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Editorial Article

Save Water Save Life

Krishan Kumar Saini

Water is one of the prime elements responsible for life on earth, human body consisting of 75 percent of it and two thirds of the earth's surface is covered by water. Water circulates through the land just as it does through the human body, transporting, dissolving and replenishing nutrients and organic matter, while carrying away waste material. Further in the body, it regulates the activities of fluids, tissues, cells, lymph, blood and glandular secretions.

Water is also used as religious symbol since antiquity, to express devotion and purity. Some cultures worship gods who were thought to live in and command the waters. Cities have been built by considering the location and availability of pure drinking water.

Contrary to the past, our recent developed technological society has become indifferent to this miracle of life. Our natural heritage (rivers, seas and oceans) has been exploited, mistreated and contaminated. The population decline of the marine and riparian life, the appearance of green algae in the rivers and the stench, slime that comes as a result of putrefaction in the water, are clear signs of the depth and extent of disruption that has been caused to this intricate ecosystem. Government and water authorities will have to make us believe that it is 'safe' and we should not worry about this global alarm. Awareness and action lies entirely upon us, as we need to become our own educators, physicians and innovators. Socrates had once said: "an unexamined life is not worth living....", Jesus took it a step further: "seek, and you shall find.....the truth shall set you free..." So questioning everything and anything that anyone tells you until it makes sense, is of uppermost importance. If it is the truth it will feel right, set you free and lead you on the road of discovery and recovery.

Today our drinking water is far from being pure. It contains some two hundred deadly commercial chemicals. Add to that bacteria, viruses, inorganic minerals (making the water hard) and you have a chemical cocktail that is unsuitable (if not deadly) for human consumption. Chlorine, Giardia & cryptosporidium (unicellular organisms), aluminium sulphate, sodium fluoride, lead, Nitrates from fertilisers are the cause serious diseases like cancer, intestine infection, loss of

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memory, tooth decay and many more. Inorganic minerals (minerals not suitable for human consumption) such as calcium carbonate, are unable to be assimilated and they store in between joints, muscles, bones, nerves, inside arteries and become partners in many crippling diseases, such as arthritis, hardening of the arteries, gall stones, kidney stones, gout, tinnitus and perhaps even stroke and neuralgia.

'You are whatever you drink, so make sure what you drink is pure and safe' Ten years ago the prospect of drinking only purified or bottled water was a fiction, or a novelty for most people. Nowadays, it is becoming a necessity in maintaining and preserving good health. Finding pure water is becoming more than just food for thought and with our brain being 85 percent water, we better start thinking of the choices.

Rain water is no longer the best available option with today's pollution. Water is a hungry solvent and as the rain falls, it begins to collect hundreds of potentially harmful substances, such as radioactive isotopes and their degradation products of atomic fission including barium, caesium and strontium from world wide atomic experiments and "accidents" which travel around the atmosphere. In addition industrial and exhaust fumes including carbon monoxide, sulphuric acid and lead are collected. That is why the sky looks so clean after a good 'acid' rain.

Spring water contains those unwanted inorganic minerals and their purity is debatable if you consider the pollution of the soil. So use it sparingly or when nothing else is available. Don't be mislead by claims about the value of inorganic minerals, the body cannot make use of any minerals unless they are derived from the plant kingdom (organic minerals). A well balanced diet will provide an abundance of organic minerals that water never could.

Reverse osmosis is by far the most advanced technology for home installation available to the public. It is based on the process by which the human cells diffuse fluids between the intracellular and extracellular spaces, by separating and selectively preventing the passage of solute molecules (through a semipermeable membrane) and allowing the passage of the solvent H_2O. Through this process almost all harmful bacteria, minerals and toxins are eliminated. Professional installation and surveillance is necessary for if the membrane is ruptured without your knowledge the final condition of the water could be worse than if it were not filtered.

Distilled water; there is a wide held view that it leaches organic essential minerals and micronutrients from our body but its emptiness works in favour. It dissolves and eliminates harmful inorganic minerals and toxic waste accumulation. Once the organic nutrients have been absorbed by the cells they cannot be taken away. Is there an inherent intelligentsia behind all this? The answer is yes! after all, what is the animating factor behind all things? but far from being just an esoteric answer, the key lies in the inherent 'instructions' of the human body's filtering system. The kidneys make sure that nothing valuable will be lost, there is a constant recycling, so even if

nutrients were to be 'stolen' they would be returned by the kidneys. But in normal distillation pollutant gases such fluorine and chlorine are also evaporated over into the condenser. Fragmented distillation and C.M.D method (Cold Molecular Distillation) are better options. C.M.D water contains no solid matter and is solely consisting of two elements, Hydrogen and Oxygen.

Another important factor is the amount of water necessary for our body to function at its peak performance. Our body looses each day about 2-3 litters of water through elimination, urination, perspiration and respiration. However, this may increase during illness, high performance, exercise, pregnancy and nursing. The beverages most people choose to consume are often counter-productive in promoting hydration. Coffee, tea, alcohol, soft and sugary drinks are all diuretics and will cause not only the loss of water they are dissolved in, but they will also draw water, the bodies reserves. In normal conditions your body needs to replace the fluids it has lost throughout the day. Most of fluids should be replaced by drinking pure water. The rest you should get from fruit, vegetables and their juices. Attention must be given that the elderly and children are meeting their daily requirements. Dry mouth is not the only indication of dehydration; in fact it is the last sign. You need to acquire the habit to drink water even when you think you don't need it and eventually your true thirst mechanisms will be reawakened. Signs to look for that identify with dehydration are constipation, headaches, indigestion, weight gain, fluid retention, dark and pungent urine, and their associated pathologies colitis, kidney stones, bladder and urinary track infections to name only a few.

Water is involved in all bodily functions: digestion, assimilation, elimination, respiration, maintaining temperature (homeostasis) integrity and the strength of all bodily structures. Today, the water is polluted with hundreds of toxins and impurities. Authorities only test for a small number of them. Our body, being primarily water, requires sufficient daily water replacement in order to function efficiently. Water treatments, that are aimed to render our drinking water bacteriologically safe, have been proven ineffective and the presence of certain pathogenic bacteria like giardia and cryptosporidium is just one of the many examples. Viewing the effects of individual chemicals, inorganic minerals and their by-products, one can see a link to today's major diseases. If you drink devitalised, impure water how can you expect vitality and health? Dehydration, due to the offensive taste of the water and the introduction of commercial sugar loaded beverages, has become another contributing factor to disease. Stop treating thirst with medications. Mineral water may be wonderful to bathe in, however, the presence of inorganic minerals makes it undesirable. Tap water has been proven unsuitable even for showering. Pure water may become the medicine of the future. 'Oxygen enriched and free of radioactive and chemical compounds' may read on the label of our bottle water in the next millennium. At this stage Reverse

Osmosis and C.M.D water are our best available options. Cost is an important issue, it should be in the reach of economically weaker section of the society.

(Krishan Kumar Saini)

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Role of Department of Science & Technology for promoting research through interaction: A case study

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Abstract

Science progresses by dissemination of scientific information and sharing of knowledge among scientists. There is best platform to present the scientific findings are conferences. Present study includes the role of Seminars/ Symposia/ Workshops and Conferences for promotion of science and technology in our country.

Keywords: Seminars | Symposia | Workshops | Conferences | Scientific Policy | Innovation

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Introduction

Science and Technology play an important role in laying foundation for social and economic development of country. Science serves and influences the society and society in turn supports the development of science. As a key to the development, science and technology are important not only for industrial development, which may bring not only economic growth, but is also bring change in behavioral, psychological, sociological, cultural and other development of scientific temper and outlook of scientific achievements, which in turn enhances international prestige. India has followed the policy of self-reliance through scientific research, to initiate, advance and accelerate national development in all segments. Given this policy initiative, India has been able to usher in to significant growth in its capacity and capability building in basic, applied, and developmental research. Its science & technology infrastructure has also become very large, comprising more than 400 universities, 400 research laboratories, 13 institutes of national importance, and 1300 in-house industrial R&D units, besides several

other government departments, private establishments, international and non-profitable institutions.

Science and Technology (S&T) are complementary in the knowledge domain, where in, Science symbolizes discovery and knowledge generation, and Technology symbolizes usable innovations using knowledge.

Thus Science → knows what

Technology → knows how

Technology is a mechanism for delivering the benefits of science to the people and society. Recognizing the importance of Science, Technology and Innovation (STI) in the economic and Industrial growth, the government of India aspires for faster sustainable and inclusive growth and reemphasized the need to view Science, Technology and innovation together in its "Science*, Technology and Innovation policy 2013*" following the "*Science and Technology Policy- 2003*" – "*Scientific Policy Resolution of* 1958" and the "*Technology Policy Statement* 1983". While stating that "the nation continues to be firm in its resolve to support Science & Technology in all its facets", the policy recognizes the central role of our S&T system "in raising the quality of life of the people, particularly the marginal section of the society. Science, Technology and Innovation (STI) is increasingly important for economic growth, business competitiveness have emerged as the major drivers of national development globally competitive in utilizing natural resources in a sustainable manner, in protecting the environment and ensuring national security is of utmost importance. Over the years, there have been serious concerns about the ability of the basic sciences to attract bright young students. The standards of university education and the miniscule contributions they make to research and development are areas of concerns. Workshops/ Seminars/ Symposia and Conferences at National and International Level in various fields provide platform to promote and develop scientific temper and foster scientific and technological researches in universities and other academic institutions and attract brightest and young persons. We will discuss in this paper the role of Seminars/ Symposia/ Workshops and Conferences for promotion of scientific activity in our country. There are many Central agencies that provide grant –in - aid to facilitate platforms of Seminars/Symposia and Conferences to share and foster research. A few prominent out of them are as below.

University Grant Commission

University Grant Commission provides financial assistance scheme to institutions for organizing Workshops/Seminars/Symposia and Conferences at National and International Level in various fields. The basic objective of the scheme is to bring together academicians and experts from different parts of the country and abroad to exchange knowledge and ideas. Further the scheme intends to provide a platform to teachers and researchers for sharing their knowledge, experiences and research in order to promote high standards in Colleges for making strong base and generating quality man power for research and

teaching. The occasion provide an in-depth analysis of subjects and update the knowledge of the participants from academic as well as research institutions. Under this scheme the financial assistance provided by the commission has Ceiling of via Regional Level Seminar/Workshop Rs. 70,000/-, State Level Conference/Workshop Rs. 80,000/- National Level Conference/Workshop Rs. 1.00 lakh, International Level Conference/Workshop Rs. 1.50 lakh.

Department of Biotechnology

Department of Biotechnology provide financial support for organizing Seminar/ Symposium/ Conference in specialized area related to biotechnology such as, tissue Culture, Seribiotechnoloy, Biofertilizers/ Biopesticides, Food Biotechnology, Medicinal & Aromatic Plants, Animal Biotechnology, Aquaculture & Marine Biotechnology, Animal Tissue Culture, Hybridoma and Cell culture-based Vaccines, Medical Biotechnology, Immunology and Immunodiagnostics, Microbial & Industrial Biotechnology, Biochemical Engineering, Downstream Processing and Process Optimisation, Pharmaceutical Biotechnology, Molecular Virology, Human Genetics and Genome Analysis, Peptide and Nucleic Acid Chemistry and Applications, Protein Research, IPR, Bioproducts and Biosafety, Bioprospecting, Biodiversity Conservation and Environmental Biotechnology, Bioinformatics etc.

Council of Scientific & Industrial Research

CSIR provide grant for the organization of a symposium/seminar/conference/workshop etc. of national character. All India societies/associations of scientists and engineers and academic institutions are eligible to apply for the Grant. The applications must be received at least three months before the event. The application is to be filled in by the Executive Authority of the Parent Organization and countersigned by the local organizing Committee and the Head of the Institution where the Symposium/Seminar is to be held.

Indian Council of Medical Research

The Council provides partial financial assistance for organizing Seminars/ Symposia/ Workshops. The sanction of grants by Council depends on the importance of the topic /subject of the Seminar/Symposium and its relevance to ICMR. All applications for grant of financial assistance should be furnished, completed in all respect with all details in the prescribed Performa (in ten copies) at least two months before the date of commencement of the Seminar/ Symposium/ Conference/Workshop.

Indian Council of Agricultural Research

The Indian Council of Agricultural Research (ICAR) a society registered under the Department of Agricultural Research and Education. ICAR provide Grant of financial assistance by ICAR for holding of Scientific Symposia/Seminars and promoting scientific excellence. For holding National/International Symposium/Seminar/Conference on the theme

chosen by them, to Scientific/Professional Societies, Public/quasi-public bodies and General Universities having post-graduate teaching and research in agriculture and allied sciences. ICAR widely circulate, including on its website, the list of such areas to all institutes (including ICAR institutes), universities including general as well as agricultural universities, and scientific societies for seeking good proposals to organize the seminar/symposium. The selection of a suitable hosting institution will be on competitive basis. Quantum of grant, for holding seminar/symposium/conference, the quantum of financial assistance to individual society/association/ institution will be determined after taking into account its relevance and performance as also merit of the proposal. The financial assistance will, however, not be more than 3.50 lakhs for holding national seminar/symposium/ conference on the topic chosen by the grantee body, and up to 5.00 lakhs on the theme identified by the Council. The amount for international event will be determined on case to case basis, but will not exceed 10.00 lakhs.

Department of Science and Technology

The S& T Professional Bodies and Academies of Science and Engineering play an important role in creating cohesiveness amongst the scientific community by arranging regular Annual Technical Meetings and Seminars, Conferences, Workshops, brain storming meet etc. and publishing scientific journals, annuals, bulletins *etc*. The department extends partial support on a selective basis, for organizing seminar/ symposia/ training programmes/ workshops/ conferences at national as well as international level. The support is provided to Research Institutes/ Universities/ Medical and Engineering Colleges and other Academic Institutes/ Professional Bodies who organize such events for the scientific community to keep them abreast of the latest developments in their specific areas. The support is generally given for encouraging participation of young scientists and research workers in such events and publication of proceedings/ abstracts for wider dissemination. A detailed analysis has been made by using data of support for last three consecutive financial years.

Regional distribution of resources

The grant-in-aid was disbursed to the science and technological organizations located in 30 states include union territory. The institution located in Tamil Nadu, Delhi, Karnataka, Uttar Pradesh, Maharashtra, west Bengal and Andhra Pradesh are the leading state in organizing, Technical Meetings and Seminars, Conferences, Workshops, etc.. The trend of financial support, have been given in Fig. 1 and Fig.2, Tamil Nadu is most active state regarding awareness of science and technology application in terms of organizing events, while maximum financial support were given to Karnataka, States such as Bihar, Himanchal Preadesh, Goa, States in NE region, J & K are required special attention to foster science & technology activities

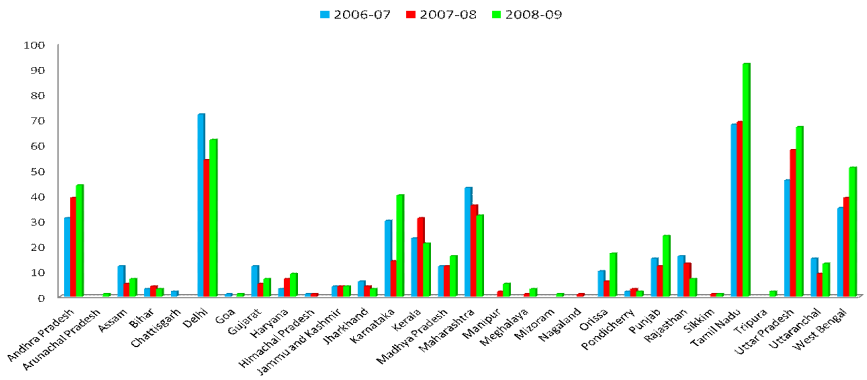

Fig.1: State wise number of applications supported

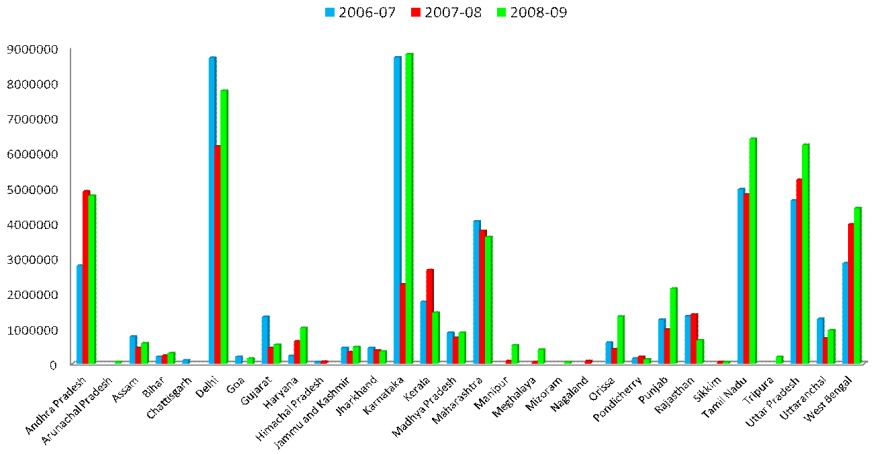

Fig.2: State wise actual funds distribution

Institution Profile

Universities and Government Institutions were forefront in organizing symposium/ seminar/ conference/ workshop *etc*. Though the country has witnessed phenomenal growth in the number of university and college imparting science education, a majority of them still do not have enough resources and infrastructural facilities. Consequently, there has been significant fall in the outturn of student from post-graduate and doctor courses. Once a basic expertise is established, the industry directly interacts with academics. Often researchers from industry are deputed in the professor's laboratory at the initial stages. State University contribution is more over on central and deemed university. The trend can be seen in

Fig.3, The participation of the universities and the research institutions on a common platform was useful to bring to centre-stage the fact, that one is not independent of the other. From Fig. 5 we may see the most active institute in country like AIIMS, New Delhi, Delhi University and Banaras Hindu University.

Growing subject area

The organization of seminar, symposia, conference, workshop through seminar, symposia scheme was successful in more ways than one. Earlier it has been a practice of holding National Conferences on central issues that deal with India's Future in Science and Technology more frequently compared to other broad subject, life science has registered better position in comparison to engineering, physical, chemical, and other allied area. There is substantial increase in life sciences. Life Science or biological science is any science which deals with living organism, their life processes and their interrelationships, such as biology medicine or ecology. It is a synthesis of several traditional disciplines including biology, zoology, botany and newer more specialized areas of study such as biophysics, biochemistry, microbiology, biotechnology etc. Essentially, life science is the scientific study of the living world as a whole. Greater attention is increasingly being placed on global environmental change, biodiversity conservation, environmental toxicology, integrated solid waste management. Another important area like Physics is a "*flesh and blood*" science, observed a well-known academician The Intellectual climate from which the discipline originated can be emphasized to make more awareness in society. The trend may be seen in Fig. 4

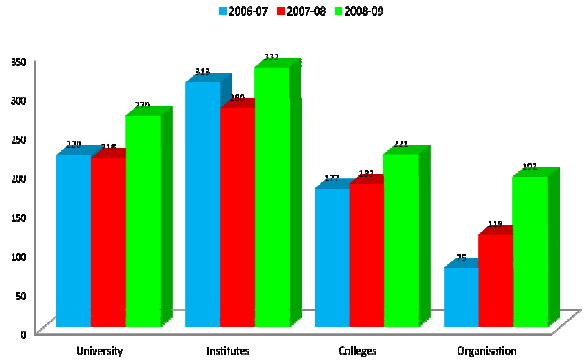

Fig.3: Institutional category wise applications received

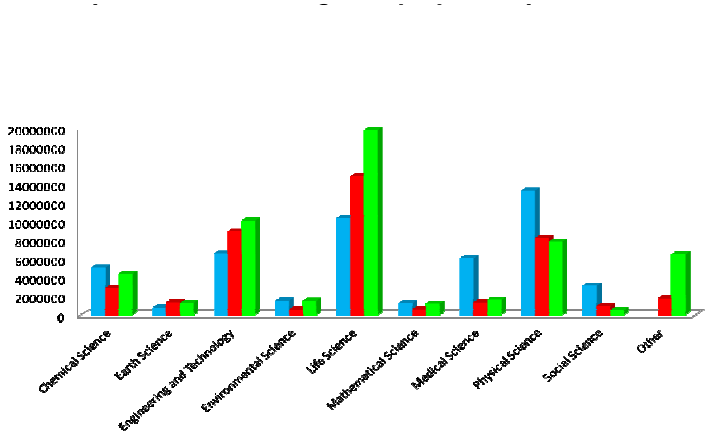

Fig.4: Subject wise funds distribution

Active Institutions Assessment:

The demand for excellence has increased even as the larger science and technology education interest of youngster declines, and now many colleges and universities have begun to offer courses in sustainability. To attract the leading experts and best talents in the area concerned

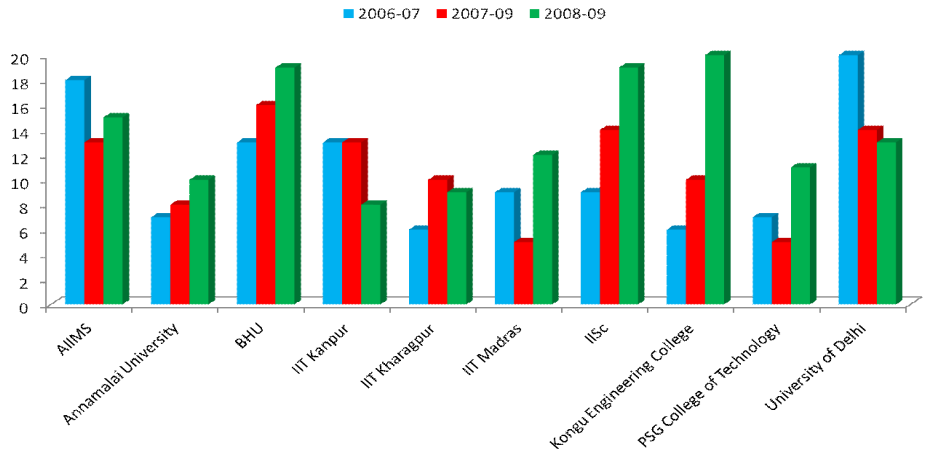

Fig.5: Top 10 most active Institute

of science and technology. A goal of the active Institute is to foster growth and expansion of science and its laboratory-research wide. The Institute intends to have a major impact on the overall research, and to play a crucial role in the formulation of new research directions and future initiatives. Its activities aim to benefit individual groups, both theoretical and experimental level. The Institutes' have to catalyzes interdisciplinary interactions and collaboration by organizing Seminars, workshops, conference, symposia *etc.* on wider basis on , well focused, and highly interactive character of the research and allows tackling a rich variety of the most urgent and topical scientific problems. In order to assess the ten most active institutes based on spirit organizing Seminars, workshops, conference, symposia are shown in Fig. 5. All India Institute of Medical Science (AIIMS) New Delhi. Delhi University Delhi (DU) Banaras Hindu University (BHU) are clear winner. These activities attract the most talented scientists, advance the current projects, and contribute enormously to the creative and stimulating atmosphere of the Institute. Fig. 5 Show 10 most active Institutes as per their role in organizing these activities.

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PCR-SSCP and sequence analysis of three populations of *Microtermes obesi* (Order: Isoptera; Family: Termitidae) from Chandigarh (India) on the basis of partial *16Sr RNA* and *ND1* gene

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Abstract

In the present work three populations of Microtermes obesi collected from Chandigarh, India were studied. PCR-SSCP and sequencing techniques have been applied to characterize the partial sequences of two mitochondrial genes *i.e.*, 16Sr RNA and 16Sr RNA tRNA leu ND1 of these populations. SSCP analysis revealed two types of conformational patterns for each gene. Types-I and –II were found in 419 bp long 16Sr RNA and Types-A and -B in 532 bp long 16Sr RNA tRNA leu ND1. A+T content was seen to be high for each gene which was above 60%. Stretches of As were more in 16Sr RNA, while in 16S rRNA tRNA leu ND1, stretches of Ts were more. For 16Sr RNA the percent diversity was found to be zero within M. obesi individuals. In case of 16Sr RNA tRNA leu ND1 it ranged between 0.2 to 0.7 %. Thus, in this study ND1 gene was found to be evolving faster than 16Sr RNA.

