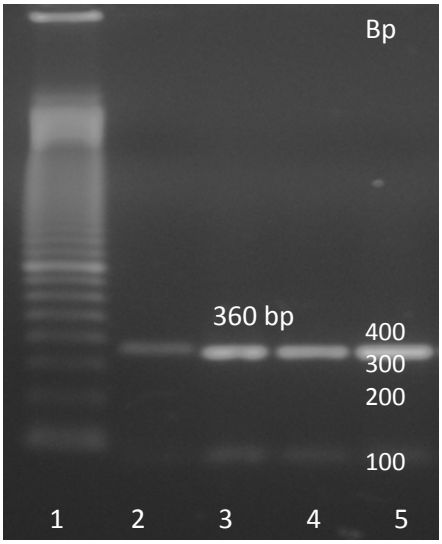

Image (1) the agarose gel electrophoresis of the obtained isolates of *M. bovis*

1- 100bp marker
2- 5- *Mycoplasma bovis* isolates

The taken serum samples were used for the studying the biochemical changes accompanying the case. In table (2), it was found that mycoplasma infection cause a significant decrease in total protein, albumin, total globulin and consequently in A/G ratio. Also the liver enzyme alanine aminotransferase (ALT) was significantly decreased while aspartate aminotransferase (AST) did not changed.

The decrease noted in total protein caused by the decrease both in albumin and globulin, a similar finding were recorded by Eissa et al. (2007). Science hypoalbuminemia may be due to increased catabolism as a result of tissue damage or inflammation as stated by Limidi and Hyde (2003), that agreed with Wise and Evans (1975), who mentioned that serum albumin concentrations, were markedly reduced in poults with *M meleagridis* and also, suggested that low serum albumin concentrations may play a primary role in the pathogenesis of mycoplasma. UIvund (1990) observed hypoalbuminaemia in mycoplasma mastitic cows and revealed it to the inadequate protein synthesis as a result of Mastitis which badly affected the hepatic parenchyma leading to the failure of protein synthesis as recoded by Coles (1986).

Immunosuppression caused by *M. bovis* had been observed by the results obtained by Thomas et al. (1986) who recorded low levels of IgA antibody in sera three and four weeks after Mycoplasma infection, and Postepski et al. (2003) who found low titer of IgG and IgM with patients of *Mycoplasma pneumonia.*

Table (2): The biochemical changes accompanying *Mycoplasma bovis* infection

Items tested	control	diseased	LSD
Albumin (mg %)	3.74±0.11 a	2.60±0.14 b	0.4419***
Total globulin (mg %)	4.10±0.11a	3.160±0.260b	0.688*
Total protein (mg %)	7.83±0.20 a	5.74±0.268b	0.8179***
AG Ratio	0.91±0.02 a	0.737±0.096b	0.141*
ALT (u/L)	58.25±6.76 a	30.00±4.34 b	19.661*
AST (u/L)	142.75±15.69	121.00±14.40	52.121ns

*: Significant variation between groups by one ways ANOVA at $P \leq 0.05$.

Serum ALT decrease may be due to alternation in the metabolic rate resulted from nutritional deficiency in mastitic buffaloes as reported by UIvund (1990) and Abd El- Ghany et al. (2007). On the other hand mycoplasma inside cells produces ammonia and oxidized compound (hydrogen peroxide, peroxide compound) which all toxic to cells (Nicholson et al., 1998).Rao and Murthy 1992 stated that activities of ALT decreased in hyperammonemic states.

Mycoplasma infection in cows may cause serious problems in herd not only due to respiratory distress but also to the immunosuppressive effect that may lead to combined infection.

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Effect of Growth Regulators on Meristem-tip Development and *in vitro* Multiplication of Potato Cultivar 'Kufri Himalini'

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Abstract

In the present study meristem tips of potato (*Solanum tuberosum)* were cultured on Murashige and Skoog (MS) medium, supplemented with different hormonal combinations i.e. MSGN1 (0.25 mg/l GA_3 and 0.01 mg/l NAA), MSGN2 (0.25 mg/l GA_3 and 0.03 mg/l NAA), MSGN3 (0.25 mg/l GA_3 and 0.04 mg/l NAA), MSKN1 (0.01 mg/l Kinetin and 0.1 mg/l NAA), MSKN2 (0.001 mg/l Kinetin and 0.1 mg/l NAA) and MSKN3 (1 mg/l Kinetin and 0.1 mg/l NAA), which affected *in vitro* propagation of potato. After 35-40 days of culture shoot height, number of nods, root length, shoot and root fresh weight were measured. Shoot height in M.S. medium with GA_3 and NAA combination showed better result in comparison to M.S. medium with Kinetin and NAA. Shoot height in MSGN1 combination reached 8.28 (±0.5) cm. with 11.9 (±1.1) cm. root length and 9.4 (±1.0) nodes while in MSKN1 shoot height reached 6.4 cm. (±0.6) with 8.2 (±0.5) cm. root length and 5.0 (±0.7) nods. MSKN2 and MSKN3 reached low shoot height respectively 5.3 cm. (±1.2) with 4.2 (±0.8) nods and 4.0 cm. (±0.6) with 2.7 (±0.7) nods in comparison to all combinations. MSGN2 and MSGN3 combinations reached respectively 7.15 (±0.5) cm. with 8.2 (±1.0) nodes and 6.15 (±0.6) cm. with 6.3 (±0.9) nodes. Result showed that lower concentration of auxin (0.01 mg/l NAA) with Gibberelic Acid (0.25 mg/l GA_3) is best for development of complete plantlets and multiplication from meristem tips. [Nature and Science, 2009;7(9):31-34]

Keywords *In vitro*, meristem tip, *Solanum tuberosum* and Kufri Himalini

Introduction

The ICAR has identified a new hybrid variety of potato Kufri Himalini. Nearly 8% of the total area under Potato in the country lies in the hills, where potato is an important cash crop. This species is best for commercial cultivation in hilly regions. The new variety, with medium maturity of 110-120 days has been recommended for cultivation in the north- western and eastern hills during summer. It provides a yield advantage of over 10% over Kufri Jyoti and Kufri Giriraj. In the plains and its keeping quality is better then all the cultivars develop so far for hill regions (Anonymous, 2005).

Micro propagation is the alternative to conventional propagation of potatoes (Chandra *et al.*, 1994). *In vitro* propagation methods using meristem tips, nodal cuttings and micro tubers are more reliable for maintaining genetic integrity of the multiplied clones since de-differentiation and the subsequent organogenesis/ embryo genesis with the accompanying genetic changes have been reported (Wang and Hu, 1982). Meristem culture provides a reproducible and economically viable method for producing pathogen free plants. As meristem tips are free from viruses, elimation and generation of virus free plants are possible through meristem culture (Jha and Ghosh, 2005). Through several workers have reported the use of MS medium without hormones during proliferation stage (Aburkhes *et al.*, 1984; Rosell *et al.*, 1987; Gopal *et al.*, 1980) but the growth was slow and it took 3-4 weeks to grow 30-50 high shoots (Hussey and Stacey, 1981). Improvement has been made possible by addition of growth regulators to the medium. Gas stimulated development of nodal cutting on MS but at high concentration it produce narrow and elongated shoot (Novak *et al.*, 1980) depending on genotypes. Longest main shoot and highest node numbers are reported to be obtained in medium containing NAA and BAP (Yousef *et al.*, 1997). Pennazio and Vecchiate (1976) used MS medium supplemented with GA and NAA for proliferating meristem tip. The main aim of this study was to see the effect of different hormonal combinations of GA_3: NAA and Kinetin: NAA with MS medium on *in vitro* shoot regeneration of potato cv. Kufri Himalini using meristem tips.

Material and Method

The present investigation was carried out with the objective; to study the effect of two hormonal combinations i.e. GA_3+ NAA and Kinetin + NAA with MS medium on shoot regeneration and multiplication using meristem tips of potato cv. Kufri Himalini.

For obtaining sprouts, the tubers were cut into pieces; these pieces were dipped in a solution of 0.1% Bavistin, for 2-3 minuts and sown in sand filled plastic pots. These were grown under poly house conditions following optimum cultural practices. After 25-30 days of growth meristem tips were ready for inoculation. For inoculation of explants different media with hormonal combinations were prepared properly. MS media supplemented with different combinations of GA_3+ NAA and Kinetin + NAA (Table-1), were autoclaved at 15 psi for 20 minute. The hot medium was immediately dispensed in to culture flask (30 ml medium in each flask) and covered with autoclaved cotton plug in Laminar Air Flow Cabinet. The segment of about 0.5-1 cm. size were collected in a water filled beaker from the mother plant of Kufri Himalini, and kept under running water prior to sterilization in the laminar air flow cabinet. The explants were treated by sodium hypochlorite with 8 minutes, followed by 5 minute wash of savolon, and 30 second wash of alcohol, at last 6-7 wash of distil water was done. After sterilization, explants were inoculated in MS medium supplemented with different hormonal combinations and shifted to culture growth room at $25^0 \pm 1^0$ c and 16 h photoperiod. Best combination of GA_3+ NAA and Kinetin + NAA with MS medium was selected on the basis of cultures growth performance i.e. shoot height, number of nodes, root length, shoot and root fresh weight, after 35-40 days. The mean values were calculated of cultures growth of all the combinations. The selected combination was used for sub culturing of plantlets also.

The shoot development was studied in terms of the parameter given above. The best combination of hormones with MS medium was selected and which cultures showed higher growth were further sub-cultured on its parent medium by cutting it in to small pieces in a way that each subsection have at least 1-2 nodes.

Result and Discussion

In hilly regions, late blight in potato crop has become more frequent and intends last few years and the resistance to late blight was found eroding in existing varieties Kufri Jyoti and Kufri Giriraj. To overcome this, the new hybrid variety Kufri Himalini has been developed which has high level of resistance to late blight was used in the study. Indian Council of Agriculture Research (ICAR) has identified Kufri Himalini for commercial cultivation in hilly regions, a new variety with medium maturity of 110-120 days has been recommended for cultivation in the northwestern and eastern hills during summer. Kufri Himalini provides a yield advantage of 10% over Kufri Jyoti and Kufri Giriraj (Anonymous, 2005).

Different combinations of GA_3+ NAA and Kinetin + NAA with MS medium influenced *in vitro* shoot regeneration from meristem tip culture. Shoot height in M.S. medium with GA_3 and NAA combination showed better result in comparison to M.S. medium with Kinetin and NAA (Table- 1).

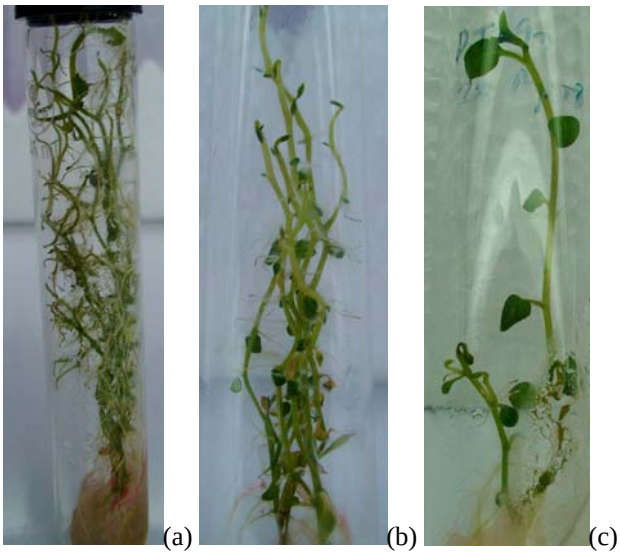

Fig. 1 **(a) to (c):** Meristem development on different hormonal combinations with M. S. medium **(a) and (b):** MS+ 0.25 mg/l GA3 +0.01 mg/l NAA **(c):** MS+0.01 mg/l Kn +0.1 mg/l NAA

The combination of Kinetin and NAA had consistently given good result for improving shoot height. The MSKN2 (0.001 mg/l Kinetin and 0.1 mg/l NAA) having low concentration of Kinetin and NAA and MSKN3 (1 mg/l Kinetin and 0.1 mg/l NAA) combinations having higher concentration of Kinetin (1 mg/l) 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 meristem tips.

After 35-40 days of incubation, shoots in MSH1 (0.25 mg/l GA_3 and 0.01 mg/l NAA) reached 8.28 cm with 9.4 nodes. These results are comparable or even better than the most rapid node production (x8 to x10 per month) reported earlier using agar (Hussey and Stacey, 1981). The combination of GA_3 + NAA showed best result for

improving all the parameters of the study (Table-2). The MSGN2 (0.25 mg/l GA_3 and 0.03 mg/l NAA) and MSGN3 (0.25 mg/l GA_3 and 0.04 mg/l NAA) combinations respectively having higher concentration of NAA responded the least mean Shoot height and number of nodes. This could be attributed to the fact that higher concentration of NAA inhibit root and shoot growth (Pennazio and Vecchiati, 1976). Result showed that lower concentration of auxin (0.01 mg/l NAA) with Gibberelic Acid (0.25 mg/l GA_3) is best for development of complete plantlets from meristem tips with avoiding callus and satisfactory root formation.

In the present comparative study the conclusion is that GA_3 + NAA (MSGN1) combination is best for shoot regeneration and multiplication of potato cv. Kufri Himalini in comparison to the combination Kinetin + NAA with M. S. medium.

Table-1: 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	Root length (cm)
GA_3	NAA	Kn	Symbol used			
0.25	0.01	0	MSGN 1	8.2 ± 0.5	9.4 ± 1.0	11.9 ± 1.1
0.25	0.03	0	MSGN 2	7.1 ± 0.5	8.2 ± 1.0	10.6 ± 1.0
0.25	0.04	0	MSGN 3	6.1 ± 0.6	6.3 ± 0.9	9.4 ± 1.0
0	0.1	0.01	MSKN 1	6.4 ± 0.6	5.0 ± 0.7	8.2 ± 0.5
0	0.1	0.001	MSKN 2	5.3 ± 1.2	4.2 ± 0.8	6.8 ± 0.8
0	0.1	1	MSKN 3	4.0 ± 0.6	2.7 ± 0.7	5.3 ± 0.9

Table-2: 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
MSGN 1	0.501 ± 0.05	0.296 ± 0.05	1.76 ± 0.5
MSGN 2	0.364 ± 0.04	0.234 ± 0.01	1.56 ± 0.2
MSGN 3	0.348 ± 0.04	0.212 ± 0.008	1.64 ± 0.2
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

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8/5/2009

INVOLUTION SIGNS DURING THE POSTNATAL LIFE IN THE PINEAL TISSUE OF BUFFALO AND CAMEL

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Abstract: The regressive changes of pineal tissue in buffalo and camel were not studied before. The signs of involution in the form of calcium deposit or brain sand seemed curious in the buffalo pineal. Calcium deposit started early after birth but it was few. Calcium infiltration increased by age where the highest level was reached at the ages of 1 - 10 years. The calcium precipitation occurred within the walls of blood vessels and inside it and increased gradually leading to complete calcification of some blood vessels. Therefore the structure that was so called brain sand in the past not more than completely calcified blood vessels and indicating involution of the pineal. This will decrease the blood supply of the pineal and directly affecting its growth and function. The involution features in the camel pineal, including a striking physiological degeneration and hyalinization of blood vessels and accumulation of melanin pigment in all samples of all ages. The degeneration and hyalinization were moderate and usually happen in the smaller blood vessels. However high extent of hyalinization including large blood vessels sometimes occurred at the ages of 3-10 years; it was associated with severe depletion of pinealocytes. Blood vessels were lost by hyalinization. It is supposed that the function and growth of the pineal will be reduced. The regressive changes of the pineal may refer to that its main function is carried out during the prenatal life. [Nature and Science. 2009;7(9):35-44]. (ISSN: 1545-0740).

Key words: Pineal; Involution; Brain sand; Hyalinization; Buffalo; Camel

1. Introduction

Any attempt to summarize the confusing features of pineal tissue of different species and its relation to the pineal role is fraught with perils (Golan et al., 2002; Nimmagadda et al., 2006). This is because papers may simply not be able to draw any convincing conclusions in that respect. Also the most commonly encountered information was concluding that the pineal is fundamentally involved in nearly many functions in the postnatal life through its melatonin hormone (Hales and Fawcett, 1993; Miguez et al., 1996; Guimaraes et al., 1997; Khan et al., 1997; Pacchierotti et al., 2001; Lewczuk et al., 2004; Kus et al., 2004) on the other side pinealectomy in sheep seemed to be not serious (McCloghry et al., 1992; Regodon et al., 2001). itself as a hormone was studied with many biological functions (Omer et al., 2004; Claustrat et al., 2005; Jaworek et al., 2005, Maertroni et al., 2005, Peters et al., 2005). Light photoperiod was still a field of interest of some scientists in the last decade (Tosini et al., 2000; Engel et al., 2005). Brain sand or corpora arenacea was considered as one of the landmarks of the pineal tissue of mammals (Vigh et al., 1998; Koshy and Vettivel 2001). Brain sand or calcium deposit was also found in buffalo (Lalitha and Seshadri, 1992). Moreover it was found as excretory plugs in the cerebrospinal fluid of dog (Garma - Avina, 2000). Structure of camel pineal was also studied (Taher et al., 1975; Abbas and Ewais, 1982). There were no study could be obtained clarifying the role of brain sand and other features of blood vessels in pineal involution in animals. So the present investigation selected two species as an example of ruminants, the buffalo and camel to study that respect which will indicate indirectly when the pineal show its

main function.

2. Materials and methods

The pineals of 18 males of both buffalo (*Bos bubalis L.*) and camel (*Camelus dromedarius*) were obtained from Damanhour and Kom-Hamada abattoirs in winter. All animals were living under natural light conditions. After slaughter the head was removed then dentition was applied according to Miller and Robertson (1959) and Banerjee (1991). The ages of the buffaloes were 1- 6 months, 1 - 3 years and age of 7 - 10 years. The ages of camels were 1 year, 2 years, 3 years, 5 years, 7 years, and 10 years. The brain was dissected and 2 pineals were collected for each age. The pineals were fixed in neutral buffered formalin, and then processed. Paraffin serial sections of 4-5 micrometers (μm) were prepared and stained by Harris Hematoxylin and Eosin (H&E), Tungsten Hematoxylin (PTAH), Modification of von Kossa's method for calcium with counterstaining by Van Gieson and Masson Trichrome and Dimethylaminobenzaldehyde - Nitrite method (DMAB-Nitrite method) for tryptophan the raw material of melatonin. Bleaching with hydrogen peroxide was carried out to confirm melanin. The average weight of the pineal was obtained. All the methods were reported by Woods and Ellis (1994).

3. Results

3.1. Buffalo

The pineal of buffalo was roughly pea-shaped in young animals with slight elongation in older ones (Fig. 1). It is situated in the deep mid-depression between the two thalami cranially and rostral colliculi caudally. The average weight of buffalo pineal was about 110 mg in 1-6 months, 337.5 mg in 1- 3 years and 160 mg in 7-10 years. The size of the buffalo pineal quantitatively did not increase widely by age however it was variable and sometimes seemed to be regressed (Fig.1). Generally the cells of buffalo pineal were including pinealocytes and glial cells occasionally astorcytes. The pinealocytes had pale stained cytoplasm; the prominent euchromatin and the presence of several nucleoli were characteristic (Fig. 2). The astrocytes nuclei were smaller and with darker chromatin (Fig. 2). Cell processes were of the characters of the cellular elements of the pineal (Fig. 12).

The signs of involution in the form of calcium deposit or brain sand seemed curious in the buffalo pineal. Sometimes the amount of calcium showing great differences even within the same age. Calcium deposit started so early at 1-6 months age after birth but it was few (Fig. 3). Calcium infiltration increased by age where the highest level was reached at the ages of 1-10 years (Figs. 4 & 5).

The calcium precipitation occurred within the walls of blood vessels associating its collagen fibers in a manner exhibiting a correlation (Figs. 6 & 7). The calcium deposit started by formation of small calcium granules then the granules increased gradually in number and size (Fig.7). The granules coalesced with each other resulting in complete calcification of the walls of some blood vessels (Fig. 8). The calcification progressed till cluttering up such blood vessels completely (Fig. 9). In some cases calcification started inside the lumen of blood vessels hence calcium masses appeared attached into the internal walls of blood vessels (Fig. 10). Then the calcium masses increased in size until partially or fairly obliterating such blood vessels (Figs. 2, 10 & 11).

Therefore the calcium deposit appeared in the form of patches and elongated well identifiable structures in the same locations and courses of blood vessels. Hence the structure that was so called brain sand in the past not more than completely calcified blood vessels and indicating involution of the pineal. This will decrease the blood supply of the pineal and directly affecting its growth. The pineal function is suggested to be affected.

Occasionally the blockage of blood vessels by brain sand was associating with decrease of pinealocytes amount. So the amount of pinealocytes decreased by age because they were crowded at the ages of 1-6 months but loosely arranged at the ages of 1-10 years (Figs. 6, 8 & 12). The pigment granules could not be observed in buffalo pineal.

The cytoplasm of pinealocytes was showing neither characteristic elements nor reaction of tryptophan the raw material of melatonin (Fig. 13).

3.2. Camel

The average weight of camel pineal were 90, 300, 130 mg at the ages of 1-3, 5 and 7-10 years. The quantitative characters of size, shape, situation and basic cellular elements of camel pineal were not far differ than those of buffalo, however the nuclei of camel pinealocytes may be more primitive. The involution features were including a striking physiological degeneration and hyalinization of blood vessels and accumulation of melanin pigment in all samples of all ages.

The degeneration and hyalinization were in the form of foci in crossly cut blood vessels or extend along with the direction of blood vessels (Fig. 15). Firstly dissolution of the wall of blood vessel and its content of blood cells occurred (Fig. 14) and then hyalinization started (Fig. 15). Hyalinization began at the center of the dissolute blood vessels and could be seen surrounded by remnants of the degenerated wall (Fig. 15). Blood vessels were lost as in buffalo, but with a new method, the degeneration and hyalinization. The quantity of degeneration and hyalinization was moderate at the age of 1 year (Fig. 14). However areas of high extent of hyalinization occurred by advancement of age, at 3 - 10 years; these areas elaborated severe depletion of pinealocytes (Fig. 16). Occasionally small intermittent areas showing few or even free from pinealocytes were recognized at all ages (Fig. 14 & 15). Generally the amount of pinealocytes decreased by age concomitantly with the lost of blood vessels (Figs. 15 & 16). So it is supposed that the pineal function and growth are reduced actually.

The melanin pigment were scattered allover the cellular elements of pineal tissue. They were more frequent in the periphery of the pineal and around the blood vessels. Most of the pinealocytes of all ages had melanin pigment. Some pinealocytes, glial cells and even fibroblasts were so packed with the pigment granules that they obscure the nuclei (Fig. 17). Aggregations of pigment granules may be observed in the intercellular spaces (Fig. 17), they may be for degenerated pinealocytes. Melanin pigment was confirmed by bleaching method. Heretofore no functional role has been assigned to the pineal pigment, so it may indicates non specialization of pinealocytes or a function regression. Calcium deposit was rare and the reaction of tryptophan did not differ than that of buffalo. The early regressive changes of the pineal may refer to that its main function is carried out during the prenatal life.

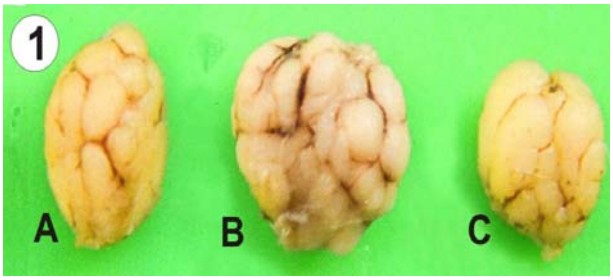

Fig. 1: Buffalo pineal at the age of 1 month (A); 2 years (B) and 7 years (C). The shape can be recognized. Regression seemed at the age of 7 years.

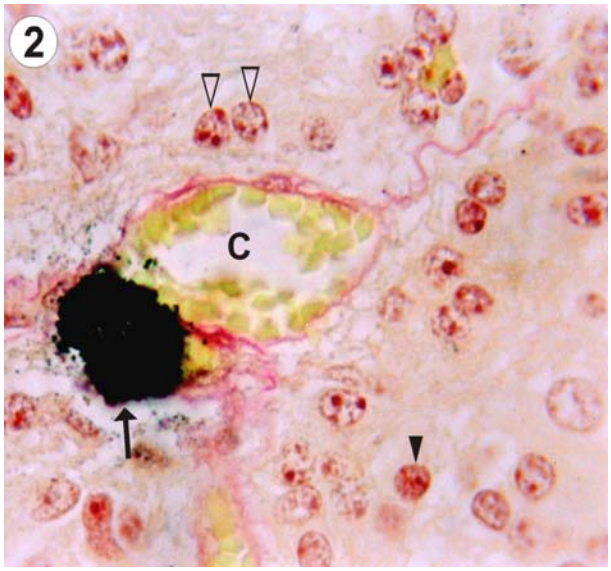

Fig. 2: Buffalo pineal at the age of 1 month showing pinealocytes (white arrow head), astrocytes (black arrow head) and partially obliterated blood vessel (C) by a calcium mass (arrow). (von Kossa's method & Van Gieson; x 1000).

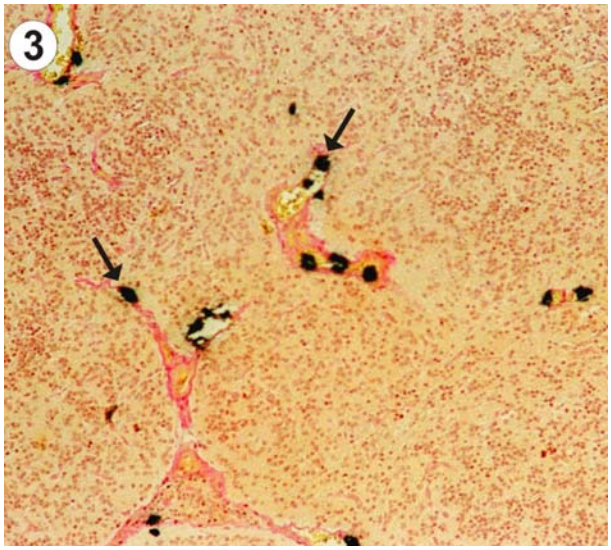

Fig. 3: Buffalo pineal at the age of 1 month showing few calcium deposits (arrows). (von Kossa's method & Van Gieson; x 100).

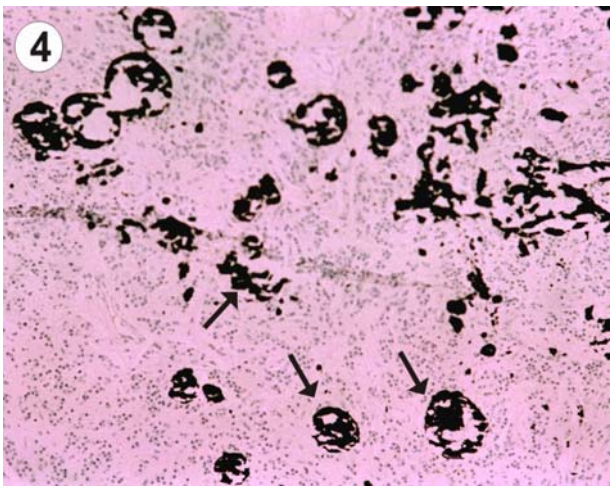

Fig. 4: Buffalo pineal at the age of 1 year showing highest level of calcium infiltration (arrows). (von Kossa's method & Van Gieson; x 100).

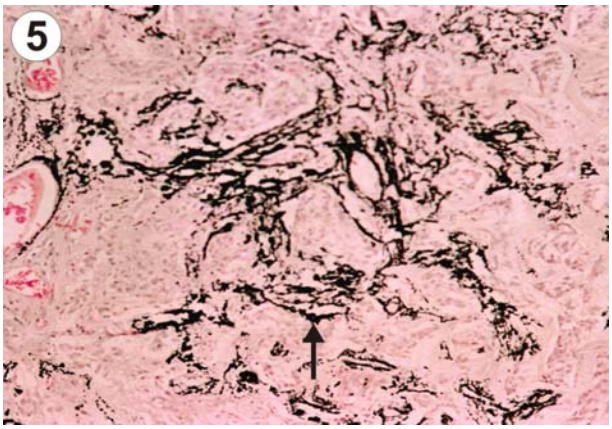

Fig. 5: Buffalo pineal at the age of 10 years showing highest level of calcium infiltration (arrows). (von Kossa's method & Van Gieson; x 100).

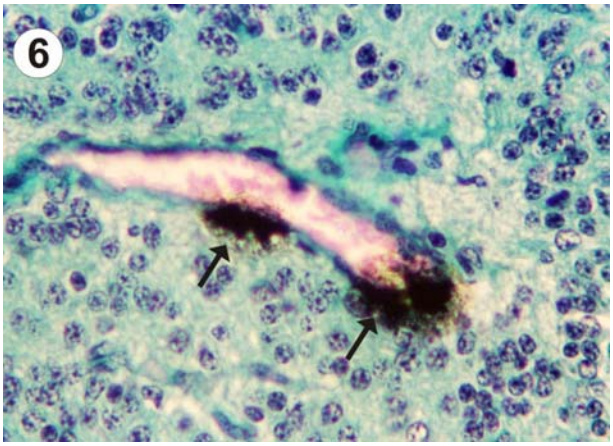

Fig. 6: Buffalo pineal at the age of 1 month showing calcium precipitation (arrows) within the wall of blood vessel, the pinealocytes are crowded. (von Kossa's method & Masson trichrome; x 400).

