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PREFACE

*We are delighted to publish our book entitled "**Ecology Research (Volume I)".** This book is the compilation of esteemed articles of acknowledged experts in the fields of ecology providing a sufficient depth of the subject to satisfy the need of a level which will be comprehensive and interesting. It is an assemblage of variety of information about advances and developments in ecology. With its application oriented and interdisciplinary approach, we hope that the students, teachers, researchers, scientists and policy makers will find this book much more useful.*

The articles in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. We thank our publisher Bhumi Publishing, India for compilation of such nice data in the form of this book.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

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BIOCHEMICAL GENETIC MARKERS FOR MONITORING AQUATIC GENOTOXICITY AND ENVIRONMENTAL MANAGEMENT: SINCE FIVE DECADES

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Abstract:

Application of biochemical genetic markers in their molecular forms i.e. - allozymes and isozymes, has played decisive role to resolve taxonomic and population status of many organisms including fishes. This is also significant for monitoring of fish environment. More than half of the known enzymes occur as isozymes which are used to analyze genotypes of organisms and are not influenced by environmental factors. These are established as biochemical genetic tools in aquaculture since 1960s providing various criteria to clarify taxonomic status of species; evolutionary relationships of populations, species and higher taxa and also in genetic toxicology studies which have been proved as quite significant for fish environmental monitoring. This article aims to present an extensive review of utilities of some of these isozymes like esterase (EST), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G-6-PDH) and super oxide dismutase (SOD) with special reference to fish, fisheries as well as their significance to fish ecology with special reference to genotoxicology.

Keywords: Ecology; Electrophoresis; Fisheries; Isozymes; Toxicology

Introduction:

Genetic variations in a species are key materials for applied biology where biochemical genetics has significant contributions in consistence. Observed phenotypic variations in various organisms including fishes could be explained based on population structuring revealed by biochemical markers since their inception. These markers are used for study of genetics and molecular biology where the product(s) of a gene rather than the DNA sequence is examined for enzyme activity. Allozymes represent enzymes from different alleles of the samegene where as isozymes are enzymes from different genes that process or catalyse the same reaction. Isoenzymes are multiple forms of enzymes that differ in amino acid sequence but catalyze the same chemical reactions. Their genes are expressed

in co-dominant pattern which help to analyze the genotype and are not influenced by the environmental factors. The ease with which Mendelian variants could be detected by electrophoresis resulted in proliferation of descriptive studies of proteins in many organisms where fishes are not exceptions. These markers are extensively used in fisheries since 1970s. These enzymes are expressed by distinct gene loci with high degree of genetic variability involved in important physiological processes, reproduction, developmental stages, nervous impulse control, xenobiotics tolerance and detoxification.

Electrophoretically detectable loci were reported in natural populations of brown trout (*Salmo trutta* L.) and the expression of the loci coding these enzymes were described and interpreted to serve as a basis for detailed examination of genetic variation in their population (Allendorff *et al.*, 1977). As per Ferguson (1980), genetic markers like proteins and enzymes pertinent to allozymes and isoenzymes have played decisive role in determining the taxonomic and population status of many organisms. Whitt (1987) mentioned the significance of isozyme analysis in species differentiation and analysis of systematic and evolutionary trends. Utter *et al.* (1989) studied isozymes for fish genetics by interpreting genetic variation through electrophoresis where as Utter (1991) reported development and applications of biochemical genetic tools to solve problems of fishery management.

The technique of isozyme/allozyme analysis is suitable for population studies as it is a fairly rapid procedure to perform on a large scale and a huge number of loci dispersed throughout the genome can be screened simultaneously (Park and Moran, 1994). Analysis of isozymes/allozymes is used for discrimination of species and clarification of taxonomic status in many taxa (Rossi *et al.*, 1998; Gusmao *et al.*, 2000 and Manchenko *et al.* 2000). According to Di Giulio and Meyer (2008) some of the isozyme forms the most important enzyme systems for detoxification of reactive oxygen species in all organisms which include superoxide dismutase (SOD), catalase (CAT) and glucose 6-phosphate dehydrogenase (G6PDH). This article aims to present an extensive review of utilities of isozymes like esterase (EST, EC: 3.1.1.), lactate dehydrogenase (LDH, EC: 1.1.1.27), malate dehydrogenase (MDH, EC: 1.1.1.37), glucose-6-phosphate dehydrogenase (G6PDH, EC: 1.1.1.49) and super oxide dismutase (SOD, EC: 1.15.1.1.) with special reference to fish genetics and management of environmental toxicology in aquaculture.

Isozymes in Fishes:

With reference to use of isozymes as biochemical markers in fishes, several investigations since 1970s have been concerned with the characterization of tissue and

organ specific isoenzyme patterns (Seimiya *et al.*, 1997; Li *et al.*, 2001 and Shahjahan *et al.*, 2008). Kurutas *et al.* (2009) studied aquatic levels of pollution indicator parameters and observed their effects on various oxidative stress biomarkers in gill and liver tissues of spotted barb (*Capoeta barroisi* Lortet, 1894) analyzing superoxide dismutase (SOD) and glucose-6-phosphate dehydrogenase (G6PD). Kurutas *et al.* (2009) informed that, the SOD-catalase (SOD-CAT) system provides the first defense against oxygen toxicity. Osman *et al.* (2010) investigated the alteration in the activity of Glucose-6-Phosphate Dehydrogenase (G6PDH) and Lactate Dehydrogenase (LDH)] and the histological changes on liver and gills of the African catfish *Clarias gariepinus* and concluded that the altered activities of G6PDH and LDH could be useful biomarkers of water pollution.

1. Esterase (EST, EC: 3.1.1.):

Arunachalam *et al.* (1985) electrophoretically analysed the liver and muscle of *Channa Punctatus* subjected to maximum sub-lethal concentration of carbaryl and reported only three fractions of esterase. According to Berrebi *et al.* (1990), fish esterases are among the most difficult enzymes using starch gel electrophoresis. Several toxicity tests on different agrochemicals employing isozymes were done on different species of *Puntius* (Gill *et al.* 1990). Esterases are the lipid hydrolyzing enzymes which split into an acid and an alcohol in a chemical reaction with water involving the hydrolysis of ester having a great significance in the field of genetics and toxicology (Callaghan *et al.*, 1994). Esterases are reported to be involved in functioning of nervous system and development of resistance to insecticides (Karunaratne *et al.,* 1999), which may be used as bio-indicators to monitor pollutants in the environment (Vanda *et al.,* 2003). These enzymes have various industrial applications, in food, detergent, fine chemical, waste water treatments, biodiesel production and pharmaceutical industries and in bio-remediation (Sharma *et al.*, 2001, Bornscheuer *et al.,* 2002, Reetz 2002, Cammarota and Freire, 2006; Hasan *et al.,* 2006). Expression of tissue specific esterase isozymes shows differential banding pattern. It is used in toxicological study as well as for the development of molecular markers. Ghazala *et al.* (2016) evaluated the effect of commercial formulation of triazophos on esterase activity in the liver, kidney, brain, blood and muscles of *Catla catla, Labeo rohita* and *Cirrhinus mrigala* fingerlings. Choudhurya *et al.* (2017) assessed antioxidant biomarkers and protein levels in tissues of *Oreochromis mossambicus* and *Channa punctatus* exposed to toxicity by fungicides using antioxidant enzymes like lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and peroxidase (Pox) as biomarkers. Tripathy and Sarangi (2014, 2018) reported esterases profile in backcrosses of *catla* and *rohu* and significance of esterases in fishes as biochemical markers in genetics and toxicological studies.

2. Lactate Dehydrogenase (LDH, EC: 1.1.1.27):

Lactate dehydrogenase is one of the chief enzyme of carbohydrate metabolism which catalyses the oxidation of lactate and reduction of pyruvate during anaerobic glycolysis. Lactate dehydrogenase (LDH, lactate; NAD-oxidoredutase, EC 1.1.1.27) is among the most extensively studied glycolytic enzyme. This cytoplasmic enzyme is widely used as marker of organ or tissue lesions in toxicology and in clinical chemistry (Ramesh *et al.*, 1993; Das *et al.,* 2004 a, b). It is the terminal enzyme of anaerobic glycolysis located in the cellular cytoplasm used for estimating anaerobic capacity which commonly reflects the metabolic capacity of tissues after long term exposure to contaminated water bodies (Pelletier *et al.*, 1994, Gagnon and Holdway, 1999). In most vertebrates, LDH is encoded by two gene loci, Ldh-A and Ldh-B which synthesize two subunits, A and B or M and H (Heinova and Blahovec, 1994; Tsoi and Li, 1994, Abdelmordy, 1999). It functions as link between the protein and carbohydrate metabolism and serves as indicators of stress (Abhijith *et al.* 2016). Manna and Chakraborty (2012) reported LDH of some selected major carps of Calcutta by cellulose acetate electrophoresis to assess the effect of pollutants and reported that, the profile for all tissues of three fish species of Indian major carps like *Catla catla, Labeo rohita* and *Cirrhinus mrigala* were consistently similar with little variation in the expression levels of muscle LDH of mrigala and kidney LDH of rohu. LDH in the serum of *Cirrhinus mrigala* increased significantly following the treatment of sub-lethal dose of methyl parathion (Ray and Sinha, 2014). Ray and Sinha (2016) reported increase in LDH activity with increased anaerobic conditions under the influence of methyl parathion to meet the energy demand in *Labeo rohita*.

3. Malate Dehydrogenase (MDH, EC:1.1.1.37):

MDH is an enzyme that reversibly catalyzes the oxidation of malate to oxaloacetate using the reduction of NAD^+ to NADH. This reaction is part of many metabolic pathways, including the citric acid cycle. Malate dehydrogenase catalyzes the reversible oxidation of malate to oxalacetate requiring NAD+ as a cofactor. It is involved in gluconeogenesis, lipogenesis and in the malate-aspartate shuttle during aerobic glycolysis. Sullivan and Somero (1980) reported much higher and more variable activity of MDH relative to citrate synthetase suggests that, in addition to its function in the citric acid cycle, MDH may play an important role in redox balance in fish white muscle. It is a Kreb's cycle enzyme and a reduction in its activity is an indicator of reduced metabolic activity (Yengkokpam *et al.*, 2013). Tiwari and Singh (2009) observed decrease in MDH values in tissues of *Clarias batrachus* on exposure to endosulfan. Afsar *et al.* (2012) analyzed MDH activity in *Anabas testudineus* recovered from lead intoxication and reported that, it acts as the energy

supplier in metabolic pathways and biochemical reactions under extreme stress conditions. Exposure to fungicides and insecticides caused decline in MDH in all fishes (Chauadhurya *et al.*, 2017). Fucoidan significantly reduced malate dehydrogenase activity in *Labeo rohita* liver tissue (Gora *et al.*, 2018).

4. Glucose-6-phosphate-dehydrogenase (G6PDH, EC: EC: 1.1.1.49)

G6PDH is the first and key enzyme of the pentose phosphate pathway which catalyzes the first reaction of the PPP and whose main physiological function is to produce NADPH used in lipid biosynthesis and in the protection of the cell from oxidative stress and 5-phosphoribosyl pyrophosphate used in RNA synthesis. It has long been recognized as an antioxidant enzyme (Nogae and Johnston 1990; Pandolfi *et al.*, 1995; Reiter *et al.*, 1997, Salvemini *et al.,* 1999) and is relevant as markers for carcinogenesis in mammals and pollution induced carcinogenesis in fish. Walzem *et al.* (1991) reported that, G6PDH showed preferential increases in its activity as food intake increased. Erdouan *et al.* (2004) investigated inhibitory effects of some antibiotics on glucose 6-phosphate dehydrogenase from the erythrocytes of rainbow trout.

5. Superoxide Dismutase (SOD, EC: 1.15.1.1.)

Superoxide is produced as a by product of oxygen metabolism and its uncontrolled production causes many types of cell damages. Superoxide dismutase (SOD) alternately catalyzes the dismutation of superoxide ($O2^{-}$) radical into less damaging species like molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). SOD is one of the sub cellular radical scavenging enzyme which clears superoxide ($O2^{-}$) radical to delay the process of cellular aging and apoptosis. Alteration of SOD activities were reported by various authors in different species of fishes due to exposure to various chemicals such as Dimitrova *et al.*, (1994) in *Cyprinus carpio* after exposure to zinc and lead, Farombi and Adelowo (2008) on *Clarias gariepinus* treated with butachlor, Stara *et al.* (2012) in common carp *Cyprinus carpio* treated with simazine, Hemalatha *et al.* (2015) in freshwater fish *Cyprinus carpio* treated with Quinalphos and Desai and Bhilave (2018) in *Cirrhinus mrigala* subjected to acute and chronic exposure to Methanol. Zikic *et al.* (2001) reported activities of superoxide dismutase and catalase in erythrocytes and plasma transaminases of goldfish exposed to cadmium. According to Droge (2002), superoxide dismutase (SOD) and catalase (CAT) are powerful antioxidant enzymes in fishes which are widely distributed among different tissues and it greatly accelerates the conversion of $O2^{-}$ to water and H_2O_2 and the efficacy of SOD relies on its co-operation with catalase (CAT) and glutathione peroxidase (GPx) as per Parihar *et al.* (1997) and Pandey *et al.* (2003). Vutukuru *et al.* (2006) reported acute effects of copper on superoxide dismutase in *Esomus danricus.* Jordanoska *et al.* (2008) studied

antioxidant enzyme activities of *Barbus petenyi* Heck to establish possible environmental impact of toxic effect on anthropogenic pollution on Lake Ohrid by measuring SOD activity by ferricytochrome c method using xantine/xantine oxidase as source of superoxide radicals. Campa-Cordova *et al.* (2009) reported SOD activity in juvenile *Litopenaeus vannamei* and *Nodipecten subnodosus* exposed to the toxic dinoflagellate. Desai and Bhilave (2018) reported toxicological studies of methanol on superoxide dismutase (SOD) activity of *Cirrhinus mrigala.* As per Gora *et al.* (2018), the liver and gill superoxide dismutase activity was significantly reduced in the fucoidan fed groups of *Labeo rohita*. Pichardo *et al.* (2018) reported changes of superoxide dismutase in tilapia exposed to extracts of microcystin containing cyanobacteria.

Conclusion:

The review of literature signifies importance of isozymes in many fields of fishery research including systematics, phylogeny, genetics, physiology, toxicology and many more related to fish ecology although they have little disadvantages such as representing only the expressible parts of the genome and their pattern cannot detect point mutations hence do not reveal all genetic variations. Handling of isozymes/allozymes during in vitro analysis needs more accurate, experienced, expert and skilled workers as these are highly perishable after their extraction due to their protein nature. In spite of all these pros and cons of isozyme analysis, it proves as a suitable and strong tool like any other molecular marker tool in fishery management and fish genetics. Isozyme/allozyme analysis in form of biochemical markers are till existing as significant tool in fishery research which is evidenced from hundreds of upcoming researches, analysis, reports and investigations throughout the globe reported day to day. These tools have touched several aspects of biological researches including microbes to vertebrates including fishes where ecology, genotoxicology and analysis of other environmental parameters are also correlated.

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NO VEHICLE DAY - A WISE STEP TO REDUCE AIR POLLUTION: A CASE STUDY IN S. R. T. M. UNIVERSITY'S CAMPUS

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Abstract:

Air pollution is one of the major environmental affairs since bioaerosols and many other air pollutants are causing sever adverse effects on public health such as cancer, cardiovascular diseases and high mortality rates in human. Bioaerosols are crucial indicators of air pollution that contained a mixture of different components, including bacteria, fungi and virus etc. Therefore everyone needs to become more conscious about the effects of all types of pollution. In this context, employees and visitors of Swami Ramanand Teerth Marathwada University, Nanded do perceive 'no vehicle day' on every Thursday. This campaign is an idea of Hon. Vice-Chancellor Udhav V. Bhosle. In this regard we got an idea to perform a case study on enumeration of one of the component of bioaerosol i.e. bacteria on 'no vehicle day' within the campus. In present study, nutrient agar plates were exposed to the air of Nalanda gate and School of social science sites of university campus. Encouraging results were recorded that showed and confirmed 75.85% average reduction in bacterial components of bioaerosol on 'non vehicle day'. In conclusion, taking a cue from the university's initiative, 'no vehicle day' should be perceived in all educational institutions that would be a positive step towards maintaining Green Earth.

Keywords: air pollution, bioaerosol, no vehicle day, respiratory diseases, SRTM University

Introduction:

Air pollution has become worse over the past several years and for this situation many factors contribute that include vehicle exhaust fumes, fossil fuel-based power plants, exhaust from industrial plants and factories, construction and agricultural activities, household activities and some other natural causes such as cyclones, volcanoes and storms

(Goyet *et al.,* 2006). Hence human beings are now more conscious about the extent of not only air but all types of pollution and their severe effects are increasing day-by-day. Everyone is now aware about the problems of polluted air that can cause difficulty in breathing, flare-ups of allergy or asthma, and other lung problems. Long-term exposure in polluted air can raise the risk of heart diseases and cancer too (Kim *et al.,* 2018). The outbreak of various diseases due to air, water and soil pollution has created the risk for the well survival of plants, animals and human beings (Shao *et al.,* 2020).

In present investigation, we selected two sites from the campus of Swami Ramanand Teerth Marathwada University, Nanded. There are two big and crowded hospitals around this university namely 'Shakarrao Chavan government hospital and medical college' and 'Nanded rural dental college and research center'. We know that hospital surrounding environment is always contaminated with human pathogens like Staphylococci and many other species. Under these circumstances there is high possibility of bearing a medical burden attributes in bioaerosol of the university's campus environment. By considering all afore-mentioned critical affairs about environmental safety, Dr. Udhav V. Bhosle, Hon. Vice-Chancellor of this university decided to campaign against air and noise pollution and put forth a step towards maintaining Green Earth. In this regard, in the meeting of management council held on 31st Dec. 2019, it was decided to perceive 'no vehicle day' on every Thursday in the university campus (595 acres area) and at university sub-centres Latur (22 acres area) and Parbhani along with the university-managed degree college at Hingoli. This was carried into effect from the first day of New Year i.e. 1st January, 2020 (SRTM, 2019).

In this context we got an opportunity for comparative evaluation of microbial load in low dust environment. The dust particles are one of the carriers of microbes and there is direct relationship between occurrence of dust in air and presence of microbial load. Therefore our study was aimed at assessment of bacterial component of bioaerosol on 'no vehicle day' within the campus of S.R.T.M. University, Nanded.

Materials and methods:

Selection of study area:

The area of Nalanda gate and school of social sciences of SRTM University campus was selected for study of microbial load on 'no vehicle day' and 'normal day'. These are the two main entry points from which one can enter in the university campus. Therefore we decided assessment of number of bacterial components in bioaerosol of these areas.

Preparation of nutrient agar plates:

We selected a general purpose medium nutrient agar since it supports luxurious growth of a wide range of bacteria. Composition of nutrient agar was 5 g peptone, 3 g beef or yeast extract, 5 g sodium chloride, 25 g agar powder and 1 L distilled water. These ingredients were mixed and then sterilized by autoclaving, at 121^0C for 15 minutes. Then the medium was cooled down to around 47-50^0C and poured into sterilized Petri plates which were covered by the lid immediately in aseptic condition. The plates were left over on the surface of table overnight for sterility testing and then used in further part of the experiment (Aneja, 2007, Pathak and Rathod, 2014, 2015, Rathod and Pathak, 2014, 2016).

Enumeration of bacterial colonies:

The nutrient agar plates were exposed for 10 seconds in the air of selected study area sites at 10:00 a.m. from 13th Jan. 2020 to 17th Feb. 2020. The selected time was the peak of rush for entry of employments and visitors. The exposed plates were incubated at 37^oC in a bacteriological incubator (Kumar make, Mumbai) for 24 hr. (Aneja, 2007). Number of bacterial colonies appeared on normal day was assumed as 100% and percent occurrence of bacterial colonies on every 'no vehicle day' was calculated. Further, Percent Reduction was calculated by subtracting percent occurrence of bacterial colonies from 100. For this, 0% reduction in bacterial component of bioaerosol was assumed on 'normal day'.

Results:

Enumeration of microorganisms:

The data for enumeration of bacterial component of bioaerosol have been presented in Table 1 and Fig. 1. Selected photographs of incubated nutrient agar plates after exposure at selected study sites are shown in Fig. 2. Percent reduction in bacterial component of bioaerosol from Nalanda gate and school of social sciences study sites of SRTMUN are shown in Fig. 3 and Fig. 4 respectively. Maximum 72 and 79.7% reduction in bacterial colonies were recorded on 4th attempt of 'no vehicle day' at study sites at Nalanda gate and school of social science of SRTMUN respectively. Percent Reduction and percent occurrence of bacterial colonies from Nalanda gate and school of social sciences of SRTMUN on 4th attempt of 'no vehicle day' are shown in Fig. 5 and Fig. 6 respectively. There is holiday for administrative employees on every 1st and 3rd Saturday. Hence reduced microbial load was observed on Saturday. These findings discriminates the occurrence of microbial load on 'no vehicle day' and 'normal day'.