Keywords: *16Sr RNA tRNA leu ND1* | PCR - SSCP analysis | mitochondrial genes | conformational patterns | percent diversity | sequence variations

Introduction

Termites of the genera *Odontotermes* and *Microtermes* cause the heaviest destruction of seasoned timber both within buildings and extramurally. Losses due to termites run to several millions of rupees in agricultural crops alone. About 10-25 per cent loss is estimated in most field and forest crops. Severe loss in different regions of India has been recorded on highly susceptible crops such as wheat and sugarcane in northern India, maize, groundnuts, sunflower and sugarcane in southern India, tea in North-Eastern India and cotton in western India. Termite problems in agriculture in Southeast Asia largely affect

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perennial tree crops. The most economically important genera throughout Southeast Asia are *Microtermes, Coptotermes, Odontotermes, Macrotermes, Trinevitermes* and *Heterotermes.*

Though a good deal of work has been carried out on the taxonomy of Indian termites based on their morphological aspects, identifying workers and separating soldiers of different species is very difficult and in spite of using precise measurements, overlap may occur (Scheffrahn and Su, 1994). They, thereby, need to be characterized at molecular level. Nowadays, molecular methods have revolutionized insect systematics (Roderick, 1996; Caterino *et al*., 2000), and they are increasingly being applied to diverse groups of insects.

The population structure of *Coptotermes gastroi* (Wasmann) was characterized by using microsatellite markers by Yeap *et al.* (2011). Mitochondrial DNA is a valuable marker that is being used to study the insects phylogeny. Sharma *et al.* (2013) and Singla *et al.* (2013) used the partial fragments of mt. genes *COI* and *COII* and *12S* to determine the phylogenetic positions of various Indian termites. Similarly, partial fragments of two mitochondrial genes *viz*., *16SrRNA* and *ND1* were used in present study to find the extent of genetic relatedness/variations in three populations of *Microtermes obesi* (Isoptera: Termitidae). *16Sr RNA* is specific to a given species. Black and Piesman (1994) using *16Sr RNA* investigated the phylogeny of ticks at the family level and concluded that this gene is useful for phylogenetics of ticks at or below this level. They also suggested that *16S* in combination with another gene would give a more fully resolved tree. In 2010, Yeap *et al.* (2010) used the information from partial sequence of mitochondrial genes *16S, 12S and COII* together with morphometric measurements to determine the relationship between *Coptotermes heimi* and *C. gastroi* and found these species to be conspecific.

CP analysis of genome is another approach introduced by Orita *et al*. (1989). The technique can detect single base pair mutations in a PCR product without any prior sequence knowledge beyond that needed for the PCR amplification. The product is denatured to form single stranded DNA and snapcooled to form folded structure, which affects the mobility of the strands in a non-denaturing gel. Hence two bands are expected from homozygotes and four from heterozygotes. Two identically migrating bands cannot be assumed to have identical sequences, because not all mutations will affect mobility.

Molecular diagnosis, therefore, is just one way that might gain insight as to the origin of newly introduced species of termites, so that intervention may be directed in the most economically effective manner. The information so obtained can further be used to denitrify the source of introduction of termites, to discriminate the species for the application of corrective treatment measures and for cataloguing species with the intent to correctly classify them that would likely be discovered later. Molecular diagnostics are expected to yield genetic information from the collections, which will further be used as an integral

component of phylogenetic studies.

Materials and Method

Collection and storage

Termites were collected from three locations separated by about 10-15 km of distance within Chandigarh and its surrounding areas from North-Western part of India (Table 1) and preserved in 100% ethanol. Voucher specimens were put in 70% ethanol mixed with a few drops of glycerol and maintained in the Department of Zoology, Panjab University, Chandigarh (UT), India.

Identification of termites

Soldier specimens of all the populations collected (packed in scintillation vials in 70% ethanol) were sent to the Isoptera Section of the Zoological Survey of India (ZSI), Kolkata, for their identification. The details of collection site, date of collection, source and name of the authors were mentioned on each vial.

Isolation of genomic DNA

Genomic DNA was extracted from termites regardless of their castes by using standard phenol: chloroform extraction method (Sambrook *et al.,* 1989). The whole insect was homogenized in 1.5 µl eppendorf tube in 500 µl of TE (Tris EDTA, pH 8) with hand pestle and the homogenate was centrifuged at 7000-10,000 rpm for 10 minutes in cooling centrifuge (4°C). The supernatant was discarded and the pellet dissolved in 500 µl of lysis buffer (400 µl of TE and 100 µl of 10% SDS) followed by the addition of 6 µl of Proteinase K and the solution was incubated at 65°C for 1 hour in water bath. After the addition of 120 µl of phenol : chloroform : isoamyl alcohol (25 : 24 : 1), the tubes were vortexed for 30 seconds and then centrifuged for 5 min at 10,000 rpm in cooling centrifuge. The upper aqueous layer was carefully transferred to fresh tube and 500 µl of isopropanol was added to it and stored at 4°C for overnight and then centrifuged at 7000 rpm for 10 minutes. The supernatant was discarded and the pellet was washed with 70% ethanol. The alcohol was drained out, the pellet dried and dissolved in 30 µl of TE and stored at – 20°C after checking in 0.8% agarose gel. The concentration of DNA was then quantified by using-UV visible scanning spectrophotometer.

Amplification of *16SrRNA* gene

The region between nucleotides 7902 and 8321 in *Bombyx mori* (GenBank, AB070264) (Yukuhiro *et al*., 2002) of *16SrRNA* was amplified by using the universal primers LR-J-13007 and LR-N-13398 in the same termites to give a 419 bp long fragment (Table 2, Fig.1).

Amplification of *16Sr RNA-tRNA leu-ND1* gene

A 532 bp long fragment of *16Sr RNA-tRNA leu-ND1* gene from nucleotides 136 to 668 in *Reticulitermes banyulensis* (GenBank, AY510537) (Kutnik *et al*., 2004) was amplified by using the appropriate primers (Table 2, Fig. 2).

Single strand conformation polymorphism for *16Sr RNA* AND *ND1* genes of termites

SSCP analysis was carried out by using protocol of Vega *et al.* (1997) with slight

modifications. Five microlitres of PCR product was resuspended in an equal volume of formamide loading buffer in separate 0.2ml PCR tubes.

Electrophoresis to detect SSCPs

Electrophoresis was carried out in a vertical unit (BioRad Protean II system) by casting non-denaturing polyacrylamide gels using well clean glass plates. Gels were made from 12% acrylamide solution and 0.5X TBE; polymerization was initiated by adding 40µl of TEMED and 400µl of ammonium per sulphate (10% w/v). Gels were electrophoresed in 1X TBE buffer. Pre run was done and after that, the upper lid was removed and the shark toothcomb was inserted between the glass plates taking care that it just pierces the top of the gel and complete wells were formed. The samples were denatured by placing them in thermocycler at 95°C for 5 minutes and the tubes were cooled by keeping them immediately on the ice. Denatured samples (3.0µl each) were loaded in each well and the apparatus was run at 350V for 12 hours at 15°C. These were ideal conditions for detection of variations in *16SrRNA* and *ND1* genes. These conditions in fact allowed detection of fragments up to 550 bp length.

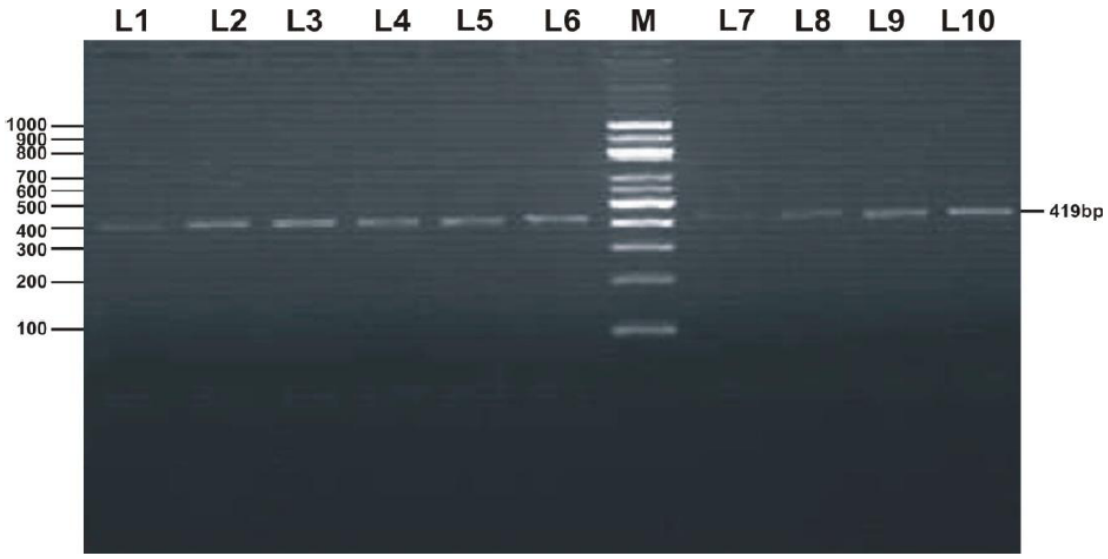
S. No.	Date	Species	Collection Site	Source	Sample Code*
1.	24.06.2004	*Microtermes obesi*	Hallomajra, Chandigarh	Tree	H
2.	26.06.2004	-do-	Darba, Chandigarh	Dampwood	D
3.	22.08.2004	-do-	Outskirts of Sukhna Lake, Chandigarh	Dead Tree Trunk	S

*H, D and S designate the initials for the name of places from where collections were made

Table 1: Collection data of termite species and their populations

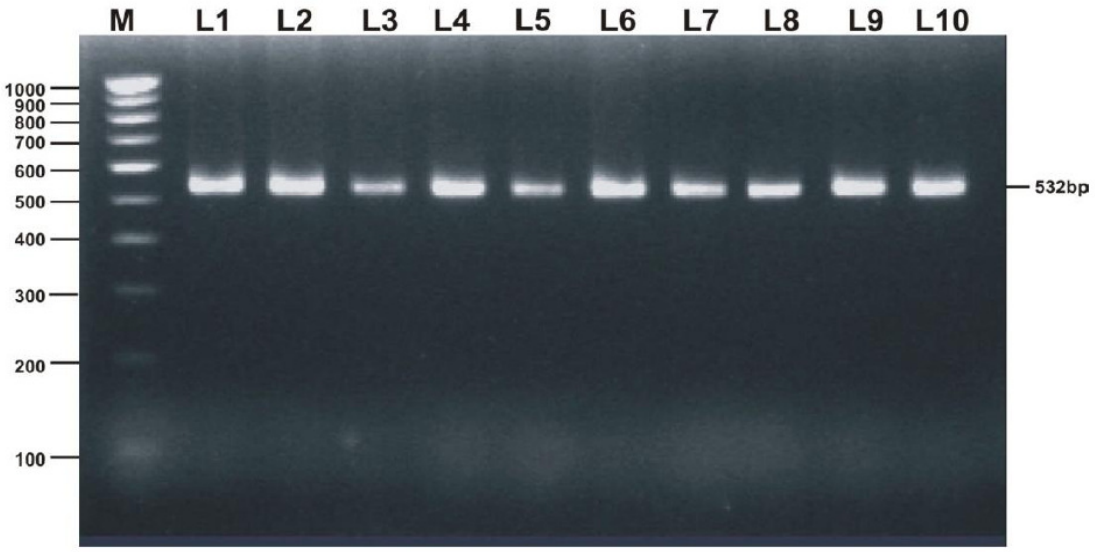
Primers	Primer Sequences	Length Melting	Annealing Temperature (Tm)	Temperature (Ta)
LR-J-13007-F(*16S*)	5'TTACGCTGTTATCCCTAA-3'	18	58.4°C	49°C
LR-N-13398-R(*16S*)	5'-CGCCTGTTTATCAAAAACAT-3'	20	57.3°C	49°C
ND1-F	5'TATTTTGGCAGATAAGTGCGTTAG -3'	24	53°C	53°C
ND1-R	5'AAAATAAAGGCCAATCTTACCTC A-3'	24	53°C	53°C

Table 2: Sequences and the conditions of the primers used to amplify mitochondrial DNA genes i.e. *16Sr RNA* and *ND1* in termites



Lane M: 100bp DNA leader
Lane L1-L10: Amplified products (419bp)

Fig. 1: PCR amplified products of *16Sr RNA* gene in various populations of termites.



Lane M: 100bp DNA ladder
Lane L1-L10: Amplified products

Fig. 2: PCR amplified products of *16Sr RNA-tRNA leu-ND1* gene in various populations of termites

Species	Populations	Total number of individuals studied	Pattern Type	Number of individuals with a haplotype	Frequency (%)
Microtermes obesi	P1	10	I	10	100
-do-	P2	10	I	10	100
-do-	P3	10	I	8	80
			II	2	20

Table 3: Frequencies of conformational pattern types obtained in various populations of *Microtermes obesi* studied for *16Sr RNA* gene

Silver staining

Gels were stained in clean shallow plastic trays. They were fixed in 2000 ml of 10% glacial acetic acid for 20 minutes. Washed thrice with double distilled water with agitation and dipped in 2000 ml of silver nitrate solution (2g silver nitrate and 3 ml formaldehyde in 2000 ml of double distilled water) for 30 minutes. The gels were agitated in between twice or thrice. They were removed from staining solution, kept in double distilled water for 10 seconds and then in 2000 ml of developer (60 gm sodium bicarbonate, 400µl sodium thiosulphate and 3 ml 37% formaldehyde in distilled water) with agitation until the first band appeared. They were then immediately kept in stopper or fixative *i.e.*, 10% glacial acetic acid and rinsed with double distilled water and kept for drying in cellophane sheet for preservation and further analysis.

Sequencing

Sequence interpretation

After the completion of the electrophoresis (run), chromatograms drawn by data collection software were used to extract the sequences of each individual. The sequences were subjected to multiple alignments with CLUSTAL V and further adjusted manually. For CLUSTAL V alignment 'Meg Align' program of 'Lasergene' software package (DNASTAR Inc., USA) was used. Edited sequences were compared against non-redundant nucleotide database from NCBI using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1997) algorithm from National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The edited nucleotide sequences were aligned and compared amongst the various haplotypes obtained across the species/populations of the order Isoptera included in the present study. Percent divergence was determined by computing the number of base differences for the total length of the gene sequence. The sequences were aligned in pairs, followed by aligning between pairs. Phylogenetic tree based on neighbor-joining method and divergence/similarity matrix were also drawn using the same Lasergene software.

Results and Discussion

For these species/populations, no prior genomic information is available. Fragments of two mitochondrial genes *i.e.*, *16Sr RNA* and *16Sr RNA tRNA leu ND1* genes were subjected to PCR amplification by using appropriate primers across all three populations (Table 3). Each individual yielded fragments of specific base pair length (Fig.1 and Fig.2). The amplified fragments were than subjected to polyacrylamide gel electrophoresis (SSCP analysis) in order to find variants within each group of species (Fig.3 and Fig.4). This helped in screening the samples for sequencing.

Single strand conformation polymorphism

After optimization of the parameters that affect the detection of SSCPs, the PCR products from 30 individuals (10 from each of the 3 populations studied were run on polyacrylamide gel) were analyzed for *16Sr RNA* and *16Sr RNA tRNA leu ND1* gene under modified conditions.

Single strand conformation polymorphism detection of 16Sr RNA gene

Figure 3 shows the SSCP analysis of 419 bp fragment of the *16Sr RNA* gene. Two conformational patterns (I, II) were detected for this fragment (Table 3).

All the three populations of this species showed very high frequency *i.e.,* 100, 100 and 80% of Type-I pattern for P10, P11, P12 respectively. Type-II was seen only in P12 with frequency of 20% only.

Single strand conformation polymorphism detection of 16Sr RNA tRNA leu ND1 gene

Figure 4 shows the SSCP analysis of 532 bp long fragment of *16Sr RNA tRNA leu ND1* gene.

In three populations (P10, P11 and P12) of *M. obesi* studied, two types of conformational patterns *i.e.*, Types-A and -B were observed. All the individuals of populations P1 and P2 revealed Type-A pattern only. This pattern also had high frequency in P3 (80%). Twenty percent of the individuals showed Type-B pattern.

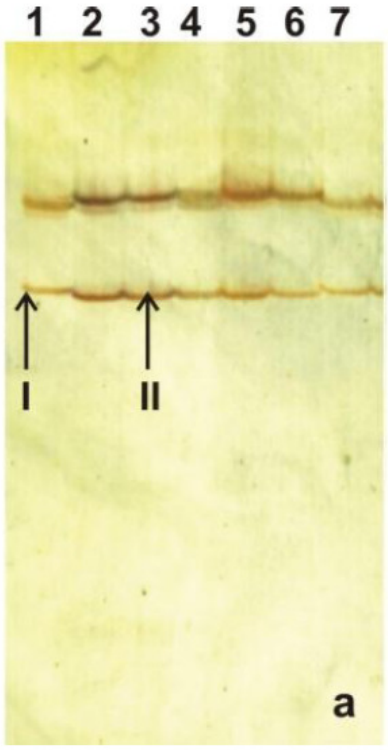


Fig. 3: SSCP analysis of 419bp fragment of *16Sr RNA* gene. Electrophoresis was performed by running 3 l of denaturated samples in a 12% acrylamide gel at 350V and 15°C for 12 hours. The frequencies of the conformational patterns detected were 93.33% for pattern I, 6.67% for pattern II in *Microtermes obesi*.

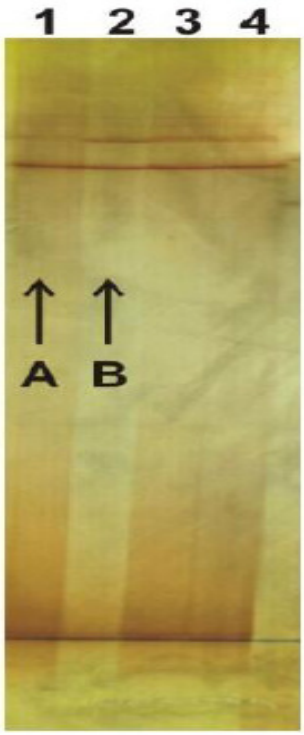


Fig. 4: SSCP analysis of 532bp fragment of *16Sr RNA-tRNA leu-ND1* gene. Electrophoresis was performed by running 3 l of denaturated samples in a 12% acrylamide gel at 350V and 15°C for 15 hours. The frequency of Type-A, 93.33% i and Type-B was 6.67% in *Microtermes obesi*.

Species	Populations	Total number of individuals studied	Pattern Type	Number of individuals with a haplotype	Frequency (%)
Microtermes obesi	P1	10	A	10	100
-do-	P2	10	A	10	100
-do-	P3	10	A	8	80
			B	2	20

Table 4: Frequencies of conformational pattern types obtained in various populations of *Microtermes obesi* studied for *16Sr RNA-tRNA leu-ND1* gene

Sequence analyses of 16Sr RNA gene

One individual of each haplotype pattern was sequenced and the variation was observed only at one position in the form of indel. The A+T content was 63.1% and that of G+C, it was found to be 36.9%. The percent diversity between the two haplotypes was zero. The average base frequencies were observed as A (0.420%); G (0.104%); T (0.217%) and C (0.257%). Stretches of As were more commonly seen in the sequences of the amplified fragment.

The sequence divergence of the Indian haplotypes of *Microtermes obesi*-I and *M. obesi*-II (Family: Termitidae) with both outgroup members of the same family *i.e. N. ephratae* and *N.costalis* from Guadeloupe (Schefrahn *et al*., 2005), retrieved from NCBI public database (Table 5), was found to be 14.6 and 15.3% respectively (Table 6).

Species	Family	Subfamily	Isolates/Country	Accession Number
Nasutitermes ephratae	Termitidae	Nasutitermitinae	Guadeloupe	AY623089
Nasutitermes costalis	Termitidae	Nasutitermitinae	Guadeloupe	AY623099

Table 5: List of species whose sequences were retrieved from Gene Bank public database and included in the analysis

	***M. obesi*-I**	***M. obesi*-II**	***N. ephratae***	***N. costalis***
M. obesi-I	0			
M. obesi-II	0.0	0		
N. ephratae	14.6	15.3	0	
N. costalis	14.6	15.3	1.4	0

Table 6: Pair wise data matrix showing percent diversity with out group taxa

Sequence analyses of 16Sr RNA tRNA leu ND1 gene

From the sequence alignment data and data matrix showing pairwise percentage divergence, it was apparent that the sequence divergence of all *Microtermes obesi* haplotypes was low. The sequences of all the three individuals were seen to be almost identical

showing approximately 0.64% variations among themselves. 0.43% transition substitutions were revealed, while transversions were 0.21% (Table 7). The A+T content was 65.03% and that of G+C, it was 34.97%. The average base frequencies were observed as A (0.153%); G (0.241%); T (0.499%) and C (0.109%). In this case Stretches of T_s were more common.

M. obesi-A1 and -A2 showed 0.2 % divergence among themselves. *M. obesi*-B had the divergence value of 0.7% from *M. obesi*-A1 and 0.4 % from *M. obesi*-A2 (Table 8).

The sequence divergence within the Indian haplotypes of *Microtermes obesi* ranged from 0.2-0.7%, while with outgroup members of the family Rhinotermitidae *i.e.*, *R. flavipes* and *R. grassei*, retrieved from NCBI public database (Table 9), the percent divergence was found to be 16.6-16.9 and 15.6-15.8 % respectively (Table 10).

Haplotypes	**Variations**		
M. obesi-A1	G	G	C
M. obesi-A2	G	G	T
M. obesi-B	A	T	T

Table 7: Variant sites in *Microtermes obesi* haplotypes for *16Sr RNA-tRNA leu-ND1* gene

	***M. obesi*-B**	***M. obesi*-A1**	***M. obesi*-A2**
M. obesi-B	0		
M. obesi-A1	0.7	0	
M. obesi-A2	0.4	0.2	0

Table 8: Matrix showing pairwise percentage divergence amongst two haplotypes of *M. obesi* of North-West India (Isoptera: Termitidae) in *16Sr RNA-tRNA leu ND1* gene.

Species	**Family**	**Subfamily**	**Isolates/Country**	**Accession Number**
R. flavipes	Rhinotermitidae	Rhinotermitinae	Raleigh (USA)	AY101831
R. grassei	-do-	-do-	Aranda de Duero (Spain)	AY101828

Table 9: List of species from Rhinotermitidae family whose sequences were retrieved from Gene Bank public database and included in the analysis.

	***M. obesi*-B**	***M. obesi*-A1**	***M. obesi*-A2**	***R. flavipes***	***R. grassei***
M. obesi-B	0				
M. obesi-A1	0.7	0			
M. obesi-A2	0.4	0.2	0		
R. flavipes	16.9	16.9	16.6	0	
R. grassei	15.8	15.8	15.6	6.6	0

Table 10: Pair wise data matrix showing percent diversity with out group taxa.

Among the three collections, P3 revealed two types of conformational patterns for each gene, while no variations were observed in the rest of the two populations in each case. The existence of multiple mtDNA haplotypes within a single collection was an unexpected result as mtDNA is maternally inherited in animals (Brown *et al.*, 1983) and cooperative colony found by multiple females is not common in termites and if such cases are there then the multiple founders appeared to involve sisters, which maintain the single maternal lineage (Thorne, 1982). The observed polymorphism might be due to the heteroplasmic nature of the mtDNA for more than one haplotype. Tokuda *et al.* (2012) analyzed the complete mitochondrial genome sequence of *C. formosanus* collected from three isolated islands in the Ryukyu Archipelago of Japan and found 99.9% similarity among these populations.

Nucleotide analyses of *16Sr RNA* gene

Because of its moderate size and range of evolutionary rates across sequences, *16Sr RNA* has great importance in phylogenetic studies across wide range of insects (Simon *et al.*, 1994). The sequences of the two *Microtermes obesi* individuals examined in the present study were almost 100% identical within this region. In Similar study carried out by Jenkins *et al.* (2002), it was observed that Formosan subterranean termites did not show any genetic polymorphism for *16S* marker. This lack of variations in *16Sr RNA* made it ideal for molecular diagnosis. The application of the *16Sr RNA* had been applied to identify *Reticulitermes* populations from the south-central United States (Austin *et al.*, 2004a, b, c) and across North America (Austin *et al.*, 2005a). This marker has tremendous potential for molecular diagnosis of *Reticulitermes*, with increased accuracy of positive species identifications (Szalanski *et al.*, 2003) and clarifying the identities of exotic introductions around the world (Austin *et al.*, 2005a, b). The genome of *Microtermes* has a high A+T contents (63.1%). Having high A+T content in mitochondrial genome is a general observation in insects (Xiang and Kocher, 1991; Kambhampati, 1995). The application of *16Sr RNA* proved to be reliable and easy to use for clarification between numerous *Reticulitermes* groups, particularly within *R. flavipes* from North America (Austin *et al.*, 2004a,b,c) and from Europe (Marini and Mantovani, 2002; Luchetti *et al.*, 2004).