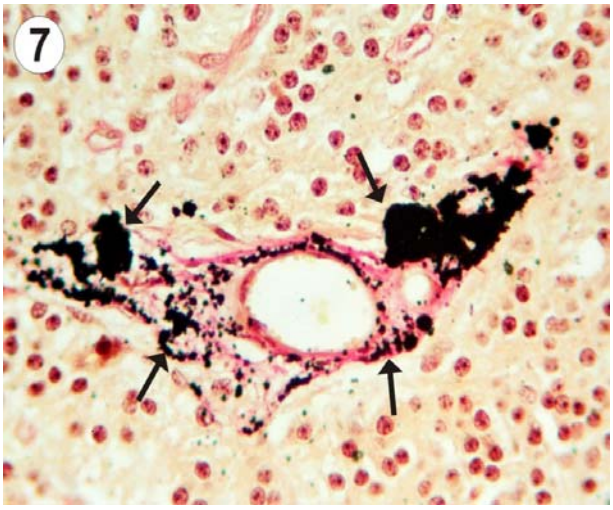

Fig. 7: Buffalo pineal at the age of 2 years showing increased calcium granules in number and size (arrows) associating the collagen of blood vessel wall. (von Kossa's method & Van Gieson; x 400).

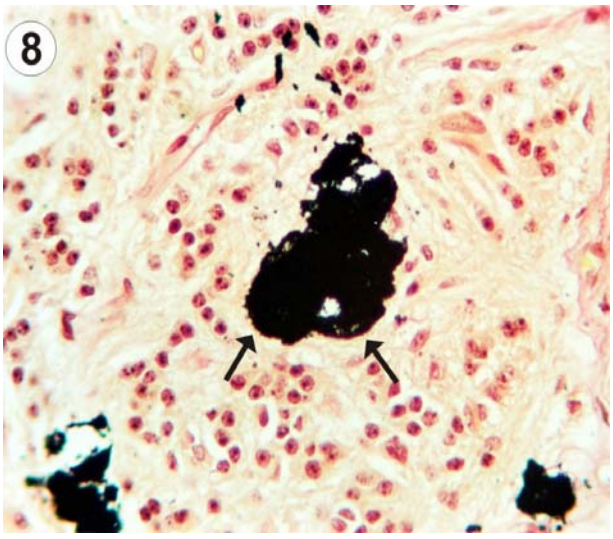

Fig. 8: Buffalo pineal at the age of 2 years showing complete calcification of blood vessel's wall (arrows); the amount of pinealocytes decreased. (von Kossa's method & Van Gieson; x 400).

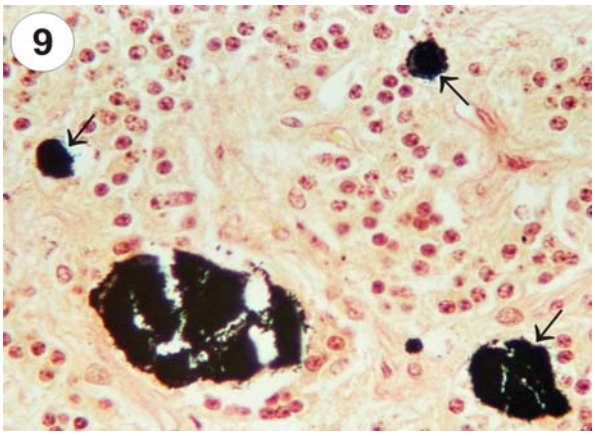

Fig. 9: Buffalo pineal at the age of 2 years showing calcification cluttering up some blood vessels completely (arrows). (von Kossa's method & Van Gieson; x 400).

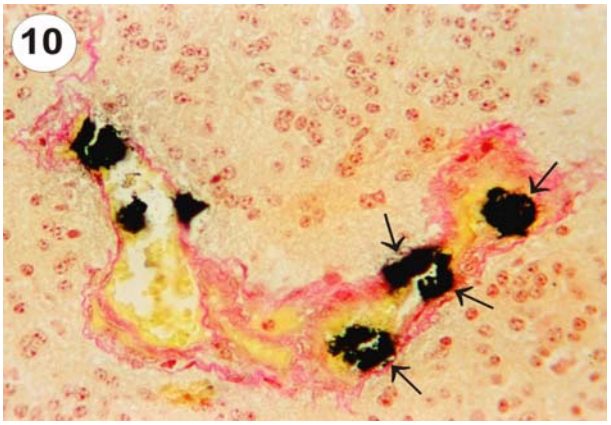

Fig. 10: Buffalo pineal at the age of 1 month showing calcium masses in the internal wall of blood vessel (arrows) and partial obliteration. (von Kossa's method & Van Gieson; x 400).

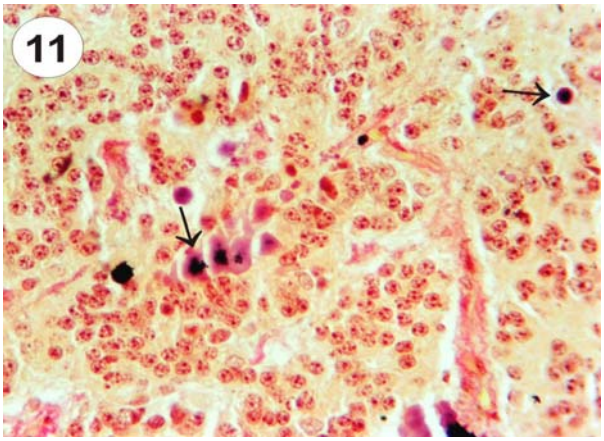

Fig. 11: Buffalo pineal at the age of 6 months showing completely obliterated blood vessels (arrows) by calcium masses. (von Kossa's method & Van Gieson; x 400).

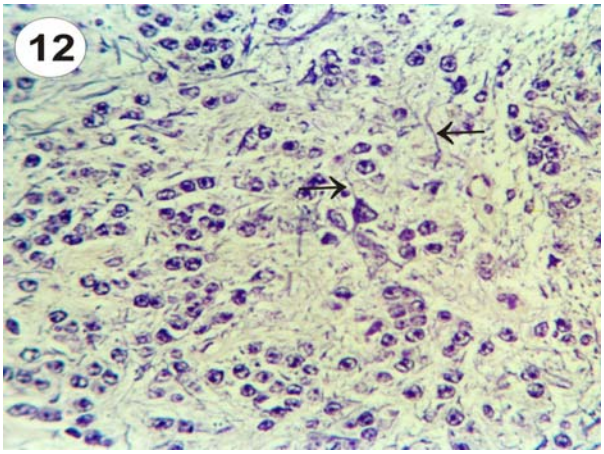

Fig. 12: Buffalo pineal at the age of 3 years showing loosely arranged pinealocytes and cell processes (arrows) (PTAH,x 400).

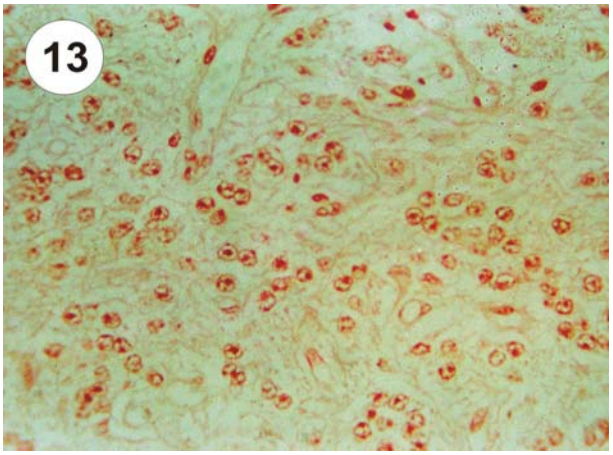

Fig. 13: Buffalo pineal at the age of 2 years showing no reaction of tryptophan. (DMAB-Nitrite method; x 400).

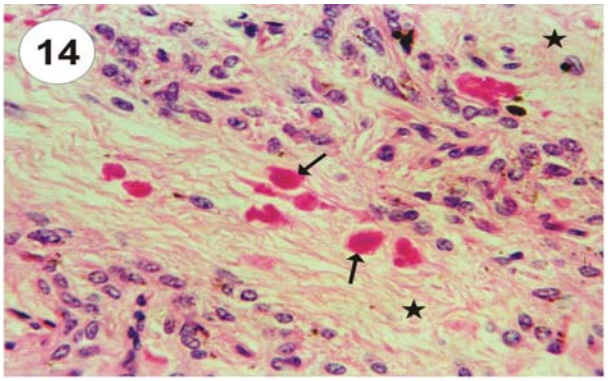

Fig. 14: Camel pineal at the age of 1 year showing dissolute blood vessels (arrows) and intermittent areas free from pinealocytes (asterisks). The quantity of degeneration is

moderate. (H & E; x 400).

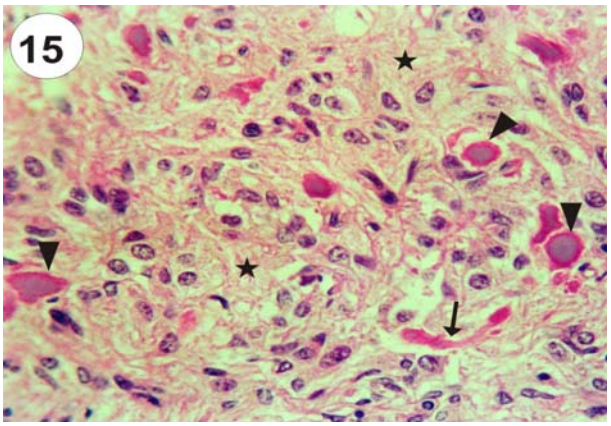

Fig. 15: Camel pineal at the age of 3 years showing hyalinized crossly cut blood vessels (arrow head) surrounded by remnants of degenerated wall; degeneration extends along with the direction of blood vessel (arrow); intermittent areas free from pinealocytes (asterisks), the amount of pinealocytes decreased. (H & E; x 400).

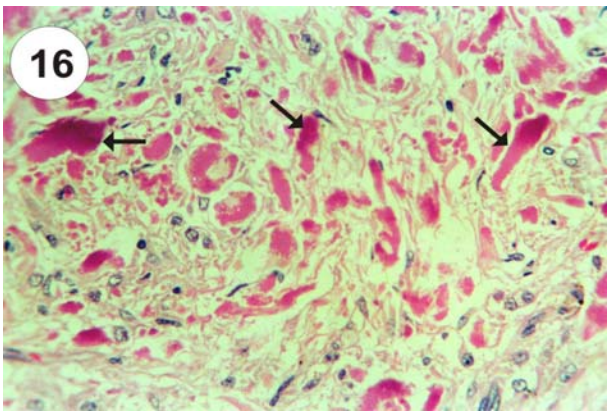

Fig. 16: Camel pineal at the age of 10 years showing high extent of hyalinization (arrows) with severe depletion of pinealocytes. (H & E; x 400).

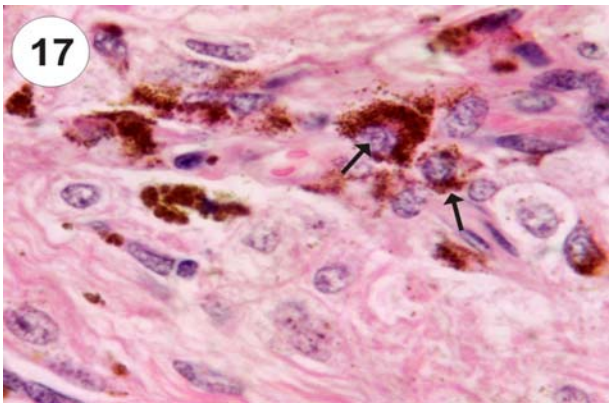

Fig. 17: Camel pineal at the age of 5 years showing packed pinealocytes with pigment granules (arrows) and pigment in the intercellular space. (H & E; x 1000**4.**

Discussion

Involution of the pineal was not studied on a large scale except very few papers in the past and it was restricted to morphometric data of some species, where involution was stated to occur periodically during the months of july and august, however the statistical weight study of the human pineal body was indicating that involution nearly inexistent (Legait and Legait, 1980). In the present study the size of the buffalo and camel pineals quantitatively did not increase widely by age however it was variable and sometimes seemed to be regressed. In addition the weight of the pineal increased in the middle aged animal and decreased in older ages which in a line with that recorded by Venzke (1975) in ruminants.

Brain sand or concretions was considered one of the familiar structures of pineal of many mammals including buffalo (Kawamura et al., 1986; Lalitha and Seshadri, 1992). Pinealocytes were shown to have a role in the start point of progressive steps of concretion in gerbil (Milin, 1998). The results of some investigations suggested that the cell organelles were involved in the genesis of the concretion (Welsh, 1984; Humbert and Pévet, 1995 b). Krstic (1986) explained the steps of concretion where the initial intracellular calcification sites occur in the cytoplasmic matrix, vacuoles, mitochondria and the endoplasmic reticulum of certain pinealocytes. These loci and particularly those within the cytoplasmic matrix, transform into acervuli (concretion) by further addition of hydroxyapatite crystals. The cells gradually degenerate, die, break down, and the acervuli reach the extracellular space. Galliani et al., (1989) suggested another idea that the cytoskeleton has a possible role to promote the brain sand. The intracellular calcium concentration is evoked by noradrenergic and cholinergic receptors resulting the release of calcium from the intracellular stores and by the influx of calcium from the external medium (Marin et al., 1996; Korf et al., 1997). So there are data suggesting that sympathetic impute to the pineal is necessary for the formation of pineal concretion (Vaughan et al., 1986). The present study described the brain sand in the pineal of buffalo as a curious signs of involution. This is because the calcium precipitation occurred within the wall or inside the lumen of some blood vessels; started by formation of small calcium granules which increased gradually in number and size, coalesced and progressed till cluttering up such blood

vessels completely. Therefore the calcium deposit appeared in the form of patches and elongated well identifiable structures in the same locations and courses of blood vessels, this will decrease the blood supply of the pineal and directly affecting its growth. The findings of Nikonorov and Makarov (1990) that during the pineal involution in human, the volume of its intraorganic vascular bed decreases essentially support the present study. The association of calcium with the collagen fibers of blood vessels wall in buffalo pineal, exhibiting a manner of correlation. This correlation was accorded by Humbert et al., (1997) where they suggest that collagen fibrils are involved in the genesis and growth of extra cellular concretion located in the connective tissue. The involution signs in camel including a striking physiological degeneration and hyalinization of blood vessels. Firstly dissolution of the wall of blood vessel and its content of blood cells occurred then hyalinization started at the center of dissolute blood vessel and could be seen surrounded by remnants of the degenerated wall. Blood vessels were lost by dissolution and hyalinization and this is shown as a new method of involution. In human the cause of decrement of vascular bed was the sclerosing process; this results in certain disturbances of blood supply and affects functional activity of the organ (Nikonorov and Makarov, 1990).

The present study denied some papers (Welsh, 1984; Galliani et al., 1989) and we reported new information wherethrough the structure which was so long termed brain sand in the past is completely calcified and blocked blood vessels and indicating involution of the buffalo pineal. Although, our results, were in accordance with recent investigations (Humbert and Pévet, 1995 a; Vigh et al., 1998; Luke, 2001) wherewith the calcification (brain sand) process is interpreted as being as age-related phenomenon. Hence we stated the beginning of calcium infiltration in buffalo to be as early as 1-6 months age after birth and the highest level was reached at the age of 1-10 years. Consequently the degree of degeneration and hyalinization of blood vessels in camel was moderate at the age of 1 year however areas of high extent of hyalinization occurred at the ages of 3-10 years. On the contrary the brain sand and calcium an ion in Mongolian gerbil and human does not appear to be age related and its involvement in the secretory activity rather than in gland atrophy is also suggested (Krstic, 1986; Galliani et al., 1989, Redecker et al., 1996).

Occasionally the lost of blood vessels in buffalo and camel was associating with decrease of pinealocytes amount. So the amount of pinealocytes decreased by age because they were crowded at the ages of 1-6 months but loosely arranged at the ages of 1-10 years in buffalo. Also in camel, severe depletion of pinealocytes was elaborated in the areas of high extent of hyalinization in advanced ages (at 3-10 years). The degeneration that was apparent in the parenchymal cells of buffalo pineal (Lalitha and Seshadri, 1992) potentiate the present study. Moreover similar features of pinealocytes number were recorded in rat (Humbert and Pévet; 1995 a). Also in human pineal an age related phenomena were studied (Luke, 2001). On the other side no important difference between pineals of young and advanced aged rats were found (Prosenc and Cervós - Navarro, 1994) and the human pineals do not necessarily degenerate progressively after involution (Hasegawa et al., 1987).

Pigment cells were usually found in the pineal body of the dromedary and also its fetus (Taher et al., 1975). In addition the pigment granules were constant elements of the dog pineal in both puppies and adult (Abou-Easa, 1997). Ultrastructurally the pigment cells were identified as a special type of pinealocytes (Calvo et al., 1988). The pineal pigment was histochemically identified as melanin (Calvo et al., 1992). These data support the present study that the melanin pigment were scattered allover the cellular elements of pineal tissue of camel. Most of the pinealocytes of all ages had contained melanin pigment. According to the species, the pigment-containing cells increase in number with increasing age in goat pineal (Ohshima and Matsuo, 1987). Some pinealocytes, glial cells and even fibroblasts in camel were so packed with the pigment granules that they obscure the nuclei, but no pigment granules could be observed in buffalo. Melanogenesis was attributed to the multipotency of the pineal cells (Orii et al., 1994). Also the multipotency of pinealocytes may be proved by the presence of striated muscle fibers in the pineal of some domestic animals, e. g. swine (Hayano et al., 1976) and rat (Prosenc and Cervós-Navarro, 1994). Heretofore no functional role has been assigned to the pineal pigment, so it may indicates non specialization of camel pinealocytes. It was known that pineal hormone, melatonin is biologically active derivative of tryptophan (Devlin, 1986) but we could not detect tryptophan.

The involution signs of pineal in buffalo and camel

will decrease the blood supply of the pineal and directly affecting the pineal growth and it is strongly hypothesized to reduce its function. The same hypothesis was accomplished in rat (Humbert and Pévet, 1995 a) and human (Nikonorov and Makarov, 1990). Therefore we affiliate to Redondo et al., (1996) who had suggested in a prenatal study of sheep pineal, that the pineal has a secretory function in uterine life). Some more recent study try to find the function of certain genes in the camel pineal (El Allali et al., 2008). But also some recent studies interested in the relation of the pineal function and melatonin to brain (Lahiri et al. 2004; Yun et al., 2004; Engel et al., 2005; Kim et al., 2005; peters et al., 2005).

Finally the present findings suggesting that the main function of the pineal is still doubtful; if the main function of the pineal is ever carried out in the postnatal life, it should not showing any sign of involution. So we suggest that the main function of the pineal may be in the prenatal life and may be related to maturation of neurons.

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8/8/2009

Occurrence of Parasitic Watermolds in Selected Forest Soils of Nainital, Indian Central Himalaya

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Abstract: Information regarding parasitic watermolds of forest soils and decomposing leaf litter is rare. Therefore, the present study was undertaken in hitherto unexplored forest ecosystems of Indian Central Himalaya. The present paper deals with isolation and taxonomic description of *Olpidiopsis* species from Indian Central Himalayan forest ecosystems of Kumaon region. Five species of *Olpidiopsis* were isolated from forest soils and decomposing leaf litter of cypress, mixed oak and chir pine forest ecosystems. *O.achylae* and *O.fusiformis* were isolated for the first time from Central Himalayan forest soils. Similarly *O. saprolegniae* var.*saprolegniae* and *O.varians* were isolated from decomposing leaf litters, while *O.pythii* was isolated from different soils and decomposing leaf litters. [Nature and Science. 2009;7(9):45-48]. (ISSN: 1545-0740).

Key Words: decomposing leaf litter; forest soils; Indian Central Himalaya; parasitic watermolds

1. Introduction

Some species of *Olpidiopsis* have been reported from India from tropical waters and soils by Butler (1907), Thirumalachar (1947), Das Gupta and John (1953) , Srivastava and Bhargava (1963), Srivastava (1964,1975), Dayal and Thakurji (1968) and Khulbe (1985, 2001).But the information regarding forest soils and decomposing leaf litter is little. Therefore, the present study was undertaken in hitherto unexplored Indian Central Himalayan forest soils and decomposing leaf litter.

During the course of the present study on parasitic watermolds, three temperate forest ecosystems viz., cypress (*Cupressus torulosa*, 2610m asl), mixed oak (*Quercus leucotrichophora* and *Q. floribunda*, 2150m asl) and chir pine (*Pinus roxburghii*, 1600m asl) were investigated. The characteristics of study sites are as follows:

Table 1. Characteristics of Study Site

Study Site	Latitude	Longitude	Elevation (m.)	Dominant Herbs	Dominant Shrubs	Dominant Trees
China Peak	29°23'9"	79°26'4"	2610	Erigeron karvinskianus, Anaphalis busua, Swertia pulchella, Oxalis dehradunensis, Achyranthes bidentata, Viola canescens, Nepeta leucophylla, Thalictrum foliolosum	Boenninghausenia albiflora, Myrsine africana, Rumex hastatus, Plectranthus japonicus, Colquehounia coccinea	Cupressus torulosa, Quercus floribunda, Cornus macrophylla
Pines	29°27'	79°23"	1600	Gerbera gossypina, Micromeria biflora, Setaria glauca, Commelina bengalensis,	Elsholtzia fruticose, Pyracantha crenulata, Berberis asiatica,	Pinus roxburghii, Rhododendron arboreum

				Polygonum chinense	Spiroea canescens, Aechyranthera goyypina, Euphorbia roylei,	
Tiffin Top	29°23'04"	79°28'36"	2150	Valeriana jatamansi, Impatiens amphorata, Flemingia involucrata, Polygonum nepalense, Salvia lanata, Anemone vitifolia Picea scripta	Rosa moschata, Rhamnus virgatus, Berchemia lineata, Caryopteris grata, Sarcoccoca hookeriana, Synotis rufinervis	Q. leucotrichophora, Q. floribunda

2. Materials and Methods

2.1 Method of collection:

To facilitate the isolation of aquatic fungi from soil samples, about 5 g soil of each sample was diluted in 100 cc of sterilized distilled water. The soil suspension was poured into five sterile Petri dishes and then baited with sterile hemp seed halves. The gross cultures were incubated at room temperature (18-22^{0}C). The colonized baits were taken out from the gross culture and washed thoroughly with sterile distilled water for several times and placed into freshly sterilized small Petri dishes containing sterile distilled water. Potassium tellurite (0.01% w/v), a bacterial suppressant was added in each culture to check the bacterial contamination. The colonized baits were examined under microscope. Pure cultures of host and parasites were identified with the help of keys provide by Coker (1923), Coker and Matthews (1937), Johnson (1956), Sparrow (1960), Scott (1961) and Khulbe (2001).

2.2 Fungi Collected

Olpidiopsis Cornu

Ann. Sci. Nat. Bot., V, 15: 114 (1872).

Thallus at first naked, later surrounded by a membrane, endobiotic, holocarpic; sporangium spherical or ellipsoidal, smooth walled or spiny, non-tubular and unlobed, with one to several discharge tubes; zoospores formed within the sporangium, biflagellate without well defined diplanetism, resting spores thick-walled, smooth or spiny, formed asexually.

Olpidiopsis achlyae McLarty

Bull. Torrey Bot. Club, 68: 62 (1941).

Specimen examined: INDIA, UTTARANCHAL, Nainital, Pines, 29^{0}27'N and 79^{0}26'E, 1600m a.s.l., hempseed halves, 22nd February, 2001, Manisha Upadhyay, Olp/a.

Zoosporangia solitary to numerous usually in terminal hyphal swelling of host, spherical, oval, variable in size, ranging from 16.0-120.0 x 105.0-560.0 μm; exit tubes 1-3; zoospores numerous, hyaline, small, spherical, oval, 3.5-6.0 μm in diameter, resting spores spherical, 30.0-120.0 μm, brown; companion cells may or may not present.

The fungus was parasitic on *Achlya* species. It was isolated for the first time form forest soils of Kumaun region of Indian Central Himalaya.

In India, it was isolated by Das-Gupta & John (1953), Dayal & Thakurji (1968), Manoharachary & Rao (1999) and Khare (1992). In an artificial inoculation experiment, this species could also infect *Thraustotheca clavata* (Khulbe 1985).

Olipidiopsis fusiformis Cornu

Ann. Sci. Nat. Bot. V, 15: 147 (1872).

Specimen examined: INDIA, UTTARANCHAL, Nainital, Pines, 29^{0}27'N and 79^{0}26'E, 1600m a.s.l., hempseed halves, 25th June, 2001, *Manisha Upadhyay*, Olp/b.

Thallus endobiotic, holocarpic; zoosporangia one to many in terminal or intercalary swelling of the host hyphae; spherical, oval, fusiform, ellipsoidal, cylindrical, 35.0-350.0 μm long and 12.5-95.0 μm in diameter, wall smooth; exit tubes 1-3; zoospore

numerous, ovoid; resting spores spherical, 40.0-80.0 *µm* in diameter; outer surface covered with spines, up to 7.0 *µm* long; companion cell 1-2 per resting spore, spherical, smooth walled, 20.0-30.0 *µm* in diameter.

It has been reported to be parasitic on *Achlya americana*. In India it was isolated by Srivastava (1966), Khulbe (1985), Rai and Misra (1981), Gupta and Mehrotra (1988, 1992). It was isolated for the first time form forest soils of Kumaun region of Indian Central Himalaya.

Olpidiopsis pythii (Butler) Karling

Simple Holocarpic Biflagellate Phycomycetes, p. 47 (1942).

Specimen examined: INDIA, UTTARANCHAL, Nainital, China Peak, Pines, Tiffin Top, $29^0$23'N and $79^0$26'E, $29^0$27'N and $79^0$23'E,$29^0$23N and $79^0$28'E respectively, 2610 m,1600 m and 2150m a.s.l., respectively, hemp seed halves, and decomposing leaf litter of cypress on 26th February 2000, pine (soil sample) on 24th February 2000 and 25thJuly 2001, and oak 22th June and 28th August 2000, and 22nd February and 22nd June 2001, Manisha Upadhyay, Olp/c.

Thallus endobiotic, holocarpic; zoosporangia one to many in terminal or intercalary swellings of the host hyphae, spherical, 12.0-38.0 *µm* in diameter, wall smooth, exit tubes 1-3; zoospore numerous, oval; resting spores spherical, sometimes oval; 9.5-35.0 *µm* in diameter, outer surface brown covered with spines; companion cells absent.

This fungus was parasitic on hyphae of *Pythium monospermum.*

It was interesting to isolate it for the first time from different soils and decomposing leaf litters of the Indian Central Himalayan forests. In India, it was earlier isolated by Khulbe (1985), Joshi (1993), Kaur (1996) and Nayal (1999) from different habitats in India.

Olpidiopsis saprolegniae var .***saprolegniae*** (Braun) Cornu

Indian Phytopathology, 16:271-274 (1963).

Specimen examined: INDIA, UTTARANCHAL, Nainital, China Peak and Tiffin Top, $29^0$23'N and $79^0$26'E and $29^0$27'N and $79^0$23'E, respectively, 2610 m and 2150m a.s.l., respectively, hemp seed halves, and decomposing leaf litter of cypress on 23rd July 2000 and 23rd August 2001, and Oak on 25th June 2000 and 28th August 2001, Manisha Upadhyay, Olp/d.

Thallus endobiotic, holocarpic; zoosporangia one to many, usually formed in terminal swellings of the host hyphae, sometimes intercalary, spherical, ovoid or ellipsoidal, 50.0-195.0 *µm* in diameter; wall smooth, discharge tubes 1-3, narrowly cylindrical; zoospore oval to elongate, 3-4 *µm* in length; resting spores spherical, thick, spiny, slender, 6-12 *µm* long; companion cells 1-2 per resting spore, usually spherical, 25.0-30.0 *µm* in diameter.

The fungus was parasitic on the hyphae of *Saprolegnia diclina*. This species was also isolated for the first time from different decomposing leaf litters (cypress and mixed oak) of Indian Central Himalayan forests.

Olpidiopsis varians Shanor

Indian Phytopathology, 17(3):249-253(1964).

Specimen examined: INDIA, UTTARANCHAL, Nainital, Pines, $29^0$27'N and $79^0$26'E,1600 m a.s.l., hemp seed halves and decomposing leaf litter of pine, 26th June and 24th July 2000, and 26th July 2001, Manisha Upadhyay, Olp/e.

Thallus endobiotic, holocarpic; zoosporangia one to many, usually formed in terminal or occasionally intercalary swellings of the host hyphae, spherical to oval, 35.0-75.0 *µm* in diameter; wall smooth, discharge tubes 1-3; zoospore oval, up to 5 *µm* in length; resting spores spherical, spiny, 20.0-52.5 *µm* in diameter (with spines); companion cells 1-2 per resting spore, spherical, 12.0-26.0 *µm* in diameter.

It was found parasitic on *Achlya flagellata*. This species was isolated for the first time from decomposing leaf litter of chir-pine forest in Indian Central Himalaya.

3. Results

Five parasitic watermolds viz., *O. lpidiopsis achylae*, *O. fusiformis*, *O. pythii*, *O.saprolegniae* var .*saprolegniae* and *O. varians* have been reported from temperate forest ecosystems of Nainital, Indian central Himalaya. Of these *O. achylae*, *O. fusiformis*, *O. pythii*, were isolated for the first time from chir-pine forest soils. *O. varians* was isolated from decomposing leaf litter of chir-pine forest for the first time. *O. pythii* and *O. saprolegniae* var. *saprolegniae* were isolated from soils (mixed oak) and decomposing leaf litters of both cypress and mixed oak forest ecosystems. All the cultures were deposited in Aquatic Fungal Culture

Collection Centre, Aquatic-Mycopathology laboratory, D.S.B Campus, Kumaon University, Nainital, Uttarakhand, India.

4. Acknowledgements

The authors are thankful to the Head, Department of Botany, Kumaon University, Nainital for providing laboratory facilities and encouragement during the course of this study. The guidance received from Prof. R.D Khulbe, who passed away in January 2002 is gratefully acknowledged. The authors are also grateful to Director, Herbal Research & Development Institute,Gopeshwar, Chamoli, Uttarakhand for providing facilities and help.

