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Cover: The crest of St. Xavier's College Mumbai., Designed in 1929 by Fr. T. Molina. Shows an eagle teaching its young ones to fly. Above it, on the left, is the emblem of the Society of Jesus which consists of the Greek initials of the name of Jesus set in a sun; on the right is a chequered moon, taken from the arms of the house of Xavier. The motto in Latin is taken from the bible and refers to the eagle who encourages (its young ones) to soar aloft.

From the Managing Editor's desk

The sixth volume, and the first as a separate science edition, this issue of 'Xplore – The Xavier's Research Journal' is in the truest sense a peregrination within the biological, chemical, earth and physical sciences. With research articles covering almost all the prominent silos of sciences and attempting to bridge them through applied research, this issue contains nine research papers and one review article.

The positive correlation between research and the unraveling of truth is irrefutable, as research re-establishes and reiterates the credibility of scholarly endeavours. Research by reigniting an individual's academic curiosity, rejuvenates the pursuit of the ultimate knowledge of a discipline.

Since its inception in 2010, Xplore has fostered collaboration among researchers from various institutions and subject domains, which is also evident in this issue. We have research articles with collaborators from university colleges, state university departments and also from international institutions. Xplore has followed a transparent and ethical system of peer review, which has helped us maintain standards. We acknowledge the efforts of the panel of peer reviewers for their contribution in enhancing the quality of these articles. We are also grateful for the financial support extended by the University Grants Commission (UGC) for the publication of this research journal as well as for the support given towards the academic enrichment of our faculty under the 'College of Excellence (CE) Scheme'.

Dr. Agnelo Menezes
Managing Editor : Xplore - The Xavier's Research Journal.
And
Principal
St. Xavier's College (Autonomous), Mumbai.

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Review Article

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Phenological and ethnobotanical data extraction from herbarium specimens: A case study on Mumbai and Thane Using Blatter Herbarium

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Abstract:

This paper lists a short communication on first-hand information of a few phenological and ethnobotanical notes recorded on the labels of the herbarium sheets and highlights the importance of label information. The authors consulted Blatter Herbarium (BLAT) which is located in St. Xavier's College, Mumbai, India. The aim of the consultation was to study collections from Thane District so that an effective field trip to collect plant specimens could be planned in the near future based on the phenology information from the herbarium sheets and secondly to check how many specimens had ethnobotanical information recorded on them.

The authors have accounted ethnomedicinal uses mentioned on the herbarium sheets and have studied the collections mainly from Mumbai and Thane focusing on a few medicinally important plant species used to treat asthma, cold, cough and fever. The authors have databased and imaged the herbarium specimens. In all 328 specimens have been databased in Excel and Botanical Resource Herbarium Management System (BRAHMS) and are listed under results.

Keywords: Ethnobotany, Herbarium, Phenology

Introduction

Phenology is the study of the times of recurring natural phenomena, especially in relation to climate. Examples include the date of emergence of leaves and flowers which vary from year to year depending upon the weather. Robert Marsham (b.27 January 1708 d. 4 September 1797), an English naturalist is the founding father of modern phenological recording. He recorded "indication of spring" on his estate at Stratton Strawless, in Norfolk from 1736. These were in the form of dates of the first occurrence of events such as flowering, bud burst, emergence or flight of an insect. Same events were recorded consistently for many years by generations of the same family eventually ending with a family member's death in 1958. His records showed that between 1850-1950 a long-term trend of gradual climate warming was observable (Sparks & Carey 1995). Phenologists record such behaviour to see longer terms trends whether these correspond with recorded changes in climate(http://data.kew.org/wild/phenology/more_info.html). In the present paper, phenology refers to the flowering and fruiting phenomena.

Ethnobotany deals with the total natural relationship of man with plants (Jain 1989). The prehistoric man used plants for food and curing ailments by trial and error. The history of herbal medicine in India dates back to the oldest documented Hindu Scriptures like Rigveda (4500 -1600BC), Atharveda (1500 B.C.), Upanishads (1000-600B.C.), Mahabharata and Puranas (700-400 B.C), Sushrut Samhita (ca 500 B.C) and Charak Samhita (ca 100 A.D.). Charak Samhita (written in Sanskrit) has the text of the teachings of Sage Punarvasu Atreya recorded about 1900 years ago and to date remains the most respected work on Ayurveda (the knowledge for long life) (Patil 2012).

Within the last fifty years, botanists have undertaken organized field work among the tribals of different areas. Botanical Survey of India (BSI) has undertaken ethnobotanical studies in various states; for example, Uttar Pradesh, Madhya Pradesh, Maharashtra, Mizoram and also Andaman & Nicobar Islands (Jain & Mudgal 1999). These states are rich in tribal communities representing more than thirty different tribes. Recorded ethnobotanical data include plants used for medicine, food, fodder, house building, fuel,

oil, seeds, narcotics, magico-religious purposes, musical instruments and veterinary medicines (Jain & Mudgal 1999). Till date, over 9500 wild plants species used by tribals have been recorded under the All India Coordinated Research project on ethnobiology involving BSI, National Botanical Research Institute (NBRI), Birbal Sahni Institute of Palaeobotany, Central Drug Research Institute (CDRI), Tropical Botanical Garden and Research Institute (TBGRI), several regional research laboratories and departments of various institutions and universities across India(Jain 1986; Jain & Mudgal 1999).

There are other agencies involved in documenting the traditional knowledge. For example, Foundation for Revitalization of Local Health Tradition (FRLHT) founded in 1993 focuses on research on medicinal plants and traditional knowledge. Traditional Knowledge Digital Library (TKDL) set up by the Government of India in 2001 serves a repository of 1200 formulations of various Indian system of medicine (ISM), such as Ayurveda, Unani and Siddha and 1500 Yoga postures (asanas) and is available in public domain in five different languages viz. English, German, French, Spanish and Japanese.

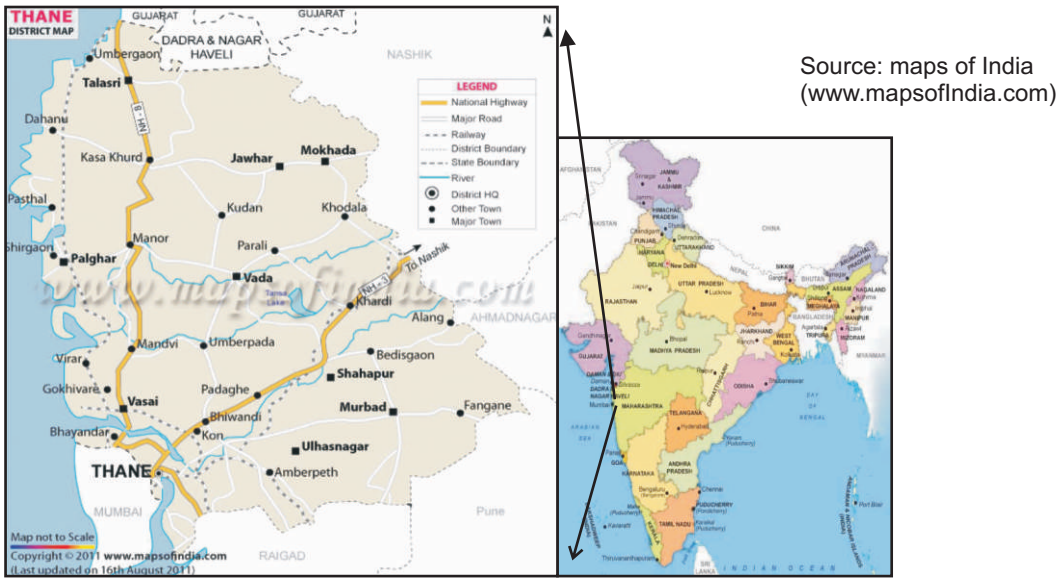
The present communication gives information on the uses of a few plant species which has been extracted from the herbarium. A herbarium (dried/pressed plant specimen preserved) serves an important useful resource and at least 72 uses have been reported by Vicky Funk (2003). The herbarium specimens are also useful to plan field trips effectively based on the flowering and fruiting information recorded on the sheets (Tiwari, 2006).

The Blatter Herbarium (BLAT) located within St. Xavier's College, Mumbai is 109 years old housing about 1,50,000 specimens of flowering plants, especially from Western Ghats of India. There are collections from other parts of India as well. The oldest specimen from the herbarium dates back to 1876. Some of the important collections are of Charles McCann, L. Sedgwick, Fr. Blatter and Fr. Santapau. There are also collections deposited by Almeida, T. Cooke, N. Dalzell and N. Wallich. The Herbarium specializes in the vascular plants of western India; algae, mosses, and fungi of Mumbai; seed samples of medicinally and economically important plants of Maharashtra and wood samples of Maharashtra.

The authors have accounted ethnomedicinal uses mentioned on the herbarium sheets and have studied the collections mainly from Mumbai focusing on a few medicinally important plant species used to treat asthma, cold, cough and fever found in Thane District.

Study area

Thane district lies between 18°42'N to 20°20'N latitudes and 72°45'E to 73°48'E longitudes. Covering an area of 9558 sq. km. is home to 2,486,941 (more than 2.4 million) people and is the most populated district of the nation (Source: As per Population Census 2001 of India). Please see Fig.1 for the location of study area.



Source: maps of India (www.mapsofIndia.com)

Fig 1. Thane District, study area

The district is rich in tribal areas and the ST (Scheduled Tribe) population is more than 50% as per population census 2011. The STs are socially disadvantaged people who are remotely located in rural areas. Some of the important tribes found in the district are Koli, Konkana, Katkari, Mahadev Koli, Thakur, K-Thakur, M-Thakur and Varli. These tribes have their own dialects and speak the Marathi language. Their folklore, culture and traditions show that they possess a unique heritage. Their main occupation is agriculture. Some of them work in the fields, in road building projects or some of them go to cities to earn their living. Generally they have poor living standards and are reliant on local resources.

Vegetation: The vegetation of Thane District area comes under Konkan Vegetation Zone and consists of tropical semi-evergreen to evergreen forests, tropical moist deciduous forests and tropical dry deciduous forests (Singh & Karthikeyan 2000, Prasad *et al* 2011). These forests abound in twiners, climbers or lianas, evergreen and deciduous species and grasses. The flora of North Mumbai is richer than that of Southern Mumbai. "Studies on the vegetation and flora of Thana District, Maharashtra state" was written by Billore (1972). The climate in this region has seasonal rainfall and has high humidity throughout the year. The average rainfall is 2576 mm. Most of the annual rainfall is received between June-September. Temperature varies from 17.5 - 34.4 degree Celsius.

Major rivers are Ulhas, Vashisthi, Kunadalika, Shastri, Urali, Gad, Ter etc. Coconut, betel nut and mangroves grow well on the banks and depressions. The coastal line is covered by mangroves like *Avicennia marina, A.officinalis* and *Rhizophora mucronata*.In sandy soils and where the coast is not muddy, plants like *Cressa cretica, Cyperus bulbosus, Pandanus tectorius* and *Tribulus terrestris* occur.

Literature Review

In India, studies in Ethnobotany were initiated by Janaki Ammal in mid-fifties who made intensive studies on the food plants of certain tribes. S.K. Jain and his associates contributed a lot of information on plants used by the tribals. These studies have had a great influence on ethnobotany which is clearly apparent from the various publications (Singh & Karthikeyan 2000).

George Watt between 1885-1894 compiled a book 'The Commercial Products of India". A corrected and abridged version was later brought out in 1908. This book contains products which are of prospective industrial or commercial importance. Some of the plants mentioned in this work are still being used in native medicine to treat cold, cough, asthma, and bronchitis, e.g. *Abrus precatorius, Acacia catechu, Adhatoda vasica, Withania somnifera, Zingiber officinale* and *Zizhypus jujuba.*

Studies on ethnobotany in the region were initiated by Malhotra & Moorty (1973). Vartak & Gadgil (1980) started the study of sacred groves and its relation to ethnobotany. Other important works dealing with ethnobotany of the region include those of Shah *et al.* (1983), Kumbhojkar *et al.* (1996), Tosh (1996) and Kulkarni & Kumbhojkar (1997).

Information about the fuel, wood, fodder, non-wood and socioeconomic plant resources used by the tribal of Western Maharashtra have been provided by Ghate (1992) and Kulkarni & Kumbhojkar (1992 a,b,c, 1993, 1996). Plants used by tribals for the medicinal purpose have been recorded by Upadhye *et al*. (1987, 1994).
Some important books of ethnobotany of Maharashtra include Ethnobotany of Nasik District (Patil & Patil 2006), Ethnobotany of Jalgaon District (Patil & Pawar 2008), Ethnobotanical studies on Korku and Pawra tribes of Satpura regions of Maharashtra (Jagtap & Deokule 2010) and Ethnobotany of Buldhana District (Patil *et al*. 2011). The plant uses in Thane district have also been recorded by Natrajan & Paulsen (2000) and Marathe & Bhaskar (2011).On reviewing the literature from the regions listed above, it is observed that the findings are based on interviewing tribals. However, it might have been much more helpful if the data collected was scientifically validated and comparative uses amongst various tribes were shown. Natarajan and Paulsen (2000) have listed 51 uses of plants and tried to fill the gap by substantiating some of the claims by incorporating information from the literature on the bioactive compounds.

One of the important resource book, Indian Materia Medica (Nadkarni 2009) lists over 2000 drugs. The book "Tribal medicine" by Marie D'Souza (1998) lists 150 plants used in traditional medicine. It mentions the plants and parts used for medicines, the

preparation/dosage and also acknowledges the informant/healer. Plants used to treat cold, cough, asthma mentioned in this book are: *Solanum xanthocarpum, Carica papaya, Terminalia belerica, Adhatoda vasica, Achyranthes aspera, Abrus precatorius, Cymbopogon citratus, Ocimum sanctum, Aloe barbadensis, Rungia repens, Solanum verbascifolium, Piper nigrum* and *Zizhypus oenoplia*. This book is a good comparative study of plants used for diseases and their preparation methods/dosage in another district - Ahmednagar of Maharashtra. Some of these plants used to treat diseases and preparation methods/dosage are different in tribal areas of Thane District. This book is available in 2 languages: English and Marathi and is a good source of information in terms of enhancing the knowledge on traditional uses of plants.

Dictionary of specific epithets (Almeida & Almeida 2008) is a good attempt to check the Sanskrit and local names of the plants and their botanical equivalents in Maharashtra. Handbook of diseases and their remedies in Maharashtra (part I & II) by Almeida & Almeida (2010) lists diseases, botanical names and parts used.

Materials and Methods

Blatter Herbarium (BLAT) has collectors index with their collection number and families. One can easily locate these collections in the cupboards which are arranged as per families and then genera and species. The species folders have sheets which have accession numbers arranged numerically in descending order.

An initial list of plants to be studied was made from Cooke's Flora (1901-1908), Flora of Maharashtra State (Almeida 1996-2009, Singh & Karthikeyan 2000, Singh et al 2001), The Commercial Products of India (Watt 1896, 1908) and various online search engines on medicinal plants on the internet (http://www.ncbi.nlm.nih.gov/pubmed).

Each entry consists of the accession number, scientific name of the plant followed by collector number, collector name, a place where the plant was collected, collection date, phenology (flowering and fruiting) information and remarks. The plant names were checked for latest accepted taxonomy and the correct authority (www.ipni.org;www.tropicos.org). The authors have databased and imaged the herbarium specimens (Please see Fig. 2) especially found in Thane District focusing the above-mentioned diseases found in Mumbai region. These 328 specimens have been databased in Excel and Botanical Resource Herbarium Management System (BRAHMS) and are listed under **Results** as **Table 1**. Ethnobotanical records have been highlighted in bold. 'Economic Botany Data Collections Standard' (Cook 1995) was also consulted in databasing the information from herbarium sheets. A few of these species where no herbarium specimens from Thane District were available have not been data based.

Based on the specimens phenology information (see Graph 1) an effective field trip is being planned in the near future.

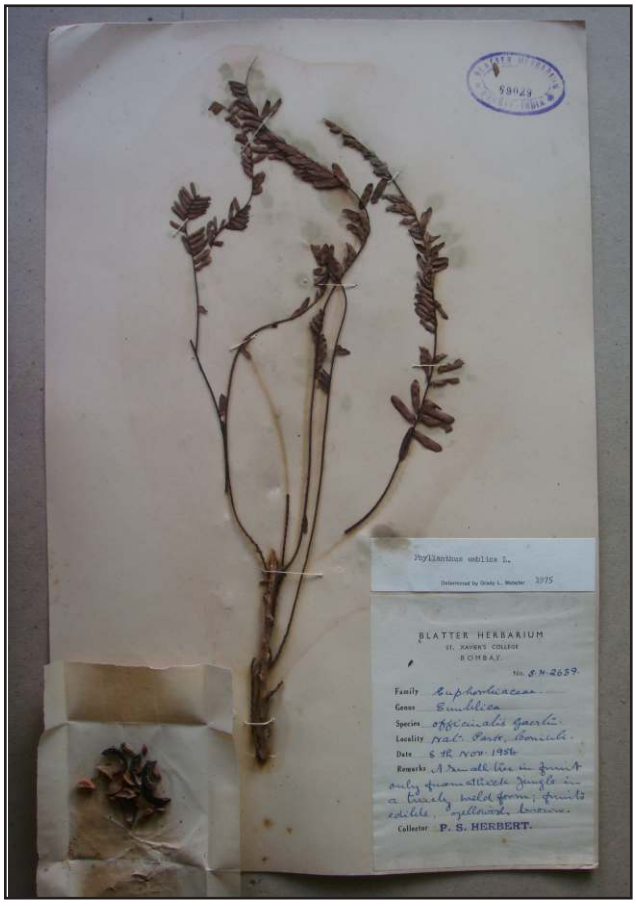
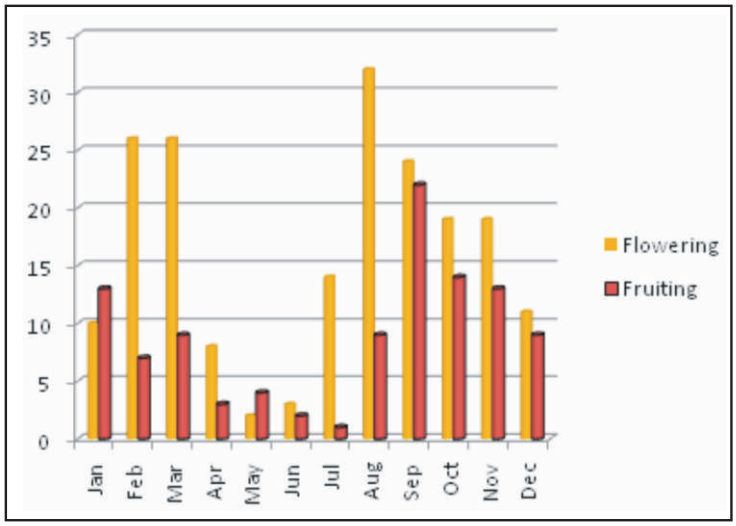


Fig. 2. Herbarium sheet
(Source: Blatter Herbarium (BLAT)

Graph 1. Phenology information



(Source: herbarium specimens, BLAT)

Discussion and Conclusion.

Although the specimens studied are well known for their uses and there is nothing new to report, it is worth mentioning that hardly any labels from the specimens mention the ethnobotanical/ethnomedicinal uses. Of the total 328 specimens studied, only 11 specimens had some ethnobotanical information. "It is estimated that less than 1% of herbarium labels contain any ethnobotanical information. None the less, it is desirable that this information is made as widely available as possible" (Vickery 1990). In future, it will be worth to validate these findings with field visits and documenting the ethnobotanical notes in detail and this study also shows how documentation of field notes can be improved on. Due to rapid changes in modernization and rural areas becoming modernized at a fast pace, in few years time- there is a danger of losing this knowledge and hence it is crucial that field botanists record the labels in greater depth e.g. the exact usage/dosage/preparation of plant uses elaborated and the names of the informants must also be recorded (Jain & Mudgal, 1999). This way the knowledge will be documented and conserved for future generations. The information collected / data based can also be validated with ethnopharmacological studies so as to find the bioactive compounds thereby leading to drug inventions. However, intellectual rights must always be retained with the tribal/indigenous people and undue advantage of traditional knowledge needs to be discouraged. Efforts in this direction are being implemented by the National Biodiversity Authority (NBA) India, and Bijoy's Kani Model (2007) is a good case study on access and benefit sharing. Following the study of specimens at Blatter Herbarium, an effective field trip in collaboration with Blatter Herbarium is being planned in the near future and this shows that herbarium resource especially the data from the labels provide a verygood source of information e.g. the phenology data is used to plan the itinerary for field trip in the near future.

Acknowedgements:

We are thankful to the Principal, St. Xavier's College and Dr. Mrs. Ujwala Bapat, Director - Blatter Herbarium and Head, Department of Botany, St. Xavier's College for providing the facilities at the Blatter Herbarium. We are also thankful to Mr. Nitin Lokhande for helping to extract the plant specimens from the herbarium cabinets for our study and laying them back in cabinets.

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Results: Table 1. Ethnobotanical notes (highlighted in bold) recorded from herbarium sheets at Blatter Herbarium (BLAT), Mumbai

Accession No	Family	Genus	Species	Collector No	Collector Name	Locality	Date	Phenology (Fl & Fr)	Remarks
54812	Acanthaceae	*Acanthus*	*ilicifolius* L.	18056	H. Santapau, S.J.	Mumbra	13/02/1954	fl	Rare in flower or bud, abundant in leaf. Spines on leafless stems very painful. BSI, Flora of India Project (Cal). Vidit. Debatri Panja 03.08.2007
54813	Acanthaceae	*Acanthus*	*ilicifolius* L.	29591	Blatter, E.	Versova, Salsette Island	01/06/1917	fl	(flowers only in capsule).
54814	Acanthaceae	*Acanthus*	*ilicifolius* L.	14861	Blatter, E.	Sion, Bombay	01/07/1917		BSI, Flora of India Project (Cal).
54815	Acanthaceae	*Acanthus*	*ilicifolius* L.	K.V.S.2395	Shenoy, K.V.	Mumbra	25/03/1954	fl	(fl).
54816	Acanthaceae	*Acanthus*	*ilicifolius* L.	152.4 H	D' Almeida	Ghodbunder Creek Salsette Island	30/03/1941		No notes
54817	Acanthaceae	*Acanthus*	*ilicifolius* L.	31099	Vakil, B.N.	From bunds in the creeks leading to Anacardium hillock.	01/05/1919		(sterile specimen)
54818	Acanthaceae	*Acanthus*	*ilicifolius* L.	11020	H. Santapau, S.J.	Mumbra-Parsik Hill	11/06/1950	fl	(fl)
54819	Acanthaceae	*Acanthus*	*ilicifolius* L.	152.72	H. Santapau, S.J.	Versova Juhu Shores	12/04/1942	fl & fr	(flowers& fruits).
54820	Acanthaceae	*Acanthus*	*ilicifolius* L.	152.6 H	H. Santapau, S.J.	Versova	01/05/1941	fl	Flowers blue. In marshy swamps
54823	Acanthaceae	*Acanthus*	*ilicifolius* L.	K.V.S.33	Shenoy, K.V.	Mumbra	07/07/1953	fl	(fl)
54824	Acanthaceae	*Acanthus*	*ilicifolius* L.	K.V.S.2184	Shenoy, K.V.	Mumbra	22/02/1954		A glabrous shiny shrub growing in marshy places. BSI-Flora of India Project(Cal), Vidit. Debatri Panja 03.08.2007
54840	**Acanthaceae**	***Adhatoda***	***vasica* Nees**	**K.V.S.2166**	**Shenoy, K.V.**	**Mumbra**	**18/02/1954**		**A shrub planted as a hedge plant in the village. Leaves are used as medicine against cold.**
54845	Acanthaceae	*Adhatoda*	*vasica* Nees	K.V.S.2162	Shenoy, K.V.	Mumbra	18/02/1954	fl	Cultivated as a hedge plant. Flowers white.
54846	Acanthaceae	*Adhatoda*	*vasica* Nees	K.V.S.2164	Shenoy, K.V.	Mumbra	18/02/1954	fl	Flowers white in simple racemes (spikes).
54848	Acanthaceae	*Adhatoda*	*vasica* Nees	P.D.501	Divakar, P.	Mora-Uran	14/02/1960	fl	Shrubs. Flowers white with rosy streaks. Common along hedges.
54849	Acanthaceae	*Adhatoda*	*vasica* Nees	K.V.S.5558	Shenoy, K.V.	Mumbra	06/12/1954	fl	(fl)
54851	Acanthaceae	*Adhatoda*	*vasica* Nees	S.D.20	D'souza, S.	Victoria Gardens, Bombay	23/11/1957	fl	(fl)
54852	Acanthaceae	*Adhatoda*	*vasica* Nees	K.V.S.2351	Shenoy, K.V.	Mumbra	23/12/1954	fl	(fl)
54859	Acanthaceae	*Adhatoda*	*vasica* Nees	P.D.580	Divakar, P.	Uran	17/03/1960	fl	Flowers white, common.
54860	Acanthaceae	*Adhatoda*	*vasica* Nees	PD 5661	Divakar, P.	Uran	20/12/1960	fl	Flowers white, common.
54861	Acanthaceae	*Adhatoda*	*vasica* Nees	Shah 1667	Shah, G.L.	Malad Ghodbunder Road	19/02/1955	fl	Flowers white.

Accession No	Family	Genus	Species	Collector No	Collector Name	Locality	Date	Phenology (Fl & Fr)	Remarks
(No acc. No)	**Acanthaceae**	***Adhatoda***	***vasica* Nees**	**60**	**Coutinho, S.**	**Aarey Milk Colony**	**01/11/1998**	**fl**	**Erect shrub. Leaves simple, flowers white, leaves and roots remedy for cough and cold. Dye is obtained from leaves.**
30361	Apiaceae(Umbelliferae)	*Centella*	*asiatica*(L.)Urb.	14142		St. Xavier's College, Bombay		fr	(fr)
30362	Apiaceae(Umbelliferae)	*Centella*	*asiatica*(L.)Urb.	14138		Bombay Island		fl & fr	(fl & fr)
30369	Apiaceae(Umbelliferae)	*Centella*	*asiatica*(L.)Urb.	PD 2685	Divakar, P.	Mora-Uran	03/10/1961	fl	Creeping herb. Flowers pinkish. Common in moist shady places.
30695	Apiaceae(Umbelliferae)	*Centella*	*asiatica*(L.)Urb.	s.n.	H.Santapau, S.J.	St. Xavier's College, Bombay	30/01/1953	fr	(fruiting)
30772	Apiaceae(Umbelliferae)	*Centella*	*asiatica*(L.)Urb.	s.n.	H.Santapau, S.J.	St. Xavier's College, Bombay	30/01/1953	fr	(fruiting)
No acc. No	**Asteraceae**	***Artemisia***	***nilagirica*(C.B.Clarke)Pamp.**	**85**	**Ms. Coutinho, S.**	**Kalsubai, Igatpuri**	**22/12/1996**	**fl**	**A shrubby plant. Stem is ribbed. Flowers small. Plant has stomachic properties. Medicinally important plant. Plant flowering.**
No acc. No	**Asteraceae**	***Artemisia***	***nilagirica*(C.B.Clarke)Pamp.**	**KMM1870**	**Mathew, K.M, S.J.**	**Kodaikanal**	**17/01/1960**	**fl**	**A hedge plant. The oil is extracted from the leaves by steam distillation.**
36399	Asteraceae	*Elephantopus*	*scaber* L.	K.V.S.2425	Shenoy, K.V.	Mumbra	25/03/1954	fl	flowering
36400	Asteraceae	*Elephantopus*	*scaber* L.	K.V.S.1622	Shenoy, K.V.	Mumbra	10/12/1953	fl	flowering
36401	Asteraceae	*Elephantopus*	*scaber* L.	K.V.S.1621	Shenoy, K.V.	Mumbra	10/11/1953	fl	(Lots of specimens by K.V.Shenoy in Mumbra locality), flowering.
36402	Asteraceae	*Elephantopus*	*scaber* L.	K.V.S.1242	Shenoy, K.V.	Mumbra	08/11/1953	fl	(flowering)
36403	Asteraceae	*Elephantopus*	*scaber* L.	K.V.S.1240	Shenoy, K.V.	Mumbra	08/11/1953	fl	(flowering)
36404	Asteraceae	*Elephantopus*	*scaber* L.	K.V.S.1241	Shenoy, K.V.	Mumbra	08/11/1953	fl	(flowering)
36405	Asteraceae	*Elephantopus*	*scaber* L.	K.V.S.993	Shenoy, K.V.	Mumbra	24/10/1953	fl	(flowering)
36406	Asteraceae	*Elephantopus*	*scaber* L.	k.V.S.986	Shenoy, K.V.	Mumbra	24/10/1953	fl	(flowering)
36408	Asteraceae	*Elephantopus*	*scaber* L.	14444	Blatter, E.	Matunga, Bombay(Mumbai)	01/11/1916	fl	(flowering)
36411	Asteraceae	*Elephantopus*	*scaber* L.	s.n.	Blatter, E.	Ghodbunder	01/11/1924	fl	Flowers red-purple.
36412	Asteraceae	*Elephantopus*	*scaber* L.	A.R.412	Randeria, A.J.	National Park, Borivali	27/09/1952		No notes
36413	Asteraceae	*Elephantopus*	*scaber* L.	A.R.566	Randeria, A.J.	National Park, Borivali	14/11/1953		No notes
36418	Asteraceae	*Elephantopus*	*scaber* L.	s.n.	Savant, B.G.	Thane	23/08/1971	fl	Voucher specimen for antifertility experiments from Dr. M.H.Shah, Chemotherapy Dept, Haffkine Inst. Mumbai-12. (An erect plant, corolla violet, typical shade loving plant, common in shady places).

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Accession No	Family	Genus	Species	Collector No	Collector Name	Locality	Date	Phenology (Fl & Fr)	Remarks
36419	Asteraceae	*Elephantopus*	*scaber* L.	s.n.	Savant, B.G.	Thane	23/08/1971	fl	Voucher specimen for antifertility experiments from Dr. M.H.Shah, Chemotherapy Dept, Haffkine Inst. Mumbai-12. (An erect plant, corolla violet, typical shade loving plant, common in shady places).
36423	Asteraceae	*Elephantopus*	*scaber* L.	21099	Blatter, E.	Igatpuri	01/09/1917	fl	Flowers purple.
106258	Asteraceae	*Elephantopus*	*scaber* L.	SMA 3028	Almeida, S.M.	Otavana	14/09/1980		A common plant in shady undergrowth in forest areas.
54429	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	CS 6885	Saldanha, C.S.J.	Varwada, Talasari, Thane Dist.	30/08/1961	fr	Several trees in a forest near the top of the hill. (fr).
54430	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	CS 6884	Saldanha, C.S.J.	Varwada, Talasari, Thane Dist.	30/08/1961	fr	Several trees in a forest near the top of the hill. (fr).
54431	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	CS 6885-A	Saldanha, C.S.J.	Varwada, Talasari, Thane Dist.	30/08/1961		Several trees in the forest on top of the hill. Part of the large compound leaf.
54432	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	NYD 3558	Das, N.Y.	Chandip(Tungar)	06/08/1961		A small slender tree with large pinnate leaf (a portion of the alrge terminal leaf mounted here). Corolla pruplish, common at plains.
54433	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	CS 6884-A	Saldanha, C.S.J.	Varwada, Talasari, Thane Dist.	30/08/1961		Several of these trees in the forest.
54434	Bignoniaceae	*Oroxylum*	*indicum* (L.)Kurz	NYD 3558	Das, N.Y.	Chandip(Tungar)	06/08/1961	fl	A small slender tree with large pinnate leaf (here no flowers; stalk is mounted here), corolla purplish, common at the plaings.
54435	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	NYD 3560	Das, N.Y.	Chandip (Tungar)	06/08/1961	fl	(flowers only)
54437	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	NYD 3561D	Das, N.Y.	Chandip (Tungar)	06/08/1961		(leaves only)
54438	Bignoniaceae	*Oroxylum*	*indicum* (L.)Kurz	NYD 216	Das, N.Y.	Shivansai(Tungar)	16/08/1959		(sterile specimen)
54438	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	2348	H. Santapau, S.J.	Borivili Kanheri Caves, Salsette	08/08/1943	fl & fr	Young fruits and flowers.
54441	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	NYD 218	Das, N.Y.	Shivansai(Tungar)	16/08/1959	fl	(fl only)
54443	Bignoniaceae	*Oroxylum*	*indicum* (L.)Kurz	22573	E.B.	Karjat, Colaba Dist	01/03/1917	fr	Poisoned 18.3.1941. (Fruit only and seeds).
54445	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	2348	H.Santapau, S.J.	Borivili Kanheri Caves, Salsette	08/08/1943	fl & fr	Young fruits and flowers.
54449	Bignoniaceae	*Oroxylum*	*indicum* (L.)Kurz	A.R.364	Randeira, G.L.	National Park, Borivali	26/08/1952	fl	(flower only)
54450	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	T2092	Tavakari, S.C.	Central Dairy to U& and behind it	18/11/1958	fl & fr	Tree in fl. Buds, fls and fruits, tree about from 10'-18'tall; rare.
54451	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	T1771	Tavakari, S.C.	Behind U30 back to U7 Aarey Milk Colony, Goregaon, Bombay	17/09/1958	fl	Tree about 25 feet tall inleaves and flowers, flowers red; fairly common.
54452	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	T2091	Tavakari, S.C.	Central Dairy to U7 and behid it, Aarey Milk Colongy, Goregaon	18/11/1958	fl & fr	Tree in fl. Buds and flowers and fruits, tree about from 10'-18' tall, rare.

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54453	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	22604	H. Santapau, S.J.	Kanheri Caves, Salsette	00/08/1917	fl	Cream, turning purple after falling off.
54454	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	150.2H	H. Santapau, S.J.	Talasari, Thana Dist.	00/05/1941	fr	Seeds only and leaves.
54456	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	A.R. 612	Randeira, G.L.	National Park, Borivili	03/03/1954	fr	With dehisced capsules
54457	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	K.V.S.4099	Shenoy, K.V.	Mumbra	26/08/1954		Leaves compound, often more than 1m in length
54458	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	K.V.S.4165	Shenoy, K.V.	Mumbra	26/08/1954		(leaves only)
54459	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	K.V.S.4100	Shenoy, K.V.	Mumbra	26/08/1954	fl	(flowers only)
54460	Bignoniaceae	*Oroxylum*	*indicum*(L.)Kurz	K.V.S.4105	Shenoy, K.V.	Mumbra	26/08/1954	fl	(flowers only)
54461	Bignoniaceae	*Oroxylum*	*indicum* (L.)Kurz	K.V.S.4103	Shenoy, K.V.	Mumbra	26/08/1954	fl	(flowers only)
15419	Caesalpiniaceae	*Cassia*	*absus* L.	K.V.S.4311	Shenoy, K.V.	Mumbra	08/09/1954		No notes
15433	Caesalpiniaceae	*Cassia*	*absus* L.	T1839	Tavakari, S.C.	Central Dairy to U.5	25/09/1958	fl	Herb from 1'-2" tall in flowers, fruits and leaves, flowers yellow. Fairly common and abundant.
15434	Caesalpiniaceae	*Cassia*	*absus* L.	S.H.1905	Herbert, P.S.	National Park, Borivili	04/08/1956	fl	Flowers in short racemes, fruits densely hairy when young.
15435	Caesalpiniaceae	*Cassia*	*absus* L.	Bole 1365	Bole, P.V.	Kasara-Igatpuri	03/09/1955	fr	(Fruiting), densely hairy.
15436	Caesalpiniaceae	*Cassia*	*absus* L.	T160	Tavakari, S.C.	Sakainaka to Powai Lake	16/11/1957	fl & fr	Erect herb about 2 feet tall in flowers and fruits. Flowers yellow, seeds black. Fairly common.
15438	Caesalpiniaceae	*Cassia*	*absus* L.	226	Nanda, U.	Karjat	02/10/1961	fr	(Fruiting)
15439	Caesalpiniaceae	*Cassia*	*absus* L.	Usha 262	Nanda, U.	Karjat	02/10/1961	fr	An erect herb about 40 cm high, Only in fruits.
15440	Caesalpiniaceae	*Cassia*	*absus* L.	248	Nanda, U.	Karjat	02/10/1961	fl & fr	An erect herb in flowers and fruits. Flowers pinkish yellow.
15441	Caesalpiniaceae	*Cassia*	*absus* L.	227	Nanda, U.	Karjat	02/10/1961		No notes
15442	Caesalpiniaceae	*Cassia*	*absus* L.	Usha 189	Nanda, U.	Marol	11/10/1961	fr	Erect herb up to 1.5 m high, only frts.
15443	Caesalpiniaceae	*Cassia*	*absus* L.	Usha 224	Nanda, U.	Karjat	02/10/1961	fl	A small herb about 40-60 cm high, flrs. Common, yellow.
15444	Caesalpiniaceae	*Cassia*	*absus* L.	Usha 188	Nanda, U.	Marol	11/10/1961	fr	An erect herb upto 1.5 m high, only in frts.
15445	Caesalpiniaceae	*Cassia*	*absus* L.	Usha 138	Nanda, U.	On way to Tulsi via Aarey Colony	16/09/1961	fl & fr	Small herb upto 40 cm high. In flr and frts. Fls. Yellow with pinkish tinge.
15446	Caesalpiniaceae	*Cassia*	*absus* L.	Usha 136	Nanda, U.	On way to Tulsi via Aarey Colony	16/09/1961	fl	Small herb upto 40 cm tall. In flr and fr. Fls pinkish yellow, common.
15447	Caesalpiniaceae	*Cassia*	*absus* L.	Usha 133	Nanda, U.	On way to Tulsi via Aarey Colony	16/09/1961	fl	A small herb about 40-50 cm high. In frts. Sometimes even in fls. Fls pinkish yellow, common.
15448	Caesalpiniaceae	*Cassia*	*absus* L.	Usha 132	Nanda, U.	Aarey Colony to Tulsi Lake	16/09/1961	fl & fr	Small erect herb in open places. Found both in fl& frt. Fls bright yellow with pinkish tinge.