Nucleotide analysis of *16Sr RNA tRNA leu ND1* gene

The diversity among various haplotypes of *Microtermes* individuals ranged between 0.2-0.7% revealing close association among different colonies. By using the *16Sr RNA tRNA leu ND1* gene, Uva *et al.* (2003) found association among colonies of Italian populations of *R. lucifugus*. They had detected four haplotypes (A, B, C, D) in the individuals of 13 colonies and reported two evolutionary groups within the species studied. Like *16S* gene fragment *ND1* gene fragment too had high A-T contents. It was between 60.13-65.52% for all the individuals studied.

From the present study it has been revealed that no information of any gene sequences

from *M. obesi* is available in the data bank till now. Thus, the sequence data generated and submitted to the Gene Bank in this study will act as baseline data for future studies regarding comparison and diagnosis of this species.

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Public awareness towards global warming with special reference to HP University, Shimla

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Abstract

Climatic change is the single best environmental and humanitarian crisis of our time. The Earth's atmosphere is overloaded with heat trapping carbon dioxide, which threatens large scale disruptions in climate with disastrous consequences. The increased volumes of carbon dioxide and other greenhouse gases released by the burning of fossil fuels, land clearing, agriculture, and other human activities, are believed to be the primary sources of the global warming that has occurred over the past 50 years. Scientists from the Intergovernmental Panel on Climate carrying out global warming research have recently predicted that average global temperatures could increase between 1.4 and 5.8 °C by the year 2100. Changes resulting from global warming may include rising sea levels due to the melting of the polar ice caps, as well as an increase in occurrence and severity of storms and other severe weather events. There is also the experience of new forms of illnesses, epidemics unheard of and increasing morbidity rate, increasing incidences of malaria *etc*. While there is any number of studies on environment related issues, very little has been studied specifically about the awareness of the public regarding their contribution towards the phenomenon of global warming, as well as of its impact on their lives. The researchers were keen to study what people of an ecologically rich and sensitive place like Shimla thought and knew about this all encompassing phenomena. The research is mainly focused on the centre of education in Shimla, the HP University itself, which is expected to have the highest level of awareness regarding such issues.

Keywords: Global warming | Climate change | Awareness | Shimla

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Introduction

Global warming is the increase in the average temperature of the earth's near-surface air and oceans, the global surface temperature increased 0.74±0.18 ºC during the last century. Green house gases are responsible for most increase in the surface temperature which was noticed by Intergovernmental Panel on Climate Change (IPCC) (IPCC, 2007). Increasing global temperature will cause sea level to rise and will change the amount and pattern of precipitation, which probably includes expansion of sub-tropical level (Gabriel & Thomas, 2007). The other effects include shrinkage of Amazon rain forest and Boreal forest, increase in the intensity of extreme weather events, change in agricultural yield and species extinction (Hegerl & Gabriele *et al.,* 2007). As the oceanic layers are disturbed, a disturbance in the food web is noticed due to which human society that depends on marine ecosystem services is also impacted (Jennifer, 2007).Border effects are expected due to global warming which includes glacial retreat, Arctic shrinkage, rise in worldwide sea level, changes in crop yield, change in the range of disease vectors, addition of new trade routes and species extinction (Jennifer, 2007). Along with this effect there also arise, scarcity of water in some regions and increased precipitation in others, changes in mountain snowpack, and adverse health effects from warmer temperatures (McMichael, Woodruff and Hales, 2006). Due to reduction in the ozone layer, the spread of the diseases such as malaria, dengue fever (Parry, 2007), lyme disease, hautavirus infection, bubonic plague and cholera (American society of Microbiology, 2008), is noticed. A wide variety of measures have been suggested for adaptation to global warming, this includes installation of air conditioning equipments, major infra structure projects such as abandonment of settlement threatened by sea level rise, water conservation projects (John, 1997), changes in agricultural practices (Adam, *et al,* 1990), construction of flood defences (Nicholls, 2004), changes in medical care (Kovats & Martens, 2004) and interventions to protect threatened species (Hulme, 2005).

Importance of Public Awareness of the Impending Disaster

This scientific knowledge has to become part of the general awareness which alone can bring about effective remedial action with public involvement. The situation undoubtedly demands change in life-style of humanity as a whole. This requires a critical awareness of the issue and its implications.

Various steps are taken in this regard by the state, by various voluntary initiatives, and through the education system, especially at the school level. However, it is still doubtful, whether these efforts go beyond advertisements and information to the level of preventive and remedial action. Every little step in this direction counts.

Methodology

People have been experiencing differences in weather conditions in Shimla – what one calls erratic. No snowfall in 2006, last year (2008) 46 cm snowfall, no snowfall till February in

2009. The temperature in Shimla has risen by 2 to 3°C more than what is normal. 2006 recorded a temperature of 21.1°C, highest in 15 years. "In the last 20 years, a lot of years have gone by either without any snow or with minimal snowfall between December and March. It's fast becoming a common pattern," says Manmohan Singh, director of the meteorological office in Shimla (www.merinews.com).

While there is any number of studies on environment related issues, very little has been studied specifically about the awareness of the public regarding their contribution towards the phenomenon of global warming, as well as of its impact on their lives.

The research work was focused on the centre of education in Shimla, the HP University itself, which is expected to have the highest level of awareness regarding such issues. It was hoped that the university could take some initiative on the basis of the study to give lead to this vital change process.

Samples

40 students

10 faculty members

Sampling Method:

Stratified sampling. An effort was taken to ensure equal representation of the various segments of the academic community – faculty, PG, UG students; male and female; arts, science and commerce streams.

Tools of Data Collection

1. Questionnaires: A questionnaire with 14 questions was designed. While 10 questions were close ended with options to be filled in by the respondents, four questions were deliberately kept open ended, so as to get a more realistic assessment of the awareness level of the respondents.
2. Survey of Secondary data: Though, documentation by government departments was aimed to study the variations in climatic conditions of Shimla, this was not possible. The researchers had to be satisfied with the minimal information available on the net.
3. Informal Interactions: Informal interactions with key resource persons (knowledgeable and familiar with Shimla) were employed to get some qualitative dimensions of the issue under study.
4. Observation: Observation of natural environment was employed to understand the causes and/or impact of global warming.

Analysis

The responses of the respondents were given weighttage in order to arrive at a score that would be indicative of their awareness level. The weights have not been standardized; however, this was done in consultation with people having expertise in the field.

A maximum score of 54 could be obtained by a respondent. When the scores of the respondents were calculated they fell in the range of 21 to 48. Standard deviation was

calculated as 6.559 approximated to 7. Subtracting one standard deviation from the mean score of 34, score indicating lower awareness level was identified in the range of 21-27; and adding one standard deviation higher level of awareness was identified ranging between 41 and 48. The range of 28-40 was identified as the medium awareness level. The respondents were classified into high, medium and low awareness level groups based on their scores, and the various independent variables were cross tabulated with the dependent variable – awareness level - to examine their inter-relationship.

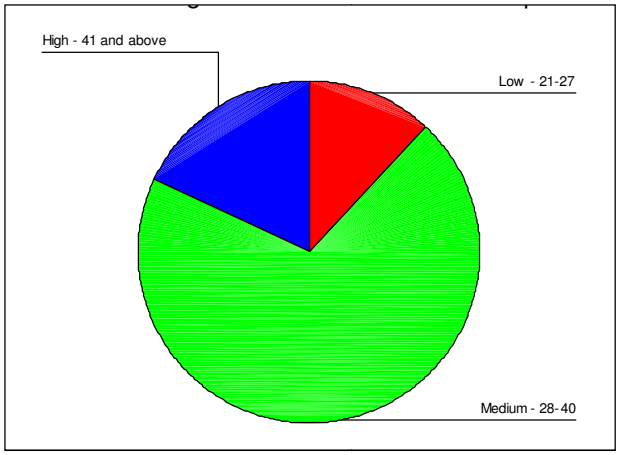

Fig. 1 Awareness Level of the Respondents

S. No	Awareness Level	Frequency	Percentage
1	**Low - 21-27**	6	12.0
2	**Medium - 28-40**	35	70.0
3	**High - 41 and above**	9	18.0
	Total	50	100.0

Table 1: Awareness Level

The vast majority of the respondents fall in the category of medium awareness; a high level of awareness is found in 18% of the respondents, and 12% of the respondents showed low awareness level.

S. No	Academic Stream		Awareness Level			
			Low	Medium	High	TOTAL
1	**SCIENCE**	**Frequency**	1	14	4	19
		Percentage	5.3%	73.7%	21.1%	100.0%
2	**COMMERCE**	**Frequency**	1	9	4	14
		Percentage	7.1%	64.3%	28.6%	100.0%
3	**ARTS**	**Frequency**	4	12	1	17
		Percentage	23.5%	70.6%	5.9%	100.0%
	TOTAL	**Frequency**	6	35	9	50
		Percentage	12.0%	70.0%	18.0%	100.0%

Table 2: Academic Stream and Awareness Level

The various academic streams at higher education level did not seem to significantly influence the awareness level. However, the data show that there were more of arts students in the low awareness category, and more of science as well as commerce students in the higher awareness category. When it comes to the medium level awareness, the three streams appeared more or less at par.

A one-way ANOVA test was conducted in this regard, to examine whether there is a statistically significant difference between the awareness levels of the three groups.

S. No	Academic Streams	N	Mean	S.D	F ratio	Stat. Significance
1	**Science**	19	2.1579	.5015	2.580	P>0.05 Not Significant
2	**Commerce**	14	2.2143	.5789		
3	**Arts**	17	1.8235	.5286		
	Total	50	2.0600	.5500		

Table 3: One way anova awareness level and the academic streams

P value is > .05, implying that the difference in the awareness level among the three groups is not statistically significant.

Thus, the hypothesis, that there will be a significant difference between the awareness level of the respondents belonging to different streams of studies is found to be not valid in this case, and hence rejected.

Sl. No	Occupational Status		Awareness Level			
			Low	**Medium**	**High**	**TOTAL**
1	**Faculty**	**Frequency**	1	4	5	10
		Percentage	10.0%	40.0%	50.0%	100.0%
2	**PG (& Post PG) Students**	**Frequency**	3	18	4	25
		Percentage	12.0%	72.0%	16.0%	100.0%
3	**UG Students**	**Frequency**	2	13	0	15
		Percentage	13.3%	86.7%	0.00%	100.0%
	Total	**Frequency**	6	35	9	50
		Percentage	12.0%	70.0%	18.0%	100.0%

Table 4: Occupational Status and Awareness Level

The data indicated a relatively high level of awareness among the faculty members (high 50% & medium 40%). The PG & Post-PG section also showed a relatively higher percentage of deeper awareness, with 16% at the higher awareness level. The UG students had no body in the higher awareness level category, and had a relatively higher percentage in the lower awareness level category

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S. No	Age Group		Awareness Level			
			Low	**Medium**	**High**	**TOTAL**
1	**18 -25**	**Frequency**	4	26	3	33
		Percentage	12.1%	78.8%	9.1%	100.0%
2	**26 – 35**	**Frequency**		5	2	7
		Percentage		71.4%	28.6%	100.0%
3	**36 and above**	**Frequency**	2	4	4	10
		Percentage	20.0%	40.0%	40.0%	100.0%
	TOTAL	**Frequency**	6	35	9	50
		Percentage	12.0%	70.0%	18.0%	100.0%

Table 5: Age Group and Awareness Level

While overall, age-wise groupings did not show significant difference in their awareness level, it can be observed that the higher age group among the respondents showed a higher level of awareness (40%), while for the younger group it was only 9.1%.

A plausible explanation could be that as most of the senior group respondents were academicians, it was likely that they were more alert towards this matter.

S. No	AGE GROUPS	N	Mean	S.D	F ratio	Stat. Significance
1	**18 – 25**	33	1.9697	.4667	1.380	P>0.05 Not Significant
2	**26 – 35**	7	2.2857	.4880		
3	**36 and above**	10	2.2000	.7888		
	Total	50	2.0600	.5500		

Table 6: Awareness level and the age group

Based on 't' test, the hypothesis, that there will be a significant difference between the awareness level of the respondents based on their age groups.

			Awareness Level			
S. No	**Gender**		**Low**	**Medium**	**High**	**TOTAL**
1	**Female**	**Frequency**	4	17	4	25
		Percentage	16.0%	68.0%	16.0%	100.0%
2	**Male**	**Frequency**	2	18	5	25
		Percentage	8.0%	72.0%	20.0%	100.0%
	Total	**Frequency**	6	35	9	50
		Percentage	12.0%	70.0%	18.0%	100.0%

Table 7: Gender and Awareness Level

In the higher awareness level, male respondents were found to be slightly more than female respondents, and vice-versa, in the case of lower awareness level. However, in general, the group had a relatively high awareness level, with around 88% having a score above 50% of the total score.

Sl. No	Groups	N	Mean	S.D	t-value	Stat. Result
1	Male	25	2.1200	.5260	.768	P > 0.05 Not Significant
2	Female	25	2.0000	.5774		

Table 8: Awareness level of the respondents male-female

Based on 't' test, the hypothesis, that there will be a significant difference between the awareness level of the respondents based on their gender, is rejected.

Conclusion

The awareness level of the HP University Campus, Shimla, as indicated by the responses of the sample of this study, shows a high awareness level on the whole, i.e., having an awareness score of more than 60% for more than 60% of the respondents. The various category wise calculations show that variables such as academic stream, age-group, gender

etc. did not have much influence on the awareness level of the respondent group. The statistical tests of 't' and 'anova' rule out such influences in this study.

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Strategic implementation of site specific crop management in Indian agriculture for biodiversity conservation

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Abstract

Site specific crop management is based on a set of resources that allow field variability management. The main idea is to identify areas which present different levels of productivity, and offer an individual treatment for each of them, managing these differences. Site specific farming basically depends on measurement and understanding of variability, the main components of site specific crop farming management system must address the variability. It requires the requisition, management, analysis and output of large amount of spatial and temporal data. In Indian prospective for sustainable Bio diversity through precision farming the changes in agricultural policies are also necessary to promote the adoption of precision farming. There are basically two policy approaches: regulatory policies and market based policies. The former refer to environmental regulations on the use of farm inputs and later refer to taxes and financial incentives aimed at encouraging growers to efficiently use farm inputs. Along with the policy measures efficient and productive agricultural land use, ensuring a sufficient income for farmers, can be brought in line with nature conservation objectives as a result of which farmers are prepared to adapt their farming methods to enhance biodiversity and that ecoinnovation, Also Indian farmers need financial as well as non-material support to better align their economic interests with biodiversity targets.

Keywords: Strategic implementation | Environmental Adoption | Agri-environmental measures | biodiversity conservation

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Introduction

The Site specific crop farming management is the application of technologies and principles to manage spatial and temporal variability associated with all aspects of agricultural

production for improving production and environmental quality. The success in site specific agriculture depends on the accurate assessment of the variability, its management and evaluation in space-time continuum in crop production. The agronomic feasibility of precision agriculture has been intuitive, depending largely on the application of traditional arrangement recommendations at finer scales. The agronomic success of precision agriculture has been quite convincing in crops like sugar beet, sugarcane, tea and coffee. The potential for economic, environmental and social benefits of site specific agriculture is largely unrealized because the space-time continuum of crop production has not been adequately addressed. Successful implementation of precision agriculture depends on numerous factors, including the extent to which conditions within a field are known and manage, the adequacy of input recommendation and the degree of application control. The enabling technologies of site specific agriculture can be grouped in to fine major categories: Computers, Global Positioning System (GPS), Geographic Information System (GIS), Remote Sensing (RS) and Application control. The various aspects of precision agriculture encompass a broad array of topics including variability of the soil resource base, weather, plant genetics, crop diversity, machinery performance and most physical, chemical and biological inputs used in crop production. The present paper tried to show the strategic implementation of Site specific agriculture for farmers in developing country like India to achieve efficient crop production with sustainable bio diversity conservation. Site specific crop management farm practices collects and interprets huge amount of data from the field so as to understand the causes of variability and propose strategies for field management, biological species geographic distribution models, based on ecological niche concepts, combine species presence and absence points with environmental biotic and abiotic data, in order to generate models that describe probabilistic distributions of that species – represented as geographical distribution maps for biodiversity conservation.

Conceptual Framework

Site specific crop management is Defined as Information Technology Based, Relatively Better Management System that Identifies, Procures, Analyzes & Manages, Natural Variability Amongst the Fields &Optimizes Productivity, Profitability, Sustainability, which Protects the Land Resources and bio diversity

Review of Literature

Site specific crop management is based on a set of resources that allow field variability management. The main idea is to identify areas which present different levels of productivity, and offer an individual treatment for each of them, managing these differences. This concept dates from the 80s and started a revolution in the resources management (Robert, 2002). Since site-specific management, site-specific farming, or precision farming, alternative names for precision agriculture, has been directed to

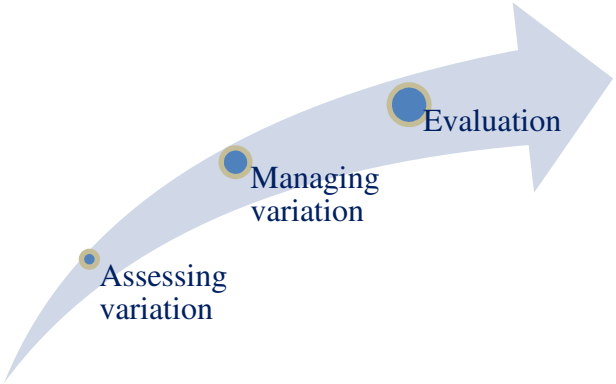
intensive data and technology usage, resulting in relevant researches, such as those presented in Plant (2001), Zhangh et al. (2002) and Korduan et. al (2004), and a large amount of products, systems and devices for rising production profitability, improving production quality and helping environment protection. Some examples of precision agriculture purposes include soil properties study for the application of fertilizers in variable rate, and the main aims and other potential benefits of its adoption are to increase productivity, sustainability, crop quality, food safety, rural welfare and economic development. Its users have many information systems to choose from, but usually the systems are monolithic and able to perform specific tasks only, e. g.Productivity management or soil fertilizer mostly furnished by equipment manufactures and with no relationship between each other. Nevertheless, precision agriculture requires the integration of the tools used in all steps performed, requiring from the user the expertise to deal with many software packages, with different GUIs (Graphical User Interface) and data formats, and sometimes demanding other software packages for data conversion, in order to use the output of a package as the input for the next (Murakami, 2006).There are also more complete packages, which incorporate database and field equipment connectivity, GIS (Geographical Information System) functionalities and other useful characteristics for precision agriculture. Despite that, many authors identified relevant requirements that they do not cover (Saraiva, Massola, Paz, 1997; Saraiva, Massola, Cugnasca, 1998; Lütticken, 2000; Sorensen et al., 2002; Pedersen et al., 2003; Korduan, Bill, Böling, 2004; Adrian, Norwood, Mask, 2005): decision support systems and management must be designed for meeting producers specific needs; systems should have GUIs that could be customized for different user profiles, because a friendly interface is paramount for users with little software expertise; easy and automated methods, programmable according to the user rules, should be able to be included, and the user should be able to control and access analysis functions and parameters, in order to be able to try new and more applicable solutions; rule-based knowledge should be possible, so as to refine and adapt the system to local practices and preferences, reducing learning curve and technical support needs; systems should be interoperable with other software packages, local or remote, via Internet, using open patterns – these are fundamental for integration with distributed and legacy systems; systems should have scalability, metadata support and low cost.

Accomplishments of Site specific crop management in Bio Diversity conservation

- Address Poverty Alleviation, Enhance Quality of Life & Food Security
- Socio Economic Need for Enhanced Productivity per Unit of Land, Water and Time.
- Increased Land Degradation, Depletion of Water Resources in India,
- Environment Pollution due to Increased Use of Fertilizers and Chemicals.

- Improved Crop Yield by Efficient Application of Chemical, Fertilizer & Energy Costs.
- Increase Profit Margin & Enable Better Management Decisions

Basic Steps in Adoption of site specific crop management farm practices



Strategies for implementation of site specific crop management farm practices

A strategy for implementation of site specific crop management in bio- diversity conservation includes:

- Predictive approach: based on analysis of static indicators (soil, resistivity, field history, *etc*.) during the crop cycle.
- Control approach: information from static indicators is regularly updated during the crop cycle by:
  - sampling: weighing biomass, measuring leaf chlorophyll content, weighing fruit, *etc*.
  - remote sensing: measuring parameters like temperature (air/soil), humidity (air/soil/leaf), wind or stem diameter is possible thanks to Wireless Sensor Networks
  - Proxy-detection: in-vehicle sensors measure leaf status; this requires the farmer to drive around the entire field.
  - Aerial or satellite remote sensing: multispectral imagery is acquired and processed to derive maps of crop biophysical parameters.

Obstacles in adoption of Site specific crop management farm practices

There are many obstacles to adoption of site specific farming in developing countries in India are as follows.

- Culture and perceptions of the users
- Small farm size
- Heterogeneity of cropping systems and market imperfections
- Land ownership, infrastructure and institutional constraints
- Lack of local technical expertise
- Knowledge and technical gaps

Conclusion

In Indian prospective for sustainable Bio diversity through precision farming the changes in agricultural policies are also necessary to promote the adoption of precision farming. Along with the policy measures efficient and productive agricultural practices must be adopted like:

- Agriculture must create biodiversity conservation.
- Genetic diversity in Agriculture must be maintained.
- Farmers must be made partners in nature conservation.

- The competitiveness of farms must not suffer.
- Ensure contractual nature conservation on a voluntary basis and Agri-environmental measures.
- Provide financial incentives and advice for biodiversity-friendly practices.
- Farm practices must integrate biodiversity protection into farming.
- Assure standard solution for maintaining biodiversity.
- Promote agricultural research and innovation serving biodiversity.

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Assessment of ground water quality for drinking and irrigation suitability in Jaunpur District (U.P.) India

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Abstract

The physico-chemical status of ground water samples from 21 blocks major part of locality in Jaunpur district was assessed. The sampling points were selected on the basis of irrigation and drinking purpose.The major hydro-chemical parameters for determine the quality of water as pH, Electrical conductivity, Turbidity, TDS, TS, Acidity, Alkalinity , Chloride , Bicarbonate, sulphate, Dissolved Oxygen, Total Hardness, Major cations (Ca^{++}, Mg^{++}, Na^{+}, k^{+},) and major anions (Cl^{-}, F^{-},NO_3^{-}, PO_4^{-} SO_4^{-} were analysed and compared with WHO Standards. The pH varies from 7.5 to 8.9, indicating alkaline nature. The electrical conductivity (EC) value varies between 484 and 3120 ($\mu s/cm^{-1}$) in the ground water. TDS varied from 443 to 2434 (mg/l) and higher concentration of dissolved ions was observed in the water samples. High values of salinity, sodium absorption ratio (SAR), Na%, residual sodium carbonate (RSC) and permeability index (PI) of ground water in some blocks of Jaunpur district were found unfit for drinking and irrigation purpose.

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Keywords: Physico-chemical parameters | Drinking quality | Irrigation suitability | Ground water

Introduction

The groundwater chemistry have contributed important information on the suitability of the groundwater for drinking and agricultural purposes, and presently its contamination has been recognized as one of the most serious water pollution problems in the world (Adams *et al*. 2001; Jalali 2007, 2009, Djabri *et al*. 2007). Groundwater is the only available water resource for human consumption, as well as for drinking and agriculture uses. It is estimated that approximately one-third of the world's population use groundwater for drinking (UNEP, 1999). Urbanization and unregulated growth of the population have altered local topography and drainage system directly affected both quality and quantity of the ground water.

Water is essential to the existence of human beings and other living organisms. Water is the precious gift of nature to the human being. The

quality of water is of vital concern for mankind since it is directly linked with human health, protection of the environment and sustainable development. Groundwater occurs almost everywhere beneath the earth surface not only in a single widespread aquifer, but also in thousands of local aquifer systems and compartments that have similar characters. Man's activities such as food production, nutrition are dependent on water availability in adequate quantities and good quality.