Table 1: Enumeration of bacterial components of bioaerosol appeared on nutrient agar plates when exposed on 'no vehicle day' and 'normal day' at selected study area site

Type of day	No. of bacterial components of bioaerosol from Nalanda gate (%)	Reduction percentage of bacterial components of bioaerosol from Nalanda gate	No. of bacterial components of bioaerosol from school of social science (%)	Reduction percentage of bacterial components of bioaerosol from school of social science
Normal day	Assumed 100	Assumed 00	Assumed 100	Assumed 00
1st attempt of no vehicle day	36	64	55.1	44.9
Saturday (Low rush)	80	20	46.4	53.6
2nd attempt of no vehicle day	32	68	27.55	72.45
3rd attempt of no vehicle day	40	60	23.2	76.8
4th attempt of no vehicle day	28	**72**	20.3	**79.7**
5th attempt of no vehicle day	36	64	27.55	72.45

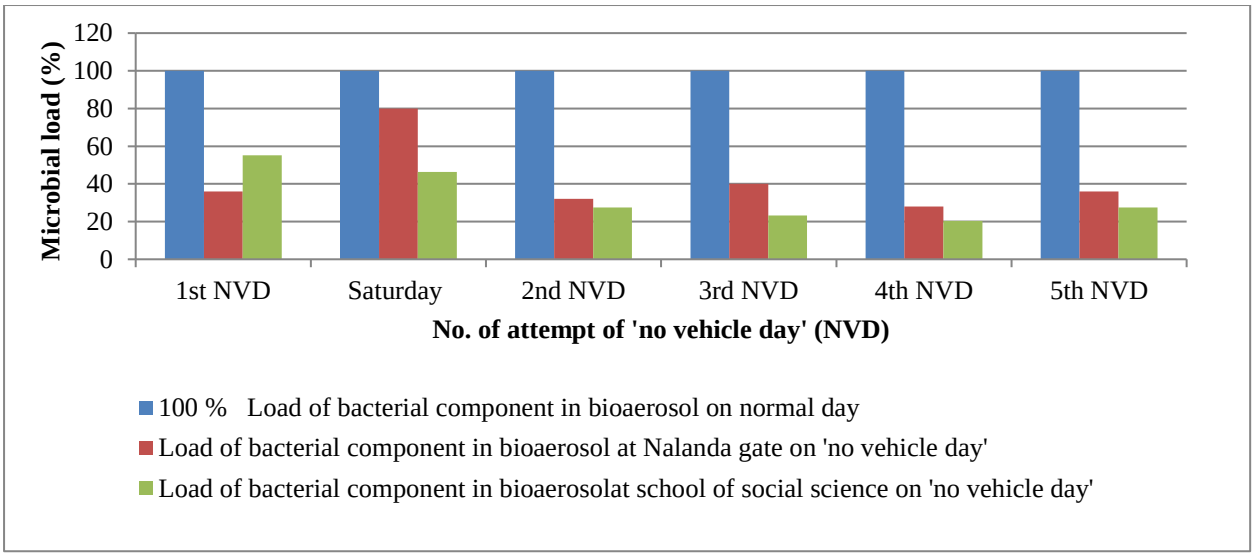

Figure 1: Enumeration of bacterial components of bioaerosol on 'no vehicle day' and 'normal day'

A B C

Figure 2: Bacterial colonies on nutrient agar plates appeared after exposure in selected study area sites (A: Exposure at normal day, B and C: Exposure at 'no vehicle day')

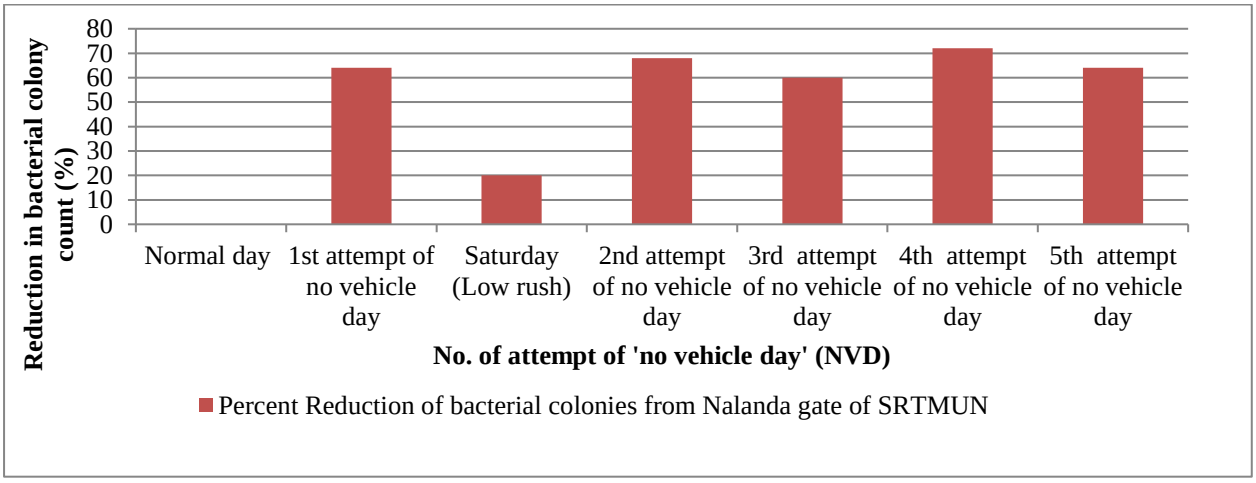

Figure 3: Reduction in bacterial component of bioaerosol from Nalanda gate study site of SRTMUN

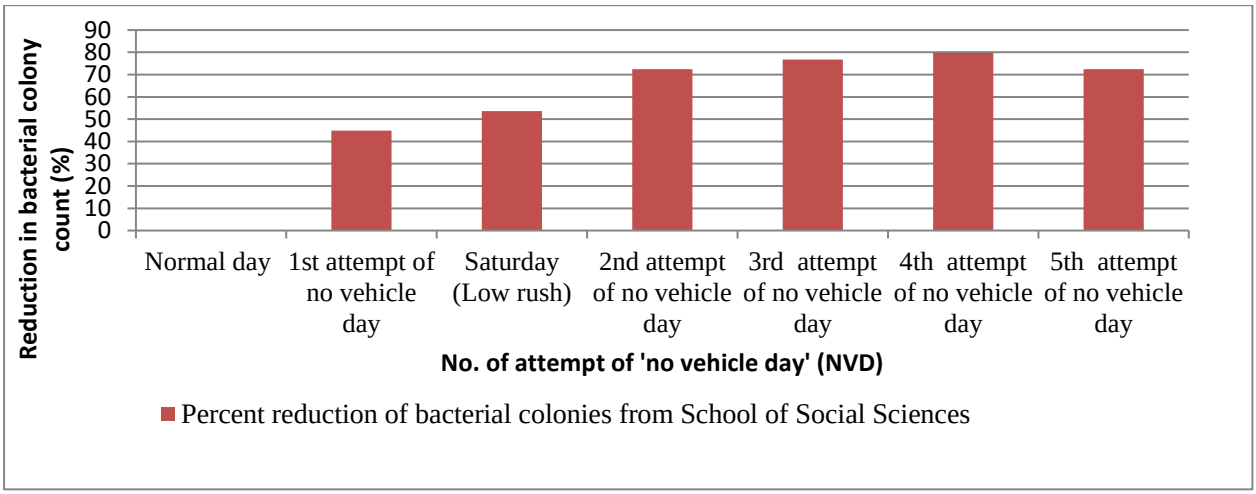

Figure 4: Reduction in bacterial component of bioaerosol from School of social sciences study site of SRTMUN

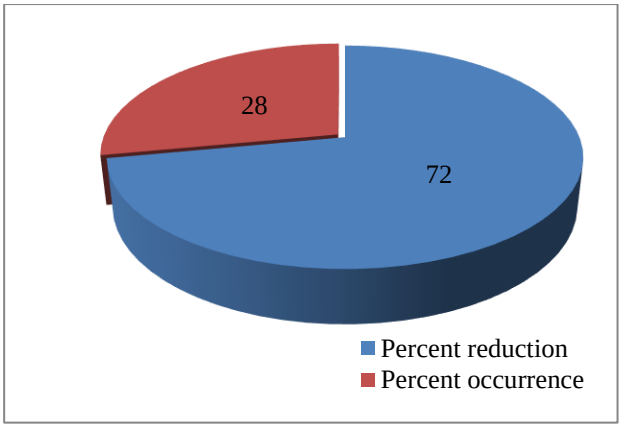

Figure 5: Percent Reduction and occurrence of bacterial colonies from Nalanda gate of SRTMUN on 4th attempt of 'no vehicle day'

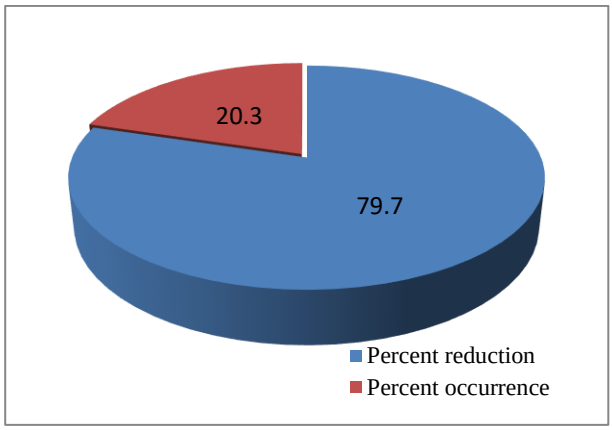

Figure 6: Percent Reduction and occurrence of bacterial colonies from School of social sciences of SRTMUN on 4th attempt of 'no vehicle day'

Discussion:

Jiayu *et al.* (2019) reviewed on microbiology community structure in bioaerosols and very precisely explained it's components i.e. viruses (enteric viruses, including Noro-and rotaviruses, and some respiratory virus such as influenza and coronaviruses), bacteria (staphylococci, legionellae, tuberculous and non-tuberculous), bacterial spore formers (*Clostridium difficile* and *Bacillus anthracis*), non spore formers (*Micrococcus luteus*, *Staphylococcus epidermidis*, *Streptococcus* sp., *Diphtheroid* sp., *Micrococcus roseus*, *Propionibacterium* sp., *Corynebacterium* sp., *Lactobacillus* sp. and *Pseudomonas* sp) and fungi (*Aspergillus* sp., *Penicillium* sp., and *Cladosporium* sp. and *Stachybotrys chartarum*). Moreover, they have clarified relevant risk of bioaerosol to public health due to respiratory

diseases including coughing, runny nose, irritated eyes or throat, allergic rhinitis, aggravation of asthma and fatigue as well as some infectious diseases such as tuberculosis and Legionnaire's Disease.

Bacteria are the major community structure of bioaerosol and this reduced bacterial burden will protect to staff and students of campus from the diseases such as Q fever, pulmonary tuberculosis, Legionnaire's diseases, Pontiac fever, asthma, allergic rhinitis, bronchitis, atypical conjunctivitis, organic dust toxic syndrome, hypersensitivity pneumonitis, mucous membrane irritations, and chronic obstructive pulmonary disease. Reduced bacterial load due to 'no vehicle policy' will protect university's employees from afore-mentioned diseases. Reduced air pollution will help to flourish diverse flora and fauna of this area.

Conclusions:

In conclusion, all the bacterial colonies appeared on nutrient agar plates were obvious pathogens as experiments were performed at 37°C. Remarkable reduction i.e. in average 75.85% in bacterial components of bioaerosol was recorded on 'no vehicle day' in comparison with 'normal day' at selected study sites. The reduced dust containing environment could favor for decrease in pathogenic bacteria community structure of bioaerosol.

Acknowledgement:

We are extremely thankful to Dr. Udhav V. Bhosle (Hon. Vice Chancellor, SRTMUN) for implementing 'no vehicle day' activity in the campus.

Conflict of interest: The authors declare there is no conflict of interest.

Author Contributions: As per the sequence in which names of authors appeared.

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POTENTIAL AND PRACTICAL APPLICATIONS OF SOMATIC EMBRYOGENESIS

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Abstract:

Somatic embryogenesis has a potential application in plant improvement. Since both the growth of embryogenic cells and subsequent development of somatic embryos can be carried out in a liquid medium, it is possible to combine somatic embryogenesis with engineering technology to create large-scale mechanised or automated culture systems. Such systems are capable of producing propagules (somatic embryos) repetitively with low labour inputs. In this process of repetitive somatic embryogenesis (also referred to as accessory, adventive, or secondary somatic embryogenesis) a cycle is initiated whereby somatic embryos proliferate from the previously existing somatic embryo in order to produce clones.

Keywords: Cloning zygotic embryos repetitive somatic embryogenesis potential.

Introduction:

A wide range of soybean genotypes, have been tested for their ability to undergo auxin-stimulated somatic embryogenesis during cloning of zygotic embryos. All of them are reported to form somatic embryos provided appropriate nutrients are provided in the medium (Ranch *et al.,* 1986, Parrot *et al.,* 1988, Gaj, 2001). Evaluated a diverse group of 33 soybean genotypes and observed that the genotype with the highest regeneration capacity had an average number of somatic embryos per explanted cotyledon (SE/COT) from immature zygotic embryos equivalent to 2.09. This figure was l00x higher than the SE/COT of the worst responder. The role of genotypes in conferring regeneration capacity is further supported by studies on zygotic embryo cloning of wheat, rice and maize. Diallel analysis of various cultivars demonstrated that the regeneration capacity of these crops was directly affected by non-additive, additive and cytoplasmic factors. Raising somaclonal variations in tree species

Embryos formed directly from PEDCs appear to produce relatively uniform clonal material, whereas the indirect pathway involving lEDCs generates a high frequency of somaclonal variants. Mutation during adventive embryo- genesis may give rise to a mutant embryo which on germination would form a new strain of plant. Nucellar embryos, like shoot tips, are free of virus and can be used for raising virus-free clones, especially from some tree species (Schiedero, 1978, Parrot *et al.,* 1988, Ahloowalia, 1991, Railev *et al.,* 1993) e.g., poly- embryonate *Citrus,* where shoot tip culture has not been successful (Lazzori *et al.,* 1985, Chazi and Kerns, 1986). For clonal propagation of tree species, somatic embryogenesis from nucellar cells may offer the only rapid means of obtaining juvenile plants equivalent to seedlings with parental genotype. Clonal propagation through somatic embryogenesis has been reported in 60 species of woody trees representing 25 families.

Potential and Applications of Synthetic/Artificial Seeds:

There is considerable worldwide interest in the development of methods for encapsulation of somatic embryos to enable them to be sown under field conditions as 'synthetic' or 'artificial' seeds (Lin *et al.,* 1996, Wang 2006). Research programmes on production of artificial seeds via somatic embryogenesis in respect of commercially important crops would not only contribute to increased agricultural production, but also add to our basic knowledge of the regulatory mechanisms which control plant growth and differentiation (Redenbergh *et al.,* 1986, Perry *et al.,* 1999). Synthetic seeds, consisting of somatic embryos enclosed in a protective coating, have been proposed as a 'low-cost-high-volume' propagation system (White, 1963, Schenk, 1972, Rode *et al.,* 1989, Zimmerman, 1993). [Plate – I (Fig-3)]. The inherent advantages of synthetic seeds are the production of many somatic embryos and the use of conventional seed-handling techniques for embryo delivery. The objective is to produce clonal 'seeds' at a cost comparable to true seeds.

Source of Regenerable Protoplast System

Embryogenic callus, suspension cultures and somatic embryos have been employed as sources of protoplast isolation for a range of species (Klercker *et al.,* 1892). Cells or tissues in these systems have demonstrated the potentiality to regenerate in cultures and, therefore, yield protoplasts that are capable of forming whole plants. Embryogenic cultures are especially valuable in providing a source of regenerable protoplasts in the graminaceous, coniferous and citrus species (Gleddies *et al.,* 1989). Attempts to achieve regeneration of callus or even sustained divisions in mesophyll-derived protoplasts of

Gramineae proved unsuccessful until Vasil and Vasil (1979) turned to embryogenic cultures obtained from immature embryos of pearl millet *(Pennisetum purpureum)* as the source of protoplasts. Protoplasts from these cultures were induced to divide to form a cell mass from which embryoids, and even plantlets, regenerated on a suitable nutrient medium. Similar success was subsequently reported by other workers with embryogenic suspension of *Panicum maximum, Pennisetum purpureum, Oryza saliva, Saccharum officinarum, Lolium perenne, Festuca arundinaceae* and *Dactylis glomerata.*

Somatic embryogenesis in dicotyledonous cultures:

Totipotent embryogenic cells have been most commonly obtained from explants of embryonic or young seedling tissues (Schenk *et al.,* 1972). Excised small tissues from young inflorescences (before maturation of floral primordia) are equally effective for the induction of somatic embryogenesis in cultures (Latha and Venkateshwarlu, 2019). Other explants used are the scutellum, young roots, petioles, immature leaf, and immature hypocotyl In Ranunculus *sceleratus* various floral (including anthers) and vegetative tissues proliferate to form a callus on a medium containing coconut milk (10%) with or without IAA. Within 4 weeks, numerous embryos appear from the peripheral and deep-seated cells of the callus. A high yield of embryogenic calli can also be obtained from isolated fully differentiated mesophyll cells or protoplasts in a defined culture medium. *Citrus* nucellar cells have a natural potential for somatic embryogenesis, which is also manifested in their cultures (Plate-I, Fig-2).

Somatic embryos germinate *in situ* or when they are excised and cultured individually on a fresh semi-solid medium. A special and noteworthy feature may be the development of a fresh crop of.adventive embryo (numbering 5-50) which originates from single epidermal cells on the stem surface of the plantlets obtained from germinating embryos (Singh *et al.,* 2000, Tomar and Tiwaris, 2006, Venkateshwarlu *et al.,* 2018). Age, physiological state, genotype and orientations of the explant, while in contact with the medium, influence the induction of somatic embryogenesis (Odelu *et al.,* 2015). These aspects govern the disruption of explant tissue integrity, callus friability, isolation of cells and other requirements in order to enhance somatic embryogenesis in various species.

Somatic embryogenesis in monocotyledonous cultures:

Many monocotyledonous plants are of agricultural and medicinal importance. Unlike dicots, the vegetative parts of a monocot plant do not readily proliferate in cultures (Ugender *et al.,* 2018). Therefore, explants are best taken from embryogenic or

meristematic tissues (young inflorescences and leaves) (Morel and Martin, 1952, 1965). The procedures have been developed to induce somatic embryogenesis in suspension cultures of other monocot species, such as *Dioscorea (D. floribunda, D. bulbifera).*

Young caryopses (10-15 days after pollination) or seeds are sterilised by a 30 s rinse in 70% ethanol, followed by 10-20 min soak in 20% commercial bleach to which a few drops of detergent have been added as a wetting agent. Some species may require a further 30-60 s rinse in mercuric chloride (0.01-0.1%) to eliminate the contamination problem. Explants are then washed at least three times in sterile distilled water and zygotic embryos removed aseptically with the unaided eye (maize embryo) or using a dissecting microscope (for young and immature embryos). Excised embryos are now transferred to a culture vial containing MS medium supplemented with BAP, Kn, NAA, 2, 4-D (1.5-3.5 mg l^{-1} in the case of cereals or 18 μM for *Dioscorea*) and sucrose (2-6%). Cultures are incubated in diffused light or complete darkness. A small and slow-growing callus will appear in 4-6 weeks. In gymnosperm (conifer) species, immature zygotic embryos have embryogenic tissue arising from cells in suspensor region which is initiated into embryogenic cultures. Ammirato (1983)

Premeiotic inflorescences with the primordia of the individual florets just beginning to protrude, have been observed to be the most suitable material in some systems. The inflorescences, generally 1-2 cm in length, are sterilised according to the procedure described for zygotic erpbryos. Following sterilisation, each inflorescence is exposed by a vertical incision through the surrounding leaves and then cut into 1-2 mm thick segments. Individual segments are then cultured on a medium containing 2, 4-D for proliferation and initiation of an embryogenic callus.

Somatic embryogenesis:

In somatic embryogenesis the embryos regenerate from somatic cells, tissues or organs either *de novo* or directly from the tissues (adventive origin), which is the opposite of zygotic or sexual embryogenesis. Various terms for non-zygotic embryos have been reported in literature such as adventive embryos (somatic embryos arising directly from other organs or embryos), parthenogenetic embryos (those formed by the unfertilized egg), androgenetic embryos (formed by the male gametophyte). However, in general context, somatic embryos are those which are formed from the somatic tissue in culture, i.e. *in vitro* conditions. In sexual embryogenesis, the act of fertilization triggers the egg cell to develop into an embryo. However, it is not the monopoly of the egg to form an embryo. Any cell of the gametophytic (embryo-sac) or sporophytic tissue around the embryo-sac may give rise to an embryo.'Cells

of the nucellus or inner integument of members of Rutaceae family (e.g. *Citrus)* may develop into embryos (Murashige, 1974, Kim *et al.,* 1989).

There are examples of embryos arising from endospermal cells also. However, occurrence of asexual embryogenesis is generally restricted to intraovular tissues. What is particularly striking about embryogenesis in plant cultures is the development of embryos from somatic cells (epidermis, parenchymatous cells of petioles or secondary root phloem) in addition to their formation from unfertilized gametic cells and tissues typically associated with *in vivo* sexual embryogenesis (e.g. nucellus). Somatic embryogenesis differs from organogenesis in the embryo being a bipolar structure with a closed radicular end rather than a monopolar structure. The embryo arises from a single cell and has no vascular connection with the maternal callus tissue or the cultured explant. Further, induction of somatic embryogenesis requires a single hormonal signal to induce a bipolar structure capable of forming a complete plant, while in organogenesis it requires two different hormonal signals to induce first a shoot organ, then a root organ.