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8/11/2009

Eco-Phytochemical Studies of Plants in a Crude Oil Polluted Terrestrial Habitat Located at Iwhrekan, Ughelli North Local Government Area of Delta State

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Abstract: Eco-phytochemical studies and analyses of soils from crude oil impacted and unimpacted sites at Iwhrehkan in Ughelli North Local Government Area of Delta State were carried out. The vegetation was sampled using a 1m x 1m quadrat along the diagonals of the heavily impacted area. A total of 3952 plants belonging to six families (Poaceae-4 species; Asteraceae-2 species; Melastomataceae-2 species; Convolvulaceae, Euphorbiaceae and Tiliaceae 1 species each) were encountered in the field. *Paspalum vaginatum* was most abundant and had a frequency of 75.1% while *Ipomoea involucrata* had the least frequency of 13%. Heavy metallic ionic concentrations of Zn, Cd, Fe, Co, Cr, As and Hg were significantly higher in crude oil polluted soil compared to the control. In contrast, sulphate, phosphate, nitrate and ammonium ions concentrations were higher in the unimpacted soil samples. Polycyclic aromatic hydrocarbons (PAH) concentrations were similar in unimpacted soil samples while they varied from one to the other in the polluted soil. Tissue analyses of mature cassava root tubers from crude oil contaminated soil showed lower concentrations of SO_4^{-2}, PO_4^{-2} and NO_3^- but higher NH_4^+ compared to the control. [Nature and Science. 2009;7(9):49-52]. (ISSN: 1545-0740).

Key words: Eco-phytochemical, crude oil, terrestrial habitat.

1. Introduction

Crude oil is a naturally-occurring hydrocarbon compound used by humans in a variety of ways: fuelling of cars, lorries and trucks; heating of homes, cooking gas and other fractions utilized in the manufacture of synthetic products. Industrialization coupled with an ever-increasing demand for petrochemicals have resulted in prospecting for more oil wells with consequent pollution of the environment. Okoloko (1974) reported the main causes of oil pollution in Nigeria to include discharge from sludge, production tests, drilling mud, spills from pipelines, well-blow outs, gas flaring and sabotage.

The effects of oil pollution vary according to the type and amount of oil involved, time of the year, degree of its weathering and age of the plant species concerned (Ajiwe, *et al* 1996). Crude oil is phytotoxic because it creates unsatisfactory conditions for plant growth ranging from heavy metal toxicity to inhibited aeration of the soil. Heavy metals such as Cu and Zn are essential for normal plant growth, although elevated concentrations of both essential and non-essential metals can result in growth inhibition and toxicity symptoms (Hall, 2002). Toxicity symptoms observed in plants exposed to oil pollution include chlorosis, necrosis, stunted growth, suppression of leaves, enormous reduction in biomass to stomatal abnormalities (Baker, 1970).

Contamination of soils by crude oil does not seem to have any adverse effect on *Fusarium moniliform* (Okafor, 1987). This species of fungus is able to utilize crude oil hydrocarbon as a source of carbon and energy for growth. Plants may tolerate sites polluted with petroleum hydrocarbon by creating a soil environment rich in microbial activity that can change the availability of organic contaminants or enhance their degradation (Cunningham and Lee, 1995).

This study is aimed at: i) identifying plant species that can tolerate crude oil polluted sites; ii) ascertain the levels of polycyclic aromatic hydrocarbons and heavy metals in impacted soils, and iii) absorption of heavy metals by cassava – the staple food of the people.

2. Materials and Methods

2.1 Study area. Ughelli, one of the big towns in Delta State is located at latitude 5.30^0N and longitude 5.58^0E. Iwhrekan, where the spill occurred is a small farming Community about 10 km from Ughelli. The bushes around Iwhrekan are cross-crossed with oil pipelines conveying crude oil to an oil terminal at Escravos. The major crop cultivated by the people is cassava; the natural vegetation is evergreen tropical rainforest but presently transiting to derived savanna as a result of excessive cultivation, reduced fallow period caused by increased population pressure. The spillage occurred on the 14th of April, 2006 due to vandalization, covered an area of 15 hectares (both heavily and slightly impacted areas). This study was carried out between August and September, 2006; no

treatment was applied to the field before the samples were collected.

2.2 Sampling techniques. A heavily impacted area measuring 500 m x 500 m with the source of the spill at the centre was marked out with pegs. Two line transects were made diagonally each passing through the spot where the pipeline was vandalized. The vegetation was sampled using a 1 m x 1 m quadrat, laid along the transects at intervals of 10 m. In all, 128 quadrats were laid. Sampling along the diagonals was aimed at ensuring proper coverage of the sampled area. The data were analyzed using three quantitative measures: frequency, abundance and density.

Frequency was the number of quadrats in which a species occurred expressed as a percentage of the total number of quadrats. Density was recorded as the number of plants per m^{-2} for each plant expressed over all quadrats laid; while abundance referred to the total number of plants encountered in the field for each of the species.

Ten soil samples each collected at a depth of 10 cm from the heavily impacted and unimpacted sites were taken with a soil corer. Each sample lot was properly mixed to form homogenous mixtures. The soil samples were analyzed for polycyclic aromatic hydrocarbons (PAH), ionic concentrations of some heavy metals, phosphate, sulphate, nitrate and ammonium ions.

Tuberous roots of cassava also from heavily polluted and unpolluted sites were uprooted and analyzed for some heavy metallic ion concentrations. The soil and tuberous roots of cassava were analyzed using standard laboratory procedures as outlined by AOAC (1984).

3. Results and Discussion

A total of three thousand, nine hundred and fifty two (3, 952) plants belonging to six families were encountered in the field. The families consisted of Asteraceae–2 species, Convolvulaceae–1 species, Euphorbiaceae–1 species, Melastomataceae–2 species, Poaceae–4 species and Tiliceae–1 species (Table 1). *Paspalum vaginatum* (Sw) was most abundant among all the species with a frequency of 75.1% and a density of 10.41 m^{-2} , indicating that this species had the greatest potential to survive in oil polluted soils. *Eragrostis tenella* Linn was encountered in 64 quadrats with a frequency of 50 % compared to *Dissotis erecta* (Guill and Pers.) Dandy which occurred in 64 quadrats, had an abundance of 183 and a frequency of 50%. *D. erecta* had the same distribution with *Eragrostis tenella* Linn but a lower density of 1.42 (Table 1).

Of importance was the localized occurrence of *Digitalia longiflora* (Retz.) Pers. in only 32 quadrats with second highest abundance of 577 and a density of 4.5. This limited spread may be due to the topography of the land – which was sloppy and the species was found mainly at the upper end of the slope which did not permit the seepage of the crude deeply into the soil. The least abundant plant in the field was *Ipomoea involucrata P. Beauv* with a frequency of 13% and a density of 0.2 (Table1). Soils contaminated with crude oil contain polycyclic aromatic hydrocarbons (PAH) and heavy metals that are toxic to plants. The adverse effects of petroleum and its compounds on plant growth had earlier been reported by Gill *et al*, (1992) on *Chromolaena odorata* (L) R.M. King & Robinson. Wang and Lau (1985) had reported phytoxicity of heavy metals to the roots of *Cynodon dactylon* (Linn.) Pers. and *Eleusine indua* Gaertn. Also, the inhibition of plant growth by harmful metallic ions present in petroleum was reported by Winter, *et at* (1976).

The concentrations of anions and cations present in impacted and unpolluted soil samples varied considerably (Table 2). Phosphate, sulphate and nitrate ionic concentrations were higher in the control (unimpacted soil) compared to polluted site (Table 2). This indicated that crude oil spillage could make vital plant nutrients unavailable to plants. Total petroleum hydrocarbon (TPH) content was higher in impacted soil samples (Table 2). The toxic nature of crude oil and its components was responsible for the low number of plant families encountered in the field. Iwherekan is located in the heart of tropical rain forest known for high species diversity; many other taxa had been smothered by the spill.

The survival of cassava *(Manihot esculenta Gantz)* in the heavily impacted area was significant as it was the stable food of the people. Cassava stands smothered by crude oil were observed regenerating just below the soil surface. This showed that the economic crop was able tolerate oil-polluted site. The results of the comparative analyses of tuberous cassava roots from both crude oil impacted and unimpacted sites are presented in Table 3. Nitrate, sulphate and phosphate ions assayed in the tuberous roots of cassava from impacted soil were significantly lower than their concentrations in the unimpacted area. This showed that crude oil may have reduced the uptake and availability of these ions to cassava (Table 3). The concentrations of Cr, As, Co and Hg were similar in both cassava samples even through they were higher in impacted soil; this indicated that cassava may have a mechanism of excluding or detoxifying heavy metals thereby ensuring its survival in the polluted soil.

Baker (1980) had reported two basic strategies to heavy metal tolerance: metal exclusion and metal detoxification. The excluders prevent metal uptake into roots, avoiding translocation and accumulation in shoots (De Voss *et al.* 1991). The plants have a low potential for metal extraction but could be used to stabilize the soil. Conversely, hyperaccumulators absorb high levels of heavy metals in their cells.

About 400 species of plants that hyperaccumulate heavy metals had been reported by Prasad and Feitas (2003). Among the families documented by them to be heavy metal accmulators were Asteraceae, Brassicaceae, Caryophylaceae, Cyperaceae, Cunouniaceae, Fabaceae, Poaceae, Violaceae, Lamiaceae, Euphorbiaceae and Flacourtiaceae. In this study, members of the family Poaceae were most abundant and frequent and most tolerant to crude oil contamination. Gibson and Pollard (1988) reported that heavy metal tolerance was common in the family Poaceae. This confirmed the dominance by Poaceae in the polluted site.

The results of the study suggested that plant species documented in this report were tolerant to crude oil contamination and by extension some heavy metals which are usually high in crude oil polluted habitats. It had presented a short list of plants likely to be used in revegetating crude oil polluted lands.

Table 1. Plant species encountered in the field: their abundance, frequency (%) and density (m^2)

Plant Species	***Family***	***Number of quadrats species occurred***	***Abundance***	***Frequency (%)***	**Density (m^{-2})**
Minihot esculenta Crantz	Euphirbiaceae	64	288	50	1.78
Pasalum vaginatum (Sw)	Poaceae	96	1333	75.1	10.41
Eragrotis tenella Linn	Poaceae	64	525	50	4.10
Dissotis erecta (Guill & Perr) Dandy	Melastomataceae	64	183	50	1.42
Clappertonia ficifolia (Wild)	Tiliaceae	32	123	26	0.96
Leersia hexandra (Sw)	Poaceae	80	448	63	3.50
Aspillia africana (Pers) C.D. Adams Adams	Asteraceae	64	131	50	1.02
Digitalia longiflora (Retz.) Pers	Poaceae	32	557	26	4.5
Bidens pilosa Linn	Asteraceae	32	99	26	0.77
Dissotis rotundifolia (Sm) Triana	Melastomataceae	47	219	37	1.7
***Ipomoea involucrata* P. Beauv.**	Convolvulaceae	16	26	13	0.2

Table 2. Polycyclic aromatic hydrocarbon (PAH), Chemical properties and total petroleum hydrocarbon (TPH) contents of soils from impacted and unimpacted sites.

PAH	***Unimpacted soil sample (ppm)***	***Impacted soil sample (ppm)***	***Chemical properties***	***Impacted soil sample (ppm)***	**Unimpacted soil sample (ppm)**
Acenaphthalene	<0.001	0.085	pH	5.60	4.95
Benzo (k) fluoranthene	<0.001	0.000	Zn^{++}	16.70	8.70
Anthracene	<0.001	0.145	Cd^{++}	2.50	0.42
Benzo (a) anthracene	<0.001	0.112	Fe^{++}	36.75	21.06
Benzo (b) fluoranthene	<0.001	0.105	Co^{++}	19.40	6.10
Benzo (g) perylene	<0.001	0.000	Mn^{++}	65.80	51.70
Benzo (a) pyrene	<0.001	0.075	Cr^{++}	6.15	1.16
Chrysene	<0.001	0.036	As^{++}	0.21	<0.01
Dibenz (ah) anthracene	<0.001	0.000	Hg^{++}	0.11	<0.01
Fluranthene	<0.001	0.000	SO_4^{2-}	345.60	417.80
Flurine	<0.001	0.000	PO_4^{3-}	0.89	22.75
Indoeno (1,2,3) pyrene	<0.001	0.000	NO_3^-	116.30	267.26
Phenthrene	<0.001	0.000	$NH_4^+{}_4$	216.40	579.30
Pyrene	<0.001	0.000	TPH	8625.4	<1.0
perylene	<0.001	0.714			

Table 3. Concentration of ions in the tuberous roots of cassava obtained from impacted and unimpacted sites.

Ions	*Impacted soil (ionic concentrations ppm)*	Unimpacted soil (ionic concentrations ppm)
Zn^{++}	20.8	54.7
Cd^{++}	<0.01	<0.01
Fe^{++}	188.6	128.0
Co^{++}	<0.01	<0.01
Mn^{++}	885.6	228
Cr^{++}	<0.01	<0.01
As^{++}	<0.01	<0.01
Hg^{++}	<0.01	<0.01
SO_4^{2-}	3043.36	17722.9
PO_4^{3-}	478.36	975.7
NO_3^-	38.42	93.5
$\mathbf{NH_4^+}$	96.05	8.75

1. Acknowledgement

We are grateful to Petroleum Training Institute (PTI) Staff, Warri who assisted in the analyses of the samples collected.

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7/9/2009

Environmental geological assessment of a Solid Waste Disposal site: a case in Ilorin, Southwestern, Nigeria.

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Abstract: This study was carried out to evaluate the effects of leachate from open landfill in the Ita-Amo area of Ilorin, southwestern Nigeria. It is also aimed at determining the possibility of upgrading the site to a modern waste containment facility (Sanitary Landfill) by evaluating the geotechnical properties of soils obtained over the site. A total of ten water samples and four representative soil samples were analyzed. Results of water analyses showed that the P^H range from 6.6 to 7.1; Electrical Conductivity range from 107µs/cm to 241µs/cm; TDS range from 61mg/l to 971mg/l ; Ca^{2+} range from 7.4mg/l to 64.0mg/l, Mg^{2+} range from 17.0mg/l to 61.0mg/l; HCO_3^- range from 93.5mg/l to 250.0mg/l; SO_4^{2-} range from 98.0mg/l to 1400.0mg/l; Cl^- range from 56.4mg/l to 880.0mg/l during the wet season. In the dry season, the concentration of ca^{2+} range from 41.3mg/l to 117.2mg/l; Mg^{2+} from 50.0mg/l to 100.4mg/l, K^+ range from 2.1mg/l to 14.0mg/l; HCO_3^- range from 91.8mg/l to 234.0mg/l; SO_4^{2-} range from 91.7mg/l to 1730.0mg/l; and Cl^- range from 43.7mg/l to 1347.0mg/l. The concentrations of Pb range from 41.2µg/l to 53.4µg/l and 40.8µg/l to 61.7µg/l; Zn^{2+} range from 57.0µg/l to 60.0µg/l and 15.0µg/l to 67.1µg/l at wet dry season respectively while Ni was 30.3µg/l as measured in the wet season only. Generally, the concentrations of these parameters are higher than WHO recommendations and decrease away from the centre of the waste dumpsite which is suggesting anthropogenic influence on the water chemistry. Geotechnically, the soils have 0.75% gravel, 32% sand, 48% clay and 19% silt in a compactible sandyclay soil. The result of the Atterberg limits tests showed that the soils are absolutely inorganic clay of low plasticity and average clay activity value of 0.39 which is suggesting non-reactive kaolinitic clay. The dry density of the soils are 1.80t/m^3 and 2.1t/m^3 when compacted at standard and modified Proctor energies respectively while the coefficient of permeability of the soils are in the order of $1x10^{-9}$m/s and $1x10^{-11}$m/s respectively. These results are found favorably compared to recommendations of several researchers. Thus, the soils satisfied the requirements for use as mineral seals in sanitary landfills. The higher energy of compaction (Modified Proctor) is recommended because it offered lower values of coefficient of permeability. [Nature and Science. 2009;7(9):53-62]. (ISSN: 1545-0740).

Keywords: Sanitary Landfill; Leachate; Coefficient of Permeability; Mineral Seals; Solid waste

1. Introduction

Human population is increasing on daily basis, so is the corresponding quantity of waste contending for space with man and its effect impairing the quality of the environment. It is therefore very common to find waste dumps within built-up areas and cities in bags along roads and streets. Attempts by Nigeria government, groups and individual to check these problems include composting, open burning and river dump of refuse. These attempts had severally failed because of their inadequacies (Ige, 2003 and Asiwaju-Bello, 2004).

The city of Ilorin falls into southwestern (Figure 1) and Northcentral on geological and political classifications respectively the capital town of kwara state, Nigeria. It has a total land coverage of over 400km^2 (Africa Atlases, 2007) and a population estimate of 2,185,494 people (NPC, 2007) which are responsible for the generation of waste often deposited in open spaces, river banks, road side etc. thereby degrading the quality of the environment. In attempt to alleviate environmental pollution within the city, three (3) final waste disposal sites (unengineered) were prepared and located strategically at the outskirt of the city (Figure 2). However, the selection, design, construction and operational activities of these sites did not consider the geology and impacts on the adjacent environment. One of the deposal sites, along Ilorin –

peke village has been investigated as presented in this study.

This study is therefore aimed at assessing the effects of leahate that is generated from dumpsite on the shallow groundwater of the study area to ascertain the extent of influence on the qualities of such water resources. The geotechnical and environmental issues were also investigated for the possible upgrading of the site to a modern solid waste containment facility such as sanitary landfill.

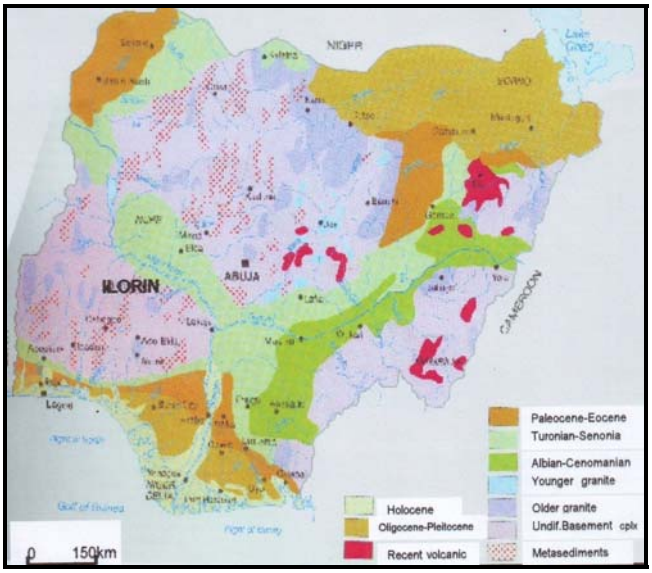
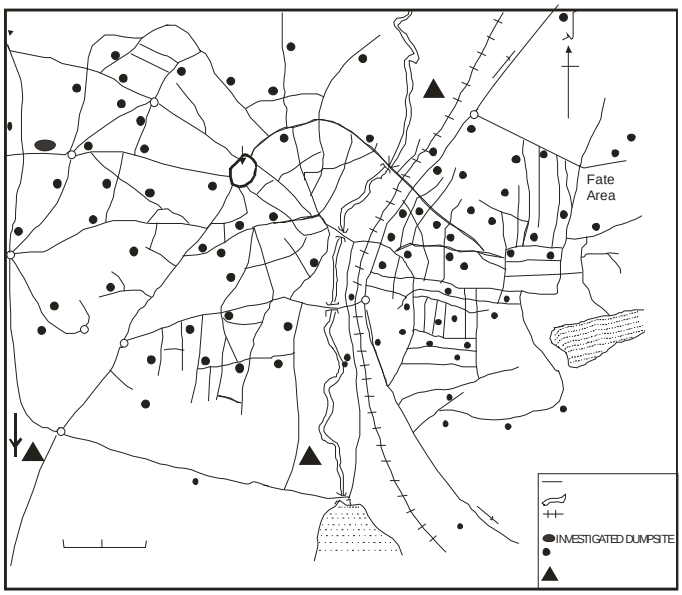


Figure 1: Geological map of Nigeria showing Ilorin (Modified from Africa Atlases, 2007)



2. Study area

The study area (Ita-Amo waste disposal site, Ilorin) is located within latitude 8^0 25^0 N and 8^0 30^0 N and longitude 4^0 20^0 E and 4^0 30^0E. The approximate area extent of the dumpsite is $3.63x10^6m^2$ with average dump thickness of about 7.7m. The site inhabits and still occupying several farm land area. Geologically, the area lies in the Precambrian Basement complex of southwestern Nigeria and is underlain by rock of metamorphic and igneous type. However, migmatite predominantly underlies the waste dumpsite and characterized by weathered regolith which vary in thickness from place to place. The hydrologic setting of the area studied is typical of what is obtained in other Basement complex area where the availability of water is a function of the presence of thick-little clay overburden material and presence of water filled joints, fracture or faults within the fresh Basement rocks. The humid tropical climate of Ilorin has particularly encourage relatively deep weathering of the near surface rocks to produce porous and permeable material that allows groundwater accumulation as shallow aquifer which is recharged principally through infiltration of rainwater. At the investigated dumpsite, the waste leachate may also infiltrate to pollute the shallow ground water.

3. Material and Methods

10 water samples were used in this study. 4 shallow water wells were dug around the solid waste deposition centre (1within the dump, 3 outside the dump and a river water sample). The well within the dumpsite is labeled 1while wells at distances "devoid of hydraulic interconnectivity" away from dumpsite and the river water sample and labeled 2,3,4 and 5 (50m, 70m, 120m and 327m away from dumpsite) respectively. Each of the well was dugged below the groundwater table to provide column for water accumulation. The water sampling was done in the well and dry season to monitor pollution (if any) with respect to season. Each of the water samples was collected into 2-litre container for the determination of anion concentration while 1-litre was used for cation concentration determination. The cation containers were acidified with 1-2 drops of HCl acid to prevent adhering to the surface of the container. The sensitive physical parameter such as total dissolve solid (TDS),
Electrical conductivity (Ec) and P^H were determined in the field using portable digital conductivity and PH meters also measure on the field are colour and turbidity. The analyses of the chemical constituents of the water sample were carried out at the water laboratory UNICEF office in Ilorin, Nigeria. Major cations (Mg^{2+}, Na^{+}, Ca^{2+}, K^{+}) and anions (HCO_3^{-}, SO_4^{2-}, Cl^{-}) were analyzed using the flame photometry and atomic absorption photometry method while calorimetric, gravimetric and titrimetric methods were used for the determination of the anions.

Also four soils were recovered from the shallow wells at different depth within the lateritic zone. The variation in depth of soil sample was necessary to know the geotechnical properties of the whole laterite zone which may be useful as a mineral seals in the construction of modern waste containment facility (sanitary landfill). All the soil samples were analyzed with respect to their grain size distribution, Atterberg limit, moisture content-density relationship and the coefficient of permeability (***K***) characteristics at the soil laboratory of the Yaba College of technology, Lagos, Nigeria according to the BS 1377: 1990 standard. The results obtained were later compared with the recommendation of several previous researchers and regulatory agencies.

4. Results and Discussions

4.1 Physical Properties of Water Samples

Summary of the physical properties (and some site characteristics) of water sample is presented in Table 1. The P^H value range between 6.8 - 7.9 at both seasons. This range falls within the acceptable boundary of (WHO, 1993) for water usage in drinking. The colour value range between 15Hazen - 61Hazen units in the wet season and 50Hazen - 92Hazen in dry season with highest value observed at well 1. Turbidity is higher in dry season than in wet season, probably due to dilution activities in the wet season, and decrease away from the dumpsite area (Table 1).

Table1: Physical Properties of Water Sample and other site Characteristics.

Properties	W1	W2	W3	W4	SW
Static water level (m)	5.71	6.30	6.57	4.9	-
Depth of Well (m)	6.47	6.47	7.15	6.71	-
Thickness of horizon(m)	3.90	3.58	3.84	3.79	-
Depth of soil sampling(m)	1.15	2.70	4.50	3.10	-

W1- 4: Well Number **SW: Surface Water**

4.2 Chemical Properties of Water Samples

The relative abundance of the elements is shown in Table 2. The calcium ion concentration range from 7.4 mg/l - 64mg/l and 41mg/l -121.4mg/l during wet and dry season respectively. While the magnesium ion concentration range from 17.0mg/l - 61.0 mg/l and 17.0mg/l-100.4mg/l during wet and dry season respectively. Concentration of sodium ion range from 12.0mg/l – 57.5mg/l in the wet season, and 23.0 mg/l to 109.4mg/l in the dry season while potassium ion concentration range from 0.78mg/l - 2.70mg/l and 1.30mg/l -14.0 mg/l during wet and dry season respectively. Calcium concentration falls below the maximum permissible level (MPL) of their concentration in water to be useful for drinking purpose (WHO, 1993). This may be due to little or no interaction of water with the underlying basement rock (the principal source of calcium and magnesium ion) since all water samples were collected within vadose zone. However, concentrations above allowable level (AL) were noticed at wells1, 2 and 3 in dry season and decreases with distance away from the centre of the dumpsite (Figures 3-12).

For the anions, bicarbonate concentrations range from 93.5mg/l - 250mg/l in the wet season and from 91.8mg/l - 234.0mg/l in dry season. The sulphate range from 98.0-1400.0mg/l and 91.7mg/l - 1730.4mg/l in the wet and dry season respectively. The chloride ion concentration range from 56.4mg/l - 880.0mg/l ion in the wet and from 43.7mg/l - 1347.0mg/l in the dry season. The nitrate ion concentration (where determined) range from 17.0mg/l - 31.0mg/l and 20.1mg/l-53.4mg/l at the two season respectively. All the parameters (anions) were observed to be above MPL at the wells (1-4) during the two seasons and decrease away in concentration from dumpsite centre (Table 2). This may be connected to the liquification and leaching of decomposed dominantly domestic, industrial and commercial waste within the dumpsite as a result of the action of heat, pressure and presence of water. Generally, low concentrations of these parameters were observed which may be due to pollutant filtering capability of the underlying soil, season and age of dumpsite.