The rapid depletion of ground water quality through leaching of open dumping of solid wastes and thus the estimation of ground water quality of extremely importance for proper assessment of the associated health hazards (Mathur and Maheswari, 2005; Warhate *el al*, 2006). Groundwater resource in Jaunpur district is widely exploited for irrigation and other domestic purposes in addition to drinking purpose. The Jaunpur resident mainly depends on groundwater for their drinking and irrigation purpose. Sources of water pollution in the study area occurred mainly due to run off agriculture fertilizer, sewage, hospital waste etc. Concentration of pollutants more than permissible limits in drinking water leads to health problems, such as water borne diseases, like fluorosis, typhoid, jaundice, cholera, premature baby and other problems, especially in infants (Spalding and Exner, 1993).

Materials and Methods

Study area

Jaunpur district is one of the important districts of eastern part of Uttar Pradesh, India. It lies between 25^0 24' N and 26^0 12' N latitude and 81^0 19' E and 82^0 27' E longitude and located at of 261 to 290 meter from sea level with covering an area of about 4,038 km^2. According to the 2011 census, district had a population of 4,494204 with the rural and urban area both Population depend upon agriculture for their livelihood. The climate of district is extreme nature temperature deep 5 to 7 ^{0}C in winter season and 45 to 47 ^{0}C in summer season. Average rainfall in Jaunpur district is 987 mm.

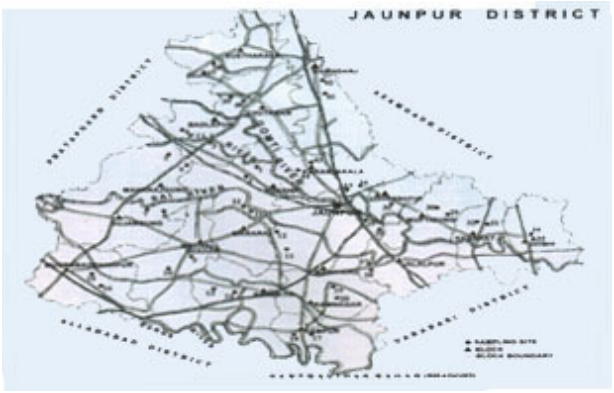

Fig.1: **Jaunpur district showing sampling sites**

Analytical methods

The ground water samples were collected using acid washed polypropylene bottles to avoid unpredictable changes in quality characteristic as per standard procedures. At the time of sampling, bottles were thoroughly rinsed two to three times before sampling. The measurements of physico-chemical parameters analyzed including Electrical Conductivity (EC), pH, Total Dissolved Solids (TDS), Alkalinity, Acidity, Total Suspended Solids (TSS), total hardness were determined as per (APHA,1998) and major cations (Ca^{2+}, Mg^{2+},Na^+, K^+) and major anions (Cl^-, F^-, NO_3^- ,PO_4^-,SO_4^-) measured by UV

spectrophotometers and Some measurement of agricultural purpose for the suitability of irrigation like Sodium absorption ratio (SAR), Permeability index (PI), Residual sodium carbonate (RSC) calculated by following formula as.

Sodium absorption ratio (SAR)

Sodium absorption ratio is an important parameter to determine the suitability of irrigation water and was calculated by (Richards, 1954).

$$S.A.R = \frac{Na^{+}}{\sqrt{\frac{1}{2}(Ca^{2+} + Mg^{2+})}}$$

Permeability index (PI)

Permeability index was calculated as per the method suggested by (Doneen, 1964)

PI= $[(Na^{++}HCO3^{-})/(Ca2^{++}Mg2^{++}Na^{+})]$ 100

PI was used to evaluate the sodium hazards of irrigation water.

Residual sodium carbonate (RSC),

The concept of residual sodium carbonate is employed for evaluating high carbonate waters and was calculated by (Aghazadeh and Mogaddam, 2010) the formula given below.

RSC= $(CO_3^{-}+HCO_3^{-})-(Ca^{2+}+Mg^{+2})$

Results and Discussion

pH

pH is a term used universally to express the intensity of the acid or alkaline condition of water. Most of the ground water samples were slightly alkaline due to presence of carbonates and bicarbonates. The pH values of water samples were measured maximum and minimum value between 7.8 to 8.9. The average pH value was found 8.32 of ground water from Jaunpur district. The range 5.5 to 8.5 pH of ground water is suitable for drinking and irrigation purpose according to (WHO, 2002).

Electrical Conductivity

Electrical conductivity of water is generally related to the amount of dissolved solid or minerals ions and represents the ability of water to conduct an electric current. The minimum (484 µS/cm) and maximum (3120 µS/cm) concentrations of EC were recorded from the samples of ground water. High range of EC in ground water area was indicated the enrichments of salts in the ground water.

Total Dissolved Solids

The TDS value of ground water ranged as minimum value (443 mg/l) and maximum value (2434 mg/l) and mean value was ranged 1140.8 mg/l. ground water contain less than 500 mg/l of total dissolved solids is desirable for drinking uses but water contain more than 1,000 to 3,000 mg/l of TDS value is not recommended for drinking as well as for other domestic purpose. Such water may be use for irrigation, according to classification of (Davia and DeWiest, 1966). TDS value of the ground water of some blocks i.e. Badalapur, Rampur, Jaunpur, Sircony and Darmapur were fall in the class three and the quality of ground water indicated that water may be used for only irrigation purpose not for drinking shown in Table. 1.

Class	TDS (mg/l)	Classification
1	500	Desirable for drinking
2	500–1,000	Permissible for drinking
3	1,000–3,000	Useful for irrigation
4	> 3,000	Unfit for drinking and irrigation

Table 1: Groundwater classification based on TDS (Davis and DeWiest, 1966)

Total Hardness

Water hardness is caused primarily by the presence of cations such as calcium and magnesium and anions such as carbonate, bicarbonate, chloride, and sulphate in ground water. The total hardness (as CaCO3) values ranged between 212 - 1094 mg/l and average value 471 mg/l were found shown in Table 2. The ground water of Jaunpur district was indicated that 80 % of water samples were out of permissible limit for drinking purpose (WHO, 2002).

Major cations

The present results demonstrate that calcium concentration in ground water ranged between 14.5 to 212.5 mg/l as average values 68.74 mg/l were found shown in Table. 2. The high concentrations of calcium in water samples were not hazardous both on human health and agricultural purpose. High concentration of Calcium may be attributing to the passage through or deposits of limestone, dolomite and gypsum (APHA, 1992). The magnesium concentrations of ground water were found 23.6 to 178.4 mg/l and average 78.11 mg/l. The concentration of manganese in Jaunpur city and Dharmapur block were out of permissible limit according to (WHO, 2004). The sodium concentrations were found 10.7 to 557.9 mg/l and average concentration 157.61 mg/l and concentration of potassium 2.4 to 11.8 mg/l and average concentration 4.98 mg/l shown in the Table. 2.

Major anions

The chloride concentration of ground water ranged between 11.1 to 685 mg/l and average concentration 196.11 mg/l were found shown in Table 2. The concentration of chloride in ground water of some blocks i.e. Badalapur, Rampur, Sircony and Jaunpur city were out of permissible limit (WHO, 2002). The chloride concentration in ground water was caused injurious effects to people suffering from diseases of heart and kidney. The fluoride concentration in ground water ranged between 0.15 to 1.95 mg/l and average concentration was found 1.02 mg/l. Fluoride affects mainly dental caries at low concentrations and higher concentration of fluoride causes serious problems such as dental and skeletal fluorosis (Schafer *et al*. 2010). Fluoride is one of the main trace elements in groundwater, which generally occurs as a natural constituent. In fact, fluoride related to groundwater has been studied intensively during the past decades (Roberston 1986; Zhaoli *et al*. 1989; Travi and Faye 1992; Hitchon 1995; Subba Rao 2003; Coetsiers *et al.* 2008). These studies showed that concentration of fluoride was increased with the process of leaching from minerals in various aquifers with different lithological process. Bedrocks containing fluoride minerals are generally responsible for high concentration of fluoride in groundwater (Handa 1975; Wenzel and Blum 1992; Bardsen *et al.* 1996. Subba Rao, 2003). Nitrate

Blocks	Physico-Chemical Parameters				Major Anions						Major Cations		
	pH	EC	TDS	TH	Cl^-	F^-	NO_3^-	HCO_3^-	SO_4^-	Ca^+	Mg^+	Na^+	K^+
Buksha	8.3	647	557	365	29.7	0.89	3.3	403	3.7	33.8	68.2	10.7	4.3
Badalapur	8.3	3000	2434	651	475.9	1.72	3.2	749	454.8	71.5	114.8	557.9	5.6
Maharajgang	8.5	1588	1368	559	208.4	1.95	11.8	741	59	75.5	90.1	178	2.4
Sujangang I	8.1	484	443	213	17.8	0.9	4.3	310	17.2	46.3	23.6	20.1	3.1
Mariyahoo	8.4	1153	1008	212	75.8	0.15	39.8	512	119.5	40	51.8	165	4.9
Barsathi	8.5	853	780	313	31.5	1.19	13.8	481	55.1	28.5	37.6	127.5	3.7
M.Badsahpur	8.7	1017	979	793	22	1.7	13.3	589	115.4	61	58.3	114	3.9
Rampur	8.9	2700	2084	226	685.9	1.54	2.3	473	325.8	14.5	72.1	500.5	8.4
Ramnagar	8.3	826	758	333	17.7	1.63	26.2	525	18.3	62.5	58.4	44.5	3.3
Sikrara	8.1	1345	1220	396	269.5	0.73	12.8	496	140.6	68.5	116	113.5	2.6
Jaunpur	8.4	3120	2263	500	532	1.06	48.9	523	466.1	140	175.9	364	11.6
Sircony	8.0	2800	1992	648	391	0.38	1.5	329	748	212.5	112.5	191.5	5.5
Jalalpur	7.8	945	846	994	149	1.0	20.7	449	7.0	81	87.6	45.5	4.5
Muftigang	8.1	518	492	329	21.2	0.86	1.9	336	7.8	59.8	48.3	11.9	4.0
Kerakat	8.3	581	550	563	11.1	0.89	28.6	368	4.5	46.2	52.5	34.4	3.9
Dobhi	8.3	555	519	376	43.7	0.32	2.5	317	27.1	39.8	48.8	37	2.9
Darmapur	8.6	3100	2188	300	611	0.67	119.6	655	103	144.5	178.4	364.5	11.8
Suithakala	8.4	751	645	1094	87.3	0.99	41.5	356	11.8	26.5	48.7	68.5	4.0
Kutahan	8.1	579	551	266	45.6	0.87	1.5	355	3.5	53.7	40.5	45.7	4.4
Shahgang	8.4	763	680	301	49	1.87	1.4	438	17.1	33.5	48.3	89.5	1.8
Minimum	**7.8**	**484**	**443**	**212**	**11.1**	**0.15**	**1.5**	**310**	**3.5**	**14.5**	**23.6**	**10.7**	**2.4**
Maximum	**8.9**	**3120**	**2434**	**1094**	**685.9**	**1.95**	**119.6**	**749**	**748**	**212.5**	**178.4**	**557.9**	**11.8**
Average	**8.32**	**1398**	**1140.8**	**471.6**	**196.11**	**1.02**	**20.92**	**471.94**	**141.48**	**68.74**	**78.11**	**157.61**	**4.98**

Table 2: Water quality characteristics of ground water of Jaunpur district

EC (dS/m at 25°C)	Water class	Interpretation
<0.25	Low salinity (C1)	Safe with no likelihood of any salinity problem Developing
0.25 – 0.75	Medium salinity (C2)	Need moderate leaching
0.75 – 2.25	High salinity (C3)	Cannot be used on soils with inadequate drainage, since saline conditions are likely to develop
2.25 – 5.0	Very high salinity (C4)	Cannot be used on soils with inadequate drainage, since saline conditions are likely to develop

Source: Santhi. 2003

Table 3: Interpretation of irrigation water quality based on EC measurement

concentrations of the ground water samples ranged from 1.5 to 119.6 mg/l and average value was found 20.92 mg/l. The nine ground water samples of the study area were found above the permissible limit (WHO, 2004). The excessive nitrate content in drinking water can

cause 'blue baby syndrome' in infants (Fewtrell 2004). The minimum values of sulphate were found 3.5 mg/l and maximum value 141.48 mg/l and average concentration was found 141.48 mg/l in the ground water samples. The sources of sulphate contamination in ground water by leaching of agricultural fertilizers hence increased through runoff in ground water of Jaunpur district.

Sodium absorption ratio (SAR)

Sodium absorption ratio is the most commonly used for evaluating groundwater suitability for irrigation purposes (Ayers and Westcot, 1985). The classification of SAR content of alkali hazard, which is normally expressed in Sodium adsorption ratio (Rao, 2005, Hem, 1991). Sodium hazard of irrigation water can be well understood by calculation or the value of SAR index. This index quantifies of the proportion of sodium (Na^{+}) to calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions in water samples. In ground water samples, SAR values varied 0.24 to 11.93 and the classification of groundwater samples from the study area with respect to SAR was represented in Fig.3.

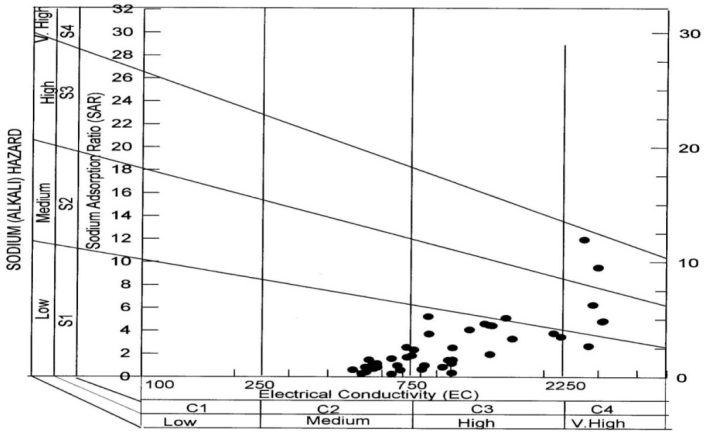

Fig. 3: Plots of calculation values of SAR and EC of ground water sample (Rechards, 1954)

USSL diagram

The SAR and EC values for groundwater samples of the study area were plotted in the USSL graphical diagram of irrigation water. Based on USSL diagram, the water quality showed that the majority of the samples falls in the C2-S1 and C3-S1 medium to high salinity with low sodium alkalinity hazard and two samples falls in the field of C4-S2 as very high salinity with low sodium alkalinity and C4-S3 was showed very high salinity and high alkalinity shown in Fig 3. The C4-S3 class water was not suitable for without proper treatment.

Residual Sodium Carbonate (RSC)

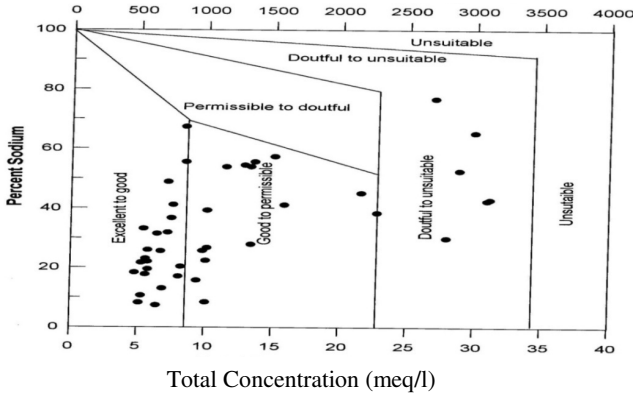

Fig. 4: Plot of sodium percent versus electrical conductivity

The concentration of bicarbonate and carbonate also influences the suitability of water for irrigation purpose. One of the empirical approaches is based on the assumption that all Ca and Mg precipitate as carbonate. Considering this hypothesis proposed the concept of residual sodium carbonate (RSC) showed for the quality assessment of high carbonate in water. A high value of RSC in water leads to an increase in the adsorption of Na in soil. Irrigation water having RSC values greater than 5 meq/1 are

considered harmful to the growth of plants. The most of the analysed water samples, RSC value was measured above 5 meq/1 making it unsuitable for irrigation uses. Only five ground water sample fall in doubtful to unsuitable for irrigation shown in Fig 4.

Permeability Index (PI):

Doneen (1964) classified waters quality characteristics based on the Permeability Index (PI) for irrigation and evolved a criterion for assessing the suitability of water for irrigation based on the permeability index. The classification mainly based on sodium, calcium, magnesium and bicarbonate concentration in ground water. The ground waters can be classified as class I, Class II and Class III orders by Doneen's chart (Domnico & Schwartz 1990), implying that the ground water of Jaunpur district, was good quality for irrigation purposes about 75% of ground water fall in class I and class II indicate that maximum permeability. Only two groundwater samples belong to class-III, i.e. water unsuitable category for the irrigation shown in Fig 5.

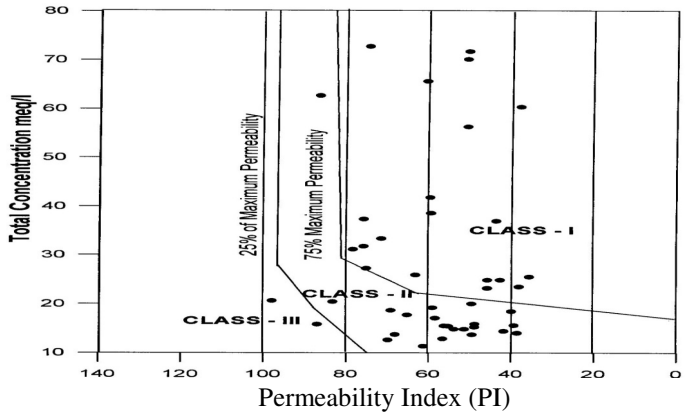

Fig. 5: Permeability Index verses Total Concentration of ion.

Suitability for drinking and general domestic uses

The most of ground water samples of study area were under the suitable index for drinking and domestic uses but few exceptions, as most of the parameters are within the permissible limits. The values of TDS & EC exceed the permissible limit of thirteen ground water samples, indicating the higher ionic concentration. Concentration of sulphate and nitrate in ground water on some sites was exceeding the permissible limits. High concentration of nitrate levels can cause methemoglobinemia in infants and high sulphate may contribute to the corrosions effect on human health system. The ground water was restricted for direct uses for drinking purpose in some particular block of Jaunpur..

Suitability for irrigation

The parameters like total hardness (TH), residual sodium carbonate (RSC), total dissolved solids (TDS), sodium absorption ratio (SAR) and permeability index (PI) which affects the quality of water for irrigation purpose were also computed and results were furnished of the important hydro-chemical properties of ground water to determine its suitability for irrigation. The calculated value of SAR ranged from 0.24 to 11.9 in ground water. The plot of data on the USSL salinity diagram, in which the EC is taken as salinity hazard and SAR as a alkalinity hazard showed that most of the water samples fall in the category C3S1and C2S1, indicating medium to high salinity and low alkaline water. High saline water cannot be used for irrigation with

restricted drainage and requires special management for salinity control. About 11% analysed water samples fall in the very high saline category shown in Fig. 3. The very high saline water is not suitable for irrigation under ordinary conditions but may be used occasionally under very special circumstances. SAR indicates that the effect of relative concentration in the water, thus sodium adsorption ratio is a more reliable method for determination of the irrigation water as per the) classification (Richards, 1954).

Conclusion

The present study revealed that the ground water samples of Jaunpur district were under good quality. Although some of the quality parameters exceeded from World Health Organization guideline values, most of the analyzed physico-chemical parameters were satisfactory for drinking and irrigation purposes. The cations and anions concentration exceeded only 15% of ground water sample out of permissible limit according to (WHO, 2002, 2004), and 2- 3% of water samples were found unsuitable for irrigation. The classification of sodium absorption ratio (SAR) indicated that 78% of water samples were under good category for irrigation suitability and the permeability index indicated that 75% of ground water samples were accepted for irrigation. According to analyzed water samples indicated that the ground water samples of Dharmapur, Sircony, Rampur block and Jaunpur city were found unsuitable category for irrigation under normal condition and requires proper water purification management.

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Evaluation of drinking water quality of Navsari District (Gujarat)

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Abstract

Navsari district is located in the south eastern part of Gujarat state in the coastal lowland along Purna River in India. Its geographical coordinates are 20° 51' 0" North, 72° 55' 0" East. In the present study, the physico-chemical parameters of Navsari district (Gujarat, India) have been analyzed regarding their suitability for drinking purpose. The study was carried out by collection of water samples from six sampling sites. These samples are analyzed for turbidity, pH, total solids, total suspended solids, total dissolved solids, total hardness, magnesium hardness, calcium hardness, phenolphthalein alkalinity, total alkalinity. The analyze results is compared with permissible limits as prescribed by WHO, GPCB for drinking water quality.

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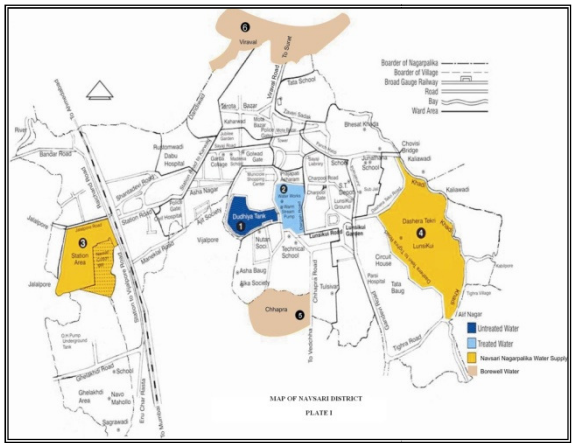
Keywords: Drinking water | Hardness | Total solids | Pollution | Navsari

Introduction

Water is the most beautiful and precious gift of nature without which no life could survive on earth (Dara, 1998; Kumar and Kakrani 2000). Water takes many different shapes on earth and to study water a new science evolved named as "Hydrology" which is the science to know the properties, distribution and behavior of water in nature (Fair and Geyer, 1958). Among the various needs of water, the most essential need is drinking. Surface water and ground water are two major sources for the supply of drinking water. Surface water comes from lakes, reservoirs, and rivers. Groundwater comes from wells that the water supplier drills into aquifers (Park, 1997). Maintaining the quality of water is the most important one for human being since it is directly linked with his daily life (Gosh, 2002). Thus, proper and managed study of water, especially freshwater is essential to understand the relationship and

interdependence of various constituents of any habitat.

The town of Navsari is approximately about 2000 years old. The city is situated at southeastern Gujarat state, west-central India. It is situated in the coastal lowland along the Purna River.The district covers an area of 2,211 square kilometers and has population of 1,229,463 of which 27.36% is urban. It lies between 72.5 east latitude and 65.3 west longitudes. Weather is pleasant almost all the year around, sunny from September to May, rainy from June to August. There are two lakes in the city namely Dudhiya Talao and Sarbatiya Talao. The main source of Nagarpalika Water Works Supply in Navsari city comes from Kakarapar through a canal and is stored in a small reservoir called "Dudhiya Talao" (Patel *et al*., 2000)**.** The kakrapar wier is constructed across the river Tapi and down stream of Ukai dam. To monitor the potable water quality, total selected six sampling sites shown in Plate 1 are untreated water of Dudhiya Talao (Site 1), treated water of Navsari water works (Site 2), Station area (Site 3), Lunsikui area (Site 4), Chhapra village (Site 5) and Viraval village (Site 6).



Materials and Method

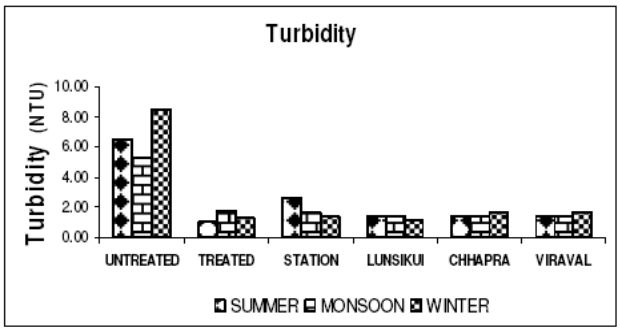
In the present study, six sampling sites were selected. The sampling was done on seasonal pattern. Composite sampling method was particularly adopted in Dudhiya Talao (Site 1). The taps were kept open for 2-3 minutes while collecting samples from pipeline supply to remove the possible impurities in water through pipes. Water samples were collected at fixed time to maintain the consistency in the results. Care was also taken for collection timing depending on water supply from Navsari Nagar Palika.