The initiation and development of embryos from somatic tissues in plant culture was first recognized by Steward *et al.* (1958) in cultures of *Daucus carota.* In addition to the development of somatic embryos from sporophytic cells, embryos have been obtained from generative cells, such as the classic work of Guha and Maheshwari (1964) with *Datura innoxia* microspores. Although the list of species from which somatic embryogenesis has been reported is so long, clear-cut examples are far less. In addition to the members of Umbellifereae and Solanaceae, a range of dicotyledonous families have produced somatic embryos. The Leguminoseae and many monocots of Gramineae family, which are so important agronomically, have proven difficult to grow in culture and regenerate somatic embryos. Though there are reports of successes in these species but manipulation is not as easy as with Solanaceous crops. Somatic embryos should closely resemble their bipolar nature as in the case of zygotic embryos (Shyamkumar *et al.,* 2003, Ugender *et al.,* 2017)

There should be appropriate root, shoot and cotyledonary development. There should be no vascular connection with the mother tissue. In this chapter embryogenesis will be restricted to sporophytic tissue; the discusssion on androgenesis will be dealt separately

Rode *et al.* (1989) described two routes to somatic embryogenesis.

1. Direct embryogenesis*:* The embryos initiate directly from the explant tissue in the absence of calius proliferation. This occurs through 'Pre-Embryogenic Determined Cells' (PEDC) where the cells are committed to embryonic development and need only to be released. Such cells are found in embryonic tissues (e.g. scutellum of cereals), certain tissues of young *in vitro* grown plantlets (e.g. hypocotyl in *Daucus*

carota, Ranunculus scleratus, Linum usitatissimum, Brassica napus), nucellus and embryo-sac (within ovules of mature plants).

2. Indirect embryogenesis*:* Cell proliferation, i.e. callus from explant takes place from which embryos are developed. The cells from which embryos arise are called embryogenicaliy determined cells and forms embryos which are induced to do so, also called as 'Induced Embryogenic Determined Cells' (IEDC), e.g. secondary phloem of carrot, leaf tissues of coffee, *Petunia, Asparagus,* etc. In majority of cases embryogenesis is through indirect method. Here, specific growth regulator concentrations and/or cultural conditions are required for initiation of callus and then redetermination of these cells into the embryogenic pattern of development. When the conditions are suitable these embryos germinate to produce plantlets.

For some species any part of the plant body serves as an explant for embryogenesis (e.g. carrot) whereas in some species only certain regions of the plant body may respond in culture e.g. Cereals. Floral or reproductive tissue in general has proven to be an excellent source of embryogenic material. The physiological state of the plant from which the explant is taken is also extremely important, as is the season during which the material is removed. Somatic embryogenesis encompasses various stages from callus initiation to embryo development and maturation and subsequently plantlet formation. Equally important is the sequence of media and especially the growth regulators. For many species, one media is used for initial callusing and for the maintenance of callus, a second medium is used for somatic embryo maturation, and a third to allow their growth into plants. An elaborate sequence of media is essential where somatic embryogenesis is lacking or difficult.

The presence of auxin in the medium is generally essential for embryo initiation. Tissue or calluses maintained continuously in an auxin-free medium generally do not form embryos. The callus is initiated and multiplied on a medium rich in auxin which induces differentiation of localized group of meristematic cells called embryogenic clumps. Somatic embryo development generally follows the transfer of cells or callus to media lacking auxin, or with reduced levels of the same auxin, or with similar or reduced levels of a weaker auxin. When transferred to a medium with low auxin or no auxin, the embryogenic clumps develop into mature embryos. In a number of species, somatic embryo initiation and maturation occurs on the primary medium. Transfer to a secondary medium is needed for their growth into plants. Some variables that affect somatic embryogenesis and maturation of embryos in culture are discussed.

Somatic embryos have been grown on a range of media from the dilute White's medium to very high salt MS medium, but the latter has been extensively employed. The

addition of reduced nitrogen in the medium helps in both embryo initiation and maturation. The sources of reduced nitrogen have already been explained. Of all the amino acids, L-glutamine seems to play a special role. Another factor is the chelated form of iron in the media. In the absence of iron, embryo development fails to pass from the globular to the heart-shaped stage.

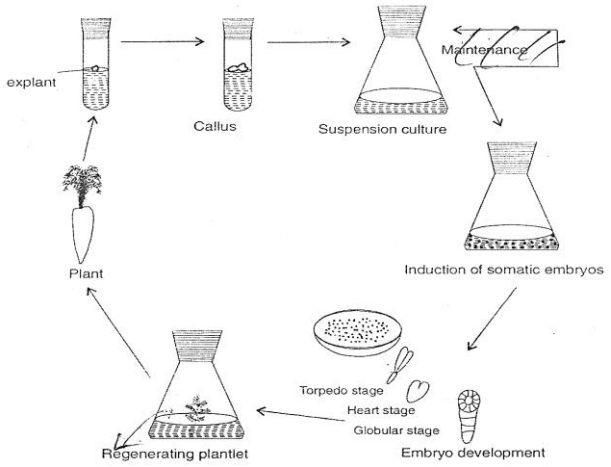

Figure 1: Protocol for somatic embryogenesis in carrot

Growth regulators in the medium, especially auxin or auxin in combination with cytokinin appear essential for the onset of growth and the induction of embryogenesis. Of all the auxins, 2, 4-D followed by NAA has proven to be extremely useful. Effective concentration ranges are 0.5-27.6 μM for 2, 4-D and 0.5-10.7 μM for NAA. The auxin for the primary (callus initiation) and secondary media (embryo development) may be same or different. One auxin or several may be used in the same medium. Cytokinins have been important in a number of species. Cytokinins have been used in the primary medium invariably during embryogenesis of crop plants. The effective concentration range for kinetin is 0.5-5.0 μM. Cytokinins are important in fostering somatic embryo maturation and especially cotyledon development (Tomor, 2006). Cytokinins are sometimes required for growth of embryos into plantlets. Gibberellins are rarely incorporated in primary culture media.

Their addition to culture media may permit somatic maturation to proceed under conditions when it normally would not occur. The addition of activated charcoal to the medium has proved to be useful for somatic embryo development. Charcoal media shows lower levels of phenylacetic acid and p-OH benzoic acid compounds, which inhibit somatic embryogenesis. Also, it absorbs 5-hydroxymethyl furfural, an inhibitor formed by sucrose degradation during autoclaving. Environmental conditions of light, temperature, and

density of embryogenic cells in medium are important. Regarding culture vessel, the position of embryos (floating or submerged) and the physical state of the medium (semisolid or liquid) have little effect.

Plate I: Potential and practical applications of Somatic Embryogenesis

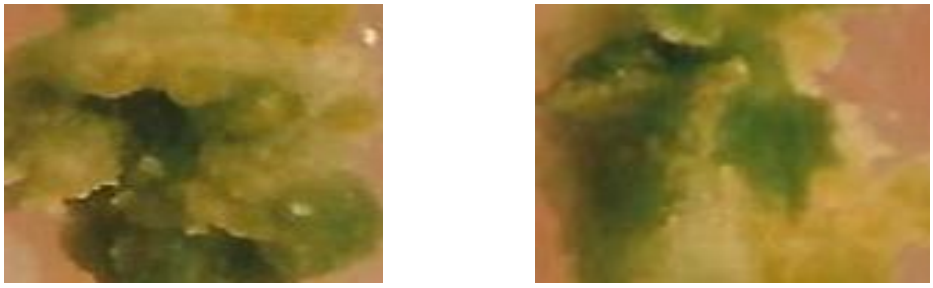

Figure 2: Somatic Embryos in vitro cultures

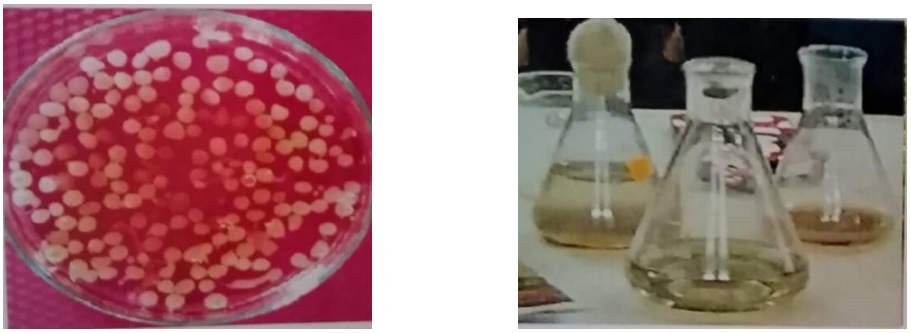

Figure 3: Artificial seeds (Sodium alginate, $Cacl_2$ combination)

Somatic embryogenesis as a means of propagation is seldom used because:

i. There is a high probability of mutations arising.
ii. The method is usually rather difficult.
iii. The chances of losing regenerative capacity become greater with repeated subcultures.
iv. Induction of embryogenesis is often very difficult or impossible with many plant species.
v. A deep dormancy often occurs with somatic embryogenesis that may be extremely difficult to break.

Genetic and molecular aspects:

Nuclear changes as polyploidy, aneuploidy and chromosomal mutations in cultured cell may be responsible for the loss of organogenic or embryogenic potential in prolonged

cultures. This loss is generally irreversible. The understanding of loss of morphogenic potential in cultures has been facilitated by identification of biochemical markers in the process of somatic embryogenesis. Isolation and characterization of drug-resistant mutants also enable a search for biochemical markers. Some interesting results have been obtained from the characterization of temperature-sensitive mutants in which the embryo development process is impaired. For example, phosphorylation seems to cause a defect in one or more peptides of 'heat shock' proteins induced at higher temperature in carrot mutant cell line Ts 59. From this, one can speculate that (a) heat shock proteins are important in several steps of the development program, (b) phosphorylation is a signal for activation of particular function and (c) kinases have strict specificity. Another mutant line (tsllc) at non-permissive temperatures is unable to acquire polarity because the somatic embryo reaches the globular stage and subsequently enlarges to form secondary embryos or monstrosites.

In orchardgrass and carrot somatic embryogenesis is genetically controlled by a dominant trait. Cytoplasmic factors (Mitochondrial) have also been implicated in control of somatic embryogenesis. About 21 'embryo-specific' or 'embryo enhanced' genes have been cloned and it is likely that some of these genes may be useful markers for early embryo development. AGL 15-specific antibodies found to accumulate in microspore embryos in oilseed rape and somatic embryos of alfalfa and are found to participate in regulation of programs active during the early stages of embryo development.

Several genes have been isolated which express during somatic embryogenesis. They are classified in three categories: (a) genes involved in cell division (21D7 and CEM1), (b) genes involved in organ formation at globular and torpedo stage embryos), and (c) embryo-specific genes (CHB2 and CEM6). Expression and regulation of the gene action of these genes are described in detail by Komamine (2001

Competitive hypothesis:

In the complex multicellular explant only a few cells are able to give rise to embryogenic clumps while the remaining cells are non-totipotent. According to the competitive hypothesis, the non-embryogenic cells of the explant will increase under conditions favorable to their growth, resulting in a gradual loss of embryogenic component during repeated subcultures. Restoration of embryogenesis in such cultures is impossible, - but if the culture carries few embryogenic cells which are not able to express their totipotency due to the inhibitory effect of the non-embryonic cells, it should be possible to

restore the morphogenic potential of these cultures by altering the composition of the medium in such way that selective proliferation of the embryogenic totipotent cells occurs.

Disadvantages with artificial seeds:

1. Facilities required are costly and economic considerations may not justify their use in commercial propagation of many kinds of plants.
2. Special skills are required to carry out the work.
3. Errors in maintenance of identity, introduction of an unknown pathogen, or appearance of an unobserved mutant may be multiplied to very high levels in a short time.
4. Specific kinds of genetic and epigenetic modifications of the plant can potentially develop with some cultivars and some systems of culture.

Protocols:

Meristem and Node Culture of Potato *(Solatium tuberosum)*

Plant material*: Potato tuber*

1. Soak potato tubers in 0.03 M gibberellic acid (GA_3) for 1 h or warm potato tubers at room temperature for 2 days before using to break dormancy.
2. Rinse the potato tubers with tap water. Surface sterilizes the tubers by soaking in 10-20% commercial sodium hypochlorite solution for 10-20 min.
3. Place whole tubers or cut into 2-3 cm^2 of 20-30 g sections on the surface of sterile moist vermiculite and keep them in a growth chamber.
4. Harvest sprouts when they reach 2-5 cm in length.

Establishment of *in vitro* plantlets from plant material:

1. Excise nodal sections 1-2 cm from third and fourth nodes from the stem apex with a scalpel and detach their leaves.
2. Surfaces sterilize the nodal tissue by soaking in 10% commercial sodium hypochlorite for 10 min. Rinse 3x with sterile distilled water. Re cut the base of tissue with a scalpel.
3. Place the nodal sections or sprouts on MS medium.
4. Allow growth to 4-6 nodes/stem stage.

Propagation by nodal cutting

1. In the laminar flow hood with the help of large forceps remove a plantlet from the culture vessel and place it on a sterile filter paper or sterile Petri dish.
2. Cut the main stem above and below each node into sections of 1-2 cm length. Place one node in each culture vessel containing MS medium with 1-2 mg/l BAP/KIN.

Propagation by meristem:

1. Take nodal cuttings from third and fourth nodes of *in vitro* raised plants obtained from plants or sprouts.
2. In the laminar flow hood. Peel away protecting leaves on the buds under a dissection microscope at 10-25x.
3. Dissect out meristem domes with one subjacent leaf primordium -0.5 to 0.75 mm.
4. Culture the meristem domes on the surface of MS agar medium supplemented with KIN (0.1 mg/l = 0.046 μM) and GA_3 (0.1 mg/l = 0.29 μM).

Proliferation of Axillary Buds (Strawberry *Fragaria chiloensis)*

Plant material: Runner shoots tips of strawberry

1. Collect runners from strawberry plants.
2. Surface sterilize the runners in 10-20% commercial sodium hypochlorite for 10 min and then rinse 4x with sterile distilled water. It is preferable to sterilize further with 0.02% HgCI, for 3-4 min followed by washing with 1% KCI for 1 min in order to neutralize the mercury ions.
3. Dry the runners in a sterile petri dish.
4. Cut the runners into 1-2 cm or peel the shoot tips and then cut the tip portion of 0.1-0.5 mm size. Inoculate on the establishment medium:
   MS + 0.5-1.0 mg/l BAP + 0.1-0.2 mg/l IBA
5. Incubate the cultures at 25°C under 16 h photoperiod.
6. After 3-4 weeks on this medium, axillary buds will appear.
7. The buds can be proliferated or multiplied if cultured on the following medium:
   MS + 0.8-1 mg/l BAP + 0.2 mg/l IBA + 0.2 mg/l GA_3
8. The developed shoots alongwith buds can be further multiplied on the same proliferation medium after 3-4 weeks of culture. Or shoots are separated and transferred to MS medium with auxin (0.5-1 mg/l) IBA. The development of axillary buds ceases and the young plantlets with roots are developed. Profuse rooting should occur within 4-5 weeks.

9. Rooted plantlets can be transferred to soil and follow the procedure of plant establishment and hardening .

Organogenesis: Adventitious Shoot Formation

Plant material: Leaf explants *tobacco* or African vivolet

1. Take leaf explants from *in vitro* grown plants or potted plants.
2. If leaves are taken from potted plants, gently wash them with a mild detergent and then rinse under tap water. Dip each leaf into 70% alcohol. Leaves are then surface sterilized with 10% commercial sodium hypochlorite for 5-10 min followed by rinsing 3x in sterile distilled water.
3. Place the leaves in sterile petri dish. Cut leaves into approximately 1 cm^2 sections using sterile forceps and scalpel, without vein in the explant.
4. Place the leaf sections on the following media:
   African violet. MS + 1.0 mg/l KIN + 0.1 mg/l IAA
   Nicotiana: MS + 1-2 mg/l BAP + 0.1-0.2 mg/ I NAA
5. Seal the cultures. Incubate at 25°C under 16 h photoperiod at 1000-lux light intensity.
6. Observe the shoot formation after 3 weeks and record the data at regular intervals.
7. Cut tissue masses into small pieces for shoot multiplication, each piece should include at least one tip or rosette, and place them on their respective media.
8. For rooting, transfer the well developed shoots to MS medium without growth regulators or little concentration of auxin (0.5 mg/l) to enhance root production.
9. Transfer the rooted plantlets to soil and follow the procedure of plant establishment and hardening (Protocol 5.1).

Organogenesis via Callus Formation *(Nicotiana)*

Plant material*:* *Nicotiana*

1. Follow Steps 1-4 of Protocol 6.'3. Place the leaf sections on the following medium: MS + 1-2 mg/l 2,4-D
2. Incubate the cultures in dark at 25°C. Callus will be produced in 3-4 weeks.
3. For shoot induction, subculture pieces of callus approximately 0.5 cm^2 to the medium: MS + 1-2 mg/l BAP + 0.1-0.2 mg/l NAA
4. Follow Steps 5 to 9 of Protocol 6.3.

Organogenesis via Callus Formation (Cereals - Wheat, Barley, Maize, Rice, etc.)

Plant material: Immature embryos of Cereals: *(wheat, barley, maize, rice)*

1. Collect spikes (for example wheat/barley) 14-15 days after anthesis from plants. Separate each developing seed and remove the lemna and palea.
2. Surface sterilizes the seeds in 10-20% commercial sodium hypochlorite containing 1-2 drops of Tween 20 for 10 min. Rinse 3-5x with sterile distilled water.
3. Dissect out immature embryos (1-1.5 mm diameter) from the seeds under dissection microscope in the hood of laminar flow.
4. Place the immature embryos with scutellum side up on the following medium for callus induction: MS + 2 mg/l 2,4-D
5. Incubate the cultures in darkat25°C. Initially the embryos will show swelling followed by callus proliferation from the scutellum in 3-4 weeks. (Embryos bigger than 2 mm diam. will show precocious plant formation.)
6. Scutellar callus can be multiplied at least once on the same callus initiation medium by breaking into 2-3 pieces after 4-6 weeks of initiation of callus.
7. Place hard and compact calli of approximately 0.5 cm^2 on the following shoot induction medium: MS + 2 mg/l BAP + 0.2 mg/l NAA or MS without growth regulators.
8. Incubate the cultures at 25°C under 16 h photoperiod with ~2 klux light intensity. Shoots would emerge in 3-5 weeks.
9. Transfer shoots to MS medium with 1 mg/l of auxin (IAA/IBA/NAA) for root induction under the same cultural conditions.
10. Transfer rooted plantlets to soil and follow the procedure of plant establishment and hardening (Protocol 5.1).

Embryogenesis (Carrot) (Fig-I)

Plant material: Hypocotyl of *Carrot* Seedling

1. Wash seeds by submerging in water with a few drops of detergent in a beaker and shake by hand, or wrap seeds in two layers of cheese cloth/muslin cloth/nylon pouch and then wash with water.
2. Submerge the seeds in 70% alcohol for 30-60 s. Decant the alcohol.
3. Transfer the seeds to a flask or beaker containing 20-40% commercial sodium hypochlorite for 15-20 min. Rinse 4x with sterile distilled water.
4. Place 2-3 seeds per culture vessel on the surface of MS agar medium.

5. Incubate the cultures at 25°C under 16 h photoperiod with ~1000lux light intensity for 1-2 weeks.
6. Collect the germinated seedlings when the cotyledons are fully expanded. Place each seedling on a sterile petri dish and excise the hypocotyl from each' seedling and cut them transversely into two parts.
7. Place the hypocotyl sections on the following medium: MS + 1-2 mg/l 2,4-D
8. Incubate the cultures in dark at 25°C for 4-8 weeks.
9. Maintain the callus by subculturing small pieces on fresh medium every 3-4 weeks. Callus will contain pro-embryo initial cells as well as minute microscopic embryos in the early stages of development.
10. Place 0.5 to 1 cm^2 callus pieces on MS agar medium without growth regulators and incubate the cultures at 25°C under 16 h photoperiod with -1000 lux light intensity. Within 2-3 weeks cultures will exhibit embryos and green plantlets.
11. Tease out individual or group of plantlets from the callus mass and transfer on half strength MS medium under 16 h photoperiod with high light intensity of ~ 5 klux. Within 4-5 weeks the cultures will resemble seedling carrots.
12. Transfer the plantlets to small pots containing sterile peat moss and vermiculite in a 1:1 ratio. Enclose the piantlets with plastic containers to maintain high humidity.
13. Transfer the plants to soil and follow the procedure of plant establishment and hardening .

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ETHNO-ECOLOGICAL ATTRIBUTES OF SOME WEEDS OF FAMILY ASTERACEAE IN CROP FIELDS OF BHANDARA DISTRICT (M.S)

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Abstract:

The present investigation is focused on weed diversity of Asteraceae members in crop fields and their folklore information gathered from tribal and rural people. Many members of the large and diverse plant family Asteraceae have become serious agricultural weeds, many of which frequent in vegetable fields. In view of this, some ethno-ecological aspects were studied for identifying the status of each species of the Asteraceae members in the present study site.

Keywords: Asteraceae, Diversity, Ethno-ecology, Tribal, Weed

Introduction:

In the total population of Bhandara district, 85% is residing in rural area and their source of income is Agriculture. The major cultivated crops are paddy, wheat, soybean, chick pea, pigeon pea, horticulture and vegetable crops. The main source of irrigation is canals, open wells, tanks, lift irrigation and drip irrigation methods. The district is occasionally drought and flood prone. But it is not affected by cold, frost and hail storm.

The present investigation is focused on weed diversity of Asteraceae members in crop fields and their folklore information gathered from tribal and rural people. Asteraceae is the one of the largest families of the Angiosperms. This family includes annuals, biennials, and perennials with or without taproots, rhizomes, or tubers. Many weeds in this family have strongly lobed or divided leaves, which grow in basal rosettes during the vegetative phase of the plant's lifecycle. They are generally easy to distinguish from other plants, mainly because of their characteristic inflorescence and other shared characteristics.