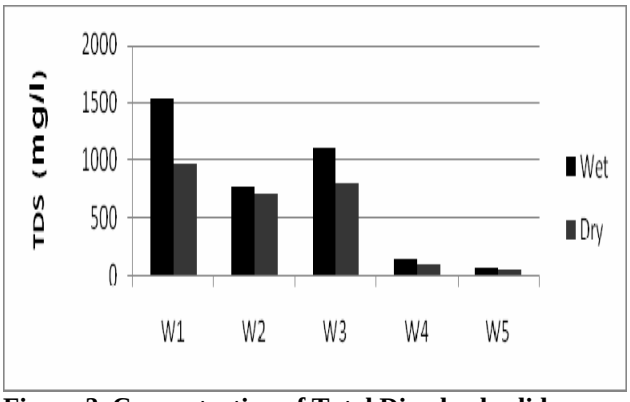


Figure 3. Concentration of Total Dissolved solid (TDS)

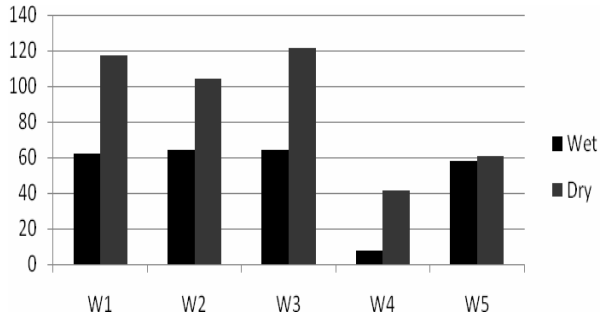


Figure 4. Variation of calcium concentration in the wells

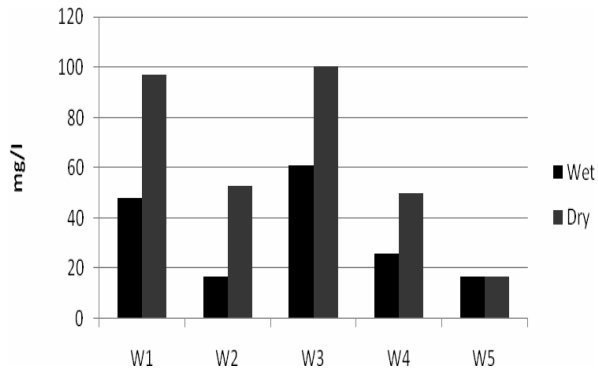


Figure 5. Variation of magnesium ion concentration in the wells

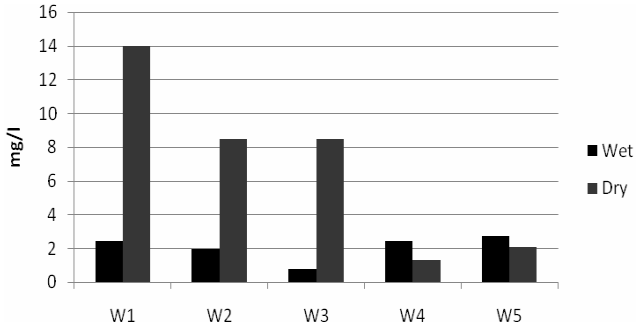


Figure 6. Variation of Potassium ion concentration in the wells

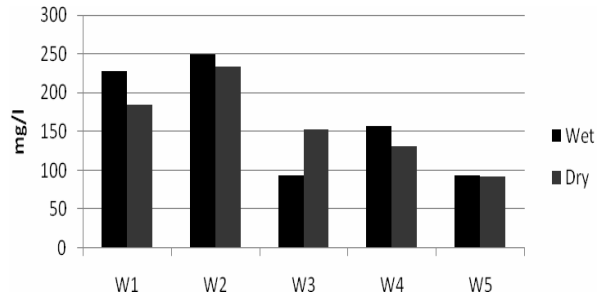


Figure 7. Variation of Bicarbonates ion concentration in the wells

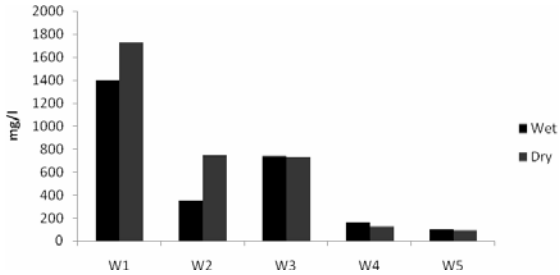


Figure 8. Variation of Sulphate ion concentration in the wells

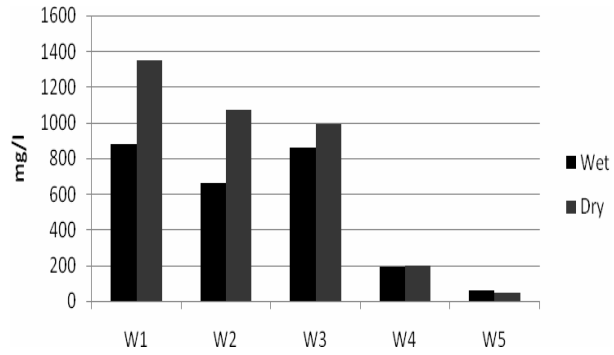


Figure 9. Variation of Chloride ion concentration in the wells

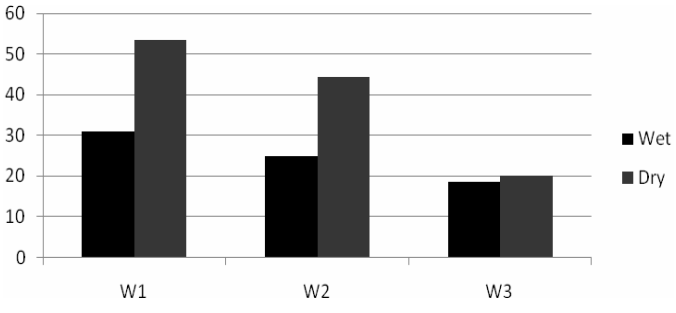


Figure 10. Variation of Nitrate ion concentration in the wells

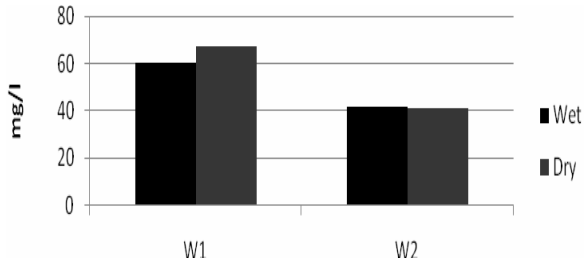


Figure 11. Variation of Zinc ion Concentration in the wells

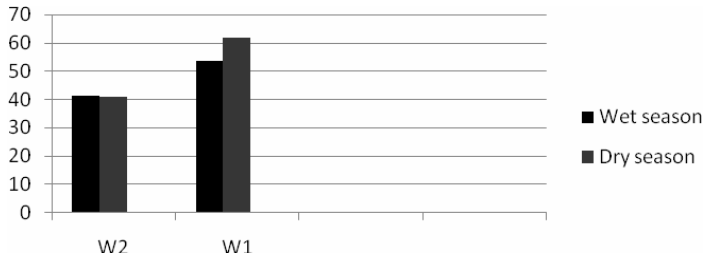


Figure 12. Variation of Lead ion concentration in the wells

T able 2: Chemical Properties of Water Samples

Parameters	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	AL	MPL
PH	7.1	6.6	7.2	6.8	7.6	7.1	7.4	7.1	7.9	7.0	-	
Ec (µs/cm)	3320	2411	1585	1034	2360	1917	500	439	160	107	-	400
TDS (mg/l)	1530	971	785	704	1110	816	142	101	74	61	-	1000
Calcium (mg/l)	62.0	117.2	64.0	104.4	64.0	121.4	7.4	41.3	58.0	61.0	75.0	200.0
Magnesium(mg/l)	48.0	97.2	17.0	53.0	61.0	100.4	26.0	50.0	17.0	17.0	50.0	150.0
Sodium (mg/l)	12.0	49.4	57.5	109.4	12.0	61.1	23.0	27.0	25.0	23.0	20.0	200.2
Potassium (mg/l)	2.4	14.0	1.95	8.5	0.78	8.5	2.4	1.3	2.7	2.1	10.0	12.0
Bicarbonate(mg/l)	228.1	184.2	250.0	234.0	93.5	153.3	157.4	131.0	94.1	91.8	variable	variable
Sulphate (mg/l)	1400	1730.4	350.0	742.7	740.1	733.6	160.0	120.0	98.0	91.7	250.0	400
Chloride (mg/l)	880.0	1347.0	664.1	1074.1	860.0	994.4	193.9	200.7	56.4	43.7	250.0	600
Nitrate (mg/l)	31.0	53.4	24.7	44.4	18.4	20.0	ND	ND	17.0	20.1	25.0	50.0
Zinc (µg/l)	60.0	67.1	57.2	61.0	ND	15.4	ND	ND	-	-	-	50
Lead (µg/l)	53.4	61.7	41.2	40.8	ND	ND	ND	ND	-	-	-	50
Nickel (µg/l)	30.3	33.1	ND	17.4	ND	ND	ND	ND	-	-	-	50
Colour (Hazen)	61.4	92.3	53.4	68.1	51.4	60.7	15.5	50.6	31.4	56.1	5	50
Turbidity	21.7	28.2	17.0	33.9	9.3	20.3	7.7	7.3	12.8	7.2	-	

5.0 GEOTECHNICAL PROPERTIES OF SOIL

Several limits have been proposed by various researchers with respect to geotechnical properties of soils to be useful as barriers in landfills. Such limits have been compared with the results of the investigated parameters and presented as follow

5.1 Grain Size Distribution

In the soils investigated the largest grain has diameter ≤ 6.3mm. This is very small compare to 63mm suggested by ÖNORM S2074 (1990) and less than 50mm suggested by Daniel 1993. The percentage of clay contained in the soil range between 41% - 51% Table 3. These values are much higher than 15% proposed by ÖNORM S 2074 (1990) and less than 30% suggested by Daniel1993, Bagchi(1994), Benson *et al* (1994), Rowe (2005) and Mohammedzein (1998). Oeltzcshner (1992) preferred soils with clay fraction of greater than 20%.

The percentage of gravel recommended by Daniel 1993, Bagchi (1994) and Rowe (1995) is less or equal 30% of the soil mass. The highest proportion of gravel from the investigated soil sample is 2% with an average value of 0.75% over the whole area. The specific gravity also ranges between 2.61% and 2.69% which is better than 2.2 recommended by ÖNORM S2074 (1990).

Table 3: Grain size analysis of soil samples

S/N	Well No	Depth(m)	Natural Density(t/m^3)	Specific Gravity	Grain Size (%)	Sand (%)	Clay (%)	Silt (%)	Fine (%)
1	W1	1.15	2.04	2.69	2	28	48	22	70
2	W2	2.70	1.67	2.64	1	34	45	20	65
3	W3	4.50	1.60	2.63	0	28	51	2`	72
4	W4	3.10	1.91	6.61	0	36	49	15	64
		Average	1.81	2.64	0.75	32	48.3	19	68

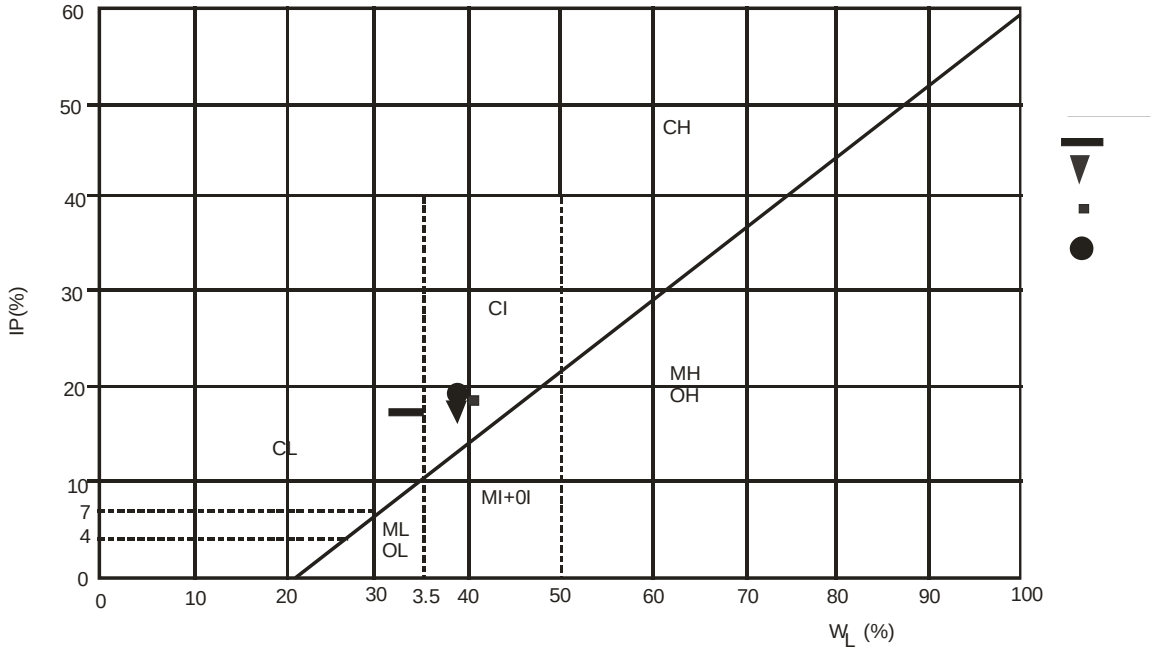
5.2 Atterberg Consistency Limits

The results of the liquid limits tests (L_l) for the soil range between 35.34% and 40.56% while the index of plasticity (Ip) range between 17.15% and 20.55% (Table 4). These are higher than recommendations of several previous workers. From the results, the Casangrande plasticity chart was plotted for all the soils. All the soil samples fall within the inorganic clay of intermediate plasticity (**Fig. 13)**. This is a good result when compared to the recommendations of Bagchi (1994), Hughes *et al* (2005) and Jagger and Ash (2008). Clay activities (A_C) of the soils were also evaluated to determine the reactivity of the soil. The values range between 0.35 and 0.40. It therefore suggested that the soil contain essentially kaolinitic clay mineral type which are non-reactive and non-expansive (Lambe 1951, Bagchi 1994 and Withlow (1998). They are less attack by chemical and withstand volumetric shrinkage (Taha and Kabir, 2006).

Table 4: Atterberg Consistency limits of the soil samples

Symbol	WL (%)	WP (%)	IP (%)	Plots on Plasticity chart	Ac
W1	35.34	18.19	17.15	CL	0.35
W2	39.14	20.80	18.34	CI	0.41
W3	39.80	21.31	18.49	CI	0.39
W4	40.56	20.01	20.55	CI	0.42
Average	38.71	20.00	18.63	CI	0.39

KEY: WL = Liquid Limit WP = Plastic Limit
IP = Index of Plasticity Ac = Activity of Clay



5.3 Moisture **Content - Density Relationship**

The results of compaction tests carried out at different energies of compaction to obtain the soils optimum moisture content (OMC) and the corresponding maximum dry density (MDD) is presented in the Table 5. Benson and Trast (1995) reported that the coefficient of permeability is sensitive to compactive effort and molding water content. The soil MDD values for standard Proctor energy of compaction range between 1.77t/m^3 and 1.84t/m^3 while MDD at modified Proctor energy range between 1.9t/m^3 and 2.2t/m^3. These values are higher than 1.7t/m^3 stipulated in ÖNORM S 2074 (1990). They are also better than 1.45t/m^3 (standard Proctor) and 1.64t/m^3 (modified Proctor) of MDD recommended by kabir and Taha (2006) for soils produced from Basement complex rocks to be useful as barrier in landfills.

5.4 Coefficient of Permeability (*k*)

The coefficient of permeability is the key parameter affecting most soils to be useful as barrier in landfill (Benson, 1990). Thus great attention is focused at ensuring a low permeability is achieved. Several investigators and waste management agencies have recommended 1x10^{-9}m/s as the minimum allowable value for soil to be useful for this purpose. From table 5, values lower than recommendation of several authors (e.g Mark 2002; Joyce 2003; Fred and Anne 2005 and Jagger and Ash, 2008) were obtained from all the soil investigated with both standard and modified Proctor energies. It was also observed that the coefficient of permeability decreases with increased compactive energy (Table 5). This is because there is a decrease in the frequency of pores resulting from the structural rearrangement of soil particle in the soil mass (Acar and Oliveri, 1990). For the purpose of sanitary landfill, the least achievable coefficient of permeability on the field is preferred. Therefore the higher energy (modified Proctor) of compaction is recommended.

Table 5: Maximum Dry Density and Coefficient of Permeability of the soil Samples.

WELL SYMBOL	STANDARD PROCTOR		MODIFIED PROCTOR		COEFFICIENT OF PREMEABILITY(*K*)cm/s	
	OMC (%)	MDD (t/m³)	OMC (%)	MDD (t/m³)	STANDARD PROCTOR	MODIFIED PROCTOR
W1	13.4	1.84	10.8	2.2	$1.1x10^{-9}$ m/s	$3.4x10^{-11}$m/s
W2	14.0	1.78	10.3	1.9	$4.0x10^{-9}$ m/s	$5.1x10^{-11}$m/s
W3	14.2	1.77	10.0	2.1	$5.3x10^{-8}$ m/s	$3.6x10^{-11}$m/s
W4	16.7	1.80	12.5	2.0	$3.7x10^{-9}$ m/s	$2.3x10^{-11}$m/s
AVERAGE	14.6	1.80	10.9	2.1	$3.53x10^{-8}$ m/s	$1.6x10^{-11}$m/s

OMC = Optimum Moisture Content MDD = Maximum Dry Density

6. Conclusion

The following conclusions were made on the hydrogeological and geotechnical evaluation of the Ita-amo waste dumpsite in, Ilorin, Nigeria.

The physical properties of the water samples are quite lower than the allowable recommended by the WHO for drinking and irrigation purpose. However, there is seasonal influence on the cationic concentration in the dry season with wells 1, 2 and 3 having concentration above the WHO permissible level. Also, Liquification and leaching activities of the deposited large volume of domestic and industrial waste at site may have been responsible for the high increase in the ionic concentration at both seasons. Ionic concentration decreases with increase distance away from the waste dump centre.

The overall engineering characteristics of the soil samples recovered from test pits, irrespective of the depth of recovery, show that the soils are inorganic clay with low to medium plasticity. Generally, these types of soils possess desirable characteristics to minimize hydraulic conductivity of compacted soils. The indices properties (liquid limit, plastic limit, percentage fine, percentage gravel, activity etc) of the soil samples satisfy the basic requirements as barrier materials in landfills. They are inactive clayey soils. Thus, the soils will be less affected by waste chemical and also less susceptible to shrinkage. The soils have hydraulic conductivity of less than 1×10^{-7} cm/s when compacted with both modified and standard Proctor compaction efforts.

This result compared favorably with the recommendations of several researchers. Also higher energy of compaction is recommended because it gives lower values of coefficient of permeability for the compacted soils.

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8/2/2009

Floristic structure and phytodiversity along an elevational gradient in Peepalkoti-Joshimath area of Garhwal Himalaya, India

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Abstract: The present study was conducted in temperate Himalayan forests of Joshimath area in Chamoli district of Uttarakhand to understand the effect of altitudinal variation on structure and composition of the vegetation and to record the floristic diversity and economic utilities of the plants in the study area. Three altitudinal zones viz., upper zone (U) = 2000-2200m asl, middle zone (M) = 1800-2000m asl and lower zone (L) = 1600-1800m asl were selected for the study. In the present floristic survey the total of 74 families (72 Angiospermous and 2 Gymnospermous), 149 Genera (145 Angiospermous and 4 Gymnospermous) and 177 species (173 Angiospermous and 4 Gymnospermous) were recorded in the study area. Out of these 177 species identified in the study area 100, 47, 20 and 10 were herbs, shrubs, trees and climbers respectively. Rosaceae was the dominant family recorded with 16 species in the study area followed by the Asteraceae (15), Lamiaceae (11), Fabaceae (11) and Caryophyllaceae (5). In Ethnobotanical survey very useful information was recorded about the economic utility of the plants species present in the study area. Uses recorded were medicinal, fuel, fodder, edible and timber. Tree Species richness (SR) decreased from lower altitude to higher altitude. Species diversity (richness) and dominance (Simpson index) were found to be inversely related to each other. Tree density decreased from lower altitude to upper altitude, whereas TBC showed reverse trend. [Nature and Science. 2009;7(9):63-74]. (ISSN: 1545-0740)

Keywords: Phytosociology, Floristic composition, Diversity indices, Economic utility, Altitudes.

1. Introduction

The Indian Himalayan region occupies a special place in the mountain ecosystems of the world. These geodynamically young mountains are not only important from the stand point of climate and as a provider of life, giving water to a large part of the Indian subcontinent, but they also harbor a rich variety of flora, fauna, human communities and cultural diversity (Singh, 2006). The biodiversity which few years ago was considered unimportant by ecosystem ecologists, has now been shown to be significantly important for many aspects of ecosystem functioning. Diversity at all organizational levels, ranging from genetic diversity within populations to the diversity of ecosystems in landscapes, contributes to global biodiversity. The biodiversity has long been a source of amazement and scientific curiosity and increasingly a source of concern. Understanding of forest structure is a pre-requisite to describe various ecological processes and also to model the functioning and dynamics of forests (Elourard et al. 1997).

Species diversity has functional consequences, because the number and kinds of species present in any area determine the organismal traits, which influence ecosystem processes. The components of species diversity that determine the expression of traits include the number of species present (species richness), their relative abundance (species evenness), presence of the particular species (species composition), the interactions among species (non-additive effects), and the temporal and spatial variation in these properties. In addition to its effects on current functioning of ecosystems, species diversity influences the resilience and resistance of ecosystems to environmental changes (Chapin et al. 2000).

The altitude and aspect play a key role in determining the temperature regime and atmospheric pressure of any site. Within one altitude the cofactors like topography, aspect, inclination of slope and soil type affect the forest composition (Shank and Noorie, 1950). The micro-environment of different aspects of hill slopes is influenced by the intensity and duration of available sunlight (Yadav and Gupta, 2006). This type of ecological knowledge is fundamental for conservation and sustainable utilization, and may provide important information for the policy makers for drafting management plans of fragile mountain ecosystems. Under the backdrop of the aforesaid facts, the present study was undertaken in temperate Himalayan forests of Peepalkoti-Joshimath area of Garhwal Himalaya, 1) to record plant species present in the study area along with their economic uses and 2) to understand the effect of altitude on the structure and composition of the vegetation of natural forests.

2. Material and Methods

The present study was conducted in temperate Himalayan forests of Joshimath area in Chamoli

district of Uttarakhand in year 2008. After the reconnaissance survey three altitudinal zones viz., upper zone (U) = 2200-2000m asl, middle zone (M) = 2000-1800m asl and lower zone (L) = 1800-1600m asl were identified to study the effect of altitudinal variation on structure and composition of the vegetation. The climate of the study area is typical temperate type. The year is represented by three main seasons; the cool and relatively dry winter (December to March); the warm and dry summer (mid-April to June); and a warm and wet period (July to mid-September) called as the monsoon or rainy season. The rainy season accounts for about three-quarters of the annual rainfall. Apart from these main seasons, the transitional periods interconnecting rainy and winter, and winter and summer are referred to as autumn (October to November) and spring (February to March). The mean annual rainfall was recorded as 1500mm and mean annual temperature between 5°C to 28°C.

The composition of the forest along the altitudinal gradient was analysed by using nested quadrate method or centre point quadrat method for trees, shrubs and herbs species as per Kent and Coker (1992). Three vegetation layers, (i.e., trees, shrubs and herbs) were analyzed for species richness, density and diversity. A total of 60 plots (twenty plots in each forest type) measuring 10m X 10m each were sampled. Trees (≥10cm dbh) were analyzed by 10m x 10m sized quadrats, whereas shrubs by 5m x 5m sized quadrats. Further, quadrats of 1x1m size were randomly laid out with in each 10x10m sized quadrat at each site, to study plants in the herb layer. Circumference at breast height (cbh= 1.37m) was taken for the determination of tree basal area and was calculated as πr^2, where r is the radius. Total basal area/cover is the sum of basal area/cover of all species present in the forest. The data were quantitatively analyzed for density, frequency and abundance following Curtis and McIntosh (1950). Species Richness was simply taken as a count of number of species present in that forest type. Basal area (m^2/ha) was used to determine the relative dominance of a tree species. Importance Value Index (IVI) was the sum of relative frequency, relative density and relative dominance (Phillips, 1959). The diversity (H') was determined by using Shannon-Wiener information index (Shannon and Weaver, 1963) as: $H' = - \sum n_i /n \log_2 n_i /n$; where, n_i was the IVI value of a species and n was the sum of total IVI values of all species in that forest type. The Simpsonôs concentration of dominance (Simpson, 1949) was measured as: $Cd= \sum Pi^2$, where, $\sum Pi = \sum n_i /n$, where, ni and n are same as in Shannon-Wiener diversity index. Simpsonôs diversity index (Simpson, 1949) was calculated as: D = 1-Cd, where, D = Simpsonôs diversity and Cd = Simpsonôs concentration of dominance. Species heterogeneity was calculated as under root of concentration of dominance (Cd).

To study the phytodiversity in the study area, regular field trips were undertaken in different seasons i.e., rainy, winter and summer, to collect the specimens of higher plants (Gymnosperms and Angiosperms). Identification of the specimens was done with the help of the existing Herbariums of Botany Department HNB Garhwal University (GUH), Forest Research Institute (DD) and Botanical Survey of India, Northern Circle (BSD). After identification, the enumeration of plants was done according to Bentham and Hookerôs system of classification (1862-1883). The plants were divided into categories of common and uncommon according to their occurrence in the study area. An Ethnobotanical survey was also conducted in the villages nearby the study area to know the economic utility of various plant species encountered.

3. Results

Forest community structure and composition: Results of forest community structure and composition are given in tables 1 to 3.

Trees: At upper altitude *Cedrus deodara* was the dominant tree species with highest density (170 Ind/ha), TBC (98.82 m^2/ha) and IVI (155.96). At middle altitude *Pinus wallichiana* was the dominant tree species with highest density (180 Ind/ha), TBC (84.41 m^2/ha) and IVI (120.59). At lower altitude *Alnus nepalensis* was the dominant tree species with highest density (340 Ind/ha) and IVI (85.90), whereas highest TBC (3.78 m^2/ha) at this altitude was recorded for *Quercus semecarpifolia*. Tree Species richness (SR) decreased from lower altitude to higher altitude with highest SR at lower (19) altitude followed by middle (8) and upper (3) altitude. Highest (800 Ind/ha) tree density was recorded at lower zone followed by middle (600 Ind/ha) and lower (330 Ind/ha) altitudinal zone, where as highest (181.5 m^2/ha) TBC was recorded at upper altitude followed by middle (143.05 m^2/ha) and lower (9.63 m^2/ha) altitudes. Tree density decreased from lower altitude to upper altitude, whereas TBC showed reverse trend. Cd was found to be highest (0.4328) on upper altitude followed by middle (0.2561) and lower (0.1958) altitude whereas Simpsonôs diversity index showed reverse trend with highest (6.80) value at lower altitude followed by middle (6.74) and upper (2.57) altitude. Value H' was found to be highest (0.67) at upper altitude followed by middle (0.28) and lower (0.15) altitude.

Shrubs: At upper altitude *Rabdosia rugosa* was the dominant shrub species with highest density (520 Ind/ha) and TBC (0.3600 m^2/ha), whereas highest IVI (82.38 m^2/ha) at this altitude was recorded for *Corairia nepalensis*. At middle altitude *Rabdosia rugosa* was the dominant shrub species with highest density (680 Ind/ha), TBC (0.4310 m^2/ha) and IVI (89.98). At lower altitude *Desmodium elegans* was the dominant shrub species with highest density (440 Ind/ha), TBC (0.1300 m^2/ha) and IVI (70.27). Shrub Species richness (SR) decreased from lower altitude to higher altitude with highest SR at lower (22) altitude followed by middle (10) and upper (7) altitude. Highest (2420 Ind/ha) density was recorded at middle altitude followed by lower (2020 Ind/ha) and upper (1620 Ind/ha) altitudinal zone, where as highest TBC (1.21 m^2/ha) was recorded at middle altitude followed by upper (0.75 m^2/ha) and lower (0.39 m^2/ha) altitudes. Cd was found to be highest (0.1996) on middle altitude followed by upper (0.1896) and lower (0.1138) altitude, whereas H' was found to be highest (0.17) at middle altitude followed by upper (0.14) and lower (0.06) altitude. Simpsonôs diversity index varied between 15.89 (lower altitude) to 7.81 (upper altitude).

Herbs: At upper altitude *Galium* sp. was the dominant herb species with highest density (15000 Ind/ha), TBC (0.0183 m^2/ha) and IVI (54.36). At middle altitude *Geranium* sp. was the dominant herb species with highest density (28750 Ind/ha), TBC (0.0760 m^2/ha) and IVI (70.52). At lower altitude *Pilea umbrosa* was the dominant herb species with highest density (16250 Ind/ha), TBC (0.0191 m^2/ha) and IVI (44.31). Herb Species richness (SR) decreased from lower altitude to higher altitude with highest SR at lower (19) altitude followed by middle (16) and upper (7) altitude. Highest (174375 Ind/ha) density was recorded at middle altitude followed by lower (136250 Ind/ha) and upper (112500 Ind/ha) altitudinal zone, where as highest TBC (0.17 m^2/ha) was recorded at middle altitude followed by lower (0.08 m^2/ha) and upper (0.06 m^2/ha) altitudes. Cd was found to be highest (0.0961) on middle altitude followed by upper (0.0777) and lower (0.0711) altitude, whereas H' was found to be highest (0.05) at middle altitude followed by upper (0.03) and lower (0.02) altitude. Simpsonôs diversity index varied between 21.90 (middle altitude) to 18.93 (lower altitude).

Phytodiversity: In the present floristic survey the total of 74 families (72 Angiospermous and 2 Gymnospermous), 149 Genera (145 Angiospermous and 4 Gymnospermous) and 177 species (173 Angiospermous and 4 Gymnospermous) were recorded in the study area (table 4). Out of these 177 species identified in the study area 100, 47, 20 and 10 were herbs, shrubs, trees and climbers respectively. Rosaceae was the dominant family recorded with 16 species in the study area followed by the Asteraceae (15), Lamiaceae (11), Fabaceae (11) and Caryophyllaceae (5). Families with only one species were Agavaceae, Anacardiaceae, Aquifoliaceae, Araliaceae, Asclepidaceae, Berberidaceae, Betulaceae, Buxaceae, Cannabinaceae, Chenopodiaceae, Coriariaceae, Crassulaceae, Cucurbitaceae, Cuperasaceae, Cuscutaceae, Dioscoreaceae, Dipsacaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Gentianaceae, Geraniaceae, Hippocastanaceae, Hydrangeaceae, Juglandaceae, Lythraceae, Malvaceae, Meliaceae, Mimosaceae, Nictaginaceae, Orchidaceae, Oxalidaceae, Philadelphaceae, Phytolaccaceae, Plantaginaceae, Polygalaceae, Primulaceae, Rhamnaceae, Rutaceae, Saxifragaceae, Smilacaceae and Vitaceae. Families with two species were Boraginaceae, Brassicaceae, Campanulaceae, Caprifoliaceae, Onagraceae, Salicaceae, Thymelaeaceae, Ulmaceae, Urticaceae and Violaceae. Families with three species were Acanthaceae, Amaranthaceae, Araceae, Balsaminaceae, Cyperaceae, Moraceae, Oleaceae, Pinaceae, Rubiaceae, Scrophulariaceae and Solanaceae. Families with four species were Apiaceae, Hypericaceae, Poaceae, Polygonaceae and Ranunculaceae. In Ethnobotanical survey of the plant species present in the study area, very useful information was recorded about the economic utility of the plants. Uses recorded were medicinal, fuel, fodder, edible and timber and results are shown in the Table 4.

Table 1: Analytical characters for different forest types.