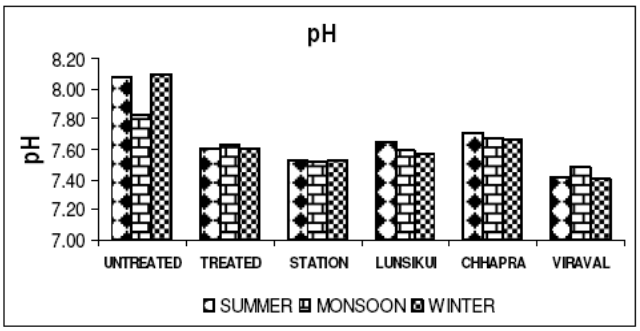
The methods of APHA (1995) and Trivedi and Goel (1986) were followed for water analysis. The parameters such as turbidity, pH, total solids, total suspended solids, total dissolved solids, total hardness, magnesium hardness, calcium hardness, phenolphthalein alkalinity, total alkalinity were brought for further analysis.

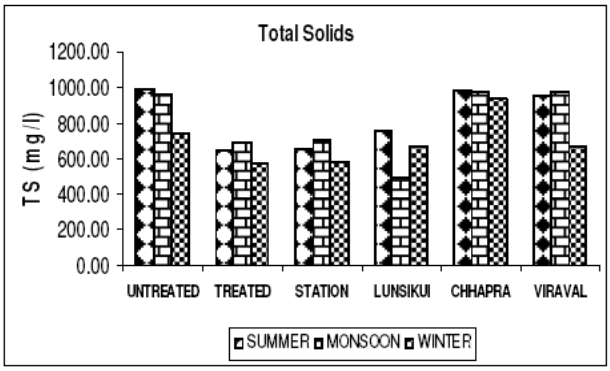
Results

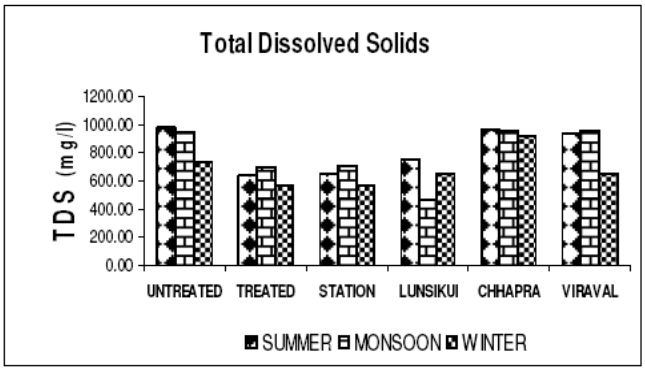
The results of physico-chemical parameters of average of six sampling sites are shown through graph. Turbidity of drinking water of Navsari district was higher from untreated water (site 1) and reduced drastically after treatment (site 2). More or less same value was recorded from remaining sampling sites3-6 in all the three seasons except in summer season from (site 3).

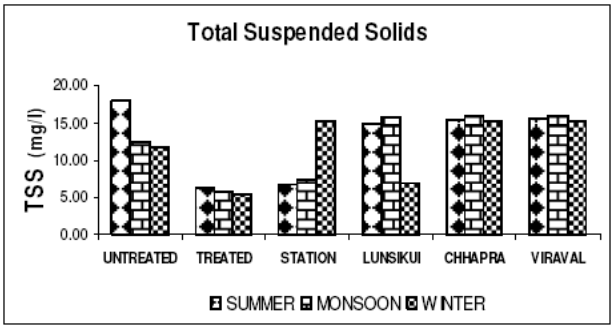
The pH was predominantly alkaline ranged between 7.48 to 7.83 in monsoon season, 7.41 to 8.10 in winter season and 7.41 to 8.08 in summer season throughout the study from all the six sampling sites of Navsari district. pH of

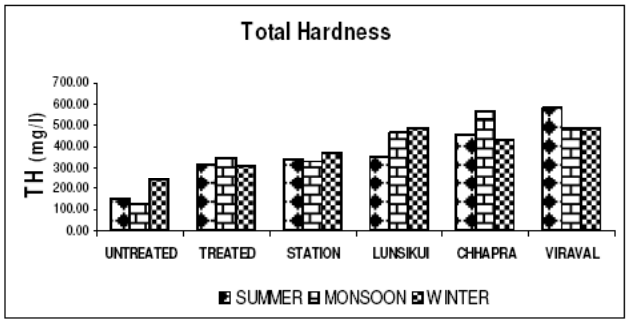

Turbidity
Turbidity (NTU)
10.00
8.00
6.00
4.00
2.00
0.00
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER MONSOON WINTER

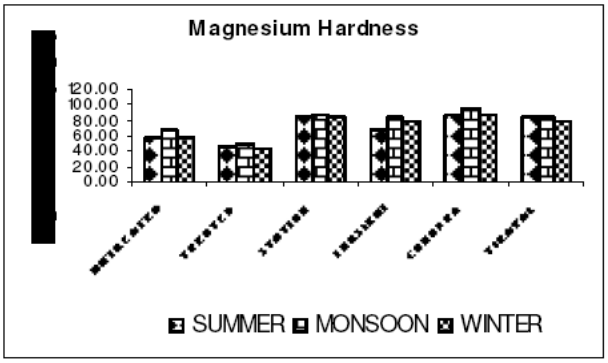

pH
pH
8.20
8.00
7.80
7.60
7.40
7.20
7.00
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER MONSOON WINTER

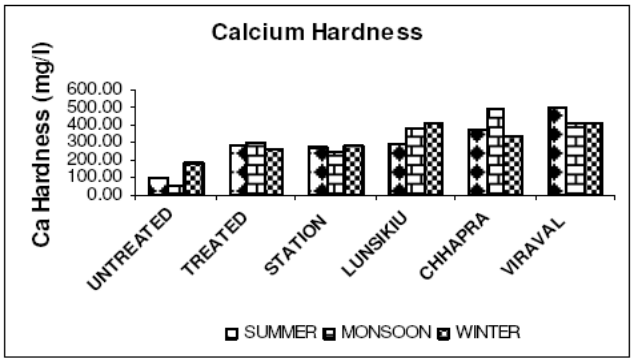

Total Solids
TS (mg/l)
1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER MONSOON WINTER

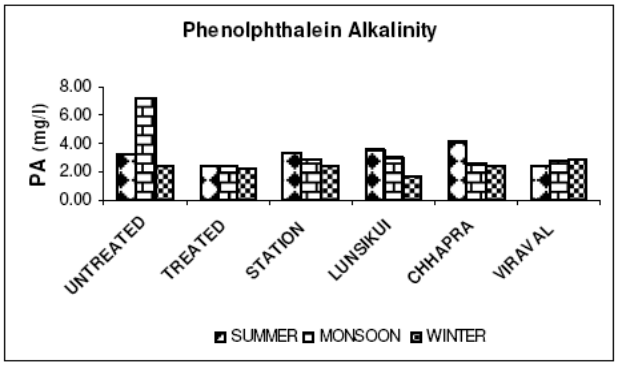

Total Dissolved Solids
TDS (mg/l)
1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER MONSOON WINTER

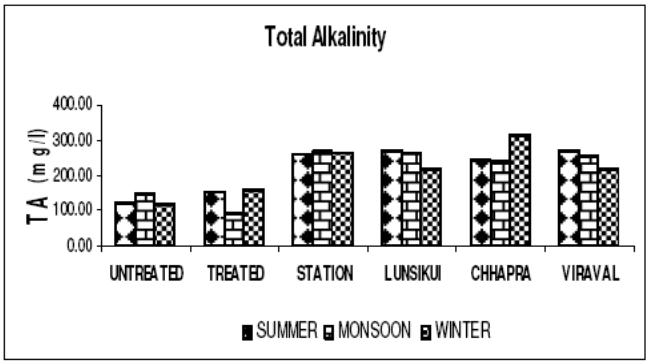

Total Suspended Solids
TSS (mg/l)
20.00
15.00
10.00
5.00
0.00
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER MONSOON WINTER

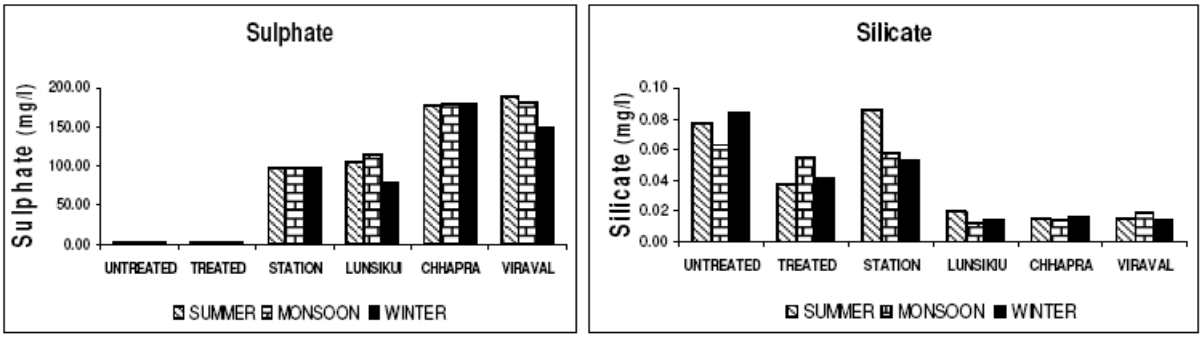

Total Hardness
TH (mg/l)
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER MONSOON WINTER


Magnesium Hardness
120.00
100.00
80.00
60.00
40.00
20.00
0.00
SUMMER MONSOON WINTER

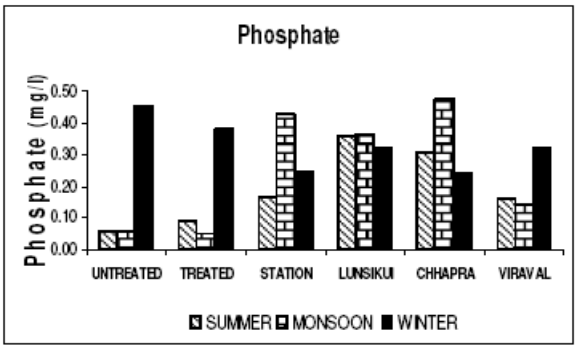

Calcium Hardness
Ca Hardness (mg/l)
600.00
500.00
400.00
300.00
200.00
100.00
0.00
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER MONSOON WINTER

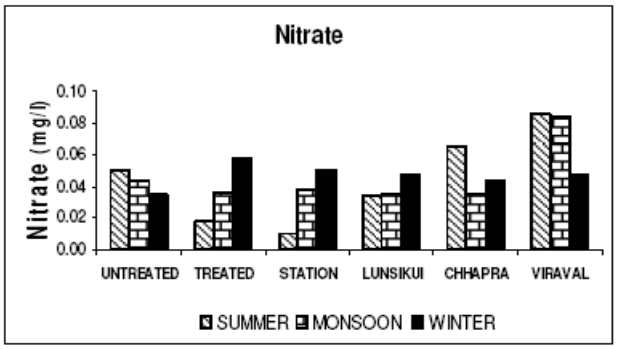

Phenolphthalein Alkalinity
PA (mg/l)
8.00
6.00
4.00
2.00
0.00
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER MONSOON WINTER

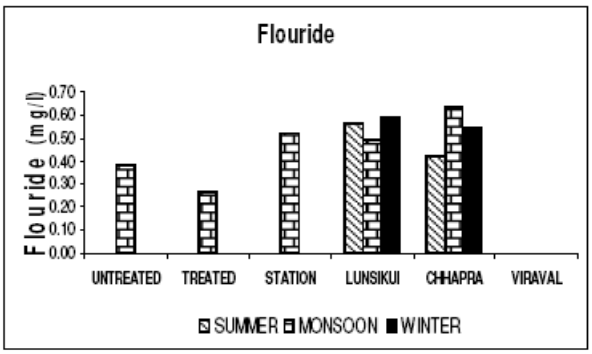

Total Alkalinity
TA (mg/l)
400.00
300.00
200.00
100.00
0.00
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER MONSOON WINTER

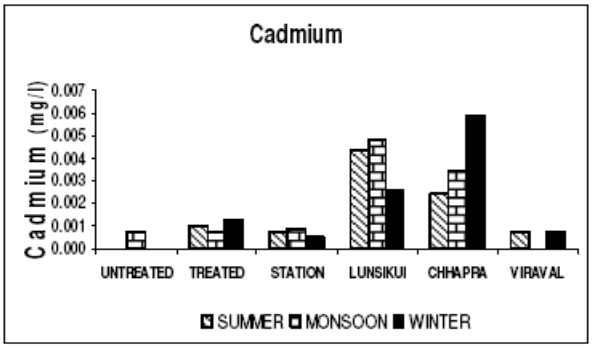

Sulphate
Sulphate (mg/l)
200.00
150.00
100.00
50.00
0.00
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER
MONSOON
WINTER

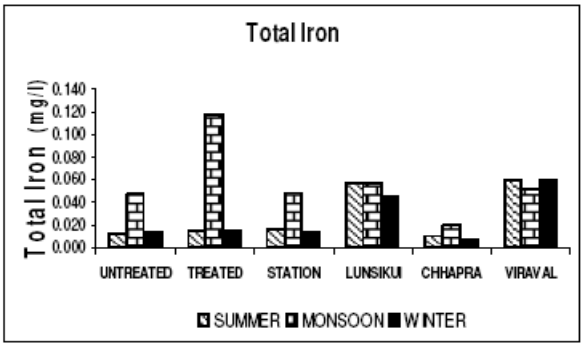
Silicate
Silicate (mg/l)
0.10
0.08
0.06
0.04
0.02
0.00
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER
MONSOON
WINTER

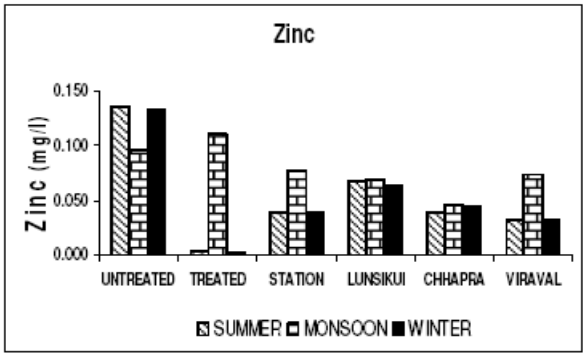

Phosphate
Phosphate (mg/l)
0.50
0.40
0.30
0.20
0.10
0.00
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER
MONSOON
WINTER

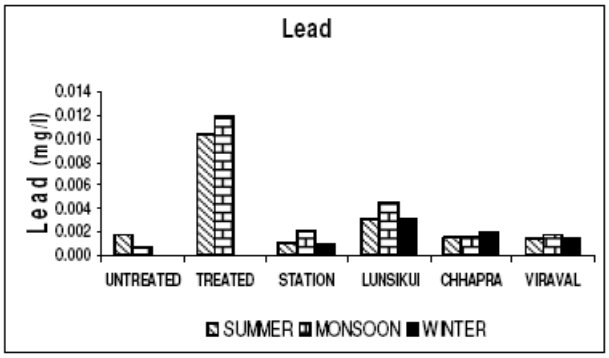

Nitrate
Nitrate (mg/l)
0.10
0.08
0.06
0.04
0.02
0.00
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER
MONSOON
WINTER


Flouride
Flouride (mg/l)
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER
MONSOON
WINTER


Cadmium
Cadmium (mg/l)
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER
MONSOON
WINTER


Total Iron
Total Iron (mg/l)
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER
MONSOON
WINTER


Zinc
Zinc (mg/l)
0.150
0.100
0.050
0.000
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER
MONSOON
WINTER


Lead
Lead (mg/l)
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
UNTREATED
TREATED
STATION
LUNSIKUI
CHHAPRA
VIRAVAL
SUMMER
MONSOON
WINTER

drinking water was found highest in untreated water (site 1) whereas lowest in Viraval village (site 6) during all the three seasons. The pH of treated water (site 2), station area (site 3), lunsikui area (site4) and chhapra village (site 5) were more or less same in all three seasons.

Total solids were recorded maximum in the range of 664.47-990.50mg/l from untreated water (site 1), chhapra village (site 5), viraval village (site 6), and found to be minimum 488.16 mg/l from lunsikui area (site 4) in monsoon season whereas it was recorded more or less same from sites 2 and 3 during all the three seasons. Total dissolved solids of untreated water (site 1), chhapra village (site 5), viraval village (site 6) were recorded in range of 649.19 - 972.50 mg/l in all three seasons whereas in treated water (site 2), station area (site 3) and lunsikui area (site4) were more or less same in range between 472.31 - 749.38 mg/l.

The results of total suspended solids were found to be higher from untreated water (site 1) and reduced drastically after the treatment (site 2). More or less same value was recorded from chhapra village (site 5) and Viraval village (site 6) whereas in station area sampling site3 and lunsikui area sampling site4 results were close to sampling sites 5 and 6.

Total hardness of untreated water (site 1) was found to be minimum during all three seasons. It was recorded in the range 306.50-486.47 mg/l from treated water (site 2), station area (site 3) and lunsikui area (site 4) whereas it was found to be in similar range of 426.85-565.36 mg/l from sampling sites 5 and 6. The results of calcium hardness, in untreated water (site 1) was far below than the treated water (site 2) in summer and monsoon seasons whereas it was found in same range between 241.82 - 499.66 mg/l from station area (site 3), lunsikui area (site 4), chhapra village (site 5) and Viraval village (site6). Magnesium hardness were recorded in range 58 - 67.78 mg/l from untreated water (site 1) and was minimum from treated water (site 2) whereas it was more or less similar range from 67.09 - 95.63 mg/l from station area (site 3), lunsikui area (site 4), chhapra village (site 5) and Viraval village (site6) during all the three seasons.

Total alkalinity of untreated water (site 1) and treated water (site 2) was found minimum during all the three seasons in range 93.50-156.50 mg/l whereas it was more or less similar range from 217.69 - 315.03 mg/l from station area (site3), lunsikui area (site 4), chhapra village (site 5) and Viraval village (site 6) during all the three seasons as shown in above graph. Phenolphthalein alkalinity were recorded in the range of 1.66 - 4.06 mg/l from all the sampling sites 2 - 6 except during monsoon season from untreated water (site 1) it was highest in range of 7.25 mg/l was depicted in graph.

Discussion

In the present study, turbidity was highest in untreated water and was reduced in all the sites and maintained well. The highest turbidity in untreated water was due to presence of clay, silt brought with runoff of water from

Kakrapar canal and did not crosses the standard limits.

pH is the measure of the intensity of acidity or alkalinity and measures the concentration of hydrogen ions in water (Mackee and Wolf, 1963). pH value of 7 is considered to be the best and most ideal (Sawyer and Mc Carty, 1967)**.** During the present study pH was found to alkaline range between 7.4-8.10 which was under the desirable limit.

The survey regarding the taste threshold level of TDS was done by Bruvold and Ongerth (1969) and was concluded that the range between 658-758mg/l was good enough and the range between 1283 - 1333 mg/l unpalatable for drinking. So, water with presence of high level of TDS was not used by the consumers. In the present study TDS was found in the range 472.31 - 972.50 mg/l which was within the desirable limit.

Hardness is defined as the concentration of calcium and magnesium ions content of water (Kumar and Kakrani, 2000)**.** Most natural water supplies contain at least some hardness due to dissolved calcium and magnesium salts (Fulvio and Olori, 1965)**.** Hardness was higher from sampling sites 3-6 compared to untreated and treated. However the value did not cross the limits.

Calcium is important as a nutrients, its deficiency causes rickets (Trivedi and Goel, 1986). High concentrations of calcium are not desirable in washing, laundering and bathing. Scale formation in boilers takes place by high calcium along with magnesium (Park, 1997)**.** In the present study, calcium was found highest from sampling sites 5 and 6 due to bore well water.

Magnesium also occurs in all kinds of natural waters with calcium, but its concentration remains generally lower than the calcium (Purohit and Saxena, 1990) So if calcium and magnesium is high in water than it may cause kidney disease (Taylor, 1958)**.** In the present study magnesium was found below the desirable limit.

Alkalinity in natural waters is due to free hydroxyl ions and hydrolysis of salts formed by weak acids and strong bases. Water with low alkalinity is more likely to be corrosive, which could cause deterioration of plumbing and an increasing chance for lead in water if present in pipe, solder or plumbing fixtures (Frank, 1987)**.** In the present study, alkalinity was high from sampling sites 3-6, this may be due to corrosion in distributing pipes and the bore well supply but were found in normal range.

Conclusion

All the physico-chemical fall within the permissible limit. This indicates that the water of Navsari district and its vicinity is suitable for drinking purpose.

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Screening of pesticide from contaminated water using Molecular Imprinted Polymer-Solid phase extraction method

H.V. Pant[1], K. P. Singh[2], S. Kumar[3] and P. Singh[4]

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Abstract

The quality of natural water is deteriorating continuously due to the accumulation of undesirable constituents into it. The main sources of the contamination are industrialization, domestic activities, agricultural activities and other environmental changes. These activities and changes, if improperly controlled, can destroy the quality of our environment.Molecularly imprinted polymers (MIPs) with recent advancements have created synthetic materials that can mimic the function of chemical and biological receptors but with less stability constraints. These polymers can provide high sensitivity and selectivity while maintaining excellent thermal and mechanical stability. In the present study molecularly imprinted membrane has been fabricated for the specific recognition of pesticide like Deltamethrin which is being used widely to control insects in crop management system. The composite membranes have been prepared by using methacrylic acid (MAA) as functional monomer, selected by electrostatic interactions based computational simulation and ethylene glycol dimethacrylate (EGDMA) as cross linker. The garbing of deltamethrin MIP on membrane matrix was confirmed by the SPE, UV-VIS spectrophotometer and FTIR.

Keywords: Sensor | molecularly imprinted polymer (MIP) | SPE

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Introduction

In order to increase the food production, uses of pesticides in agriculture become a general thing. But excessive and uncontrolled uses of pesticides contaminate water. These pesticides like Deltamethrin have very severe health effects. Deltamethrin may come in contact with human body through inhalation, ingestion and the dermal routes of eye and skin. Each of

these pathways can possibly lead to acute health effects. Allergic reactions have included the following effects: anaphylaxis, bronchospasm, eosinophilia, fever, hypersensitivity, pneumonia, pallor, polyposis, sweating, sudden swelling of the face, eyelids, lips and mucous membranes and tachycardia.

In the last decade, one of the most promising technical applications based on the use of MIPs has been molecularly imprinted solid-phase extraction (MISPE). The technique for the first time was used for making MIP by Sellergren et al. for imprinting small target molecules. With the same concept, Nematollahzadeh *et al*. developed the techniques based on MIP. Most of the studies performed have focused on extracting compounds from biological samples. Bio fluids have been the samples analysed in most of the biological studies with only a few papers reporting the extraction of analyte from tissue samples. In the last few years, MISPE has also been applied to extracting compounds from other matrices, such as environmental Samples (water and soils), food, plants and tobacco, although the number of studies of some of these sample types is limited. There are many recent reports of molecularly imprinted polymer which are used to develop detection systems for pharmaceuticals and environmental contaminants. Nematollahzadeh et al. developed a general technique, so-called polymerization packed bed, to obtain a hierarchically structured high capacity protein imprinted porous polymer beads by using silica porous particles for protein recognition and capture. MIPs show a promising future in the developing knowledge and application in food sciences.

In the present paper a plan is designed by using MISPE to extract Deltamethrin from Deltamethrin contaminated water. SPE in particular is widely applied in this area due to its simplicity, important savings in time and cost, and, last but not least, because it is an environmentally friendly technique compared to classical solvent extraction.

Materials and Method

Preparation of Template

A template of Deltamethrin (1.006 gm) was taken in a 15 ml reaction vial and solvent (Acetonitrile, 5 ml), cross linker (EGDMA, 4.8ml), functional monomer (Methacrilic acid, 0.5ml) and initiator [4, 4'- azo-bis (4-cyanovaleric acid) - 0.05 gm] were added to it. All the components were thoroughly mixed. Blank polymer without Deltamethrin was also prepared. After that reaction vials were kept at 70-85^{0}c in oven for overnight for complete polymerization. The polymer was transferred from the vial to mortar, pistol, grinded and filtered with methanol. The polymers of 40-125 µm were collected through methanol solvent extraction. Remaining residue filtrate was also collected for future use. An amount of 400 mg of MIP were packed in cartridges with frits with the help of frit settling rod.