Many members of the large and diverse plant family Asteraceae have become serious agricultural weeds, many of which frequent in vegetable fields. In view of this, some ethno-ecological aspects were studied for identifying the status of the each species of the Asteraceae members in the present study site.

Material and Methodology:

Periodical field trips were under taken in various crop fields of Bhandara District. The author concentrated on Asteraceae members grown in crop fields as weeds. Weed phyto-ecological parameter were taken from 1.0× 1.0 m quadrate placed randomly in crop fields. Twenty quadrate samples were taken from each field for the study of ecological aspects (Relative Abundance, Relative Density, Relative Frequency and IVI) by using the following principle as presented.

$$\text{Frequency (F)} = \frac{\text{Number of quadrates in which species occurred}}{\text{Total number of Quadrates studied}} \times 100$$

$$\text{Relative Frequency (RF)} = \frac{\text{Frequency of individuals of a species}}{\text{Total number of Quadrates studied}} \times 100$$

$$\text{Density (D)} = \frac{\text{Total number of individuals of the species}}{\text{Total frequency of all species}}$$

$$\text{Relative Density (RD)} = \frac{\text{Density of individuals of a species}}{\text{Total density of all species}} \times 100$$

$$\text{Abundance (A)} = \frac{\text{Total number of individuals of the species}}{\text{Number of quadrates in which species occurred}}$$

$$\text{Abundance (RA)} = \frac{\text{Abundance of individuals of a species}}{\text{Total abundance of all species}} \times 100$$

Importance Value Index (IVI)= *Relative frequency+ Relative density+ Relative Abundance*

The utilization of some medicinal plants of Asteraceae was recorded from farmers, village heads, and senior women by questionnaires to them. The communication with these

people was in Marathi and Hindi. For some of the plants ethnobotanical information was gathered from review of literature. Data was recorded in the field book on the plant part used, their collection, processing, preparation of the drug, its dosage and administration, etc has given by the local informants. Voucher specimens were collected along with local names for authentication of information and for future reference.
(E.B.H.No.: Ethnobotanical information on Herbarium sheet with number).

Results and Discussion:

As per the Ethno-ecological studies, population of the medicinal plant species decreasing day by day due to over harvesting and over exploitation. Communities are unaware about the status of medicinal plants; ultimately plant becomes extinct. By considering this view, awareness among the people is needed. The present study helps to know the status of weeds of Asteraceae members and their medicinal values in various crop fields of Bhandara District.

A total of 21 Asteraceae members were identified and isolated in different crop fields of study site and eumerated in the form of descriptive format as given below:

1. *Ageratum conyzoides* L.

Vernacular name : Ghannera Ossadi

Habitat : Field boundaries and waste lands

Morphology : Erect, herbaceous, hairy 30-60 cm tall. Leaves opposite, crenate, ovate with serrate margin. Stem reddish or green covered with soft white hair. Flowers all tubular, white or purplish and head borne in terminal corymbs. The inflorescence is composed of a cluster of 30-50 white to purple flower. It is known as a corymb. Fruits dry, indehiscence achene with small single seed.

Phenology : June-December

E.B.H.NO : PSR-01

Part used : Whole plant

Folklore Herbal Information: Whole plant is used to treat fever, diarrhea, colds and rheumatism. The leaf paste has a quick healing effect in burn wounds.

2. *Blumea lancer* (Burm.f.) DC.

Vernacular name : Kukundarah, Hindi: Jangalimuli

Habitat : Fields, Waste lands

Morphology : An herbaceous, annual erect. The whole plant with glandular, pubescent; a strong odour of turpentine, leaves simple, alternate, the lower petiolate,

often incised or lyrate, the upper sub sessile, elliptic –oblong, flower yellow, many heads, both terminal and axillary; fruit aches, ribbed, crowed with white pappus hairs.

Phenology : November –March:

E.B.H.No : PSR-02

Part used : Leaf

Folklore Herbal Information: Acts astringent, anti-inflammatory, styptic, ophthalmic, abdominal disorder intestinal worms, liver disorders, leucorrhoea, fever, haemorrhoids haematemesis, cough, bronchitis.

3*. Blumea oblique* (L.) Druce.

Vernacular name : Gangavati

Habitat : Waste land, near fields.

Marphology : Erect or slightly procumbent, strong smelling herbs. Leaves sessile, half amplexicaul, elliptic, dentate .Heads pinkish, usually all solitary on long peduncles; ray floret pinkish, disk florets yellowish. Achenes smooth, pubescent.

Phenology : January-April.

E.B.H.NO. : PSR-03

Part used : Whole plant

Folklore Herbal Information: Whole plant is used as astringent, anti-inflammatory and for treating abdominal disorders intestinal worms, liver disorders, fever, cold, cough and bronchitis.

4. *Caesulia axillaris* Roxb.

Vernacular name : KalaMaka

Habitat : Wet land, rice field, Irrigation ditches

Morphology : Sub-erect herbs, 20-40 cms tall, stem purplish. Leaves sessile, lanceolate, acute and serrulate. Heads white or purple in the axil of leaves; involucres bracts leafy, purple. Achenes flat, obovoid, enclosed in two –lobed involucres.

Phenology : November – March

E.B.H.NO. : PSR-20

Part used : Leaf, Seed

Folklore Herbal Information: Seed oil is mixed with Brahmi or Bringaraj oil for proper growth of hair. Leaf paste acts as antifungal and antibacterial.

5*. Cirsium vulgare* (Savi) Ten.

Vernacular name : Plum Thistle

Habitat : Field boundaries and disturbed areas

Morphology : *Cirsium* commonly called Plum Thistle is a biennial , erect stem and prickly leaves, with a characteristic enlarged base of the flower which is commonly spiny. The leaves are alternate, slightly hairy and leaf base is down the stem and conspicuous. The seed has tufts of tiny hair.

Phenology : April to August

E.B.H.NO. : PSR-13

Part used : Whole plant

Folklore Herbal Information: Paste of leaves and roots of the *Cirsium vulgare* is used as a medicine to treat stiffness of neck and some nervous disorders. Leaf decoction is effective diuretic and cures fever. Flowers are used as a remedy against swollen veins.

6. *Cosmos sulphurous* Cav.

Vernacular name : Sulfur Cosmos

Habitat : Field boundaries and disturbed areas

Morphology : This species is a slightly hardy annual; grow up to 1-6 feet. Its leaves are opposite and pinnately divided. The flowers appear in shades of yellow, orange, and red.

Phenology : December –May

E.B.H.NO : PSR-12

Part used : Whole plant

Folklore Herbal Information: Whole plant is used for treating as a hepato protective effect, organoleptic and pancreatic lipase.

7. *Eclipta prostrate* (L.) L.

Vernacular name : Maka, Bhringranj

Habitat : Marshy places.

Morphology : Creeping, prostrate or slender, erect herbs when erect it is about 50 cms tall. Leaves variable, linear-oblong, lanceolate, margin slightly toothed, turning black when dry. Heads white, radiate, solitary or 2-3 together on erect pubescent stalk. Achenes narrow oblong, ribbed with pappus teeth.

Phenology : Throughout the year.

E.B.H.NO. : PSR-06

Part used : Whole plant

Folklore Herbal Information: Whole plant is used in Ayurveda medicine. Plant juice in combination with aromatic is administered for jaundice. Oil prepared from plant is used as a hair tonic.

8. *Erigeron banariensis* L.

Vernacular name : Asthma weeds

Habitat : Field boundaries and disturbed areas

Morphology : Plant grows up to 60 cm in height and leaves are covered with stiff hairs but hairs are long at the apex of the bracts. Flower heads having two colored florets- white ray florets and yellow disc florets. Seeds having tuft of hairs can easily blown and spread by wind.

Phenology : August-December

E.B.H.NO. : PSR-07

Part used : Whole plant

Folklore Herbal Information: A decoction of the whole plant has been used to treat bleeding piles, treating rheumatic joints.

9. *Gnaphalium luteo-album* L.

Vernacular name : English-Jersey Cow weed, or Cat'spaw

Habitat : Rice Fields

Morphology **:** A prostrate or ascending, white-woolly herb. Leaves oblong or subspathulate, half-amplexicaul. Head golden-yellow on leafless stalk, a dense, corymbose, shining clusters heterogamous. Achenes brown, oblong, papillose.

Phenology : February -April

E.B.H.NO. : PSR-19

Part used : Leaves

Folklore Herbal Information: The leaves are used as an astringent and for treating high blood pressure, stomach ulcers, diarrhoea, gut infections.

10. *Grangea maderaspatana* (L.)Poir.in Lamk.

Vernacular name : English: Madras Carpet, Marathi :Mustaru, Godri

Habitat : Rice fields

Morphology : A prostrate, tufted, leafy herb, forming circular patches, leaves sinuate pinnatifid, inflorescence thick head yellow, disciform, heterogamous, Pappus copular.

Phenology : February-April

E.B.H.NO. : PSR-17

Part used : Leaves

Folklore Herbal Information: Leaves have antispasmodic properties and are prescribed as an infusion and in hysteria.

11. *Launaea procumbens* (Roxb.) and Ramayya and Rajgopal

Vernacular name : Hindi-Jangli Gobi, Marathi-Pathari

Habitat : Fields, Waste lands

Morphology : Creeping *Launaea* is a perennial creeper, 20-50 cm tall. Stem glaberous and branched. The whole plant secretes a yellow juice. Long leaves, 5-25 cm, arise from the roots, have white margins and are spinous toothed. Yellow flower-heads are 1-1.5 cm across, and occur either singly or in small racemes on short stalks along the branches.

Phenology : September-January

E.B.H.NO. : PSR-05

Part used : Whole plant

Folklore Herbal Information: Ground plant is boiled in water and the extract is applied on the area of infection for the treatment of ringworm infection.

12. *Parthenium hysterophorus* L.

Vernacular name : Congress grass, Hindi: Gajargawat, English-Wild carrot, Marathi: Chatak Chandani

Habitat : Fields, roadsides, waste lands

Morphology : Profusely branched herbs, Leaves sessile, dissected, pubescent; lobes entire, acute. Heads many, white, panicles radiate. Outer florets 5, Achenes black.

Phenology : August-December

E.B.H.NO. : PSR-16

Part used : Whole plant

Folklore Herbal Information: This weed is considered to be a cause of allergic respiratory problems, asthma, and bronchitis contact dermatitis. The whole plant

decoction is used in traditional medicine to treat fever, diarrhea, urinary tract infections, dysentery and neurologic disorders.

13. *Senecio vulgaris* L.

Vernacular name : Ground Sel

Habitat : Field boundaries and disturbed areas

Morphology : It is an erect herbaceous annual plant, growing up to 50 cm tall. Leaves are pinnately lobed and are covered with soft and fine hairs. Open clusters of 10 to 20 small cylinder shaped yellow flower heads without ray florets. The seeds are achene,

Phenology : January-May

E.B.H.NO. : PSR-14

Part used : Whole plant

Folklore Herbal Information: The whole plant is antiscorbutic, anthelmintic, diaphoretic, diuretic, purgative and also useful in treating sickness of the stomach menstrual disorders and nose bleeds

14. *Sonchus oleraceus* L.

Vernacular name : Mhatara

Habitat : Moist place and cultivated field

Morphology : Erect, annual, 60-100 cm tall; stem glaucous. Leaves obovate oblanceolate, rounded or acute, lamina dentate but not spinous, that of cauline leaf thin, semi-amplexicaul, auricle acute. Head few to many in corymbs; generally glabrous. Ligulate corolla yellow. Achenes brown, obovoid, ribbed, minutely muricate.

Phenology : March-April

E.B.H.NO. : PSR-28

Part used : Whole plant

Folklore Herbal Information: It is widely used in Ayurvedic system of medicine to treat headaches, liver infection, menstrual problems, fever, cough, hepatitis, salmonella infections, general pain, diarrhea eyes problem, ,inflammation and rheumatism.

15. *Sphaeranthu sindicus* Linn.

Varnacular name : English: East Indian Glow, Hindi: Chhagul, Marathi - Gorakhmundi

Habitat : Rice fields

Morphology : Prostrate or ascending, aromatic herb, branching from near base; stem narrowly winged; wing toothed. Leaves ovate to obovate, acute to round at tip, sharply toothed. Compound heads globous purplish; bracts lanceolate, acute, toothed along margin, membranous.

Phenology : January-April

E.B.H.NO. : PSR-15

Part used : Whole plant

Folklore Herbal Information: It is widely used in Ayurvedic system of medicine to treat epilepsy, hemicrania, liver disorders, mental illness, leprosy, fever, cough, hernia, haemorrhoids, dyspepsia, gastropathy, diabetes and skin diseases.

16. *Spilanthes paniculata* Wall.

Vernacular name : Phakpet, Marathi-Akkalkhada

Habitat : Fields, open areas

Morphology: Stout herbs. Leaves broadly ovate, acute, sub-entire.Heads in axillary and terminal panicles. Bracts oblong elliptic, fimbricate. Floret white. Achenes of the outer floret trigonous, thickened along margin and densely ciliate along these; those of inner florets dorsally compressed, ciliate along margin; both black, verrucose.

Phenology : November-February

E.B.H.NO. : PSR-22

Part used : Flower heads

Folklore Herbal Information: Used to relieve toothache and infections of throat and gum, arthritis, stomatitis, ulcer and general weakness.

17. *Sylibum marginatum* (L.) Gaertn.

Vernacular name : Milk thistle

Habitat : Field boundaries and disturbed areas

Morphology : Milk thistle can grow up to 2 meters tall, and appear as conical shape.The stem is grooved and is covered in a light white powder. The stem is hollow. The leaves are oblong to lanceolate and pinnately lobed, with spiny edges shiny green, with milk-white veins.The flower heads are red-purple colour. The fruits are achenes.

Phenology : June to December

E.B.H.NO. : PSR-27

Part used : Whole plant

Folklore Herbal Information: A decoction of the whole plant has been used externally to treat bleeding piles and treating rheumatic joints.

18. *Tridex procumbens* (L) L.

Vernacular name : Kambarmodi

Habitat : Field boundaries, Waste lands

Morphology : Procumbent herbs creeping and ascending. Petiole short, hairy; leaf blade ovate, acute, deeply dentate, sometimes 3-lobed; lobes acute; hair thick-based. Heads 1 to 1.2 cm; outer bracts ovate, acute, herbaceous, green, pubescent; inner scarious, pinkish above middle.Corolla yellowish white.

Phenology : All seasons.

E.B.H.NO. : PSR-09

Part used : Leaves

Folklore Herbal Information: Traditionally, *Tridax procumbens* has been used for wound healing and as an anticoagulant, antifungal, and insect repellent. The juice extracted from the leaves is directly applied on wounds. Its leaf extracts were used for liver disorders, hepato-protection, and gastritis.

19. *Vernonia cinerea* (L.) Less.

Vernacular name : Sahadevi.

Habitat : Road side, paddy fields

Morphology : Annual herb, Leaves ovate, acute, cuneate below, crenate hairy. Bracts ovate- lanceolate, acute-acuminate, hairy, the outer shorter. Corolla pink; lobes ovate, acute. Achenes 2-3 angled densely hairy; pappus.

Phenology : May-December

E.B.H.NO. : PSR-22

Part used : Seeds

Folklore Herbal Information: Plant possesses anti-cancerous property. Seeds cure diseases caused by round worms and thread worms, coughs, intestinal colic, dysuria, leucoderma, psoriasis and other chronic skin-diseases. The seed paste is used for controlling pediculi.

20. *Vicoa indica* (L.) DC.

Vernacular name : Sankuli, Sonuli.

Habitat : Field boundaries and disturbed areas

Morphology **:** It *is* a slender and erects, rigid, rough or sofyly hairy stem with 30-90-cm tall. Spines about 1 metre height. Plant Leaves are dark green, sessile, ovate-lanceolate, acute, auricled, at base. Heads yellow, heterogamous, involucral bracts many linear, tips recurved, hairy. Achenes small, cylindrical, silky pubescent annual herbaceous.

Phenology **:** October-May

E.B.H.NO. **:** PSR-28

Part used **:** Whole plant

Folklore Herbal Information: A decoction of the whole plant has been used externally to treat bleeding piles, treating rheumatic joints and sore jaws.

21. *Xanthium strumarium* L.

Vernacular name **:** Ardharis

Habitat **:** All over, waste land and road side.

Marphology **:** Stout herbs or under shrubs, 90-120 cms. Tall. Leaves alternate, triangular, cordate or ovate or 3 lobed, irregularly toothed. Heads monecious in terminal and axillary recemes. Achenes obovoid, think, enclosed in hardened involucral cells. Fruiting involucres clothed with hooked prinkles.

Phenology **:** October.-December

E.B.H.NO. **:** PSR-10

Part used **:** Whole plant

Folklore Herbal Information: Whole plant acts as a laxative, antipyretic, improves appetite, voice, complexion , Antirheumatic, appetizer, diaphoretic, diuretic, sedative and anthelmintic.

Weed interference in annual cropping systems can be highly variable from year-to-year, as well as spatially heterogeneous (Mcdonald *et al.*, 2009). The most persistent and abundant weeds are easily dispersed and persist a long time in the soils as dormant seeds (Bukun, 2004). In the present study, a total of 21 weeds belonging to Asteraceae family were isolated in crop fields (Table 1). Importance Value Index (IVI), which determine the overall importance of each species in the weed community structure of the crop fields in Bhandara district.

Table1: Ecological attributes of some weed flora of Asteraceae members in crop fields of Bhandara District

Sr. No.	Name of the Species	F	D	A	RF	RD	RA	IVI
1.	***Caesulia axillaris* Roxb.**	**22**	**1.16**	**0.42**	**0.74**	**1.68**	**1.31**	**3.73**
2.	*Sonchus oleraceus* (L.) L.	25	1.16	0.42	0.74	1.68	2.31	4.73
3.	*Vicoa indica* (L.) DC.	24	1.68	0.45	0.79	1.74	2.40	4.93
4.	*Cosmos sulphureus*	85	5.50	1.09	2.91	2.29	2.78	7.26
5.	*Erigeron banariensis*	84	5.50	1.09	2.91	2.29	2.78	7.98
6.	*Sylibum marginatum*	85	5.50	1.09	2.91	2.29	2.78	7.98
7.	*Xanthium strumarium* L.	84	5.50	1.09	2.91	2.29	2.78	7.98
9.	*Blumea oblique* (L.) Druce	83	5.50	1.09	2.91	2.29	3.78	8.98
8.	*Tridex procumbens* (L) L.	84	5.40	1.09	2.90	3.28	3.77	9.95
10.	*Parthenium hysterophorus* L.	75	4.83	2.06	3.61	2.75	3.79	10.15
13.	*Senecio vulgaris*	42	2.70	1.42	2.49	3.39	4.67	10.55
12	*Eclipta prostrata* (L.) L.	42	2.70	1.65	2.89	3.93	4.41	11.23
11.	*Launaea procumbens* (Roxb.) and Ramayya and Rajgopal	90	5.80	2.75	4.81	3.06	4.22	12.09
14.	*Gnaphalium luteo-album* L.	75	4.83	2.06	3.61	3.75	4.79	12.15
16.	*Ageratum conyzoides* L	56	3.61	2.56	4.49	4.58	6.13	15.20
17.	*Cirsium vulgare*	56	3.61	2.56	4.49	4.58	6.13	15.20
15.	*Vernonia cinerea* (L.) Less.	100	6.50	4.08	7.15	4.08	5.62	16.85
18.	*Grangea maderaspatana* (L.)Poir.in Lam.	100	5.94	4.65	8.15	5.06	6.97	20.18
19.	*Spilanthes paniculata* Wall. ex. DC.	92	5.94	4.65	8.15	5.06	6.97	20.18
20.	*Blumea lancer* (Burm.f.) DC	100	6.50	4.50	7.89	5.50	7.20	20.59
21.	*Sphaeranthus indicus* Linn**.**	**100**	**6.50**	**5.75**	**10.07**	**5.75**	**7.92**	**23.74**

F- Frequency, D- Density, A– Abundance, RF- Relative Frequency, RD- Relative Density, RA- Relative Abundance, IVI- Importance Value Index

In the present investigation, all weed species are herbs, so that here instead of Relative Dominance, Relative Abundance is considered for calculating the IVI of the species

of the Community. It provides the idea of the ecological structure of a family in its totality in the community. These ecological attributions revealed that the less dominant species in the present investigation were *Caesulia axillaris, Sonchus oleraceus and Vicoa indica and most dominant species is Spheranthus indicus.* The different environmental conditions determine the specific weed spectrum, composition and population of each region (Memon *et al.*, 2007). The reduction in yield due to weed-crop competition mainly depends on weed species and their densities as well as crop species (Muhammad Tauseef *et al.,* 2012). As the distribution and infestation intensity of each weed is different, so the extent of crop yield reduction will mainly depend on the number and kind of weeds found in the field (Frisbie *et al.*, 1989). Mostly weeds with small population are unimportant but they share the habitat resources on the other hand, the weed species with high IVI and frequency might compete better to reduce growth and yield of associated crop (Ranjana Kumara, 2016).

Conclusion:

The present study suggests that a variety of weeds are infesting the crops quite heavily in agricultural fields that may cause losses to yield of different crops. So the phonological study of weeds in a particular crop is compulsory for a weed control.

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PHOTOCHEMICAL BLEACHING OF TEXTILE DYE DIRECT BLACK 155 AND REACTIVE RED 152 BY PHOTOCATALYST SnO_2

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Abstract:

Textile waste production is one among the foremost polluting. The effluent contains several sorts of chemicals like dispersants, levelling agents, acids, alkalis and various dyes. Production processes not only generate heavily polluted waste water, but also waste heat, solid waste and exhaust gas. Photocatalytic degradation of Direct black 155 and Reactive red 152 dye were examined by using U.V. light in Photochemical reactor with SnO_2 photocatalyst. The consequences of varied operational parameters like catalyst dose, sort of dye and dye concentration were also investigated. The progress of treatment stages was examine by spectrophotometrically at different wavelengths.