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15449	Caesalpiniaceae	*Cassia*	*absus* L.	131	Nanda, U.	On way to Tulsi via Aarey Colony	16/09/1961	fr	(Fruiting)
15455	Caesalpiniaceae	*Cassia*	*absus* L.	S.H.833	Herbert, P.S.	National Park, Borivili	20/10/1955	fr	Common flowering period just over, fruits plenty leaflets always 2 pairs.
15459	Caesalpiniaceae	*Cassia*	*absus* L.	1253		Borivili	07/05/1905		No notes
15460	Caesalpiniaceae	*Cassia*	*absus* L.	Shah 7384	Shah, G.L.	Madh Island	26/08/1956	fl & fr	Common shrub. Flowers cream or pale yellow. Plenty of fruits.
15462	Caesalpiniaceae	*Cassia*	*absus* L.	226	Kapadia, Z.J.	Borivili	26/09/1953	fr	Common along the road side. In fruit.
15465	Caesalpiniaceae	*Cassia*	*absus* L.	S.H.810	Herbert, P.S.	National Park, Borivili	16/10/1955	fl & fr	Flowers few, common in fruits.
15466	Caesalpiniaceae	*Cassia*	*absus* L.	K.V.S.4372	Shenoy, K.V.	Mumbra	08/09/1954	fr	(Fruiting)
15467	Caesalpiniaceae	*Cassia*	*absus* L.	K.V.S.4273	Shenoy, K.V.	Mumbra	02/09/1954	fl & fr	(Fl & Fr)
15468	Caesalpiniaceae	*Cassia*	*absus* L.	K.V.S.4091	Shenoy, K.V.	Mumbra	26/08/1954	fl	(Flowering)
15469	Caesalpiniaceae	*Cassia*	*absus* L.	K.V.S.4439	Shenoy, K.V.	Mumbra	23/09/1954	fl & fr	(Fl & Fr)
15470	Caesalpiniaceae	*Cassia*	*absus* L.	K.V.S.607	Shenoy, K.V.	Mumbra	25/08/1953	fl	(Flowering)
15471	Caesalpiniaceae	*Cassia*	*absus* L.	K.V.S.745	Shenoy, K.V.	Mumbra	13/10/1953	fl & fr	(Fl & Fr)
15472	Caesalpiniaceae	*Cassia*	*absus* L.	S.H.1941	Herbert, P.S.	National Park, Borivili	10/08/1956	fl	Erect undershrub branched or unbranched, flowers pale yellow, small.
15475	Caesalpiniaceae	*Cassia*	*absus* L.	S.H.2552	Herbert, P.S.	National Park, Borivili	24/10/1956	fr	Small undershrub in undergrowth, in fruit only.
15723	Caesalpiniaceae	*Cassia*	*mimosoides* L.	S.H.2247	Herbert, P.S.	National Park, Borivili	14/09/1956	fl	Erect, flowers with long pedicels, leaflets more than 40 pairs.(fr)
15727	Caesalpiniaceae	*Cassia*	*mimosoides* L.	20300	H. Santapau, S.J.	National Park, Borivili	19/11/1955	fl & fr	Erect, flowers, rare, fruits, common among grasses in plains.
15735	Caesalpiniaceae	*Cassia*	*mimosoides* L.	16085	H. Santapau, S.J.	National Park, Borivili	26/09/1953	fr	(fruiting). Erect, slender plant upto 1 m. tall, bright yellow flowers. Plenty of fruits. Abundant along path in plains. Stamens 10, long and short, anthers with apical pores only. Nos 16088-89 are parts of the same plant.
15743	Caesalpiniaceae	*Cassia*	*mimosoides* L.	172	Nanda, U.	On way to Tulsi via Aarey Colony	16/09/1961	fl & fr	Herb about 1/2 m high. In flrs and fruits. Flowers yellow. Stamens 9.
15744	Caesalpiniaceae	*Cassia*	*mimosoides* L.	167	Nanda, U.	On way to Tulsi via Aarey Colony	16/09/1961	fl & fr	(Fl & Fr)
15745	Caesalpiniaceae	*Cassia*	*mimosoides* L.	168	Nanda, U.	On way to Tulsi via Aarey Colony	16/09/1961		No notes
15746	Caesalpiniaceae	*Cassia*	*mimosoides* L.	166	Nanda, U.	On way to Tulsi via Aarey Colony	16/09/1961	fl & fr	(Fl & Fr)

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15749	Caesalpiniaceae	*Cassia*	*mimosoides* L.	169	Nanda, U.	Aarey to Tulsi, lake on way	16/09/1961	fl & fr	(Fl & Fr)
15750	Caesalpiniaceae	*Cassia*	*mimosoides* L.	170	Nanda, U.	On way to Tulsi via Aarey Colony	16/09/1961	fl & fr	(Fl & Fr)
15755	Caesalpiniaceae	*Cassia*	*mimosoides* L.	130	Nanda, U.	Aarey to Tulsi	16/09/1961	fl & fr	(fr & fl)
15759	Caesalpiniaceae	*Cassia*	*mimosoides* L.	S.H.2581	Herbert, P.S.	National Park, Borivili	27/10/1956	fl	Erect herb or undershrub, in flowers and fruits, stem yellowish.
15764	Caesalpiniaceae	*Cassia*	*mimosoides* L.	20301	H. Santapau, S.J.	National Park, Borivili	19/11/1955	fl	Erect, flowers rare, fruits common, among grasses in plains.
20301	Caesalpiniaceae	*Cassia*	*mimosoides* L.	20301	H. Santapau, S.J.	National Park, Borivili	19/11/1955	fl	Erect, flowers rare, fruits common, along grasses in plains.
O2005	Capparidaceae	*Capparis*	*zeylanica* L.	41.2H	H. Santapau, S.J.	Uran, on walls of dry tank between landing stage and Uran Village	01/03/1941	fl & fr	Stamens and filaments purple (fl &fr)
O2006	Capparidaceae	*Capparis*	*zeylanica* L.	Shah 9720	Shah, G.L.	Malad Hills	02/02/1958	fl	Common scandent spinous shrub; flowers creamy white, filaments at first white, purple later on.
O2007	Capparidaceae	*Capparis*	*zeylanica* L.	Shah 9720	Shah, G.L.	Malad Hills	02/02/1958	fl	Flowers in no. 9719 (sterile).
O2009	Capparidaceae	*Capparis*	*zeylanica* L.	K.V.S.1876	Shenoy, K.V.	Mumbra	05/01/1954	fr	(fr)
O2010	Capparidaceae	*Capparis*	*zeylanica* L.	Shah 9866	Shah, G.L.	Malad, Quarry Hills	28/06/1958	fr	A common climber, fruits plenty; ripe fruits deep red or purple.
O2011	Capparidaceae	*Capparis*	*zeylanica* L.	Shah 9596	Shah, G.L.	Marve Madh Road, Malad	07/02/1957	fl	Common on nedges as twiners on small trees or shrubs (fl).
O2012	Capparidaceae	*Capparis*	*zeylanica* L.	23563	H. Santapau, S.J.	National Park, Borivili	18/02/1960	fl	Large climbing shrub in flower in the entrance to National Park.
O2013	Capparidaceae	*Capparis*	*zeylanica* L.	PD 413	Divakar, P.	Uran	17/01/1960	fl	Extensively armed climber-flowers whitish or pinkish with purple filaments. Common along hedges and on trees.
O2014	Capparidaceae	*Capparis*	*zeylanica* L.	23564	H. Santapau, S.J.	National Park, Borivili	18/02/1960	fl	Large climbing shrub in flower in the entrance to National Park.
O2015	Capparidaceae	*Capparis*	*zeylanica* L.	401	Merchant, Y.A.	Kanheri Caves, Borivili.	04/11/1957	fl	A large climber in flowers.
O2016	Capparidaceae	*Capparis*	*zeylanica* L.	K.V.S.51895	Shenoy, K.V.	Mumbra	05/01/1954	fr	(fr)
O2017	Capparidaceae	*Capparis*	*zeylanica* L.	Shah 8604	Shah, G.L.	Malad-Kandivali Ghodbunder Road	04/02/1957		see 8603
O2025	Capparidaceae	*Capparis*	*zeylanica* L.	K.V.S.2657	Shenoy, K.V.	Mumbra	09/04/1954	fr	(fr)
O2030	Capparidaceae	*Capparis*	*zeylanica* L.	T805	Tavakari, S.C.	Central Dairy to U 23 via Goregaon Calf Rearing Unit	09/03/1958	fl	A diffuse prostrate shrub; in flowers; flowers handsome, corolla yellow, filaments red, ovary on gynophore. Rare.
O2033	Capparidaceae	*Capparis*	*zeylanica* L.	NYD 2897	Das, N.Y.	Mandvi(Tungar)	10/02/1960	fl	(fl)
O2034	Capparidaceae	*Capparis*	*zeylanica* L.	NYD 2896	Das, N.Y.	Mandvi(Tungar)	10/02/1960	fl & fr	(fl & fr)
O2036	Capparidaceae	*Capparis*	*zeylanica* L.	Shah 10301	Shah, G.L.	Malad	03/04/1959	fr	Fruits, infected by fungus; rare.
O2040	Capparidaceae	*Capparis*	*zeylanica* L.	Shah 1137	Shah, G.L.	Malad Ghodbunder Road	03/12/1954	fr	(fr)

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O2044	Capparidaceae	*Capparis*	*zeylanica* L.	T1011	Tavakari, S.C.	Aarey Milk Colony, near Staff Quarters	02/04/1958	fl	Climbing shrub; flowers red; stamens purple(filaments), rare.
O2046	Capparidaceae	*Capparis*	*zeylanica* L.	PD 414	Divakar, P.	Uran	17/01/1960	fl	Armed climber with creamy white flowers. Common.
O2049	Capparidaceae	*Capparis*	*zeylanica* L.	K.V.S.1872	Shenoy, K.V.	Mumbra	05/01/1954	fr	(fr)
O2050	Capparidaceae	*Capparis*	*zeylanica* L.	K.V.S.1873	Shenoy, K.V.	Mumbra	05/01/1954	fr	(fr)
O2051	Capparidaceae	*Capparis*	*zeylanica* L.	K.V.S.1874	Shenoy, K.V.	Mumbra	05/01/1954	fl & fr	(fl & fr)
O2052	Capparidaceae	*Capparis*	*zeylanica* L.	NYD 2894	Das, N.Y.	Mandvi(Tungar)	10/12/1960	fl	(fl)
O2053	Capparidaceae	*Capparis*	*zeylanica* L.	NYD 2895	Das, N.Y.	Mandvi(Tungar)	10/12/1960	fr	(fr)
O2054	Capparidaceae	*Capparis*	*zeylanica* L.	NYD 3085	Das, N.Y.	Chandip(Tungar)	19/02/1961	fl	(fl bud)
O2055	Capparidaceae	*Capparis*	*zeylanica* L.	NYD 3084	Das, N.Y.	Chandip(Tungar)	19/02/1961	fr	(fr)
O2056	Capparidaceae	*Capparis*	*zeylanica* L.	NYD 3083	Das, N.Y.	Chandip(Tungar)	19/02/1961	fr	(fr)
O2057	Capparidaceae	*Capparis*	*zeylanica* L.	NYD 3082	Das, N.Y.	Chandip(Tungar)	19/02/1961		(sterile specimen)
O2058	Capparidaceae	*Capparis*	*zeylanica* L.	Shah 9720	Shah, G.L.	Malad Hills	02/12/1958	fl	A common spinous twiner, flowers plenty, creamy white with purple filaments; buds rusty brown.
O2063	Capparidaceae	*Capparis*	*zeylanica* L.	K.V.S.1893	Shenoy, K.V.	Mumbra	05/01/1954	fr	(fr)
O2064	Capparidaceae	*Capparis*	*zeylanica* L.	K.V.S.1887	Shenoy, K.V.	Mumbra	05/01/1954	fr	(fr)
O2065	Capparidaceae	*Capparis*	*zeylanica* L.	K.V.S1875	Shenoy, K.V.	Mumbra	00/05/1941	fl	(fl)
O2066	Capparidaceae	*Capparis*	*zeylanica* L.	41.1.H	H.Santapau, S.J.	Talasari, Thana Dist.	02/02/1960	fl	"Tarati". Scarlet fruit, flower and fruit supraaxillary.
O2067	Capparidaceae	*Capparis*	*zeylanica* L.	PD476	Divakar, P.	Karanja-Uran	01/03/1960	fl	Climbers with white or pink flowers. Filaments purplish. Common.
O2069	Capparidaceae	*Capparis*	*zeylanica* L.	PD536	Divakar, P.	Karanja-Uran		fl	Extensive climber, flowers creamy white with purple filaments. Common.
25615	Combretaceae	*Terminalia*	*bellerica*(Gaertn.)Roxb.	Shah 9541	Shah, G.L.	Madh Island	22/12/1957		No notes
25616	Combretaceae	*Terminalia*	*bellerica*(Gaertn.)Roxb.	Shah 9642	Shah, G.L.	Madh Island	22/12/1957		2 to 3 trees on hills along Cart Road. Fruits.
25617	Combretaceae	*Terminalia*	*bellerica*(Gaertn.)Roxb.	Shah 9640	Shah, G.L.	Madh Island	22/12/1957	fr	(sterile specimen)
25628	Combretaceae	*Terminalia*	*bellerica*(Gaertn.)Roxb.	Shah 9718	Shah, G.L.	Malad	02/02/1957	fr	Common tree, on plains. Tree up to 30' tall, fruits, occasionally.
25630	Combretaceae	*Terminalia*	*bellerica*(Gaertn.)Roxb.	109.14 H	H. Santapau, S.J.	Mudgaon, Salsette Islands.	15/06/1941		(sterile specimen)
25631	Combretaceae	*Terminalia*	*bellerica*(Gaertn.)Roxb.	T789	Tavakari, S.C.	Near Central Dairy	09/03/1958	fr	Tree in fruits. Rare.

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25633	Combretaceae	*Terminalia*	*bellerica*(Gaertn.)Roxb.	K.V.S.2361	Shenoy, K.V.	Mumbra	23/03/1954	fl	(fl)
25634	Combretaceae	*Terminalia*	*bellerica*(Gaertn.)Roxb.	K.V.S.2363	Shenoy, K.V.	Mumbra	23/03/1954	fl	(fl)
25635	Combretaceae	*Terminalia*	*bellerica*(Gaertn.)Roxb.	K.V.S.2364	Shenoy, K.V.	Mumbra	23/03/1954	fl	(fl)
46918	Convolvulaceae	*Cuscuta*	*reflexa*Roxb.	NYD 7425	Das, N.Y.	Usgaon	11/03/1962	fl	An extensive twiner on *Streblus asper*, stem greenish yellow, flowers white, not very common.
46949	Convolvulaceae	*Cuscuta*	*reflexa*Roxb.	13835	H. Santapau, S.J.	Versova	11/10/1941	fl	(fl)
46955	Convolvulaceae	*Cuscuta*	*reflexa*Roxb.	Bole	Bole, P.V.	Ghodbunder	01/01/1954		On *Streblus asper*.
46956	Convolvulaceae	*Cuscuta*	*reflexa*Roxb.	5573	H. Santapau, S.J.	Dahisar near Borivili Salsette	17/12/1944	fl	(fl)
46961	Convolvulaceae	*Cuscuta*	*reflexa*Roxb.	17503	H. Santapau, S.J.	Mumbra	28/11/1953		Stems deep dark green, creamy. On *Carissa congesta*. Not common.
46965	Convolvulaceae	*Cuscuta*	*reflexa*Roxb.	Shah 8591	Shah, G.L.	Madh Island, Bombay	02/02/1957	fl	A parasite on Streblus, plenty of flowers, flowers white or cream.
46971	Convolvulaceae	*Cuscuta*	*reflexa*Roxb.	Shah	Shah, G.L.	Malad Ghodbunder Road			A parastie on *Mussaenda frondosa*.
46977	Convolvulaceae	*Cuscuta*	*reflexa*Roxb.	VP 1624	Patel, V.M.	Tansa Lake Dist	18/12/1955		same as VP 1623
46995	Convolvulaceae	*Cuscuta*	*reflexa*Roxb.	13859	H. Santapau, S.J.	Versova	13/02/1942	fl	On *Bougainvillea spectabilis*, wild. Flowers creamy white, plant yellowish green.
46997	Convolvulaceae	*Cuscuta*	*reflexa*Roxb.	VP1304	Patel, V.M.	Borivili National Park	29/01/1955	fl & fr	Found in flowers and fruit, growing on *Clerondendron inerme* along the roadside.
28266	Cucurbitaceae	*Coccinia*	*grandis*(L.)Voigt	5611H	H. Santapau, S.J.	Sion Hill, Bombay	01/07/1941	fl	Flowers white.
28271	Cucurbitaceae	*Coccinia*	*grandis*(L.)Voigt	12964	H. Santapau, S.J.	Worli Hill, Bombay	14/07/1951		No notes
28272	Cucurbitaceae	*Coccinia*	*grandis*(L.)Voigt	GK 40	Kaliamiwalla, P.G.	National Park, Borivili	23/08/1951	fl	Perennial scandent, dioecious herb, male flowers solitary, white, female flowes not seen. Not common.
28273	Cucurbitaceae	*Coccinia*	*grandis*(L.)Voigt	GK 303	Kaliamiwalla, P.G.	National Park, Borivili	15/07/1952	fl	In flower.
7565	Cucurbitaceae	*Coccinia*	*indica*Wight& Arn.	7565	H. Santapau, S.J.	Rozi near Jamnagar	17/10/1945	fl	(fl)
28267	Cucurbitaceae	*Coccinia*	*indica*Wight& Arn.	14941		Worli, Bombay	01/02/1917		No notes
28268	Cucurbitaceae	*Coccinia*	*indica*Wight& Arn.	5620	H. Santapau, S.J.	Versova	11/10/1941	fl & fr	Flowers white, fruits not bitter.
28269	Cucurbitaceae	*Coccinia*	*indica*Wight& Arn.	31175	Vakil, B.N.	Bandra, Bombay	01/07/1919	fl	Front shore, gravelly shore, climbing on Opuntias, white flowers.
28274	Cucurbitaceae	*Coccinia*	*indica*Wight& Arn.	Shah 8863	Shah, G.L.	Madh Island, Bombay	27/07/1957	fl & fr	Prostrate herb on sand, flowers and fruits, note the galls on the stem. Rare.
28276	Cucurbitaceae	*Coccinia*	*indica*Wight& Arn.	T650	Tavakari, S.C.	Veterinary Hospital, Parel, Bombay	30/01/1958	fl & fr	Climber in flowers and fruits. Female flowers, white in colour.
28277	Cucurbitaceae	*Coccinia*	*indica*Wight& Arn.	PD2582	Divakar, P.	Mora-Uran	17/09/1961	fl	Climbing herb, flowers white, common.

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28278	Cucurbitaceae	*Coccinia*	*indica*Wight& Arn.	Shah 7213	Shah, G.L.	Madh Island	29/07/1956	fl	A very common twiner with white herb; flowers collected from sasndy beach, twiner on *Vitex negundo.*
28279	Cucurbitaceae	*Coccinia*	*indica*Wight& Arn.	S.H. 563	Herbert, P.S.	National Park, Borivili	22/02/1955	fl & fr	Flowers white, only female flowers and fruits seen, collected from the hills next after caves, near Saduhi's [Sadhu's] Ashram.
28287	Cucurbitaceae	*Coccinia*	*indica*Wight& Arn.	3940	H. Santapau, S.J.	Bombay Reclamation	4?/4/1944	fl	Flowers white, probably same as 3939, but leaves less lobed.
28289	Cucurbitaceae	*Coccinia*	*indica*Wight& Arn.	21	Merchant, Y.A.	Worli Hill, Bombay	20/07/1957	fl	Flowers white, occasional on hedges near the top of the hill.
29275	Cucurbitaceae	*Trichosanthes*	*palmata*Roxb.	SKW 6798	Wagh, S.K.	Shivdurg(Nellore)	01/08/1957		Common near the pond.
29276	Cucurbitaceae	*Trichosanthes*	*palmata*Roxb.	SKW 6799	Wagh, S.K.	Shivdurg(Nellore)	01/08/1957		Common near the pond.
67292	Euphorbiaceae	*Euphorbia*	*antiquorum* L.	R 4017	Fernandez, R. R.	Cumbala Hill (Mumbai)	25/04/1957	fl	(flowering)
101253	Euphorbiaceae	*Euphorbia*	*antiquorum L.*	947	Shinde, R.	Khandgaon (Satara)	01/12/1986	fl	(flowering). Flora of Nandur Madhmeshwar
69028	Euphorbiaceae	*Phyllanthus*	*emblica* L.	K.V.S.2607	Shenoy, K.V.	Mumbra	06/04/1954	fl	(fl)
69029	**Euphorbiaceae**	***Phyllanthus***	***emblica* L.**	**S.H. 2639**	**Herbert, P.S.**	**National Park, Borivili**	**08/11/1956**	**fr**	**A small tree in fruit,only from a thick jungle in a truly wild form, fruits edible, yellowish brown.**
69033	Euphorbiaceae	*Phyllanthus*	*emblica* L.	NYD 7306	Das, N.Y.	Parol(Tungar)	06/03/1962	fl	A large tree with spreading branches, flowers yellow, common.
69036	Euphorbiaceae	*Phyllanthus*	*emblica* L.	K.V.S. 2537	Shenoy, K.V.	Mumbra	30/03/1954	fl	(fls)
69037	Euphorbiaceae	*Phyllanthus*	*emblica* L.	S.H. 1494	Herbert, P.S.	National Park, Borivili	17/03/1956	fl	A deciduous tree,large or small, flowers very small, numerous, clustered in axils, mainly of fallen leaves.
69038	Euphorbiaceae	*Phyllanthus*	*emblica* L.	S.H. 1495	Herbert, P.S.	National Park, Borivili	17/03/1956		A deciduous tree, found occasionally.
69039	Euphorbiaceae	*Phyllanthus*	*emblica* L.	S.H. 1496	Herbert, P.S.	National Park, Borivili	17/03/1956	fl	A deciduous tree, large or small, found occasionally, flowers pale reddish, numerous in axils, mainly of fallen leaves.
69042	Euphorbiaceae	*Phyllanthus*	*emblica* L.	K.V.S. 2608	Shenoy, K.V.	Mumbra	06/04/1954	fl	(fl)
69063	Euphorbiaceae	*Phyllanthus*	*emblica* L.	NYD 1977	Das, N.Y.	Usgaon(Tungar)	21/08/1960	fr	A deciduous middle sized tree, common above 300ft. Fruit edible.
69064	**Euphorbiaceae**	***Phyllanthus***	***emblica* L.**	**NYD 1979**	**Das, N.Y.**	**Usgaon(Tungar)**	**21/08/1960**	**fr**	**A deciduous middle sized tree, common above 300ft. Fruit edible.**
69065	Euphorbiaceae	*Phyllanthus*	*emblica* L.	NYD 1978	Das, N.Y.	Usgaon(Tungar)	21/08/1960	fr	A common tree in open placees and above 300 ft. Fruit edible.
69066	**Euphorbiaceae**	***Phyllanthus***	***emblica* L.**	**S.H. 2638**	**Herbert, P.S.**	**National Park, Borivili**	**08/11/1956**	**fr**	**2638-39 same, a small tree in fruit only; from a thick jungle in a truly wild form; fruits edible, yellowish brown.**
44600	Gentianaceae	*Enicostema*	*littorale* Blume	12952	H. Santapau, S.J.	Worli Hill, Bombay	14/07/1951	fl	(fl)
44601	Gentianaceae	*Enicostema*	*littorale* Blume	14385		Sewree, Bombay	01/07/1917	fl	(fl)

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44602	Gentianaceae	*Enicostema*	*littorale* Blume	35326	Vakil, B.N.	Bandra, Bombay	01/08/1919	fl	(fl), Abundantly growing on the pipeline. Bund behind goods yard-Bandra.
44619	Gentianaceae	*Enicostema*	*littorale* Blume	8132	H. Santapau, S.J.	Versova, near Andheri.	01/12/1945	fl	(fl)
44624	Gentianaceae	*Enicostema*	*littorale* Blume	ACK 717	Aclaud, R.D.	Matunga, Bombay Railway line	01/07/1925		Buds only.
44634	Gentianaceae	*Enicostema*	*littorale* Blume	Shah 4731	Shah, G.L.	Kandivali, near Home Guards ground	29/07/1955	fl	Flowers white.
44641	Gentianaceae	*Enicostema*	*littorale* Blume	Shah 1075	Shah, G.L.	Marve Road, Malad	18/11/1954	fl	(fl)
101032	Gentianaceae	*Enicostema*	*littorale* Blume	476	Shinde, R.	Khandgaon	01/08/1985	fl	Flowers white, syn. *E. littorale* (Flora of Nandur Madhmeshwar)
65106	Lauraceae	*Cinnamomum*	*zeylanicum* Blume	17797	H. Santapau, S.J.	Castlerock, along forests on either side of railway line	21/12/1953	fl	large tree, flowers yellowish, plenty in terminal panicles. Common in forests.
65107	Lauraceae	*Cinnamomum*	*zeylanicum* Blume	s.n.	P.S.K.	Castlerock	01/1891	fl	(fl). Flora of Bombay
65108	Lauraceae	*Cinnamomum*	*zeylanicum* Blume	JF 1453	Fernandez, J.	Old Guud Village	13/05/1950	fr	(fr)
65119	Lauraceae	*Cinnamomum*	*zeylanicum* Blume	17796	H. Santapau, S.J.	Castle Rock, along forests on either side of railway line	21/12/1953	fl	Large tree, flowers yellowish, plenty in terminal panicles. Common in dense forests.
65124	Lauraceae	*Cinnamomum*	*zeylanicum* Blume	JF 1453	Fernandez, J.	Old Guud Village, N. Kanara.	13/05/1950	fr	(fr)
65130	Lauraceae	*Cinnamomum*	*zeylanicum* Blume	JF 826	Fernandez, J.	Jog, N. Kanara	19/01/1950	fl	(fl)
12786	Leguminosae	*Abrus*	*precatorius* L.	10228	H. Santapau, S.J.	Talasari, Thana Dist.	16/05/1941	fr	(fr)
12787	**Leguminosae**	***Abrus***	***precatorius* L.**	**NYD 2063**	**Das, N.Y.**	**Usgaon (Tungar)**	**11/09/1960**	**fl & fr**	**A climber, flowers light white with pinkish tinge. Seeds are red with black eye. Black smiths are using these seeds as weights. Fresh leaves are used along with Pan. Local people using these leaves as a good vegetable. Roots are used in Indian liquorice.**
12788	Leguminosae	*Abrus*	*precatorius* L.	P.D.866	Divakar, P.	Karanja	17/07/1960	fl	Climber with pinkish flowers. Seeds light red with a black spot. Common near hedges and edges and jungles.
12795	Leguminosae	*Abrus*	*precatorius* L.	DP 2147	Pathanki, D.P.	Powai	08/12/1954	fr	A climber with dehisced dry fruits only. On hedges along the roadside.
12797	Leguminosae	*Abrus*	*precatorius* L.	S.H.2198	Herbert, P.S.	National Park, Borivili	14/09/1956	fr	Very common large twiners on hedges. (fr).
12798	Leguminosae	*Abrus*	*precatorius* L.	S.H.2405	Herbert, P.S.	National Park, Borivili	29/09/1956	fr	In fruit only.
12804	Leguminosae	*Abrus*	*precatorius* L.	s.n.	Pathanki, D.P.	Borivili	09/10/1984	fr	(fr).
12807	Leguminosae	*Abrus*	*precatorius* L.	K.V.S.1860	Shenoy, K.V.	Mumbra	23/12/1953	fr	(fr).
12808	Leguminosae	*Abrus*	*precatorius* L.	K.V.S.5214	Shenoy, K.V.	Mumbra	06/11/1954	fr	(fr).

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12809	Leguminosae	*Abrus*	*precatorius* L.	S.H.309	Herbert, P.S.	National Park, Borivili	08/11/1954		A very common twiner during early monsoon, flowers absent.
12830	Leguminosae	*Acacia*	*catechu*(L.f.)Willd.	Shah 6741	Shah, G.L.	Ghodbunder Road, Malad	16/01/1956	fr	(fr)
12886	Leguminosae	*Acacia*	*catechu*(L.f.)Willd.	7068	H.Santapau, S.J.	Borivili Kanheri Caves	25/08/1945	fl	(fl)
12888	Leguminosae	*Acacia*	*catechu*(L.f.)Willd.	G.K.1052		National Park, Borivili	17-18/08/1952	fl	(fl)
12889	Leguminosae	*Acacia*	*catechu*(L.f.)Willd.	2338	H.Santapau, S.J.	Borivili Kanheri Caves, Salsette	08/08/1943	fl	Flowers white, slightly fragrant.
12890	Leguminosae	*Acacia*	*catechu*(L.f.)Willd.	2337	H.Santapau, S.J.	Borivili Kanheri Caves, Salsette	08/08/1943	fl	Flowers whit,slightly fragrant.
12902	Leguminosae	*Acacia*	*catechu*(L.f.)Willd.	T2130	Tavakari, S.C.	Aarey Colony and near U 30	19/12/1958	fr	Tree, spiny in fruits; about 10' tall, fairly common.
16302	Leguminosae	*Clitoria*	*ternatea* L.	102.79	H. Santapau, S.J.	Jogeshwari	14/10/1941	fl & fr	Flowers dark blue, climber.(fr).
16302	Leguminosae	*Clitoria*	*ternatea* L.	S.H.114	Herbert, P.S.	National Park, Borivili	29/09/1954	fl	A large twiner, flowers white, no fruits.
16305	Leguminosae	*Clitoria*	*ternatea* L.	S.H. 2832	Herbert, P.S.	National Park, Borivili	04/09/1957		A rare slender twiner, on the fence, at the entrance of the park.
16316	Leguminosae	*Clitoria*	*ternatea* L.	Shah 8706	Shah, G.L.	Marve-Madh Road, Malad	01/03/1957	fl & fr	Flowers and fruits; now rare.
16318	Leguminosae	*Clitoria*	*ternatea* L.	Shah 7177	Shah, G.L.	Madh Island	23/07/1956	fl	A common twiner in hedges along road side, flowers blue.
16319	Leguminosae	*Clitoria*	*ternatea* L.	Shah 414	Shah, G.L.	Malad-Marve Road	31/08/1954		Common in hedges. Also Malad Quarry Hills along main road to C.O.D.Camp.
16321	Leguminosae	*Clitoria*	*ternatea* L.	DP 1446 b	Pathanki, D.P.	Victoria Gardens, Bombay	16/07/1954	fl	A climber with blue flowers, a papilionaceous type. Tow buds were present in the axil.
16325	Leguminosae	*Clitoria*	*ternatea* L.	DP 1447 b	Pathanki, D.P.	Victoria Gardens, Bombay	16/07/1954	fl	A climber with showy blue flowers. Petals crumpled.
16326	Leguminosae	*Clitoria*	*ternatea* L.	DP 1447 C	Pathanki, D.P.	Victoria Gardens, Bombay	16/07/1954	fl	A climber with bright blue flowers.
16328	Leguminosae	*Clitoria*	*ternatea* L.	NYD 7793	Das, N.Y.	Chandip(Tungar)	26/08/1962	fl	A thorny twiner on hedges, flowers white, not common.
16329	Leguminosae	*Clitoria*	*ternatea* L.	NYD 7794	Das, N.Y.	Chandip(Tungar)	26/08/1962	fl	A tiwner on hedges; flowers white, not common.
17904	Leguminosae	*Dalbergia*	*sissoo*Roxb. ex DC.	10846		Victoria Gardens, Bombay	03/1899	fl & fr	"Shisham". (fl &fr)
17906	Leguminosae	*Dalbergia*	*sissoo*Roxb. ex DC.	R2713	Fernandez, R. R.	Prabhadevi, Bombay	02/09/1956	fr	(fr)
105919	Meliaceae	*Azadirachta*	*indica*A.Juss.	4381	Almeida, S.M.	Sawantwadi	08/05/1982		Commony cultivated in the garden.
No acc. No	**Meliaceae**	***Azadirachta***	***indica*A.Juss.**	**181**	**Coutinho, S.**	**Veermata Jijabai Udyan, Byculla**	**10/10/1977**	**fl**	**A deciduous tree. Flowers lilac. Fruit globuse drupe. Tree yeilds valuable timer. Leaves, bark have insect repellant properties.**
O9341	Meliaceae	*Azadirachta*	*indica*A.Juss.	3961	H.Santapau, S.J.	Sophia College, Bombay	09/04/1944	fl	flowers and leaves
O9529	Meliaceae	*Azadirachta*	*indica*A.Juss.	s.n.	H.Santapau, S.J.	Victoria Gardens, Bombay	10/08/1960	fr	(fr)