Solid Phase Extraction

A rapidly growing application area for MIPs is what has become known as molecularly imprinted polymer solid-phase extraction (MISPE). The use of MIPs as the stationary phase in solid phase extraction makes it

possible to perform specific enrichments to facilitate analysis of substances available only at trace levels in samples. Obvious areas of interest for this technique are the analysis of drugs and environmental pollutions. Solid-phase extraction is by far the technique in which MIPs have found most of their applications, mainly due to its speed, robustness, and simplicity .While traditional SPE stationary phases offer generic selectivity related to the hydrophobic/hydrophilic and/or ionic character of the targeted compounds, MIPs introduce inherent selectivity for a specific analyte or family thereof, to the stationary phase, greatly improving its performance in demanding separations. For this reason, MI-SPE protocols have been developed, and, in most cases, have successfully substituted traditional SPE sorbents, for analysis of environmental pollutants, clean-up of biological fluids, and analysis of pharmaceuticals and food samples.

Similar to conventional SPE, MI-SPE usually comprises of few steps, depicted in Figure- 1**.** Initially the column is conditioned with an appropriate solvent/buffer and the sample is loaded. The so-called 'molecular recognition' step follows, whereby the loaded column is washed with a solvent that will promote specific interactions between the stationary phase and the imprinted analyte, while disrupting nonspecific binding. An additional washing step is usually added, followed by elution of the selectively bound analyte(s) with a strong eluting system.

Apart from the traditional offline SPE protocol discussed above, column switching and pulsed elution have been implemented in inline SPE. The inline approaches have become

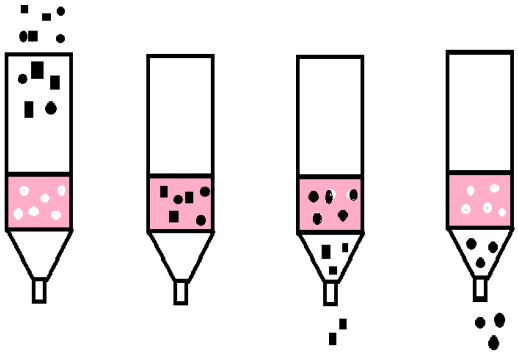

Figure 1: Principle of Solid phase extraction with an imprinted polymer. The sample is loaded on to the imprinted Polymer reulting in binding of both the analyte and contaminants (step 1). The firste elution steps removes the contaminants and the analytes remains the specific binding site (step 2). The analyte is then eluted from the solution (step 3).
■ Other sample components ● analyte

increasingly popular, because they offer possibilities for automation of the whole combined sample pre-treatment/enrichment and analytical process. Here, an MI-SPE column is coupled with an analytical column via a switching valve. Initially, the MIP column is loaded with the sample of interest and all necessary intermediate washing steps are performed using a secondary valve and pump. The MIP column is then eluted and the analyte(s) of interest are transferred into a holding loop. After valve switching, the sample is injected into the analytical column for separation . The most widely used organic modifiers so far have been acetic acid or its analogue, trifluoroacetic acid. Once all the MISPE steps have been optimised, a problem can sometimes occur, especially when using highly sensitive detection systems such as MS. This is known as bleeding of the cartridge. This problem, as mentioned previously, is a consequence of an inefficient removal of the

template molecule used during the synthesis of the polymer once the MIP is obtained. Therefore, when the sorbent is used in a MISPE protocol, remaining template molecules might still elute from the cartridge, thus masking the final result obtained. All the above-mentioned stages are essential steps in any MISPE protocol, although, as stated previously, when loading the MIP with an organic solvent, the clean-up step can sometimes be avoided. There are basically two distinct protocols to perform all these stages: MISPE offlineand MISPE on-linegenerally coupled to liquid chromatography (LC). MISPE off-line is the mostly used technique. In this case, the useful particles are generally suspended in a solvent and then poured into an empty polyethylene cartridge. As in the conventional SPE cartridge, the particles are held between two frits to avoid any losses. The cartridge is then connected to a SPE manifold and the sample is percolated through by negative pressure. The most widely used mass of sorbent for MISPE applications normally ranges from 40 to 200 mg of suitable particles. Their particle size and shape depends on the polymerisation approach taken during the synthesis of the MIP. In the synthetic protocols aiming to deliver spherical particles, the particles obtained are ready to be used, with no need for any further processing. In the case of traditional polymerisation, in which MIPs are obtained in a monolithic form, there is a need of further processing so that the particles for MISPE applications are in the range of between 20 and 60 μm. This range is the right balance because the particle size is low enough to enable both a proper flow and mass-transfer of the analytes present in the mobile phase on to the sorbent.

Preparation of MIP Composite Membrane with Template

The reactive mixtures were prepared by mixing of selected monomer (Methacrilic acid, 0.5ml) with inert porogen (acetonitrile 5 ml), suitable template (Deltamethrin, 1.006 gm) and crosslinker (EGDMA, 4.8 ml). After addition of initiator [4, 4'- azo-bis (4-cyanovaleric acid)] (0.05 gm) reactants were mixed and degassed. The yield of properly created MIPs is limited by the capacity to effectively wash the substrate from the MIP once the polymer has been formed around it.

Removal of Template and Confirmation by UV-Visible Spectrophotometer

Specific binding is confirmed by SPE and UV-Vis spectrophotometer. The MIPs were washed five times with methanol and five times with water to remove the print molecules. All the extraction experiments were performed using a SPE vacuum unit. Imprinted template was eluted with methanol. The fractions eluted from each cartridge were collected separately. The removal of template was also confirmed with UV-Visible spectrophotometer.

Fourier Transform Infrared Spectroscopy Study

FTIR provides quantitative analysis of the binding modes of a substrate molecule to the polymer site by empirical calibration of FTIR. The technique gives a consistent representation in which the target analyte binds to the

polymer site. The analysis also provides an opportunity to quantify site isolation within the polymer and the fidelity of functionalized site is maintained by the network polymer.

Results

Characterization by FTIR Spectroscopy

The FTIR spectra of imprinted polymers can be readily acquired and then applied in a similar fashion to elemental micro-analysis to extract quantitative information on the composition of the polymer. The method is of particular value when the different chemical environments in the sample (e.g. arising from the functional monomer and cross linker in an imprinted polymer) give rise to well resolved, diagnostic signals. It is also possible to use FTIR to probe non-covalent interactions, e.g. hydrogen bonds, although the insensitivity of the technique sets limits on its utility in this regard.

Figure- 2 shows FTIR spectra of polymeric matrix with Deltamethrin template. This shows peak at the wave number 440 cm^{-1}, 460 cm^{-1}, 521 cm^{-1}, 656 cm^{-1}, 811 cm^{-1}, 873 cm^{-1}, 950 cm^{-1}, 1040 cm^{-1}, 1159 cm^{-1}, 1261 cm^{-1}, 1375 cm^{-1}, 1469 cm^{-1}, 1633 cm^{-1}, and 1727 cm^{-1} clearly showing bonds of template molecule and hydrogen bonding with polymer matrix. The template is surrounded by polymer matrix and can be easily resolved out by proper extraction with solvent methanol shown later. The FTIR spectra of polymer without template are prepared. The porogen used was the acetonitrile. The important peaks at 419 cm^{-1}, 1016 cm^{-1}, 1465 cm^{-1}, 1731 cm^{-1}, 2360 cm^{-1}, 2924 cm^{-1} and 3648 cm^{-1} clearly shows the polymerisation of monomer. There is no sign of hydrogen bonding peaks, which shows the absence of template in the spectra. The FTIR spectra of the reference polymer i.e. blank polymer without template using the DMF as porogen are prepared. It shows peak at 3843 cm^{-1}, 2360 cm^{-1}, 1517 cm^{-1}, 1424 cm^{-1}, 1212 cm^{-1}, 1077 cm^{-1}, 1004 cm^{-1}, 971 cm^{-1}, 754 cm^{-1}, 668 cm^{-1}, 521 cm^{-1}, 480 cm^{-1}, and 435 cm^{-1}. These peaks exhibits the IR spectra of blank polymer matrix free of template.

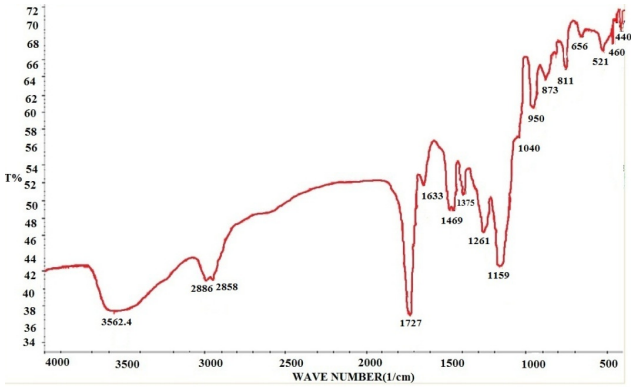

Figure- 2

Studies with SPE Column Preparation and Removal of Template

Removal of the template from the SPE cartridges was confirmed by UV-Visible spectrophotometer. The MIPs were washed five times with methanol and five times with water to remove the print molecules. UV-Visible spectra of elute was taken after first and fifth wash with methanol and first and fifth wash with water. Figure - 3 shows the absorbance and wavelength graph after first and methanol and water washing of Deltamethrin template.

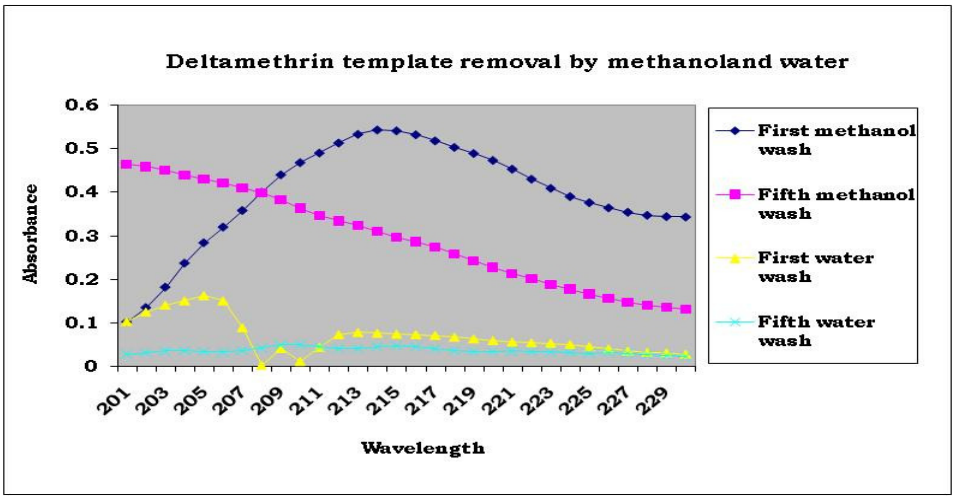

Figure - 3

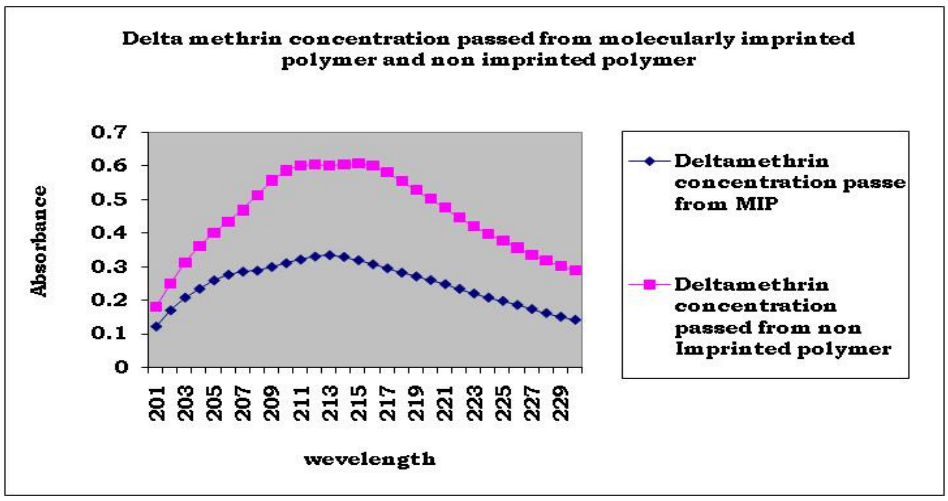

Figure - 4

Binding of Template with MIPs

Binding of the template was done by passing the concentration 20 µg/ml of template from imprinted polymer and non-imprinted polymer. MIP has specific cavities for template as concentration was passed from MIP, its shows sensitivity to template. Sensitivity of MIP confirmed by UV-visible spectra of elutes. MIP elutes shows lower absorbance than non-imprinted polymer. It confirms that MIP has sensitivity to template. Figure - 4 is showing the graph between absorbance and wavelength of Deltamethrin concentration passed from MIP and Deltamethrin concentration passed from non-imprinted Polymer.

Deltamethrin MIPs sensing and separation

When Deltamethrin concentration was charged in SPE cartridges, MIP with template Deltamethrin shows absorbance 0.218 at wave number 201, 0.189 at 202, 0.154 at 203, 0.12 at 204, 0.93 at 205 and 0.078 at 206, which clearly shows Deltamethrin MIP has sensitivity to template Deltamethrin. After the Deltamethrin MIPs sensing in contaminated water its extraction is done by Solid Phase extraction as discussed above.

Conclusion

According to molecular imprinting approach, cross-linked polymers are formed around a template molecule. The template is then removed, thus leaving molecular cavities capable of binding the template molecules back. Moreover, the synthesis of MIPs is a straightforward and inexpensive procedure. The selectivity of the imprinted membrane was due to the formation of molecular cavities inside the polymer matrix and the molecular cavities capable of binding the template molecules back. The imprinted membrane was treated with methanol for the removal of template molecules and this confirmed by taking absorbance of eluted template in methanol and it also confirmed by UV-Visible spectrophotometer and FTIR by comparing the imprinted and composite membrane.

The next step of experiment was making sensor using the MIPs. For this, SPE unit was used. For sensing, concentration of the template Deltamethrin was prepared in methanol and water (methanol to water ratio 1:9). Deltamethrin concentration was loaded in the SPE cartridges and the fractions eluted were collected separately. UV-visible spectra of elute taken, the concerned MIP (MIP with template Deltamethrin) shows lower absorbance than others.

In the present study, the synthesis of molecularly imprinted polymer matrices specific to Deltamethrin pesticides were synthesized and these MIP matrices were also used for the fabrication of MIP composite membranes. The MIP matrices were also used for specific separation and recognition of targeted analyte which are pesticide molecules with structurally related molecules. This study can resolve the problem of rising of natural receptor against these hazardous molecules by replacing with artificial receptor. These artificial receptor and equally specific and more durable than the natural were having characteristic of high stability in mild environment conditions. The identification, sensing and separation of these template molecules through SPE is a cost effective and easier way of commercial usage.

Acknowledgement

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L-Ascorbate mediated detoxification of Lambda Cyhalothrin induced histopathological changes in the gill epithelium of an experimental model, fresh water bivalve, *Lamellidens Marginallis*

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Abstract

The present study was conducted to evaluate the effectiveness of L-ascorbic acid in Lambda Cyhalothrin-induced toxicity in an experimental model, the fresh water bivalve, *Lamellidens marginallis*. Histopathological changes were recorded in the gill of fresh water bivalve, *Lamellidens marginallis* after acute exposure to Lambda Cyhalothrin alone & in combination with 50mg/L of L-ascorbic acid. Due to Lambda Cyhalothrin intoxication damage to the gill was extensive resulting epithelial shrunkage, necrosis, hypertrophy in secondary gill lamellae & connective tissue core at 24 hours of exposure. The severity of gill damage was progressed with increase in exposure period. After 96 hours of exposure to Lambda Cyhalothrin histopathological changes like shrunkage, necrosis, degeneration of the epithelial cells along with sub epithelial spaces in the secondary gill lamellae were noted. Exposure to Lambbda Cyhalothrin in combination with 50mg/L of L-ascorbic acid showed considerable reduction in nature of damage.The pre-exposed bivalve to Lambda Cyhalothrin alone showed fast recovery in presence of L-ascorbic acid than the recovery in the untreated fresh water. The probable cause of protection by the L-ascorbic acid in Lambda Cyhalothrin induced toxicity will be discussed in the paper.

Keywords: *Lamellidens marginallis* | Lambda Cyhalothrin | gill | histopathology | L-ascorbic acid

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Introduction

The pollution of rivers and streams with chemical contaminants has become one of the most critical environmental problems of the century. Some of these chemicals are biodegradable and quickly decay into harmless or less harmful forms, while others are non-biodegradable and remain dangerous for a long time. The use of various classes of pesticides

as organophosphate, organochlorine, carbamate and pyrethroids have been increased many fold for the last 10 years (Wolansky *et al*., 2006). Lambda Cyhalothrin is one of the class of chemicals called Pyrethroids and its trade name includes Karate. It is classified as Restricted Use Pesticide (by Environmental Protection Agency) because of its acute toxicity to humans. It is used for foliar treatment of vegetable, fruit and field crop, cotton, commercial, ornamentals and in and around poultry houses and dairies. Lambda Cyhalothrin is highly toxic to aquatic invertebrates, when absorbed through the mucous membrane of the respiratory tract, resulting in systemic intoxication. The bivalves are considered as the suspension filter feeders and influenced by the organization and fluctuations of the ecosystem (Akarte *et. al*., 1987). Molluscs, the lamellibranch bivalves constitute the important aquatic invertebrate biota which is of considerable research interest since they are universally distributed and have specific ecological adaptations. Pathological biochemical disturbances in aquatic organisms like mollusc due to pesticide toxicity are well documented (Waykar and Lomte, 2002; 2004).. Histopathological changes are mostly confined to organs directly involved in their metabolism and detoxification (Rashatwar and IIyas, 1994). Lamellidens marginalis is an economically important bivalve. It circulates large quantity of water through respiratory surface to obtain food and oxygen. Thus, it suffers a great risk of pesticide poisoning. Therefore, it is considered as a bio-monitoring tool in toxicological studies. There are few reports on the effects of xenobiotics on respiratory rate and gill histology of bivalves with no published data on histopathological alterations in L. marginalis after lambda cyhalothrin exposure. When pesticide enter into the body of molluscs, it create physiological as well as histopathological changes in the body of molluscs. Therefore, a study was conducted to determine possible respiratory hazards to L. marginalis following sub-lethal exposure to lambda cyhalothrin. A simple but effective way to prevent degenerative changes would be to prevent oxidative damage. The cells major defense against ROS damage includes antioxidants like ascorbic acid. For different physiological acts Vitamins are essential.

Materials and Method

Experimental Design: Set-I

1. Group ‘A’ was maintained as control.
2. Group ‘B’ animals were exposed to subacute treatment (LC 50/2 values of 96 hrs) of lambda cyhalothrin (0.75 PPM) upto 96 hrs
3. Group ‘C’ animals were exposed to subacute treatment of lambda cyhalothrin (0.75 PPM) along with 50 mg / litre L-ascorbic acid upto 96 hours.
4. Group ‘D’ animals were exposed to subacute treatment of lambda cyhalothrin (0.75 PPM) along with 100 mg/ litre L-ascorbic acid upto 96 hours.

Experimental design for recovery studies: Set- II

1. Group 'B' animals exposed to lambda cyhalothrin for 96 hours from set-I were divided into three groups for recovery study
2. Group 'E' animals pre-exposed to lambda cyhalothrin were allowed to self-cure normally in untreated fresh water up to 15 days.
3. Group 'F' animals pre-exposed to lambda cyhalothrin, were allowed to cure in 50 mg / litre ascorbic acid in fresh water up to 15 days.
4. Group 'G' animals pre-exposed to lambda cyhalothrin were allowed to cure in 100 mg/ litre ascorbic acid in fresh water up to 15 days.

During experimentation animals were fed on fresh water algae. After 24 and 96 hours interval, animals from set-I and after 5 days, 10 days and 15 days interval, animals from set -II were dissected and their gills were taken out. Tissues were fixed in the aqueous Bouin's fluid for 24 hrs and were processed by usual microtechnique method and serial sections of six micron thickness were stained with Mallory's triple stain.

Results and Discussion

The gross histopathological effects of acute dose of Lambda cyalothrin alone and with 50 mg/L and 100 mg/L of L-ascorbic acid and recovery responses studied in an experimental model fresh water bivalve, Lamellidens marginallis are shown in plate no 1 to 2.

Gills under Lambda cyalothrin intoxication

As compared to gills of control Lamellidens marginallis, after acute exposure to Lambda cyalothrin for 24 h, the Interlamellar connective tissue and muscle fibres surrounding the gill lamellae were damaged; hypertrophy of gill lamellae, loss of inter lamellar junctions, vacuolization of basal epithelium in the gills. Some cells showed very irregular vacuolated appearance, basement membrane of the epithelium of gill lamellae was found damaged at some places (Fig. b of plate 1). The severity of damage of gills progressed with longer exposure to Lambda cyalothrin. After 96 h exposure to Lambda cyalothrin, the degenerative changes such as pycnotic nuclei, necrosis of connective tissue and irregularly distributed tubular lesions were observed throughout the gill lamellae. In the gill lamellae, epithelial cells were separated from the basement membrane and the number of epithelial cells was reduced. The gill lamellae appeared to be collapsed due to damage of epithelial cells. Generalized reduction of cell and nuclear size were observed (Fig. b of plate 1).The result of microscopy showed that epithelial tissue was probably a primary target of the pesticide intoxication. In combined exposure to Lambda cyalothrin along with 50mg/L of L-ascorbic acid after 24 h showed damages at few places in the basement membrane of the tubules, slight shrinkage of epithelial cells and gill lamellae lumen (Fig. c of plate 1). After 96 h of exposure, histopathological changes in gill lamellae were relatively more, as compared to those of 24 h of exposure, but the intensity of

damage was relatively less as compared to those exposed to Lambda cyalothrin alone after 96 h of exposure (Fig. d of plate 1). In combined treatment of Lambda cyalothrin together with 100mg/L L-ascorbic acid after 24 h exposure retained most of the normal histopathological structure of gill lamellae. There were normal stratified epithelial cells arranged regularly on the basement membrane. There was slight shrinkage of tubules of the gill lamellae along with epithelial cells; the epithelial cells were slightly taller with few atrophied changes. The gill lamellae were more or less comparable to control (Fig. e of plate1). After 96 h of exposure, histopathological changes were relatively more as compared to those of 24 h exposure but less as compared to those exposed to same dose of Lambda cyalothrin after 96 h Severity of gill lamellae damage was much reduced (Fig. f of plate 1).

Recovery study

Animals pretreated to Lambda cyalothrin when allowed to cure in normal water and with L-ascorbic acid showed the restoration of normal structure of Gills. In histological section after 5 days of recovery in normal water, exhibited regeneration of connective tissue, basement membrane, epithelial cells and reduction in necrosis and vacuolization (Fig. a of plate 2). This is more evident after 10 days of recovery. The interlamellar connective tissues and muscle fibres surrounding the gill lamellae were regenerated. The tubular lesions occurred at all exposure times and persisted even after 10 days of recovery (Fig. b of plate 2). After 15 days of recovery, more restoration of epithelial cells with normal shape and size were observed but still necrotic lesions were seen (Fig. c of plate 2). When animals pretreated to acute dose of Lambda cyalothrin were allowed cure in 50 mg/L of L-ascorbic acid in freshwater, Gills of bivalve showed the restoration of normal Structure. Histological sections of Gills after 5 days of recovery showed normal shape and size of epithelial cells. Regeneration of interlamellar connective tissues and muscle fibres surrounding the lamellae was observed. In some regions of lamellae, necrotic changes were observed (Fig. d of plate 2). After 10 days of recovery more restoration of histology of Gills was observed (Fig. e of plate 2). After 15 days of recovery histological section exhibit normal histological structure likes that of control animals (Fig. f of plate 2). When animals pretreated to acute treatment of Lambda cyalothrin were allowed to cure in 100 mg/L of L-ascorbic acid in freshwater for 5 day of recovery, normal architecture of Gills, with slight tubular lesions was observed (Fig. g of plate 2). After 10 days of recovery almost all damages were recovered and structure of Gills was like those of control animals (Fig. h of plate 2). The histopathological changes as observed on Lambda cyalothrin exposure were reported after exposure to different pesticides (Thoser *et al*., 2001; Omiama, 2004; Waykar, 2006; Saraswathy *et al*., 2010). In present study on combined exposure to acute dose of Lambda cyalothrin along with 50mg/L and 100 mg/L of L-ascorbic acid showed reduction in damages, indicating the protective effect of L-ascorbic acid. Thus the result of the present study

clearly demonstrates protective ability of ascorbic acid against pesticide toxicity.