Keywords: Textile dyes, Direct black 155, Reactive red 152, SnO_2 , Photochemical reactor.

Introduction:

The main problem stems from waste coming from industry and agriculture, despite the very fact that the population also plays a crucial role in environmental contamination. Phenols, pesticides, fertilizers, detergents, dyes and other chemical products are disposed of directly into the environment, without being treated, via discharging, controlled or uncontrolled and without a treatment strategy. During this general context, it's very clear that the strategy to continue within the search of solutions to the present problem that each day presents a sensitive growth, mainly within the developing countries (Abdessemed and Nezzal, 2002, Ameta and Ameta, 2016).

Large quantity of water is consumed within the washing of cloth at the top of every process there by producing huge amount of waste water (Cifci and Meric, 2015). So as to grasp the effluent problems facing the textile industry it's necessary to be conversant in the processes which end in effluent production. Textile mills are major consumers of water and consequently one among the most important groups of industries causing intense pollution. The extensively use of chemicals and water leads to generation of huge quantities of highly polluted waste water (Gultekin and Nilsun, 2004, Meena and Meena, 2020).

Advanced oxidation processes are chemical treatment given to such sort of pollutants, which couldn't be treated by conventional treatment methods like coagulation/flocculation, membrane separation (ultrafilteration, reverse osmosis) activated charcoal adsorption and biological treatment. Advanced oxidation processes oxidize or mineralize the pollutants into their simpler forms, which are easily biodegradable then it's facilitating their treatments in conventional processes, which are having a plus of being cheaper than the other process. AOP's are often homogeneous and heterogeneous in nature (Kuo, 1992, Meena and Dadheech, 2019).

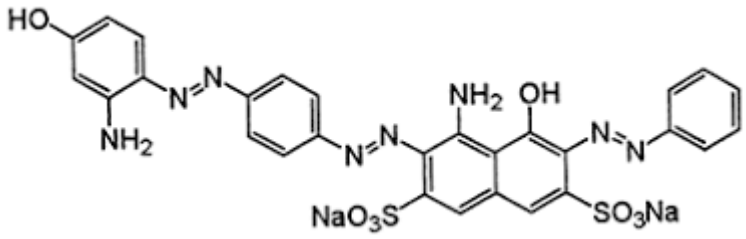
Photochemistry improved quantum efficiency of ultraviolet treatment for both the destruction of organic species and therefore the disinfection of water. Development of ultraviolet processes, to treat potable water and wastewater in distribution pipes and sewers. Environmental chemistry fate of emerging contaminants, particularly pharmaceuticals and nanoparticles, is known and risk analysis is administered. Photochemistry is that the study of the physical process of chemical changes which occurs in molecules on absorption of suitable radiation changing from 200 to 800 nm (Mills and Hunte, 1997, Meena and Meena, 2014, Pamecha *et al.,* 2016).

Material and Methods:

Dyes:

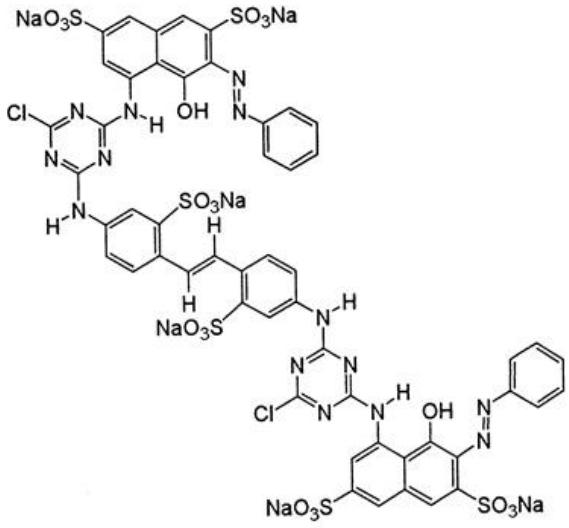
Direct Black 155:

Formula $C_{28}H_{20}N_8Na_2O_8S_2$ Weight 706.62



Reactive Red 152:

Formula $C_{52}H_{30}Cl_2N_{14}Na_6O_{20}S_6$ Weight 1752.11, λ_{max}-550 nm**.** Structure of dye shown below



Photocatalyst:

The SnO_2 catalyst was a GR grade material of 99% purity and was used without any further treatment (Raliya *et al.,* 2017).

Power of light:

The electromagnetic UV wave was used for the degradation of dyes (Rauf and Ashraf, 2009).

UV	Visible	Infrared
100-400nm	400-770nm	770-1050nm

Instruments:

Photochemical degradationwas carried out in a photochemical reactor equipped with UV tubes of 254nm. Constant stiring of solution was insured by using magnetic stirer. The spectra were taken with UV-VIS spectrometer, pH meter was used to adjust the pH of the solution (Shabudeen, 2011).

Procedure and Analysis:

To carry out the chemical reaction 1000 ml of dyes solution of desired concentration (1 x 10^{-3}M) was prepared in water. Thus both stock solutions of Direct black 155 and reactive red 152. Initial absorbance of dye solutions was observed with the assistance of UV-VIS Spectrophotometer. Before the runs, dye solutions were stirred for 10 min after the addition of catalyst SnO_2 to permit the physical adsorption

of dye molecules on catalyst surface to succeed in the equilibrium. About 3ml of sample of suspension was withdrawn with the assistance of syringe in beaker and reaction mixture was prepared by adding the catalyst (initially 0.01 gm). The entire volume of reaction mixture was made 100 ml by adding double water within the beaker. The concentration of the dye during a reaction mixture of Direct black 155 was $2X10^{-5}$ M and for Reactive Red 152 was $3X10^{-5}$ M. The experiments were administered under UV light within the photochemical reactor with UV tubes of 254 nm. Then the absorbance of dye in each degraded sample decided with spectrophotometer at λ max 664nm for Direct black 155 and λ max 550nm for reactive red 152 at different time intervals (10 min) after filtration through the centrifugal machine. The speed of reaction of decrease of colour with time was continuously monitored (Vandevivere *et al.,* 1998).

Results and Discussion:

The rate of decrease of color with time interval was continuously monitored. The result of photocatalytic degradation of Direct black 155 and reactive red 152 graphically presented in Table 1-2 and Fig 1- 2.

Table1: Photo bleaching of textile dye direct black 155 by SnO_2 reagent

Time (Hour)	Absorbance
0.0	0.500
0.5	0.460
1.0	0.420
1.5	0.310
2.0	0.200
2.5	0.185
3.0	0.170
3.5	0.140
4.0	0.123
4.5	0.080
5.0	0.015

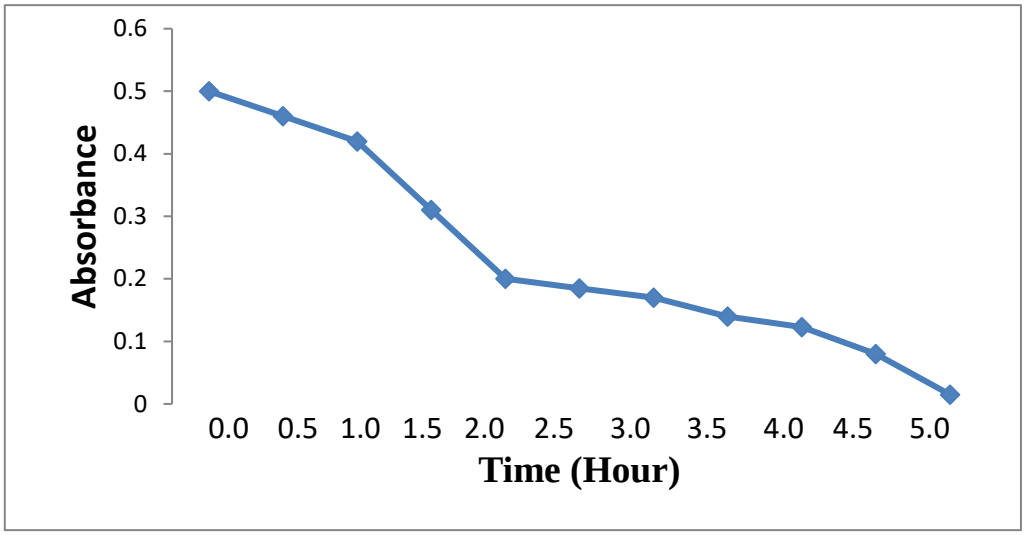


Figure 1: Photo Bleaching of Textile dye Direct Black 155 by SnO_2 Reagent

Table 2: Photo bleaching of textile dye reactive red 152 by SnO_2 reagent

Time (Hour)	Absorbance
0.0	0.480
0.5	0.430
1.0	0.350
1.5	0.310
2.0	0.200
2.5	0.180
3.0	0.160
3.5	0.150
4.0	0.123
4.5	0.280
5.0	0.040

Control Experiments confirmed the necessity of photocatalyst, to follow the photocatalytic degradation of Direct Black 155 and reactive red 152.

The photocatalytic degradation of Direct Black 155 were studied at λ_{max}-664nm and Reactive Red 152 at λ_{max}-550nm.

The optimum conditions observed for the removal of dye Direct Black 155 Concentration =2.0 x 10^{-5} M, pH= 7.5, SnO_2 = 0.1 gm

The optimum conditions observed for removal of dye (Reactive Red 152) Concentration (RR152) =3.0 x 10^{-5} M, pH= 8.0, SnO_2= 0.1 gm

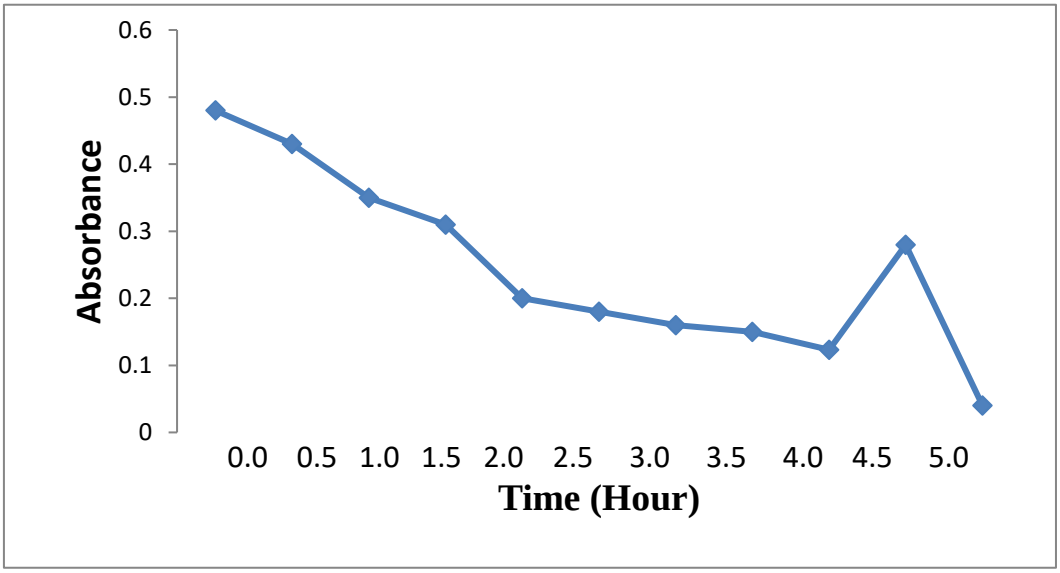


Figure 2: Photo Bleaching of Textile dye Reactive red 152 by SnO_2 Reagent

The rate of reaction was determined by using the expression:

Rate constant k (Direct Black 155) =2.303 x slope= 2.03 x 10^{-3} sec^{-1}

Rate constant k (reactive red 152) =2.303 x slope=8.4 x 10^{-4} sec^{-1}

The plot of 3+log O.D. found to be straight line suggesting that degradation of both dyes by SnO_2 follows a pseudo first order rate law. The effect of variation in reaction parameters has been studied like catalyst concentration, initial dye concentration, pH on the rate of degradation.

Effect of amount of photocatalyst:

The amount of SnO_2 powder can also affect the method of dye degradation. Keeping all the factors identical like initial dye concentration, pH, different amount of photocatalyst varying from 0.01g to 0.12 g/100 ml. it had been observed that the speed of dye decolorisation increases with increasing catalyst level up to 0.1g and beyond this the speed of reaction becomes almost constant. Same in Reactive Red 152 all the factors identical like initial dye concentration, pH, different amount of photocatalyst varying from 0.01g to 0.18 g/100 ml. it had been observed that the speed of dye decolorisation increases with increasing catalyst level up to 0.1 g and beyond this the speed of reaction becomes almost constant.

This may be observed thanks to the very fact that at the starting of reaction the quantity of catalyst increases the amount of catalyst active sites on the surface that in term increases the amount of OH and O_2- radicals. As a result the speed of degradation is increased. After a particular level of catalyst availability with an

equivalent concentration of dye, further dye molecules aren't available for adsorption. The extra catalyst particles aren't involved within the catalytic activity. Hence the degradation remains constant.

Effect of concentration of dye:

The effect of the dye concentration on the degradation of Direct Black 155 and Reactive Red 152 was studied at different concentrations varying from $0.20X10^{-5}M$ to $2.5 X10^{-5}M$ (D.R.) and $0.25X10^{-5}M$ to $6.0X10^{-5}M$ for reactive red 152 keeping all other factors identical. The result reveals that initial rate of increase with increase in concentration of dye. the very best rate of reaction was observed for $2.0 \times 10^{-5}M$ dye solution (Direct Black 155) and $3 .0 \times 10^{-5}M$ dye solution (Reactive Red 152). Further the speed of photo degradation of dyes decreases with the rise within the concentration of dye. The rationale behind it's that the rise in initial concentration of dye lies within the consistency of the hydroxyl radicals concentrations for all the dye molecules and thus the speed of decolorisation increase. Dye molecules adsorbed on catalyst surface and degradation occurs. On increasing the concentration of dye keeping catalyst doze constant, catalyst surface get saturated. Simultaneously intense color of dye doesn't permit light to succeed in photocatalyst.

Conclusion:

Photocatalytic degradation of two dyes Direct Black 155 and Reactive Red 152, has been investigated using SnO_2 catalyst. It was found that degradation depends upon various reaction parameters like amount of catalyst, dye concentration, pH. The photochemical degradation of active Red 152 by SnO_2 was effective within the removal of those dyes from solution. Additionally to the removal of colour reaction also proceed with the partial oxidation and it also completely degrade the toxic dye products. The photochemical efficiency has been generally found to extend with increase in catalyst loading up to a limiting value, decrease in initial concentration, increase in pH and UV candlepower. Therefore this easy technology of photochemical degradation of the coloured effluent has the potential to enhance the standard of the waste water from textile and also useful for other industries. The economy and faster rate of reaction could also be further improved by using certain modifications. This process has proved its superiority to other conventional methods of waste water treatments, within the presence of biorecalcitrant compounds. It results in complete destruction of

hazardous contaminants and avoids transfer of pollutants from one phase to a different. Photocatalytic process is dear thanks to application of UV light and catalyst.

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PHYTOSOCIOLOGICAL STUDY OF HERBACEOUS PLANT COMMUNITY IN NORTH-EAST PART OF MALEGAON FOREST, DISTRICT NASHIK (MAHARASHTRA)

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Abstract:

The quantitative analysis of phytosociology of North-East Malegaon forest based on frequency (%), density and abundance data is given for 4 localities and North-East Malegaon forest as a whole. The vegetation of the Malegaon forest is of a dry deciduous type with thorny species .The dominant communities are variable in different localities. It is *Phyllanthus-Achyranthus-Senna-Parthenium-Euphorbia* as a whole forest. A study of frequency classes shows that the vegetation is homogeneous for all localities and the forest as a whole. The variation of frequency classes in different localities may be due to biotic interference, occurrence of numerous sporadic or accidental species.

Keywords: Phytosociology, frequency, density, abundance, Malegaon forest.

Introduction:

Phytosociology deals with the qualitative study of the structure of the vegetation with an emphasis on quantitative relationship of a few species which are judged to be dominant on the belief that these largely control the community and thereby the occurrence of a large number of rare species. There are detailed accounts on the Phytosociology of Chhotaudepur forest (Shah *et al.,* 1979), Panchmahals, Dang forest (Yadav, 1979) and from Maharashtra Tryambakeshwar, Vani and Saptshringi (Jadhav, 2004) Talegaon (Jadhav, 2016) Sapgaon (Jadhav, 2018) Tryambakeshwar (Jadhav, 2019) Saptashringi forest (Jadhav, 2020). A similar investigation is carried out in Malegaon forests with a view to study the communities in different localities and to analyse them objectively with reference to frequency (%), density and abundance and to note variations.

Materials and Methods

40 quadrates of 5m X 5m were laid down in different directions in each locality, so that quadrats represented almost all species in the area. All together 40 plots covering 1000 Sqm were laid down. By using the formulae of Raunkiaer (1934), the frequency, density, abundance and heterogeneity of vegetation were calculated.only the frequency is tabulated in table 1 for each species in locality tp reduce the size of the table without affecting the merits of our obertvations. The commuties are also named after the species having higher percentage of frequency (Table 2). When two species have equel frequency percentage, abundance is also taken into consideration.The species are divided into five frequency classes (Raunkiaer, 1934):

Class A 1-20%; Class B 21-40%; Class C 41-60%; Class D 61-80 %; Class E 81-100 %.

The quadrates are studied at Malegaon taluka forest (stand 1-4). The selection of the quadrate sight in a locality also depended on the density and diversity of the vegetation.

Study area:

The Nashik district is located in the western ghats, is situated at 19° 33' – 22° 53' N and 73° 16' – 75° 06' E. Malegaon lies 18° 25' 12" N 77° 31' 48" E. The study area is 115 km away from district headquarter Nashik. Total geographical area of study is 773 hectares (7730000sqm).

Soil and Geology:

Northern, Western and Southern parts of Malegaon Tahasil are surrounded by hills. It is sloping area towards the eastern side. All the rivers in this region flow from west to east. The variation of lands can be observed here. Hills, forests and plains are found in Malegaon Tahasil. The height of plains is from 600 to 600 meters while the hilly areas from 600 to 900 meters from sea level. Except the northern and south-west parts, the whole area of Malegaon is covered by plains. Due to Girana, Mosam Panjan,Parsul and some small rivers, the central part of Malegaon is more fertile than the hilly regions. According to the geographical structure, Malegaon taluka have black, brown and medium type of soil.

In the southern part from Galna fort to Satmala ranges, the entire area comes in the valley of Sahyadri ranges.has formed by igneous rocks. Therefore the soil is black , soft and suitable for cultivation. The eastern part of Malegaon is covered by plains. The middle part from Daregaon hill to Lallimg area is barren. In eastern part the soil is hard which creats problems in the way of cultivation of land. All the land covered with small hills.

Climate, Temperature and Rainfall:

Malegaon is located about 350 km away from Arabian Sea towards the East of Sahyadri ranges. Consequently the climate of Malegaon is hot in summer and cold in the winter and slightly humid in the rainy season. As a result, both the summer and winter seasons are severe. Generally, Malegaon is grouped under the monsoon region. The seasons are divided as follows:

(i) Summer - from March to May

(ii) Rainy Season - from June to October

(iii) Winter - from November to February.

May is the hottest month of the year. The maximum temperature in May goes upto 44º C. On the contrary temperature comes down as low as upto 7o C to 5o C in winter in December and January which are the coldest months. The following table shows the maximum and minimum temperature of Malegaon.

Sr. No.	Seasons	Max. Temp	Min. Temp
1.	Summer	44º C	35º C
2.	Rainy Season	30º C	20º C
3.	Winter	34º C	20º C

The maximum temperature was recorded 47.7º C in the year 1916, while the minimum temperature was recorded 0.6º C in January 1935. With the growth of population, several new industries, slum areas, housing colonies and traffic increased on a large scale. On the other hand, farming lands, trees and forests are cleaned up for the new construction. As a result, the problem of pollution has emerged as a major problem in the city. Hence, the weather of Malegaon has been extreme.

The rainy season is from June to October. Malegaon is located in the leeward of Sahyadri ranges, therefore, it does not receive more rain. The western part of Malegaon, which is surrounded by forest and is situated on the hills, receives more rain than the rest part of Malegaon.

Observations:

From the Tables 1 and 2, it is seen that *Phyllanthus-Achyranthus-Senna-Parthenium-Euphorbia* though form a dominant community for the Malegaon North- East as a whole forest . It is not so for different localities , where the dominant communities differ even among various localities .

Phyllanthus-Achyranthus- Senna – Parthenium- Euphorbia are the members of the dominant community in most of the localites . The other members frequently found in the dominant community composing are *Desmodium* and *Passiflora* wheras Boerhavia *and*

Portulaca in a few localites. *Phyllanthus-Achyranthus* has the highest frequency at all four stands, but it has equal frequency with *Parthenium-Senna* in stand 1and 2 . It is also interesting to note that it most abundant at stand 3 and 4. At other places it is *Euphorbia* at stand 3 and 4.