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O9538	Meliaceae	*Azadirachta*	*indica*A.Juss.	ACK 152	Aclaud, R.D.	Bombay streets	00/03/1925	fl	"Bakan", stamen tube dark purple, petals lilac turning white.
40124	Myrsinaceae	*Embelia*	*ribes*Burm.f.	2377	Bole, P.V.	Dholi's Falls to Lodwick Point, on way right hand side	14/03/1960		Climber, 30'-40', leaves only. Rare.
40125	Myrsinaceae	*Embelia*	*ribes*Burm.f.	2377	Bole, P.V.	Dholi's Falls to Lodwick Point, on way right hand side	14/03/1960		Climber, 30'-40', leaves only. Rare.
40126	Myrsinaceae	*Embelia*	*ribes*Burm.f.	23422	H. Santapau, S.J.	Mahabaleshwar	14/03/1960		Powerful climber in leaf, leaves simple. Along Dhobrie's ride.
40127	Myrsinaceae	*Embelia*	*ribes*Burm.f.	23421	H. Santapau, S.J.	Mahabaleshwar	14/03/1960		powerful climber inleaf, leaves simple, along Dhobrie's ride.
40128	Myrsinaceae	*Embelia*	*ribes*Burm.f.	2086	Almeida, M.R.	Ponmudi, Kerala		fl	(fl)
40130	Myrsinaceae	*Embelia*	*ribes*Burm.f.	2258	Almeida, M.R.	Mahabaleshwar	29/09/1972		(sterile specimen)
26085	Myrtaceae	*Eugenia*	*jambos* L.	14018		Victoria Gardens, Bombay	01/02/1917		No notes
26086	Myrtaceae	*Eugenia*	*jambos* L.	14006		Victoria Gardens, Bombay	01/03/1918		No notes
26087	Myrtaceae	*Eugenia*	*jambos* L.	13995		Mount Petit, Bombay	01/11/1917	fl	Flowers rose coloured. (No flowers on sheet).
26089	Myrtaceae	*Eugenia*	*jambos* L.	13988		Victoria Gardens, Bombay			No notes
26093	Myrtaceae	*Eugenia*	*jambos* L.	ACK 443	Aclaud, R.D.	Dapoli	01/03/1922	fl	Flowering.
26094	Myrtaceae	*Eugenia*	*jambos* L.	19857		Bombay Presidency			Escape.
26096	Myrtaceae	*Eugenia*	*jambos* L.	11939	H. Santapau, S.J.	Old Mahabaleshwar	10/11/1950	fl	Flowers white or cream, cultivated in gardens in old Mahabaleshwar.
26098	**Myrtaceae**	***Eugenia***	***jambos* L.**	**Bole 1675**	**Bole, P.V.**	**Mahabaleshwar Guest House Compound**	**20/06/1958**	**fr**	**Tree 20 or more. Said to be cultivated. The fruits are edible, fragrant and sweet, very palatable. Worthy of further propagation.**
16161	Papilionaceae	*Cicer*	*arietinum* L.	DP 2161	Pathanki, D.P.	Powai	08/12/1954	fr	A herb thrown on the roadside. Neither wild nor cultivated in this area. In fruits only.
16181	Papilionaceae	*Cicer*	*arietinum* L.	PD3487	Divakar, P.	Uran	27/12/1961	fr	(fr)
100913	Papilionaceae	*Cicer*	*arietinum* L.	95	Shinde, R.	Khandgaon-Karanji Road	17/12/1984	fr	(fr). 'Harbara', cultivated.
17842	Papilionaceae	*Dalbergia*	*latifolia*Roxb.	K.V.S.2569	Shenoy, K.V.	Mumbra	02/04/1954	fl	(fl)
17844	Papilionaceae	*Dalbergia*	*latifolia*Roxb.	K.V.S.4140	Shenoy, K.V.	Mumbra	26/08/1954	fl	A large deciduous tree found on top of the hills. (Fl)
17847	Papilionaceae	*Dalbergia*	*latifolia*Roxb.	JF261	Fernandez, J.	Karjat	12/04/1949	fl	(fl)
17849	Papilionaceae	*Dalbergia*	*latifolia*Roxb.	K.V.S.2392	Shenoy, K.V.		25/03/1954	fr	(fr)
17850	Papilionaceae	*Dalbergia*	*latifolia*Roxb.	11480	E. Blatter. S.J.	Above Kanheri Caves	01/11/1918	fr	(fr)

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17852	Papilionaceae	*Dalbergia*	*latifolia*Roxb.	K.V.S.4143	Shenoy, K.V.	Mumbra	26/08/1954	fl	A large deciduous tree found on the slopes.(fl)
17853	Papilionaceae	*Dalbergia*	*latifolia*Roxb.	11434	H. Santapau, S.J.	Borivili Kanheri Caves	01/11/1918	fr	(fr)
17857	Papilionaceae	*Dalbergia*	*latifolia*Roxb.	S.H.213B	Herbert, P.S.	National Park, Borivili	22/10/1954	fl	Small or big tree, flowers white, fruits are not seen in Borivali probably due to infertitilisation.
17858	Papilionaceae	*Dalbergia*	*latifolia*Roxb.	K.V.S.2563	Shenoy, K.V.	Mumbra	02/04/1954	fl	(fl)
17860	Papilionaceae	*Dalbergia*	*latifolia*Roxb.	K.V.S.979	Shenoy, K.V.	Mumbra	24/10/1953	fl	(fl)
17862	Papilionaceae	*Dalbergia*	*latifolia*Roxb.	K.V.S.2547	Shenoy, K.V.	Mumbra	30/03/1954	fl	A deciduous tree (fl)
17863	Papilionaceae	*Dalbergia*	*latifolia*Roxb.	K.V.S.2393	Shenoy, K.V.	Mumbra	25/03/1954	fr	Fr (a moderate sized tree)
17828	Papilionaceae	*Dalbergia*	*sissoo*Roxb. ex DC.	R2711	Fernandez, R. R.	Mahim, Bombay	02/09/1956	fr	In fruit. Rachis of leaves, zig zag where young, leaves variable.
17911	Papilionaceae	*Dalbergia*	*sissoo*Roxb. ex DC.	R3745	Fernandez, R. R.	Victoria Gardens, Bombay	21/02/1957	fl	(fl)
17912	Papilionaceae	*Dalbergia*	*sissoo*Roxb. ex DC.	R2714	Fernandez, R. R.		02/09/1956	fr	road side (fr).
17983	Papilionaceae	*Dalbergia*	*volubilis*Roxb.	S.H.1438	Herbert,P.S.	National Park, Borivili	19/02/1956	fl	A climber, flowers rose red.
18008	Papilionaceae	*Dalbergia*	*volubilis*Roxb.	JF66	Fernandez, J.	South of Karjat	29/01/1949	fl	(fl)
18014	Papilionaceae	*Dalbergia*	*volubilis*Roxb.	5969	H. Santapau, S.J.	Mumbra	08/02/1945	fl	(fl)
18018	Papilionaceae	*Dalbergia*	*volubilis*Roxb.	JF92	Fernandez, J.	Hillsides South of Karjat	10/02/1949	fl	(fl)
18022	Papilionaceae	*Dalbergia*	*volubilis*Roxb.	18130	H. Santapau, S.J.	Badlapur near Kalyan	27/02/1954		Large erect shrub with large sarmentose branches or subscandent. In hedges. Only one clump seen in open country.
18023	Papilionaceae	*Dalbergia*	*volubilis*Roxb.	T664	Tavakari, S.C.	Central Dairy along Borivili Road via U.7	09/02/1958	fl	Woody tree; climber by which young twigs climb, flowers pink, rare.
18025	Papilionaceae	*Dalbergia*	*volubilis*Roxb.	30601	H. Santapau, S.J.	Virar, Thane District	01/02/1919	fl	large ?ambler?. Flowers bluish purple.
18030	Papilionaceae	*Dalbergia*	*volubilis*Roxb.	S.H.539	Herbert, P.S.	National Park, Borivili	12/02/1955	fl	(fl)
18031	Papilionaceae	*Dalbergia*	*volubilis*Roxb.	S.H.1428	Herbert, P.S.	National Park, Borivili	19/02/1956	fl & fr	Flowers rose red, a common large climber. (fl & fr)
18033	Papilionaceae	*Dalbergia*	*volubilis*Roxb.	57/236		Near Atgaon	21/03/1893	fl & fr	(fl & fr)
84221	Poaceae	*Eleusine*	*coracana*(L.) Gaertn.	KVS 5290	Shenoy, K.V.	Mumbra	12/11/1954	fl & fr	(fl&fr).
84222	Poaceae	*Eleusine*	*coracana*(L.) Gaertn.	S.H.960	Herbert, P.S.	National Park, Borivili	15/11/1955	fr	A rare wet herb, in fruit only.
84226	Poaceae	*Eleusine*	*coracana*(L.) Gaertn.	R930	Fernandez, R. R.	National Park, Borivili	19/10/1952	fl & fr	(fl& fr).
84227	Poaceae	*Eleusine*	*coracana*(L.) Gaertn.	5235		Sion, Bombay	01/11/1916	fr	(fr).

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Accession No	Family	Genus	Species	Collector No	Collector Name	Locality	Date	Phenology (Fl & Fr)	Remarks
84233	Poaceae	*Eleusine*	*coracana*(L.) Gaertn.	R930	Fernandez, R. R.	National Park, Borivili	19/10/1952	fl & fr	(fl& fr), cultivated, also found in wild state, rare tall herb.
84241	Poaceae	*Eleusine*	*coracana*(L.) Gaertn.	S.H.1049	Herbert, P.S.	National Park, Borivili	19/11/1955	fr	Rare tall herb, mainly in fruit.
84242	Poaceae	*Eleusine*	*coracana*(L.) Gaertn.	K.V.S.2267	Shenoy, K.V.	Mumbra	08/03/1954	fr	(fr)
31745	Rubiaceae	*Cephaelis*	*ipecacuanha*(Brot.) A. Rich.	s.n.	Rao, T.S.N.	Nougpoh, K+J Hills, Assam	28/06/1962	fl	(fl)
31756	Rubiaceae	*Cinchona*	*calisaya*Wedd.	KMM 1633	Mathew, K.M, S.J.	Perumal, Kodaikanal	19/10/1962	fl	Exotic, flowers whitish.
31748	**Rubiaceae**	***Cinchona***	***officinalis* L.**	**KMM 1628**	**Mathew, K.M, S.J.**	**Shembaganur (Palni Hills), S.H. College**	**05/09/1960**		**Exotic, remnants of a plantation earlier to 1887! (fl). First effective drug quinine is prepared from this plant which is used for Malaria treatment. Imagine, if this plant was not discovered and correctly identified by the plant taxonomists, chemist would not have been able to extract quinine and many would have perished by the attack of Malaria. However, this drug is not effective now, hunt for new drug is on.**
53415	Scrophulariaceae	*Striga*	*gesneroides* (Willd.)Vatke	9582	D'Almeida	Bandra, Land's End	17/09/1942		Parasite on roots of *Lepidagatthis* sp. (*cristata*?)
53423	Scrophulariaceae	*Striga*	*gesneroides* (Willd.)Vatke	K.V.S.4107	Shenoy, K.V.	Mumbra	26/08/1954	fl	(fl)
53425	Scrophulariaceae	*Striga*	*gesneroides* (Willd.)Vatke	145.17	H. Santapau, S.J.	Tungar Hill	29/09/1941	fl	Alt. 470 m. Root parasite, dark reddish stems (fl).
53432	Scrophulariaceae	*Striga*	*gesneroides* (Willd.)Vatke	5487	H. Santapau, S.J.	Khandala, Sausages Top	04/11/1944		parasite on roots of *Lepidagatthis* sp.
53436	Scrophulariaceae	*Striga*	*gesneroides* (Willd.)Vatke	9582	D'Almeida	Bandra, Land's End	17/09/1942		Parasite on roots of *Lepidagatthis* sp. (*cristata*?)
50251	Solanaceae	*Solanum*	*surattense*Burm.f.	P.D.6178	Divakar, P.	Uran	13/03/1963		As in PD 6177. (fl)
50252	Solanaceae	*Solanum*	*surattense*Burm.f.	P.D.6177	Divakar, P.	Uran	13/03/1963	fl	Herbs with purple flowers. Common.
50254	Solanaceae	*Solanum*	*surattense*Burm.f.	P.D.2482	Divakar, P.	Karanja-Uran	06/02/1961	fr	Armed herb mostly in fruits. Fruits yellowish. Common.
50256	Solanaceae	*Solanum*	*surattense*Burm.f.	NYD 2219	Das, N.Y.	Parol[Parel]	06/03/1962	fl	A prostrate herb, yellowish spines, petals violet, anthers yellow, common on plains.
50257	Solanaceae	*Solanum*	*surattense*Burm.f.	NYD 5221	Das, N.Y.	Mandvi	08/12/1961		Prostrate spreading herb; fairly common.
50258	Solanaceae	*Solanum*	*surattense*Burm.f.	PD 565	Divakar, P.	Karanja-Uran	01/03/1960	fl	Herbs with violet flowers. Common.
50259	Solanaceae	*Solanum*	*surattense*Burm.f.	NYD 5222	Das, N.Y.	Mandvi	08/12/1961	fl	Prostrate spreading herb; fairly common (fl).
50260	Solanaceae	*Solanum*	*surattense*Burm.f.	NYD 5103	Das, N.Y.	Chandip	25/11/1961	fr	(fr)
50352	Solanaceae	*Solanum*	*surattense*Burm.f.	VP 1246	Patel, V.M.	Borivili National Park	15/01/1955	fl & fr	Found mostly in flower. Flowers showy and large at this time of the year (fr).

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Accession No	Family	Genus	Species	Collector No	Collector Name	Locality	Date	Phenology (Fl & Fr)	Remarks
O6265	Tiliaceae[now Malvaceae]	*Corchorus*	*actangulus* Lam.	K.V.S.4270	Shenoy, K.V.	Mumbra	24/09/1954	fl	(fl)
O6266	Tiliaceae[now Malvaceae]	*Corchorus*	*actangulus* Lam.	K.V.S. 721	Shenoy, K.V.	Mumbra	15/09/1953		(sterile specimen)
O6267	Tiliaceae[now Malvaceae]	*Corchorus*	*actangulus* Lam.	K.V.S.4087	Shenoy, K.V.	Mumbra	26/08/1954		(sterile specimen)
O6268	Tiliaceae[now Malvaceae]	*Corchorus*	*actangulus* Lam.	15599	Shenoy, K.V.	Dadar, Bombay	00/08/1916	fr	(fr)
O6282	Tiliaceae[now Malvaceae]	*Corchorus*	*actangulus* Lam.	220	Merchant, Y.A.	Mumbra	07/09/1957	fl	Common, small plants, in flower or young fruit.
O6290	Tiliaceae[now Malvaceae]	*Corchorus*	*actangulus* Lam.	194	Kapadia, Z.J.	Borivili National Park, by the roadside	26/09/1953	fr	(fr)
O6291	Tiliaceae[now Malvaceae]	*Corchorus*	*actangulus* Lam.	S.H. 768	Herbert, P.S.	National Park, Borivili	08/10/1955	fl	A small undershrub, flowers few, fruits plenty.
O6292	Tiliaceae[now Malvaceae]	*Corchorus*	*actangulus* Lam.	S.H. 1518	Herbert, P.S.	National Park, Borivili	26/03/1956	fl	Same as S.H. 1517. A clearly prostrate herb, flowers yellow, few, in open grassland.
O6293	Tiliaceae[now Malvaceae]	*Corchorus*	*actangulus* Lam.	S.H.1517	Herbert, P.S.	National Park, Borivili	26/03/1956	fl	A clearly prostrate herb with numerous branhces in all directions, flowers yellow, few, collected from open grasslands.
O6294	Tiliaceae[now Malvaceae]	*Corchorus*	*actangulus* Lam.	S.H.1945	Herbert, P.S.	National Park, Borivili	10/08/1956	fl	Very common and sometimes abundant herb. Flowers yellow, solitary; extra axillary.
O6301	Tiliaceae[now Malvaceae]	*Corchorus*	*actangulus* Lam.	NYD 1980	Das, N.Y.	Usgaon(Tungar)	21/08/1960	fl	Annual, much branched plant, flowers leaf opened, common in wet places and at the bank of the running waters.
O6331	Tiliaceae[now Malvaceae]	*Corchorus*	*actangulus* Lam.	NYD 1804	Das, N.Y.	Mandvi(Tungar)	26/08/1960	fl	An erect herb, leaves pubescent, flowers yellow, common in plains.
O7042	Tiliaceae[now Malvaceae]	*Corchorus*	*actangulus* Lam.	NYD 4013	Das, N.Y.	Mandvi(Tungar)	28/09/1961	fl	An erect much branched herb, petals yellow, capsule 6 angled out of which 3 sides are winged. Common in open moist places.
59963	Verbenaceae[now Lamiaceae]	*Vitex*	*negundo* L.	NI 2889	Irani, N.I.	Rugby Park, Matheran	12/02/1959	fl	Erect shrub 4-12 feet high, growing in clumps in the open. Flowers bluish.
59991	Verbenaceae[now Lamiaceae]	*Vitex*	*negundo* L.	142-1	H.Santapau, S.J.	Mobar, near Malwan	00/03/1941		Near sea shore, on sandy ground.

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Carrier detection of Mucopolysaccharidosis I by assay of α – L – Iduronidase: A report in South Indian Population

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Abstract :

Mucopolysaccharidosis type I (MPS I) is an autosomal recessive, progressive, multi system lysosomal storage disorder caused by mutations in the α - L - Iduronidase (IDUA) gene. These mutations lead to the deficiency of the glycosidase, α - L - Iduronidase (IDUA), which is required for the degradation of the glycosaminoglycans (GAGs) heparan sulphate and dermatan sulphate and hence, their storage in the lysosomes. At present the disease is not curable, hence, carrier testing is the service most often requested by the affected families, second only to the demand for effective therapy. The present study is a report of a study undertaken in this perspective, in south Indian population. 102 clinically diagnosed patients were biochemically analysed and 11 of them were confirmed to be IDUA deficient patients (MPS I). The activity of IDUA was estimated in leukocytes for 34 controls (14 adults and 20 children) and found to have a mean specific activity of 27.2± 7.5 nmol/hr/mg protein in adults and 30.1± 9.7 nmol/hr/mg protein in children. The levels of the enzyme in 18 obligate heterozygotes were estimated and the mean specific activity was found to be 9.17± 4.07 nmol/hr/mg protein which was found to be little less than 50% of the mean in controls. Thus carrier detection of MPS I is clearly possible and recommends this method for examination of potential carrier status of this disorder in our population.

Key words: Mucopolysaccharidosis I, Lysosomal storage disorder, Glycosaminoglycans, α - L - Iduronidase, Carrier detection

Abbreviations used: GAGs-Glycosaminoglycans, IDUA - α - L - Iduronidase, MPS - Mucopolysaccharidosis

Introduction :

Mucopolysaccharidosis I (MPS I, MIM 252800) is an autosomal recessive disease that is caused due to the deficiency of the lysosomal glycosidase α - L - Iduronidase (IDUA, EC 3.2.1.76). It was one of the first of the ten disorders of the mucopolysaccharide metabolism described so far (Hurler, 1919). It is considered as the archetype MPS, and is the most common of the MPS in majority of the populations (between 1 in 100,000 and 1 in 150,000 live births (Lowry et al 1990, Meikle et al 1999, Poorthuis et al 1999).

MPS I is clinically delineated into three phenotypes namely Hurler, being the most severe Hurler-Scheie, with intermediate and Scheie with mild manifestations. However, currently the phenotypes are recognized as a continuous spectrum ranging from severe to mild phenotypes with progressive involvement of central nervous system, corneal clouding, skeletal abnormality, hepatosplenomegaly etc. These phenotypes are caused due to reduced or absence of IDUA activity required for degradation of glycosaminoglycans dermatan and heparan sulphate in the lysosomes. Progressive accumulation of these GAGs in lysosomes results in multi organ dysfunction and excess of them are excreted in urine of the patients (Ru et al. 2011; Beck et al., 2014).

Preliminary diagnosis of the patients is based on detection of GAGs in urine by qualitative and

quantitative methods. However, the definitive diagnosis is based on assay of the enzyme in leukocytes or fibroblasts. Families with MPS I often request for detection of carrier status. However, the analysis of IDUA activity does not provide definitive carrier information as there could be overlap between carrier and normal enzyme values. Unfortunately, heterogeneity of mutations that underlie MPS I and the technologies available to assess gene mutations do not currently allow for carrier detection by molecular methods (Neufeld and Muenzer, 2001).

The present study is a report of a study undertaken in this perspective in south Indian population. 102 clinically diagnosed patients were biochemically analysed and 11 of them were confirmed to be IDUA deficient patients (MPS I). The levels of the enzyme were estimated in 18 obligate heterozygotes and were found to be little less than 50% of the mean activity in controls. The results give an indication that carrier detection of MPS I by assay of IDUA in leukocytes is possible and recommends the use of this method for examination of potential carrier status of this disorder in our population.

Materials and Methods

Materials:

Alcian Blue 8GX, chondroitin - 4 - sulphate (bovine trachea), chondroitin - 6 - sulfate (shark cartilage), heparan sulfate (bovine kidney), dermatan sulfate (bovine mucosa) and keratan sulfate (bovine cornea), 4 - methylumbelliferyl β - D - glucuronide, 4 - methylumbelliferone, p - nitrocatechol, p - nitrocatechol sulphate were purchased from Sigma chemicals Co, St Louis, MO, USA. 4-methylumbelliferyl-α-L-iduronide was procured from Calbiochem Novabiochem Corporation, CA, USA. Dextran grade A (MW 200,000 - 275,000) was from BDH laboratories, Poole, England. Cellulose acetate membranes were purchased from Schleicher and Schuell, New Hampshire, USA. Precoated cellulose plates (cat. no.5552) were obtained from E-Merck, Darmstadt, Germany. All other chemicals used were of analytical grade. Glass double distilled water was used in all experiments.

Subjects:

Patients suspected to be suffering from MPS based on their clinical and radiological features were referred to our laboratory by different hospitals of Chennai city. These hospitals are national referral centres for patients with genetic disorders, hence the patient population were from different parts of south India and from different communities an economic background. Urine samples without any added preservative were collected from these patients and age and sex matched healthy children (Gospel Vision Ministry, Chennai; Meenakshi Clinic, Kanchipuram) and stored frozen at -70°C until analysis. Heparinized (15 IU/ml blood) venous blood (3-5 ml) was collected and transported to the laboratory at room temperature and processed immediately. Enzyme assay was carried out in patient blood samples. After confirming the MPS I patients by IDUA assay, heparinized venous blood was collected from the obligate heterozygotes (parents of MPS I patients) and sibs of the patients. Blood samples were also collected from age and sex matched normal children and normal adults to get the range of the enzyme values in normal individuals.

Urinary GAG analysis:

Isolation of Urinary GAGs: Urinary GAGs were isolated from 8 ml of centrifuged urine of controls and patients as described (Elango et.al., 1998; Mahalingam et.al., 2004). The final GAG pellet was dissolved in 120 µl distilled water and stored frozen at -20°C until analysis which was usually within a week.

Qualitative and quantitative analysis of urinary GAGs:

The amount of GAGs isolated from urine was estimated by acid alcian blue complex formation method (Elango *et.al.,* 1998; Mahalingam *et.al.,* 2004) and the results expressed as mg GAG/ mmol creatinine. The isolated GAGs were subjected to cellulose acetate membrane electrophoresis (Elango *et.al.,* 1998) and sequential multisolvent thin layer chromatography (Mahalingam *et.al.,* 2004; Dembure and Roesel,1991) to identify the type of GAGs present in the urine and hence classify the patients based on it. The enzyme assays were carried out on patients who

showed urinary excretion of heparan sulfate and dermatan sulfate.

Enzyme assays:

Various lysosomal enzymes were assayed in the leukocytes. Leukocytes were isolated from heparinized blood by dextran sedimentation method (Shapira *et al*., 1989). The final leukocyte pellets were washed with 0.85% sodium chloride and suspended in 0.4 ml distilled water. For the assay, the frozen leukocytes were thawed on ice and sonicated using a 3 mm probe at 25,000 g for 30 sec pulses for four times with 10 sec intervals. 0.1 ml of the direct lysate was kept aside for IDUA assay and the remaining was centrifuged at 12,000 rpm for 15 min at 4ºC. The supernatant was used to assay arylsulphatase B, β - D- glucuronidase and acid phosphatase. Protein was estimated in both aliquots (Lowry *et al.,* 1951).

The leukocyte IDUA was assayed by the fluorimetric method (Hopwood *et al*., 1979) using the substrate 4- methylumbelliferyl α-L- iduronide. Saccharic acid D - lactone was omitted in the assay and 15µg protein was used. β - D – Glucuronidase and acid phosphatase were also assayed using fluorimetric substrates namely 4- methylumbelliferyl β- D-Glucuronide and 4- methylumbelliferyl phosphate respectively (Shapira *et al*, 1989; Kolodny and Mumford, 1976). With each experiment a standard graph of 4 – methylumbelliferone (MU) was run. Arylsulfatase B was estimated colorimetrically using p-nitrocatechol sulphate as substrate (Shapira *et al.,* 1989) and p-nitrocatechol as standard.

Results and Discussion

Qualitative and quantitative analysis of urinary GAGs

To date, 11 enzyme deficiencies that result in 7 distinct types of MPS have been described (Neufeld *et al*., 2001). However, it is not practical to examine all known enzyme defects in every suspected case, considering the cost and time. A total of 102 clinically diagnosed patients were referred to our laboratory from different hospitals of Chennai city, over a period of five years. Urinary GAG analysis, both quantitative and qualitative analysis was carried out in all the patients. Based on the urinary GAG cellulose acetate membrane electrophoresis and sequential TLC, the patients who excreted heparan sulphate and dermatan sulphate were suspected to be MPS I/II/VI/VII. A total of 15 patients showed this pattern of urinary GAGs.

Estimation of lysosomal enzyme activities in patients

Although urinary GAG analysis helps in the differential diagnosis of MPS patients, the definitive diagnosis is based only on the lysosomal enzyme assays using artificial substrates in cultured fibroblasts or isolated leukocytes (Wraith and Jones, 2014). IDUA, arylsulphatase B (deficient in MPS VI), β-D glucuronidase (deficient in MPS VII) and acid phosphatase (control enzyme, marker enzyme of lysosomes) were assayed in the 15 patients suspected to be MPS I based on urinary GAG analysis. Of these, 11 patients showed very low or minimal residual activity of IDUA confirming them to be MPS I patients (Table 1, Fig.1). These patients showed activity in the normal range for the other lysosomal enzymes namely arylsulphatase B, β-D glucuronidase (deficient in MPS VI and VII) and acid phosphatase (Table 2, Fig.2).

Estimation of lysosomal enzyme activities in obligate heterozygotes

Enzyme assays were performed in 18 obligate carriers, ie parents of the nine enzymatically confirmed MPS I patients and seven sibs. From the results it is clear that, the obligate heterozygotes showed mean IDUA activities one third of that of the normal adults indicating them to be confirmed carriers. These individuals showed activity in the normal range for the other lysosomal enzymes (Table 1 and 2 and Fig 1 and 2). The sibs also showed values lower than the normal children indicating them to be border line carriers.

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Table 1: Mean specific activity of IDUA in normal and MPS I families

SL.No.	Group	Number Analysed	Mean Specific activity (nmol/hr/mg protein)
1	Normal Adults	14	27.2 ± 7.5 (14.6 - 39.5)
2	Fathers	9	9.7 ± 4.5 (6.2 - 21.01)
3	Mothers	9	8.6 ± 3.1 (4.8 - 15.2)
4	Normal Children	20	30.1 ± 9.7 (18.3 - 54.2)
5	MPS I patients	11	0.3 ± 0.6 (0 - 2.12)
6	Sibs	7	20.8 ± 3.6 (16.1 - 26.4)

( ) Parenthesis indicates range in each group

Fig 1. Mean specific activity of IDUA in normal and MPS I families

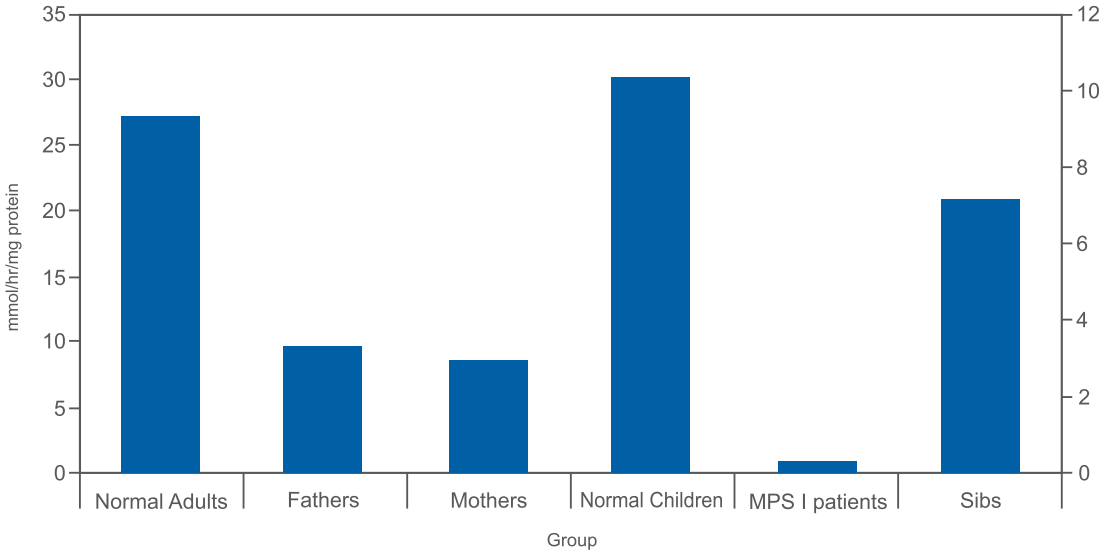
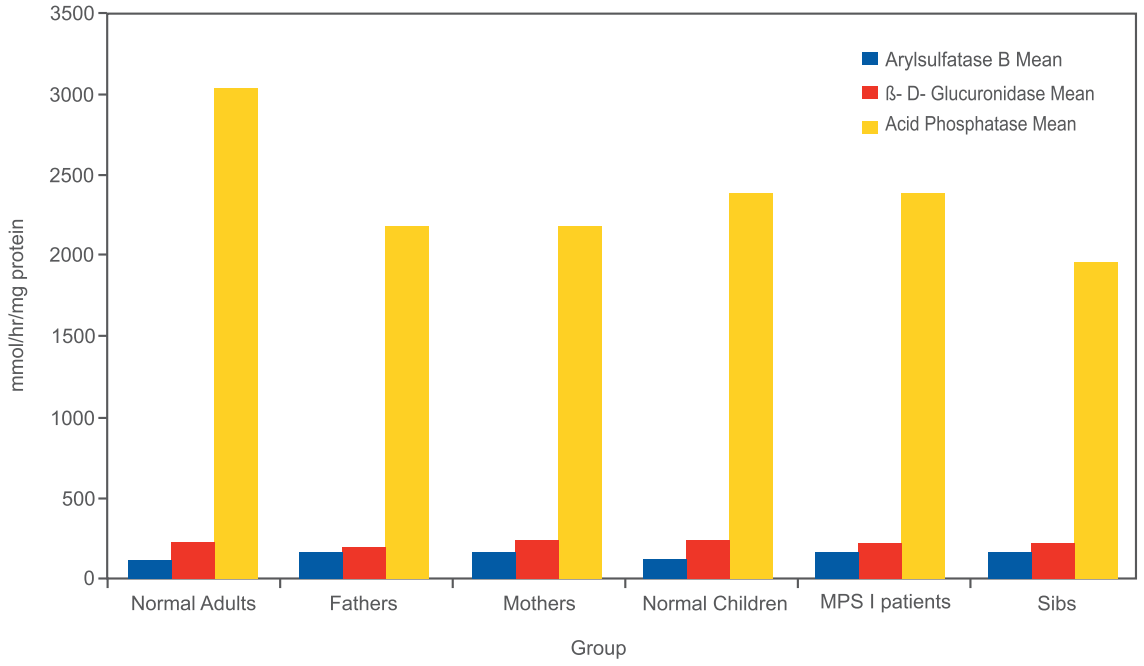


Table 2: Mean specific activities of other lysosomal enzymes in normal and MPS I families

SL.No.	Group	Number Analysed	Mean Specific activity and Range (nmol/hr/mg protein)		
			Arylsulphatase B	ß - D-Glucuronidase	Acid Phosphatase
1	Normal Adults	14	110.9± 27 (85 - 175)	110.9± 27 (85 - 175)	110.9± 27 (85 - 175)
2	Fathers	9	170.5±48.5 (80 - 220)	170.5±48.5 (80 - 220)	170.5±48.5 (80 - 220)
3	Mothers	9	165.4±41.3 (86 - 200)	165.4±41.3 (86 - 200)	165.4±41.3 (86 - 200)
4	Normal Children	20	112.4 ± 36.5 (55 - 175)	112.4 ± 36.5 (55 - 175)	112.4 ± 36.5 (55 - 175)
5	MPS I patients	11	138.8±39.7 (80 - 200)	138.8±39.7 (80 - 200)	138.8±39.7 (80 - 200)
6	Sibs	7	159.5 ± 38.5 (100 - 170)	159.5 ± 38.5 (100 - 170)	159.5 ± 38.5 (100 - 170)

( ) Parenthesis indicates range in each group

Fig 2. Mean specific activities of other lysosomal enzymes in normal and MPS I families



Conclusion:

All forms of MPS I have undetectable enzyme activity with currently available diagnostic assays. Hence, the residual enzyme activity cannot be used to predict the severity of the disease phenotype. The extent of clinical manifestation is thought to be related to the rate of turnover and the distribution of stored glycosaminoglycan in the body. Urinary glycosaminoglycan levels, although often higher in more severely affected patients, are not a reliable indicator of severity. It is widely accepted that mutational heterogeneity underlies the clinical heterogeneity of MPS I and that phenotype is largely determined by the type of mutation in the IDUA gene (Muenzer et al., 2009). However, the large number of single-occurrence mutations underlying MPS I has limited the predictive value of genotype for many patients (Beck *et al.,* 2014). Similarly, mutation analysis gives the clear indication of the carrier status of individual. However, due to lack of data on hotspots mutations being available in the Indian population (Verma *et al.* 2012, Jayesh *et al.,* 2013) it is difficult to detect the carrier status of individuals, making enzyme assay the alternate option. Hence, the study provides considerable evidence and recommends the assay of IDUA to detect the carrier status of individuals in our population.

Informed Consent

All procedures followed were in accordance with the ethical standards and informed consent was obtained from all patients and family members for being included in the study.

Acknowledgement

We thank the doctors from various hospitals in Chennai city who referred the clinically suspected MPS patients to our laboratory. Thanks are also due to Dr. Paula, The Gospel Vision Ministry, Chennai, and Dr. A. Meenakshi, of Meenakshi Clinic, Kanchipuram for providing samples from healthy children. Priya Sundarrajan and E.M. Elango would like to thank the Council of Scientific and Industrial Research, New Delhi, for financial assistance.

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A Multi-Criteria Decision Making (MCDM) Approach to Demarcating Potential Groundwater Zones Around Nandurbar City, Maharashtra, India

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Abstract :

Groundwater potentiality of an area depends on many factors such as topography, elevation, geomorphology, hydrogeological characteristics of the rocks and lineaments. In the present study the focus is on delineating the groundwater potential zones of around 640 sq.km area in and around Nandurbar, which is located close to Narmada-Tapi Lineament. Basaltic flows, which constitute major part of the lithology of the study area, are dissected by numerous dykes majority of them trending ENE-WSW direction. Various thematic maps were generated based on geology, dyke density, geomorphology, slope, drainage density, water level fluctuation and land use were used to analyze the data using Multi Criteria Decision Making (MCDM) techniques. Analytical Hierarchy Process (AHP) was used in systematically assigning weights for different parameters. Integration of all these parameters in Geographical Information System (GIS) platform helps to generate a groundwater prospect map of the area using weighted overlay analysis. The resultant map of the area was classified according to its groundwater potential in to 'good' 'moderate' and 'poor' zones. It is concluded that 172.8sq.km (27%) of the area falls under good category followed by 307.2sq.km (48%) falls in the moderate category and 160sq.km (25%). falls under moderate and poor category respectively. Such an attempt would help to formulate the policies for better management of groundwater resources in similar areas.

Keywords : Groundwater, MCDM, Nandurbar

Introduction :

Groundwater is one of the major source of water used to meet the domestic, industrial and agricultural requirements in India. Maharashtra being an agrarian state in India with diversity in topography and rainfall depends heavily on groundwater, i.e. 71% of total irrigation in the state is groundwater assisted (Duraiswami, 2008). Remote sensing and GIS techniques are widely used in recent times in groundwater studies (Meijerink et al., 2007; Waters et al., 1990). Data obtained from such sources are of great use for better management and exploration of groundwater resources. The synoptic view associated with the remotely sensed data enable people to interpret the indirect evidences on occurrence of groundwater such as: lineament, geomorphology, vegetation, drainage pattern etc.

Remote sensing (RS) data along with Geographical Information System (GIS) was used to interpret surface features and their relation to groundwater recharge and discharge (Corgne et al., 2010; Dar et al., 2010; Krishnamurthy et al., 2000; Mabee et al., 1994; Mahmood, 1996; Meijerink et al., 2007; Sander et al., 1997). A detailed review by Jha et al., (2007) list the core areas of focus for RS and GIS application in groundwater and also the application of such techniques in conjunction with field investigation to optimize the expanding potential of RS and GIS approach.

GIS based approach using hydrogeomorphological mapping was applied to suggest the site specific artificial recharge techniques in Deccan volcanic province (Ravi Shankar and Mohan, 2005; Saraf and Choudhury, 1998). Groundwater quality zonation mapping in Panvel basin was carried out using GIS techniques while delineating the areas vulnerable to contamination which helped to device area specific better management strategies (Anbazhagan and Nair, 2004).