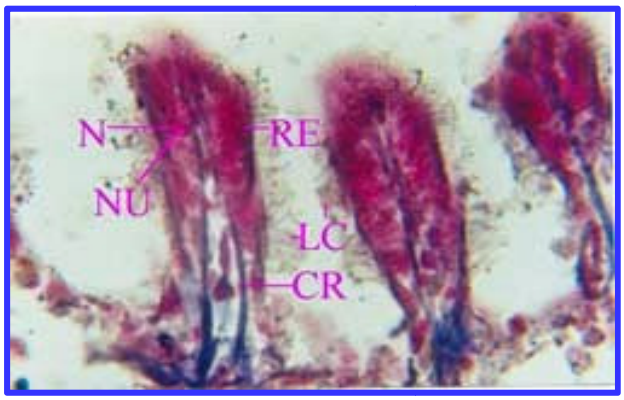

Plate 1a. L.S.of gill X 400 - Control gill

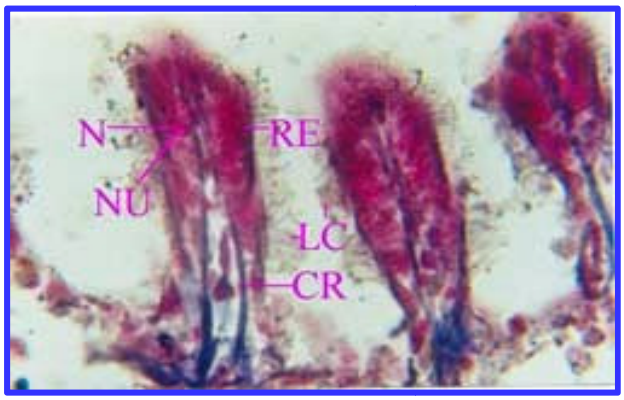

Plate 1b. L.S.of gill X 400 -After acute exposure to lambda cyhalothrin after 96hrs

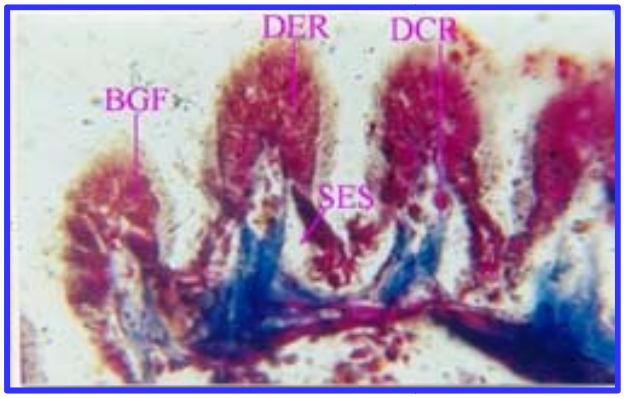

Plate 1c. After sublethal exposure to lambda cyhalothrin with 50 mg/l ascorbic acid at 24 hr

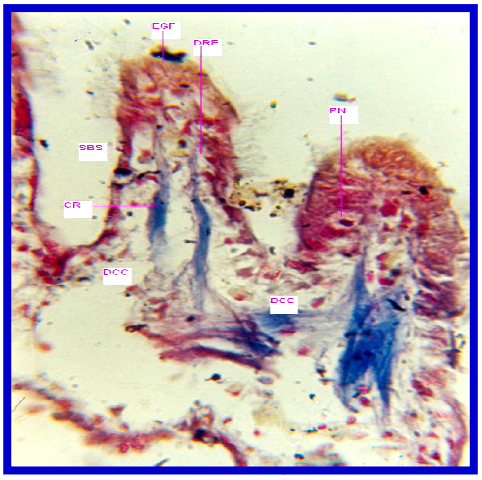

Plate 1d. After sublethal exposure to lambda cyhalothrin with 50 mg/l ascorbic acid at 96 hr

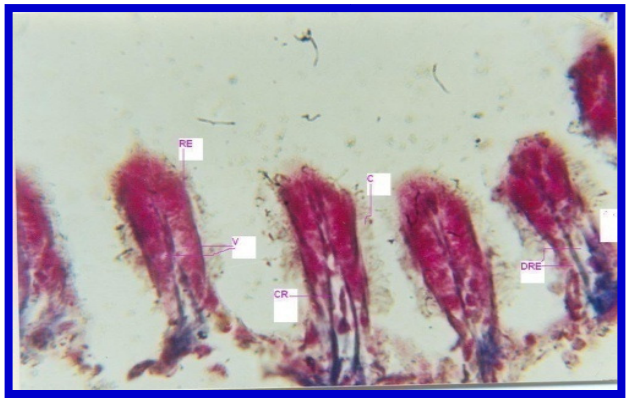

Plate 1e. After sublethal exposure to lambda cyhalothrin with 100 mg /l ascorbic acid at 24 hrs

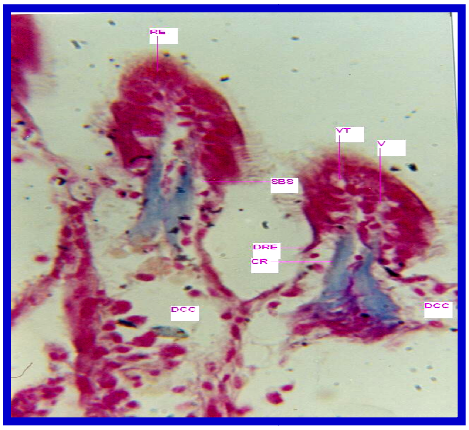

Plate 1f. After sublethal exposure to lambda cyhalothrin with 100 mg /l ascorbic acid at 96 hrs.

Plate1: Microphotographs showing L. S. of Gills (X-400) of Lamellidens marginallis on acute exposure to Lambda cyalothrin alone and in combination with 50mg/L and 100 mg/L of L-ascorbic acid

Recovery Study

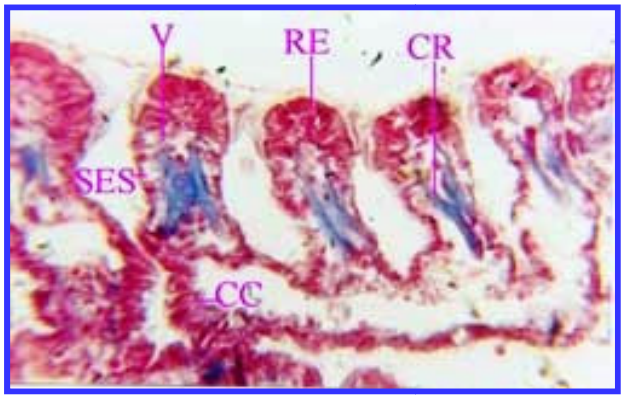

Plate 2a. Curing gills in normal fresh water in 5 days

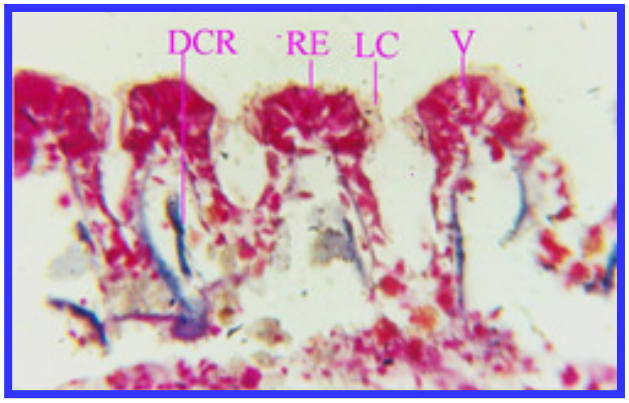

Plate 2b. Curing gills in normal fresh water at 10 days

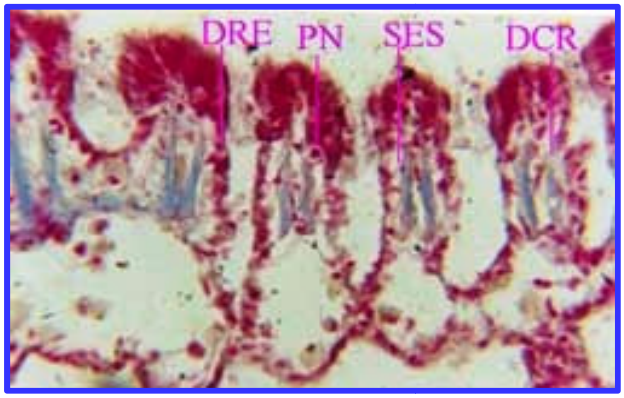

Plate 2c. Curing gills in normal fresh water at 15 days

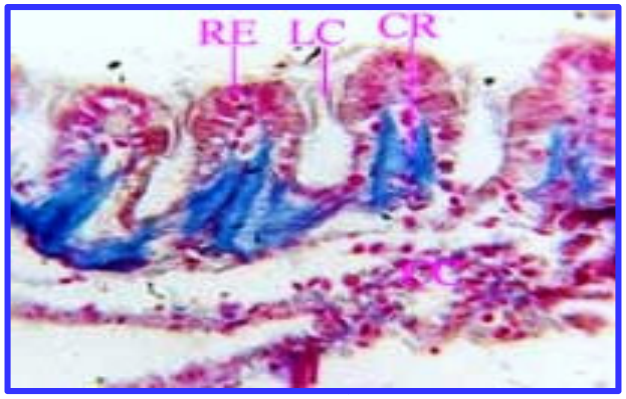

Plate 2d. Curing gills in 50mg/l ascorbic acid in fresh water at 5 days

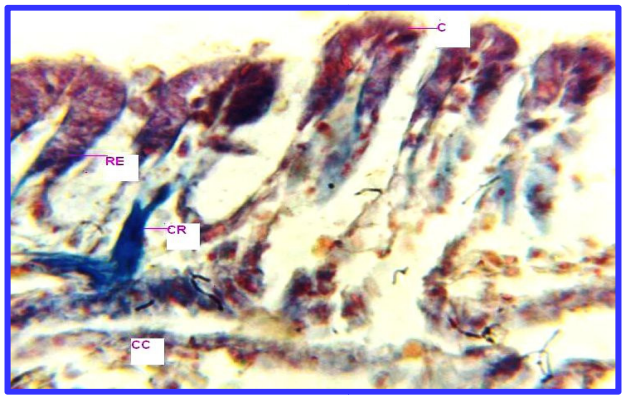

Plate 2e. Curing gills in 50mg/l ascorbic acid in fresh water at 10 days

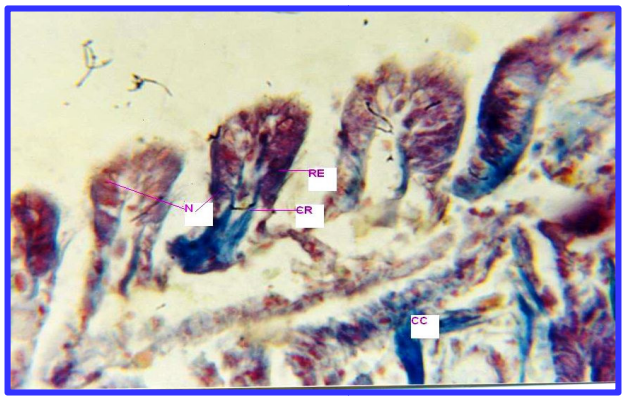

Plate 2f. Curing gills in 50mg/l ascorbic acid in fresh water at 15 days

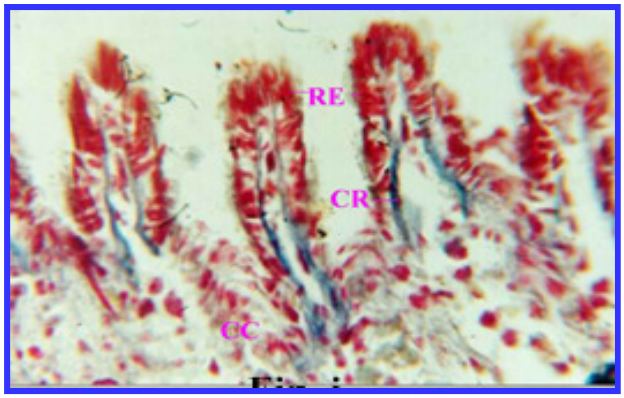

Plate 2g. Curing gills in 100mg/l ascorbic acid in fresh water in 5 days

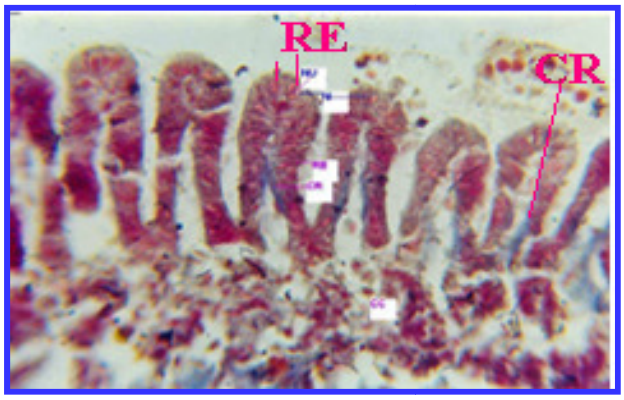

Plate 2h. Curing gill in 100mg/l ascorbic acid in fresh water in 10 days

Plate 2: Microphotographs showing L. S. of Gills (x 400) of pre-exposed Lamellidens marginallis to Lambda cyalothrin during recovery in normal fresh water ,50mg/L and 100 mg/L of L-ascorbic acid in freshwater.

Abbreviations C- Ciliary's border, CR- Chitinous rods, GF-Gill filament, IFS- Interfilamental space, N- Nucleus , Nu – Nucleolus, RE -Respiratory epithelium **,** DRE - Degenerating respiratory epithelium DCR - Degenerating chitinous rods , SW - Swollen tip of gill filaments IC - Interfilamental space

Conclusion

This study clearly indicated protective and wound healing property of ascorbic acid in pesticide induced tissue damage in experimental model. Thus it is evident that Vitamin C not only confirms protection against pesticide toxicity but can also perform therapeutic role against pesticide toxicity.

Acknowledgement

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Effect of chromium on the foliar epidermal pattern in different accessions of *Vigna angularis*

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Abstract

In the present work two accessions of *Vigna angularis* V1 and V2 from Chaubattakhal 1600m asl and Uttarkashi 1200m asl respectively) a hilly local crop were subjected to ten molar concentrations (10^{-1} M to 10^{-10} M) treatments of Cr. The seeds were first treated with various Cr concentrations for 24 and 48 hours). The number of stomatal cells decreased with increase in Cr concentrations. The mean number of epidermal cells in different concentrations of Cr were found to vary from 571 to 759 per mm^2 on adaxial and 540 to 702 per mm^2 on abaxial surface, number of stomata vary from 270 to 326 per mm^2 on adaxial surface and 246 to 300 per mm^2 on abaxial surface and number of subsidiary cells vary from 77 to 105 per mm^2 on adaxial leaf surface and 74 to 125 per mm^2 on abaxial leaf surfaces. The GCI (Guard Cell Index) was found to be increased with increase in Cr concentrations on adaxial leaf surface in both the accessions of *Vigna* while it decreased with increase in Cr concentration on abaxial leaf surface.

Keywords: Chromium | foliar epidermis | guard cell index | *Vigna angularis*

Introduction

Among large number of industrial pollutants, heavy metals occupy considerably lethal position because of threat to human health, owing to their toxicity. For millions of years, rocks at the earth surface were the only resource, releasing heavy metals into water and soil by way of weathering caused by rain, wind and other similar processes. The heavy metals were absorbed by living organisms and were released back into soil and water. This natural cycle in the beginning of present century was severely disturbed with the several fold increase in

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dependence upon heavy metals for fast industrial and agricultural developments.

Barcelo *et al.* (2005) analysed the water relations of Cr (VI) treated bush bean plants *(Phaseolus vulgaris* L. cv. contender) under both normal and water stress conditions. The distribution of Chromium, Nickel, and Cobalt in different parts of plant species and soil in mining area of Keban has been worked out by Sasmaz and Yamar (2006).

The legumes have been under cultivation throughout the world since time immemorial. They occupy a significant position among food crops as source of pulses, vegetables, oils, etc., (Khanna and Gupta, 1988). The legumes have a unique property of maintaining and restoring soil fertility through bacterial nodules, which are formed on their roots. India has the distinction of being world's largest producer of legumes (pulses and oil yielding) occupying about 13 % of area under cultivation and producing 22-23 million tons of grains annually (Tiyagi and Alam, 1992).

The *Vigna angularis* (Willd.) Ohwi & Ohashi is known as **Soonthiya** or **Rayans** in Garhwal. It belongs to the family Papilionaceae. Its English name is **Adzuki bean**. It is often cultivated; rarely met as an escape. *Vigna* flowers and fruits from August to November. The genus *Vigna* has about 150 species in tropics and 25 species in India (Gaur, 1999).

Materials and Method

The foliar epidermal patterns were analysed in all the control and treated plants. For epidermal analysis, the mature leaves were fixed in FAA. The adaxial and abaxial epidermis was studied. Following epidermal parameters were analysed for quantifying the variability in different accessions of *Vigna angularis*:

a) Number of epidermal cells per mm^2,
b) Number of stomata per mm^2,
c) Guard cell index (GCI), and
d) Size (length X Breath) of stomata (µm).

Results and Discussion

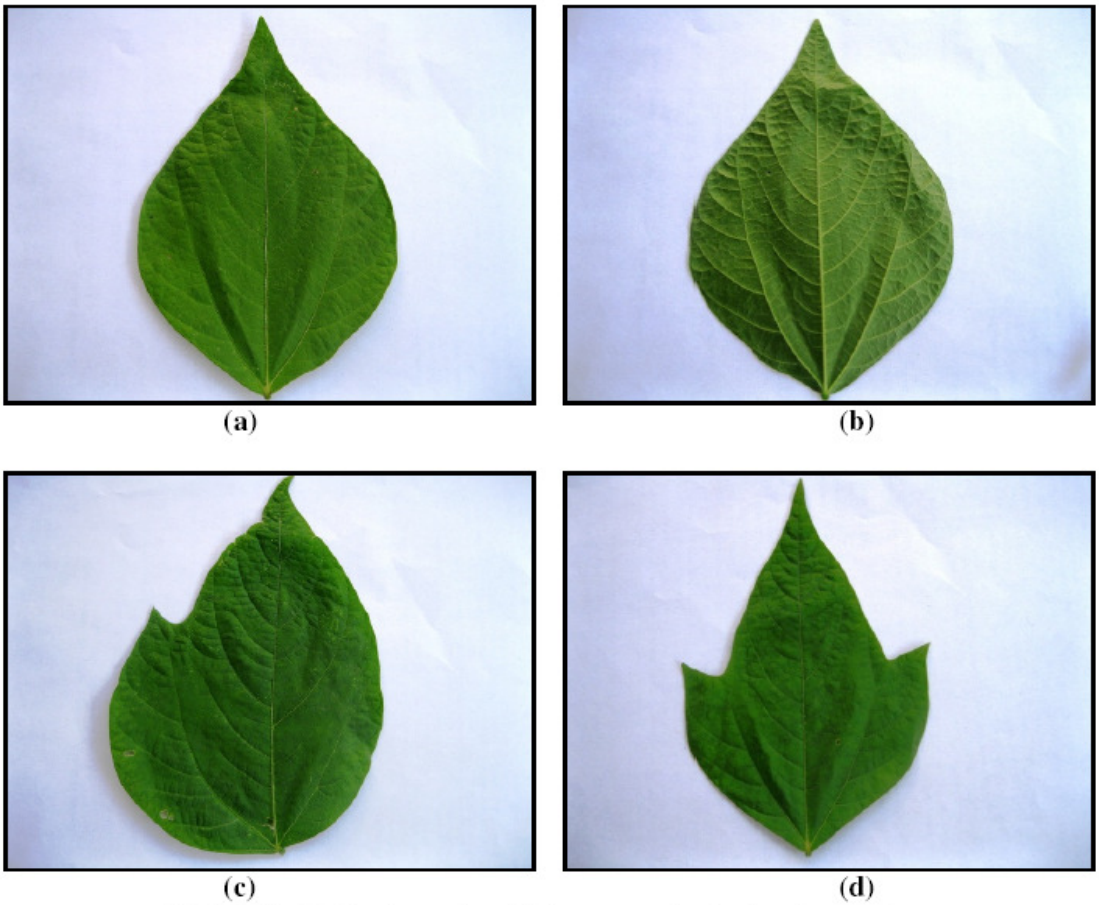
The leaves of *Vigna* were compound and leaflets were ovate-rhomboid, 5-8X0.4-0.8 cm, acute or acuminate, rounded-cuneate at base, hairy, stipules suborbiclar. (plate 1). The data related to mean number of epidermal cells, stomatal cells and subsidiary cells on abaxial and adaxial surface is given in Table 2. The mean lengths (µm) and breadths (µm) data of stomatal cells in both the accessions of *Vigna* are given in Table 3. The guard cell index (GCI) data is given in Table 4.

Species	Procurement place	Abbreviation used
Vigna angularis	Chaubattakhal, 1800 m asl	V1
	Uttarkashi, 1200 m asl	V2

Table 1: List of *Vigna angularis* accessions

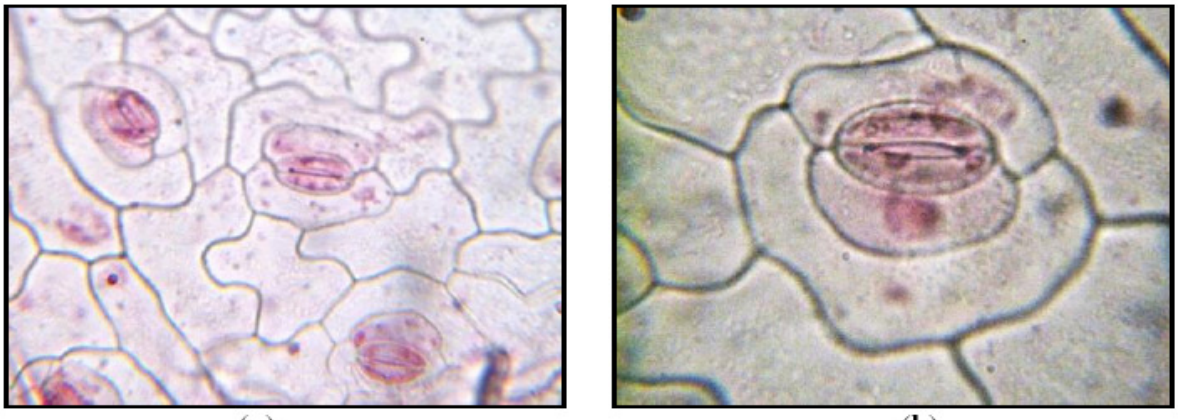
The mean number of stomata in V1 was found to be lower on the abaxial surfaces in control and treated plants. The stomata were of meso-paracytic type in nature (plate 502 a-c). The number of stomatal cells decreased with increase in Cr concentrations. The mean number of epidermal cells in different concentrations of Cr were found to vary from 571 to 759 per mm^2 on adaxial and 540 to 702 per mm^2 on abaxial surface, number of stomata vary from 270 to 326 per mm^2 on adaxial

surface and 246 to 300 per mm^2 on abaxial surface and number of subsidiary cells vary from 77 to 105 per mm^2 on adaxial leaf surface and 74 to 125 per mm^2 on abaxial leaf surfaces in V1. In V2 the mean number of epidermal cells in different concentrations of Cr were found to vary from 488 to 736 per mm^2 on adaxial and 590 to 719 per mm^2 on abaxial surface, number of stomata ranged from 271 to 349 per mm^2 on adaxial surface and 249 to 287 per mm^2 on abaxial surface and number of subsidiary cells vary from 74 to 105 per mm^2 on adaxial leaf surface and 74 to 125 per mm^2 on abaxial leaf surface.