	Density (Ind/ha)			TBC (m^2/ha)			IVI		
Trees	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**
Aesculus indica	-	40	-	-	3.41	-	-	19.58	-
Alnus nepalensis	30	120	340	0.28	3.76	0.96	25.91	33.15	85.80
Cedrus deodara	170	140	-	98.82	49.32	-	155.96	78.86	-
Celtis australis	-	-	30	-	-	0.35	-	-	15.72
Lyonia ovalifolia	-	40	50	-	1.20	1.26	-	18.03	27.67

Pinus wallichiana	130	180	-	82.40	84.41	-	118.13	120.59	-
Populus ciliata	-	-	40	-	-	0.84	-	-	22.06
Pyrus pashia	-	30	80	-	0.25	1.49	-	10.44	37.97
Quercus semecarpifolia	-	-	190	-	-	3.78	-	-	79.67
Salix alba	-	50	70	-	0.70	0.95	-	19.35	31.12
	330	600	800	181.50	143.05	9.63	300.00	300.00	300.00
Shrubs	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**
Berberis sp	-	120	60	-	0.0040	0.0040	-	10.55	8.26
Buddleja paniculata	-	-	80	-	-	0.0100	-	-	10.80
Corairia nepalensis	340	440	-	0.3300	0.3100	-	82.38	59.61	-
Cotoneaster baccilaris	-	60	-	-	0.0020	-	-	5.28	-
Cotoneaster microphyllus	40	-	60	0.0004	-	0.0100	8.40	-	9.81
Daphne retusa	-	-	80	-	-	0.0050	-	-	11.64
Dapnae sp.	100	140	-	0.0020	0.0050	-	35.85	14.09	-
Desmodium elegans	360	520	440	0.3600	0.4300	0.1300	75.82	72.84	70.27
Deutzia compacta	-	-	160	-	-	0.0200	-	-	23.73
Elaeagnus conferta	-	-	140	-	-	0.0600	-	-	28.82
Lonicera quinquelocularis	-	-	40	-	-	0.0030	-	-	4.88
Princepia utilis	-	60	100	-	0.0020	0.0200	-	7.91	16.50
Rabdosia rugosa	520	680	320	0.0500	0.4310	0.0500	47.55	89.98	39.40
Rhamnus persica	-	-	60	-	-	0.0040	-	-	8.26
Rhamnus sp.	40	60	-	0.0010	0.0040	-	20.25	8.07	-
Rhamnus virgatus	-	-	40	-	-	0.0020	-	-	4.62
Rubus foliolosus	-	-	40	-	-	0.0030	-	-	4.88
Rubus niveus	-	-	80	-	-	0.0050	-	-	11.64
Sorbaria tomentosa	120	200	240	0.0100	0.0200	0.0600	20.50	20.45	35.90
Wikstroemia canescens	100	140	80	0.0010	0.0020	0.0010	9.25	11.21	10.60
	1620	2420	2020	0.7544	1.2090	0.3870	300.00	300.00	300.00
Herbs	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**
Ajuga paviflora	-	-	5000	-	-	0.0008	-	-	9.25
Arisaema sp.	2500	1875	-	0.0004	0.0002	-	5.49	3.44	-
Artemisia capillaris	-	-	2500	-	-	0.0004	-	-	5.40
Bidens pilosa	-	-	5000	-	-	0.0018	-	-	8.93
Chenopodium album	3750	-	-	0.0006	-	-	9.33	-	-
Chenopodium sp.	-	5000	2500	-	0.0016	0.0004	-	9.19	5.40
Circium sp.	-	3750	-	-	0.0011	-	-	6.09	-
Circium verutum	2500	-	-	0.0004	-	-	5.49	-	-
Clinopodium sp.	6250	5000	4375	0.0030	0.0016	0.0008	15.63	8.15	7.26
Conyza japonica	-	-	4375	-	-	0.0011	-	-	7.62
Cynoglossum glochidium	3750	7500	8750	0.0006	0.0046	0.0062	8.14	12.41	20.09
Elsholtzia sp.	5000	8750	-	0.0018	0.0062	-	12.48	12.01	-
Eriophorum comosum	-	-	6250	-	-	0.0029	-	-	11.18
Fragarea sp.	5000	-	-	0.0016	-	-	12.14	-	-
Fragaria nubicola	-	5625	-	-	0.0011	-	-	8.21	-
Galium sp.	15000	17500	10000	0.0183	0.0183	0.0046	54.36	27.54	20.61
Geranium sp.	11250	28750	-	0.0058	0.0760	-	27.42	70.52	-
Hypericum elodeoides	-	3750	-	-	0.0008	-	-	4.88	-
Impatiens sp.	6250	8750	7500	0.0029	0.0062	0.0050	16.65	14.08	17.72

Lactuca sp.	2500	2500	-	0.0004	0.0004	-	5.49	3.92	-
Malva verticilata	-	-	5000	-	-	0.0018	-	-	8.93
Micromeria biflora	-	-	6250	-	-	0.0023	-	-	11.99
Origanum vulgare	8750	11250	9375	0.0050	0.0103	0.0072	23.84	19.18	21.76
Oxalis acetocella	7500	23125	10000	0.0030	0.0220	0.0062	16.74	31.76	22.55
Phytolacca acinosa	5000	5000	-	0.0050	0.0026	-	17.91	9.77	-
Pilea umbrosa	-	-	16250	-	-	0.0191	-	-	44.31
Pimpinella sp.	3750	11250	8750	0.0006	0.0080	0.0046	9.33	17.80	18.15
Plantago sp.	-	2500	-	-	0.0002	-	-	3.80	-
Polygonum sp.	-	-	11250	-	-	0.0109	-	-	29.16
Prunella vulgare	7500	5000	5625	0.0050	0.0018	0.0018	21.33	8.27	12.46
Salvia moocroftiana	-	-	7500	-	-	0.0046	-	-	17.23
Salvia sp.	3750	3750	-	0.0006	0.0006	-	9.33	6.83	-
Stellarea sp.	5000	7500	-	0.0016	0.0029	-	13.34	11.40	-
Thalictrum sp.	-	2500	-	-	0.0002	-	-	3.80	-
Viola sp.	7500	3750	-	0.0023	0.0008	-	15.55	6.95	-
	112500	174375	136250	0.0589	0.1675	0.0825	300.00	300.00	300.00

Abbreviations: U= Upper altitude; M= Middle altitude; L= Lower altitude; TBC= Total Basal Cover; IVI= Importance Value Index.

Table 2: Diversity Indices of different forest types.

	Cd			SDI			H'			Heterogeneity		
Trees	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**
Aesculus indica	-	0.0043	-	-	0.9957	-	-	0.00	-	-	0.07	-
Alnus nepalensis	0.0075	0.0122	0.0818	0.9925	0.9878	0.9182	0.0021	0.00	0.08	0.09	0.11	0.29
Cedrus deodara	0.2703	0.0691	-	0.7297	0.9309	-	0.4666	0.06	-	0.52	0.26	-
Celtis australis	-	-	0.0027	-	-	0.9973	-	-	0.00	-	-	0.05
Lyonia ovalifolia	-	0.0036	0.0085	-	0.9964	0.9915	-	0.00	0.00	-	0.06	0.09
Pinus wallichiana	0.1550	0.1616	-	0.8450	0.8384	-	0.2027	0.22	-	0.39	0.40	-
Populus ciliata	-	-	0.0054	-	-	0.9946	-	-	0.00	-	-	0.07
Pyrus pashia	-	0.0012	0.0160	-	0.9988	0.9840	-	0.00	0.01	-	0.03	0.13
Quercus semecarpifolia	-	-	0.0705	-	-	0.9295	-	-	0.06	-	-	0.27
Salix alba	-	0.0042	0.0108	-	0.9958	0.9892	-	0.00	0.00	-	0.06	0.10
	0.4328	0.2561	0.1958	2.5672	6.7439	6.8042	0.6715	0.28	0.15	1.00	1.00	1.00
Shrubs	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**
Berberis sp	-	0.0012	0.0008	-	0.9988	0.9992	-	0.00	0.00	-	0.04	0.03
Buddleja paniculata	-	-	0.0013	-	-	0.9987	-	-	0.00	-	-	0.04
Corairia nepalensis	0.0754	0.0395	-	0.9246	0.9605	-	0.0688	0.03	-	0.27	0.20	-
Cotoneaster baccilaris	-	0.0003	-	-	0.9997	-	-	0.00	-	-	0.02	-
Cotoneaster microphyllus	0.0008	-	0.0011	0.9992	-	0.9989	0.0001	-	0.00	0.03	-	0.03
Daphne retusa	-	-	0.0015	-	-	0.9985	-	-	0.00	-	-	0.04
Dapnae sp.	0.0143	0.0022	-	0.9857	0.9978	-	0.0057	0.00	-	0.12	0.05	-
Desmodium elegans	0.0639	0.0590	0.0549	0.9361	0.9410	0.9451	0.0536	0.05	0.04	0.25	0.24	0.23
Deutzia compacta	-	-	0.0063	-	-	0.9937	-	-	0.00	-	-	0.08
Elaeagnus conferta	-	-	0.0092	-	-	0.9908	-	-	0.00	-	-	0.10
Lonicera quinquelocularis	-	-	0.0003	-	-	0.9997	-	-	0.00	-	-	0.02
Princepia utilis	-	0.0007	0.0030	-	0.9993	0.9970	-	0.00	0.00	-	0.03	0.06
Rabdosia rugosa	0.0251	0.0900	0.0172	0.9749	0.9100	0.9828	0.0132	0.09	0.01	0.16	0.30	0.13
Rhamnus persica	-	-	0.0008	-	-	0.9992	-	-	0.00	-	-	0.03

Rhamnus sp.	0.0046	0.0007	-	0.9954	0.9993	-	0.0010	0.00	-	0.07	0.03	-
Rhamnus virgatus	-	-	0.0002	-	-	0.9998	-	-	0.00	-	-	0.02
Rubus foliolosus	-	-	0.0003	-	-	0.9997	-	-	0.00	-	-	0.02
Rubus niveus	-	-	0.0015	-	-	0.9985	-	-	0.00	-	-	0.04
Sorbaria tomentosa	0.0047	0.0046	0.0143	0.9953	0.9954	0.9857	0.0011	0.00	0.01	0.07	0.07	0.12
Wikstroemia canescens	0.0009	0.0014	0.0012	0.9991	0.9986	0.9988	0.0001	0.00	0.00	0.03	0.04	0.04
	0.1896	0.1996	0.1138	7.8104	9.8004	15.8862	0.1435	0.17	0.06	1.00	1.00	1.00
Herbs	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**	**U**	**M**	**L**
Ajuga paviflora	-	-	0.0010	-	-	0.9990	-	-	0.00	-	-	0.03
Arisaema sp.	0.0003	0.0001	-	0.9997	0.9999	-	0.0000	0.00	-	0.02	0.01	-
Artemisia capillaris	-	-	0.0003	-	-	0.9997	-	-	0.00	-	-	0.02
Bidens pilosa	-	-	0.0009	-	-	0.9991	-	-	0.00	-	-	0.03
Chenopodium album	0.0010	-	-	0.9990	-	-	0.0001	-	-	0.03	-	-
Chenopodium sp.	-	0.0009	0.0003	-	0.9991	0.9997	-	0.00	0.00	-	0.03	0.02
Circium sp.	-	0.0004	-	-	0.9996	-	-	0.00	-	-	0.02	-
Circium verutum	0.0003	-	-	0.9997	-	-	0.0000	-	-	0.02	-	-
Clinopodium sp.	0.0027	0.0007	0.0006	0.9973	0.9993	0.9994	0.0005	0.00	0.00	0.05	0.03	0.02
Conyza japonica	-	-	0.0006	-	-	0.9994	-	-	0.00	-	-	0.03
Cynoglossum glochidium	0.0007	0.0017	0.0045	0.9993	0.9983	0.9955	0.0001	0.00	0.00	0.03	0.04	0.07
Elsholtzia sp.	0.0017	0.0016	-	0.9983	0.9984	-	0.0002	0.00	-	0.04	0.04	-
Eriophorum comosum	-	-	0.0014	-	-	0.9986	-	-	0.00	-	-	0.04
Fragarea sp.	0.0016	-	-	0.9984	-	-	0.0002	-	-	0.04	-	-
Fragaria nubicola	-	0.0007	-	-	0.9993	-	-	0.00	-	-	0.03	-
Galium sp.	0.0328	0.0084	0.0047	0.9672	0.9916	0.9953	0.0198	0.00	0.00	0.18	0.09	0.07
Geranium sp.	0.0084	0.0553	-	0.9916	0.9447	-	0.0025	0.04	-	0.09	0.24	-
Hypericum elodeoides	-	0.0003	-	-	0.9997	-	-	0.00	-	-	0.02	-
Impatiens sp.	0.0031	0.0022	0.0035	0.9969	0.9978	0.9965	0.0006	0.00	0.00	0.06	0.05	0.06
Lactuca sp.	0.0003	0.0002	-	0.9997	0.9998	-	0.0000	0.00		0.02	0.01	-
Malva verticilata	-	-	0.0009	-	-	0.9991	-	-	0.00	-	-	0.03
Micromeria biflora	-	-	0.0016	-	-	0.9984	-	-	0.00	-	-	0.04
Origanum vulgare	0.0063	0.0041	0.0053	0.9937	0.9959	0.9947	0.0017	0.00	0.00	0.08	0.06	0.07
Oxalis acetocella	0.0031	0.0112	0.0056	0.9969	0.9888	0.9944	0.0006	0.00	0.00	0.06	0.11	0.08
Phytolacca acinosa	0.0036	0.0011	-	0.9964	0.9989	-	0.0007	0.00	-	0.06	0.03	-
Pilea umbrosa	-	-	0.0218	-	-	0.9782	-		0.01	-	-	0.15
Pimpinella sp.	0.0010	0.0035	0.0037	0.9990	0.9965	0.9963	0.0001	0.00	0.00	0.03	0.06	0.06
Plantago sp.	-	0.0002	-	-	0.9998	-	-	0.00	-	-	0.01	-
Polygonum sp.	-	-	0.0094	-	-	0.9906	-	-	0.00	-	-	0.10
Prunella vulgare	0.0051	0.0008	0.0017	0.9949	0.9992	0.9983	0.0012	0.00	0.00	0.07	0.03	0.04
Salvia moocroftiana	-	-	0.0033	-	-	0.9967	-	-	0.00	-	-	0.06
Salvia sp.	0.0010	0.0005	-	0.9990	0.9995	-	0.0001	0.00	-	0.03	0.02	-
Stellarea sp.	0.0020	0.0014	-	0.9980	0.9986	-	0.0003	0.00	-	0.04	0.04	-
Thalictrum sp.	-	0.0002	-	-	0.9998	-	-	0.00	-	-	0.01	-
Viola sp.	0.0027	0.0005	-	0.9973	0.9995	-	0.0005	0.00	-	0.05	0.02	-
	0.0777	0.0961	0.0711	18.9223	21.9039	18.9289	0.0291	0.05	0.02	1.00	0.80	0.00

Abbreviations: U= Upper altitude; M= Middle altitude; L= Lower altitude; Cd= Simpsonôs Concentration of Dominance; SDI= Simpsonôs Diversity Index; H'= Shannon-Wiener Diversity Index.

Table 3: Total Diversity Indices of different forest types.

		Density (Ind/ha)	TBC (m^2/ha)	Cd	SWDI	H'	SR
	Upper	330	181.50	0.4328	2.57	0.67	3
Trees	**Middle**	600	143.05	0.2561	6.74	0.28	8
	Lower	800	9.63	0.1958	6.80	0.15	19
	Upper	1620	0.75	0.1896	7.81	0.14	7
Shrubs	**Middle**	2420	1.21	0.1996	9.80	0.17	10
	Lower	2020	0.39	0.1138	15.89	0.06	22
	Upper	112500	0.06	0.0777	18.92	0.03	7
Herbs	**Middle**	174375	0.17	0.0961	21.90	0.05	16
	Lower	136250	0.08	0.0711	18.93	0.02	19

Table 4: Details and uses of the plant species recorded in the Chamoli-Joshimath study area

Botanical Name	Family	Occurrence	Economic Utility	LF
Abelia triflora	Caprifoliaceae	UC	Fu	S
Abies pindrow	Pinaceae	C	Tm, Me	T
Achyranthes bidentata	Amaranthaceae	UC	-	H
Adenocaulon himalaicum	Asteraceae	C	Me	H
Aesculus indica	Hippocastanaceae	UC	Fo, Me	T
Agave americana	Agavaceae	C	Me	S
Agrimonia pilosa	Rosaceae	C	Me	H
Albizia julibrissin	Mimosaceae	UC	Me	T
Alnus nitida	Betulaceae	C	Fu	T
Alpuda mutica	Poaceae	C	Me	H
Amaranthes viridis	Amaranthaceae	C	Me	H
Ammi majus	Asteraceae	UC	-	H
Anaphalis contrata	Asteraceae	C	Me	H
A. triplinervis	Asteraceae	C	-	H
Andropogon controtus	Poaceae	C	-	H
Androsace sp.	Primulaceae	UC	-	H
Anemone sp.	Rosaceae	UC	Me	H
A. vitifolia	Rosaceae	UC	Me	H
Aquilegia pubiflora	Aquifoliaceae	UC	Me	H
Arabis sp.	Brassicaceae	C	Me	H
Arctium lappa	Asteraceae	C	Me	H
Arenaria sp.	Caryophyllaceae	C	-	H
Arisaema sp	Araceae	C	-	H
A.. concinnum	Araceae	UC	Me	H
Artemisia capillaris	Asteraceae	C	-	H

A. roxburghiana	Asteraceae	UC	Me	S
Astragalus chlorostachys	Fabaceae	C	Me	S
Barleria cristata	Acanthaceae	UC	Me	S
Berberis angulosa	Berberidaceae	C	-	S
Bergenia ciliata	Saxifragaceae	C	Me	H
Boerhavia diffusa	Nictaginaceae	C	Me	H
Buddleja paniculata	Scrophulariaceae	C	Fu	S
Bupleurum falcatum	Apiaceae	UC	Me	H
Campanula sp.	Campanulaceae	UC	-	H
C. pallida	Campanulaceae	UC	-	H
Canabis sativa	Cannabinaceae	C	Me	S
Carex sp.	Cyperaceae	C	-	H
Cedrus deodara	Pinaceae	UC	Tm, Me	T
Celtis australis	Ulmaceae	C	Ed, Fu	T
Cerastrium sp.	Caryophyllaceae	C	Fo	H
Chenopodium album	Chenopodiaceae	C	Ed	H
Cichorium intybus	Asteraceae	C	-	H
Circium verutum	Asteraceae	UC	Me	H
Clematis connata	Ranunculaceae	C	Me	C
Clinopodium sp.	Lamiaceae	C	-	H
Corairia nepalensis	Coriariaceae	C	Fu	S
Cotoneaster baccilaris	Rosaceae	C	Fu	S
C. microphyllus	Rosaceae	C	-	S
Cupressus torulosa	Cuperasaceae	UC	Tm, Me	T
Cuscuta reflexa	Cuscutaceae	C	Me	C
Cyathula tomentosa	Amaranthaceae	C	Me	S
Cynodon dactylon	Poaceae	C	Me	H
Cynoglossum glochidiatum	Boraginaceae	C	Me	H
C. lanceolatum	Boraginaceae	UC	Me	H
Daphne retusa	Thymelaeaceae	C	-	S
Datura sp.	Solanaceae	UC	Me	S
Delphinium danudatum	Ranunculaceae	UC	Me	H
Desmodium elegans	Fabaceae	C	Me, Fu	S
D. multiflorum	Fabaceae	C	Fu	S
Deutzia compacta	Hydrangeaceae	C	Fu	S
Dioscorea deltoidea	Dioscoreaceae	UC	Me	C
Dipsacus mitis	Dipsacaceae	UC	-	H
Elaeagnus conferta	Elaeagnaceae	C	Ed	S
Elsholtzia sp.	Lamiaceae	C	-	H

E. fruticosa	Lamiaceae	C	Fu	S
E. flava	Lamiaceae	C	Me	S
Epilobium sp.	Onagraceae	UC	-	H
Erigeron sp.	Asteraceae	UC	-	H
Eriophorum comosum	Cyperaceae	C	Fo	H
Erysimum hieraciifolium	Brassicaceae	UC	-	H
Euphorbia sp.	Euphorbiaceae	C	-	H
Euphrasia himaliana	Scrophulariaceae	C	-	H
Fagopyrum dibotryis	Polygonaceae	C	Ed	H
Fallopia pterocarpa	Polygonaceae	UC	-	H
Ficus sp.	Moraceae	UC	Ed, Fu	T
F. hederacea	Moraceae	C	Fo	C
Fragaria nubicola	Rosaceae	UC	Ed	H
F. vestita	Rosaceae	UC	Ed	H
Fraxinus micrantha	Oleaceae	C	Me	T
Galium sp.	Rubiaceae	C	-	H
Geranium sp.	Geraniaceae	C	Me	H
Girardinia diversifolia	Urticaceae	C	Me	S
Hedera nepalensis	Araliaceae	C	Fo	C
Heracleum canascens	Apiaceae	UC	Me	H
Hypericum sp.	Hypericaceae	UC	-	H
H. perforatum	Hypericaceae	UC	-	S
H. elodeoides	Hypericaceae	UC	-	H
H. uralum	Hypericaceae	UC	-	S
Impatiens sp.	Balsaminaceae	C	-	H
I. falconerii	Balsaminaceae	C	-	H
I. sulcata	Balsaminaceae	UC	Me	H
Indigofera heterantha	Fabaceae	C	Fu	S
Inula cuspidata	Asteraceae	UC	Me	H
Jasminum sp.	Oleaceae	UC	-	S
J. humile	Oleaceae	UC	Me	S
Juglans regia	Juglandaceae	UC	Ed, Me	T
Kylinga sp.	Cyperaceae	C	-	H
Leptodermis lanceolata	Rubiaceae	UC	Me	S
Lespedeza gerardiana	Fabaceae	C	Me	S
L. juncea	Fabaceae	C	Me	H
Lonicera quinquelocularis	Caprifoliaceae	UC	Fu	S
Lotus corniculatus	Fabaceae	UC	-	H
Lyonia ovalifolia	Ericaceae	UC	Me	T

Malva verticilata	Malvaceae	UC	Me	H
Mentha longifolia	Lamiaceae	UC	Me	H
Micromeria biflora	Lamiaceae	C	Me	H
Morus serrata	Moraceae	C	Me	T
Nepeta sp.	Lamiaceae	UC	-	H
N. laevigata	Lamiaceae	UC	-	H
Oenothera rosea	Onagraceae	UC	-	H
Origanum vulgare	Lamiaceae	C	Me	H
Oxalis acetosella	Oxalidaceae	C	Ed	H
Paspalum paspalodes	Poaceae	C	-	H
Peristrophe paniculata	Acanthaceae	UC	-	H
Philadelphus tomentosus	Philadelphaceae	UC	-	S
Phytolacca acinosa	Phytolaccaceae	C	Ed, Me	H
Pimpinella sp.	Apiaceae	UC	-	H
Pinus wallichiana	Pinaceae	UC	Tm, Me	T
Plantago himalaica	Plantaginaceae	C	Me	H
Polygala sp.	Polygalaceae	UC	-	H
Populus ciliata	Salicaceae	UC	Fo, Me	T
Potentilla sp.	Rosaceae	UC	Me	H
Princepia utilis	Rosaceae	C	Me, Fu	S
Pteracanthus alatus	Acanthaceae	C	-	S
Pyrus pashia	Rosaceae	C	Ed, Fu	T
Quercus semecarpifolia	Fagaceae	C	Fo, Me	T
Rabdosia rugosa	Lamiaceae	C	Fu	S
Ranunculus sp.	Ranunculaceae	UC	Me	H
Rhamnus persica	Rhamnaceae	UC	-	S
R. virgatus	Rhamnaceae	UC	Fu	S
Rhus javanica	Anacardiaceae	UC	Fo, Me	T
Rosa brunonii	Rosaceae	C	-	S
Rosularia sp.	Crassulaceae	UC	-	H
Rubia cordifolia	Rubiaceae	UC	Me	C
Rubus ellipticus	Rosaceae	C	Ed	S
R. foliolosus	Rosaceae	C	Ed	S
R. prostrata	Rosaceae	C	-	S
Rumex hastatus	Polygonaceae	C	Ed, Me	H
R. nepalensis	Polygonaceae	C	Me	H
Salix alba	Salicaceae	C	Fo	T
Salvia sp.	Lamiaceae	UC	-	H
S. mocrotianna	Lamiaceae	C	-	H

Sarcococca saligna	Buxaceae	C	-	S
Saussurea albscens	Asteraceae	C	-	S
Sedum multicaule	Crassulaceae	UC	-	H
Selinum vaginatum	Apiaceae	UC	Me	H
Senecio chrysanthamoides	Asteraceae	UC	-	H
Silene sp.	Caryophyllaceae	C	Fo	H
S. edgeworthii	Caryophyllaceae	C	-	H
Smilax aspra	Smilacaceae	UC	Me	C
Solanum sp.	Solanceae	C	-	H
S. nigrum	Solanceae	UC	Me	H
Solena heterophylla	Cucurbitaceae	UC	Ed, Fo	C
Sorbaria tomentosa	Rosaceae	C	Fu	S
Spiraea canascens	Rosaceae	C	Fu	S
Spiranthes sinensis	Orchidaceae	UC	Me	H
Stellaria media	Caryophyllaceae	C	-	H
Swertia angustifolia	Gentianaceae	UC	Me	H
Tagetus minuta	Asteraceae	C	-	H
Thalictrum sp.	Ranunculaceae	UC	Me	H
Thymus linearis	Lamiaceae	UC	Me	H
Toona serrata	Meliaceae	UC	Me	T
Trifoleum repens	Fabaceae	C	-	H
Trigonella corniculata	Fabaceae	UC	Me, Ed	H
Typhonium diversifolium	Araceae	UC	-	H
Ulmus villosa	Ulmaceae	UC	Tm, Me	T
Urtica dioica	Urticaceae	C	Me	S
Verbascum thapsus	Scrophulariaceae	UC	Me	H
Vigna sp.	Fabaceae	UC	Fo	C
Vincetoxicum hirundinaria	Asclepidaceae	UC	Me	H
Viola betonicifolia	Violaceae	C	Me	H
V. pilosa	Violaceae	UC	Me	H
Vitis sp.	Vitaceae	C	-	C
Wikstroemia canescens	Thymelaeaceae	UC	-	S
Woodfordia fruticosa	Lythraceae	UC	Me	S
Youngia sp.	Asteraceae	UC	-	H
Zanthoxylum armatum	Rutaceae	UC	Me	S

Abbreviations: C= Climber; C= Common; Ed= Edible; Fo= Fodder; Fu= Fuel; H= Herb; LF= Life Form; Me= Medicinal; S= Shrub; T= Tree; Tm= Timber; UC= Uncommon.

4. Discussion

The diversity of trees is fundamental to total forest biodiversity, because trees provide resources and habitat for almost all other forest species (Huang et al. 2003). At large scales, species diversity generally was found related to climate and productivity (Rahbek,

2005). Franklin *et al*. (1989) proposed that long-term productivity of natural forest ecosystems with high tree species diversity may be greater than that of forests with low diversity as a result of increased ecosystem resilience to disturbance. Slobodkin and Sanders (1969) opined that species richness of any community is a function of severity, variability and predictability of the environment in which it develops. Therefore, diversity tends to increase as the environment becomes more favourable and more predictable (Putman, 1994). Tree species diversity varied greatly from place to place mainly due to variation in biogeography, habitat and disturbance (Sagar et al. 2003), which have also been considered as the important factors for structuring the forest communities (Burslem and Whitmore, 1999). Srivastava et al. (2005) reported that the community characters differ among aspect, slope and altitude even in the same vegetation type. In our study we found that tree diversity decreased from lower altitude to higher altitude which means in our study area the environment at lower altitude was favourable for increasing tree diversity as compared to higher altitude.

In many other studies, the mean H' values for the other forests of temperate Himalaya varied from 0.4 to 2.8 (Singh et al. 1994), 0.08 to 1.29 (Shivnath et al. 1993) and 1.55 to 1.97 (Mishra et al. 2000), whereas in our study it varied between 0.67 to 0.15. Whittaker (1965) and Risser and Rice (1971) have reported the range of values of Cd for certain temperate vegetation from 0.19 to 0.99. The values of concentration of dominance (Cd) of the present study were more or less similar to the earlier reported values for temperate forests. Mean Cd values of 0.31 to 0.42 (Mishra et al. 2000) and 0.07 to 0.25 (Shivnath et al. 1993) were reported earlier from other parts of Indian Himalaya. The higher value of Cd in the forest growing on upper altitude was due to lower species richness. According to Baduni and Sharma (1997) the Cd or Simpsonôs index was strongly affected by the IVI of the first three relatively important species in a community. Species diversity (richness) and dominance (Simpson index) are inversely related to each other (Zobel et al. 1976).

The Himalayan region is bestowed with a variety of natural resources which have been exploited by mankind since time immemorial. The link between forest management and the well-being of communities in forested areas has traditionally been defined by forest sector employment opportunities (Sharma and Gairola, 2007). Ethnobotanical studies typically focus on recording the knowledge of traditional societies in remote places (Hodges and Bennett, 2006).Indigenous people have a vast knowledge of, and capacity for, developing innovative practices and products from their environment. Indigenous knowledge grows from close interdependence between knowledge, land, environment and other aspects of culture in indigenous societies, and the oral transmission of knowledge in accordance with well understood cultural principles and rules regarding secrecy and sacredness that govern the management of knowledge (Tripathi et al. 2000). In the present study the traditional uses of various plant species by indigenous people have been recorded, which can be utilized in the future for technological advancement, economic prosperity and providing employment opportunity to the local people.

Acknowledgements:
Authors gratefully acknowledged the Head, Department of Botany, HNB Garhwal University, Srinagar, Directors, G. B. Pant Institute of Himalayan Environment and Development and High Altitude Plant Physiology Research Centre, Principal Govt. P.G. College Gopeshawar, and Prof. D. P. Vasistha, Department of Botany, HNB Garhwal University, Srinagar for encouragement and Support throughout the study periods.

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Hedychium spicatum Buch.-Ham.: A High Valued Skin Glowing and Curing Medicinal Herb Needs Future Attention on its Conservation



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Hedychium spicatum

Family : Zingiberaceae

Local name : Van Haldi, Kapoor kachri, Sati

Trade Name : Kapoor kachri

Status : Near endemic threatened

Market value : Rhizome dry weight @ Rs. 15-60 per kg

***Hedychium spicatum* growing in natural habitat**

Himalaya is known for its rich biodiversity. Due to unique geographical setup, topography and undulant landscape the climatic conditions varied along an altitudinal gradients which attributes diversified ecological habitat ranging from tropical forest, grassland to alpine meadows with vast and diverse natural resources. Kedarkhand and Kurmanchal which is todayôs Uttarakhand is new hill state carved out of the northern part of Uttar Pradesh, and lies in 28^0 43ô to 31^0 28ô north and 77^0 32ô to 81^0 00ô east; which forms north-western part of country, starting from upper Gangatic plain in the south region and extends up to snow-claded peaks of the Himadri, marking the Indo-Tibeten boundary. The state constitutes a unique geographical and geological entity comprising a diverse social, cultural, agro-economic and environmental setup. The land comprising 13 districts of Garhwal and Kumaun with about 53,484 km^2 area and 84, 79,562 populations, has many charms to offer. If the timeless temples and joyous and colourful society lend it cultural importance, it is the abounding mountains, rivers, flora and fauna that invoke a feeling of awe in everybody. Being situated in the lap of Himalaya the region is rich in forest wealth that ranges from subtropical to alpine types. Floristically, it falls under the west Himalayan biogeographic zone and it is rife with floral diversity comparable to any other Himalayan region in the country with an estimated 4000 species of flowering plants having great economic, medicinal, aromatic, and aesthetic value.