Table 1: Frequency (%) of species represented in quadrates in different forest localities in Malegaon North East and mean frequency (%) of Malegaon North East forest as a whole. The species are arranged in order of higher frequency for the Malegaon North East forest

Sr. No.	Species	Localities				Ave Freq.
		1	2	3	4	
1	*Phyllanthus amarus*	100	100	80	100	95
2	*Achyranthes aspera*	100	100	100	80	95
3	*Senna tora*	100	100	80	80	90
4	*Parthenium hysterophorus*	100	80	80	100	90
5	*Euphorbia hirta*	100	40	100	100	85
6	*Indigofera tinctoria*	80	100	60	100	85
7	*Cynodon dactylon*	100	100	100	20	80
8	*Amaranthus viridis*	100	40	100	80	80
9	*Oxalis corniculata*	100	100	60	40	75
10	*Acalypha indica*	80	60	80	60	70
11	*Chenopodium album*	80	80	100	20	70
12	*Euphorbia hypericifolia*	40	40	100	100	70
13	*Setaria viridis*	80	80	60	60	70
14	*Sida acuta*	40	80	80	40	60
15	*Ageratum houstonianum*	60	60	60	40	55
16	*Leucas aspera*	80	60	20	60	55
17	*Ipomea hederacea*	40	00	100	60	50
18	*Desmodium gyrans*	40	40	40	60	45
19	*Passiflora foetida*	20	20	60	60	40
20	*Euphorbia milii*	40	00	40	60	35
21	*Portulaca oleracea*	00	40	20	80	35
22	*Boerhavia diffusa*	20	00	60	60	35

Table 2: Communities, frequency classes and degree of heterogeneity in different localities in Malegaon North East forest

Sr. No.	Localities	Communities	Frequency Class					Degree of Heterogeneity
	Malegaon North East forest		A	B	C	D	E	
1	Stand-1	*Phyllanthus-Achyranthus- Senna – Parthenium-Euphorbia*	9.52	23.80	4.76	23.80	38.09	2.16
2	Stand-2	*Phyllanthus-Achyranthus-Senna-Indigofera– Cynodon*	5.26	26.31	15.78	21.08	31.57	1.25
3	Stand-3	*Achyranthus-Euphorbia-Cynodon-Amaranthus-Chinopodium*	9.09	9.09	27.27	22.72	31.81	1.49
4	Stand-4	*Phyllanthus-Parthenium-Euphorbia-Indigofera Achyranthus*	09.09	13.63	36.36	18.18	22.72	0.81
5	Malegaon North East forest as a whole	*Phyllanthus-Achyranthus-Senna-Parthenium-Euphorbia*	0	18.18	22.72	31.81	27.27	1.44
6	Raunkiaer's normal frequency classes		53	14	9	8	16	1.05

Thus the species with wide range of distribution in many localities and with much higher frequency supports the visual observation that such species are common. Similarly those species which have restricted distribution in one or few localities have also higher frequency, including that their distribution is more in these localities than in others. Their non-occurrence in quadrats in other localities may be that they are rare to casual in such localities or their distribution in these localities is such that they have not been encompassed in the quadrats.

Some members of the vegetation are not represented in the quadrats. They are *Astragalus, Medicago sativa, Lamium album and Xanthium strumarium* etc. Such species are rare, at times very much restricted in distribution.

From Table II , it will be also seen that , for North - East Malegaon forest area as a whole , frequency classes E and D collectively make up 59.08 % and frequency classes B and C 40.9 % of the total frequency . The prevalence of frequency classe D is much higher (31.81%). The vegetation of the forest as a whole is much homogeneous when compared to the frequency classes for heterogeneous vegetation by Raunkiaer (1934).

In general frequency class A is absent in stand - 4.The classes D and E have higher frequency percentage than other classes in all localities. Class D and E in general also has higher frequency. The absence of class A at stand-4 suggests a much degree of disturbance in vegetation where it may be equal to either of them. A comparison of frequency classes in each locality with those of Raunkiaer (1934) suggests that the vegetation is heterogeneous except stand-1, with the digree of heterogeneity 1.00-1.5. The relatively high frequency of the species in various localities in general suggests denseness of the vegetation

Discussion:

Values of frequency classes D and E are higher and A is lower. Class D and E is higher than those of Raunkiaer (1934) frequency classes at stand 1, 2, 3 and 4 for homogeneous vegetation. However, these values for classes B, D and E are much higher and of other classes much lower than those of Raunkiaer (1934) frequency classes for the Malegaon forest showing that the vegetation of the whole forest is homogeneous.

The better representation of class E in some localities indicates that the vegetation is still not much disturbed and is more or less uniform in nature. This is clearly seen from the 100 % frequencies of first three dominant species at stand -1, 2 and 4.The absence of class A at Stand- 4 suggests some disturbance in vegetation due to factors like fire and anthropogenic conditions (Misra, 1974).

Relatively higher values of frequency class A in some localities like Stand- 1 and2 and much higher in North-East Malegaon as a whole forest and class B is higher in stand 1, 2 all localities are due to numerous sporadic or accidental species (Oosting, 1956). They bring about changes in otherwise heterogeneous vegetation. At the same time the dispersal of seeds also affect the value of class A (Pandya, 1968).

With reference to the number of species in all 4 stands, the highest number of species is at stand -3and 4(22) and lowest at stand -2 (19) . The low number of species may be attributed to destruction of forests in vicinity of these places.

Relationships between the total number of individual and frequency are also of interested. In general the following types of relationships can be assumed.

Type I – species showing both a high frequency D and E class and a comparatively high number of individuals.

Type II- species with low frequency and high density and abundance.

They predominantly occur in larger groups, clusters or patches during our study a similar situation is found with reference to some of the species represented in various stands. For the first time, phytosociological observations of herbaceous vegetation bring out considerable new information on the vegetation of North-East Malegaon forest, based on a systematic study of 4 stands, most of them not studied earlier from this view point.

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STUDY OF SOIL NEMATODES ASSOCIATED WITH GARDEN PLANTS IN AURANGABAD

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Abstract:

Present investigation deals with the plant nematodes collected from the different areas of Aurangabad. In this study the soil nematodes are found from all the sites of study areas. The nematodes collected were belonging to 7 genera and their occurrence varies with the sampling sites. Among all the sites studied the maximum number of nematodes was found in Site-III (47) and the minimum (7) number of nematodes found in Site-IV. Nematdes found belongs to genera *Helicotylenchus*, *Mesodorylaimus, Eudorylaimus, Discolaimus*, *Xiphinema, Mesorhabditis* and *Mylonchulus*. This research will also provide a platform for the upcoming researchers and also will add the knowledge regarding taxonomy of soil nematodes.

Key words: Soil nematode, Aurangabad, Garden plants, Occurrence

Introduction:

Nematodes represents a relatively small amount of biomass in soil, their key positions at most trophic levels in soil food webs are vitally important in soil environment and ecosystem processes (Barker and Koenning, 1998). Soil is rich habitat for nematodes with about 26% of described genera inhabiting soil as bacterivores, fungivores, omnivores, predators or plant parasites (Wharton, 2005). It is said that wherever there is soil there are nematodes. The nematodes constitute a dominant and an important biota of the soil ecosystem with varying kinds of feeding habits and numerous feeding interactions with co-habiting animal and plant groups.

These are microscopic roundworms are widely distributed even in extreme environments where water availability is limited (Freckman and Mankau, 1977; Treonis *et al.,* 1999; Wall and Virginia, 1999; Porazinska *et al.*, 2002.). Some soil nematode species feed on bacteria or fungi, while other are omnivores, predaceous or parasites of plant roots

(Yeates *et al.*, 1993). Nematodes play important roles in belowground carbon and nutrient cycling and influence ecosystem functions such as plant growth and decomposition (Freckman, 1988; Wardle *et al.,* 2004).

Nematodes assemblage analysis are useful in accessing soil ecosystem status and function since nematodes are ubiquitous and easy to sample and classify in feeding groups and functional guilds and they are sensitive to environmental changes. Hence nematode faunal analysis is evolving as powerful bioindicator of soil conditions and structural functional attributes of the whole soil food web (Bongers and Ferris 1999; Neher, 2001; Berkelmans *et al.,* 2003; Yeates, 2003). Subsequently, it became readily apparent that nematode communities would serve as good indicators of environmental quality in terrestrial ecosystems, whereas initially (1970s) nematode genera and total nematode abundance/copepod ratios were indicators in aquatic environments (Neher, 2001a). According to Schloter *et al.* (2003) faunal indicators in the soil food web should be ubiquitous across environments, abundant and 13 important in ecosystem function, and have high diversity

Due to the nematode infestation many changes are caused to the plants which automatically affect the yield. Thus there are economic loses to the farmers. Regarding these issues study of soil nematodes from our area is utmost important. The research will also provide a platform for the upcoming researchers and also will add the knowledge regarding taxonomy of soil nematodes.

Study area:

The study area includes four different sample collection sites of Aurangabad. Soil samples were taken from following sites:

Site-I (CIDCO Mahanagar),

Site-II (garden soil of NRB Bearings),

Site-III (garden soil from university Campus) and

Site-IV(garden soil from Deogiri College Campus).

- Weekly sample collections were done during the months of December 2018 to November 2019 in the morning hours.
- The sample size is of 200 to 250 grams and was taken from the depth of about 12-15 cm near to the plantations with a hands shovel.
- Samples were placed in plastic bags, sealed and brought back to the laboratory and stored for nematode extraction.

- Each soil sample was thoroughly mixed by shaking the plastic bags Cobbs (1918) and nematodes extracted from soil sample by Burman's funnel method Thorne. G(1961).

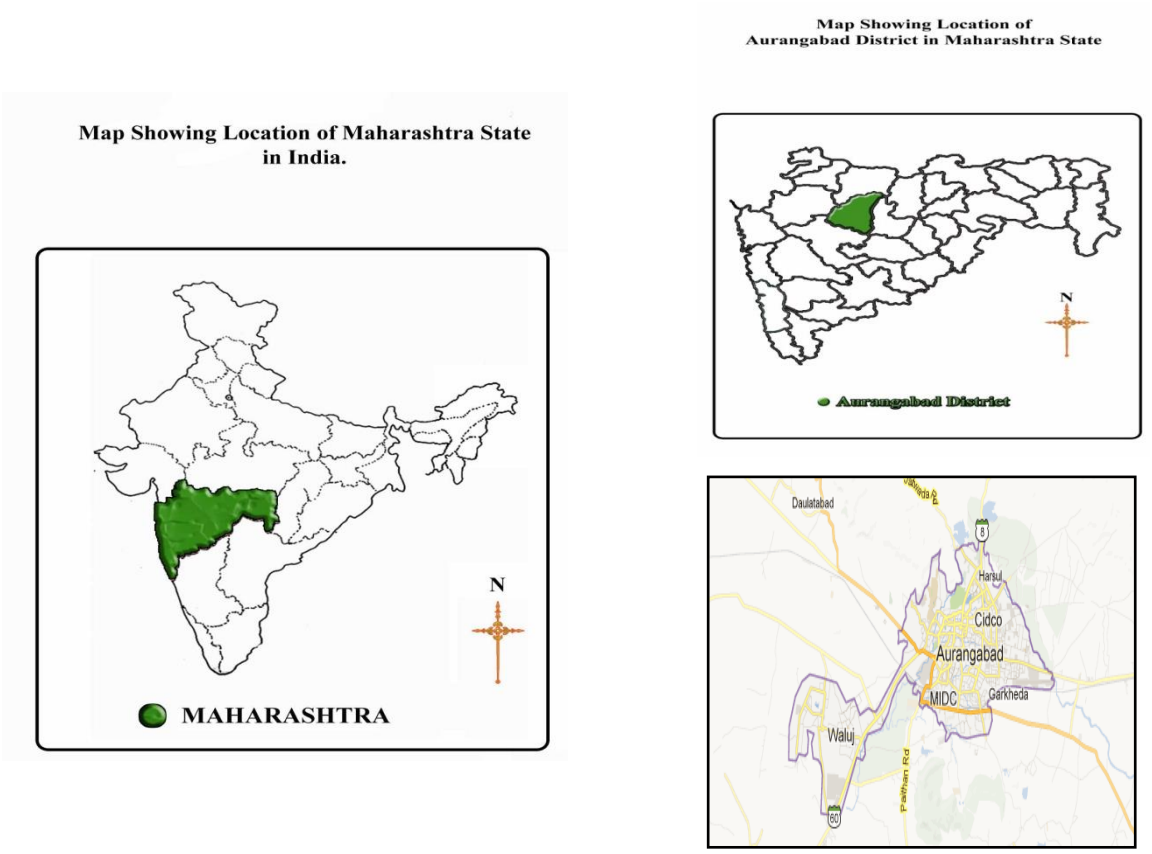


Plate 1: Map Showing the Location of Study Area

- Prior to nematode counting, vials containing nematode suspension were agitated thoroughly and an aliquot (3ml) was poured to counting dish.
- Nematodes were counted with the help of *Syracuse* counting disc under 40x magnification using the stereoscopic binocular microscope.
- Extracted sample was observed under stereoscopic binocular microscope for collection and counting of nematodes.
- Isolated nematodes were killed in hot water and fixed in FAA solution (1:1) placed in vials Steinhorst (1967).
- Selected specimens for each of the recorded species were dehydrated by Steinhorst's (1959) rapid glycerin method and mounted on glass slide in anhydrous glycerin.
- The Eppendorf tubes were labelled depend on its locality, date of collection and name of collector.
- Nematodes were fixed according to De Grissee (1969)and the slides were prepared by ring method according to Hooper (1986) and Southey, J. F. (1970).

- Based on morphological characteristics of adult and juvenile forms the nematodes were identifiedupto generic level (Mai and Lyon, 1975)
- Microphotographs were taken under Research Microscope **(**Lawrence and Mayo**)** with the help of digital camera.
- The measurements were taken by using "Occularmicometer" in µm.
- Statistical calculations were made by using following formula:

  Incidence= No. of infested samples x 100

  Total no. of samples surveyed

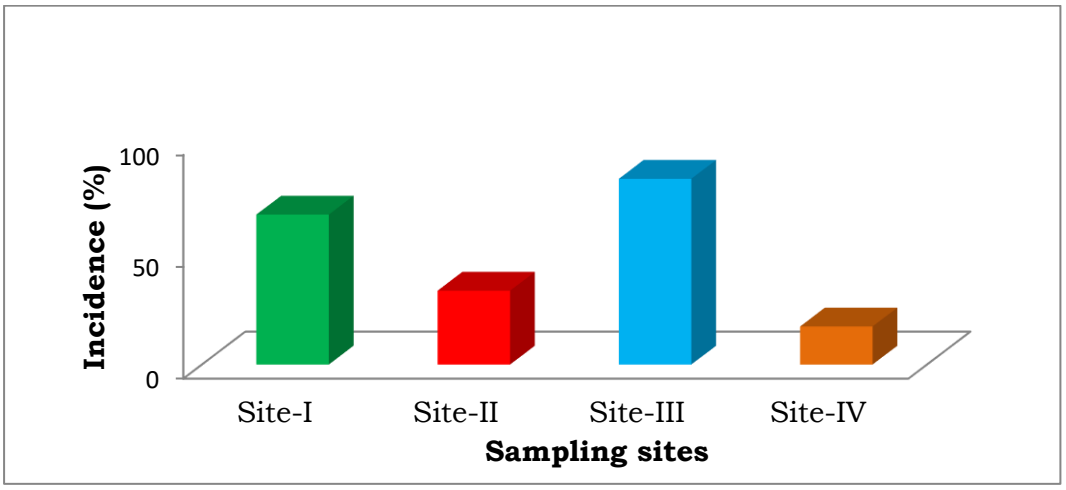
Results:

In this study the soil nematodes are found from all the sites of study areas. The nematodes collected were belonging to 7 genera and their occurrence varies with the sampling sites.In the present study among all the sites studied the maximum number of nematodes was found in Site-III (47) and the minimum (7) number of nematodes found in Site-IV.

Similarly the incidence was maximum in Site-III (83.33%) compared with other sites whereas it was minimum in Site-IV (16.66%) (Table 1). Among the 4 sites studied the Site-III having maximum amount of nematode infection (83.33%) whereas the minimum was found at Site-IV (16.66%). From all the genera found the *Xiphinema sp.* is having highest number 21(44.68%) from Site-III and the minimum number i.e. only single specimen of *Mesodorylaimus sp.*, *Eudorylaimus sp.*, *Mesorhabditis* sp. was found in Site-II and Site-IV respectively.There is also no infection of *Helicotylenchus* sp., *Discolaimus sp.* and *Mesorhabditis sp.* in Site-II and Site-IV respectively (Table 2).

Table 1: incidence of nematodes from various sampling sites of Aurangabad

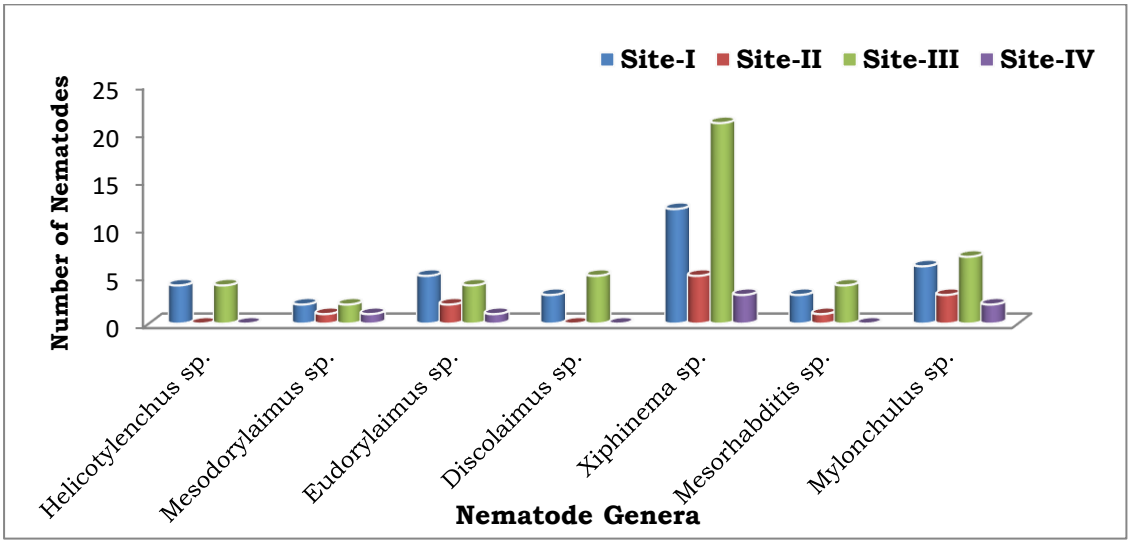
Sr. No.	Study Area	No. of samples collected	No. of infected samples	No. of nematodes	Incidence (%)
1	Site-I	6	4	35	66.66
2	Site- II	6	2	12	33.33
3	Site- III	6	5	47	83.33
4	Site -IV	6	1	7	16.66



Graph 1: Incidence of nematodes from all the sampling sites of Aurangabad

Table 2: Occurrence of nematode genera with respect to their Sampling Sites from all the study areas of Aurangabad

Sr. No.	Genera	Site-I	Site-II	Site-III	Site-IV
1	*Helicotylenchus* sp.	04(11.42%)	-	04(8.51%)	-
2	*Mesodorylaimus sp.*	02(5.71%)	01(8.33%)	02(4.25%)	01(14.28%)
3	*Eudorylaimus sp.*	05(14.28%)	02(16.66%)	04(8.51%)	01(14.28%)
4	*Discolaimus sp.*	03(8.57%)	-	05 (10.63%)	-
5	*Xiphinema sp.*	12(34.28%)	05(41.66%)	21(44.68%)	03(42.85%)
6	*Mesorhabditis sp.*	03(8.57%)	01(8.33%)	04(8.51%)	-
7	*Mylonchulus* sp.	06(17.14%)	03(25%)	07(14.89%)	02(28.57%)



Graph 2: occurrence of various nemtode Genera from different sampling sites of Aurangabad

Discussion:

In the present study the nematodes found were maximum from the Site-III (47) which is of fully irrigated as compared to the other sites and hence the moisture content is maximum in that area. The same study was done on increase in nematode populations with season would be probably due to moisture (Wallace, 1983; Vrains, 1986; Jordaan *et al.*, 1989) and ease of movement of the nematodes through the large soil pore diameter and soil particle size (Taylor and Sasser, 1978; Idowu, 1981; Prot and Van Gandy, 1981), which are typical properties ofa generally sandy soil which was the predominant soil where the study was conducted.

In the present study there is variation in the occurrence of nematodes according to the study area. The four sampling sites are having slightly variation in their temperature and humidity which influence the nematode population. The main climatic factors to exert an influence on the development of nematodes are temperature and humidity. These factors are indirectly primary act.

Seshadri (1964) studied the severity effect of soil humidity on population density of *Criconemoidesx enoplex* nematode. Nematodes which have penetrated a plant were affected indirectly through the plant by a moisture deficiency.

In this study seven genera of nematodes found are as *Helicotylenchus*, *Mesodorylaimus, Eudorylaimus, Discolaimus, Xiphinema, Mesorhabditis* and *Mylonchulus*. The observation of the genera *Ditylenchus, Helicotylenchus, Meloidogyne, Pratylenchus*, and *Scutellonema*to occur more frequently on cereals than the other genera corroborate with other reports (De Waele *et al.*, 2000; McDonald and Nicol, 2005). The presence of *Meloidogyne* sp. on cereals in Uganda suggests that root knot nematodes can be important pathogens of maize although damage caused by *Meloidogyne* spp. on cereals is greatly under estimated worldwide (De Waele *et al.*, 2000; McDonald and Nicol, 2005).

Since nematodes require a water film around soil particles in which they move, feed and reproduce (Bardgett, 2005), this extreme drought will have logically affected the nematode population adversely.Thus the nematodes found in the present study has adverse effect of climatic factors. It directly related to their population number. Here the nematodes were found maximum in number from the Site-III which was fully irrigated one and has ample amount of water so it will enhance the humidity of soil which is the main factor affecting the nematodes. In contrast to this the minimum number found in Site-II which is having less humidity in soil and also the availability of water is very less compared with the other sample sites. These findings support the results of previous workers from different parts of the world.