(a) (b) (c) (d)

Plate 1. (a-d) Photographs of *Vigna angularis* showing mature normal leaf and some variations



(a) (b)

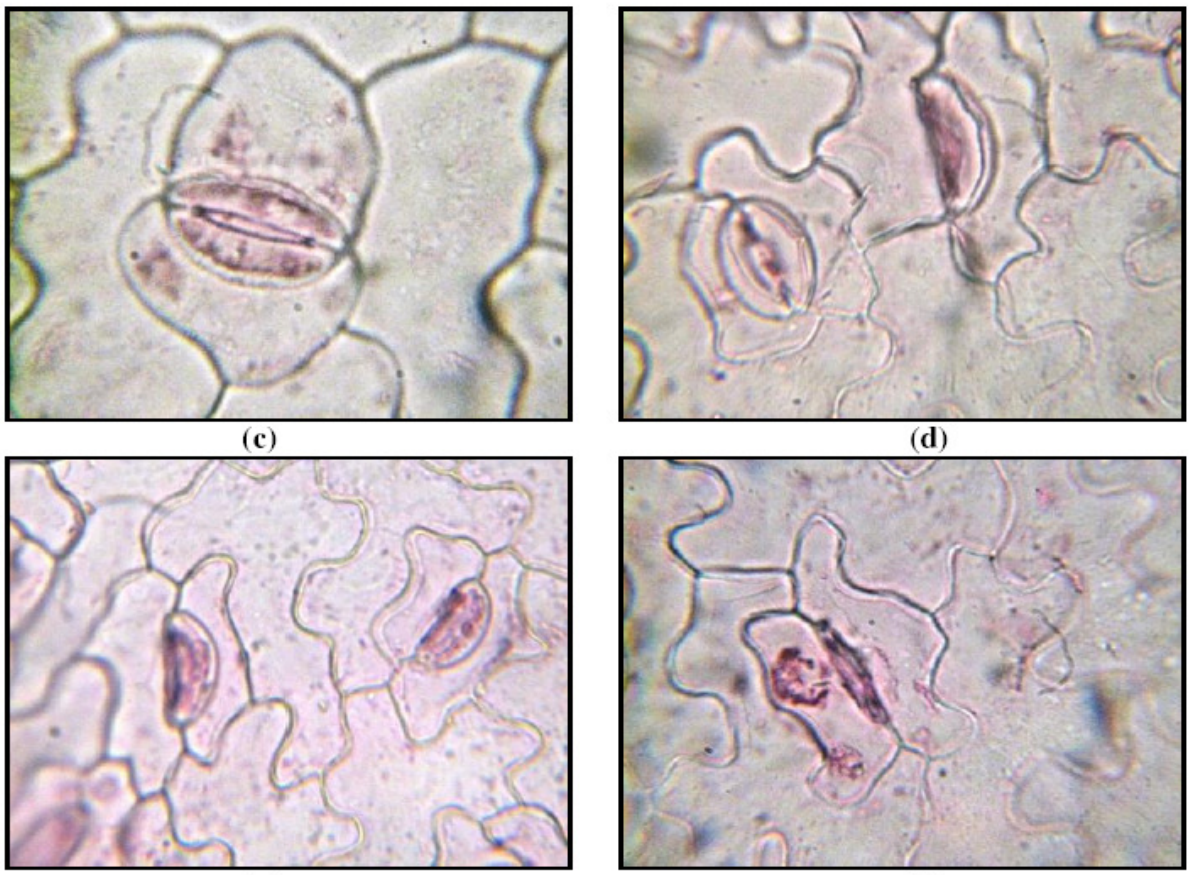


Plate 2. Microphotographs of *Vigna angularis* leaf showing:
(a) Stomata on abaxial surface (x10),
(b-c) Single stomata with subsidiary cells (x40),
(d-e) Stomata with one guard cell degenerating (x40), and
(f) Stomata with both guard cells degenerating (x40).

The mean lengths and breadth of stomata in different concentrations of Cr on adaxial and abaxial surfaces in V2 were nearly of equal size. But in V1 the abaxial leaf surface had larger stomata than on adaxial leaf surface. In V1 the mean length and breadth of stomata on adaxial leaf surface varied from 15.7 to 23.9 µm and 10.4 to 14.5 µm and on abaxial leaf surface it varied from 16.0 to 26.9 µm and 8.9 to 15.3 µm respectively. Similarly in V2, the mean length and breadth of stomata varied from 18.3 to 23.7 µm and 10.1 to 14.3 µm on adaxial leaf surface and on abaxial leaf surface it varied from 16.9 to 24.5 µm and 8.4 to 14.5 µm respectively.

The GCI was increased with increase in Cr concentrations on adaxial leaf surface in both the accessions of *Vigna* while it decreased with increase in Cr concentration on abaxial leaf surface. In treatments various anomalies like-degeneration of one guard cell (plate 2d, f), both guard cells (plate 2e) and presence of three guard cells were found on both the leaf surfaces. The distribution of various reported anomalies is shown in Table 5.

Accession	Adaxial leaf surface							Abaxial leaf surface						
	Cr Concentration	Parameter	Mean	±	SE	Range		Cr Concentration	Parameter	Mean	±	SE	Range	
V1	Control	Epi	712.68	±	12.28	661.97	- 774.65	Control	Epi	692.96	±	17.03	591.55	- 774.65
		Ns	339.44	±	9.72	309.86	- 394.37		Ns	335.21	±	13.08	267.61	- 394.37
		Nsu	83.10	±	8.52	28.17	- 126.76		Nsu	84.51	±	11.11	28.17	- 140.85
	10^{-10}M	Epi	759.15	±	9.02	718.31	- 802.82	10^{-10}M	Epi	702.82	±	17.75	619.72	- 774.65
		Ns	326.76	±	10.46	267.61	- 366.20		Ns	300.00	±	15.86	225.35	- 366.20
		Nsu	77.46	±	11.55	28.17	- 154.93		Nsu	97.18	±	11.96	56.34	- 169.01
	10^{-9}M	Epi	678.87	±	12.74	633.80	- 760.56	10^{-9}M	Epi	640.85	±	32.61	492.96	- 760.56
		Ns	318.31	±	13.64	253.52	- 380.28		Ns	308.45	±	9.02	267.61	- 352.11
		Nsu	87.32	±	13.74	28.17	- 169.01		Nsu	74.65	±	9.86	28.17	- 140.85
	10^{-8}M	Epi	657.75	±	17.57	591.55	- 732.39	10^{-8}M	Epi	540.85	±	13.48	464.79	- 591.55
		Ns	311.27	±	17.50	211.27	- 408.45		Ns	298.59	±	13.90	239.44	- 352.11
		Nsu	78.87	±	10.33	28.17	- 140.85		Nsu	107.04	±	13.31	56.34	- 169.01
	10^{-7}M	Epi	619.72	±	11.50	549.30	- 676.06	10^{-7}M	Epi	640.85	±	22.54	549.30	- 760.56
		Ns	301.41	±	13.15	225.35	- 380.28		Ns	297.18	±	9.72	253.52	- 352.11
		Nsu	84.51	±	5.94	56.34	- 112.68		Nsu	101.41	±	14.36	28.17	- 183.10
	10^{-6}M	Epi	584.51	±	11.92	521.13	- 633.80	10^{-6}M	Epi	577.46	±	12.24	521.13	- 633.80
		Ns	295.77	±	10.91	253.52	- 380.28		Ns	278.87	±	13.74	197.18	- 338.03
		Nsu	81.69	±	10.03	28.17	- 126.76		Nsu	109.86	±	12.21	56.34	- 169.01
	10^{-5}M	Epi	607.04	±	9.72	563.38	- 676.06	10^{-5}M	Epi	587.32	±	17.32	521.13	- 704.23
		Ns	315.49	±	8.96	267.61	- 352.11		Ns	290.14	±	8.19	253.52	- 338.03
		Nsu	78.87	±	11.15	28.17	- 140.85		Nsu	85.92	±	14.00	28.17	- 154.93
	10^{-4}M	Epi	628.17	±	25.21	535.21	- 774.65	10^{-4}M	Epi	566.20	±	15.11	507.04	- 647.89
		Ns	291.55	±	7.29	253.52	- 323.94		Ns	287.32	±	7.91	239.44	- 323.94
		Nsu	105.63	±	13.97	56.34	- 169.01		Nsu	116.90	±	13.62	56.34	- 183.10
	10^{-3}M	Epi	587.32	±	14.40	521.13	- 647.89	10^{-3}M	Epi	583.10	±	18.09	464.79	- 661.97
		Ns	294.37	±	6.10	267.61	- 323.94		Ns	250.70	±	11.27	197.18	- 295.77
		Nsu	95.77	±	11.27	56.34	- 169.01		Nsu	84.51	±	12.60	28.17	- 140.85
	10^{-2}M	Epi	571.83	±	9.67	535.21	- 619.72	10^{-2}M	Epi	576.06	±	19.41	450.70	- 661.97
		Ns	270.42	±	7.51	239.44	- 309.86		Ns	246.48	±	6.72	211.27	- 281.69
		Nsu	88.73	±	8.41	56.34	- 140.85		Nsu	125.35	±	13.35	56.34	- 197.18

Accession	Adaxial leaf surface								Abaxial leaf surface							
	Cr Concentration	Parameter	Mean	±	SE	Range			Cr Concentration	Parameter	Mean	±	SE	Range		
V2	Control	Epi	735.21	±	19.45	633.80	-	830.99	Control	Epi	705.63	±	21.04	591.55	-	816.90
		Ns	305.63	±	15.44	225.35	-	366.20		Ns	267.61	±	14.85	197.18	-	352.11
		Nsu	107.04	±	8.45	70.42	-	154.93		Nsu	84.51	±	11.11	28.17	-	140.85
	10^{-10}M	Epi	736.62	±	9.40	704.23	-	802.82	10^{-10}M	Epi	719.72	±	16.99	619.72	-	788.73
		Ns	277.46	+	8.15	239.44	-	323.94		Ns	273.24	+	11.54	225.35	-	338.03
		Nsu	102.82	±	9.86	56.34	-	154.93		Nsu	97.18	±	11.96	56.34	-	169.01
	10^{-9}M	Epi	681.69	±	14.88	633.80	-	788.73	10^{-9}M	Epi	659.15	±	34.61	492.96	-	774.65
		Ns	284.51	±	10.24	239.44	-	338.03		Ns	287.32	±	15.17	211.27	-	352.11
		Nsu	87.32	±	13.74	28.17	-	169.01		Nsu	74.65	±	9.86	28.17	-	140.85
	10^{-8}M	Epi	633.80	±	33.59	436.62	-	760.56	10^{-8}M	Epi	557.75	±	24.59	464.79	-	746.48
		Ns	267.61	±	13.61	197.18	-	338.03		Ns	276.06	±	13.64	225.35	-	352.11
		Nsu	78.87	±	10.33	28.17	-	140.85		Nsu	107.04	±	13.31	56.34	-	169.01
	10^{-7}M	Epi	608.45	±	18.76	549.30	-	746.48	10^{-7}M	Epi	657.75	±	21.21	549.30	-	760.56
		Ns	277.46	±	12.25	225.35	-	338.03		Ns	274.65	±	11.36	225.35	-	338.03
		Nsu	84.51	±	5.94	56.34	-	112.68		Nsu	101.41	±	14.36	28.17	-	183.10
	10^{-6}M	Epi	607.04	±	21.76	521.13	-	732.39	10^{-6}M	Epi	592.96	±	17.12	521.13	-	704.23
		Ns	294.37	±	9.49	239.44	-	338.03		Ns	259.15	±	12.81	197.18	-	323.94
		Nsu	81.69	±	10.03	28.17	-	126.76		Nsu	109.86	±	12.21	56.34	-	169.01
	10^{-5}M	Epi	618.31	±	13.52	563.38	-	704.23	10^{-5}M	Epi	600.00	±	18.80	521.13	-	704.23
		Ns	349.30	±	11.07	267.61	-	380.28		Ns	283.10	±	7.98	239.44	-	309.86
		Nsu	78.87	±	11.15	28.17	-	140.85		Nsu	85.92	±	14.00	28.17	-	154.93
	10^{-4}M	Epi	639.44	±	25.21	535.21	-	774.65	10^{-4}M	Epi	578.87	±	16.19	507.04	-	647.89
		Ns	278.87	±	9.58	239.44	-	323.94		Ns	259.15	±	11.54	197.18	-	309.86
		Nsu	105.63	+	13.97	56.34	-	169.01		Nsu	116.90	+	13.62	56.34	-	183.10
	10^{-3}M	Epi	600.00	±	16.15	521.13	-	676.06	10^{-3}M	Epi	597.18	±	12.46	549.30	-	661.97
		Ns	271.83	±	8.41	225.35	-	309.86		Ns	249.30	±	13.62	197.18	-	309.86
		Nsu	95.77	±	11.27	56.34	-	169.01		Nsu	84.51	±	12.60	28.17	-	140.85
	10^{-2}M	Epi	488.73	+	20.14	408.45	-	633.80	10^{-2}M	Epi	590.14	+	13.52	521.13	-	661.97
		Ns	295.77	±	9.62	253.52	-	338.03		Ns	249.30	±	7.58	211.27	-	281.69
		Nsu	74.65	±	8.15	28.17	-	112.68		Nsu	125.35	±	13.35	56.34	-	197.18

Table 2: Mean data related to number of epidermal cells (Epi), stomatal cells (Ns) and subsidiary cells (Nsu) on adaxial and abaxial surfaces in different accessions of *Vigna angularis*.

Accession	Adaxial leaf surface								Abaxial leaf surface							
	Cr Concentration	Parameter	Mean	±	SE	Range			Cr Concentration	Parameter	Mean	±	SE	Range		
V1	Control	L	24.93	±	0.64	21.92	-	27.40	Control	L	27.13	±	0.45	24.66	-	28.77
		B	14.93	±	0.38	13.70	-	16.44		B	15.89	±	0.37	13.70	-	17.81
	10^{-10}M	L	23.98	±	0.55	21.92	-	26.03	10^{-10}M	L	26.99	±	0.29	26.03	-	28.77
		B	14.52	±	0.30	13.70	-	16.44		B	15.34	±	0.45	13.70	-	17.81
	10^{-9}M	L	23.70	±	0.41	21.92	-	26.03	10^{-9}M	L	25.89	±	0.32	24.66	-	27.40
		B	14.11	±	0.29	12.33	-	15.07		B	14.66	±	0.29	13.70	-	16.44
	10^{-8}M	L	23.70	±	0.41	21.92	-	26.03	10^{-8}M	L	24.93	±	0.27	23.29	-	26.03
		B	14.39	±	0.23	13.70	-	15.07		B	13.56	±	0.32	12.33	-	15.07
	10^{-7}M	L	23.43	±	0.43	21.92	-	26.03	10^{-7}M	L	23.84	±	0.37	21.92	-	26.03
		B	14.39	±	0.23	13.70	-	15.07		B	13.29	±	0.29	12.33	-	15.07
	10^{-6}M	L	20.96	±	0.41	19.18	-	23.29	10^{-6}M	L	21.65	±	0.40	19.18	-	23.29
		B	13.15	±	0.22	12.33	-	13.70		B	11.92	±	0.29	10.96	-	13.70
	10^{-5}M	L	20.14	±	0.29	19.18	-	21.92	10^{-5}M	L	20.55	±	0.35	19.18	-	21.92
		B	12.88	±	0.22	12.33	-	13.70		B	11.65	±	0.23	10.96	-	12.33
	10^{-4}M	L	19.18	±	0.29	17.81	-	20.55	10^{-4}M	L	18.22	±	0.29	16.44	-	19.18
		B	12.88	±	0.22	12.33	-	13.70		B	10.28	±	0.31	9.59	-	12.33
	10^{-3}M	L	16.99	±	0.37	15.07	-	19.18	10^{-3}M	L	16.58	±	0.32	15.07	-	17.81
		B	11.51	±	0.30	9.59	-	12.33		B	9.73	±	0.25	8.22	-	10.96
	10^{-2}M	L	15.76	±	0.31	15.07	-	17.81	10^{-2}M	L	16.03	±	0.50	13.70	-	17.81
		B	10.41	±	0.30	9.59	-	12.33		B	8.91	±	0.23	8.22	-	9.59

Accession	Adaxial leaf surface								Abaxial leaf surface							
	Cr Concentration	Parameter	Mean	±	SE	Range			Cr Concentration	Parameter	Mean	±	SE	Range		
V2	Control	L	16.03	±	0.21	15.07	-	16.44	Control	L	16.17	±	0.27	15.07	-	17.81
		B	11.37	±	0.29	9.59	-	12.33		B	11.37	±	0.29	9.59	-	12.33
	10^{-10}M	L	15.76	±	0.23	15.07	-	16.44	10^{-10}M	L	15.89	±	0.22	15.07	-	16.44
		B	11.51	±	0.30	9.59	-	12.33		B	11.51	±	0.30	9.59	-	12.33
	10^{-9}M	L	15.76	±	0.23	15.07	-	16.44	10^{-9}M	L	15.76	±	0.23	15.07	-	16.44
		B	10.96	±	0.35	9.59	-	12.33		B	10.96	±	0.35	9.59	-	12.33
	10^{-8}M	L	14.11	±	0.29	12.33	-	15.07	10^{-8}M	L	14.52	±	0.22	13.70	-	15.07
		B	10.55	±	0.29	9.59	-	12.33		B	10.55	±	0.29	9.59	-	12.33
	10^{-7}M	L	13.97	±	0.27	12.33	-	15.07	10^{-7}M	L	14.11	±	0.21	13.70	-	15.07
		B	10.55	±	0.29	9.59	-	12.33		B	10.55	±	0.29	9.59	-	12.33
	10^{-6}M	L	13.77	±	0.24	13.02	-	15.07	10^{-6}M	L	13.84	±	0.30	12.33	-	15.07
		B	9.73	±	0.22	8.91	-	10.96		B	10.07	±	0.21	9.59	-	10.96
	10^{-5}M	L	13.15	±	0.20	12.33	-	13.70	10^{-5}M	L	12.88	±	0.20	12.33	-	13.70
		B	8.97	±	0.22	8.22	-	9.59		B	9.45	±	0.09	8.91	-	9.59
	10^{-4}M	L	11.44	±	0.21	10.96	-	12.33	10^{-4}M	L	11.71	±	0.24	10.96	-	13.02
		B	7.40	±	0.20	6.85	-	8.22		B	8.70	±	0.18	8.22	-	9.59
	10^{-3}M	L	10.69	±	0.29	9.59	-	12.33	10^{-3}M	L	11.51	±	0.20	10.96	-	12.33
		B	7.26	±	0.23	6.17	-	8.22		B	8.08	±	0.30	6.85	-	9.59

Table 3: Mean data related to length (L) and breadth (B) of stomata cells in µm on adaxial and abaxial leaf surfaces in different accessions of *Vigna angularis*.

Accession	Cr Concentration	Adaxial surface						Abaxial surface					
		Mean	±	SE	Range			Mean	±	SE	Range		
V1	Control	46.01	±	0.62	41.90	-	52.10	47.90	±	1.22	39.58	-	55.10
	10^{-10}M	45.98	±	1.17	36.19	-	51.40	47.10	±	1.06	37.25	-	54.25
	10^{-9}M	45.22	±	1.11	40.91	-	50.50	46.80	±	1.42	38.46	-	53.93
	10^{-8}M	44.95	±	1.68	34.09	-	53.21	45.75	±	1.44	40.91	-	53.76
	10^{-7}M	44.05	±	1.32	40.00	-	55.10	44.90	±	1.57	37.50	-	54.95
	10^{-6}M	43.10	±	1.01	41.76	-	53.47	43.80	±	1.45	38.20	-	52.27
	10^{-5}M	42.80	±	0.99	42.22	-	52.27	43.20	±	0.61	43.18	-	48.98
	10^{-4}M	42.05	±	0.82	39.56	-	49.50	42.85	±	1.00	37.78	-	48.72
	10^{-3}M	41.20	±	1.11	37.45	-	51.69	41.95	±	1.80	36.14	-	53.33
	10^{-2}M	40.00	±	1.00	38.11	-	50.57	41.20	±	1.33	34.88	-	48.10
V2	Control	42.65	±	1.47	35.85	-	49.02	44.85	±	1.80	32.56	-	50.00
	10^{-10}M	41.20	±	0.94	35.29	-	45.10	43.80	±	1.30	34.04	-	46.60
	10^{-9}M	39.90	±	1.00	36.17	-	48.48	43.10	±	1.89	31.91	-	51.76
	10^{-8}M	38.60	±	2.06	34.09	-	54.55	42.50	±	1.71	37.36	-	53.76
	10^{-7}M	38.20	±	1.18	37.89	-	50.00	42.15	±	1.46	35.56	-	49.41
	10^{-6}M	36.50	±	1.35	37.78	-	50.55	41.40	±	1.77	34.15	-	52.27
	10^{-5}M	36.20	±	1.45	40.43	-	55.32	40.70	±	0.80	40.48	-	48.89
	10^{-4}M	35.00	±	1.02	35.79	-	47.31	38.30	±	1.54	34.15	-	48.72
	10^{-3}M	33.00	±	0.55	41.67	-	46.91	36.00	±	1.70	34.11	-	50.57
	10^{-2}M	31.20	±	0.81	47.62	-	55.00	35.14	±	1.14	33.48	-	45.57

Table 5. Data related to frequency of various stomatal anomalies in different accessions of *Vigna angularis*.

Accession	Cr concentration	Adaxial Surface			Adaxial Surface		
		Type			Type		
		I	II	III	I	II	III
V1	Control	1.08	0.00	0.00	1.19	0.00	0.00
	10^{-10}M	0.56	0.00	0.56	0.62	1.30	0.00
	10^{-9}M	1.15	0.00	0.00	1.27	0.00	0.00
	10^{-8}M	0.85	0.00	0.00	0.67	0.70	0.00
	10^{-7}M	1.66	0.63	0.00	1.78	0.68	0.00
	10^{-6}M	0.59	1.22	0.00	0.00	1.18	0.59
	10^{-5}M	0.55	1.15	0.00	0.51	1.05	0.00
	10^{-4}M	1.14	0.62	0.57	1.24	0.00	0.62
	10^{-3}M	1.70	0.64	0.00	1.21	0.66	0.60
	10^{-2}M	1.21	0.66	0.61	1.69	0.00	0.56
V2	Control	0.55	0.57	0.00	1.34	0.00	0.00
	10^{-10}M	0.60	0.63	0.00	0.66	0.69	0.00
	10^{-9}M	0.57	1.18	0.00	0.60	1.26	0.00
	10^{-8}M	1.21	0.00	0.00	1.30	0.00	0.00
	10^{-7}M	1.17	0.64	0.00	1.25	0.69	0.00
	10^{-6}M	0.00	0.65	0.65	0.00	1.38	0.00
	10^{-5}M	0.60	1.25	0.00	0.61	0.00	1.22
	10^{-4}M	1.15	0.63	0.58	0.00	1.97	0.00
	10^{-3}M	0.00	1.35	0.68	0.68	0.71	0.68
	10^{-2}M	1.37	0.00	0.69	0.68	0.00	1.36

I = stomata with one degenerating guard cell
II = stomata with both degenerating guard cells
III = stomata with three guard cells

Table 4. Guard cell index (GCI) in different accessions of *Vigna angularis*.

Leaves occupy maximum portion of the aerial component of the terrestrial plants. The stomata present on leaves are inlets and outlets for gases and water vapours. Cr phyto-toxicity has been considered to be inhibitory for plant growth. Growth changes are the first most obvious reactions of plants under stress. Cr in high doses causes metabolic disorders. The overall negative influence of heavy metal on plant resulted in the retardation of normal growth (Küpper *et al*., 1996) with abnormalities.

In the present investigation significant decrease in epidermal cells, stomatal cells, subsidiary cells and length and breadth was found in treated over control plants both in *Vigna angularis* .The degeneration of guard cells may be due to disturbance in normal functioning of epidermal cells as consequences of heavy metal toxicity because heavy metals disrupt the metabolic pathways in plants. Excessive level of Cr adversely affects the leaf development and other morphological characters due to alteration in biomolecules level of cells. It also interferes with the activities of many key enzyme related to normal metabolic and developmental processes (Parmer *et al*., 2005; Jayakumar *et al*., 2008; Ogundiran, 2007).

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