Deccan basaltic province (DVP) is considered to be of multi-aquifer system due to the heterogeneity in the aquifer characteristics of successive flows

(Duraiswami et al., 2012; Kulkarni et al., 2000). Presence of dykes in some areas of DVP makes the situation more complex. Study area being heavily riddled with dykes, an approach using MCDM techniques using GIS was attempted to meet the main objective of the paper i.e. delineating the groundwater prospect zones in the study area.

Study area :

Nandurbar is primarily an Adivasi (Tribal) district located in the North-Western part of Maharashtra (Fig. 1), which was part of Dhule district till 1998. The area under study is located around the Nandurbar city, which is the district headquarter; between East Longitude 74° 05' 00" to 74° 25' 00" and 21° 15' 00" to 21° 25' 00" North Latitude. The total areal extent of the study region is approximately 640 sq. km and included within the Survey of India (SOI) topographical map numbers 46 K/3 and 46 K/7. The area is characterized by hilly regions in the south with maximum elevation of around 500m above mean sea level (msl). Towards north banks of Tapi river marks the boundary of the study area and the elevation is about 140m above msl. Numerous dykes having the major trend parallel to the Narmada-Tapi Lineament (ENE-WSW) stands out as ridges due to their resistance to weathering, which makes the topography undulated in the central and southern part of the study area. This part of Nandurbar receives an average annual rainfall of 797 mm. There are three cropping seasons in the area, viz, Kharif, Rabi and Zaid. Rabi and Zaid crops mainly depend on ground water availability.

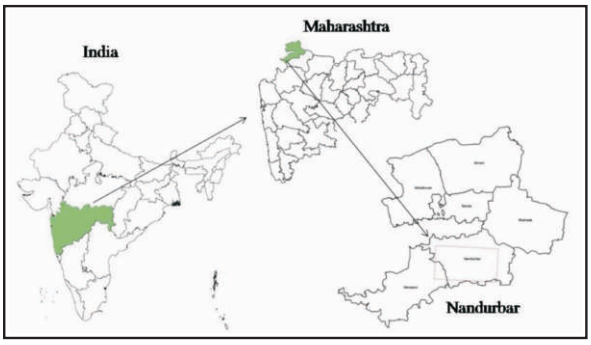


Fig. 1: Location map of the study area.

Data used and methodology:

Survey of India Topographical maps (46 K/3 and 46 K/7) were scanned in the TIFF (Tagged Image File Format) format and geo-referenced into Universal Transverse Mercator (UTM) spheroid and WGS-1984 datum. In the present study the Landsat satellite imageries (Path and Row: 147-45, acquisition date 05/03/2000) along with Indian Remote Sensing (IRS-P6) satellite LISS III (Linear Image Self Scanning Sensor) imageries (Path-Row: 95-57, acquisition date 18/10/2008, 11/3/2009, 28/04/2009) were used for extracting information related to groundwater, which was also re-projected onto UTM. Open access satellite data from Google Earth software of very high resolution was helpful in cross checking and interpreting the satellite data while digitizing various thematic layers. Satellite data interpretation was mainly carried out using visual and digital interpretation techniques. Prepared thematic maps were cross checked and validated with the subsequent field visit. ArcGIS 10.1 software was used for creation of digital database and integration of the various factors in Geographical Information System (GIS) platform.

Development of thematic layers and model for demarcating groundwater potential zones:

Groundwater potential of an area is based on various factors such as geology, lineaments, slope, geomorphology etc. Various thematic maps were prepared using the data collected during field work, topographical maps and interpretation of satellite imageries. Seven thematic layers based on geology, dyke density, geomorphology, slope, drainage density, water level fluctuation and land use were prepared digitally for better integration and evaluation. A model depicting the scheme while preparing the Groundwater potential zonation map is given in fig. 2. Classes under each thematic map were assigned values in a scale ranging from 1 to 10, where one is poor and 10 is considered excellent.

Assigning weights using AHP

Each thematic map was assigned a weight depending on its influence on their control on groundwater conditions. In hydrogeological investigation many parameters need to be taken in to account and quantification of the influence of each parameters is a great challenge for decision making. Many

researchers used knowledge based method for assigning weights for different thematic layers (eg. Dinesh Kumar et al., 2007; Jaiswal et al., 2003; Preeja et al., 2011; Rekha et al., 2011). A well established methods for assigning weights for different parameters for decision making is Saaty's Analytical Hierarchy Process (AHP)(Saaty and Vargas, 1991; Saaty, 1990, 1980)is used for this analysis. Saaty, (1990) proposed an absolute scale with a 1 to 9 index (Table: 1) for comparing the importance of parameters in decision making. According to this scheme of decision making a problem is divided in to various parameters, arranging them in a hierarchical structure, making judgments on the relative importance of pairs of elements and synthesizing the results (Saaty and Vargas, 1991).

This scheme of decision making involves two levels: first level is to set the goal (i.e. here groundwater potential zonation map) of the analysis and selection of the respective parameters (here thematic maps). Main objective of the second level is to derive the pair wise comparison matrix based on the thematic layers using Saaty's nine point scale of importance. Table: 2 shows the matrix comparing the seven themes in order to attain the priority along with the Eigen values. Consistency ratio and principal Eigen values are determined to check the inconsistency in judgments (Saaty and Vargas, 1991). Saaty suggest that ideally consistency ratio is <0.10 and if it is more than suggested value then it is recommended to go back and revise the comparison to minimize the inconsistency. The consistency Ratio for the pair wise comparison matrix for this analysis is 0.08 and the maximum Eigen value is 7.52. Detailed table below shows the distribution of weights and ranks for each thematic layer (Table: 3).

Results and Discussion

Geology :

Geology of the area is of great importance while evaluating the potentiality of an area for groundwater exploration and management. Geological map of the study area was prepared using extensive filed work (Fig. 3a). The area is predominantly composed of basaltic rocks and basic dykes of basaltic or doleritic composition. Quaternary alluvial deposits overlying the Deccan basalts were mapped in the north eastern part of the study area which is mainly deposited on the banks of Tapi River. Dug wells tapping the alluvial aquifers were found to be having better groundwater potential; however which also depends on the thickness and extent of alluvial formation. While assigning weights for the classes under lithology

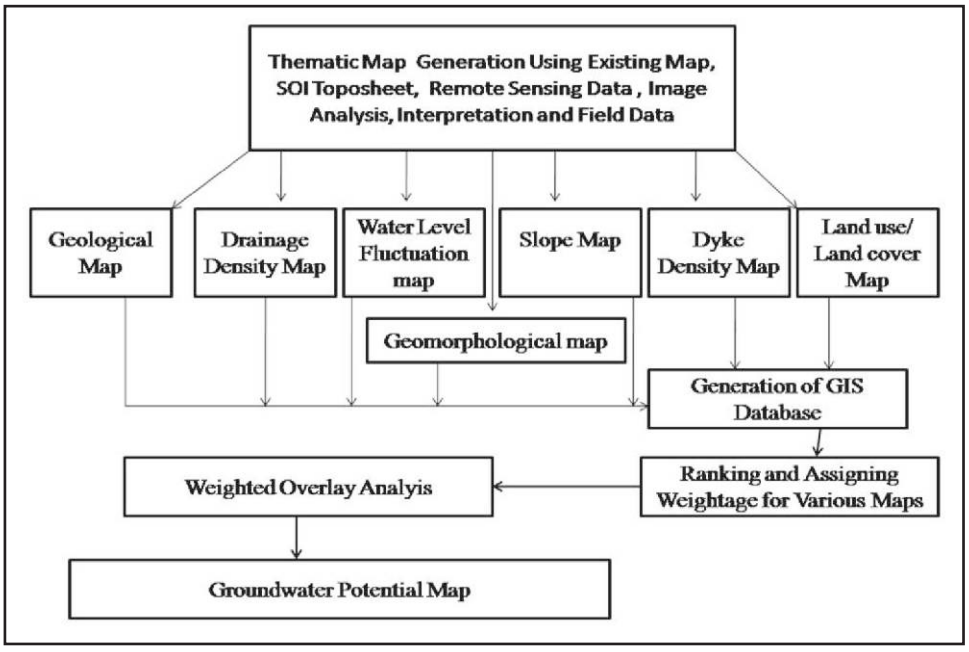


Fig. 2: Flow chart showing work plan used for deriving groundwater potential map

Table: 1 Saaty's Scale of importance

Intensity of Importance on an absolute scale	
1	Equal importance
3	Moderate importance of once over another
5	Essential or strong importance
7	Very strong importance
9	Extreme importance
2,4,6,8	Intermediate between the two adjacent judgments
Reciprocals	If activity ***i*** has one of the above numbers assigned to it when compared with activity ***j***. then ***j*** has the reciprocal value when compared with ***I***

Table 2: Pair wise comparison matrix and weightage assignment

	Geo	DrD	WLF	Slp	Geom	LuLc	DyD	Weights (Eigen vectors)
Geo	1	2	1	1/3	1/3	3	1/2	0.09859
DrD	1/2	1	1	1/4	1/3	1/2	1/2	0.0601
WLF	1	1	1	1/4	1/7	1/2	1/5	0.04989
Slp	3	4	4	1	1/3	3	1/2	0.17627
Geom	3	3	7	3	1	5	3	0.3497
LuLc	1/3	2	2	1/3	1/5	1	1/4	0.06610
DyD	2	2	5	2	1/3	4	1	0.19925

*Geology (Geo), Drainage density (DrD), Water Level Fluctuation (WLF), Slope (Slp),Geomorphology (Geom), Landuse Landcover (LuLc), Dyke Density (DyD).

alluvial formations were categorized as moderate based on the field observation. Secondary porosity in basalts are the main factor controlling the aquifer characteristics of basalt and is not uniform across the study area, so they were categorized as poor.

Slope:

Digital elevation model of the area was generated using the digitized contours from survey of India Toposheets which in turn used to prepare the slope map (Fig. 3b) of the study area. Rate of change of elevation (slope) has an important role in controlling the surface run-off and recharge. Slope map was further classified in to five classes based on the slope in degrees(Rao and Jugran, 2003; Ravi Shankar and Mohan, 2005) from <1° (Plain or nearly level), 1°-3° (Gently sloping), 3°-10° (Moderately sloping), 10°-20° (Steeply sloping) , >20° (Very steeply sloping). Steeply sloping areas favours run off and were considered poor areas for groundwater development with respect to moderately to flat areas which enhances infiltration. Plain areas were categorized as excellent and the other categories were given moderate to least importance with increasing slope values.

Geomorphology:

Geomrophological map (Fig. 3c) of the area was

prepared by the visual interpretation of the satellite imageries based on the classification scheme proposed by National Remote Sensing Centre (NRSC, 2008). Landforms in the area can be broadly classified based on their origin in to three types, i.e. structural, denudational and fluvial. These classes are further divided in to subclasses. Landforms of structural origin are further classified into low dissected hills and valleys (which include the dykes ridges) and the moderately dissected lower plateau. Denudational landforms are classified into moderately dissected lower plateau and pediment pediplain complexes. Landforms of fluvial origin are located mainly in the north-eastern part of the study area. Landforms of fluvial origin were categorized as excellent based on their relation to groundwater potential. Pediment-pediplain complex of denudational origin is the most dominant geomorphic unit in the study area. Weathered zones in this landform are potential zones of groundwater development. Landforms of denudational origin was characterized as moderate to good. Landforms of structural origin are mainly characterized by run-off zones hence are categorized as poor.

Land use/ land cover :

The scheme of classification proposed by National Remote Sensing Centre (NRSC, 2008)was used while preparing the Land use/ Land cover map (Fig.3d). The study area is composed of seven classes: Crop land/ Fallow land, Water bodies, Scrub land, reserved forest, Ravinous land, rural and urban areas. While assigning weights water bodies were given the top priority purposefully, keeping in mind that these are located at the gap in the dyke ridges. Crop land/fallow land was given moderate and the least values were given for scrub land. There are many rural settlements in the study area and are given higher weightage than and the urban area. Nandurbar city is located between two dyke ridges and groundwater resources in this region are overexploited and the city is heavily dependent on pipe water supply.

Drainage density :

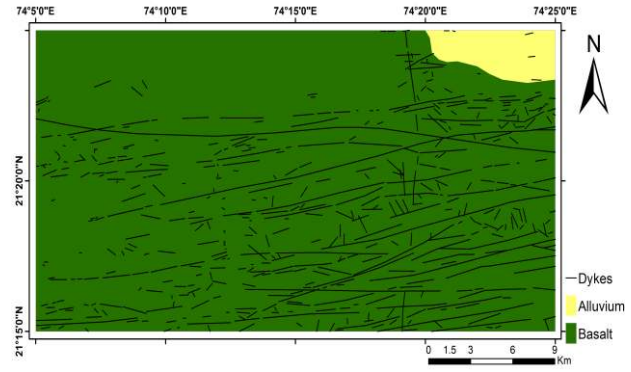
Drainage density is defined as the ratio of the total stream length to total area of the drainage basin (Strahler, 1964). Drainage density is important factor controlling the recharge characteristics of the terrain and can be correlated with permeability. Drainage density map (Fig. 3e) was prepared using the digitized drainage network data from topographical map. Drainage density of the area ranges from 0- 7.9 km/sq.km and was further classified in to five classes. Classes having low drainage density to high drainage density values were assigned values ranges from excellent to poor respectively.

Dyke density:

This area is characterized by the abundance of dykes and the major lineament in this area. Dyke map was prepared using onscreen digitization from the satellite imageries and data collected from field work. Dyke density map (Fig.3f) was prepared using the same method as in the case of drainage density map. Dyke density map was classified in to five classes. Lineaments and the surrounding areas are considered to be an excellent groundwater prospect zones. Hence the classes having high dyke density values were considered excellent compared to the classes with least values as poor. Hydrogeological conditions in this area are controlled by the existence of dykes as the dykes in this area are almost orthogonal to the regional hydraulic gradient.

Water level fluctuation :

Water level fluctuation studies for this area helped to understand the re-charging conditions over the years. Entire study area shows fluctuation in water levels in the range of few centimetres to maximum of 12.8 metre during adjacent two seasons (i.e. post-monsoon 2011 to pre-monsoon 2012 season). It ranges from few centimetres to maximum of 12.1 metres during post-monsoon 2012 to pre-monsoon 2013. The results are shown in Fig. 3g. It is evident that the rainfall has a direct correlation with water level fluctuations. Basaltic flows being multi-aquifer system, based on the field observations low fluctuation dug wells were giving low yield or rather dry during summer but the higher fluctuation wells were supplying water even during summer season. Hence higher values were given for greater fluctuation low values for less fluctuation.



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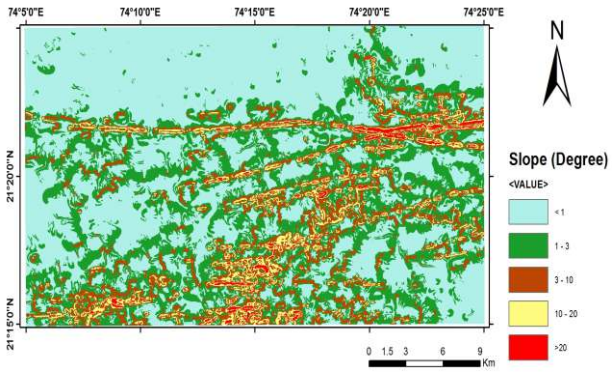

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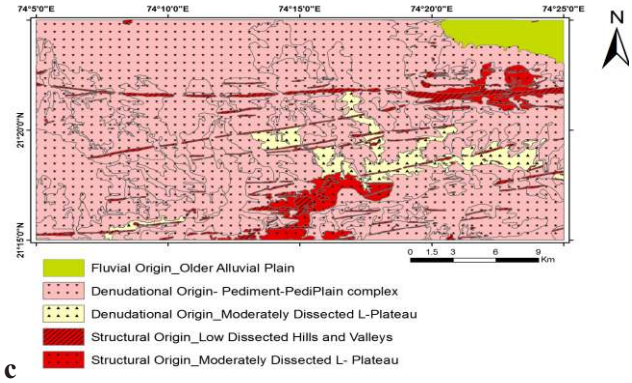

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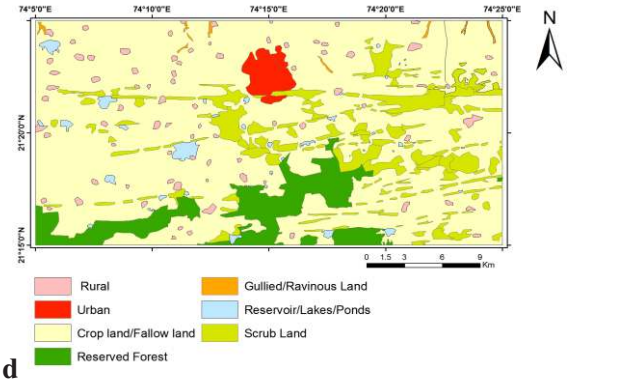

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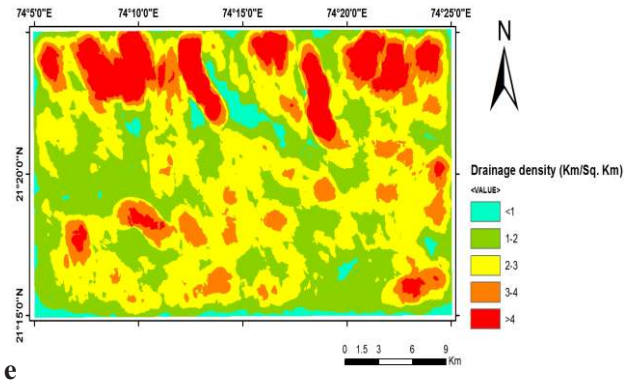

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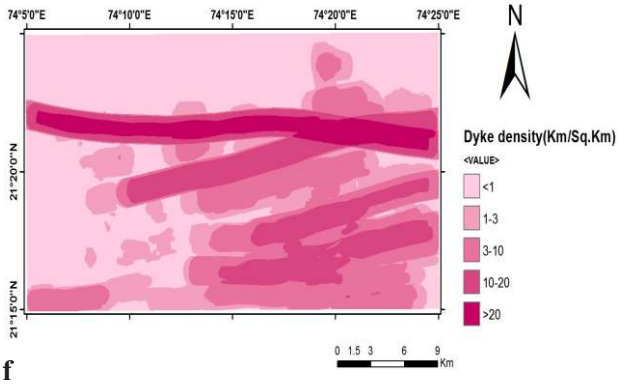

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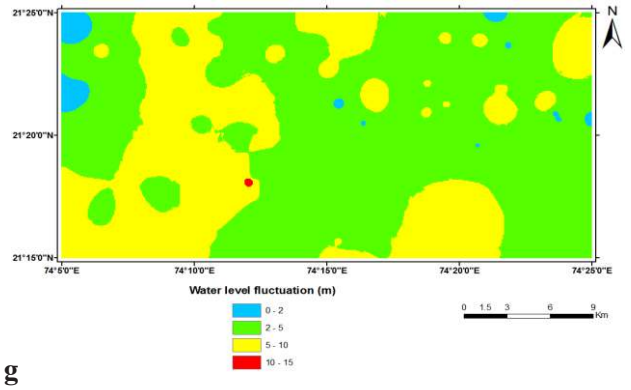

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Fig. 3: Thematic maps of various parameters

a. Geology
b. Slope
c. Geomorphology
d. Landuse land cover
e. Drainage density
f. Dyke density
g. Water level fluctuation

Table 3: Weights of individual themes and feature scores used for groundwater prospecting

Sl No	Thematic Layers	Map weight (Wt)	Individual features	Feature score (Wi)
1	Geology	10	Basalt	3
			Alluvium	5
2	Drainage density	6	<1	10
			1-2	9
			2-3	7
			3-4	5
			>4	3
3	Water Level Fluctuation	5	<1	5
			1-3	6
			3-6	8
			>6	10
4	Slope	18	<1	10
			1-3	8
			3-10	5
			10-20	3
			>20	1
5	Geomorphology	35	Fluvial- Older Alluvial	9
			Denudational-Pediment/pediplain	7
			Denudational–Moderately dissected	4
			Structural- Low dissected	3
			Structural-Moderately dissected	2
6	Land use/Land cover	7	Rural	4
			Cropland/ Fallow Land	5
			Urban	3
			Reserved Forest	5
			Gullied and Ravenous Land	4
			Reservoir/ Lake/ Pond	9
			Scrub Land	2
7	Dyke Density	19	<1	4
			1-3	5
			3-10	6
			10-20	8
			>20	10

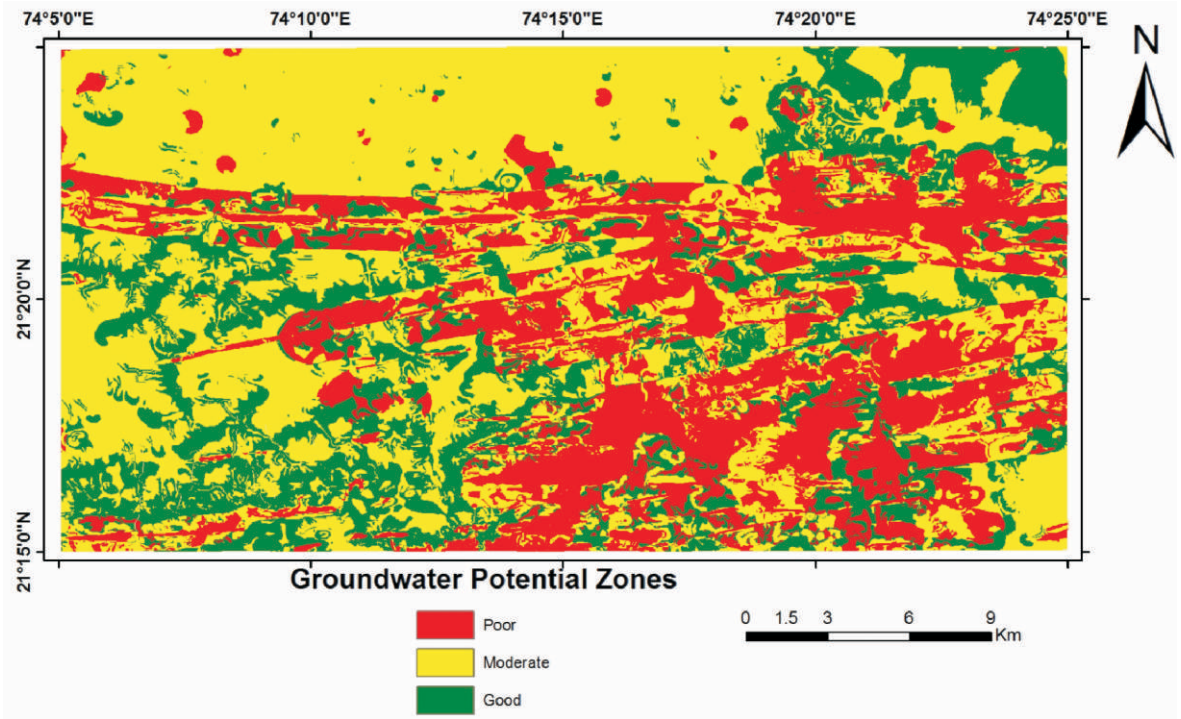

Fig. 4: Groundwater potential zonation map

Weighted overlay analysis:

The seven thematic maps were reclassified and assigned weights as per the mathematical calculation discussed above (Table 3). Individual features in each thematic map were given knowledge based ranking as discussed. Reclassified thematic maps were overlaid using weighted overlay function in spatial analyst module of ArcGIS 10.1. to prepare the final map showing the groundwater prospect zones (Fig. 4).

Conclusions

The data gathered from 88 observation wells and the field observations were used while preparing the thematic maps. Conventional methods would ideally based on one or two themes while analyzing the results. MCDM techniques were very useful to formulate and interpret the results in a GIS platform. Dykes being the major lineaments in the study area it is observed that they have a greater control on the hydrogeological set up of the area. Good groundwater prospect zones in resulted map also match with the topographical lows in dykes which were validated by the field observations.

The resultant map was reclassified qualitatively to classify the area based on the groundwater potential into good (27%), moderate (48%) and poor zones (25 %). Groundwater potential map prepared using MCDM technique was validated in the field and it corroborate with the field observations. Dykes in this areas can also be used effectively for the managing the groundwater recharge structures. This in turn can help to formulate the strategies to improve the groundwater prospects of moderate to poor areas in the study area. Such an attempt is of great use while planning the groundwater development and management in similar areas around the world.

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Isolation and study of Cellulolytic Alkalophiles from soil.

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Abstract :

Cellulases, a group of enzymes which catalyze the hydrolysis of cellulose and related cello-oligosaccharide derivatives, is considered a potential tool for industrial saccharification of cellulosic biomass. An economic process for its production is thought to be critical for the successful utilization of cellulosic materials. Towards this end, work was initiated to isolate bacteria with high cellulolytic activity, from sources such as soil. Samples were plated on Avicel/ CMC Congo Red -agar plates at pH 9.0 and the cellulose-degrading bacteria were detected by the zones of clearance around the colony. Out of 19 isolates six of them showing very good zone of clearance were selected for further study. All the isolates grew at temperature ranging from 4 - 60°C. Some of the isolates showed observable enzymatic activity at high pH and temperature. The extent of utilization of various substrates like avicel, bagasse and paper by these isolates was studied by measuring the amount of free reducing sugar in the medium. Sugarcane Bagasse was utilized well whereas paper did not prove to be a good substrate. The three isolates which could tolerate pH of 10 and could grow at 60°C have the potential to be used in industry and also in waste treatment.

Keywords: Cellulolytic organisms, Carboxy methyl cellulose, Avicel, Congo red staining.

Introduction:

Plant structural polysaccharides provide a major source of nutrients for ruminant livestock, and have the potential, through microbial fermentations, to provide renewable substrates for the chemical, pharmaceutical and feed industries (Coughlan MP, 1985 a,b; Bhatt and Bhatt, 1997). The potential cellulase market has been estimated to be as high as US$400 million per year (van Beilen and Li, 2002). The large market potential and the important role that these enzymes will play in the future energy and bio-based product industry drives researchers to develop better cellulase preparations for plant cell wall cellulose hydrolysis. There is a need to find cellulases that have characteristics necessary for biorefineries, such as higher catalytic efficiency on insoluble cellulosic substrates, increased stability at elevated temperature and at a certain pH, and higher tolerance to end-product inhibition. There are three major types of cellulose-degrading enzymes: Endo-β-1,4-glucanase (1,4-β-D-glucan-4-glucanohydrolase; EC 3.2.1.4), Exocellobiohydrolase CBH (1, 4-β-D-glucan glucohydrolase; EC 3.2.1.74) and β-glucosidase (β-D-glucosideglucohydrolase; EC 3.2.1.21). The endoglucanase randomly hydrolyzes the β-1,4 bonds in the cellulose molecule, and the exocellobiohydrolases in most cases release a cellobiose unit which is subsequently converted to glucose by a β-glucosidase (Bhatt and Bhatt, 1997).

Materials and methods:

Soil Samples : Soil samples were collected from agricultural farms located in South Karnataka.

Isolation of cellulolytic bacteria

Soil samples were 1:10 diluted and 100uL of the same were streaked on sterile ACR (Avicel Congo Red agar peptone 0.2%, $MgSO_4$ 0.025%, K_2HPO_4 0.03%, Na_2CO_3 0.02%, avicel 0.2%, Congo red 0.02%) and CCR (composition same as above with avicel replaced with carboxymethyl cellulose, CMC) pH 9.0 plates, incubated at 37°C for 48 hours. The cellulolytic bacteria showed clearance around their colonies. Six isolates were selected based on their size and clarity of the zones. They were further sub cultured on sterile ACR as well as sterile nutrient agar to obtain pure culture and to study their colony characteristics.

Alternate methods for screening of cellulose degraders:

The isolates were streaked on Avicel broth (ACR broth without Congo Red) and incubated at 37°C overnight. The plates were then stained with 0.2% Congo red for

30 min and destained with 1M NaCl. Alternate staining methods tried include using lactophenol blue and 0.1% trypan blue for 30 min followed by destaining with distilled water.

pH and Temperature tolerance

The selected isolates were screened for growth at different temperature and pH. They were streaked onto a ACR plates at pH ranging from 8 to 10.5 and incubated at different temperatures overnight. The pH of the media was adjusted using 0.1N HCl and 2N NaOH. The temperature range was from 4°C to 60°C.

Supernatant assay

The six isolates were grown in Avicel broth at 37^{0}C for 48 hours on the shaker at 150rpm. One milliliter of each of the culture was spun at 12,000rpm for 10 minutes at 4^{0}C. Supernatants were collected and frozen at -20^{0}C. Cell pellet was treated with 100µl of 0.1% tween 80, incubated for 30minutes at room temperature and centrifuged at 5000 rpm for 10 minutes at room temperature. The pellet-supernatant thus obtained was collected and stored at -20^{0}C. The supernatant and cell pellet suspensions were spot plated on CMC and Avicel assay plates of pH 6, 7, 8.2, 9, and 10 (CMC/ Avicel assay agar : 0.05M citrate phosphate buffer at pH 6, citrate phosphate buffer pH 7.0, Carbonate-Bicarbonate buffer pH 10.0, Bicarbonate-NaOH buffer pH 11.0, 2% Agar, 0.2% avicel/ CMC). The plates were incubated for 3 hours and were stained with Congo red for 30 min followed by destaining with 1M NaCl. (Patel et al., 2004).

Cellulase assay

Each of the isolates were inoculated in to Avicel broth and incubated at 37°C on shaker for six days. The culture supernatants were then assayed for free reducing sugars using DNSA method (Wood and Bhat, 1988).

One unit (U) of FPase, Avicellase and CMCase activity was defined as the amount of enzyme, which under the conditions used releases an amount of reducing sugars equal to 1 µmole of glucose in one minute. Dextrose 1 mg/ml was used to as a standard with a working range of 50µg to 500µg. The standard assay was performed as given in Table 1 below:

Enzyme assay using sugarcane bagasse and filter paper as substrate

Each of the six isolates were inoculated into 10 ml medium containing 500mg of sugarcane bagasse and filter paper (containing $MgSO_4$ 0.025%, K_2HPO_4 0.03%, Na_2CO_3 0.02%, Peptone 0.2%). After incubation for 6 days on shaker at 37°C the supernatants were assayed for free reducing sugars using DNSA method.

Table1 : DNSA assay with standards.

Concentration of sugar (µg)	Dextrose 1mg/ml(µl)	Distilled water (ml)	DNSA (ml)		Distilled water (ml)
Blank	- -	1.0	1.0	Heat for 10minutes in boiling water bath and cool	3.0
50	50	0.95	1.0		3.0
100	100	0.90	1.0		3.0
200	200	0.80	1.0		3.0
300	300	0.70	1.0		3.0
400	400	0.60	1.0		3.0
500	500	0.50	1.0		3.0

Results and Discussion:

Isolation and screening of cellulase degraders

The soil suspension was spread plated on ACR and CCR plate of pH 9, incubated for overnight at 37^0C.Twenty two isolates were obtained from CCR plates and 19 isolates were obtained from ACR pH 9 plates with two fungi isolates. ACR plate showed strongly cellulase positive organisms (Ref fig 1). From the grid plate 6 bacterial and 2 fungal isolates were selected. The isolates were named as S52, S511a, $S_5$11b, $S_5$20, $S_5$21, S_5C, $S_5$3F and S_5F. S_5F and $S_5$20 showed blue coloration around the colony which indicates that the organism produces some acid which changes the color of the pH sensitive Congo red to blue colour.

Isolates were streaked on ACR, ATB (Avicel Trypan blue is the same as ACR in composition but Congo red stain is replaced with 0.01% Trypan blue (R Berlemont et al 2009))and Avicel medium plate to compare other dyes with Congo red for selection (Ref fig 2). Out of the three screening and isolation methods, ACR plate showed observable zone of clearance followed by Avicel plate stained with Lacto phenol cotton blue and then ATB plate which showed clearance just at the edges of the culture growth. Trypan blue can also be used to screen the organisms, however the concentration used in this case was very less, so for further standardization with higher concentrations of Trypan blue is needed.

pH and Temperature tolerance

This experiment was done to test the temperature and pH at which the isolates can survive. In this, the isolates were streaked on ACR plates at different temperatures and different pH, incubated for 24hours.

Qualitative assay for endocellulases:

Spot assays done with culture supernatants and their processed pellets gave us an insight about the nature of the enzyme, whether it is secreted out or it remains intracellular. Enzyme activity was found in both pellets as well as the supernatants. Tween-80 added in the negative control also showed a zone of clearance, hence there is a need to replace the Tween-80 with an alternative agent for cell lysis (Ref fig 3).

Table 2: pH and temperature tolerance of the six isolates

$S_5$2:

Temperature (^{0}C) pH ↓ →	4	25	37	50	60
8	-	+	+	+	++
9	-	+	++	++	++
10	-	+	+++	++	+
10.5	-	-	-		

$S_5$11a:

Temperature (^{0}C) pH ↓ →	4	25	37	50	60
8	-	+	+	+	+
9	-	+	+	-	+
10	-	+	+	-	-
10.5	-	-	-		

$S_5$11b:

Temperature (^{0}C) / pH ↓ →	4	25	37	50	60
8	-	+	+	+	++
9	-	+	++	++	++
10	-	+	+++	++	+
10.5	-	-	-		

$S_5$20:

Temperature (^{0}C) / pH ↓ →	4	25	37	50	60
8	-	+	+	+	+
9	-	+	++	+	+
10	-	+	+++	+	+
10.5	-	-	+		

$S_5$21:

Temperature (^{0}C) / pH ↓ →	4	25	37	50	60
8	-	+	++	-	+
9	-	+	++	-	-
10	-	-	-	-	+
10.5	-	-	-		

S_5C:

Temperature (^{0}C) / pH ↓ →	4	25	37	50	60
8	-	+	+	-	-
9	-	+	+	-	-
10	-	-	++	-	-
10.5	-	-	-		

\+ : Very light zone of clearance seen
++ : Very clear zone of clearance seen
\- : No zone of clearance seen

All the isolates can be classified as mesophiles as they could grow in the range of 20-40^{0}C. $S_5$2, $S_5$11a, $S_5$11b, $S_5$20 and $S_5$21 could grow up to 60^{0}C. All six isolates could tolerate and grow at the alkaline pH of 10.0

Quantitative estimation of Cellulases : Sugar estimation by DNSA

Each of the isolates were inoculated in to Avicel broth and incubated at 37°C on shaker for 48 hours. The culture supernatants were then assayed using DNSA method. $S_5$3F produced maximum amount of sugars followed by $S_5$11b, with least amount being $S_5$2 (Ref Fig 4).

Determination of the extent of utilization of sugarcane bagasse and filter paper as substrate by the isolates

The utilization of bagasse and filter paper was tested to find out that how far the isolates could shift to industrial use and for waste management. The isolates were inoculated in 10ml of medium containing 500 mg bagasse and filter paper. The tubes were incubated on shaker at 37°C for six days. The culture supernatants were assayed for free reducing sugars using DNSA method. Sugarcane bagasse was found to be utilized better than filter paper.

Table 3: Quantitative estimation of Cellulases : Sugar estimation by DNSA

Supernatant of isolates	Absorbance at 540nm	Amount of sugar produced (µg/ml)
$S_5$2	0.01	21.27
$S_5$11a	0.02	30.36
$S_5$11b	0.06	48.54
$S_5$20	0.04	66.72
$S_5$21	0.049	56.72
S_5C	0.012	23.09

Table 4: Enzyme assay to determine extent of utilization of sugarcane bagasse and filter paper as substrates.

Culture	Reducing sugar (µg) generated in medium containing	
	Sugarcane bagasse	Filter paper
$S_5$2	1.777	0.101
$S_5$11a	1.986	0.026
$S_5$11b	1.842	0.072
$S_5$20	1.895	0.044
$S_5$21	1.753	0.080
S_5C	0.481	0.061
$S_5$3F	1.935	0.033
S_5F	1.798	0.036
Control	0.329	0.015

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Colour Plates

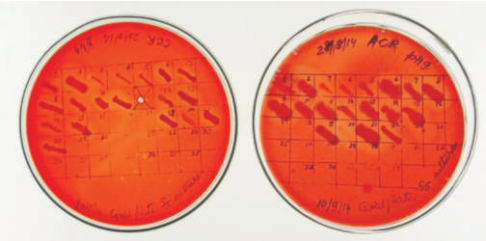

Fig.1: Grid plate of the isolates on ACR and CCR pH9.