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A STUDY ON FERTILIZER RESIDUES IN SOIL SAMPLES OF AGRICULTURAL AREAS FROM PANDAVAPURA TALUK, MANDYA DISTRICT

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Abstract:

The research on fertilizers is currently focusing on reducing the harmful environmental impacts of fertilizer usage and find new, less expensive sources of fertilizers. Supplying fertilizer in a form which is less susceptible to runoff and making more concentrated mixtures. Materials added to the soil or applied directly to crop foliage to supply elements needed for plant nutrition. These materials may be in the form of solids, semisolids, slurry suspension, pure liquids, aqueous solution or gases. The chemical elements are Nitrogen, Phosphorus and Potassium which are the macronutrients, or primary fertilizer elements which are required in greatest quantity. Sulphur, calcium and magnesium are called as secondary fertilizer elements. In the present study, the soil samples were collected in different locations.and subjected for analysis. From the experimental results, it was observed that, inmost of the soil samples, the organic carbon showed the higher percentage. It indicates the fertility of soil in that area is good for cultivation and for better yield. The Calcium, Sodium, Potassium and Phosphate concentrations are within the normal range, which implies that, the soil samples in the study area were not polluted with any inorganic contaminants of salts and acids.

Keywords: Fertilizers, Soil, Urea, DAP, Organic carbon.

Introduction:

Chemical fertilizers are the type of nutrients which are applied to promote pant growth. They play a significant role in the efficient management in quality and quantity of food production [1].The major nutrients applied in the form of synthetic fertilizers are Nitrogen, Phosphorus and Potassium and other nutrients are added in smaller amounts. Chemical fertilizers are normally applied either by surface broadcast method or foliar spray

[2, 3 and 4]. In Western Australia and in many other countries, deficiencies of micronutrients are identified as a major factor for the conversion to unproductive lands in the 1940's and 1950's [7, 9]. Excessive use of chemical fertilizers has many environmental complications. It results in depletion of soil nutrients, soil acidity, destruction of beneficial soil micro flora, heavy metal pollution, air and water pollution problems [10, 11 and 12]. With this concept, the present work is undertaken with an objective to assess chemical fertilizers residues and its effect on soil physico-chemical characteristics.

Materials and Methods:

Study area:

Pandavapura is one among theTaluks of Mandya district, Karnataka, India. It is located at 12.5^{0}N 76.67^{0}E. The taluk has elevation of about 709m. The town is enclosed with large acres of agricultural lands. The major cultivable crops in this region are sugarcane with other seasonal crops. The inhabitants of this town are mainly dependent on agriculture as major occupation. The study area is enclosed by many villages and it is considered as the central point in the town. The Visweshwaraiah Canal pass through the town,. The water for this canal is sourced from the Krishnaraja Sagara, which is the reservoir across the river Cauvery. During the cropping seasons, the different types of major and minor nutrients are added in the form chemical fertilizers.

Sample collection:

The soil samples were collected in different locations of Pandavapurataluk. The soil was collected by zigzag manner in the agricultural fields at the depth of 15 cm in a hectare. Then, the soil samples were allowed to dry at room temperature up to 5 days and the soil was not directly exposed to sunlight. The collected soil sample was sieved and preserved for further experimental analysis.

Analysis of soil samples:

The soil sample were analyzed for pH, Electrical conductivity, Organic carbon, Chloride, Phosphate, Nitrate, Nitrite, Sodium and Potassium, Bulk density, Particle density, Porosity, Ammonia Nitrogen, DAP and Urea. Urea is determined by 2 M PMA-KCL methods. The soil pH was determined by pH meter and Electrical conductivity was recorded by using conductivity meter. The Organic carbon was determined by using walky and black method. The Chloride was determined by titrimetric method. The Sodium and Potassium were determined by flame photometric method. The Phosphate was determined by Stannous chloride method. The Nitrate was determined by Brucine method. The Nitrite was determined by sulphanilic acid method. Bulk density and Particle density were determined by volumetric flask method. Ammonia nitrogen is determined by Nessler's reagent method.

Result and Discussion:

pH: The measurement of pH shows the acidity and basicity or alkalinity of the Soil. The Soil with pH greater 8.5 is generally called as sodic soil. During the present study, the pH values varied from 3.3 to 6.3. Highest values were recorded in S-1 and lowest were recorded in S-3. In comparison with normal range, the pH values were found to be below and the values reflects the acidic nature of soil.

Table 1: Soil sampling places, crops cultivated and types of fertilizers applied in Pandavapura taluk Mandya district

Sr. No.	Soil samples	Name of the Place	Crops cultivated	Type of fertilizers applied
1	S-1	Singrigowdanakoppalu	Cucumber	Urea; DAP; 20:20
2	S-2	Bellaale	Tomato	DAP; ZINC; 20:20
3	S-3	Mahadeshwarapura	Paddy	Urea; 20:20
4	S-4	Beer shettihalli	Tomato	Urea; Potash
5	S-5	Kuppalli	Beans	Urea; 20:20
6	S-6	Lakshmisagara	Sugarcane	Urea; DAP
7	S-7	Hosakote	Mariegold(yellow)	DAP; 20:20
8	S-8	Kodaala	Ragi	Urea
9	S-9	KereThonnur	BT-Brinjal	20:20; DAP
10	S-10	Eeregowdanahalli	Capsicum	DAP; 20:20

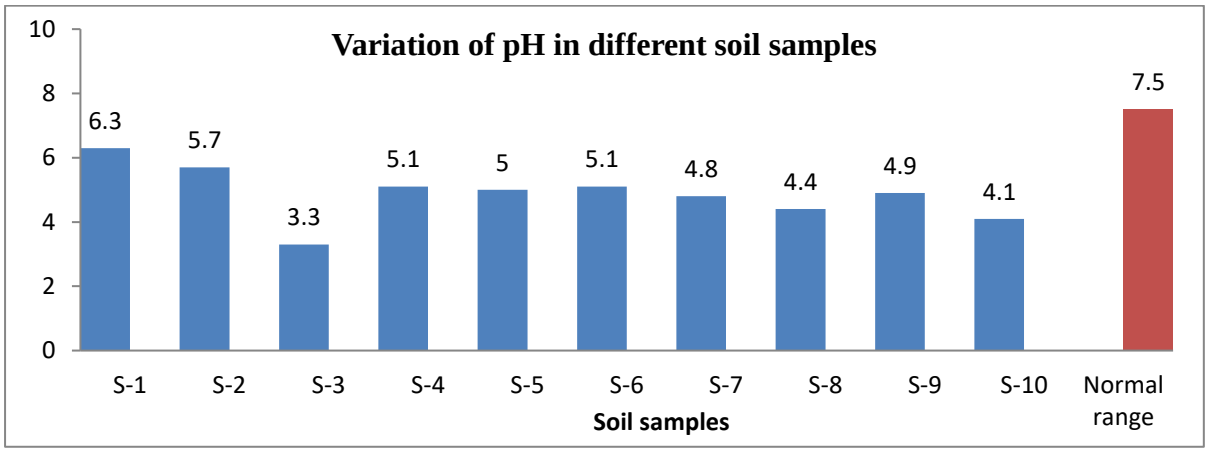

Figure 1: Graphical representation and variation of pH in different soil samples

Electrical Conductivity: In the presentstudy, the conductivity values ranged from 0.01 to 0.14 ms^{-1}. Higher values were recorded in S-10 and lower values were recorded in S-2. In comparison with normal range, all the soil samples were found to be within the normal range. So, the soil samples in the agricultural areas are found to be safe for cultivation of crops.

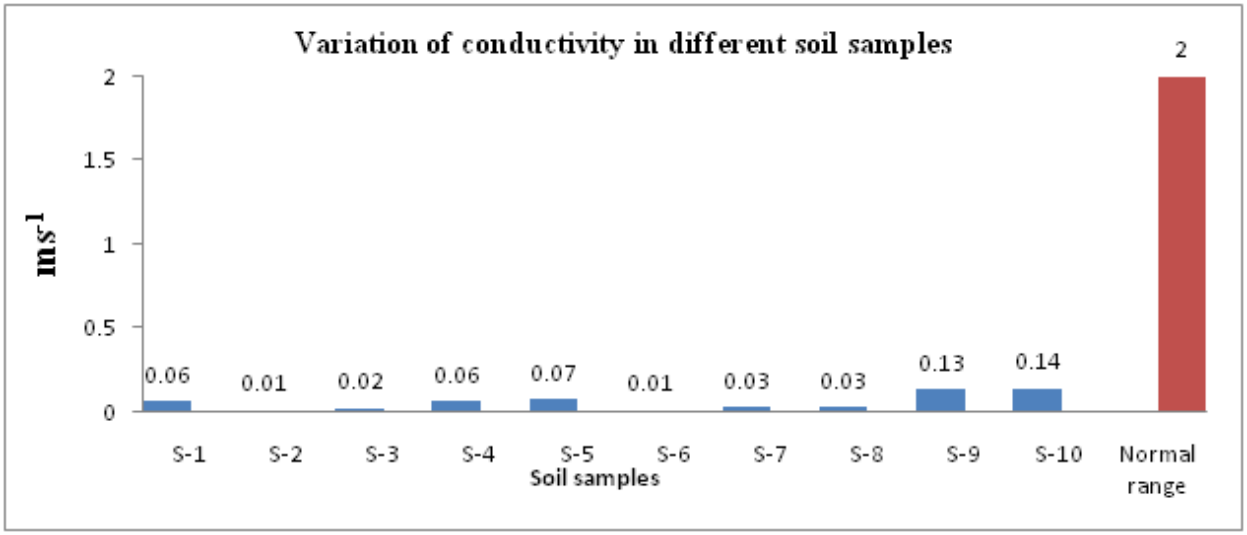

Figure 2: Graphical representation and variation of Conductivity in different soil samples

Chloride: Excess accumulation of salts makes soil alkaline. Salt contain ions responsible for salinization of soil. Due to accumulation of ion such as chloride, the soil becomes sodic. According to the normal range, the permissible limit is 0.020-0.120 %. So, the soil sample of the study area was found to be above the normal range which is due to the geological processes. The higher values are due to the input of excess sodium chloride presence in the soil complex.

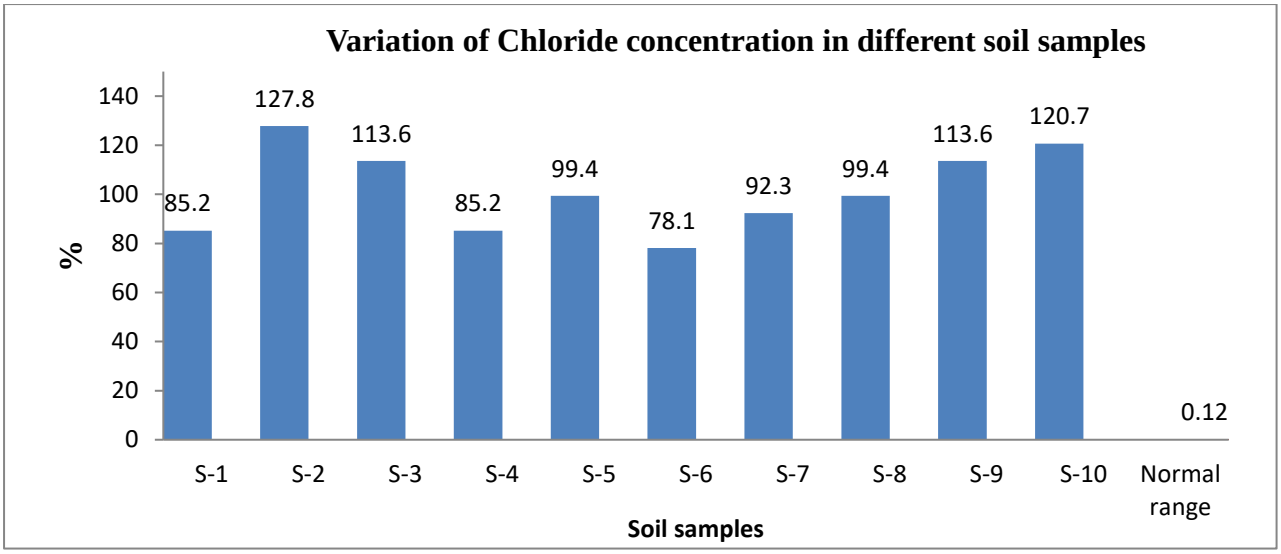

Figure 3: Graphical representation and variation of Chloride concentration in different soil samples

Organic Carbon: Organic carbon content is very much essential for the stability of soilparticals. The normal range for organic carbon is 0.50-0.75 %. The results shows, higher percentage of organic carbon except S-6. The higher amount is due to the application of soil amendment such as, animal manure, crop residues and green manures.

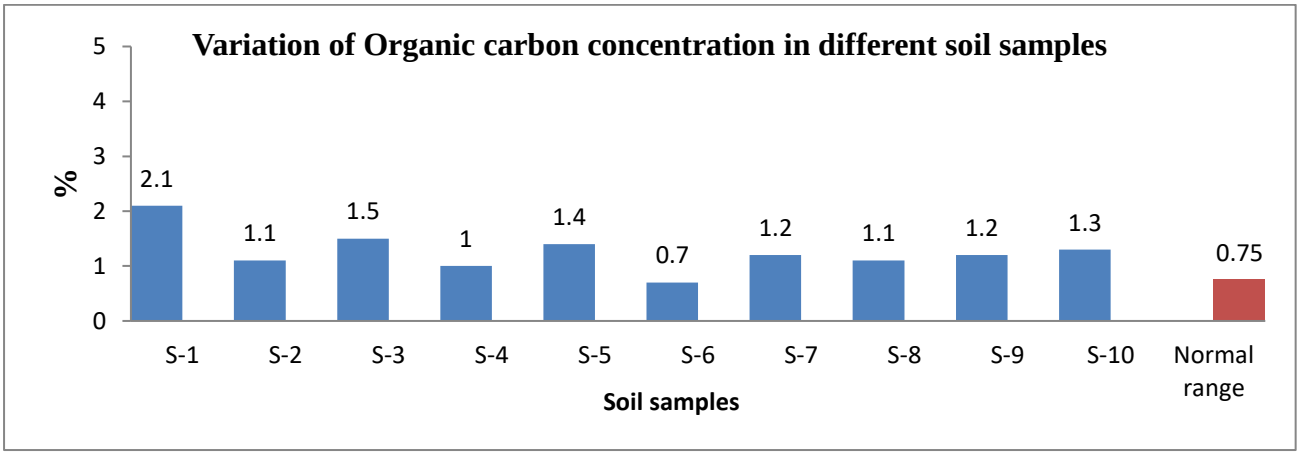

Figure 4: Graphical representation and variation of Organic carbon concentration in soil samples

Calcium and Magnesium: Calcium and Magnesium are very important elements for plants life. These are however required in comparatively small amounts and are known as secondary nutrients. Calcium accumulates in plants as calcium acetate and is essential for the growth of root tips at higher concentrations. Magnesium is widely distributed in soils and is mainly involved in the activation of many enzyme complexes and also in phosphorus transfer process.The normal range for calcium and magnesium in agricultural soils is 10-30 Meq/L (for Ca^{2+}) and 5-10Meq/L (for Mg^{2+}). The present investigation shows experimental

values within the normal range. In case of magnesium, all the soil samples were found to be above the normal range.

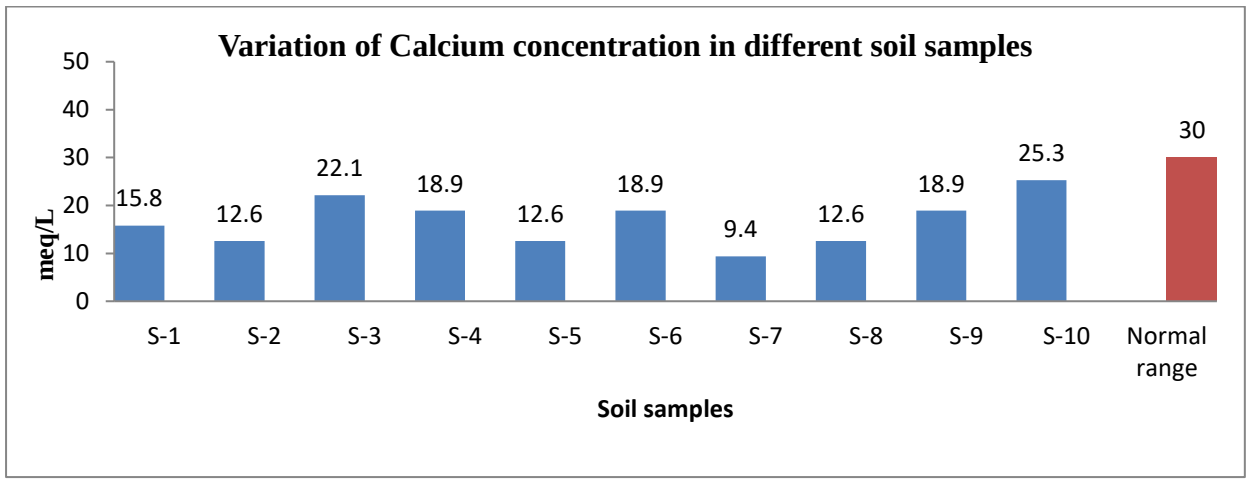

Figure 5: Graphical representation and variation of Calcium concentration in different soil samples.

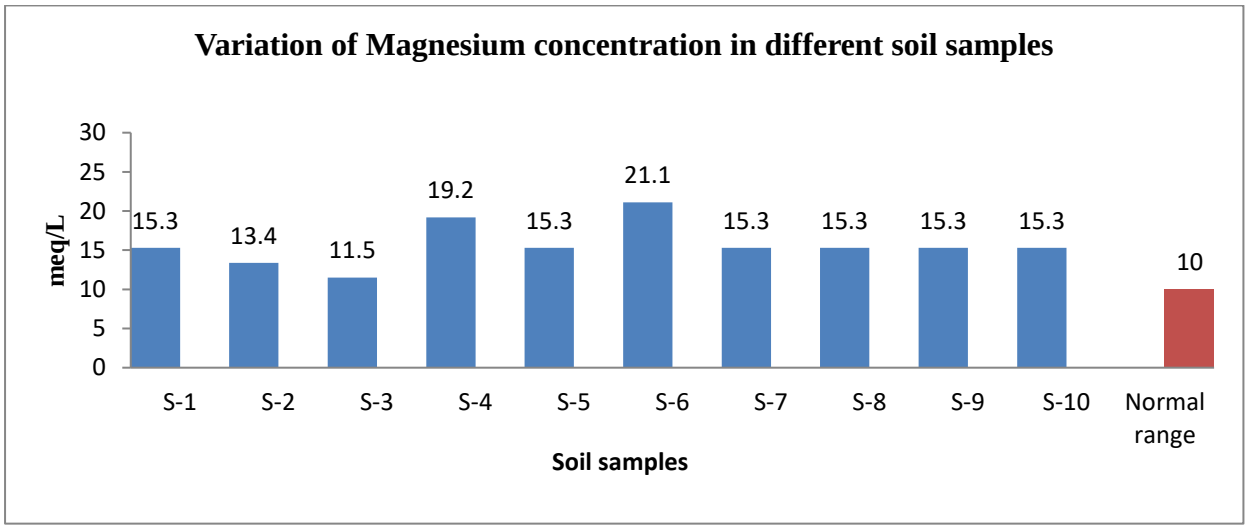

Figure 6: Graphical representation and variation of Magnesium concentration in different soil samples

Nitrate: Nitrate is one among the essential element for growth and development plants. In the present study, the values ranged from 1.2 to 7.0 ppm. Higher values were recorded in S-7 and lower values were recorded in S-5. The variation in concentration is depends on soil enzyme activity.

Phosphate: Phosphate compounds in soil store energy created from photosynthesis and carbohydrate metabolism, which will be used for plants growth and reproductive process. But high concentration of phosphate leads to Eutrophication. The normal range is 5-10 ppm. In comparison with the normal range, the phosphate values in all the soil samples were found to be within the normal range. Among the soil samples highest values were

recorded in S-7. It is because of application of amounts of Diammonium phosphate to the soil.