In western Himalaya about 500 species of medicinal plants are used for curing illness from time immemorial. Anthology also reveals that the life saving drug Sanjivani was taken from Dronagiri peak of the Himalaya, which is 3rd highest peak of western Himalaya, on the direction of a celestial physician Dhanvantri. The Uttarakhand has rainfed conditions and the people have very limited life supporting resources and are either dependent on subsistence agriculture and tourism as their primary livelihood. Hence traditional agriculture is uneconomical

and can not support the poor farmers of the region. Nearly 75-78% population of the state is dependent for their food energy on horticultural crop and terrace faming. Though the maximum population involved in agricultural activities but due to subsistence agriculture ecosystem, the output to feed the needs of people is not sufficient and monetary gain is also very low which forces the youth of the region to migrate in the metropolitan cities in search of better livelihood. However, the state is home of many traditional medicinal resources which have been extracted for modern medicinal uses in the recent years at a large scale from all over the Uttarakhand and many of which remain unexplored for their trade potential and nutritional value and become a subject of great interest recently. The ethno-cultural diversity in the Uttarakhand is quite pronounced and folk medicinal practices evolved parallelly using the native medicinal plants, may become viable and sustainable economic generating option for the region. A conservative society, week economy, inaccessibility and consequently lack of modern medical facilities in the rural areas of the state are the factors leading to interest toward the indigenous medicinal plants for new and sustainable economic generating option. There is virtually no cultivation cost involved, and therefore is a good source of extra income for farmers. Several medicinal plants (*Taxus baccata*, *Acorus calamus Bergenia ciliata, Swertia angustifolia, S. chiriyita, Berberis* species, *Aconitum atrox, A. heterophyllum, Nardostachys jatamansi, Plantago depressa, Podophyllum hexandrum, Rauvolfia serpentina, Picrorhiza kurrooa, Rheum emodi*, *Dioscorea deltoidea,* etc.) are well known for their medicinal value in world market. But the resources are dwindling fast due to lack of any significant conservation efforts. The local people are by and large aware of the nutritional value and medicinal properties of these plants. Therefore, the medicinal plants of the region requires an immediate attention; listing of the medicinal plants and documenting the inherited knowledge passed through several generations. At the same time due consideration is also required for conservation of diversity protecting the threatened status of herbs for reckless exploitation.

Identification and cultivation of new wild medicinal and economically viable crops adapted to wide range of climatic and soil conditions are essential for sustainable agriculture in arid and semiarid regions, for higher economic return. It can replace the existing cultivation which is largely uneconomical. It can also play a vital role in sustaining natural resources and restoration of degraded lands and thus help the upgrading the natural resource and quality of poor farmers life. However, recently the Uttarakhand Govt. has declared itself an herbal state and the Herbal Research and Development Institute Gopeshwar (HRDI) have been created as a nodal agency for the furtherance of this cause. Besides the Govt. establishing the several nurseries all out the state, the participation of local traditional knowledge can not be ignored, which have may valuable practice unknown to outside world so far. The sufficient scientific skill, agricultural manpower and traditional knowledge of indigenous medicinal use of Uttarakhand has a great scope to exploit the global market of plant derived drugs and botanicals as raw ingredient of herbal products and can get considerable size of market both nationally and internationally. It is estimated that world market of plant derived drugs is about 2 lakhs crores. The new opportunities generated by recent technological advances in medical and aromatic plants cultivation, processing and business can make farmers capable for becoming viable industry of tomorrow. Out of several medicinal herbs reported from Himalayan region, *Hedychium spicatum* a high valued skin glowing and curing medicinal herb is one of them.

Hedychium spicatum Buch-Ham. belonging to family Zingiberaceae locally known as Ban-Haldi is a tall perennial herb with leafy stems that grow the beneath of forest cover on marginal land and growing up to 1.5m by 0.7m. The leaves oblong or oblong-lanceolate, reaching 30 cm or more. Spikes sometimes 30 cm, densely flowered, bracts large, oblong, obtuse, calyx shorter than bract flower white ascending and closely imbricate type. Stamen rather shorterthan lip, anther linear, capsule glabrous, globose. It flowers in October. The flowers are hermaphrodite (has both male and female organs).

Hedychium spicatum grows well in moist soil, sunny position and wide range of climatic conditions of forest margins from 1500 to 2800m asl. It tolerates temperature down to about -2°C and has been known to withstand temperatures down to -16°C. It can be grown at the foot of a south-facing aspect if given good mulch in the winter. Plants seem to be immune to the predations of rabbits. The tubers should only just be covered with soil. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils. The plant thrives better in acid, neutral and basic (alkaline) soils. It cannot grow in the shade and susceptible to frost.

The fleshy rhizomes running at or just below the soil surface which have a so-called indeterminate growth habit The leafy shoots that emerge above ground and carry the spiky leaves that make *Hedychium* such striking plants even when not in flower. The flowers are borne from midsummer to autumn at the top of the leafy stems although not every stem will produce a flower spike or inflorescence. On the inflorescence the flowers emerges in the axils of green leafy bracts that in some species roll in

on themselves to form a tube from which the flowers emerge. Deep dry mulch will help protect against the worst of the winter cold. It is suggested that *Hedychium* should be grown under light shade except in marginal gardens where they are best in full sun. Keeping plants well watered in summer can mitigate the tendency for flowers to go over quickly in full sun. *Hedychium* resent being lifted annually and stored as dry rhizomes over winter. *Hedychium* should preferably be left in situ in the garden over winter. If you are concerned about losing the plant to frost then it should be mulched for the. Alternatively whole clumps can be lifted and potted for the winter. In each case the plants are over-wintered under protection still in leaf so that they continue to grow slowly in winter.

Medicinal Uses: The roots and leaves are used in several Ayurvedic preparations and has great potential for exports. The powder as well as decoction of root is carminative; digestive; emmenagogue. A small cup of root decoction twice in a day is expectorant; stimulant; stomachic; Tonic; Vasodilator. The one spoonful powder of root thrice in a day is useful in the treatment of liver complaints, and is also used in treating fevers, vomiting, diarrhoea, inflammation, pains and snake bite. The root is used in Tibetan medicine, it is said to have an acrid taste and two spoonful powders twice in a day is given for heating potency to the female. It is used in the treatment of indigestion and poor circulation due to thickening of the blood. The rootstock is acrid, bitter, pungent, heating, and astringent. The root powder 3-4 gm 2 times in a day is used in asthma, foul breath, bronchitis, hiccough, vomiting, tridosha, diseases of blood. Root powder is laxative to bowel and decoction is tonic to brain.

Besides above medicinal properties the fruit cooked and eaten in savoury dishes with lentils and also used for **e**ssential oil and incense. Crushed dried root both the bruised and the dried root are very aromatic with a fragrant, somewhat pungent smell similar to orris root but more powerful. The rootstock yields 4% essential oil. This oil, which has a scent somewhat like hyacinths, is so powerful that a single drop will render clothes highly perfumed for a considerable period. The dried root is burnt as incense. The Fresh flowers are sweetly scented, the scent being most pronounced towards evening.

Propagation:The seeds of this plant should be sown just after ripening of fruit. Allow to grow large enough and keep as such at least first winter in its natural habitat and plant out in late spring in the forests margin. For the vegetative propagation dig up the clump and divide it with a sharp spade or knife, making sure that each division has a growing shoot. Larger clumps can be planted out direct into their permanent positions, but it is best to pot up the smaller divisions and grow them in its own nature until they are established. Plant them out in the summer or late in the following spring. Nature and Science, 2009;7(9):75-77 (ISSN: 1545-0740)

Acknowledgements:

Authors gratefully acknowledged the Head, Department of Botany, HNB Garhwal University, Director, High Altitude Plant Physiology Research Centre, Principal Govt. P.G. College Gopeshawar, and Project Director Aajeevika project for encouragement and support throughout the study periods.

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Thermodynamic modeling of performance of an irreversible Diesel cycle with engine speed

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Abstract: Finite-time thermodynamic analysis of an air-standard internal-combustion Diesel cycle is performed in this paper. The relations between the power output and the compression ratio, between the power output and the thermal efficiency are derived. The maximum net power output and the corresponding efficiency limit of the cycle with friction losses are also found. Detailed numerical examples are given. This paper provides an additional criterion for use in the evaluation of the performance and the suitability of a Diesel engine. [Nature and Science. 2009;7(9):78-82]. (ISSN: 1545-0740).

Key words: finite-time thermodynamics; Diesel cycle; performance optimization

1. Introduction

Today, practical engineers make use of the air-standard Diesel cycle to provide a short description of the Diesel engine. Conventional analysis of the air-standard Diesel cycle is performed using reversible thermodynamics. The development of finite-time thermodynamics (Curzon and Ahlborn, 1975; Aragon-Gonzalez et al., 2000; Chen et al., 2008), a new discipline of modern thermodynamics, provides a new and powerful tool for the performance analysis of practical engineering cycles. Thus, much work has been performed for the performance analysis and optimization of finite time processes and finite size devices (Aragon-Gonzalez et al., 2006; Chen et al., 2006; Ge et al., 2008a). Chen et al. (1982) and Aizenbud and Band (1993) determined the optimal motion of a piston fitted to a cylinder. The cylinder contained a gas pumped with a given heating rate and coupled to a heat bath during finite times. Blank and Wu (1993) examined the effect of combustion on the work or power optimised Otto, Diesel and Dual cycles. They derived the maximum work or power and the corresponding efficiency bounds. Chen et al. (1996) derived the relations between the power output and the thermal efficiency for the Diesel cycle with the consideration of the heat-transfer losses. Bhattacharyya (2000) proposed a simplified irreversible model for an air-standard Diesel cycle by using the finite time thermodynamic approach. In his study, global thermal and friction losses are lumped into an equivalent friction term, which is linear in the piston velocity. Rocha-Martinez et al. (2002) investigated the effect of variable specific-heats on the Otto and Diesel cycle performance. Chen et al. (2002) modeled the behaviors of Diesel cycle, with friction losses, over a finite period. Chen et al. (2003, 2004) determined the characteristics of power and efficiency for Otto and Dual cycles with heat transfer and friction losses. Fischer and Hoffman (2004) concluded that a quantitative simulation of an Otto-engine's behavior can be accurately achieved by a simple Novikov model with heat leaks. Hou (2004) derived the performance characteristic of Dual cycle with only heat transfer loss and studied the effects of heat transfer loss on the performance of the cycle. Ge et al. (2005) derived the performance characteristics of the diesel cycle with heat transfer and friction like term losses when the maximum temperature of the cycle was not fixed. Ozsoysal (2006) gave the valid ranges of the heat transfer loss parameters of the Otto and diesel cycles with consideration of the heat loss as a percentage of the fuel's energy. Parlak et al. (2006) defined the internal irreversibility by using entropy production, and analyzed the effect of the internal irreversibility on the performance of irreversible Dual cycle. Al-Sarkhi et al. (2006) studied the effects of variable specific heats of the working fluid on the performances of the Diesel and Miller cycles. Ge et al. (2007) studied the effects of variable specific heats of the working fluid on the performances of the Diesel cycle. Zhao and Chen (2007) performed analysis and parametric optimum criteria of an irreversible Atkinson heat engine using finite time thermodynamics. Zhao and Chen (2007) also defined the internal irreversibility by using compression and analyzed the performance of

Dual cycle when the maximum temperature of the cycle is fixed and the efficiency has a new definition. Ge et al. (2008a; 2008b; 2009) analyzed the performance of an air standard Otto, Diesel and dual cycles. In the irreversible cycle model, the non-linear relation between the specific heat of the working fluid and its temperature, the friction loss computed according to the mean velocity of the piston, the internal irreversibility described by using the compression and expansion efficiencies, and the heat transfer loss are considered. Ebrahimi (2009a) studied the effects of the temperature dependent specific heat ratio of the working fluid on the performance of the diesel cycle.

As can be seen in the relevant literature, the investigation of the effect of engine speed on performance of Diesel cycle does not appear to have been published. Therefore, the objective of this study is to examine the effect of engine speed on performance of air standard Diesel cycle.

2. Irreversible Diesel cycle

The Diesel cycle shown in figure 1 approximates the compression stroke up to ignition with the adiabatic reversible (isentropic) process $1 \to 2$; it assumes that the combustion process is represented by the reversible constant pressure process $2 \to 3$; it approximates the power stroke with the isentropic expansion process $3 \to 4$; and it assumes that the heat-removing process is the reversible constant volume process $4 \to 1$.

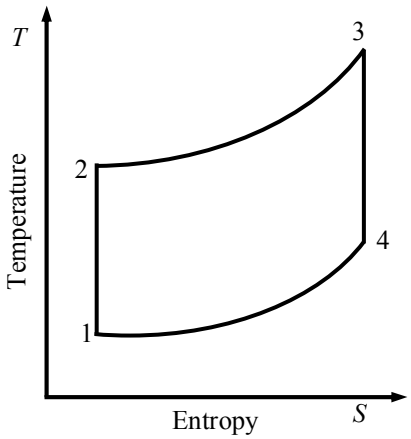

Figure 1. $T-S$ diagram of a Diesel cycle

The combustion heat input is Q_{23} and the heat rejected by the working fluid is Q_{41}. Thus, assuming that the heat engine is operated at the rate of N cycles per second, the reversible power output per second is:

$$P_{rev} = N\left(Q_{23} - Q_{41}\right) = NM\left[c_p\left(T_3 - T_2\right) - c_v\left(T_4 - T_1\right)\right] \quad (1)$$

where c_v is the constant volume specific heat, c_p is the constant pressure specific heat, M is the molar number of the working fluid and T is the absolute temperature.

The compression ratio, r_c, is defined as:

$$r_c = V_1/V_2 \quad (2)$$

For the processes $1 \to 2$ and $3 \to 4$, we have

$$T_2 = T_1 r_c^{\gamma-1} \quad (3)$$

and

$$T_4 = T_1^{\gamma-1} T_3^{\gamma} r_c^{(1-\gamma)\gamma} \quad (4)$$

Where γ is the ratio of specific heats, $\gamma = c_p/c_v$. Thus, Equation (1) becomes:

$$P_{rev} = NM\frac{R}{\gamma-1}\left[\gamma\left(T_3 - T_1 r_c^{\gamma-1}\right) - T_1^{\gamma-1} T_3^{\gamma} r_c^{(1-\gamma)\gamma} + T_1\right] \quad (5)$$

where R is the molar gas constant of the working fluid.

Taking into account the friction loss of the piston and assuming a dissipation term represented by a friction force that is a linear function of the piston velocity gives (Chen et al., 2006; Ge et al., 2007; Ebrahimi, 2009a).

$$f_\mu = -\mu v = -\mu\frac{dx}{dt} \quad (6)$$

where μ is the coefficient of friction, which takes into account the global losses, x is the piston's displacement and S_P is the piston's velocity. Therefore, the lost power due to friction is

$$P_{los} = \frac{dW_{los}}{dt} = -\mu\left(\frac{dx}{dt}\right)^2 = -\mu\left(S_p\right)^2 \quad (7)$$

Thus, the lost power is

$$P_{los} = -\mu\left(\overline{S}_p\right)^2 \quad (8)$$

where $\overline{S}_p$ is the mean velocity of the piston.

Running at N cycles per second, the mean velocity of the piston is

$$\overline{S}_p = 4LN \quad (9)$$

where L is the total distance the piston travels per cycle.

The resulting power output (P) the Diesel cycle engine can be written as:

$$P = P_{rev} - P_{los} = NM\frac{R}{\gamma-1}\left[\gamma\left(T_3 - T_1 r_c^{\gamma-1}\right) - T_1^{\gamma-1} T_3^{\gamma} r_c^{(1-\gamma)\gamma} + T_1\right] - 16\mu\left(LN\right)^2 \quad (10)$$

The efficiency of the Diesel cycle engine is expressed by

$$\eta_{th} = P/Q_{in} \quad (11)$$

Where

$$Q_{in} = NMc_p\left(T_3 - T_2\right) \quad (12)$$

Hence, the irreversible cycle efficiency is

$$\eta_{th} = \frac{MR\left[\gamma\left(T_3 - T_1 r_c^{\gamma-1}\right) - T_1^{\gamma-1} T_3^{\gamma} r_c^{(1-\gamma)\gamma} + T_1\right] - 16\mu N(\gamma-1)L^2}{MR\gamma\left(T_3 - T_2\right)} \quad (13)$$

Notice that both power and efficiency are convex functions of the compression ratio.

When r_c , T_1 and T_3 are given, the power output and thermal efficiency of the Diesel cycle engine can be obtained from Eqs. (12) and (13), respectively. Therefore, the relations between the power output, the thermal efficiency and the compression ratio can be derived.

3. Results and discussion

The following constants and parameters have been used in this exercise: $T_3 = 2200\,K$, $T_1 = 360\,K$, $L = 95\,mm$, $\mu = 12.9\,Nsm^{-1}$, $N = 3000 \rightarrow 7000\,rpm$, $r_c = 1 \rightarrow 70$, $\gamma = 1.4$ and $M = 1.57 \times 10^{-5}\,kmol$ (Chen et al. 2006; Ghatak and Chakraborty, 2007; Ge et al., 2009; Ebrahimi, 2009b). Using the above constants and range of parameters, the power output versus compression ratio characteristic and the power output versus efficiency characteristic with varying the mean piston speed can be plotted. Numerical examples are shown as follows.

Figures 2 and 3 show the effects of the variable engine speed on the cycle performance with heat resistance, internal irreversibility and friction losses (the dashed lines in the figures denote where the cycle cannot work normally). From these figures, it can be found that the engine speed plays important roles on the power output. It is clearly seen that the effect of engine speed on the power output is related to compression ratio. They reflect the performance characteristics of a real irreversible Diesel cycle engine.

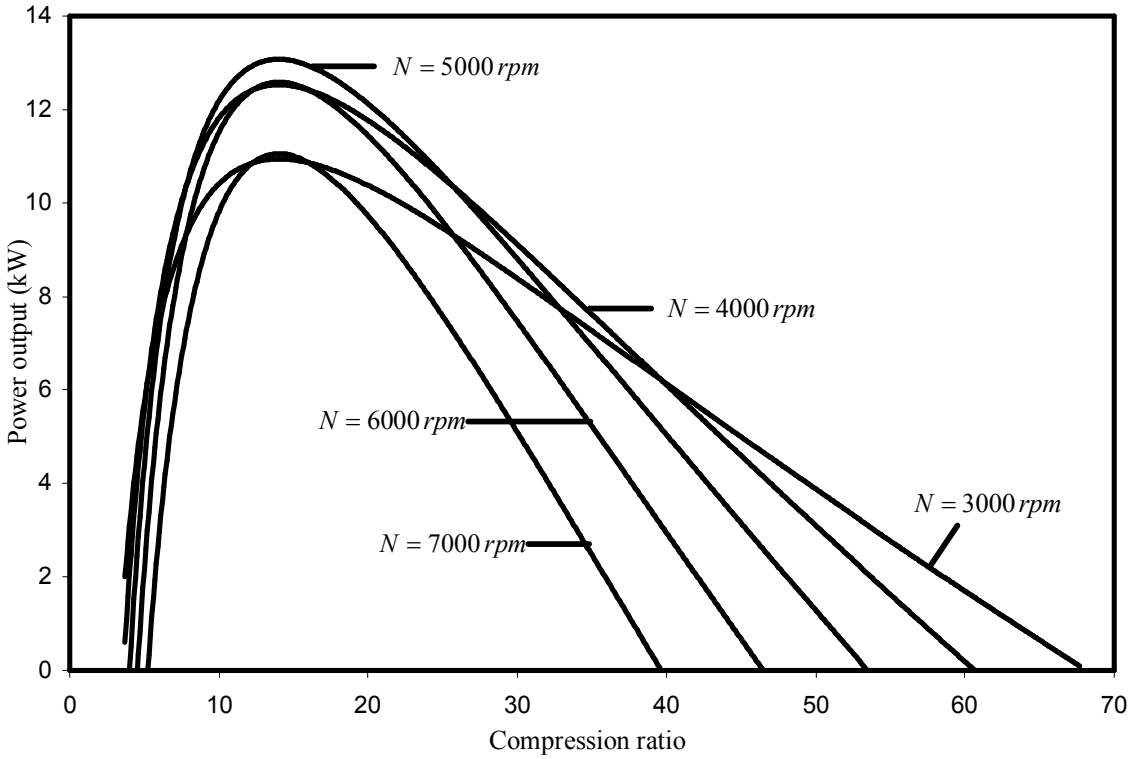

Figure 2. Effect of engine speed on the variation of the power with compression ratio

Figure 2 indicates the effects of the engine speed on the power output of the cycle for different values of the compression ratio. It can be seen that the power output versus compression ratio characteristic is approximately parabolic like curves. In other word, the power output increases with increasing compression ratio, reach their maximum values and then decreases with further increase in compression ratio. The maximum power output increases with increasing engine speed up to about $N = 5000\,rpm$ where it reaches its peak value then starts to decline as the engine speed increases. This is consistent with the experimental results in the internal combustion engine (Mercier, 2006).

The optimal compression ratio corresponding to maximum power output point remains constant with

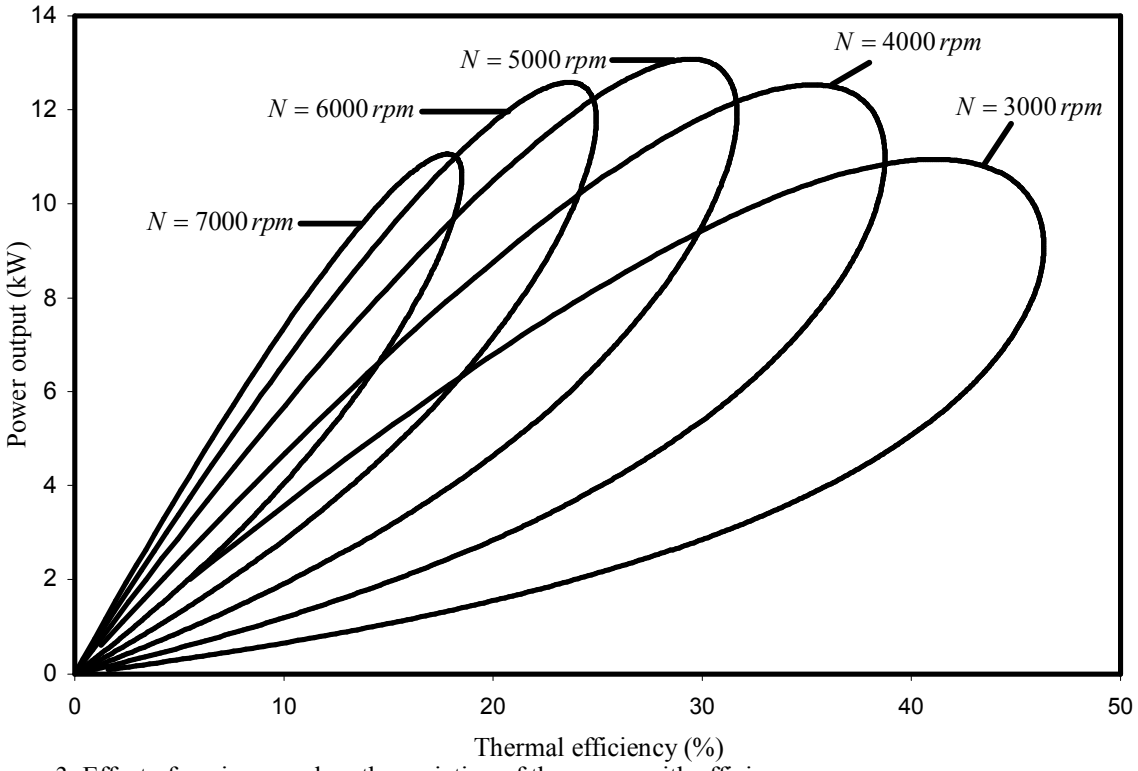

Figure 3. Effect of engine speed on the variation of the power with efficiency

increase of engine speed. The results shows that if compression ratio is less than certain value, the power output increases with increasing engine speed, while if compression ratio exceeds certain value, the power output first increases and then starts to decrease with increasing engine speed. With further increase in compression ratio, the increase of engine speed results in decreasing the power output. Numerical calculation shows that for any same compression ratio, the smallest power output is for $N = 5000\,rpm$ when $r_c \le 12.3$ or $r_c > 16$ and is for $N = 3000\,rpm$ when $12.3 < r_c \le 16$ and also the largest power output is for $N = 3000\,rpm$ when $r_c \le 5.2$ or $r_c > 39.5$, is for $N = 4000\,rpm$ when $5.2 < r_c \le 7.8$ or $25.8 \le r_c \le 39.5$ and is for $N = 5000\,rpm$ when $7.8 < r_c < 25.8$.

The influence of the engine speed on the power output versus thermal efficiency is displayed in figure 3. As can be seen from this figure, the power output versus thermal efficiency is loop shaped one. It can be seen that the power output at maximum thermal efficiency improves with increasing engine speed from 3000 to around 5000 rpm. With further increase in mean engine speed, the power output at maximum thermal efficiency decreases. It can also be seen that the thermal efficiency at maximum power decreases with increase of engine speed from 3000 to 7000 rpm.

According to above analysis, it can be found that the effects of the engine speed on the cycle performance are obvious, and they should be considered in practice cycle analysis in order to make the cycle model be more close to practice.

4. Conclusion

The effect of finite rate process and friction on the irreversible Diesel cycle is determined in this paper. The relations between net power output, efficiency, compression ratio, and the engine speed are derived. The effects of engine speed on the power output and the efficiency were analyzed by detailed numerical examples. Our above analysis provides a new theoretical basis for the performance evaluation, improvement, and optimization of practical Diesel engines.

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1/9/2009

Landscape change and sandy desertification monitoring and assessment: a case study in Northern Shaanxi Province, China

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Abstract The objective of this study is to develop techniques for assessing and analyzing sand desertification in the northern part of Shaanxi Province, China. In order to reveal the process of land degradation, especially the latest situation of sandy desertification, a method integrating remote sensing, Geographic Information System (GIS) and field survey was employed to build a sandy desertification dataset for analysis. Remote Sensing images included the Landsat Thematic Mapper (TM) images in 1987 and Landsat Enhanced Thematic Mapper Plus (ETM^+) images in 1999). Eight land- cover classes, including active sand dunes, fixed sand dunes, semi-fixed sand dunes, grass land, farm land, wet land, built up area and unused land and Normalized Difference Vegetation Index (NDVI), were identified.Results showed that the active sand dunes and semi-fixed sand dunes have increased with a change rate of 128.70 $km^2.yr^{-1}$ and 55.65 $km^2.yr^{-1}$, respectively, in the meantime the fixed sand dunes has decreased with a decreasing rate of 182.14 $km^2.yr^{-1}$. During the 1987s, the area of sandy desertified land reached 12,006.11 km^2 (57.17% of the total sandy area), of which severely desertified, medium desertified, and slightly desertified land areas were 4,442.23, 4,253.45and 3,310.43 km^2 , respectively. By the year 1999, the area of desertified land was increased to 13,782.30 km^2 (65.63% of the total sandy area), of which severely desertified, medium desertified, and slightly desertified land areas were 5,169.89, 4,918.15and 3,694.26 km^2, respectively increasing by 1,776.19 km^2. Spatial change detection based on active sand dunes showed that the expansion area was much larger than the reversion in the past two decades, and that several active sand belts has been formed, suggesting that sandy desertification of northern part of Shaanxi Province, China, will be a long-term task.
[Nature and Science. 2009;7(9):83-90]. (ISSN: 1545-0740).

Keywords: assessment; sandy desertification; NDVI; active sand dunes; GIS;China

1. Introduction

The term “desertification” should be used side by side with the term “land degradation”. Hence “desertification” in the context of assessment is land degradation in arid; semi-arid; and sub-humid areas resulting from adverse human impact. According to the United Nations Conference on Environment and Development (UNCED, 1992), defines desertification as “land degradation in arid, semi-arid and dry sub-humid areas resulting from various factors including climatic variations and human activities.

Sandy desertification is one of the main form of land degradation in China, especially in northern China (Wang, 2001), which has kept expanding since the 1950’s and has exerted severe impacts on regional socio-economic development and environmental security (Wang, 2006) .Harsh physiographic conditions (sparse vegetation coverage, sandy soil and water deficiency), irrational land-use practice and population augmentation are regarded as the forces of triggering sandy desertification (Zhu et al ,1994.Chen et al, 2005). Therefore, the sandy desertification assessment and monitoring are always concerned by researchers, the public and the policy-makers.

Desert and sandy decertified land occupy about 1.67 million km^2, or 17% of the total land area of China (Wang, 2003). Decertified sandy land increased by 25,200 km for the period from 1975 to 1987 about 40.5% of which was distributed in the semi-arid agro-pastoral regions of northern China (Zhu and Wang, 1993).

The objective of this study is to develop techniques for assessing and analyzing sand desertification in the northern part of Shaanxi Province, China, in the past decades. A method of integrating remote sensing (RS), geographic information system (GIS) and field survey was employed to generate a sandy desertification dataset of this region. Then change detection and land degradation process were performed based on the dataset. This study intends to provide useful information

for sandy desertification controlling and environmental management of the northern part of Shaanxi Province, China.