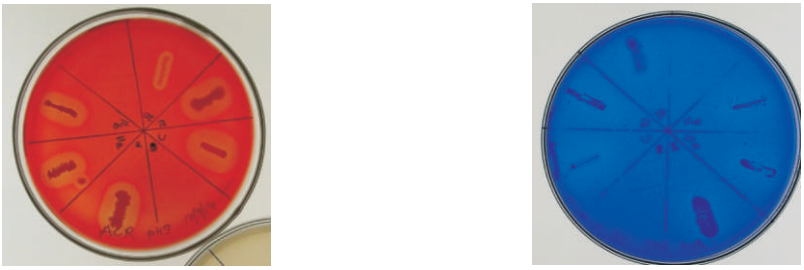

Fig2: a) ACR pH9 with 6 isolates **b) ATB plate with the six isolates.**

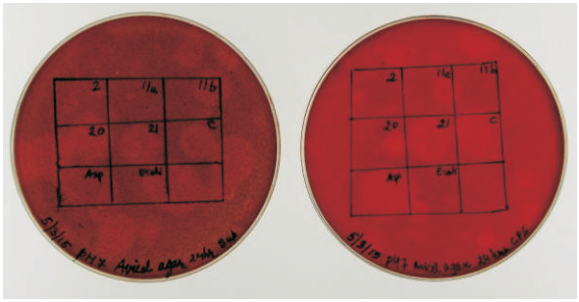

Fig3: Plate assay done on CMC plate pH 7.0 using supernatant and cell pellet suspension of 24hr grown culture

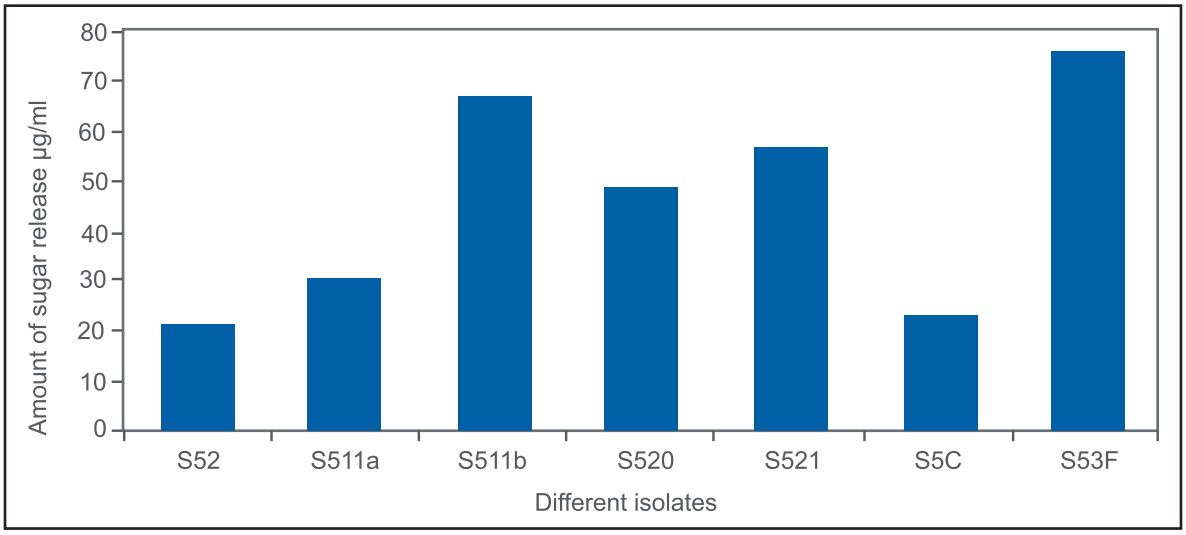

Fig.4: Cellulase assay for the six isolates.

CONCLUSION :

1. On screening of the given soil sample 19 isolates were obtained on ACR pH9 plates. Two of them being fungal isolates. Six bacterial isolates were selected from ACR plate and were named as $S_5$2, $S_5$11a, $S_5$11b, $S_5$20, $S_5$21, S_5C,

2. All the isolates grew in the temperature ranging from 25 - 40^{0}C. Only $S_5$2, $S_5$20, $S_5$21 of them showed observable enzymatic activity at high pH and temperatures.

3. The enzymes produced by these isolates are extracellular in nature, although this needs to be confirmed.

4. Avicel and Sugarcane Bagasse were used very well as substrates, whereas paper did not show promising results.

5. Lactophenol blue can be used in an similar way as Congo red for screening of cellolytic organisms. The concentrations of trypan blue to be used needs to be standardized.

Further studies need to be carried out to characterize the organisms including the fungal isolates and identify them using cultural and phylogenetic methods. The enzymes need to be purified and quantified. These strains or the enzymes can then be used for saccharification of cheap substrates to be converted to biofuels. The method is promising to meet part of the energy needs of the world in future.

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Nutritional Status of Undergraduate Students - A Case Study

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Abstract:

As part of the second Continuous Internal Assessment, students of the cross-faculty course on 'Nutrition and Reproductive Health' were asked to fill in a modified 24-hour Dietary Recall Sheet' along with levels of daily physical activity, exercise, and sleep patterns. Assessment of the recall sheets revealed erroneous eating and sleeping habits, indicating a prime cause for complaints of mental and physical fatigue prevalent amongst students of the class. Results of this study can be extrapolated to a majority of today's student generation, and are, therefore, shared in this article with the aim to generate awareness about appropriate dietary and nutritional habits.

Key words: Dietary assessment, Nutrition, Sleep, Exercise, Health

INTRODUCTION :

With 'Lifestyle Diseases' on the rise in today's young adults (Singh, 2014), a detailed study of the nutritional status of the youth has become essential. Increasing instances of Type 2 Diabetes mellitus and obesity have been noted in recent years with desk jobs and a sedentary lifestyle being the norm (Singh, 2014; Patel et al., 2015). The development of technology and medical aids, although beneficial in several respects, has led to a switch from an active lifestyle to a sedentary one, with minimal physical activity and excessive eating and drinking. Work-related stress and odd work timings lead to irregular meal times and the fad for quick, unhealthy 'on-the-go' meals. Nutritional balance is lost, leading to increasing complaints of headaches and loss of stamina indicating high levels of mental and physical fatigue (NNMB, 2000; Singh, 2014).

Common complaints of tiredness and low stamina from a majority of the students in the cross-faculty course on 'Nutrition and Reproductive Health' led to the conduction of the second Continuous Internal Assessment (CIA-2) in a unique fashion as detailed below. The exercise was an eye-opener in more ways than one. The results and a few student responses are shared herewith.

METHODS :

Sample size. 59 students from the cross faculty course on 'Nutrition and Reproductive Health', between19 -21 years of age, from differing social and economic backgrounds participated in the exercise.

Methodology. Of the several methods previously adopted to assess dietary data (Feskanich et al, 1988-1994, Gorstein, 1989; Thompson & Byers, 1994; Johnson, 2002; Yunsheng et al, 2009), the 24-hour Dietary Recall method is by-far the most popular and easy to follow, and was selected as the method-of-choice in this study. As part of their CIA-2 assignments, students were required to fill in a modified 24-hour dietary recall sheet (Figure-1) for a period of one week (7 continuous days). The daily diet (major meals and snacks, total liquid intake, alcohol consumption, if any), physical activity (standing, sitting, walking, running, climbing stairs etc.), exercise (type, duration, frequency) and sleep patterns (type – disturbed or undisturbed, duration) were recorded. Any major deviations from the normal daily routine were to be noted in the 'Other comments' section of the recall sheet. At the end of the 7-day period, the collected data were analyzed by the students in terms of food group coverage and intake quantity, water (liquid) intake, physical activity and exercise, calorie consumption and expenditure, and sleep patterns. The observed values were compared to the Recommended Dietary Allowances (RDA) specifically for the Indian population for age group 18 years and above, as per the guidelines provided by the National Institute of Nutrition, Hyderabad, an ICMR institute (Gopalan et al, 1989; NIN, 2011); and to world standards provided by the World Health Organization

(WHO, 1963) and United States Department of Agriculture (USDA website). Completed assignments were submitted online through the Moodle software. Post evaluation by the course instructor, the results of the study were discussed in class to draw meaningful interpretations to aid suitable changes in the students' diets and their lifestyle. Students were asked to consult their physicians and dieticians wherever necessary. The author's independent interpretations are presented in this research paper.

RESULTS :

The results presented below are those compiled from 58 out of the 59 students. One student had undergone a major surgery and was asked to compare and analyze pre- and post-operative diet, the results of which are not discussed here. Two students contracted chicken pox and a stomach ailment, three and four days into the assignment, respectively, after which they followed diets prescribed by their physicians. In these two cases, data corresponding to the first three and four days alone are considered.

Food group inclusion and intake quantity. It was observed that in all 58 cases, the diets were chiefly rich in carbohydrates, followed closely by proteins (Table-1). Students consuming a vegetarian diet were especially seen to lack proteins as compared to those consuming a non-vegetarian diet. A severe deficiency of vitamins and minerals was observed due to the lack of vegetables, and especially fruits in the diet. Only 5 out of 58 students (8.6%) consumed a fruit or drank a fruit juice at least once everyday. Milk and dairy products too were consumed in lesser proportions. The incidence of consuming fast food (French fries, *vadapav*, bakery products, ice creams) and carbonated drinks instead of lunch or dinner was prevalent in 48.27% cases, leading to a higher intake of fatty foods, sugar and salt. Students attributed this to the long hours spent in the College. It must be however be noted that the students made a conscious choice to consume fast food over the healthier options on offer in the College canteen. Most students observed that consuming fast food made them feel disinterested and lethargic, as compared to eating home-cooked meals. This corroborates previous observations (Kaushik, 2011; Vaida, 2013). Additionally, mixing of certain types of food like milk with citric fruits and milk with *panipuri* was found to induce stomach unease in a few cases.

The overall trend tended towards a highly unbalanced diet with most food groups not being represented in accordance with the food pyramid (Figure-2) (NIN, 2011) and in proportions/ serving sizes much below the RDA for Indians of age group 18 years and above (NIN, 2011). The complete exclusion or the deficiency of one or more food groups is detrimental to the health of an individual. One must remember that - “The major food groups shown in the three lower sections of the Food Pyramid *provide some, but not all* of the nutrients needed. Foods in one group cannot replace those in another. No one food group is more important than another – for good health, one needs them all” (USDA, 1992). Therefore, starving oneself of one or more food groups ('low carb'/ 'low fat' diets) in order to lose or gain weight is extremely injurious to the mental and physical well-being of an individual, and must be strictly avoided. The only exception might be made in the case of a person suffering from a disease or disorder wherein a trained dietician and medical practitioner specifies a certain diet to complement the health condition and the medication being given. One student was found to suffer from an eating disorder and asked to consult a trained physician and dietician with immediate effect.

Meal timings. Extremely erratic meal timings were observed in 90% of the cases studied. There was no fixed time for lunch and dinner. In several cases, dinner was eaten close to midnight. The interval between any two meals [breakfast – lunch – evening snacks – dinner] was found to be very large, in some cases as large as 7.5 (lunch at 1 PM followed by dinner at 8:30PM) and 10 hours (breakfast at 5AM followed by lunch at 3PM) on all 7 days. Erratic meal timings are a major cause of acidity problems, gastric ulcers and decreased brain activity (Mahan & Escott-Stump, 2004). As per an individual's biological clock, the sensation of hunger at particular times during the day (or night) causes the stomach to produce hydrochloric acid (< pH 2) to break down the food. However, when no food is consumed during such time, the acid starts to eat up the stomach and intestinal lining causing gastric ulcers, which in some severe cases leads to stomach cancers (Mahan & Escott-Stump, 2004). Lack of regular and timely food intake lowers blood

glucose levels and decreases brain activity as glucose is the sole source of energy to the brain (Mahan & Escott-Stump, 2004). To sustain itself throughout the day, the human body and brain require a steady supply of nutrients and therefore eating small meals at regular intervals of 2-4 hours is recommended, rather than larger meals after long intervals.15% students skipped one of the major meals - either breakfast, lunch or dinner. However, each one of these students reported a strong correlation between skipping meals and feeling tired, fatigued and incapable of performing daily chores. Those skipping breakfast noted that they felt extremely low in energy and sleepy throughout the day.

Water intake. All except one student claimed to be drinking much less than the recommended 2.2 litres of water (NIN, 2011). All agreed that their fluid intake needed to be increased considerably. Only one student mentioned consuming alcohol at a social gathering.

Physical Activity and Exercise.100% students agreed to a moderately active lifestyle. Only 28% (16 out of 58) students exercised regularly (atleast 3 times a week, 45 min – 1 hour sessions). This included working out in a gym, playing sports like football and basketball, practising yoga and going for brisk walks and jogs (Figure 3). The rest did not indulge in any type of exercise. The only physical activity for the majority 72% included walking to and from the railway station, climbing up and down stairs in the college and changing classrooms between lectures. This is a disturbing trend with implications in causing a spectrum of lifestyle diseases like Type 2 diabetes mellitus, obesity and weakening of the muscular, skeletal and immune systems of the body (NNMB, 2000; Singh, 2014; Matsuzaki et al, 2015; Patel et al, 2015).

Calorie Consumption and Expenditure. 100% students noted that they were calorie-deficient despite the erroneously presumably calorie-sufficient diets they consumed. The diets fell short of calories to account for the Specific Dynamic Action (Thermic Effect) of food (Matsuzaki et al, 2015) as also the daily physical activity and exercise routine. Figure 4 compares the daily calorie intake and expenditure (including physical activities and exercise) of students. According to the RDA values for an Indian population in the age group of 18 years and above performing moderately intensive work, the average daily calorie intake for females weighing 55kg is 2230 kcal and for males weighing 60 kg is 2730 kcal (NIN, 2011). All members in the study group fell severely short of this daily energy requirement, which when combined with vitamin and mineral deficiency and low water intake could be a prime cause of the complaints of fatigue and loss of stamina prevalent in the class. Except for the 16 students exercising on a regular basis who expended between 800-1000 kcal/day, the energy expenditure for most others was found to be very low, well below 600 kcal/day (Figure 4). A balanced increase in both, the calorie consumption and calorie expenditure, alongwith a balanced intake of other nutrients, is advisable in order to maintain a healthy and fit lifestyle.

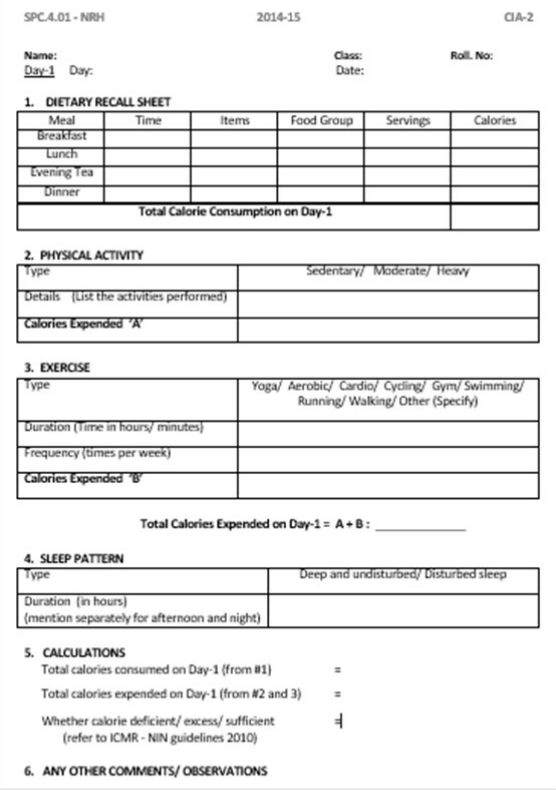
Sleep patterns. It was extremely alarming to note that 29.31% students got fewer than 4 hours of sleep in a day (Figure 5). While 50% got between 4-6 hours of sleep, it must be mentioned that this was divided between an afternoon siesta and night sleep, and was not in a continuous span of time. Most students sleeping for more than 8 hours slept at a stretch, with only 2 students taking a short afternoon nap. Additionally, 11 out of 58 students (18.96%) suffered from disturbed sleep. This was especially true for students eating heavy dinners post 10 PM. It was observed that in all such cases, the students woke up feeling exhausted and lethargic. At night, the body is tired and requires rest. Adding an excess burden of the energy-intensive digestion process leads to further bodily and mental exhaustion (Mahan & Escott-Stump, 2004). It is advisable to have a light meal about 2-3 hours prior to retiring at night. Disturbed sleep was also evident in students using electronic devices for prolonged periods before retiring to bed, and also in most students closer to CIA submission deadlines.

Student Feedback. Student feedback was taken at the end of the 7-day exercise. Excerpts from the feedback are briefly summarized here. Most students found the exercise to be beneficial as writing down what they ate over a 7-day period gave them a clear idea of the type and quantity of food they were consuming. They became aware of trends and errors in their eating, sleeping and exercise patterns, and

TABLES

Table-1. Food group coverage among the study sample. Among the 58 students who were part of the study, carbohydrate-rich food was consumed by a maximum number, followed by protein-rich food. Percentage consumption of vegetable, fruit and milk groups was less, while that of the fat, oil, sugar group was very high indicating an unbalanced diet.

Food Groups consumed	Percentage of Students
Bread, cereal, rice, pasta	77.58
Vegetable	21.68
Fruit	8.60
Milk and Dairy products	17.20
Meat, Beans, Dry nuts	36.20
Fats, Oils, Sweets	48.27



SPC.4.01 - NRH 2014-15 CIA-2

Name: Class: Roll. No:
Day-1 Day: Date:

1. DIETARY RECALL SHEET

Meal	Time	Items	Food Group	Servings	Calories
Breakfast					
Lunch					
Evening Tea					
Dinner					
Total Calorie Consumption on Day-1					

2. PHYSICAL ACTIVITY

Type	Sedentary/ Moderate/ Heavy
Details (List the activities performed)	
Calories Expended 'A'	

3. EXERCISE

Type	Yoga/ Aerobic/ Cardio/ Cycling/ Gym/ Swimming/ Running/ Walking/ Other (Specify)
Duration (Time in hours/ minutes)	
Frequency (times per week)	
Calories Expended 'B'	

Total Calories Expended on Day-1 = A + B : _____

4. SLEEP PATTERN

Type	Deep and undisturbed/ Disturbed sleep
Duration (in hours) (mention separately for afternoon and night)	

5. CALCULATIONS
Total calories consumed on Day-1 (from #1) =
Total calories expended on Day-1 (from #2 and 3) =
Whether calorie deficient/ excess/ sufficient (refer to ICMR - NIN guidelines 2010) =

6. ANY OTHER COMMENTS/ OBSERVATIONS

Figure-1. Modified 24-hour Dietary Recall Sheet used in the study. Students were required to fill in the details for 1 week (7-continuous days) and analyze the results in accordance with standard ICMR-NIN RDA values for the Indian population, and world standards according to WHO and USDA guidelines.

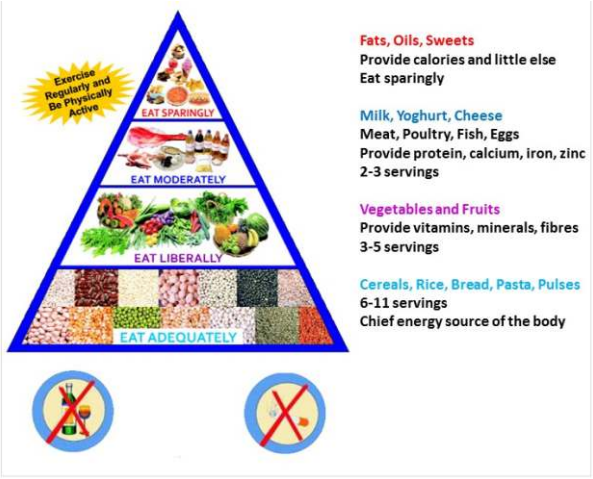


Figure-2. Food Guide Pyramid. The Food Pyramid is used to make a choice of what to eat and how much to eat of all the major food groups in order to achieve the required levels of all nutrients. Food groups at the base of the pyramid are to be consumed in larger proportions than food groups at the apex. In addition to the diet, regular physical exercise, and avoiding smoking and alcohol consumption ensures a healthy lifestyle. (Image modified from NIN Dietary Guidelines for Indians, 2011, available for free circulation at the NIN website).

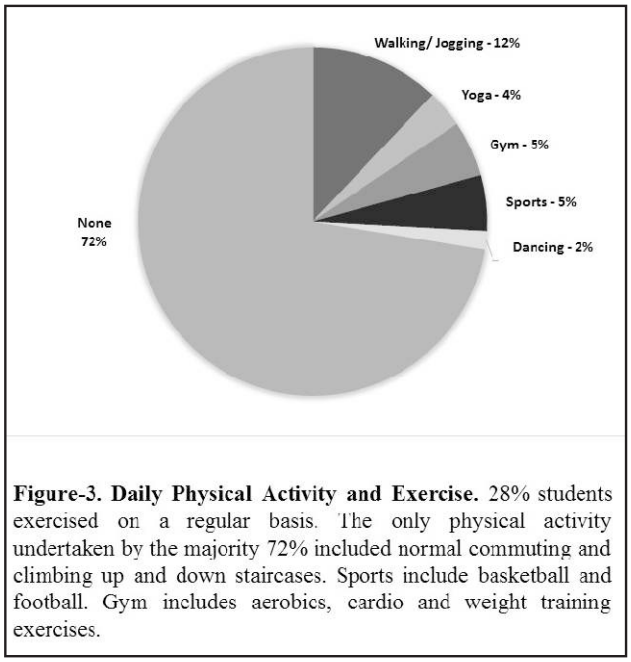


Figure-3. Daily Physical Activity and Exercise. 28% students exercised on a regular basis. The only physical activity undertaken by the majority 72% included normal commuting and climbing up and down staircases. Sports include basketball and football. Gym includes aerobics, cardio and weight training exercises.

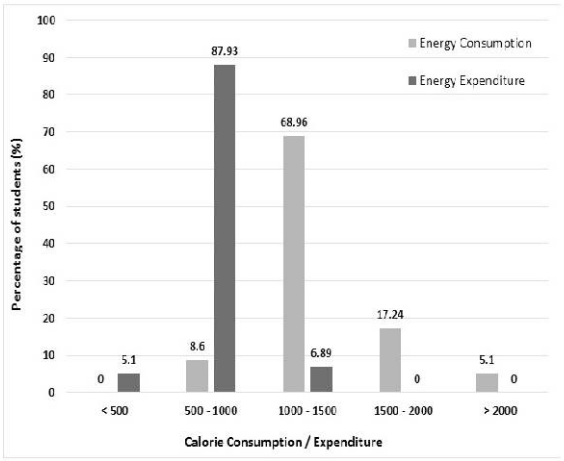


Figure-4. Daily Calorie Consumption and Expenditure. The total daily calorie consumption (green columns) for all 58 students was found to be deficient when compared to RDA values. It was insufficient to account for their daily physical activities. The average calorie expenditure per day (red columns) was found to be well below 600 kcal for most students. The 16 students who exercised regularly had calorie expenditure between 800-1000 kcal/ day.

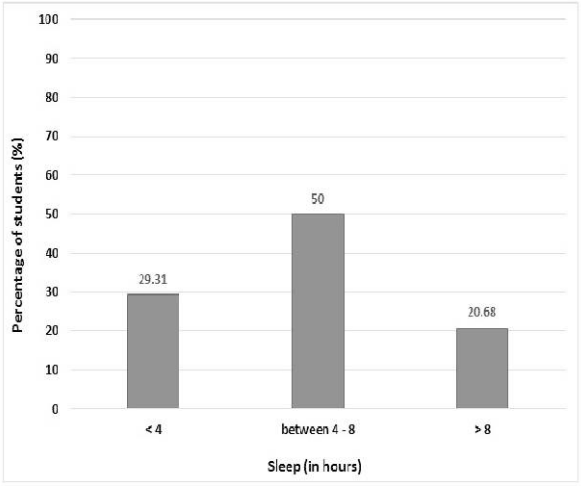


Figure-5. Sleep pattern among students. While most students slept for between 4-6 hours a day, the sleep was not in a single stretch but distributed over the afternoon and night. Only 20.68% students got adequate rest while 29.31% suffered from acute sleep deprivation for various reasons.

could relate it to their loss of stamina and physical and mental fatigue. A few students found the exercise to be tedious initially, but realized the benefits of the exercise over the one week period and specifically mentioned so in their feedback. One student further interpreted it in the social context and mentioned how fortunate some people are to get two square meals in a day, especially in a developing country like India where poverty and unemployment are rampant.

CONCLUSION

Impact. The study served as an eye opener revealing several erroneous dietary, exercise and sleep habits prevalent among the students of the class, which can be generalized to most of today's younger generation. Further, it provided probable causes of the mental and physical fatigue adversely affecting the students. It was heartening to see the participating students make keen observations and start to implement better practices with immediate effect, even as they progressed through the 7-day assignment. All-in-all, the exercise proved to be extremely effective in helping students understand the basic tenets of nutrition, and in applying them to achieve higher levels of health and fitness.

Recommendations. A similar exercise conducted on a larger scale to include different age groups will help in gaining further insights into the nutritional status of a larger population. More importantly, this exercise must be undertaken at the individual level and positive changes incorporated into the daily diet, exercise regimen, and sleeping habits to achieve better health. Nutrition-based projects for student groups and organization of lectures by experts on topics related to food, nutrition, health and fitness will help create awareness and clear misconceptions. This will encourage and facilitate individuals to make food choices wisely, exercise regularly and sleep adequately, thus enhancing their overall health.

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Brahmagupta - Bhaskara Equations

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ABSTRACT :

In this paper we follow Ancient Indian methods of Brahmagupta and Bhaskaracharya as well as modern method of continued fractions to solve equations of the type $Nx^2 \pm k = y^2$ for positive integer solutions. These equations have a lot of historical significance.

Keywords : Varga Prakruti, Brahmagupta, Bhaskara.

Introduction :

Equations of the type $Nx^2 + k = y^2$ which were studied extensively by Brahmgupta(628) and Bhaskaracharya (1150) and called VargaPrakrti, later on became known as Pell's equations in the 17th century. L E Dickson praises Brahmgupta's work and says "It is a remarkable fact that the Hindu Brahmgupta in the seventh century gave a tentative method of solving $ax^2 + c = y^2$ in integers, which is a far more difficult problem than its solution in rational numbers. "

The Two Methods :

It is well known that Pell himself had no contribution to the study of these equations. Euler called them as Pell's equations because of some confusion. Euler also used the continued fractions for solving these equations. In 1657 Fermat gave $61x^2 + 1 = y^2$ as a challenging problem to European mathematicians. He was not aware that the same problem was solved by Bhaskaracharya 500 years earlier. In the book Bijaganita written by Bhaskaracharya, complete text is available explaining the Chakrawala Method that is used in solving an auxiliary equation which leads to solution of $Nx^2 + k = y^2$ using different Bhavana.

We start by a simple example: Solve $31x^2 + 1 = y^2$.

Brahmgupta's method is based on trial. It uses the following principal.

If $N.(a_1)^2 + c_1 = (b_1)^2$ and $N.(a_2)^2 + c_2 = (b_2)^2$ then $N.(a_1b_2 \pm a_2b_1)^2 + c_1c_2 = (b_1b_2 \pm Na_1a_2)^2$.

In other words if (a_1, b_1) is a solution of $Nx^2 + c_1 = y^2$ and (a_2, b_2) is a solution of $Nx^2 + c_2 = y^2$ then $(a_1b_2 \pm a_2b_1, b_1b_2 \pm Na_1a_2)$ is a solution of $Nx^2 + c_1c_2 = y^2$.

This result generates methods called additive Bhavana when the sign is positive, subtractive Bhavana, when the sign is negative and tulyaBhavana as a special case of additive Bhavana when (a1, b1, c1) = $(a2, b_2, c_2)$.

These Bhavana are applied repeatedly to solve $Nx^2 + k = y^2$ for a suitable value of k like $\pm 1, \pm 2, \pm 4$. From this solution one can reach the solution of $Nx^2 + 1 = y^2$ using a Bhavana again.

Note that x = 1, y = 5 is a solution of $31x^2 - 6 = y^2$ and x=2, y = 11 is a solution of $31x^2 - 3 = y^2$.

Now apply additive Bhavana to get a solution of an auxiliary equation.

N	x	y	k
31	1	5	-6
	2	11	-3

1.11+2.5 = 21 5.11+31.1.2=117 (-6)(-3)=18
Hence $31.21^2 + 18 = 117^2$. Dividing this by 9 we get $31.7^2 + 18 = 39^2$

Thus x = 7, y = 39 is a solution of the auxiliary equation $31x^2 + 2 = y^2$.
Now apply TulyaBhavana

N	x	y	k
31	7	39	2
	7	39	2

2.7.39 = 546 $39^2+31.7^2=117$ 2.2=4

This gives us $31.546^2 + 4 = 3046^2$. Now divide by 4 to get

$$31.273^2 + 1 = 1520^2.$$

i.e. x = 273 and y = 1520 is a solution of $31x^2 + 1 = y^2$.

The merits of this method are described by Dutta (2003) in following words.

"Brahmagupta's methods lead to some positive integral solution of $Dx^2 + 1 = y^2$ but that need not be the minimum; and therefore the samasa (additive) bhavana will fetch only infinitely many integral solutions, but not necessarily all integral solutions. Brahmagupta's novel ideas also contain the key to the discovery of the subsequent chakravala algorithm which is a perfect method (free from trial-and-error) for obtaining, for any D, the minimum positive integral solution of $Dx^2 + 1 = y^2$. In fact, his results also aid the chakravala in rapidly arriving at this minimum solution. The samasa bhavana then generates all integral solutions from the minimum one. Thus Brahmagupta's partial solution, apart from being a remarkable landmark by itself, was also a significant step towards the grand climax".

While Brahmgupta's method was based on trial, Bhaskaracharya's Chakrawal method gave a definite procedure to form the auxiliary equation. This method is based on the result that if $Na^2 + k = b^2$ then $N[(am + b)/k]^2 + (m^2 - N)/k = [(bm + Na)/k]^2$ for any positive integer m.

According to cyclic method, to solve the equation $Nx^2 + k_0 = y^2$

1. we start by selecting any value of k (positive or negative) such that $Na^2 + k = b^2$ for some values of a and b.
2. Solve the equation (am + b)/k = t, an integer.
3. From the solutions of the above equation choose the value of m for which m^2-n is numerically minimum. Divide it by k. Call this value of $(m^2-n)/k$ as k_1.
4. We have a solution of $Nx^2 + k_1 = y^2$ given by x = (am+ b)/k and y = (bm + Na)/k.
5. If $k_1 = \pm 1, \pm 2$ or ± 4 then the above equation is suitable auxiliary equation. If not then repeat the above process to get solution of another equation $Nx^2 + k_2 = y^2$.

In this way in finitely many steps we obtain solution of a suitable auxiliary equation. Then we get the solution of the original equation using Bhavana. For example again consider the equation $31x^2 + 1 = y^2$. Clearly $31.1^2 + 5 = 6^2$. In the above notation a = 1, b = 6, and k = 5.

We consider

$31[(m + 6)/5]^2 + (m^2 - 31)/5 = [(6m + 31)/5]^2$.

Now (m+6)/5 = is an integer for m = 4, 9, 13, ….

$m^2 - 31$ is numerically least for m = 4. Hence taking m = 4 in the above equation we get, $31.2^2 - 3 = 11^2$.

This means x = 2, y = 11 is a solution of $31x^2 + k = y^2$.

Here k = -3, which is not suitable.

Apply Chakrawal method again to form the equation

$31[(2m + 11)/-3]^2 + (m^2 - 31)/-3 = [(11m + 62)/-3]^2$.

(2m + 11)/3 is an integer for m = 2, 5, 8 … etc.

$m^2 - 31$ is numerically least for m = 5. Hence taking m = 5 the above equation becomes, $31.7^2 + 2 = 39^2$.

This is the right auxiliary equation and applying Tulya Bhavana as before we get the solution.

The more recent method of solving Pell's equation as explained in Nivenuses continued fractions. We write

$$\sqrt{31} = [5; \dot{1}, 1, 3, 5, 3, 1, 1, \dot{10}] = 5 + \cfrac{1}{1+\cfrac{1}{1+\cfrac{1}{3\ldots}}}$$

The period of this expansion is 8 which is even and hence the solution is given by $x = q_8$ and $y = p_8$ where p_8/q_8 is the 8th convergent to this continued fraction. The eighth convergent to $\sqrt{31}$ is 1520/273. This gives the solution x = 273 and y = 1520.

Comparison between the two methods

Here we have two completely different approaches to solve the Pell's equations. Both are understandable to undergraduate students. In fact Pell's equations as applications of continued fractions are a part of undergraduate syllabi in many Indian universities. But students never learn the Brahmagupta - Bhaskaracharya method. Sometimes these topics are

given for a project.

Brahmagupta - Bhaskaracharya method is based on trial. How soon you will reach the answer or how many Bhavana will be required can not be predetermined. Also there is no rule that tells us whether a particular equation has a solution. For example the equation $Nx^2 + k = y^2$ has no solution when N = 31 and k = -1. i.e.$31x^2 -1 = y^2$ has no solution. This is the case whenever the continued fraction expansion of N has even period and the k is -1. Ancient methods can not predict that. In contrast the theory of continued fractions tells us whether a solution exists and gives all possible solutions in a single formula. But there are some cases when Brahmagupta - Bhaskaracharya method works remarkably well and efficiently as is illustrated in the following example : the famous $61x^2 +1 = y^2$. Some problems on Varga Prakruti are more challenging than others. $61x^2 +1 = y^2$ is computationally challenging one. We outline the steps involved in the computation - by trial we see that $61.5^2 - 4 = 39^2$.

Now use Tulya Bhavana.

N	x	y	k
61	5	39	-4
	5	39	-4

2.5.39=390 25.61+39.39=3046 16

Dividing by 2 we get x = 195 and y = 1523 as a solution for $61x^2 +4 = y^2$.

Now use additive Bhavana

N	x	y	k
61	195	1523	4
	5	39	-4

15220 118872 -16

Dividing by 4 we get x = 3805 and y = 29718 as solutions of $61x^2 - 1 = y^2$.

Now apply Tulya Bhavana

N	x	y	k
61	3805	29718	-1
	3805	29718	-1

226153980 1766319049 1

This shows that x = 226153980 and y = 1766319049 is a solution of $61x^2 + 1 = y^2$.

Conclusion :

We in India have good reasons to be proud of a rich heritage in mathematics coming to us down the ages. But the specific and exact information about the work of our ancestors is many times unknown. Bijaganita is an illustration of extraordinary intellectual curiosity that existed in India in the 12th century. The sustained development of mathematics in India in this period should be known to students and teachers of Mathematics.

The Varga Prakruti discussed here are important part of 12th century Indian Mathematics. Bhaskaracharya himself has said that anybody who solves these equations within a year is truly a mathematician.

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A study of a thermostable protease from *Brevibacillus agri* using agro industrial waste as substrate for potential use as a detergent additive

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Abstract :

A thermo-tolerant bacterium isolated from Ganeshpuri hot springs (pH 7+/-0.2, 50-60°C) in Maharashtra was investigated for thermostable protease production. A comparative study was performed to investigate the efficiency of industrial waste as a substrate for proteasefermentation. In this study, highest protease production was achieved at 55°C after 48h with whey (pH 7.0) as substrate in submerged lab scale fermentation. The protease was purified at 50% saturation ammonium sulfate precipitation. The protease enzyme was found to have optimal activity at 60°C and pH 7.0 and was stable in Sodium Dodecyl Sulfate and Triton x100. This suggests that it can resist the denaturation potential of detergents. A preliminary wash performance analysis revealed that the protease could effectively remove food stains. The bacterium was identified by 16srDNA sequencing as *Brevibacillus agri*.

KEYWORDS: Thermostable, *Brevibacillus agri*, protease, agro industrial waste, detergent additive

LIST OF ABBREVIATIONS: ANOVA: Analysis of Variance, BOD: Biological Oxygen Demand SDS: Sodium Dodecyl Sulphate, SSF: Solid state fermentation

Introduction :

Thermophilic microorganisms are known to produce thermostable enzymes. This has gained worldwide importance as these enzymes have varied applications, involving various industrial applications e.g. detergent, defeathering and pharmaceutical industries (Coolbear et al., 1992). The amount of proteolytic enzymes produced worldwide on a commercial scale is larger than that of any other biotechnologically used enzyme, currently accounting for approximately 60% of the total enzyme sales (Mala et al., 1998). 89% of the total protease sales of the market are captured by subtilins and alkaline proteases from many *Bacillus* species (Kwon et al., 1994). Thermostable proteases are of greater advantage due to their many applications that involve high temperatures. Many thermophilic bacteria have been identified from extreme environmental habitats (Ammar et al., 1991).

For the production of Protease, submerged fermentation is used. The Solid State Fermentation (SSF) process is shown to be less sensitive to contamination than submerged fermentation (Mukherjee et al., 2008; Murthy & Naidu 2010; Lazim et al., 2009; Soarese et al., 2005). Since bacterial cultivation, as against fungal, has high water activity requirement, solid state fermentation cannot be used. Protease when produced on a large scale requires economical substrates which are easily accessible in large quantities. One such source is agro-industrial waste which can be used as an alternative substrate for protease production (Gupta et al., 2002). Whey is a major by-product of dairy industry worldwide. It generally contains 4-5% lactose, 0.8-1% protein, some minerals and vitamins. Due to high Biological Oxygen Demand (BOD) whey disposal is an expensive and time consuming process. Its direct disposal into marine environments can lead to water pollution (Ashraf et al., 2008). Agrowastes like wheat bran, with high amount of organic content, consist of 50-60% of total solid wastes and are rich sources of energy and other nutrients (lignocelluloses, proteins, carbohydrates, lipids etc) which would be lost if they are disposed in open dumps and landfills. These are being used as alternative sources for production of important compounds as these are valuable raw materials (Sumantha et al., 2006). Thus, use of agro-industrial waste will not only reduce the cost of

production of an enzyme, resulting in a benefit for its commercialization, but also act as a waste management system preventing environmental pollution.

Therefore, the objective of the current study is to screen protease producing bacteria from the thermo-tolerant bacteria isolated from hot springs and to utilize agro-industrial waste as a substrate for protease production. The use of enzymes in detergent preparations increases the ability of the detergent to get rid of tough blemishes without increasing its environmental toxicity. Currently, many laundry-detergent products comprise mixtures of enzymes including proteases, amylases, cellulases, and lipases (Hmidet et al., 2009). The application of the isolated protease in detergents has also been evaluated along with other parameters required for the enzyme production.

Material and Methods :

Sample collection and isolation of thermo-tolerant strains

Soil and water samples were collected from five different Ganeshpuri hot springs located on the outskirts of Mumbai city (19°29'49"N 73°0'56"E), Maharashtra. The temperature of the hot springs was between 50-60°C and pH between 7-7.5. The samples were enriched in Thermus broth (ATCC medium 697- 0.5% peptone, 0.5% NaCl, 0.4% Beef extract, 0.2% yeast extract, pH 7.0) at 55°C and subsequently isolated on Thermus agar at 55°C (Elnasser et al., 2006). In order to determine thermo-tolerance of the bacteria, the isolated colonies, were patched on Thermus agar plates and incubated at 27°C, 37°C, 55°C and 60°C respectively. The cultures were maintained on Thermus agar slants at 4°C for short term storage and subcultured every two weeks. For long term storage, they were preserved at -20°C in nutrient broth with 50% (v/v) sterile glycerol.

Assessment of protease-production by isolates

The thermo-tolerant cultures were spotted on the surface of modified Milk Agar [10 % whole milk (Amul Gold®, tetrapack) in Thermus agar. pH 7.0]; Gelatin Agar (0.4% peptone, 0.1% yeast extract, 1.2% gelatin and 2% agar. pH 7.0) (Gomez-Gil & Roque, 2006) and modified 1% mung bean agar (0.4% peptone, 2% NaCl, 1% yeast extract, 1 % ground mung bean and 2% agar. pH 7.0) (Rosewich et al., 2002). Plates were incubated at 55°C for 48 hours and examined for zones of clearance around the spotted culture. Final selection was based on protease production i.e. clearance around the colony of the bacterial isolate on maximum number of plates containing different substrates.

Assay of Protease Activity

Protease activity was analyzed by a previously published method with some modifications (Aqel et al., 2012). The reaction mixture (total 500 µL) containing 400 µL of 1% casein solution (10 mg/mL casein in 50 mMTris–HCl buffer pH 7.2) and 100 µL of protease enzyme were incubated at 55°C for 10 min. The reaction was terminated by the addition of 1 mL 10% (w/v) trichloroacetic acid followed by centrifugation at 10,000 x g for 10 min at 4°C. The product of the enzyme substrate reaction was assayed by the Folin-Lowry method (Lowry et al., 1951). The supernatant (0.5 mL) was mixed with 2.5 mL alkaline $CuSO_4$ (10%, w/v) solution and 0.25 mL Folin-Ciocalteu phenol reagent (0.33 M). This reaction mixture was incubated at 30°C for 30 min and the absorbance of the color developed was measured at 660 nm using a colorimeter. A standard curve was prepared using tyrosine with concentrations ranging from 20 - 100 µg/mL.

Inoculum preparation

For all protease productions, inoculum was prepared as mentioned in this section. A loopful of slant culture of TH9 isolate grown in 10 mL of seed medium containing Thermus broth in a 100 mL Erlenmeyer flask with pH 7.0 was used for the preparation of inoculum. This was incubated at 55°C for 48 h. Then the absorbance was adjusted to 0.5 at 630nm using Thermus broth. 2.5mL of seed inoculum (constituting 1% v/v) was transferred into 100 mL of production medium in a 500 mL Erlenmeyer flask.

Determination of optimum conditions for protease production

To study the conditions for optimum growth and better protease production, three parameters i.e., pH, temperature and incubation period were considered. For determination of optimum temperature, 500 µL of freshly prepared culture suspension of bacterial isolate was added to 10 mL Thermus broth at pH 7 and incubated at 37ºC or 55ºC. The effect of pH on protease production by the isolate was determined by growing the cultures in 10 mL of Thermus broth at different pH values in the range of 4-9 at 55°C. To study the effect of incubation period and thus optimize maximum protease production, the culture was inoculated in to 10 mL Thermus broth at pH 7 (which was found to be optimum in the earlier study) and incubated at 55°C. The protease activity was monitored at regular time intervals of 24, 48, 72 and 96 h of duration. The protease activity was assayed at pH7 and 55°C in each of the above cases from the supernatant after 48h incubation of the fermentation medium (except in the case of optimization of the incubation period).

Production of Protease Using Industrial Waste by Lab scale Submerged Fermentation

Two different media containing Whey (0.002% FeSO4.7H2O and 0.1% $MgSO_4.7H_2O$, in 100ml whey, pH adjusted to 7.0 using 0.1N NaOH) (Francisco et al., 2008) and Wheat bran (2.5% wheat bran, 0.5% NaCl and 0.5% casein hydrolysate) (Baby & Sankarganesh, 2011) were considered and were compared with laboratory medium [0.5% (w/v) yeast extract, 1.0% peptone, 0.5 g/l glucose, 0.4 g/l Na_2HPO_4, 0.085 g/l Na_2CO_3, 0.02 g/l $ZnSO_4$, 0.02 g/l $CaCl_2$ and 0.02 g/l $MgSO_4$] (Elnasser et al., 2006). At the end of 48h incubation period at 55°C, bacterial cells were pelleted by centrifugation at 10,000 rpm for 15 min at 4°C. The cell free supernatant obtained was the crude enzyme preparation and protease assay was carried out to determine the concentration of the enzyme from each of these media.

Partial Enzyme purification

The organism was grown for 48 h in whey medium (pH 7.0) at 55°C and centrifuged (10000 xg, 15 min) to obtain cell free supernatant. The recovered supernatant was fractionated by precipitation with ammonium sulfate between 20-100% of saturation. The pellets were suspended in 50mMTris-HCl (pH 7.2) at 4°C and enzyme assay was performed as described above for both the suspended pellet and supernatant to determine the concentration of ammonium sulphate at which the protease precipitates.

Effect of pH, temperature and detergents on the activity of the enzyme

All the following studies were done with the ammonium sulphate precipitated enzyme as described above. The effect of pH on the activity of protease was determined at different pH values ranging from 4.0-9.0 by assaying the enzyme activity at these pH values. The pH was adjusted using the following buffers- 50mM Sodium Acetate (pH 4.0-5.0); 50mM Sodium Citrate (pH 6.0); 50mM Tris-HCl (pH 7.0-8.0); 50mM Glycine-NaOH (pH 9.0). Reaction mixtures were incubated at 60°C for 30 min and the activity of the protease was measured by Folin-Lowry method. Optimum temperature for the enzyme activity was determined at pH 7.2 by incubation at specified temperatures (20°C, 30°C, 37°C, 55°C, 60°C and 75°C) for 30 mins and activity of protease was measured by Folin-Lowry method (Lowry et al., 1951). The enzyme activity and stability was determined by adding 0.1% and 1% w/v Sodium Dodecyl Sulfate (SDS) and 0.1% and 1% v/v Triton-X 100 into the crude enzyme. Stability of the enzyme was checked after incubation for 1h.

Washing test with protease production

Cleansing action of protease as a detergent additive was studied on thick white muslin cloth (10X10cm) stained with 5mL of a mixture of common food items namely chocolate, tea and tomato ketchup and dried for 2 h. The study was conducted by soaking the stained cloth as follows:

1. Beaker with warm tap water (500 mL) + stained cloth.
2. Beaker with warm tap water (500 mL) + stained cloth + Wheel detergent (2mg/mL) with 20 mL enzyme solution.
3. Beaker with warm tap water (500 mL) + stained cloth + Wheel detergent (2mg/mL) without 20 mL

enzyme solution.
4. Beaker with warm tap water (500mL) + stained cloth + 20 mL enzyme solution.

The water was pre-warmed to 50-60°C. The cloth pieces were soaked for 5 mins, rinsed with water and dried. Visual examination of the various pieces exhibited the effect of the enzyme in removal of stains, as a detergent additive.

Statistical analysis

All the statistical analysis described (ANOVA and t-test) have been done using Excel or SPSS.

Identification of thermo-tolerant strain TH9

The morphological and biochemical characteristics of the thermotolerant TH9 were studied and 16srDNA sequencing conducted for the purpose of identification.

DNA extraction

Bacterial genomic DNA was extracted by perchlorate method (Johns & Paulus-Thomas, 1989). Briefly, 1ml of bacterial culture was centrifuged at 10000 rpm for 2 mins and the pellet was suspended in TE buffer along with 100 microlitre of 10% SDS and100µl of 5M sodium percholate. The suspension was heated at 60°C for 10minutes. Then, equal amounts of chloroform was added and mixed vigorously. Centrifugation was performed at 10000 rpm for 20mins.The upper layer was transferred to a new tube using a cut pipette and mixed with 800 µl of chilled ethanol. After centrifugation, the precipitated DNA was suspended in 50µl of TE buffer.

16S rRNA amplification by nested PCR: Conservative sequences of 16S rRNA of common bacterial pathogens were amplified by universal oligonucleotide primer pairs (Sauer et al., 2005) given in the following table:

Primer	Sequence
outer forward	5'- GTGTAGCGGTGAAATGCG-3'
outer reverse	5'-ACGGGCGGTGTGTACAA-3'
inner forward	5'-GGTGGAGCATGTGGTTTA-3'
inner reverse	5'-CCATTGTAGCACGTGTGT-3'

Amplicons with 709 and 287 bp for outer primer pair amplification and inner primer pair amplification were assumed to produce, respectively. Non template control was run as negative control.

The amplification of 16S rRNA with outer primer pair (first PCR reaction) and with inner primer pair (second PCR reaction) was carried out in a Cycler (ESCO) for 30 reaction cycles for first and second PCR reactions. Each PCR reaction was performed according to Sauer et al., 2005 in a total volume of 20 µL. A 1 kb DNA ladder marker (Next-Gen) was used to estimate the approximate molecular weight of the amplified products.

Sequencing analysis

Nested PCR products (287 bp band) of bacterial cultures were recovered from agarose gel by gel purification kit (Himedia, MB539). The PCR band was sequenced commercially by 1st Base-Asia. The sequences of 16S rDNA of the recovered band was checked by Chromas lite software which was then compared to highly similar sequences in the NCBI database to determine the genus and species of bacterial isolate.

Results and Discussion

Altogether 13 bacterial isolates were obtained from soil and water samples collected from hot springs of Ganeshpuri, Maharashtra. The bacterial isolates were screened for their thermo-tolerance property at different temperatures starting from 27˚C to 60˚C. Out of the 13 isolates only one could grow at a temperature of 60˚C. Rest of the isolates grew luxuriantly at 55˚C and showed slight growth at 37˚C while few strains grew even at 27˚C (See Table 1).

Assessment of protease production by isolates

All 13 isolates showing thermo-tolerance were screened for protease activity on Milk Agar, Gelatin Agar and 1% mung bean agar (See Table 2). Out of thirteen, four isolates demonstrated protease activity in the form of zones of clearance; however, TH9 and TH11 demonstrated degradation of wide range of protein sources (Table 2, Figure 1). The enzyme

Table1. Determination of thermo-tolerance of bacterial isolates from Ganeshpuri Hotsprings

Isolate	Temperature of incubation			
	27°C	37°C	55°C	60°C
TH1	-	+	+++	-
TH2	-	-	++	-
TH3	-	+++	+++	-
TH4	-	+	++	-
TH5	+	++	+++	-
TH6	-	-	++	-
TH7	+	+	+	-
TH8	-	+	+++	-
TH9	-	+	+++	-
TH10	-	+	+++	++
TH11	-	++	++	-
TH12	+	+	+	-
TH13	-	++	+	-

TH= Thermophile; - = No Growth; + = Slight Growth; ++ = Moderate Growth and +++ = Luxuriant Growth.

Table2. Screening of protease activity on different protein substrates

Isolates	10% SMA	1% Gelatin Agar	1% Moong Bean Agar
TH8	17mm	-	8mm
TH9	21mm	14mm	3mm
TH11	11mm	17mm	6mm
TH13	21mm	10mm	-

Table 2 indicates the diameters of the zones of clearance observed due to protease activity in millimeter (mm) around the bacterial growth on different media used

activities of the two strains were estimated using tyrosine standard curve obtained by Folin-Lowry method. One enzyme unit is defined as one micro mole of tyrosine produced per minute per millilitre of the enzyme. According to this analysis, TH9 gave comparatively higher activity than TH11. Therefore, TH9 was used for further studies (See Figure 2).

Determination of conditions for optimum protease production

Incubation temperature has a great influence on the growth and enzyme activity of the organisms. The optimum temperature for maximum production of protease was found to be 55°C (Figure 3). The protease production was significantly higher at 55°C compared to 37°C.The optimum pH for maximum production of protease was pH-7.0 (Figure 4). Protease production at pH 7 was significantly different from that at pH 4 and 6 but was not very different from that at pH 8.0 and pH 9.0. The maximum protease production was observed after 48h of incubation period (Figure 5). The enzyme activity gradually decreased from 48h to 96h incubation.

Production of protease using Industrial waste by Lab scale submerged fermentation

In submerged fermentation, the selection of a suitable substrate for fermentation process is a critical factor and thus screening of a number of industrial waste materials for microbial growth and enzyme production was conducted. Two different agro- industrial wastes viz., whey and wheat bran with added micro-nutrients were used as sole substrate for protease production by TH9 which was compared to that of laboratory medium. All the substrates used in the study supported growth and enzyme production. A higher proteolytic activity was obtained in the whey medium followed by the wheat bran medium as compared to laboratory medium (See Table 3) (Figure 6).Using Whey medium led to 9.5% more and 19.5% more protease production than wheat bran and laboratory medium respectively.

Thus at the end of the production optimization, the whey medium gave a 235% increase (Table 3, 53.5 U/100ml) over the original yield by TH9 (Figure 2, 15U/100ml).

Table 3. Protease activity obtained from different production medium

Medium	Proteolytic activity (U/100 mL)
Whey	53.5
Wheat bran	44
Laboratory medium	34

Partial Enzyme purification

The protease was partially purified using ammonium sulfate precipitation. When salt concentrations between 20%-100% saturation were used, results suggested that the protein precipitated between 40%-60% saturation. To confirm this, 40% saturated supernatant was sequentially precipitated further to 50% and then 60% saturation. The results suggested that 50% saturation is the optimum precipitation concentration for TH9 protease.

Effect of temperature, pH and detergents on the activity of the enzyme

It is a well-known fact that protein undergoes conformation changes or gets degraded at higher temperatures (Bashir et al., 2014). The enzyme activity increases by two fold with every 10˚C until it is degraded. Enzymes with high thermostability are more efficient at higher temperature. The crude protease enzyme showed highest activity at 60˚C whereas at 55˚C and 75˚C enzyme activity was relatively low but was stable (Figure 7). Lowest activity was observed at 20˚C. Hence, it can be said that the enzyme is stable between 55˚C-75˚C with highest activity at 60˚C.The partially purified enzyme showed highest activity at pH 7, the activity was also seen at pH 8 and pH 9 but relatively lower as compared to pH 7. A 30% reduction was seen at pH 9 while only a 13% reduction was seen at pH 8. However, it gave lowest activity at acidic pH values (Figure 8). Thus the enzyme is active at neutral to alkaline pH. The effects of SDS and Triton-X100 solutions on protease activity were investigated. Final concentrations of 0.1% and 1% of detergent were added to the crude enzyme. The results showed that the protease was stable in both the detergents. These results suggest that the protease enzyme could sustain the denaturation properties of a detergent (Figure 9).

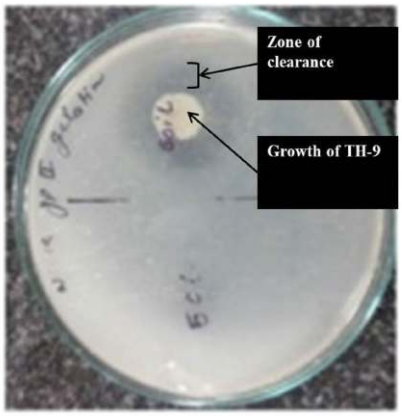

Figure 1.Determination of Proteolytic activity on a protein substrate. A representative figure showing a zone of gelatin hydrolysis by TH9 on gelatin agar

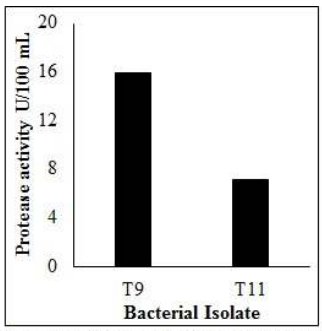

Figure 2. Protease activity of the TH9 and TH11 bacterial isolates: Protease production was set up using two of the isolates mentioned in the figure and protease was assayed from the supernatant as mentioned in materials and methods

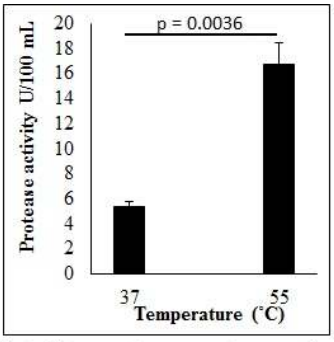

Figure 3. Effect of temperature on protease production: Protease production was studied at two different temperatures, 37°C and 55°C and protease production was measured in the medium supernatant as mentioned in the materials and methods. The exact p value was calculated using t-test.

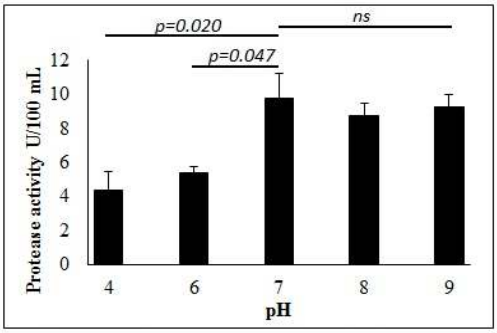

Figure 4. Effect of pH on protease production: Protease production was carried out at varying pH values mentioned above and protease production was measured in the medium supernatant as mentioned in the materials and methods. The exact p value was calculated using the ANOVA test. The overall p value for the ANOVA was 0.0071. The individual p values were calculated by applying Scheffe's post-hoc test.

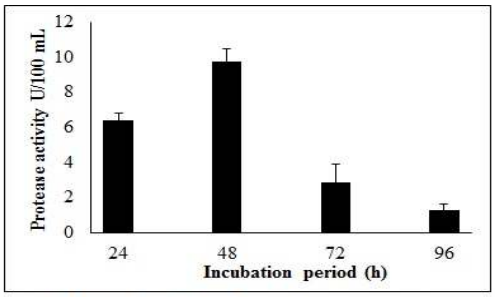

Figure 5. Effect of incubation period on protease production: Protease production was carried out and protease production was measured in the medium supernatant as mentioned in the materials and methods at different time intervals as indicated in the figure. The overall p value for the ANOVA for the four incubation periods was 0.0009. The individual p values were calculated by applying Scheffe's post-hoc test which showed that all time points were significantly different from each other except 72h and 96h.

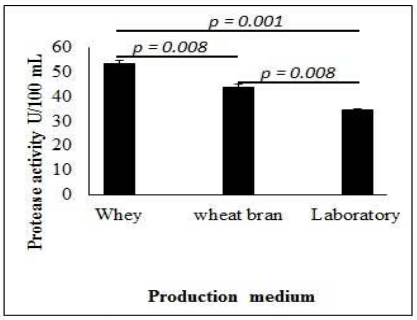

Figure 6. Effect of different media on protease production: Protease production was carried out using three different media mentioned above and protease production was measured in the medium supernatant as mentioned in the materials and methods. The exact p value was calculated using the ANOVA test. The overall p value for the ANOVA was 0.0011. The individual p values were calculated by applying Scheffe's post-hoc test.

Table 4. Ammonium Sulphate precipitation of protease obtained from whey

Step	Volume (ml)	Protein (mg/ml)	Total Protein (mg)	Activity (U/ ml)	Total activity (U)	Specific activity (U/mg protein)	Yield %	Purification (fold)
Crude	20	0.016	0.32	11.3	225.6	705	-	1
$(NH_4)_2$ SO_4 50% Saturation	1	0.010	0.01	26	26	2600	11.5	3.68

Washing test with protease production

Alkaline proteases are involved in various industrial applications e.g. detergent, defeathering and pharmaceutical industries. Microbial proteases have a number of applications. Since on characterization, the protease produced by TH9 showed thermostability, activity in alkaline pH, and detergent stability, it can be used with domestic detergent as an additive. To test whether the protease could enhance the washing capability of detergents, cloths were stained with the common food items and were treated with detergent, detergent + crude enzyme, water and crude enzyme respectively (Figure 10). The cloth treated with detergent + crude enzyme was cleaner in comparison to other treatments. Thus the enzyme produced by TH9 strain in the fermentation process is able to digest the proteinaceous stains efficiently. Hence, the enzyme produced can be used as a detergent additive.

Identification of protease-producing strain Th9

The TH9 bacterial strain was characterized by morphological observation and biochemical tests which revealed it to be a Gram positive rod and identified as *Bacillus sp.* (See Table 5). To definitively identify the organism, 287bp of the variable region of the 16srDNA was amplified using PCR (Figure 11). The 16srRNA sequencing showed that the PCR product has 99% sequence similarity with *Brevibacillus agri* based on the NCBI website (see Table 6 and Figure 12).

CONCLUSION

A protease producing thermo-tolerant bacterial strain was isolated from Ganeshpuri hot water springsand identified as a strain of *Brevibacillus agri.* Hot springs have been a source of many thermostable enzymes from *Bacillus* spp (Panda et al., 2013; Shariff et al., 2011). To enable a cost effective and feasible large scale production, the protease production was standardized for various parameters such as temperature, pH and incubation period. Cheap economic sources have been reported for protease production in the literature for bacteria and fungi (El-Shora & Metawally, 2008; Romero et al., 1998). Agro-industrial whey & wheat bran were successfully used in this study as substrates for protease production where whey gave comparatively higher production i.e. 19.5% more than laboratory medium. The highest

Table 5.Characteristics of thermo-tolerantstrainTH9.

Test	TH9
Gram stain	Gram positive rods
Motility	Motile
Endospore Production	+
Catalase	+
Glucose	Growth
Sucrose fermentation	-
Indole	-
VP	-
Citrate	-
Urease	-
Nitratase	-

Table 6.Taxonomical identity of TH9 strain.

Taxonomy ID	Max Identity	Sample Identity
ATCC 51663	99%	*Brevibacillus agri*

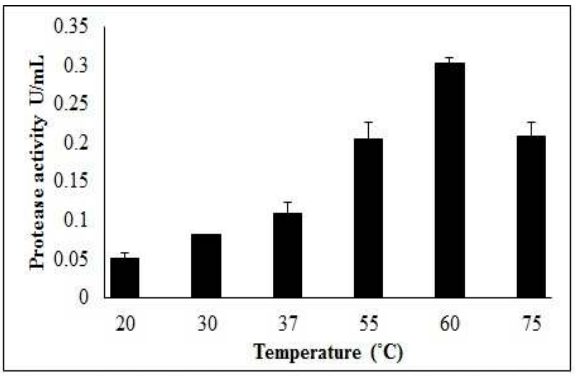

Figure 7. Effect of temperature on protease activity: The partially purified protease was used to study protease activity at different temperatures as mentioned in the figure.

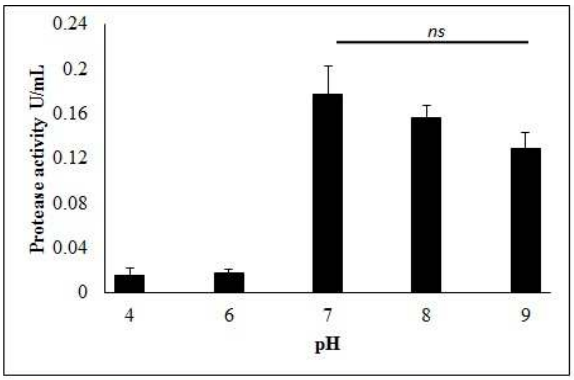

Figure 8. Effect of pH on protease activity: The activity of the partially purified protease was assayed at pH values as mentioned in the figure.

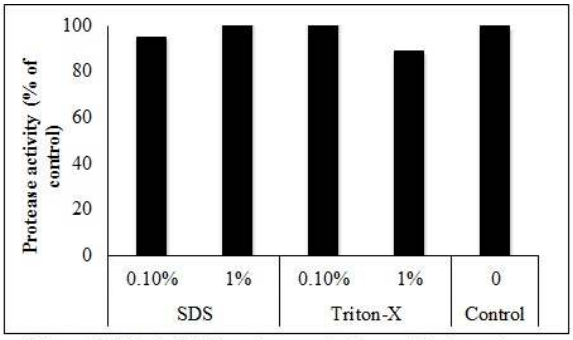

Figure 9. Effect of different concentrations of detergents on protease activity: The partially purified protease was incubated with different concentrations of detergents, SDS and Triton-X as shown in the figure. The detergent exposed enzyme was assayed for protease activity.

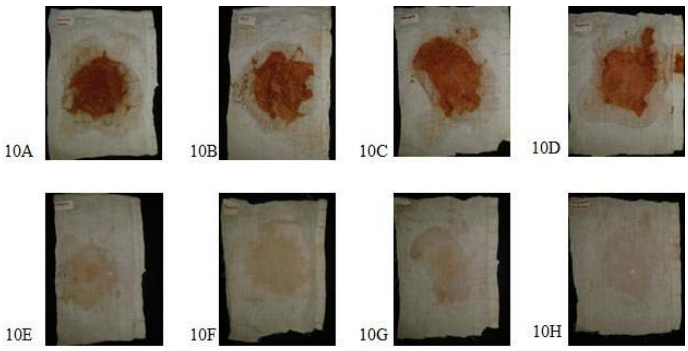

Figure 10. Efficiency of protease from *TH9* as a cleansing agent: 10A-D represents the cloths stained with food stains. 10E-H represents stained cloths washed with water only (10E); washed with detergent (10F); washed with enzyme only (10G) and washed with detergent and enzyme (10H).

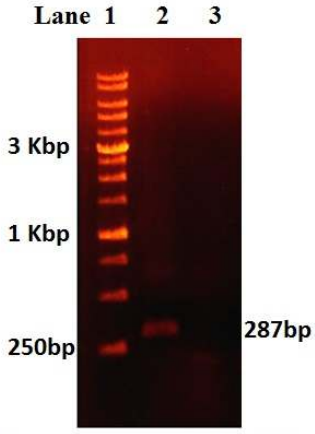

Figure 11.Agarose gel electrophoresis of 16srDNA amplification. Lane 1: 1kb DNA ladder (NEX-GEN); Lane 2 : 287bp PCR amplified DNA with the inner primers; Lane 3 : non template negative control.

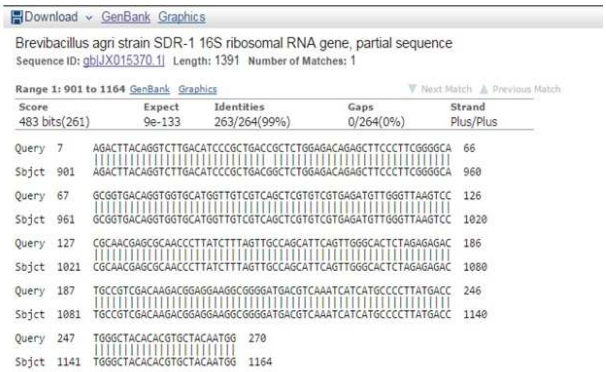

Figure 12.Results of BLAST done using the NCBI website: The query sequence is obtained from the 16srDNA sequencing done from the TH9 strain and the matched subject sequence is identified as *Brevibacillus agri.*

protease production was detected in Whey medium as a substrate (pH 7.0) at 55°C for 48h. This also acts as a waste management system as direct disposal of whey & wheat bran are not good environmental practices. The protease produced was partially purified using ammonium sulphate precipitation method. The protease was successfully used as a detergent additive in this study to enhance the cleansing efficiency of a detergent. The enzyme activity was also characterized for pH, temperature and presence of detergents. The enzyme was found to withstand higher temperatures which make it suitable for use in detergents used in higher temperature laundry washes. These are generally used to wash stained clothes. Additionally the enzyme works best at neutral to alkaline conditions and is stable in the presence of detergents. These conditions are apt for its use as a detergent additive. The strain can be further modified by various methods such as genetic engineering and mutational strain improvement techniques to obtain higher protease activity. Additional features that can make a protease more attractive as a laundry detergent such as stability towards hydrogen peroxide can be achieved using techniques like site directed mutagenesis (Estell et al., 1985).

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Video analysis of the relative distance between two projectiles

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Abstract :

In mechanics experiments with bodies interacting at high speed, keeping an error free time scale for observation has always been a challenge. This paper tries to demonstrate the use of high-speed videography using low cost mobile cameras as a means to record the time dependent data of fast moving bodies in a mechanics experiment.

Keywords : Mechanics, Projectile, Relative velocity, video analysis, high frame rate video

Introduction :

Mechanics experiments could be tedious. Measuring displacements, velocities and acceleration of the moving bodies and projectiles is not easy and is error prone. Almost all mechanics experiments require measurement of time interval. As long as the movements of the bodies are slow a normal stopwatch is sufficient. But time scale measurement is a challenge when interacting bodies are moving faster. Sometimes it requires sophisticated apparatus like stroboscope, which are generally not available in an undergraduate laboratory.

In present situation the high frame rate video cameras have become abundant and are cheaply available. They can easily be used to create the time scale. This paper aim to develops a simpler and easily accessible method for such experiments through a novel theory problem. Consider the following problem in mechanics. *"Two stones are thrown up simultaneously from a certain height with a speed of 40m/s and 10m/s respectively. Discuss the time variation of the relative position of the two bodies with respect to time."*
This paper develops a simple experimental way to solve this problem using a commonly available high frame rate camera.

Analytical solution to the problem

Let the body thrown with initial velocity 40 m/s be called body 1 (1, subscript used to identify its parameters) while other one be body 2 (2, subscript used to identify its parameters).
Their initial velocities will be u_1=40m/s and u_2 = 10m/s. Since body 2 has a lesser initial velocity it will fall earlier than body 2. Let t be the time of flight for body 1.

In time t_1 the displacement equation for the two stones could be written as follows.

$$s_1 = y_1 = u_1 t - \frac{1}{2} g t^2 \ldots 1$$

$$s_2 = y_2 = u_2 t - \frac{1}{2} g t^2 \ldots 2$$

Subtracting (1) from (2) gives the relative displacement.

$$y_1 - y_2 = (u_1 - u_2)\, t \quad = 30t$$

Since that's the equation of straight line, during this time t the relative displacement is a linear function of time giving the straight-line part of the answer.

Time of fall of first stone -
The speed of the stone 1 at the end of its flight can be obtained as follows.

$$v^2 = u^2 + 2as = 100 + 2 \times 10 \times 240 = 4900$$
$$v = 70 \text{ m/s}$$
$$\because v = u - gt$$
$$-70 = 10 - 10t$$
$$t = 8s$$

Time of fall of second stone -
The speed of the stone 2 at the end of its flight can be obtained as follows.

$$v^2 = u^2 + 2as = 1600 + 2 \times 10 \times 240 = 6400$$
$$v = 80 \text{ m/s}$$
$$\because v = u - gt$$
$$-80 = 40 - 10t$$
$$t = 12s$$

The velocity of second stone at the end of 8 minute

$$v = u - gt$$
$$v = 40 - 10 \times 8 = -40m/s$$

Motion of second stone after 8 min

$$s_2 = u_2 t - \frac{1}{2} g t^2$$

$$s_2 = -40t - 5t^2$$

After 8 min since the first stone has already hit the ground, therefore its displacement s_1 = -240m remains constant. Hence the relative displacement during this time is

$$s_2 - s_1 = -40t - 5t^2 + 240$$

Which is an equation of a parabola

Experimental Solution

Modeling of the problem - Projecting the stone with 40m/s initial velocity is logistically challenging. Fortunately a miniature model of the problem can be created. Following are the simplifications that can be easily achieved.

- Analytically to obtain the nature of the solution (i.e. graph) the any particular value of initial position along vertical axis and initial velocity is good enough.
- We can take a smooth spherical ball to minimize the air resistance.
- The two throws instead of being simultaneous can be on two different times. Acceleration due to gravity is constant with respect to time.
- Since time is an independent parameter we can assume the beginning of each throw as beginning of time.
- In order to obtain the nature of the solution we need not throw the projectile with the given velocity in the problem statement (which is huge anyway.) As long as the two throws of the ball has sufficient difference in the initial velocity it will suffice.
- The horizontal displacement of the throw does not matter, as problem requires only vertical displacement.

Experimental Requirement

1. A high frame rate video camera - Any video camera capable of taking high frame rate slow motion video will be sufficient. A normal (30frame/s) video camera will not be able to capture enough frames to analyze positions of the projectile correctly.

Since this research paper tries to develop a student level method of analysis, high-end professional cameras are ruled out. Thankfully now a days cheaper high frame rate video cameras are commonly available. However we need to be careful before selecting them because they come with their own problems.

The cheapest Casio's Exilim FS-10 is $135 and can shoot at 210, 420, and 1000 Frames per second (FPS)1.

The 420 fps the quality suffers and 1000fps requires lot of light to make it usable1.

Another alternative is iPhone camera. It offers 240 fps with decent quality. Since getting it is easier I used it to shoot the videos.

2. A person to project the projectile.

3. A mechanism to hold the camera steady.

4. Preferably a neater background.

5. A video analysis software like 'tracker'[2, 3, 4.]

Tracker is versatile video analysis software, which is freely down loadable.

Geometry of experimental set up

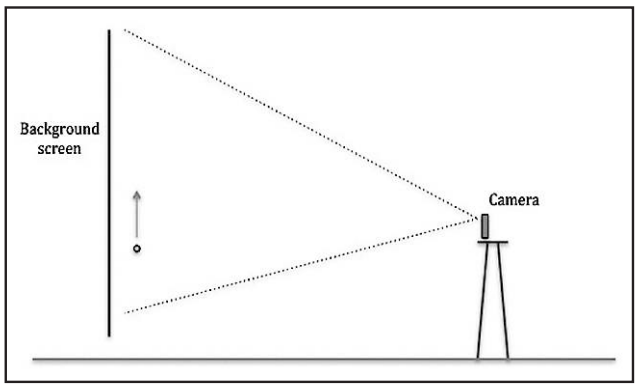


Fig. 1

Geometry of the experimental set up is explained in figure 1. A relatively cleaner backdrop is used as screen. A camera is placed at a suitable height. A ball is thrown vertically upward motion is recorded on a high FPS camera. Figure 2 shows the backdrop and person throwing the ball as seen by the camera.