2. Study area

The study area, located in the northern part of Shaanxi province, lies within longitude 108 33 to 111 24 E and from 36 97 to 39 58 N with total area of 29416.6 km^2, accounting for 18.6% of the total Shaanxi province (Figure 1).In order to study the development of sandy desertification, the counties of Dingbian, Jingbian, Hengshan, Yulin, and Shenmu have been selected as study area. Counties are situated in the northern part of Shaanxi province. Geographically, the study area is located in the transitional zone, in south and east of Mu Su Desert (Inner Mongolia) and the Loess Plateau of northern Shaanxi province in China. This area has atypical continental semi-arid climate .Annual precipitation ranges from 440 mm in the south-east to 250 mm in the north-west, of which 60-80% is concentrated in the period from June to August. The annual mean temperature is about 6.0-8.5, with monthly mean temperature of 22 in July and -11 in January (Department of Geography of Peking University, 1983) .The main prevailing wind is north-westerly, with a speed of more than 5 ms^{-1} on over 200 days of the year. The elevation is between 1000 and 1907 m a.s.l (Song l (Song and Chen ， 1991).

3. Material and Methods

3.1 Data sources

Satellite remote sensing, in conjunction with geographic information system (GIS), has been widely applied and been recognized as a powerful and effective tool in detecting landscape change (Liu et al ., 2003; Li, 1996; Cai, 2001 ; Paul et al., 1992; Allen S Hope and Douglas A Stow, 1993; Anthony Gar-On Yeh and Li ,1999). Four kinds of data were used in this study, i.e., socioeconomic data, topographic data, remote sensing data, and ancillary data. Socioeconomic data were the annual statistical data released by the National Bureau of Statistics. The data published in 1986 and 2001 were acquired. All data were amalgamated to the county level. Tow topographic maps had a scale of 1: 100,000 and 1: 50,000, respectively. The remote sensing data are landsat TM images record in 1987 and 1999.Two types of ancillary data were used, a vegetation map at a scale of 1: 500,000(Lei, 1999),and a current land use map supplied Yulin Land and Resources Bureau(2000).

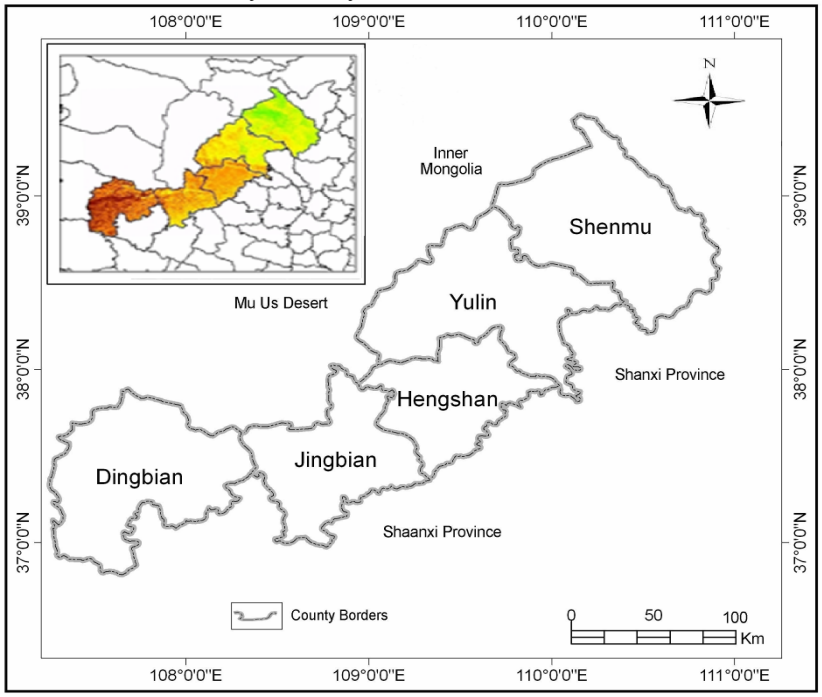

Figure 1. General location map of study area.

3.2 Data and Pre-processing

Remote Sensing images used in this study include the Landsat-5 Thematic Mapper (TM) image, and Landsat-7 Enhanced Thematic Mapper Plus (ETM^+) image, which were acquired in 24 October 1987 and 15 October 1999, respectively, the pre-processing of this dataset included geometric corrections .All images were geometrically corrected not only to eliminate geometric distortions present in the images but also to register the satellite images to ground data.. The nearest-neighbor resampling method was used in datum WGS 84 and projection UTM (49N) all images resampled to a 30 m pixel grid. In order to remove or normalize the reflectance variation between images acquired at different times, relative radiometric correction was performed to yield normalize radiometric data on a common scale (Paolini et al.,2006).Here, the histogram normalization, a simpler and more effective technique, was used to carry out the relative radiometric correction (Ding et al.,2005).

3.3 Determination of NDVI threshold

The Vegetation index can reflect a series of physics quantity of he plants, such as index of leaf's area, plant overlays degree, amount of living creature and plant category (Qingjiu et al., 1998). The index of vegetation contain many different expression methods, among them NDVI is applied in the fixed amount research of plant overlays. Therefore TM data were used to produce Normalize Difference Vegetation Index (Kidwell 1990; Dall'Olmo et al. 2002 ; Rouse et al .1974), which is defined as:

NDVI = (TM4-TM3)/ (TM4+TM3)

Where TM4 and TM3 are channels in the near infrared(NIR) and the red(R) wavelength of landsat TM data, respectively, was applied for monitoring vegetation changes in the study area within the year 1987 and 1999.

Determining the threshold of NDVI is the first step in extraction of sand desertification. Then we can use the threshold to separate vegetation information and non-vegetation information from land cover. Through comparative analysis of the NDVI images and the research area, select NDVI = 0.05 as the threshold, which can separate vegetation information and non-vegetation information from the research land cover. That is: if NDVI> 0.05, the area was considered as vegetation; instead, considered as no vegetation information.

The land desertification monitoring was performed and implemented in the GIS. Through the GIS, land desertification maps can be produced and this method can monitor areas prone to land desertification using spatial patterns from land cover classes, NDVI and main prevailing wind direction.

The correlation between NDVI and vegetation cover percentage, the selection of potential desertification areas where the vegetation cover (e.g., usually grasslands and farmlands) less than 30%, the classification of potential areas based on their position in relation to the main prevailing wind direction, and the production of land desertification areas and then the identification of land desertification changes with time.

NDVI is a greenness index and an argument can be made that greenness and vegetation cover are correlated. The vegetation cover is a primary indicator of land desertification. Before the data modelling, NDVI was density sliced. We defined that if NDVI was less than 0.078, vegetation cover percentage was less than15%; if NDVI was between 0.079 and 0.102, vegetation cover percentage was between 15–30%; If NDVI was between 0.103 and 0.138, vegetation cover was between 30–50%.

The NDVI and vegetation cover classification criteria were defined as follows: NDVI<0.079 and vegetation cover <15% as active sandy land and it is severe desertified, NDVI=0.079–0.102 and vegetation cover=15–30% as semi-fixed sandy land and it is medium desertified, and NDVI=0.103–0.138 and vegetation cover=30–50% as fixed sandy land and it is slightly desertified (Table 1). This NDVI classification was calibrated by field work and was also supported by previous studies (Guo 1990; van Genderen et al. 1993;Guo 1994; Zhang 1994).

The image pattern, tone and texture were considered to classify the land types of sandy desertification. The following four types were mapped:

1. Land severely aected by sandy desertification. This is characterized by a grayish bright color, and represent mobile sandy dunes with sickle-like shapes and high undulation which has <15% vegetation cover.

The active sand dunes are widespread, covering up to 50–70% of the total area of sandy land. The inter-dune areas are characterized by wind erosion and sand covering.

2. Land showing a medium amount of sandy desertification. This type of sandy land has a gray or grayish red color and exhibits semi-fixed sand dunes with a wave-like shape. The vegetation cover varies from 15 to 30%. The shifting sand is widespread and there are visible patches of wind erosion in farmland and grassland.

3. Land slightly aected by desertification. Grayish green and grayish red in color, it refers to fixed dunes with little shifting sand and has a vegetation cover of 30–50%

4. Land unaected by desertification. This has a uniforml saturated red color, and has no patches of shifting sand and a vegetation cover of more than 50%.

4.Results and discussion

4.1Landscape change

Generally speaking, from the 1987s to the 1999s, there was a remarkable ecological change occurred in the study area sandy land during the study period (twelve years). The sandland landscape changed significantly and desertified land has expanded rapidly .From 1987 to 1999, the fixed sand dunes, which, covered (9669.24 km^2; 32.87%) of the total area in 1987, sharply shrank to 7483.58 km^2 (25.44%) in 1999 with a decreasing rate of 182.14 $km^2.yr^{-1}$.The semi-fixed sand dunes, however, increased from 4121.27 km^2 (14.01%) in 1987 to 4789.02 km^2 (16.28%) in 1999 with a growth rate of 55.65 $km^2.yr^{-1}$.The active sand dunes ,following the same trend as the semi-fixed sand dunes, increased from 5674.46 km^2 (19.29%) in 1987 to 7218.83 km^2 (24.54%) in 1999 with a change rate of 128.70 $km^2.yr^{-1}$.The grasslands decreased from 4244.82 km^2 (14.43%) in 1987 to 2541.60 km^2 (8.64%) in 1999 with a decreasing rate of 141.94 $km^2.yr^{-1}$,while the farmlands, increased rapidly from 1723.81 km^2 (5.86%) in 1987 to 3403.50 km^2 (11.57%) in 1999 with a growth rate of change 139.97 $km^2.yr^{-1}$.The built up area increased from 1106.05 km^2 (3.76) in 1987 to 1741.46 km^2 (5.92)in 1999 ,while the unused land decreased from 2100.35 km^2 (7.14) in 1987 to 1582.61 km^2 (5.38) in 1999 ,whereas wetlands had kept with no changed mentioned during the study period. (Table 2 and Figures 2, 3, and 4). Policies have played an important role in controlling sandy desertification, this mainly happened in the study area as we can see in the rapidly increasing of farmland from 2017.98 km^2 (6.86%) in 1987 to 3697.67 km^2 (12.57%) in 1999 of the total area, on the other hand we can see the decreasing of grassland from 4244.82 km^2 (14.43%) in 1987 to 2541.60 km^2 (8.64%) in 1999 of the total area of the study area. Although land desertification has been to some extent controlled in the study area, the issue is still serious.

4.2 Severity of sandy desertification

The sandy desertification land in the study area during the two different time intervals is indicated in Fig.5.During the 1987s, the area of sandy desertified land reached 12,006.11 km^2 (57.17% of the total sandy area), of which severely desertified, medium desertified, and slightly desertified land areas were 4,442.23, 4,253.45and 3,310.43 km^2 , respectively. By the year 1999, the area of desertified land was increased to 13,782.30 km^2 (65.63% of the total sandy area), of which severely desertified, medium desertified, and

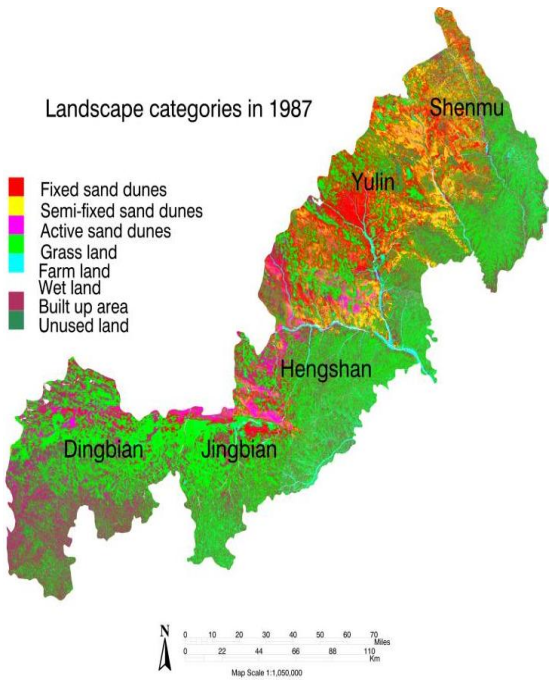

Figure 2: Landscape of the study area in 1987

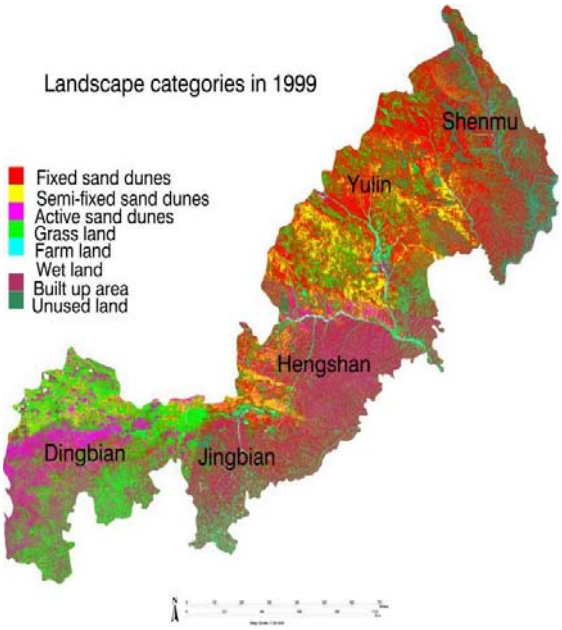

Figure 3: Landscape of the study area in 1999

slightly desertified land areas were 5,169.89, 4,918.15and 3,694.26 km^2, respectively increasing by 1,776.19 km^2. The statistics indicate that the unchanged region for the type of sandy desertification in 1987 reached 8,993.89 km^2, accounting for 42.83% of the sandy area. While in 1999 it was reached 7,217.70 km^2, accounting for 34.37% of the sandy area. It is indicated by the above data that the land desertification process was not steady and showed a trend of developing. The developed region of sandy desertification was directed mainly from the northwest towards the southeast, especially in the desert-adjacent areas.

4.3 Spatial imbalance of sandy desertification

Table 3 shows the spatial imbalance of sandy desertification during the 12 years from 1987 to 1999. The regions with serious sandy desertification are in northwestern parts whereas those slightly influenced by desertification are in the southeastern parts belonging to loess hills. This imbalance of spatial distribution varied little during the 12-year study period, and could be accounted for by the difference of physical factors and by the diversity of land-use practices. Compared to the southeast, the northwest, which is closer to the inner part of the MU US Desert, mainly under the influence of wind erosion and eolian accumulation, has an abundant sand source.

5. Conclusions

During the 1987s, the area of sandy desertified land reached 12,006.11 km^2 (57.17% of the total sandy area), of which severely desertified, medium desertified, and slightly desertified land areas were 4,442.23, 4,253.45and 3,310.43 km^2 , respectively. By the year 1999, the area of desertified land was increased to 13,782.30 km^2 (65.63% of the total sandy area), of which severely desertified, medium desertified, and slightly desertified land areas were 5,169.89, 4,918.15and 3,694.26 km^2, respectively increasing by 1,776.19 km^2. The statistics indicate that the unchanged region for the type of sandy desertification in 1987 reached 8,993.89 km^2, accounting for 42.83% of the sandy area. While in 1999 it was reached 7,217.70 km^2, accounting for 34.37% of the sandy area.

The results also demonstrate that land cover changes during the past twelve years have been caused by land desertification on farmland and grassland in the area. Such changes may not only include the development of land desertification, but also explain the causes of land desertification in the study area due to over-exploitation and inappropriate land use, as well as by the invasion of wind-blown sand.

We can conclude from the results that the sandy desertification process of the northern part of Shaanxi Province, China, during the study period (twelve years) was a severe sandy desertification, characterized by the fixed sand dunes decreasing at a high speed, and the semi-fixed and active sand dunes increasing remarkably. In most of the sand land, desertification has developed rabidly, while rehabilitation of vegetation has occurred only in marginal areas in the east and south.

Acknowledgement:
Many thanks to Professor Hu Guangdao at the Institute of geology and Remote Sensing ,faculty of earth resources ,china university of geosciences For providing the data .

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rehabilitation in China. Desertification Control Bulletin **22:** 27-30.

Table 1: Extent of desertification hierarchical list in the study area

Desertification type	Vegetation coverage	NDVI	Classification criteria
Slight desertification	30–50%	0.103–.138	No obvious blown sand activities and land surface is covered by fixed or semi-fixed sand dunes
Medium desertification	15–30%	0.079–.102	Blown sand activities are controlled significantly, and sand movement ripples exist on sand dunes
Severe desertification	<15%	<0.079	Gobi, sand dunes and sand land denuded interdunes, denuded dune residuals, , clay mounds and wind blowouts.

Table 2: Change rate of the 6 landscape patterns from 1987 to 1999

Land cover type	1987		1999		1987-1999		Change rate (+Gain,-Loss) 1987-1999
	(km^2)	(%)	(km^2)	(%)	(km^2)	(%)	($km^2.yr^{-1}$)
Fixed sand dunes	9669.24	32.87	7483.58	25.44	2185.66	7.43	-182.14
Semi-fixed sand dunes	4121.27	14.01	4789.02	16.28	-667.75	-2.27	+55.65
Active sand dunes	5674.46	19.29	7218.83	24.54	-1544.37	-5.25	+128.70
Grasslands	4244.82	14.43	2541.60	8.64	1703.22	5.79	-141.94
Farmlands	1723.81	5.86	3403.50	11.57	-1679.69	-5.71	+139.97
Wetlands	776.60	2.64	655.10	2.23	121.50	0.41	-10.125
Built up area	1106.05	3.76	1741.46	5.92	-635.41	-2.16	+52.95
Unused land	2100.35	7.14	1582.61	5.38	517.74	1.76	+43.15
Total	29416.60	100%	29416.60	100%			

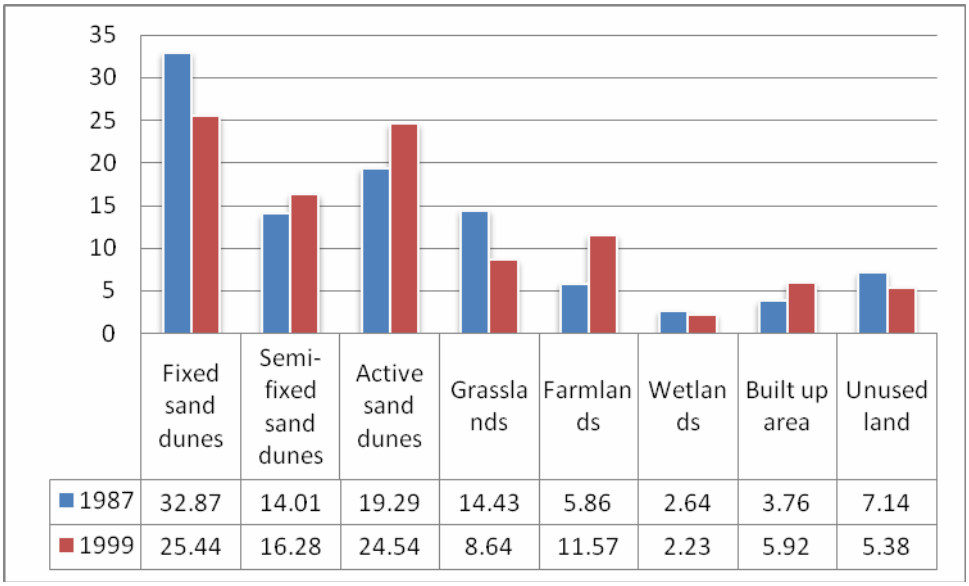

Figure 4: Area percentage of the eight landscape patterns in 1987 and 1999

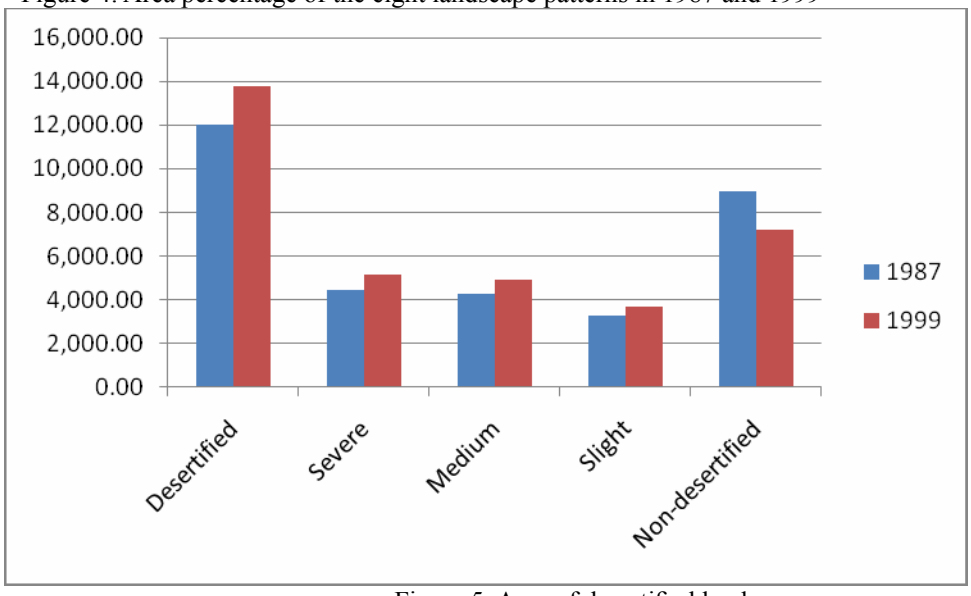

Figure 5: Area of desertified land

Table 3: Desertified land area during the study period

year	Severe desertification		Medium desertification		Slight desertification		Land unaffected by desertification	
	(km^2)	(%)	(km^2)	(%)	(km^2)	(%)	(km^2)	(%)
1987	4,442.23	36.99	4,253.45	35.43	3,310.43	27.57	8,993.89	42.83
1999	5,169.89	37.51	4,918.15	35.68	3,694.26	26.80	7,217.70	34.37

8/26/2009

Evaluation of Water Quality: Physico – Chemical Characteristics of Ganga River at Kanpur by using Correlation Study

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Abstract: We present an extensive investigation of physico-chemical parameters of water samples of Ganga river at Kanpur. Water samples under investigations were collected from Jalsansthan Benajhawar Kanpur sampling station during pre monsoon (April - May), monsoon (July - August) and post monsoon (October - November) seasons in the year 2008. Correlation coefficients were calculated between different pairs of parameters to identify the highly correlated and interrelated water quality parameters and t-test was applied for checking significance. The observed values of different physico-chemical parameters like pH, temperature, turbidity , total hardness(TH) , Iron , Chloride , total dissolved solids(TDS) , Ca^{2+}, Mg^{+2} , SO_4^{-2} , NO_3^-, F^{-1}, total alkalinity (TA) , Oxygen consumption (OC), Suspended solids (SS) of samples were compared with standard values recommended by world health organization (WHO). It is found that significant positive correlation holds for TA with Cl^-, Mg^{+2}, Ca^{+2}, TH, TDS, fluoride and OC. A significant negative correlation was found between SS with chloride, Mg^{+2}, TDS, fluoride and OC. All the physico - chemical parameters for pre monsoon, monsoon and post monsoon seasons are within the highest desirable or maximum permissible limit set by WHO except turbidity which was high while NO_3^- , Cl^{-1} and F^- are less than the values prescribed by WHO.
[Nature and Science. 2009;1(6):91-94]. (ISSN: 1545-0740).
Keywords: Physico-chemical parameters, correlation, t-test.

1. Introduction

It is a fact that good water quality produces healthier humans than one with poor water quality. Ganga River is life line of Kanpur and its water is used for domestic and agriculture purposes therefore, effective maintenance of water quality is required through appropriate measurements. Physico-chemical and micro-biological characteristics may describe the quality of water (Sinha, 1986), therefore, an analysis on physico-chemical parameters of Ganga water was made by many workers (Mehrotra, 1990;. Sinha et.al. 2000) Regular monitoring of all the parameters is very difficult and laborious task even if adequate manpower and laboratory facilities are available. Therefore, statistical correlation technique has been used for comparison of physico-chemical parameters. The present work deals with the study of 15 physico-chemical parameters like pH, temperature, turbidity , TH , Fe , Cl^- , TDS , Ca^{2+}, SO_4^{-2} , NO_3^- , F^{-1}, TA, Mg^{+2} , OC, SS of Ganga river water in Kanpur. The observed values of various physico-chemical parameters of water samples were compared with standard values recommended by World Health Organization (WHO) and are given in Table-1. The objective is to minimize the complexity and dimensionality of large set of data. Systematic calculation of correlation coefficient between physico-chemical parameters has been carried out and significant correlation has been further verified by using t- test. (Bhandari and Nayal, 2008; Garg et.al, 1990; Sarkar et. al., 2006)

2.Experimental

Water samples were collected from Jalsansthan Benajhawar Kanpur sampling station during pre monsoon(April - May), monsoon (July - August) and post monsoon (October - November) phase in year 2008. During sampling pH, temperature, and turbidity were determined using digital pH meter, thermometer and turbidimeter respectively. F^- and nitrate was estimated using colorimetric method. The laboratory analysis of samples was done using standard methods (APHA,1998), titrimetric method was used for the determination of total alkalinity and gravimetric method for total dissolved solid and total suspended solids Mohr's argentometric titration method was used for chloride (Vogel, 1978). Sulphate was estimated using turbidometric method

(Vogel,1978) Whereas Ca^{+2} , Mg^{+2} and TH was determined by EDTA titrimetric method (Vogel,1978). Atomic absorption spectrophotometer was used for determination of Fe and Cr contents. All the chemical used were of AR grade .In order to calculate correlation coefficients, correlation matrix was constructed by calculating the coefficients of different pairs of parameters and correlation for significance was further tested by applying t-test (http://www.spss.com)

Table 1. The average values of physico- chemical parameters of Ganga River water at Kanpur. HDL: Highest Desirable Limit; : MPL; Maximum Permissible Limit

NO.	PARAMETERS	UNITS	DRINKING WATER WHO Standard		Experimental Values (Range)
			HDL	MPL	
1	Temperature	^0C	- --	-----	22-30
2	Turbidity	NTU	5	10	18-470
3	pH value	-	6.5 to 8.5	No relaxation	8.4- 8.9
4	Total hardness (as $CaCO_3$)	mg/l	300	600	122-212
5	Iron	mg/l	0.3	1.0	0.2-0.8
6	Chlorides	mg/l	250	1000	7-26
7	Dissolved Solids	mg/l	500	2000	256-500
8	Calcium	mg/l	75	200	28-48
9	Sulphate	mg/l	200	400	50-91
10	Nitrate	mg/l	50	No relaxation	0-1.772
11	Fluoride	mg/l	1.0	1.5	0-0.4
12	Total Alkalinity	mg/l	200	600	13-246
13	Magnesium	mg/l	30	150	9.23-26.24
14	Oxygen Observed from $KMnO_4$ at 37^0C in 3 hrs.	mg/l	3.0	No relaxation	2.4-7.8
15	Suspended Solids	mg/l	20	150	70-280

Table 2. Different Paired Samples Correlations[9]
Note: Significant if t 0.05 >2.14

Paired Parameters		r	I t I
Pair 1	pH & OC	0.821	4.762
Pair 2	TA & TH	0.97	4.694
Pair 3	TA & Ca^{+2}	0.821	9.145
Pair 4	TA & Mg^{+2}	0.851	10.179
Pair 5	TA & Cl^-	0.91	10.511
Pair 6	TA & TDS	0.988	8.233
Pair 7	TA & OC	0.88	9.942
Pair 8	TA & Fluoride	0.836	9.85
Pair 9	TH & Mg^{+2}	0.916	9.973
Pair 10	TH & Cl^-	0.87	10.091
Pair 11	TH & TDS	0.993	8.653
Pair 12	TH & Fluoride	0.912	9.516
Pair 13	Ca^{+2} & TDS	0.816	8.711
Pair 14	Mg^{+2} & Cl^-	0.872	2.175
Pair 15	Mg^{+2} & TDS	0.889	9.179
Pair 16	Mg^{+2} & SS	- 0.935	3.261
Pair 17	Mg^{+2} & Fluoride	0.977	6.239
Pair 18	Cl^- & TDS	0.877	9.256
Pair 19	Cl^- & OC	0.985	4.639
Pair 20	Cl^- & SS	- 0.923	3.348
Pair 21	TDS & OC	0.827	9.07
Pair 22	TDS & SS	- 0.971	3.049
Pair 23	TDS & Fluoride	0.89	9.051
Pair 24	OC & SS	- 0.894	3.817
Pair 25	S.S & Fluoride	- 0.928	4.016

3. Results and Discussion:

The observed pH value ranging from 8.4 to 8.9 show that the present water samples are slightly alkaline in pre-monsoon season. These values are within maximum permissible limit prescribed by WHO.(www.lenntech.com/drinking-water-standards.htm.) Other parameters like turbidity (18 - 470 NTU), TH (122 - 212 mg/l), Fe contents (0.2 - 0.8 mg/l), Chloride (7- 26 mg/l), TDS (256 - 500 mg/l),The Ca^{2+}(28 - 48 mg/l, SO_4^{-2} (50 – 91 mg/l), NO_3^-(0-1.772 mg/l), F^{-1} (0-0.4 mg/l), TA (13 -246 mg/l) , Mg^{+2} (9.23-26.24 mg/), OC (2.4-7.8 mg/l), SS (70 -280 mg/l) are found within the highest desirable or maximum permissible limit set by WHO.(Trivedi and Goel., 1986) However, turbidity and Fe contents are observed to be on higher side in all seasons and pre-monsoon and monsoon seasons respectively.