(Fig. 2)

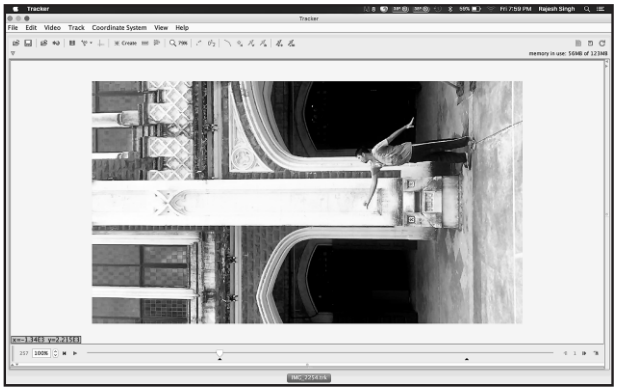


Fig. 3

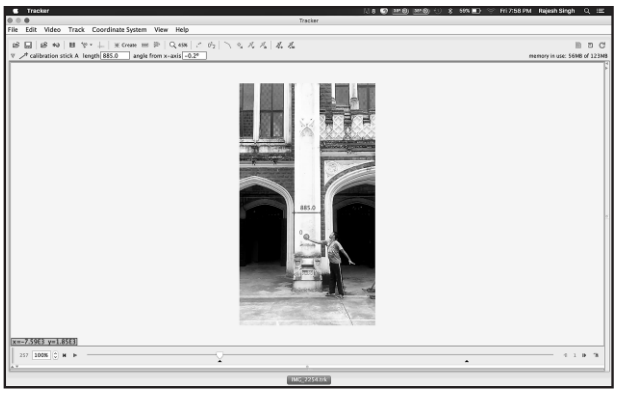


Fig. 4

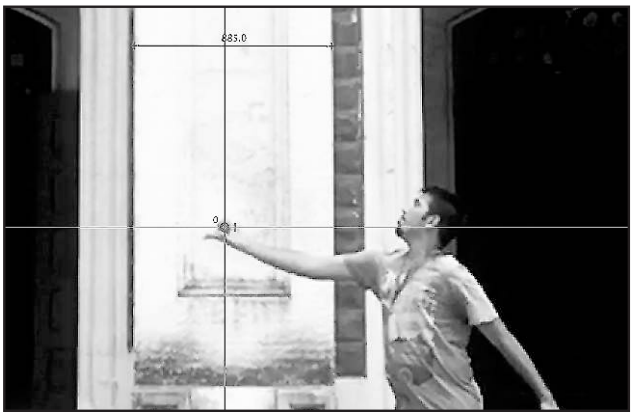


Fig. 5

Actual experimentation

A person throws the ball twice, once with a slower speed and once faster.
A steady camera records the motion of the ball at high frame rate (iPhone, 240fps).
The length measurement of a known object in the video frame is done for scaling. Larger the object used for scaling, lesser the error. In this case for scaling the width of the column in the background,885mm, is recorded.

Video processing

Video is fed to the 'Tracker', video-analyzing software.
Video can be reoriented and improved (brightness and contrast) as shown in figure 3 and 4.

Set the time origin. In this case origin is the moment when ball leaves the hand.
Set the spatial origin and frame of references as shown in figure 5.
Set the spatial scale. In this case the width of the column, 885mm in blue, in the background is recorded.

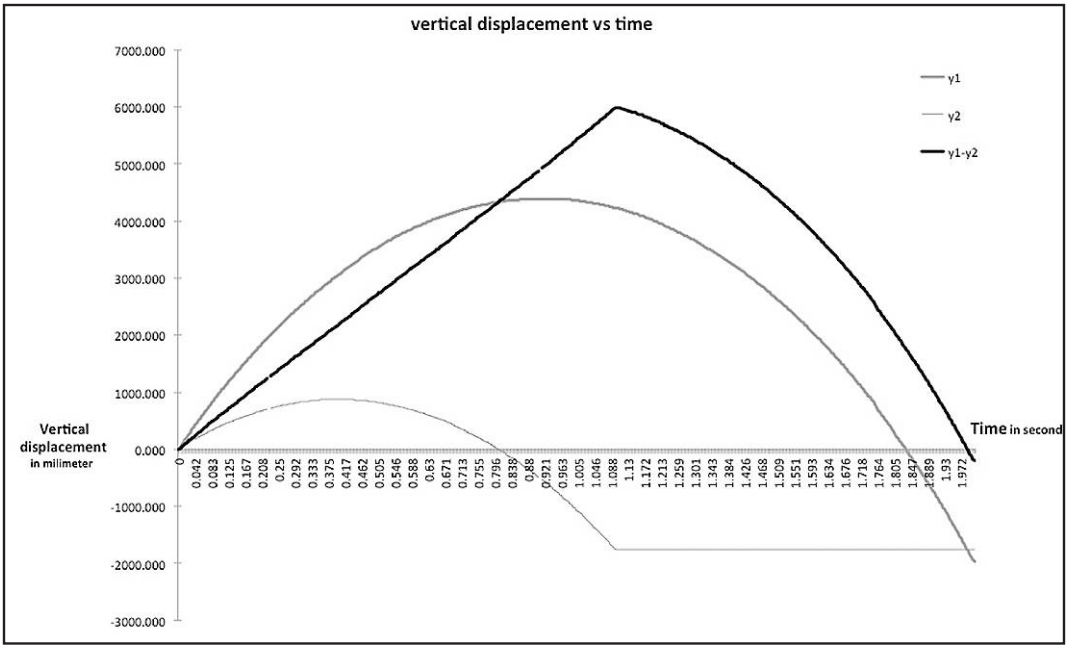
Set the tracking mass, its name, color etc. We can use either auto tracking or manual. In this case manual tracking is used. See figure 6. Data of time, x position and y position appears simultaneously in tabular form as shown in figure 7. I have preferred transferring relevant data (time and y position) to excel for processing.

Fig. 6

Table ◇ mass A

t	x	y
0	0.525	-0.262
0.004	-0.525	42.253
0.008	-1.05	83.719
0.012	2.321	125.572
0.017	-2.984	171.54
0.021	-1.216	212.205
0.025	-4.752	256.406
0.029	-4.752	297.071
0.033	-4.752	339.503
0.038	-8.288	380.168
0.042	-6.52	419.065
0.046	-8.288	461.498
0.05	-10.056	500.394
0.054	-8.288	541.059
0.058	-13.592	583.492
0.062	-11.824	620.62
0.067	-10.056	659.517
0.071	-13.592	700.182

Fig. 7



(Fig. 8)

Analysis

The initial speed of first throw

$$u_1 = \frac{42.515-0}{0.004-0} = 10628.75\ mm/s \quad = 10.62m/s$$

The initial speed of second throw

$$u_1 = \frac{21.204-0}{0.004-0} = 5301\ mm/s \quad = 5.30m/s$$

Analysis of Graph

The vertical displacent of each throw, and relative displcement of the two throws is plotted against time of throw in figure 8.

Curve y_1 represents the variation of vertical displacement in throw one w.r.t. time. Curve y_2 represents the variation of vertical displacement in

throw two w.r.t. time. Curve y_1-y_2 represents the variation of relative vertical displacement in throw one w.r.t. time.

Conclusion

- Graph y_1-y_2 is initially a straight line because $gt^2/2$ term cancels of as long as both the bodies are in flight.

- After the second body hits the ground its position becomes constant. In this part the $gt^2/2$ term survives for the first throw. Therefore the graph is parabolic in nature.
- Thus this experiment clearly demonstrates a method to solve this problem experimentally.

Possible issues in the methodology and suggested improvements

- Air resistance - Air resistance is a significant issue. Throw '1' has initial velocity of 10.62 m/s. In absence of air resistance the maximum height that must be achieved is 5.75 m. But in throw '1' the maximum height achieved is 4.39m, which is 16.69% less than the resistance less throw. A similar discrepancy is seen in the second throw. In this problem since we ignore the air resistance, it doesn't matter. However this data can be used to calculate the air resistance.

- **Squeezing of scale due camera's field of view** In the field of view of camera the any vertical displacement readings horizontal to camera level will have negligible geometrical error. Any reading for at a level different than the horizontal will have geometrical error. Although this geometrical error will be little more for the larger throw its impact is negligible on final analysis as we subtract the two readings that geometrical error get subtracted and weeded out. This error can be reduced considerably by using a camera having optical zoom lens. Such cameras must be expensive and will beat the purpose of low cost experimentation that this paper aims to establish.

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Simultaneous Quantitation of Nickel And Zinc In An Industrial Effluent Using Differential Pulse Polarography

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Abstract :

A simple and precise electroanalytical method for the quantitation of nickel and zinc simultaneously from an industrial effluent has been established using differential pulse polarography. With this technique it was possible to quantitate nickel and zinc simultaneously with a validated method. The polarogram recorded for the industrial effluent in potassium thiocyanate as a supporting electrolyte showed two cathodic peaks at -0.63 V and -0.93 V vs. saturated calomel electrode which were confirmed to be of nickel and zinc by the method of standard addition. The linear dynamic range for nickel and zinc was 0.652 µg/mL to 13.5438 µg/mL and 0.7265 µg/mL to 13.078 µg/mL respectively.

Keywords : Differential pulse polarography, Industrial effluent, Nickel, Zinc, Potassium thiocyanate.

Abbreviations used in the paper: Ni- Nickel, **Zn-** Zinc, **KSCN**- Potassium thiocyanate

Introduction :

Rapid industrialization and abnormal population growth has enhanced water pollution. Monitoring the metal ions and organic compounds in aquatic environment has been a subject of great concern over the last few decades and will continue to be so, as increasing number of metal ions in increasing amounts and a diverse array of organic compounds form a part of an industrial effluent. Metal ions are the most common electroactive species present in the industrial effluents. 'Heavy metals' is a general collective term applied to the group of metals such as Pb, Cr, Cu, Ni and Zn which are commonly associated with pollution and toxicity problems. Some of these elements may be micronutrients for many living organisms and are required in small amounts for normal healthy growth, but any metal ion in large amount will always cause acute or chronic toxicity. Trace determinations of metals by voltammetric methods with modified electrodes has been reported by Honeychurch et al (2002a, 2002b, 2002c) Complexation of the metal ions in waste water was also studied by differential pulse cathodic stripping voltammetry by Honeychurch et al (2000) and Kuruto (2003). Simultaneous determination of Zn(II) and Ni (II) was also studied in the presence of crown ethers by D.C. polarography (Kuruto 2003). Metals present in industrial effluents and sludge samples have been separated and concentrated by using other techniques like electrodialysis, coulometry and photocatalysis (Ramachandraiah 1996). However, less work has been done in the area of environmental chemistry especially on separation and quantitation of electroactive species present in industrial effluents.

Objective :

The main objective of the study was to provide a simple, rapid, efficient, precise and economical method for the simultaneous determination of Ni(II) and Zn(II) from an industrial effluent using differential pulse polarography. The developed method has been validated as per ICH guidelines ICH (ICH Q2A 1994 and ICH Q2B 1996).

Materials and Methods (Experimental) :

The Workstation - All the measurements were performed on a fully automated computerized electroanalytical workstation, an electrochemical system PG STAT 30 with 663 VA electrode stand manufactured by Metrohm (Fig.1). It includes 3 electrode system viz. hanging mercury drop electrode as a working electrode, saturated calomel electrode as a reference electrode and platinum electrode as an auxiliary electrode.

Fig. 1 a & b Metrohm PG STAT 30

Reagents

Merck A.R grade $NiSO_4.7H_2O$, $ZnSO_4.7H_2O$ and KSCN were used.

Preparation of Standard Solution

28 mg of $NiSO_4.7H_20$ and 28.75 mg of $ZnSO_4.7H_2O$ were accurately weighed and dissolved in minimum amount of double distilled water and made up to the mark in a 100 mL volumetric flask. The solution so prepared contained 58.69 µg/mL of Ni and 65.39 µg/mL of Zn respectively. All the other standard solutions containing both Ni and Zn were prepared using this stock solution.

Voltammetric method

18 mL of distilled water and 2.0 mL of1 M KSCN were placed in the dry, clean cell. The solution was purged with pure nitrogen gas for 180s. The potential scan between 0.0 V to -2.0 V vs. S.C.E was applied. The operational parameters were as follows: 1] Scan rate- 15 $mVs^{-1.}$ 2] Pulse amplitude- 50mV. After recording a polarogram of the blank, 0.4mL of standard solution of Ni and Zn were added in succession and polarograms were recorded and peak currents were measured and calibration curves were prepared (Fig. 2)

Preparation of sample solution

The sample was an effluent from an electroplating industry. The sample solution was centrifuged and filtered through Whatman paper no. 41. 50 mL of the sample was evaporated to dryness and extracted with water containing 0.2 mL of conc HCl and diluted to 50 mL in a volumetric flask with distilled water. Polarograms for the sample solutions were recorded under the same conditions used for the calibration curve. The amount of Ni and Zn were calculated from the measured peak currents and using the equation of the calibration curve. The equation of the calibration curve for Ni was y=38.2699x+4.0742 and for Zn was y = 96.5378x - 2.3310 (Fig. 3) where y is the current in nanoamperes and x is the concentration in µg/ml.

Analytical Method Validation :

System Suitability - System suitability tests were carried out to ensure reproducibility of the instrument. The system suitability test was carried out by recording polarogram for Ni and Zn at two different concentrations (2.257µg/ml and 6.288µg/mlfor Ni and 2.515 µg/ml, 7.006 µg/ml for Zn) with five replicates and the mean current was used for the calculation. The % RSD in both cases was found to be less than 2%.

Specificity - The specificity of method was confirmed by comparing the polarograms of the combined standard solutions containing Ni and Zn with the sample solution. The peak potentials recorded for the sample solution were found to be identical to those obtained for the combined standard solution of Ni and Zn. The addition of the standard solutions of Ni and Zn to the sample solution did not change the characteristics of differential pulse polarogram but enhanced the peak current. This confirms the specificity of the method.

Robustness - The robustness of the method was examined by observing the consistency of the peak height and the peak shape with the deliberately made small changes in the experimental parameters. It is a measure of the capacity of the method to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To determine the robustness of the proposed method, the

following variations were made in the analytical parameters. The Scan rate was changed by ± 0.5 $mVs^{-1}$and the Pulse amplitude ± 1.0 mV These parameters were deliberately changed one at a time and the effect of these changes on the peak shape and peak currents were studied. The proposed method was found to be robust.

Linearity and Dynamic range - The linearity for Ni and Zn in a solution containing the two was determined. In the concentration range, 0.65 ìg/mL to 13.54 ìg/mL for Ni and 0.73 ìg/mLto 13.078 ìg/mL Zn, a good linearity was obtained. The equation of the calibration curves is presented in (Table1).

Limit of Detection and Limit of Quantitation - The limit of detection (LOD) and the limit of quantification (LOQ) for Ni and Zn were fixed at signal to noise ratio of 3:1 and 10:1 respectively. Twenty replicates of the blank solution were recorded and the mean current value at the peak potential of Ni (i.e. at -0.63 V) and Zn (i.e. at -0.93 V) were calculated. The concentration at which the peak current was found three times of mean blank current was taken as the limit of detection and the concentration at which peak current was found to be ten times the mean blank current was selected as the limit of quantification. The LOD and LOQ of Ni and Zn were 0.25ìg/mLand 0.65ìg/mL and 0.28 ìg/mLand 0.73 ìg/ml respectively.

Intraday and Interday Precision - The variability of the method was tested with the intra-day and inter-day precision. It was checked by recording the polarograms of standard solutions of Ni and Zn in the concentration ranges 1.1507 ìg/mLto 8.095 ìg/mL for Ni and 1.282 ìg/mLto 9.019 ìg/mL for Zn. Intra-day precision was tested by recording the polarograms at an interval of four hours and inter-day precision twice a day with a gap of three days. The mean % RSD for intra-day and inter-day precision for Ni was found to be 0.82% and 0.95% and for Zn 0.38% and 0.54%, respectively.

Quantitation / Determination - The validated method was used for the determination of Ni and Zn. Polarograms were recorded under the optimum experimental conditions for the sample solution. Resulting peak currents for Ni and Zn were measured and the amount of Ni and Zn was calculated using calibration curve equations. The results are presented in Table 2.

Accuracy (Recovery) - The recovery technique was used to evaluate the accuracy of the method. The method of standard addition was employed for the purpose. A fixed volume of the standard Ni and Zn solution was added to the sample solutions and the mixed solutions so obtained were analyzed by the proposed method. The percentage recovery was determined at different percentage levels i.e. the added amounts ranging from 40% to 200% of the amount present in the sample. The results of the recovery analysis for Ni and Zn are presented in Table 3.

Result and Discussion

The present study provides determination of Ni and Zn from the industrial effluents using the technique of differential pulse polarography. The method was validated as per the ICH guidelines (Table 1-3). Before validation, optimization of the conditions i.e. pH, supporting electrolyte, scan rate and pulse amplitude were optimized. The polarographic response of the sample for Ni and Zn in different supporting electrolytes has been studied. With KCl as the supporting electrolyte the sample showed only one peak for both Ni and Zn. However, two separate peaks were produced with KSCN as the supporting electrolyte.

Pulse amplitude of 50mV was chosen as the optimum as there is loss of resolution at high pulse amplitude. The Differential Pulse polarogram of Ni and Zn were recorded at various scan rates. At scan rates higher than $15mVs^{-1}$ the width of peak increases, its height decreases and peak shape was distorted. At lower scan rates than $15mVs^{-1}$ peak current was lower. So a scan rate of $15mVs^{-1}$ was chosen as a best for the analysis.

Acknowledgement

We thank the Department of Chemistry St. Xavier's College for providing us all the necessary instrumentation facilities and their technical assistance.

Table 1: METHOD VALIDATION PARAMETERS FOR NICKEL AND ZINC

Parameters	Values	
	Ni	Zn
System suitability (n=5) %RSD	0.75%	0.47%
Linearity range (µg/ml)	1.1507 to 8.095 µg/ml	1.282 to 9.019 µg/ml
Slope (m) [a)]	38.2699	96.5378
Intercept (c) [a)]	4.0742	-2.3310
Correlation coefficient (R2)	0.9993	0.9992
LOD (µg/ml)	0.2527 µg mL-1	0.2815 µg mL-1
LOQ (µg/ml)	0.652 µg mL-1	0.7265 µg mL-1
Intraday precision (n=5)	0.82%	0.38%
Interday precision (n=5)	0.95%	0.54 %
Recovery	98% to 102%	98% to 102%

[a) of the equation y = mx + c, where y is peak current, m is the slope, x is the concentration and c is the intercept]

Table 2: RESULTS OF QUANTITATION STUDIES FOR NICKEL AND ZINC

Name of Metal ions	Ni	Zn
Conc in µg/ml	2141.84	1016.8
% RSD (n=5)	0.66	0.86

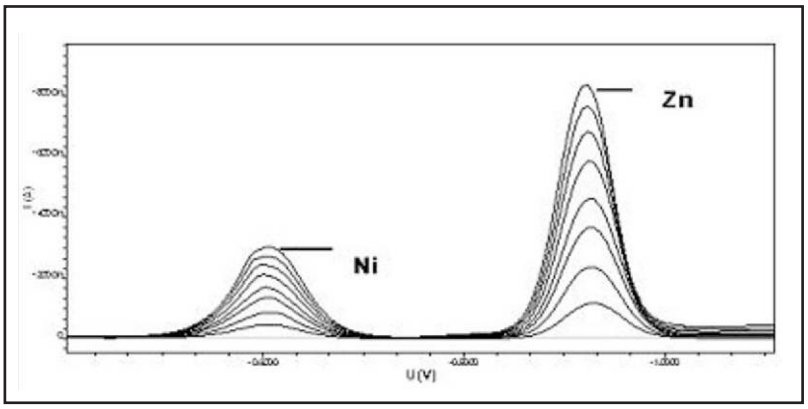

Figure-2. POLAROGRAMS OF NICKEL AND ZINC

Table 3. RESULTS OF RECOVERY STUDIES FOR NICKEL AND ZINC

Standard	Level	Conc. of std [µg/ml]	Conc. of std Found [µg/ml]	RSD (%) (n = 5)	Recovery (%)
	50%	1.684	1.6723	0.58	99.30%
Nickel	80 %	2.755	2.7959	0.57	101.48%
	100%	3.275	3.3185	0.47	101.30%
				Mean	100.69%
	40 %	0.638	0.6422	0.25	100.66%
Zinc	110%	1.877	1.8749	0.69	99.88%
	180%	3.069	3.1179	0.58	101.56%
				Mean	100.70%

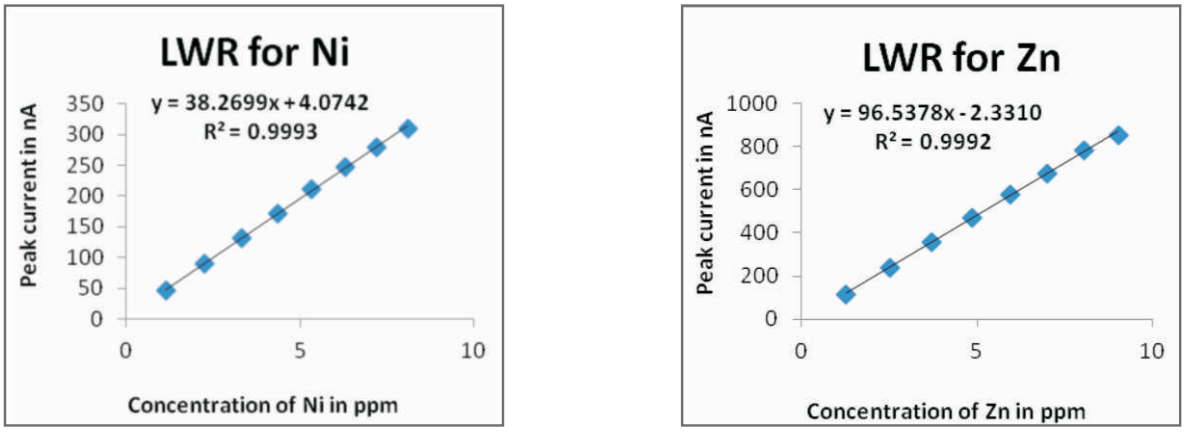

Figure-3. Linearity Graphs For STD Nickel and STD Zinc

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Ionic liquids and Nanomaterials a perfect Synergy

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Abstract

Research on new materials technology is attracting the attention of researchers all over the world. Developments are being made to improve the properties of the materials and to find alternative precursors that can give desirable properties on the materials. In this context Ionic Liquids and nanoparticles have experienced a comet-like boost in the last few years. This review highlights the synergy of ionic liquids and different nanoparticles along with some current applications of this fascinating class of new materials.

Key Words: ionic liquid, nanoparticles, green solvent

Introduction

The growing academic and industrial interest in Ionic Liquids (ILs) and nano technology has led researchers to exploit these two technologies together. Nanomaterials are not simply another step in the miniaturization of materials. They often require very different production approaches. However, one of the difficulties that nanoparticles pose as catalyst is providing stability of small nanoparticles while retaining the activity. ILs have been shown to provide "electrostatic" stabilization for metal nanoparticles and more surface area for the reaction to take place [1]. So ILs inturn control the stability and activity of nanoparticles.

Ionic liquids are generally salts of organic cations. Besides the obvious advantage of low liquid temperature, these liquids display additional attractive features. This is due to the versatility of these solvents to act both as catalyst as well as solvent. To add to it, their negligible vapour pressure, nonflammability, thermal stability, high intrinsic electrical conductivity and recyclability, trademarks ILs as universally accepted 'greener solvent'. The possibility of modulating the hydrophilic or hydrophobic character of ionic liquids leads to several potential applications in extraction technologies, generation of new materials, organic synthesis including biphasic catalysis and supported ILs. Its chemical and physical properties can be tailored as per the requirement of the reaction[2,3,4]. Ionic liquids possess all the potentials of an 'ideal solvent' that any chemist would dream of. Truly ionic liquids are 'designer solvents' for multipurpose applications, be it for organic synthesis, biphasic catalysis, extraction purposes, electrochemical studies, nanoparticle synthesis, catalysis, organo catalysts and as soluble supports for catalysts[5].

Ionic liquids (ILs) offer outstanding possibilities as media for manufacturing nanoparticles. Synthesis conditions with high reaction and nucleation rates are achievable leading to the formation of extremely small particles. The IL itself can act as an electronic as well as a steric stabiliser preventing particle growth and particle aggregation. In addition, as highly structured liquids, ILs have a strong effect on the morphology of the particles formed[6].

Ionic liquids and Nanoparticles

Srinivasan et al were the first to report the isolation of palladium nanoparticles in 2001 formed in a Heck-reaction (catalyst: $Pd(OAc)_2$, $PdCl_2$). These nanoparticles were characterized by means of transmission electron microscopy (TEM)[7].

In 2002, Dupont showed that ILs can be regarded as liquid supports for transition-metal catalysts rather than as solvents. The group synthesized iridium(0), rhodium(0), platinum(0), and ruthenium(0) nanoparticles with diameters in the 2-3-nm range and a narrow size distribution inimidazolium ionic liquids by the reduction of metal salts with molecular hydrogen or by the controlled decomposition of organometallic compounds in the zero oxidation state. The combined

intrinsic high charge plus the steric bulk of these salts was suggested to create an electrostatic and steric colloid-type stabilization of transition metal nanoparticles. Iridium nanoparticles were isolated and characterized from a biphasic hydrogenation reaction (catalyst: $[IrCl(cod)]_2$,cod = 1,5-cyclooctadiene) using imidazolium based ionic liquid $[BMI]PF_6$ as reaction media[8]. The isolated iridium nanoparticles can be reused as catalysts in $[BMI]PF_6$ ILs and the efficiency is maintained for up to at least seven recycles.

Palladium(0) nanoparticles 'embedded' in ionic liquids have also been prepared and used to catalyze the hydrogenation of 1,3-butadiene to 1-butene with excellent selectivity. These ionic-liquid-supported Pd(0) nanoparticles are also effective recyclable catalysts for carbon-carbon coupling reactions such as the Heck reaction. The group has reported that Ru(0) nanoparticles dispersed in imidazolium ionic liquids are efficient catalysts for the selective partial hydrogenation of benzene to cyclohexene under mild conditions. They can be used to develop recyclable catalytic systems and enable extra stabilization of the catalysts[9].

Reduction of $Pt_2(dba)_3$ (dba = bis-dibenzylidene acetone) with molecular hydrogen in $[BMI]PF_6$ leads to stable and isolable platinum(0) nanoaparticle, which can be used as catalyst in hydrogenation reactions with high activities and recycabilities[10].

Ionic liquids combined with various nanomaterials are applied in electrochemistry research[11,12]. The palladium nanoparticles immobilized by ionic liquid onto molecular sieve show high catalytic activity in solvent-free alkene hydrogenation[13]. Using CN functionalized pyridinum ILs, palladium nanoparticles can also be isolated from a Stille reaction process, and they are different from that isolated from non-functionalized pyridinum based ILs. The CN group in the cation can be weakly coordinated to zero covalent palladium and hence prevent the aggregation, which was observed in other reactions using conventional alkylimidazolium ILs[14].

The IL-stabilized metal nanoparticle exhibits potential advantage on catalytic reactions such as hydrogenation of various substrates over the classical "homogeneous" or "heterogeneous" catalysts, as those classical metal nanoparticles are usually prepared by the reduction of metal compounds in the present stabilizers such as functionalized ligands, polymers, or surfactants to prevent agglomeration and precipitation in solvents. This process may lead, however, to strong adsorption of the stabilizers on the active sites of the metal nanoparticles and passivation of the active sites, resulting in reduction, or worse, a loss of catalytic activity[15]. The IL-stabilized metal nanoparticles can be easily recycled and the recovered nanoparticles can be reused as a solid or redispersed in the IL several times without any significant loss in catalytic activity[16]. The metal nanoparticle-dispersing IL solution may open a novel reaction system for biphasic catalysis.

Nanoparticles as chemosensor consist of metallic nanocrystal cores and organic monolayer shells[17]. Alkyanethiol compounds are known to stabilize gold nanoparticles[18,19,20]. Nano particles synthesis in aqueous phase poses inherent problems such as ionic interaction, low reactant concentration, and difficulty in removing the residue of stabilizers after synthesis. Thiol-functionalized ionic liquids represent potentially good candidates as stabilizers since the ionic properties of ILs enables better interaction of ILs and transition metals such as Au and Pt in ionic species salts than those in conventional solvents. Additionally, they can also be easily designed to be hydrophilic or hydrophobic by combining the cations with the appropriate anions. Wei et al. also reported similar water/water-immiscible phase transferred gold nanopariticles and gold nanorods using an IL[21].

The precise control of nanoparticle size and size distribution and a better understanding of the chemical behaviour of nano particles are becoming increasingly important and have been recognized as key research tasks in order to expand their utility.

Ils with one, two, or more thiol groups were prepared in order to rationalize their effects on the size and distribution of nanoparticles (Fig. 1)[22]. In addition, thiol groups were also introduced into the anion of the imidazolium based ILs, forming a dual-functionalised system, which was also evaluated in nanoparticle synthesis.

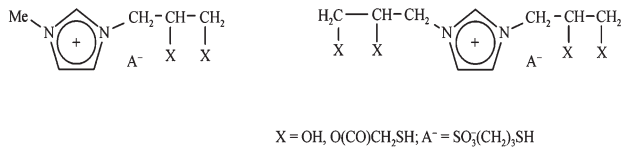
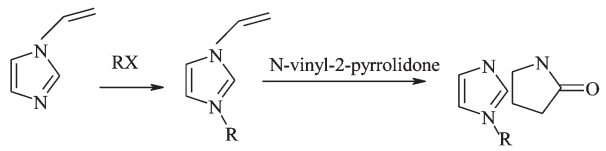


Figure 1. The ionic liquids containing thiol functionality.

Gold and platinum nanoparticles prepared from these thiol-functionalised ILs are highly dispersible in aqueous solution. The nanoparticle size and stability were affected by the position and number of thiol groups in the cation and anion, and therefore the chemical and physical interaction between the ILs and metals plays a decisive role in determining the nanoparticle structure. Moreover, the nanoparticle size could be tuned according to the nature of cation and anion. The diameter of the nanoparticles was observed to decrease as the number of thiol groups increased on the cation, and the diameter decreased when a sulfite anion with a thiol group was employed. Furthermore, the nanoparticles encapsulated by these ILs were more stable towards agglomeration. Accordingly, the IL's functional groups in the cation and anion behaved as selective gates allowing control of the size and uniformity of the encapsulated nanoparticles.

Polymer such as polyvinylpyrrolidone (PVP) is also commonly used nanoparticles stabilizer. Kou and co-workers prepared the platinum, palladium and rhodium NPs in [bmim][PF_6] (Fig.1)[23].

However, PVP's low polarity makes it hard to dissolve in high polar ionic liquids, thus limiting its application as stabilizer in IL. Kou and co-workers then designed an "ionic liquid-like" PVP polymer[24], which can be very well used as Rh NP stabilizer for high performance hydrogenation (Scheme 1). Such Rh NPs can be used as catalyst for aromatic compound hydrogenation carried out in [bmim][BF_4]. The total turnover value reached 20,000 and the synergetic interaction of modified PVP and ionic liquid is considered the reason for the long life of catalyst.



Scheme 1. PVP polymer containing ionic liquid moiety.

One of the drawbacks for the production of nanoparticles in ILs[25,26] is chloride contamination which has a significant impact on the properties of the nanoparticle. In this context, a method for the production of nanoparticle has been developed using chloride-free functional ILs[12]. Pt nanoparticles that are soluble in THF have been prepared by reduction of bis-(dibenzylideneacetone) platinum [$Pt(dba)_2$] (dissolved in THF) with molecular hydrogen in chloride free ILs 4,5-dicyanotriazolium tetraoctylammonium IL (known as “Armand's Ligand”, (Fig. 2) According to the elemental analysis it has Pt content of 15.6%. Using a mixture of $Pt(dba)_2$ and Ru(cod)(cot) (cod = 1,5 cyclooctanediene; cot = 1,3,5-cyclooctatriens) in 1:1 molar ration, mixed PtRu-nanoparticles (Pt:Ru = 1:1) can be obtained in a similar manner. All these nanoparticles show excellent methanol oxidation catalytic activities[27].

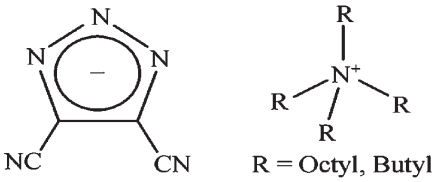


Figure 2. Halide free ionic liquids for nanoparticle preparation.

The high ionic conductivity and polarizability of ILs make them excellent microwave absorbing agents[28]. A nonpolar solvent can be used as good media for microwave-assisted chemical reaction just by addition of a small quantity of an IL[29].

Zhu et al. developed a new microwave-assisted IL (MAIL) method for the fast controlled synthesis of tellurium (Te) nanorods and nanowires[30]. Experimental results reveal that the IL favours the formation of Te nanorods and nanowires. The movement and polarization of ions of the IL under the rapidly changing electric field of the microwave reactor may result in transient, anisotropic micro-domains for the reaction system, facilitating the anisotropic growth of Te nanorods and nanowires. This shows ILs may promise as capping reagent that can direct preferential growth of the particle along particular axes of the crystal lattice.

Pd(0) nanoparticles with ~2 nm diameter, immobilized in 1-*n*-butyl-3-methylimidazolium

hexafluorophosphate ionic liquid, are efficient catalyst precursors for coupling of aryl halides with *n*-butylacrylate. In situ TEM analysis of the ionic liquid catalytic solution after the catalytic reaction shows the formation of larger nanoparticles (~ 6 nm). The palladium content in the organic phase during the arylation reaction checked by ICP-AS showed significant metal leaching (up 34%) from the ionic phase to the organic phase at low substrate conversions and doped to 5-8% leaching at higher conversions. These results strongly suggest that the Pd(0) nanoparticles serve as a reservoir of "homogeneous" catalytic active species[31].

Another review focuses on the pre-organized structure of the IL as template effect for porous inorganic nanomaterials and the intrinsic high charge and polarizability of the IL to create electrostatic and steric stabilization for metal nanoparticles and to favor phase transfer of the nanoparticles from water to water-immiscible solvent[32].

Template effect of ILs for formation of porous inorganic materials

ILs of long molecular chain exhibiting both lyotropic and thermotropic liquid-crystalline behavior in various solvents at wide temperature range has been reported. 1-hexadecyl-3-methyl-imidazolium-chloride, [C16mim]+ Cl-, 1, is a robust liquid crystal, displaying liquid crystalline property in water, acid, and tetrahydrofuran (THF), and thermo tropic liquid-crystallinity in the whole temperature range from 50°C to 230°C[33]. The employment of Fig.3 as template for the preparation of the order porous materials is expected to be interesting.

The transmission electron microscopy (TEM) image of **1**-templated monolithic silica[34] displays a large domain of a practically ordered lamellar phase of interlayer distance about 2.7 nm, i.e. the pore sizes of 1.3 nm (i.e. in the super-microporous size regime) and the thickness of the wall system of about 1.4 nm. It indicates that the long tails of the IL molecules are intensely packed in an inter digitated conformation. The corresponding Fourier diffractogram in the inset displays the typical spot pattern of crystal-like order of the produced porous nanostructure. These porous materials in the super- micropore size are very important since they bridge the gap between microporous zeolites and mesoporous materials.
Such materials exhibit the potential of size and shape selectivity for those organic molecules that are too large to have access to the pores of zeolites[35].

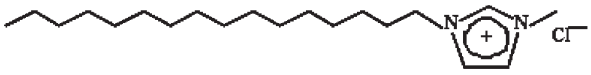


Figure 3. 1-hexadecyl-3-methyl-imidazolium chloride

The IL of long molecular chain as template can be utilized to produce the order super-microporous nanomaterials. The further results show that the variation of the molecular chain of the IL (from C_{14} to C_{18}) has only slight influence on the resulting pore size, still in the super-microporous size regime[34].

The IL, Fig.6, can also be used as solvent to synthesize very small TiO_2 nanocrystals at mild temperature and the formed TiO_2 nanocrystals was found to self-assemble into mesoporous spherical aggregates in Fig.436.