In the present study for the year 2008, pH shows significant positive correlation with OC (r = 0.821, t = 4.762). A significant positive correlation was found between total alkalinity and total hardness (r = 0.97, t = 4.694), Ca^{2+}(r= 0.821, t = 9.145), Magnesium(r = 0.851, t = 10.179), Cl^- (r = 0.91, t =10.511), TDS(r= 0.988, t = 8.233), OC(r = 0.88, t = 9.942) and fluoride(r= 0.836, t = 9.85) This shows that with increase or decrease in the values of TA ; TH , Ca^{2+}, Mg^{2+}, Cl^-, total dissolved solids, OC and F^- also exhibit decrease or increase in their values.

Total hardness bears positive correlation with Mg^{2+} (r = 0.916, t = 9.973), Cl^- (r = 0.87, t =10.091), TDS (r = 0.993, t = 8.653), and fluoride (r = 0.912, t = 9.516). It is suggested that total hardness of water samples is mainly due to the presence of the $MgCl_2$ and Magnesium fluoride (Bhoi,2005) Sulphate and Nitrate ions bear negative correlation with Ca^{+2} indicating that Calcium sulphate and Calcium nitrate may be absent in water samples. NO_3^- shows negative correlation with Mg^{+2} which indicates that $Mg(NO_3)_2$ may be absent in water samples. Similarly Fe also shows negative correlation with Cl^{-1} and SO_4^{-2} implying iron chloride and iron sulphate may be absent in water samples.

Chloride ion bears significant positive correlation with TDS (r = 0.877, t = 9.256), Mg^{2+}, (r = 0.872, t = 2.175), OC (r = 0.985, t = 4.639). It reveals that Mg^{2+} mainly remains present as $MgCl_2$. Chloride ion showed negative significant correlation with SS (r = - 0.923, t = 3.348) so with increase or decrease in the values of chloride ion, the values of TDS, Mg^{2+} and

OC increases or decreases, while suspended solids decreases or increases with increasing or decreasing n values of chloride ion.
A significant positive correlation was found between Ca^{2+} and TDS (r=0.816, t = 8.711). The magnesium content of Ganga water increases or decreases with increase or decrease in the value of TDS (r = 0.889, t= 9.179) and fluoride(r = 0.977, t = 6.239) as it shows significant positive correlation with these parameters respectively
Total dissolved solid shows significant positive correlation with OC(r = 0.827, t = 9.07) and fluoride (r = 0.89, t = 9.051), TDS shows significant negative correlation with SS (r = - 0.971, t = 3.049).SS shows significant negative correlation with OC(r = - 0.894, t = 3.817) and fluoride(r = - 0.928, t = 4.016).

4.Conclusions

A large number of factors and geological conditions influence the correlations between different pairs of physico - chemical parameters of water samples directly or indirectly. All the physico-chemical parameters of Ganga river water at Kanpur for pre monsoon, monsoon and post monsoon for year 2008 are within the highest desirable limit or maximum permissible limit prescribed by WHO except turbidity, Fe contents and pH which recorded high values in all seasons, pre monsoon and monsoon season and pre monsoon season respectively .
Ganga water recorded higher values of Ca^{2+} than Mg^{2+} in all three seasons An appreciable significant positive correlation have been found for TA with TH, Ca^{+2}, Mg^{+2}, Cl^{-}, TDS, OC and F^{-}. and Cl^{-1} with Mg^{2+}, TDS, OC and TH. Total hardness has significant positive correlation with Mg^{+2}, Cl^{-1} and F^{-1} and TDS with Mg^{+2} and F^{-1} . SS show significant negative correlation with Mg^{+2} , TDS,. Cl^{-1}, OC and F^{-1} for all three seasons for year 2008
From the results of present study we conclude that Ganga water of Kanpur is though fit for drinking purposes yet it need treatment to minimize the contamination especially turbidity and Fe contents . To minimize the contaminations of Ganga river water at Kanpur the values of correlation coefficients and their significance level will help in selecting the proper experimental methods used for treatment of water.To create increasing awareness among the people to maintain the Ganga river water at its highest quality and purity levels, the present study may prove to be useful in achieving this goal.

Acknowledgment:
ST is grateful to Dr. Pervez E. Deen, Principal Christ Church College, Kanpur for his encouragement throughout the work.

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Date of Submission: 30.8.2009

The impact of farm size on energy use and profitability of red bean production in Iran: A case study in Kurdistan province

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Abstract: In this paper, the red bean production in Kurdistan, Iran and the energy equivalences of input used in this production are investigated. The data were collected through a survey study by using a face-to-face questionnaire performed in April 2009. The aims of this study were to determine the amount of input–output energy used in red bean production, to investigate the efficiency of energy consumption, and to make an economic analysis of red bean production. We classified the land size into 3 types in this study. Type 1 (T1) is 0.1 ha, type 2 (T2) is 0.2 ha, and type 3 (T3) is 0.5 ha. We determine the energy use, productivity and profitability in the 3 types of land size to find out if they have significant difference or not. The following results were obtained at the end of the study: Irrigation energy engrossed 82.68%, 63%, and 55.41 of total energy in T1, T2, and T3 land types respectively, followed by chemical fertilizer (12.64%, 28.1%, and 35.72% respectively) during production period. The highest energy efficiency pertained T3 and it was 0.44, and its energy productivity was 0.03 in the study area. The economic analysis showed that the best profit-cost ratios of the farms pertained T3 and it was 0.97. Its net returns calculated were -228 $ ha^{-1} in the farms investigated. [Nature and Science. 2009;7(9):95-104]. (ISSN: 1545-0740).

Key words: Economic analysis, energy efficiency, Iran, Kurdistan, Land size, Red bean

1. Introduction

Red bean is indigenous to China and is traditionally used as soup ingredients for therapeutic purposes such as ameliorating symptoms of dropsy, relieving diarrhea, and as a tonic for viscera (Li, 1973). Recent studies have shown that red bean flour has functional properties comparable to the widely studied and used soybean flour (Chau and Cheung, 1998a). Protein is a major component of red bean constituting to 30.2% (dry weight) of the cotyledon and 25.2% of the whole seeds, and the salt-soluble globulin constitutes almost half of the total proteins extracted by Osborne fractionation. It was also reported that red bean protein had high level of all the essential amino acids comparing with the FAO/WHO requirement (Chau et al., 1998b).

In developing countries like Iran, agricultural growth is essential for fostering the economic development and meeting the ever-higher demands of the growing population. Energy in agriculture is important in terms of crop production and agro processing for value adding (Karimi et al., 2008). The relation between agriculture and energy is very close. Agriculture itself is an energy user and energy supplier in the form of bio-energy. At present, productivity and profitability of agriculture depends on energy consumption (Alam et al., 2005).

Energy use in agriculture has been developed in response to increasing populations, limited supply of arable land and desire for an increasing standard of living. In all societies, these factors have encouraged an increase in energy inputs to maximize yields, minimize labor-intensive practices or both (Esengun et al., 2007).

Energy in one form or another is a crucial input to agricultural production. Continually rising prices, increasing proportion of commercial energy in the total energy input to agriculture and the growing scarcity of commercial energy sources, such as fossil fuels, have necessitated the more efficient use of these sources for different crops (Singh et al., 1999).

Agriculture is both a producer and consumer of energy. It uses large quantities of locally available non-commercial energies, such as seed, manure and animate energy, and commercial energies directly and indirectly in the form of diesel, electricity, fertilizer, plant protection, chemicals, irrigation water, machinery and etc. Efficient use of these energies helps to achieve increased production and productivity and contributes to economy, profitability and competitiveness of agriculture sustainability to rural living (Singh et al., 2002).

The importance of sustaining agricultural

production to improve nutritional standards has been recognized by all countries throughout history. However, in the economic literature of the 1950s and 1960s the role of agriculture in development was considered ancillary to that of the modern industrial sector where most of the accumulation and growth was expected to take place. Subsequent theoretical investigation and the very disappointing performance of agriculture in many developing countries have led to the belief that the role of agriculture in development should be re-examined (Cornia, 1985).

Relationship between farm size and productivity in developing countries is one of the oldest issues in the academic arena for analyzing the agrarian structure (Thapa, 2007). The most frequently cited phenomenon is an inverse relation between farm size and yield per acre (Feder, 1985).

Sen explained the inverse relationship with labor dualism, where given the same technology, small-scale farmers have lower opportunity costs of their labor than operators of large farms (Sen, 1962). Deininger and Feder applied agency theory analysis on this subject. When a farm is small and labor markets are not functioning, small-scale farms use only family labor (Deininger and Feder, 2001). Hence, in the terminology of principal-agent theory, the principal and his family members supply all of the labor for the farm. These family members have a strong incentive to work because they share the farm output directly and in the long run can expect to inherit the farm. Here monitoring and incentive problems are minimal and excess family labor would push the value of the marginal product below the off-farm wage thus may result the inverse relationship (Taylor and Adelman, 2003). Bhalla and Roy and Benjamin suggested that unobserved land quality is positively related to farm productivity but inversely related to farm size, which might explain the inverse relationship between farm size and productivity as well (Bhalla and Roy, 1988; Benjamin et al., 2001).

Heltberg claimed that Bhalla and Roy's conclusions are undermined by their use of district aggregate data (Heltberg, 1998). However, using farm level data obtained in Haryana, India, Carter found a significant within-village inverse relationship between farm size and productivity (Carter, 1984).

The majority of studies of agricultural productivity in developing countries support the view that there is an inverse relationship between productivity and farm size (Berry and Cline, 1979; Barrett, 1996). If correct, land reform could contribute to improving both equity and efficiency in agriculture. Most of these studies, however, are based on partial measures of productivity such as yield which are biased in favor of small producers.

2. Materials and methods

The data were collected from 36 farmers growing red bean in Kurdistan province, Iran by using a face-to-face questionnaire in April 2009. The province is located in the west of Iran, within 34° 44´–36° 30´ north latitude and 45° 31´–48° 16´ east longitude. The total area of the Kurdistan province is 2,820,300 ha. The average rainfall of the province is 450 millimeters (Najafi, 1996). The location of Kurdistan province is shown in figure 1.

The total land area cultivated for legumes and red bean crop is 81499 and 430 ha, respectively in Kurdistan; also the total production of this crop is 11545 and 566 ton, respectively in Kurdistan. Thus about 0.05% of total legumes production in Kurdistan is obtained from red bean production (Ministry of Jihad-e-Agriculture of Iran, 2008).

We chose this area for the investigation, because according to the low yield in this province, it seems that the farmers don't use the resources in an efficient situation. Red bean is a sample for the low efficiency and there are some other crops in a same situation. If the results corroborate this hypothesis, so it would be a big alarm for the farmers and governments to use the resources in an efficient situation.

The sample size was determined using the simple random sampling method (Kizilaslan, 2009):

$$n = \frac{N * s^2 * t^2}{(N-1)d^2 + s^2 * t^2} \qquad (1)$$

In which n is the required sample size, s is the standard deviation, t is the t value at 95% confidence limit (1.96), N is the number of holding in target population and d is the acceptable error (permissible error 5%).

For the growth and development, energy demand in agriculture can be divided into direct and indirect, renewable and non-renewable energies (Alam et al., 2005). The energy efficiency of the agricultural system has been evaluated by the energy ratio between output and input. Human labor, machinery, diesel oil, fertilizer, pesticides, herbicides, fungicides, and seed amounts and output yield values of red bean crops have been used to estimate the energy ratio. The amounts of input were calculated per hectare and then, these input data were

multiplied with the coefficient of energy equivalent. Energy equivalents shown in Table 1 were used for estimation.

Figure 1. The location of Kurdistan province in Iran

Table 1. Energy equivalent of inputs and outputs in agricultural production

Particulars	Unit	Energy equivalent (MJ unit^{-1})	Ref.
A. Inputs			
1. Human labor	h	1.96	(Singh and Mittal, 1992; Erdal et al., 2007)
2. Machinery	kg		
Tractor	kg	138	(Kitani, 1999)
Plow	kg	180	(Kitani, 1999)
Disk	kg	149	(Kitani, 1999)
Harrow			
3. Diesel fuel	L	56.31	(Singh and Mittal, 1992; Erdal et al., 2007)
4.Fertilizers(N)	kg	78.1	(Kitani, 1999)
5. Seeds	kg	14.7	(Kitani, 1999)
B.Outputs (Yield)	kg	14.7	(Kitani, 1999)

Basic information on energy inputs and red bean yields were entered into Excel and SPSS 17 spreadsheets. Based on the energy equivalents of the inputs and output (Table 1), the energy ratio (energy use efficiency) and energy productivity were calculated (Mandal et al., 2002; Singh et al., 1997).

$$\text{Output - input ratio} = \frac{\text{Energy output (MJ ha}^{-1})}{\text{Energy input (MJ ha}^{-1})} \quad (2)$$

$$\text{Energy productivity} = \frac{\text{Red bean output (kg ha}^{-1})}{\text{Energy input (MJ ha}^{-1})} \quad (3)$$

Indirect energy included energy embodied in seeds, fertilizers, manure, chemicals, machinery while direct energy covered human labor and diesel used in the red bean production. Non-renewable energy includes diesel, chemical, fertilizers and machinery, and renewable energy consists of human labor, seeds, and manure. In the last part of the research, economic analysis of red bean production was investigated, and net profit and benefit–cost ratio was calculated. The net return was calculated by subtracting the total cost of production from the gross value of production per hectare. The benefit–cost ratio was calculated by dividing the gross value of production by the total cost of production per hectare (Demircan et al., 2006; Ozkan et al., 2004).

We have 3 types of land size in this study. Type 1 (T1) is 0.1 ha, type 2 (T2) is 0.2 ha, and type 3 (T3) is

0.5 ha. We determine the energy use, productivity and profitability in the 3 types of land size to find out if they have significant difference or not. Differences between mean values for the various treatments were tested by Duncan method ($P < 0.05$).

3. Results and discussion

3.1 Analysis of input–output energy use in red bean production

Amounts of inputs used and output in red bean production for each item are illustrated in Table 2. Inputs used in red bean production, energy equivalences and ratios of inputs and output are illustrated in Table 3, Table 4, and Table 5. Total energy used in various farm operations during red bean production was 105540.2 MJ ha^{-1}, 47571 MJ ha^{-1}, and 43725.4 MJ ha^{-1} in T1, T2, and T3 land types respectively. Total energy used in the first type of land size (T1) was significantly higher than the other land types at the 5% level.

Table 2. Amounts of inputs and output in red bean production

	T1 land type	T2 land type	T3 land type
Inputs	Quantity per unit area (ha)	Quantity per unit area (ha)	Quantity per unit area (ha)
Labor (h ha^{-1})	803.77	451.72	369.66
Land preparation	5.3	5.16	4.29
Planting	79.76	21.73	40.67
Irrigation	226.67	103.27	67.14
Fertilizer application	3.54	2.24	3.71
Harvesting	299.76	163.07	141.71
Threshing	173.21	145.96	108
Transporting	15.53	10.29	4.14
Machinery (h ha^{-1})	6.26	6.37	5.15
Land preparation	5.3	5.16	4.29
Transporting	0.96	1.21	0.86
Diesel (L ha^{-1})	37.81	37.42	30.55
Land preparation	33.87	32.82	27.2
Transporting	3.94	4.6	3.35
Fertilizers (kg ha^{-1})	170.83	171.15	200
Nitrogen (N)	170.83	171.15	200
Seeds	60.08	61.92	80.71
Output			
Red bean yield (kg ha^{-1})	1275	1344.23	1307.14

According to the evaluation of data in Table 2, the average human labor required in the study area was 803.77 h ha^{-1}, 451.72 h ha^{-1}, and 369.66 h ha^{-1} in T1, T2, and T3 land types respectively, and machine power was 6.26 h ha^{-1}, 6.37 h ha^{-1}, and 5.15 h ha^{-1} respectively. Almost 37%, 36%, and 38% of total human labor, in the land types respectively, was required for harvesting, because in the study area the harvesting operation was done only by human labor without using machinery. About 85%, 81%, and 83% of machine power, in the land types respectively, was consumed for land preparation, and 15%, 19%, and 17%, in the land types respectively, was for transporting the harvested red bean. The distribution of the energy input ratios in the red bean production are given in figure 2.

Table 3. Amounts of inputs and output in red bean production in type T1 land size

Inputs & output	Quantity per unit area (ha)	Total energy equivalent (MJ ha^{-1})	Percentage
A. Inputs			

1. Human labor (h)	803.77	1575.4	1.49
2. Machinery (h)	6.26	354.7	0.34
3. Diesel fuel (L)	37.81	2129.1	2.02
4. Chemical fertilizers (kg)			
Nitrogen (N)	170.83	13341.8	12.64
5. Seeds (red bean) (kg)	60.08	883.2	0.84
6. Irrigation (m^3)	14388	87256	82.68
Total energy input (MJ)		105540.2	100
B. Output			
1. Red bean (kg)	1275	18742.5	
Total energy output (MJ)		18742.5	
Output-input ratio		0.18	
Energy productivity (kg MJ^{-1})		0.01	

Table 4. Amounts of inputs and output in red bean production in type T2 land size

Inputs & output	**Quantity per unit area (ha)**	**Total energy equivalent (MJ ha^{-1})**	**Percentage**
A. Inputs			
1. Human labor (h)	451.72	885.4	1.86
2. Machinery (h)	6.37	333.8	0.7
3. Diesel fuel (L)	37.42	2107.1	4.43
4. Chemical fertilizers (kg)			
Nitrogen (N)	171.15	13366.8	28.1
5. Seeds (red bean) (kg)	61.92	910.2	1.91
6. Irrigation (m^3)	4528	29967.7	63
Total energy input (MJ)		47571	100
B. Output			
1. Red bean (kg)	1344.2	19759.7	
Total energy output (MJ)		19759.7	
Output-input ratio		0.42	
Energy productivity (kg MJ^{-1})		0.03	

Table 5. Amounts of inputs and output in red bean production in type T3 land size

Inputs & output	**Quantity per unit area (ha)**	**Total energy equivalent (MJ ha^{-1})**	**Percentage**
A. Inputs			
1. Human labor (h)	369.66	724.5	1.66
2. Machinery (h)	5.15	244.7	0.56
3. Diesel fuel (L)	30.55	1720.3	3.93
4. Chemical fertilizers (kg)			
Nitrogen (N)	200	15620	35.72
5. Seeds (red bean) (kg)	80.71	1186.4	2.71
6. Irrigation (m^3)	3186	24229.5	55.41
Total energy input (MJ)		43725.4	100
B. Output			

1. Red bean (kg)	1307.1	19214.4
Total energy output (MJ)		19214.4
Output-input ratio		0.44
Energy productivity (kg MJ^{-1})		0.03

Total energy consumed in various farm operations during red bean production in the first type of land size (T1) was 105540.2 MJ ha^{-1}. Irrigation energy consumed 82.68% of total energy followed by chemical fertilizer 12.64% during production period. Diesel energy mainly consumed for land preparation, and transportation. Total energy output was 18742.5 MJ ha^{-1}, and average annual yield of farms investigated was 1275 kg ha^{-1}. It is shown in Table 3 that machinery was the least demanding energy input for red bean production with 354.7 MJ ha^{-1} (only 0.34% of the total energy input), followed by seeds by 883.2 MJ ha^{-1} (0.84%).

Energy output-input ratio (energy use efficiency) was 0.18, and energy productivity was calculated as 0.01 in the study area. This means that 0.01 of output was obtained per unit energy.

Total energy consumed in various farm operations during red bean production in the second type of land size (T2) was 47571 MJ ha^{-1}. Irrigation energy consumed 63% of total energy followed by chemical fertilizer 28.1% during production period. Diesel energy mainly consumed for land preparation, and transportation. Total energy output was 19759.7 MJ ha^{-1}, and average annual yield of farms investigated was 1344.2 kg ha^{-1}. It is shown in Table 4 that machinery was the least demanding energy input for red bean production with 333.8 MJ ha^{-1} (only 0.7% of the total energy input), followed by human labor by 885.4 MJ ha^{-1} (1.86%).

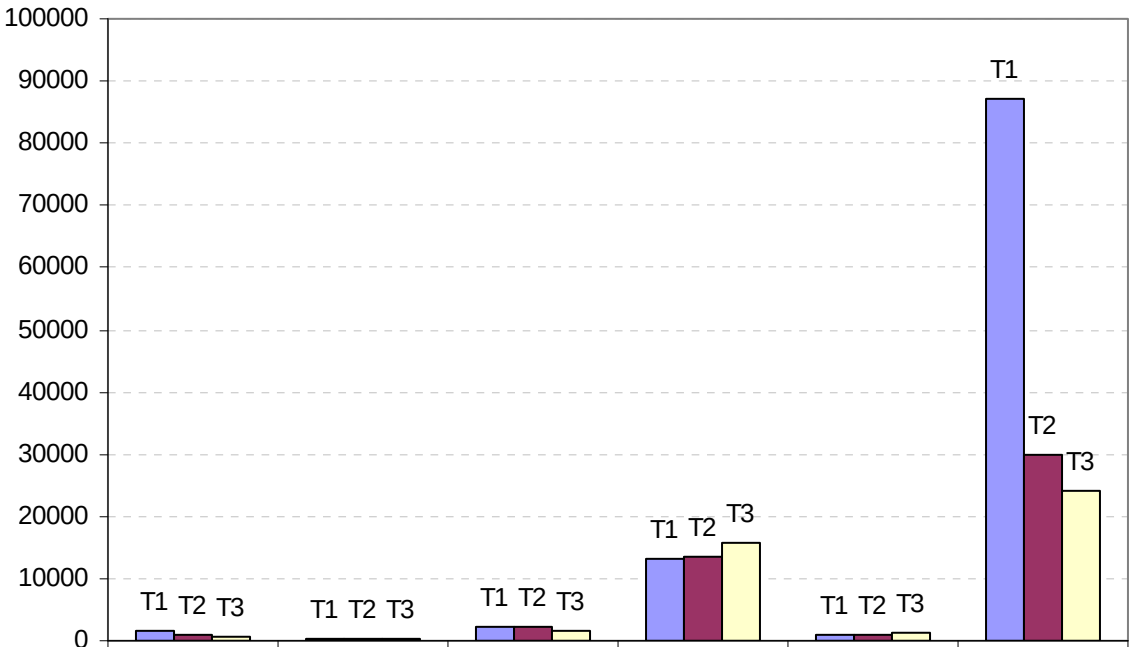
Energy output-input ratio (energy use efficiency) was 0.42, and energy productivity was calculated as 0.03 in the study area. This means that 0.03 of output was obtained per unit energy.

Total energy consumed in various farm operations during red bean production in the third type of land size (T3) was 43725.4 MJ ha^{-1}. Irrigation energy consumed 55.41% of total energy followed by chemical fertilizer 35.72% during production period. Diesel energy mainly consumed for land preparation, and transportation. Total energy output was 19214.4 MJ ha^{-1}, and average annual yield of farms investigated was 1307.1 kg ha^{-1}. It is shown in Table 5 that machinery was the least demanding energy input for red bean production with 244.7 MJ ha^{-1} (only 0.56% of the total energy input), followed by human labor by 724.5 MJ ha^{-1} (1.66%).

Energy output-input ratio (energy use efficiency) was 0.44, and energy productivity was calculated as 0.03 in the study area. This means that 0.03 of output was obtained per unit energy.

The irrigation energy used in the first type of land size (T1) was significantly higher than the other land types at the 5% level and was significantly higher than the third type of land size (T3) at the 1% level. No significant differences in yield at the 5% level by different land types were found for the red bean crops. The farmers didn't use any fungicides, pesticides, or herbicides. Overall they didn't care about crop protection and the yield is much lower than the average of the Iran.

The distribution of total energy input is shown in Table 6 as direct, indirect, renewable and non-renewable forms. As it is shown, the total energy input consumed could be classified as direct energy (3.51%, 6.3%, and 5.6% in T1, T2, and T3 land types respectively) and indirect energy (96.49%, 93.7%, and 94.4 respectively), and also renewable energy (2.33%, 3.77, and 4.4 respectively) and non-renewable energy (97.67%, 96.23%, and 95.6 respectively).



	Labor	Machinery	Diesel fuel	Nitrogen (N)	Seeds	Irrigation
T1	1575.4	354.7	2129.1	13341.8	883.2	87256
T2	885.4	333.8	2107.1	13366.8	910.2	29967.7
T3	724.5	244.7	1720.3	15620	1186.4	24229.5

Figure 2. The distribution of energy input (MJ ha^{-1}) ratios in the red bean production.

Table 6. Total energy input in the form of direct, indirect, renewable and non-renewable for red bean production (MJ ha^{-1})

	T1 land type		**T2 land type**		**T3 land type**	
Form of energy	**Quantity (MJ ha^{-1})**	**Percentage** [a]	**Quantity (MJ ha^{-1})**	**Percentage** [a]	**Quantity (MJ ha^{-1})**	**Percentage** [a]
Direct energy [b]	3704.5	3.51	2292.5	6.3	2444.8	5.6
Indirect energy [c]	101835.7	96.49	44578.5	93.7	41280.6	94.4
Renewable energy [d]	2458.6	2.33	1795.6	3.77	1910.9	4.4
Non-renewable energy [e]	103081.6	97.67	45775.4	96.23	41814.5	95.6
Total energy input	105540.2	100	47571	100	43725.4	100

[a] Indicates percentage of energy input.

[b] Includes human labor and diesel.

[c] Includes seeds, fertilizers, manure, chemicals, and machinery.

[d] Includes human labor, seeds, and manure.

[e] Includes diesel, chemical, fertilizers, and machinery.

3.2 Economic analysis of red bean production

Data obtained from economic analysis are presented in Table 7. The profit/cost ratio was found to be 0.59, 0.82, and 0.83 in T1, T2, and T3 land types respectively. The productivity in red bean production was attained as 0.53, 0.89, and 0.97 respectively. Net profit was -982.6 $ ha^{-1}, -267.6$ ha^{-1}, -228$ ha^{-1}. Net profit in the first type of land size (T1) was significantly lower than the other land types at the 5% level.

Table 7. Economic analysis of red bean production

	T1 land type	T2 land type	T3 land type
Cost and return items	Value	Value	Value
Total production costs ($ ha^{-1})	2408.4	1502.8	1342.3
Gross production value [a] ($ ha^{-1})	1425.8	1235.2	1114.3
Benefit/Cost ratio	0.59	0.82	0.83
Productivity (kg $\$^{-1}$) [b]	0.53	0.89	0.97
Net return ($ ha^{-1})	-982.6	-267.6	-228

[a] Gross production value=Red bean yield (kg ha^{-1})*Price ($ kg^{-1})

[b] Productivity (kg$-1)= Red bean yield (kg ha^{-1})/Total production costs ($ ha^{-1})

As shown in Table 7, the net return in red bean production in the studied area is negative. The reason can be due to the fact that the human labor in the region is provided by the farmer and his family and no payment is considered for the job done. In the economic analysis in this study, the human labor wage was considered as the conventional rate paid in regular agricultural operations. Therefore, in appearance, the farmer gets a false feeling of a profitable task while in reality the case is different. The outcome will influence the standard of living of the rural families involved in producing this type of crop. The economic analysis without estimating the human labor wage has shown in table 8. as it has shown, the false feeling of the farmer is obvious. Even without estimating the human labor wage, the net return in the first type of land size (T1) is negative and notwithstanding the net return in other land types is not negative, the production of this crop in the study area is not profitable.

Table 8. Economic analysis of red bean production without estimating the human labor wage

	T1 land type	T2 land type	T3 land type
Cost and return items	Value	Value	Value
Total production costs ($ ha^{-1})	1462.6	969.1	918
Gross production value [a] ($ ha^{-1})	1425.8	1235.2	1114.3
Benefit/Cost ratio	0.97	1.27	1.21
Productivity (kg $\$^{-1}$) [b]	0.87	1.39	1.42
Net return ($ ha^{-1})	-36.8	266.1	196.3

[a] Gross production value=Red bean yield (kg ha^{-1})*Price ($ kg^{-1})

[b] Productivity (kg $\$^{-1}$)= Red bean yield (kg ha^{-1})/Total production costs ($ ha^{-1})

4. Conclusions

In this study, the red bean production in Kurdistan, Iran and the energy equivalents of inputs used in this production were investigated. Irrigation energy monopolized 82.68%, 63%, and 55.41 of total

energy in T1, T2, and T3 land types respectively, followed by chemical fertilizer as (12.64%, 28.1%, and 35.72% respectively). Total energy consumption in various farm operations during red bean production was found to be 105540.2 MJ ha^{-1}, 47571 MJ ha^{-1}, and 43725.4 MJ ha^{-1} in T1, T2, and T3 land types respectively. Total energy used in the first type of land size (T1) was significantly higher than the other land types at the 5% level. No significant differences in yield at the 5% level by different land types were found in the red bean production. Total energy output attained as 18742.5 MJ ha^{-1}, 19759.7 MJ ha^{-1}, and 19214.4 MJ ha^{-1} respectively, and average annual yield was 1275 kg ha^{-1}, 1344.2 kg ha^{-1}, and 1307.1 kg ha^{-1} respectively. The highest energy use efficiency hinged T3 and was calculated as 0.44, and its energy productivity was 0.03. The machinery was the least demanding energy input in T1 for red bean production with 354.7 MJ ha^{-1} (only 0.34% of the total energy input), followed by seeds as 883.2 MJ ha^{-1} (0.84%). The total energy input consumption could be classified as direct energy (3.51%, 6.3%, and 5.6% in T1, T2, and T3 land types respectively) and indirect energy (96.49%, 93.7%, and 94.4 respectively), and also renewable energy (2.33%, 3.77, and 4.4 respectively) and non-renewable energy (97.67%, 96.23%, and 95.6 respectively). The economic analysis showed that the best profit-cost ratios of the farms hinged T3 and it was 0.97. Its net returns calculated were -228 \$ ha^{-1} in the farms investigated. Net profit in the first type of land size (T1) was significantly lower than the other land types at the 5% level. The net return in red bean production in the studied area was negative due to not considering any labor costs for family works. Without estimating the human labor wage, the highest net returns hinged T2 and calculated as 266.1 \$ ha^{-1}.

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