ILs as solvent and stabilizer for preparation of metal nanoparticles

As ILs can reach electrochemical windows of more than 4 V depending on the systems, ILs have been used as solvent for electrodeposition of nano crystalline films of a wide range of metals, alloys and semiconductors, especially a number of elements that cannot be electrodeposited from aqueous solution, such as the light and refractory metals. The related work has been reviewed[37]. ILs have also been utilized as reaction medium for synthesis of solution-stable metal nanoparticles. The present review reports the most recent literatures on the application of ILs to solution-stable metal nanoparticles.

Figure 4. 1-butyl-3-methyl-imidazolium-tetrafluoroborate

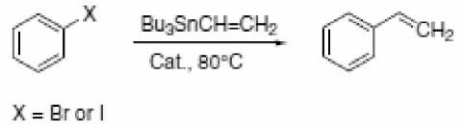
The synthesis and processing of nanoparticles consisting of metallic nanocrystal cores and organic monolayer shells promise interesting technological applications. Here, we report the synthesis of gold nanoparticles modified with ionic liquids based on the imidazolium cation. Aggregation-induced color changes of the gold nanoparticles in an aqueous solution were used as an optical sensor for anions via anion exchange of ionic liquid moiety. We also demonstrated the phase transfer of the gold nanoparticles from aqueous media to ionic liquid38.

The principle application of ionic liquids in chemistry has been as alternative solvents for synthesis and catalysis. Of the numerous task specific ionic liquids developed, a comparatively simple class of ionic liquid with alkyl-nitrile chains attached to the ionic liquid cation has been reported by Zhao[39] and his group. While the nitrile functionality is only weakly basic due to the presence of the ionic liquid cation (typically an imidazolium or pyridiniumcation), it can nevertheless weakly coordinate to metal centers[40]. The net effect of such weak coordination is four-fold:

1. To facilitate the solubility of metal salts in the ionic liquids.
2. To be sufficiently labile so as not to suppress catalytic activity by blocking coordination sites during reaction.
3. To stabilize the catalyst, facilitate the formation of transition states, and therefore increase its lifetime.
4. To enhance the immobilization of the catalyst in the ionic liquid during product extraction, thereby improving recycling.

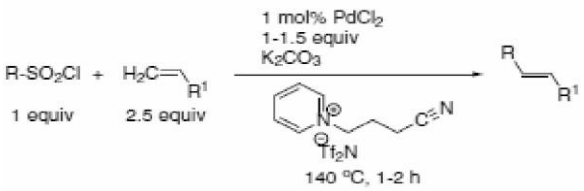
These advantages have been demonstrated to the greatest extent in palladium catalyzed C-C coupling reactions such as Suzuki, Heck, and Stille reactions[41]. Moreover, palladium nanoparticles separated after catalysis were analyzed by TEM and the images indicate that the nitrile-functionalized ionic liquid stabilizes palladium nanoparticles which are present as Pd(0) reservoirs. ICP analysis of the organic phase for palladium residues is often below the detection limit (1 ppm) suggesting that such ionic liquids could be useful in the synthesis of pharmaceuticals and liquid crystals where trace metal impurities must be extremely low.

Nitrile-functionalized ionic liquids are considerably more effective for the immobilization of palladium catalysts for the transfer of a vinyl group in Stille reactions with respect to alkyl-substituted ionic liquids (Scheme 2)[42]. Again, TEM analysis of nanoparticles extracted from the ionic liquids provide evidence for the stabilizing effect exerted by the nitrile pendant group on the metal center.



Scheme 2. Stille reaction in alkyl-substituted ionic liquids

While model substrates were used in the above examples, desulfitative Mizoroki-Heck-type arylation of alkenes using complex precursors can be performed efficiently in nitrile functionalized ionic liquids (Scheme 3), again proving superior to the commonly used organic solvents and simple ionic liquids[43].



Scheme 3. Desulfitative Mizoroki-Heck-type arylation of alkenes.

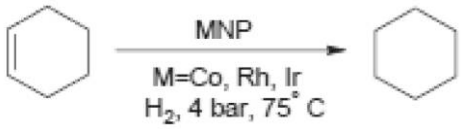
In general, these nitrile-derivatized ionic liquids can act as acetonitrile replacements wherever the advantage of having a non-volatile equivalent can be envisaged. If one searches the literature for reactions where optimum results are obtained in acetonitrile or other nitrile based solvents, then it is clear that these ionic versions offer considerable potential in synthesis and catalysis[44].

Salunkhe *et al.*[45] have demonstrated the importance of ionic liquids as superior reaction media for copper nanoparticle-catalyzed cycloaddition reaction. The group observed that only imidazole containing ILs

cannot provide sufficient stability to nanoparticles, that the use of stabilizing agents is mandatory.

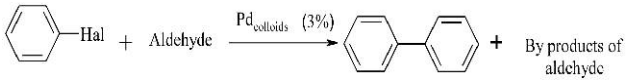
Gold nanoparticles have many optical and electrical properties that can be maximized by a high number density in solution. The best way to maximize the number density is to create a material that does not rely on solvent. Gold "nanosalts" have been prepared by functionalizing gold nanoparticles with a charged organic surfactant and balancing its charge with another bulky organic molecule. Spectroscopic evidence suggested that organic material has been attached to the gold nanoparticles and that it is an ionic liquid at room temperature. It has also been shown that the gold nanosalt is capable of forming ordered arrays and is capable of self-healing. Gold nanoparticles that were approximately 20 nm in diameter were made by reducing hydrogen tetrachloroaurate(III) hydrate with sodium citrate. The surface was functionalized with sodium mercaptoethanesulfonate. These gold nanosalts were characterized extensively. The self-healing properties of the gold nanosalt make it extremely useful for many Air Force applications including radar switches[46].

Recently, Redel and co-workers[47] have synthesized stable cobalt, rhodium, and iridium nanoparticles by thermal decomposition under argon from $Co_2(CO)_8$, $Rh_6(CO)_{16}$ and



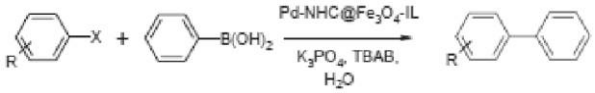
Scheme 4. Biphasic liquid-liquidHydrogenation of cyclohexene

$Ir_4(CO)_{12}$ dissolved in the ILs [bmim]BF_4, [bmim]OTf and [btmA]NTf_2 [bmim_n-butyl-methyl-imidazolium, btmA= n-butyl-tri-methyl-ammonium, OTf=O_3SCF_3, $NTf_2N[O_2SCF_3]_2$. They achieved very small and uniform nanoparticle size of about 1-3 nm in [bmim]BF_4. Increase in size was observed with increase in molecular volume of the IL anion from [bmim]OTf to [btmA]NTf_2. Importantly, among these nanoparticles the rhodium or iridium nanoparticle/IL systems function as highly effective and recyclable catalysts in the biphasic liquid-liquid hydrogenation of cyclohexene to cyclohexane (Scheme 4) with activities of up to 1900 molproduct/(molmetal) and quantitative conversion. Cal et al[48] reported the use of palladium nanoparticle for Ullmann reactions in tetrabutyl ammonium salt ILs as a reservoir of catalyst with aldehydes as the reductant (Scheme 5).



Scheme 5. Pd-nanoparticles catalysed Ullman reaction

This type of "ligand-free" catalysis is gaining considerable importance because it avoids the use of toxic or expensive phosphine ligands and allows catalyst recycling. The role of TBAA is crucial for this process, as this IL behaves simultaneously as a base, ligand, and reaction medium and has effect on the chemoselectivity of the catalysis which cannot be obtained by replacing this IL with a generic source of acetate anions. Taher and co-workers[49] reported Pd-NHC-IL matrix immobilized into IL layers coated on the surface of Fe_3O_4 (Scheme 6) by a simple process.

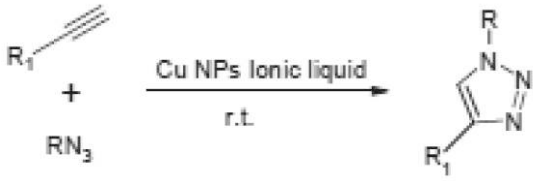


Scheme 6. Coupling reaction.

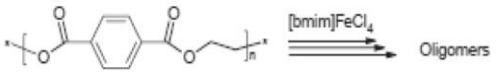
These immobilized Pd-NHC exhibited both high catalytic activity and stability for the coupling between aryl bromide and arylboronic acid in water. Importantly, this catalyst was simply recovered by an external permanent magnet and recycled without a significant loss in the catalytic activity.

The authors[50] have demonstrated the dramatic effect of ILs on shape of copper nanoparticles and their application in 1,3-dipolar cycloaddition reactions of azides and aryl- and sugar-based terminal alkynes. Change in anion has led to change in shape of the nanoparticle. It was observed that both the ILs used could not stabilize the nanoparticles and we had to use polyvinyl pyridine (PVP) as capping agent for these nanoparticles. Spherical nanoparticles were obtained in [bmim]BF_4 while cubical nanoparticles were obtained in [bmim]PF_6. Both the types of nanoparticles have shown profound effect on the cycloaddition reaction between azides and terminal alkynes

(Scheme 7). Wang and co-workers[51] demonstrated an effective depolymerization of poly (ethylene terephthalate), PET (Scheme 8), using iron-containing imidazolium-based IL 1-butyl-3-methylimidazolium tetrachloroferrate [bmim]$FeCl_4$. The advantages of using this IL are relatively low-reaction temperature, good conversion with enhanced selectivity in comparison with $FeCl_3$ and [bmim]Cl.

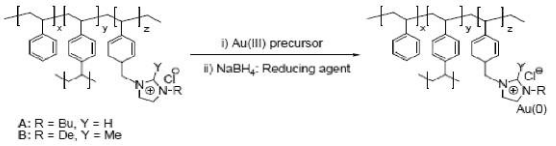


Scheme 7. Cycloaddition reaction



Scheme 8. Depolymerization of PET

Isabel and co-workershave reported the characterization and development of gold nanoparticles (AuNPs) immobilized onto two kinds of gel-supported Ionic Liquids (gel-Supported Ionic Liquid like phases, g-SILLPs) and their study as catalysts for the oxidation of organic compounds. The benchmark reaction selected for this purpose was the oxidation of 1-phenylethanol. The reactions were performed using microwave heating (as the energy source), water (as the solvent), hydrogen peroxide (as a benign oxidant agent), and supported catalysts (providing a more efficient catalytic processes and easy recovery of the catalyst)[51].



Scheme 9. Synthetic procedure for the preparation of the AuNPs-supported catalysts

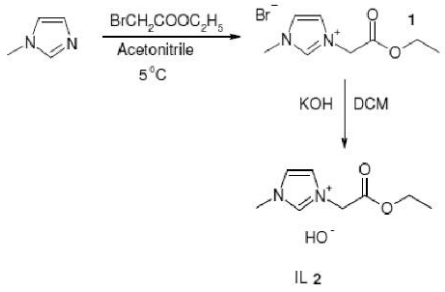
The UV-visible characterization of the catalysts confirms the presence of reduced gold species. The use of g-SILLPs on AuNPs support is a new approach to obtain an efficient catalyst for the oxidation of secondary alcohols under benign reaction conditions. This approach represents an efficient strategy towards the design of environmentally friendly processes, saving time, energy and reagents and reducing the waste generated.

Hengyao Hu and his group found a small amount of ionic liquid [bmim][BF_4] to be an efficient aid for microwave heating of nonpolar dibenzyl ether in high temperature solution-phase synthesis of monodisperse magnetite nanoparticles. It was found to act as both microwave absorber and assistant stabilizer in there active process and was recovered and reused in successive reactions. The diameter of the particles is around 6 nm. The reaction time is as low as 10 min due to the high efficiency of microwave heating. The ionic liquid can be recovered and reused in successive reactions for many times. Hence this method might be suitable for economical mass-synthesis of monodisperse magnetite nanoparticles[52].

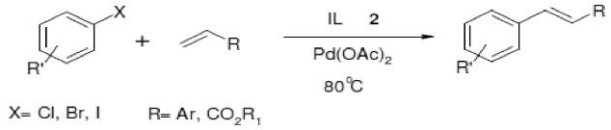
Another group has written a review on supported ionic liquid phase (SILP) catalyst system in which an ionic liquid (IL) film is immobilized on a high-surface area porous solid and a homogeneous catalyst is dissolved in this supported IL layer, thereby combining the attractive features of homogeneous catalysts with the benefits of heterogeneous catalysts. In this review reliable strategies for the immobilization of molecular catalysts in SILPs are surveyed. In the first part, general aspects concerning the application of SILP catalysts are presented, focusing on the type of catalyst, support, ionic liquid and reaction conditions. Secondly, organic reactions in which SILP technology is applied to improve the performance of homogeneous transition-metal catalysts are presented: hydroformylation, metathesis reactions, carbonylation, hydrogenation, hydroamination, coupling reactions and asymmetric reactions[53].

Sawant has reported[54] an ester functionalized, basic imidazolium based multifunctional Ionic Liquid viz. 3-methyl-1-(ethoxycarbonylmethyl) imidazolium hydroxide (IL2) prepared by reaction of 1-methyl imidazole with ethyl bromoacetate and subsequent metathesis with potassium hydroxide. He claims that this kind of ester functionalized ILs have added advantage of biodegradability an analogous behavior to their alkyl side chain analogues (like [bmim]PF_6, [bmim]OH etc.). The ionic liquid was used for palladium catalyzed Heck reaction of aryl halides and olefins. This phosphine-free Pd-IL system showed

excellent activity and reusability at relatively lower reaction temperature (80°C). There was in-situ generation of palladium nano-particles which was confirmed by TEM analysis. The high activity of this catalytic system at relatively lower temperatures could be based on in situ generation of Pd nanoparticles. Thus it was shown that the TSIL 2 plays dual role of reductant (Pd(II) to Pd(0)) and stabilizer for these generated palladium nano particles.



Scheme 10. Synthesis of IL 2



Scheme 11. An ester appending multifunctional ionic liquid for Pd(II) catalyzed Heck reaction

Vladimir etal[55] have reported an efficient green Heck reaction protocol using triethanolammonium acetate ionic liquid-palladium(II) catalytic system. The ionic liquid serves as a reaction medium, base, precatalyst-precursor, and mobile support for the active Pd species. The mechanism of the reaction of triethanolammonium acetate with $PdCl_2$ was examined using density functional theory. It was found that two Pd(II) complexes were formed, one of which acts further as a precatalyst yielding catalytically active Pd(0) complexes.

Supported ionic liquid catalysts

In order to design a new catalyst generation with high activity, selectivity, stability and efficiency supported ionic liquid phase (SILP)-catalysts were developed. These catalysts combine the advantages of homogeneous and heterogeneous catalysis, therefore expensive separation processes are not necessary, decreasing the costs of production[56].

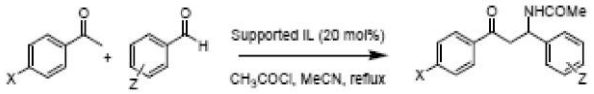
Supported ionic liquid catalysts are prepared by impregnation of a porous material with high specific surface area with an ionic liquid. Catalytic active components like metal nanoparticles or metal organic complexes are immobilized in the ionic liquid phase[57]. Due to the formation of an ionic liquid film on the surface undesirable side reactions can be inhibited, lowering the amounts of by-products and thus increasing the efficiency of the catalysis.

Supported ionic liquid catalysts can be used in fixed-bed reactors without the need for organic solvents. Due to the non-volatile ionic liquid film the catalysts can be applied in gas phase reactions[56]. They were successfully used in test reactions like hydroformylation of olefines[57], achiral hydrogenation[58], Heck-reaction[59] and hydroamination[60,61]. These applications can usually not be realized with supported organic phase[62]and aqueous phase[63,64] catalysts that were developed since 1990. The liquid film of these catalysts is volatile, so that high temperatures cannot be applied and also the stability of the catalytic active component the organic or aqueous phase is sometimes problematic[65].

Silica supported platinum catalysts coated with a thin film of 1-butyl-2,3-dimethyl-imidazolium trifluoromethanesulphonate (BDiMIm) were investigated with respect to the interactions of the ionic liquid with the oxide support and the metal clusters. IR, inelastic neutron scattering and NMR spectroscopy indicate that the vibrations of the imidazolium ring of ionic liquid are less restricted when supported on SiO_2, while the viscosity of the supported ionic liquid increased. The presence of Pt particles enhances the electron density of the ionic liquid at the nitrogen atom inducing higher basicity. The coverage of the catalyst surface and the metal particles by the ionic liquid protects the metal against oxidation. The catalysts are active and stable for hydrogenation of ethene[66].

Immobilization of 1-methylimidazoliumtrifluoroacetate ([Hmim]TFA) on silica-coated magnetic nanoparticle (SiMNP) has been reported by LalehTorkiana's team[67]. Cobalt spinel ferrite MNPs were chosen as a catalyst support based on their high magnetic susceptibility

and ability for surface functionalization. AFM image of the bare MNPs displayed aggregated nanoclusters, roughly 50 nm in diameter. β-acetamido ketones were synthesized using SiMNP with immobilized [Hmim]TFA-based supported ionic liquid catalyst.



Scheme 12. Supported IL catalyzed synthesis of β-acetamido ketones

An overview by Christian et al[68]with more than 160 references on the synthesis and stabilization of metal nanoparticles (M-NPs) from metal carbonyls, metal salts in ionic liquids (ILs) and in particular from metal carbonyls in ionic liquids has been reported. Reports say that the M-NPs can be synthesized by chemical reduction, thermolysis, photochemical decomposition, electroreduction, microwave and sonochemical irradiation. Commercially available metal carbonyls Mx(CO)y are elegant precursors as they contain the metal atoms already in the zero-valent oxidation state needed for M-NPs. No extra reducing agent is necessary. The side product CO is largely given off to the gas phase and removed from the dispersion. The microwave induced thermal decomposition of metal carbonyls Mx(CO)y in ILs provides an especially rapid and energy-saving access to M-NPs because of the ILs significant absorption efficiency for microwave energy due to their high ionic charge, high polarity and high dielectric constant. The electrostatic and steric properties of ionic liquids allow for the stabilization of M-NPs without the need of additional stabilizers, surfactants or capping ligands and are highlighted by pointing to the DLVO (Derjaugin–Landau–Verwey–Overbeek) and extra-DLVO theory. Examples for the direct use of M-NP/IL dispersions in hydrogenation catalysis of cyclohexene and benzene have been reported.

Ionic Liquids as Recycling Solvents for The Synthesis of Magnetic Nanoparticles[69]

An easy synthesis of MFe_2O_4 (M=Co, Fe, Mn, and Ni) magnetic nanoparticles MNPs has been reported by the thermal decomposition of $Fe(Acac)_3/M(Acac)_2$ by using $BMI{\cdot}NTf_2$ (1-n-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide) or $BMI{\cdot}PF_6$ (1-n-butyl-3-methylimidazoliumhexafluorophosphate) ionic liquids (ILs) as recycling solvents andoleylamine as the reducing and surface modifier agent. The growth of the MNPs is easily controlled in the IL by adjusting the reaction temperature and time. However, the thermal decomposition of $Fe(acac)_3$ performed in a conventional high boiling point solvent (diphenyl ether, bp 259 °C), under a similar Fe to oleylamine molar ratio used in the IL synthesis, does not follow the same growth mechanism and rendered only smaller NPs of 5 nm mean diameter. All MNPs are covered by at least one monolayer of oleylamine making them readily dispersible in non-polar solvents. Besides the influence on the nanoparticles growth, which is important for the preparation of highly crystalline MNPs, the IL was easily recycled and has been used in at least 20 successive syntheses.

Oliver et al[70] has reported the application of stable glow discharge plasmas as free electrodes for the generation of nanoparticles in ionic liquids. This group has studied the interaction of the plasma with the ionic liquids itself and possible influences on the production process of the particles. The focus was on the generation of noble metals and the efforts to synthesize semiconductor nanoparticles.

Shape-controlled synthesis of Ag crystals has been reported by Tae Young Kim and co-workers[71]exploiting ionic liquids (ILs) as a shape-regulating agent. The synthesis of Ag crystals involved the reduction of $AgNO_3$ by EG in the presence of ILs, specifically 1-butyl-3-methylimidazolium methylsulfate (bmim-$MeSO_4$). In accordance with non-classical crystallization growth mechanism, the primary Ag nanoparticles were formed at the early stage of the reaction, and then self-organized into 1D or 3D Ag superstructures via an IL-mediated self-assembly process. Their final morphologies were strongly dependent on the reaction conditions such as the concentration of ILs in the reaction mixture and the reaction temperature, which suggests that ILs play an important role in controlling the shape of the Ag crystals.

Burguete's group[72] has shown that crosslinked polymers containing covalently attached functional sites with chemical structures related to those present

in ILs can be advantageously applied to the preparation of gold nanoparticles (AuNPs). The size and morphology of the resulting metal nanoparticles (MNPs) could be easily modulated through a proper combination of the synthetic method used for the generation of the NPs and the structural elements of the polymer.

Gold nano-particles[73] were synthesized via a reductive reaction in ionic liquid solution containing Au^{3+} ions using a low-energy electron irradiation technique. The sizes of the primary particles increased with higher acceleration energy of the electron beam, whereas they did not depend so much on the beam current. The anion of the ionic liquid strongly affected the size and shape of the primary particles, which was due to the different local structure of the ionic liquid around the Au particles. When the thickness of the ionic liquid layer was smaller than the penetration length of the electron beam, the formation of secondary particles was suppressed. These results gave an important knowledge for controlling the size and shape of the metal particles, which is important for application of various catalyst or devices (Fig 5).

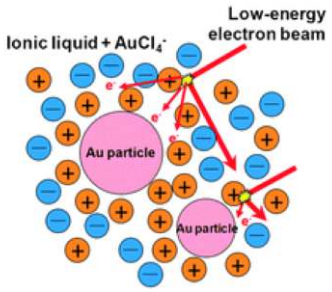


Figure 5. Synthesis of Au nanoparticle via a reductive reaction in ionic liquid solution containing Au^{3+} ions using a low-energy electron irradiation technique

Anion effect on the shape evolution of gold nanoparticles during seed-induced growth in imidazolium-based ionic liquids has been reported[74]. Shushi Suzuki[75] has reported the immobilization process of Au nanoparticles on TiO_2 by electrostatic interaction between the surface and ionic liquids. There are several reports on sputter deposited gold nanoparticles in ILs[76,77]

Eva Raluy and co-workers have a reported a smart palladium catalyst in ionic liquid for tandem processes[78]. New catalytic systems based on in situ and preformed palladium nanoparticles in ionic liquids (characterised by TEM) starting from palladium acetate or dipalladiumtris(dibenzylideneacetone) have been applied in the synthesis of 4-phenylbutan-2-one (II), a model compound for the preparation of fragrances. Imidazolium-based ionic liquidcontaining a methyl hydrogenophosphonate anion leads to an efficient Pd-catalyzed tandem coupling/reduction process, taking advantage of the multi-role of this solvent (nanoparticles stabiliser, base, hydrogen transfer agent). The influence of the mono-phosphine ligands on the catalyst has been evaluated, showing that the ligand-free palladium system turns into the most appropriate for the formation of II using $Pd(OAc)_2$ as precursor. Fine-tuning conditions involved in this multi-parameter process have led us to propose a plausible mechanism based on the hydrogen transfer coming from the methyl hydrogenophosphonate anion. Hayes[79] has reported sponge-like nanostructure in ionic liquid propylammoniumnitrate (PAN).

Polynitrogen ligands and/in ionic liquids (ILs) have been described[80] as a pertinent tandem to efficiently stabilize rhodium nanoparticles (NPs) in the size range of 2.0 nm for catalytic applications. Several N-donor ligands based on bipyridine skeleton have been used as extra protective agents in[BMI][PF_6] and compared in the hydrogenation of functionalized aromatic compounds at 80 °C and under 40 bar H_2. The hydrogenation of various oxygen-containing arenes were investigated and original results were described in the reduction of anisole and cresols as model lignin compounds, providing a significant ratio of ketone derivatives.

Kerstin et al[81] investigated the formation of Cu Clnanoplatelets from the ionic liquid precursor (ILP) butylpyridiniumtetrachlorocuprate $[C_4Py]_2[CuCl_4]$ using ascorbic acid as a reducing agent. Electron paramagnetic resonance (EPR) spectroscopy was used to evaluate the interaction between ascorbic acid and the Cu(II) ion before reduction to Cu(I). EPR spectroscopy suggests that the $[CuCl_4]^{2-}$ ion in the neat IL is a distorted tetrahedron, consistent with DFT calculations. Addition of ascorbic acid led to the removal of one chloride from the $[CuCl_4]^{2-}$anion. DFT suggested that the most stable adduct is formed when

only one hydroxyl group of the ascorbic acid coordinates to the Cu(II) ion.

Ionic liquids are a stabilizing medium for the in situ synthesis of ruthenium nanoparticles. Gorka Salas[82] and his team showed that the addition of molecular polar solutes to the ionic liquid, even in low concentrations, eliminates the role of the ionic liquid 3D structure in controlling the size of ruthenium nanoparticles, and can induce their aggregation. They have performed the synthesis of ruthenium nanoparticles by decomposition of [Ru(COD)(COT)] in 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, $[C_1C_4Im][NTf_2]$, under H_2 in the presence of varying amounts of water or 1-octylamine. For water added during the synthesis of metallic nanoparticles, a decrease of the solubility in the ionic liquid was observed, showed by nanoparticles located at the interface between aqueous and ionic phases. When 1-octylamine is present during the synthesis, stable nanoparticles of a constant size are obtained. When 1-octylamine was added after the synthesis, aggregation of the ruthenium nanoparticles was observed. In order to explain these phenomena, the molecular interactions between the different species using ^{13}C-NMR and DOSY (Diffusional Order Spectroscopy) experiments, mixing calorimetry, surface tension measurements and molecular simulations were explored. It was concluded that the behaviour of the ruthenium nanoparticles (Fig 6) in $[C_1C_4Im][NTf_2]$ in the presence of 1-octylamine depends on the interaction between the ligand and the nanoparticles in terms of the energetics but also of the structural arrangement of the amine at the nanoparticle's surface.

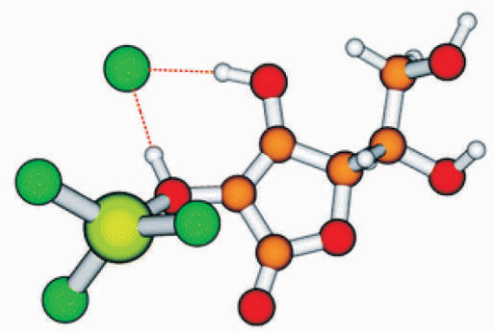


Figure 6. Ruthenium nanoparticles in ionic liquids

A novel strategy for tailoring the adsorption and structural properties of ionic liquid derived carbons has been developed. By changing the carbonization temperature and ratios of ionic liquids (ILs) containing a cross-linkable anion, such as 1-butyl-3-methylimidazolium tricyanomethanide [BMIm][C(CN)$_3$] and 1-ethyl-3-methylimidazolium tetracyanoborate [EMIm][B(CN)$_4$], boron and nitrogen-rich carbons with slit-like pores and specific surface areas exceeding 500 m^2 g^{-1} have been prepared. Furthermore, the nitrogen-rich carbons exhibit high adsorption capacity for CO_2 adsorption and selectivity for CO_2/N_2 separation[83] (Fig. 7).

The highly water-soluble palladium nanoparticles (NPs) were synthesized by using the amphiphilicpoly(ethylene glycol)-functionalized dicationic imidazolium-based ionic liquid(C_{12}Im-PEG IL) as a stabilizing agent. The aqueous dispersed palladium NPs in the range of 1.9 ± 0.3 nm were observed by transmission electron microscopy (TEM). The physicochemical properties of C_{12}Im-PEG IL in aqueous phase have been characterized by electrical conductivity, surface tension and dynamic light scattering (DLS) measurements. It was demonstrated that the amphiphilic ionic liquid can form micelles above its critical micelle concentration (CMC) in aqueous solution and the micelles played a crucial role in stabilizing the palladium NPs and thus promoted catalytic hydrogenation. Furthermore, the

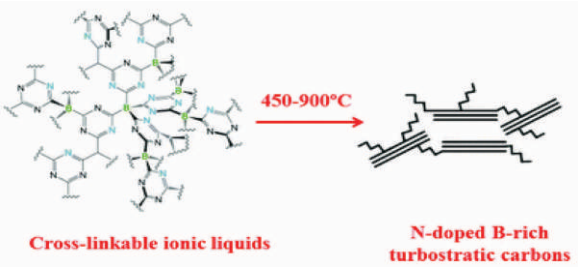


Figure 7. N-doped B-rich turbostatic carbons

dicationic ionic liquid can also act as a gemini surfactant and generated emulsion between hydrophobic substrates and the catalytic aqueous phase during the reaction. The aqueous dispersed palladium NPs showed efficient activity for the catalytic hydrogenation of various substrates under very mild conditions and the stabilizing Pd(0) nanoparticles (NPs) can be reused at least eight times

with complete conservation of activity[84].

Reports[85] show that Ionogelshave been created by blending ionic liquid-functionalized inorganic nanoparticles with an ionic liquid. A novel class of silica ionogels has been created by dispersing silica nanoparticles densely grafted with the ionic liquid (IL) 1-trimethoxysilyl propyl-3-methyl-imidazolium bis(trifluoromethylsulfonyl) imide (SpmImTSFI) in a 1-butyl-3-methyl-pyrrolidinium bis(trifluoromethylsulfonyl) imide (BmpyrTFSI) IL host. It was found that over the entire range of nanoparticle volume fractions studied the systems exist as stable suspensions of SiO_2-SpmImTFSI in the BmpyrTFSI host. Remarkably, addition of even minute quantities of SiO_2-SpmImTFSI to the BmpyrTFSI IL suppressed the crystallization of the host. The resulting disordered hybrid fluids exhibited liquid-like transport properties over a vastly extended temperature range; they open the way for facile synthesis of ILs with extended operating temperature windows. These observations are explained in terms of ionic coupling of the nanoparticle-tethered and free TFSI anions, which is thought to suppress crystallization of BmpyrTFSI (Fig. 8).

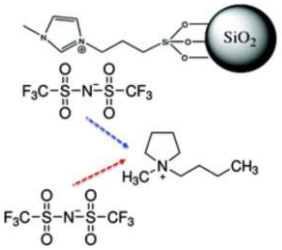
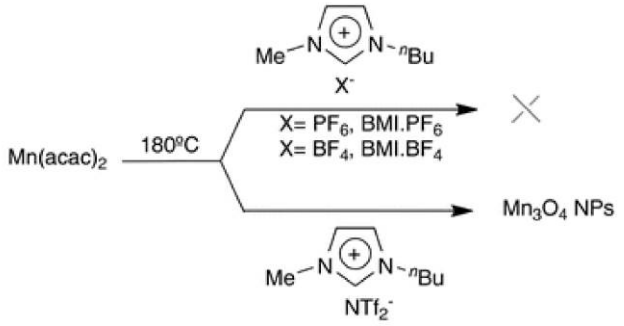


Figure 8. Ionic Liquid-Tethered Nanoparticle Suspensions: A Novel Class of Ionogels

Dewan et al[86] has reported a novel synthesis of copper nanoparticles from copper sulphate utilizing the charge-compensatory effect of ionic liquid [bmim]BF_4 and ethylene glycol. The nanoparticles were characterized and found to be stable for one year. They hypothesize that the stabilized nanoparticles should be able to catalyze one-pot multicomponent organic reactions. The group showed that the nanoparticles catalyzed Biginelli reaction at room temperature to give the product 3,4-dihydopyrimidinone (.90% yield in 15 minutes) from aldehydes, β-diketoester (ethylacetoacetate) and urea (or thiourea). Remarkably, such high yields and rapid kinetics were found to be independent of the electronic density on the reactant aryl-aldehyde. This was probably because even the surface-active particles reacted faster in the presence of ionic liquid as compared to conventional methods.

Two synthesis techniques have been developed by Paul and his group for the generation of metal nanoparticles that take advantage of the unique properties that ILs offer when compared to conventional volatile organic solvents (VOCs): microwave (MW) synthesis and physical vapour deposition (PVD). The ionic character and high polarisability of the IL renders it highly susceptible to energy uptake via MWs and extreme heating and reaction rates can be achieved. To make full use of the possibilities that ILs offer the group has designed a set of reducing ILs which can be used as direct reaction partners for the generation of metal nanoparticles. The negligible vapour pressure of many ILs makes experiments under high vacuum possible and allows for the PVD of metals into ILs[87].

Dupont et al[88] has described a simple one-step synthesis of Mn_3O_4 nanoparticles by thermal decomposition of [$Mn(acac)_2$] (acac = acetylacetonate) using imidazolium ionic liquids (ILs) and a conventional solvent, oleylamine, for comparison (Scheme 13). The Mn_3O_4 nanoparticles were characterized by XRD, ATR-FTIR, TEM, Raman, UV/VIS and magnetometry techniques. The addition of 1-n-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)amide IL (BMI·NTf_2) yielded a smaller particle size (9.9 ± 1.8 nm) with better dispersion and more regular sizes than synthesis using oleylamine as the solvent (12.1 ± 3.0 nm). Magnetometry revealed that Mn_3O_4 nanoparticles synthesized in either oleylamine or BMI·NTf_2 exhibited ferrimagnetic behavior at low temperatures, whereas they were paramagnetic at room temperature. As expected, the blocking temperature and the coercivity decreased with the size of nanoparticles. This showed that reaction conditions such as time, and the nature of the ionic liquid play important roles in determining the size of Mn_3O_4 nanoparticles.



Scheme 13. One-step synthesis of Mn_3O_4 nanoparticles by thermal decomposition of [Mn(acac)$_2$] (acac = acetylacetonate) using imidazolium ionic liquids (ILs)

Palladium nanoparticles were prepared[89] using different methods in conventional organic solvents and in ionic liquids. The deposition of these Pd nanoparticles onto multiwalled carbon nanotubes produces homogeneous dispersions of the metal over the carbon surface. The application of these new materials in C-C coupling Pd-catalyzed reaction has been tested.The catalytic C-C couplings of Heck and Suzuki type were performed using iodobenzene and methyl acrylate (Heck) or phenylboronic acid (Suzuki) under standard conditions following published procedures. The catalytic activity in standard CC couplings of these nanocomposite materials has been tested, obtaining high yields in all cases.

Stable silver nanoparticles were successfully synthesized by chemical reduction of silver nitrate in ionic liquids 1- Ethyl-3-methylimidazolium tetrafluoroborate [Emim] BF_4 and 1- Ethyl-3-methylimidazolium hexafluorophosphate [Emim] PF_6 at room temperature. The characterization of the silver nanoparticles such as their size and shape was performed by X-ray Diffraction (XRD) and Scanning Electron Microscope (SEM) techniques which indicated a size range of 50 to 55 nm. The antimicrobial activity of silver nanoparticles for three gram negative bacteria and three gram positive bacteria were investigated. It appeared that [Emim] PF_6 and its Ag nanoparticles are the most effective products against the tested bacterial strains compared with [Emim] BF_4 and its Ag nanoparticles[90].

The synthesis of noble metal nanoparticles (NMNPs) using, or in the presence of, ionic liquids (ILs) represents a burgeoning direction in materials chemistry. Processing these nanomaterials synthesized in ILs would be drastically simplified if they could be routinely dispersed into a wide variety of polar/nonpolar solvents. The phase transfer of the prepared nanoparticles from ILs to an organic or aqueous medium has been reported.. The protocol involves first mixing the noble metal sols in ILs and an ethanolic or a methanolic solution of transfer agent and then extracting the transfer-agent-stabilized NMNPs into toluene or aqueous phase. Electron micrographs reveal that the particles are fully dispersed after transfer, and the size/morphology of the NMNPs could be significantly tuned by the ILs. In particular, electrochemical measurements of the Pt nanoparticles upon methanol oxidation reaction demonstrate that the particles are dominated by low-index crystal planes[91].

Conclusion

The updated application of the IL in the synthesis of inorganic nanomaterials was briefly outlined. Since its introduction to organic, catalytic and electrochemistry, ILs have captured and held the imagination of the chemists working in these areas, and tremendous progress has occurred. Despite that the application of the ILs in inorganic nanomaterials is still in its infancy, however, many signs imply that IL is becoming an important emerging field in the nanomaterials in the coming years.

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