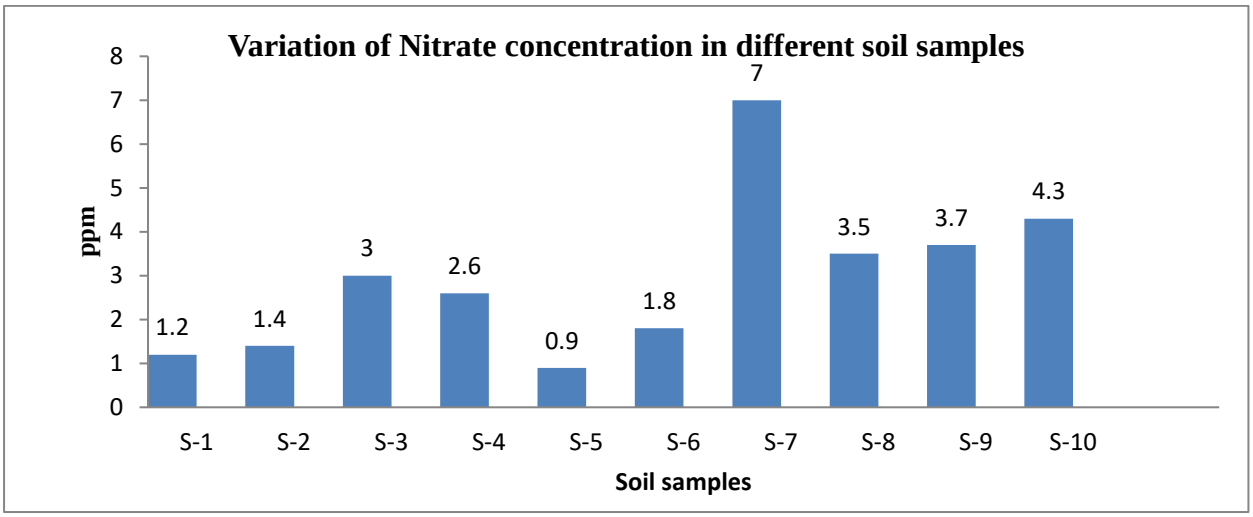

Figure 7: Graphical representation and variation of Nitrate concentration in different soil samples

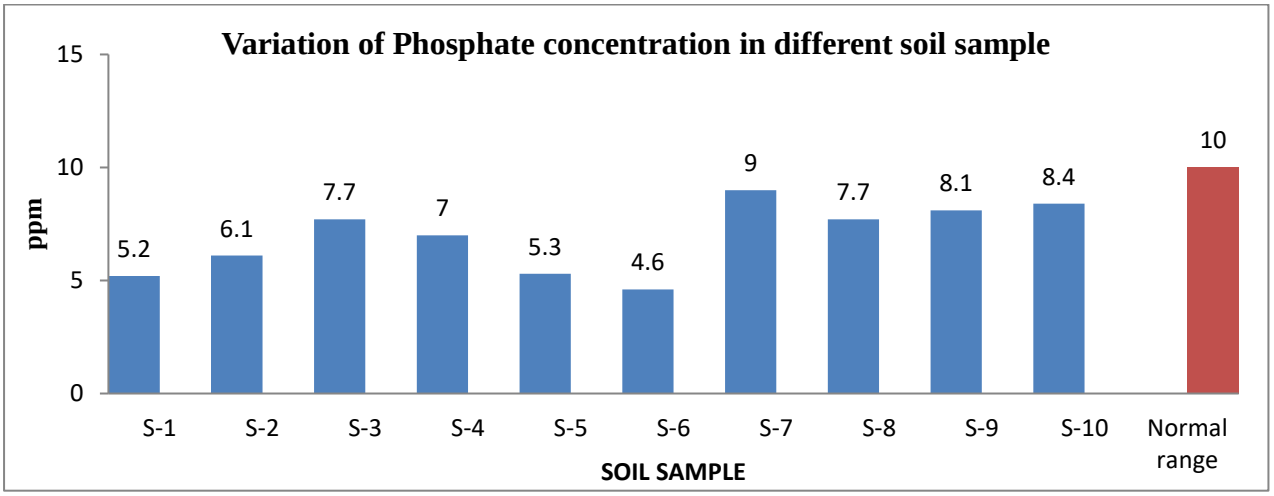

Figure 8: Graphical representation and variation of Phosphate concentration in different soil sample

Sodium and Potassium: In the present study, the sodium concentration ranged from 1.4 to 4.0 ppm. The highest values were recorded in S-6 and S-10. In case of potassium, the higher values were recorded in S-1 and S-6. The lower values are due to less amount of chemical and organic fertilizer input in the soil.

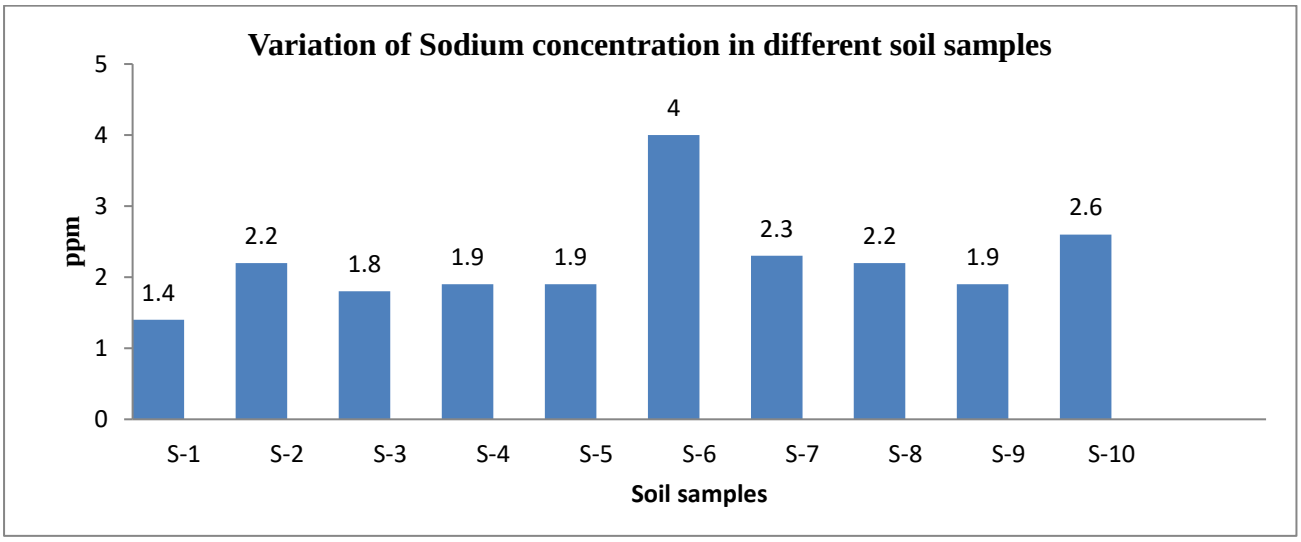

Figure 9: Graphical representation and variation of Sodium concentration in different soil samples

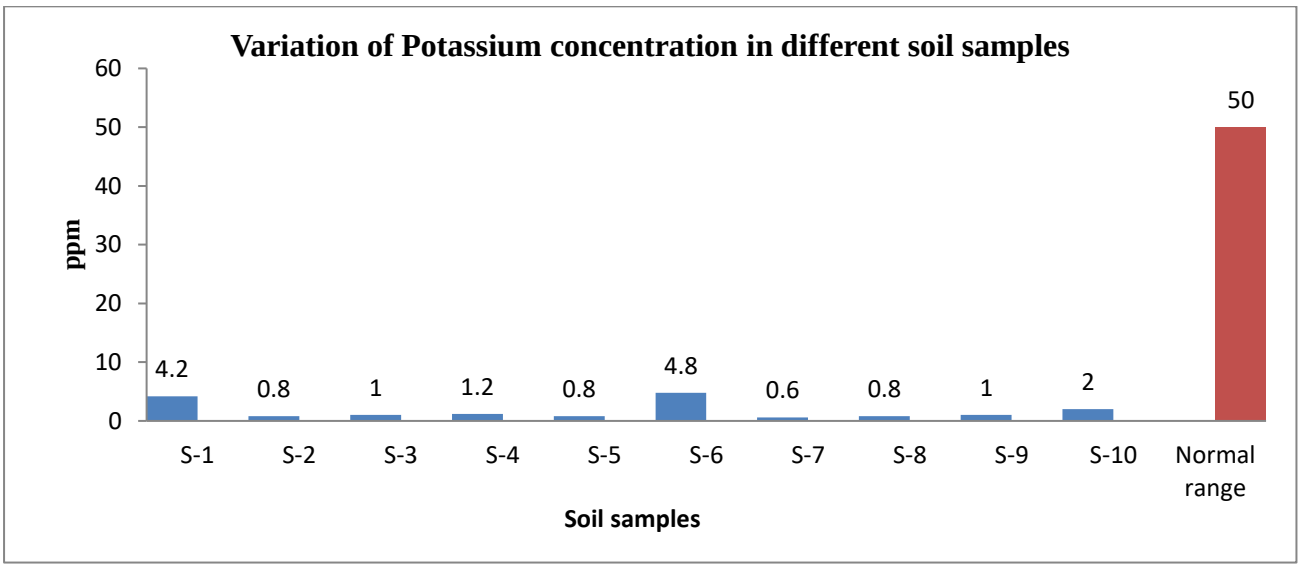

Figure 10: Graphical representation and variation of Potassium concentration in different soil samples

Bulk density: Bulk density is related to total porosity or interstitial space present in the soil for air and water movement. Lower bulk density implies greater interstitial space and improved aeration, developing a suitable environment for biological activity. In the present study all the values were found to be within the normal range.

Particle density: In the present study, the values ranged from 1.3 to 2.6 mg/$m^{3.}$ Higher values were recorded in S-6 and lower values in S-7. High particle density indicates the presence of iron in the soil.

Porosity: In the present study, the porosity values ranged from 6 to 54 %. Highest values were recorded in S-8 and lower values in S-1. The normal range of porosity of soil is 30-55

%. In comparison with the normal range, the entire soil sample was found to be with the normal range.

Urea: Urea is one of the most commonly used sources of nitrogen for worldwide agriculture. This is mainly due to high nitrogen content, solubility, low cost and rapid decomposition process. In the present study, the results show that, the soil samples have the minute quantity range and shows variation ranges in the soil samples. The higher values were recorded in S-7 and lower values were recorded in S-1. This may be influenced due to factors such as temperature, soil enzymatic activity and soil chemical properties.

Diammonium phosphate (DAP): In the present study, the soil samples ranges are varying with one another. The S-4 shows the higher value of 9.7 ppm and lower value of DAP is recorded in S-3 and in S-8. This could be due to the application of phosphate fertilizers.

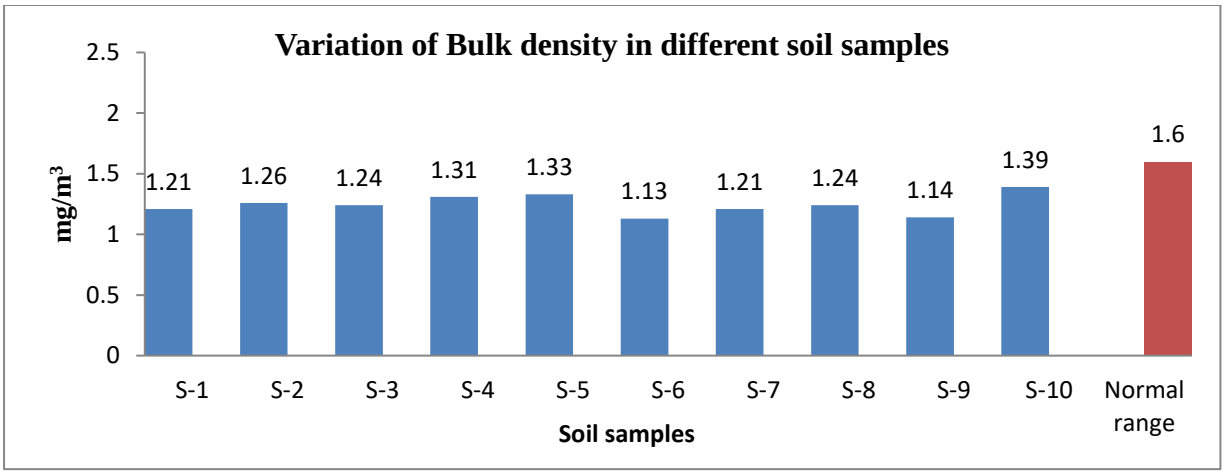

Figure 11: Graphical representation of variation of Bulk density in different soil samples

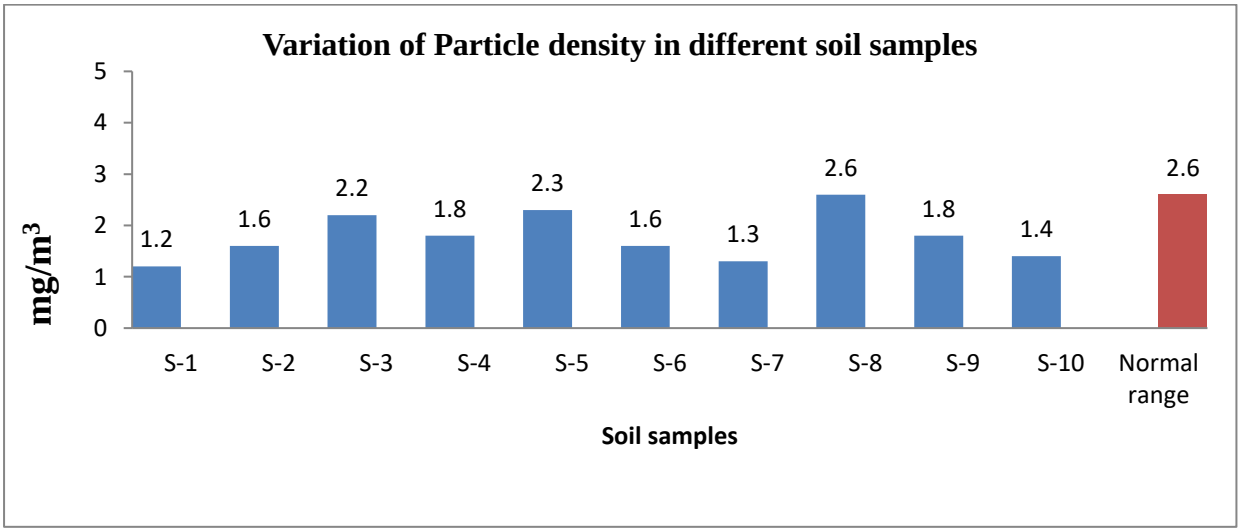

Figure 12: Graphical representation and variation of Particle density in different soil samples

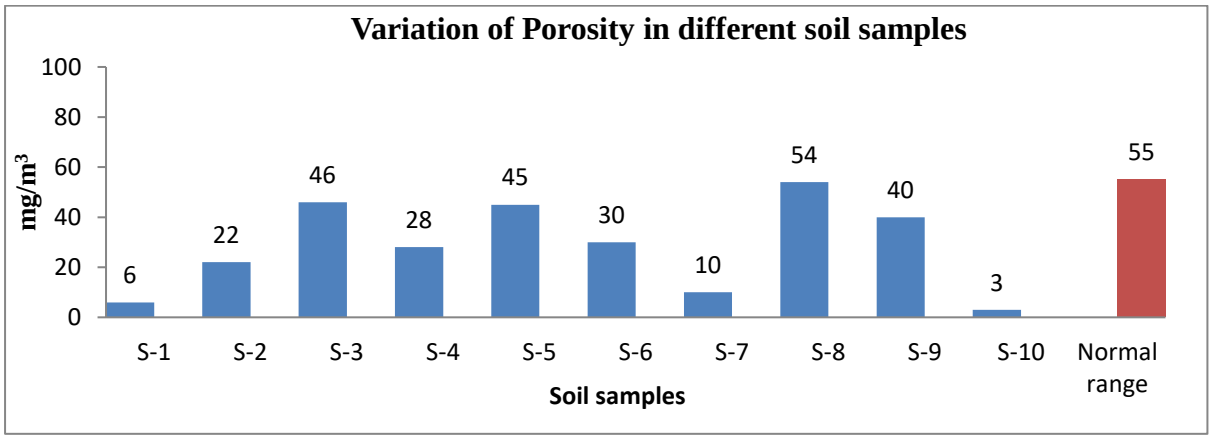

Figure 13: Graphical representation and variation of Porosity in different soil samples

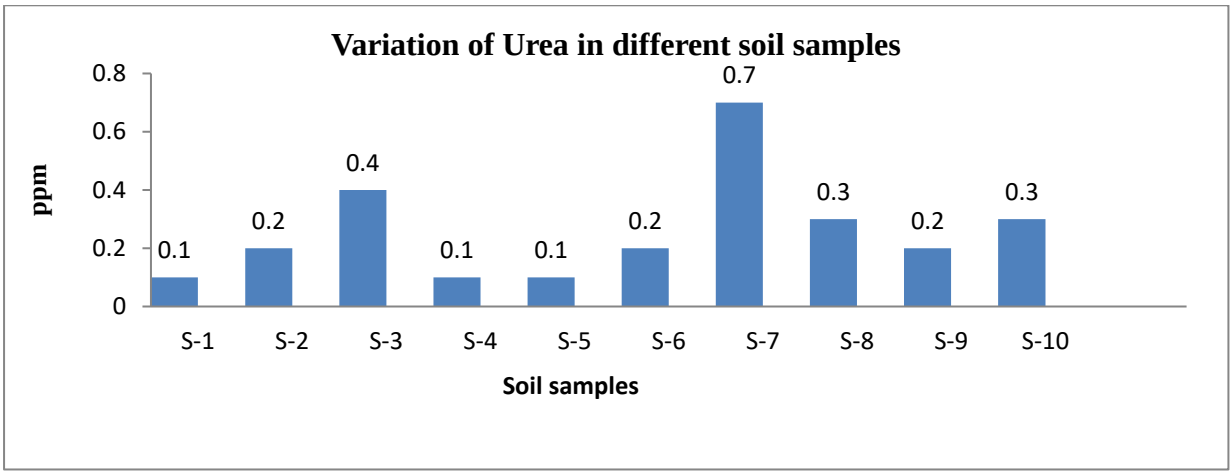

Figure 14: Graphical representation and variation of Urea in different soil samples

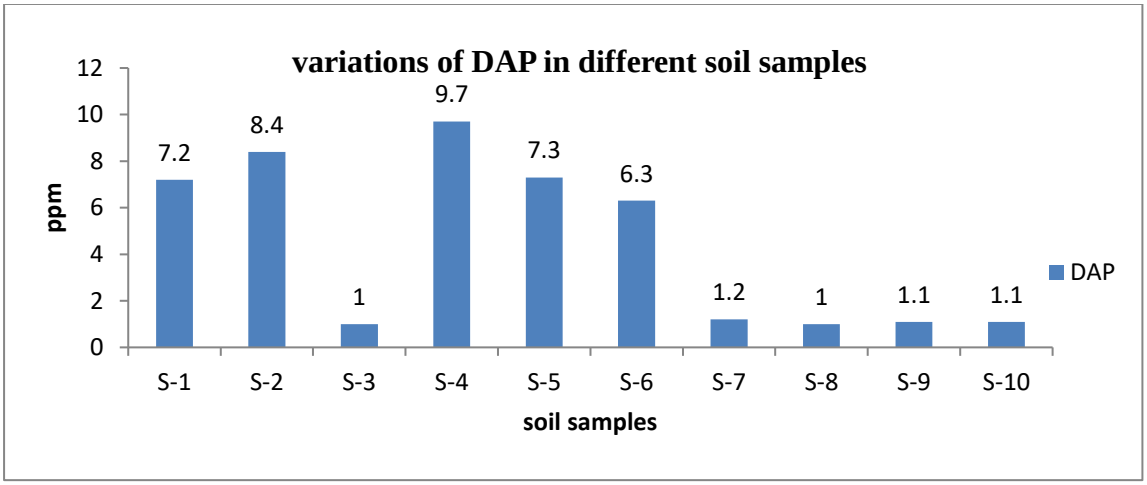

Figure 15: Graphical representation and variation of DAP in different soil samples

Table 2: Results of soil samples collected from agricultural areas of Pandavapura Taluk

	pH	EC	Ca^{++}	Mg^{++}	Cl^{-}	PD	BD	PO_4^{3-}	SO_4^{2-}	NO_2^{-}	NO_3^{2-}	Na^{+}	K^{+}	PO_4^{3-}	OC	AN	Urea	DAP
S-1	6.3	0.06	15.8	15.3	85.2	1.2	1.21	6	0.7	0.5	1.2	1.4	4.2	5.2	2.1	2	0.1	7.2
S-2	5.7	0.01	12.6	13.4	127.8	1.6	1.26	22	0.9	0.7	1.4	2.2	0.8	6.1	1.1	2.2	0.2	8.4
S-3	3.3	0.02	22.1	11.5	113.6	2.2	1.24	46	1.3	2	3	1.8	1	7.7	1.5	3.5	0.4	1.0
S-4	5.1	0.06	18.9	19.2	85.2	1.8	1.31	28	1.5	1.8	2.6	1.9	1.2	7	1.0	3.4	0.1	9.7
S-5	5.0	0.07	12.6	15.3	99.4	2.3	1.33	45	5	0.4	0.9	1.9	0.8	5.3	1.4	1.7	0.1	7.3
S-6	5.1	0.01	18.9	21.1	78.1	1.6	1.13	30	2	1.2	1.8	4	4.8	4.6	0.7	2.9	0.2	6.3
S-7	4.8	0.03	9.4	15.3	92.3	1.3	1.21	10	1.8	3.6	7	2.3	0.6	9	1.2	4.8	0.7	1.2
S-8	4.4	0.03	12.6	15.3	99.4	2.6	1.24	54	4	2	3.5	2.2	0.8	7.7	1.1	3.5	0.3	1.0
S-9	4.9	0.13	18.9	15.3	113.6	1.8	1.14	40	3.2	2.2	3.7	1.9	1	8.1	1.2	3.6	0.2	1.1
S-10	4.1	0.14	25.3	15.3	120.7	1.4	1.39	3	3	2.6	4.3	2.6	2	8.4	1.3	3.8	0.3	1.1

Abbreviations (Units for measurement):

EC= Electrical conductivity (ms^{-1}); PD= Particle density (mg/m^3); BD= Bulk density (mg/m^3); P= Porosity; OC= Organic carbon (%);

AN= Ammonia nitrogen (ppm); DAP= Diammonium Phosphate (ppm); PO_4^{3-} = Phosphate (ppm); SO_4^{2-}= Sulphate (ppm);

NO^{2-}= Nitrite (ppm); NO_3^{2-}= Nitrate (ppm); Na^{+}= Sodium (ppm); K^{+}= Potassium (ppm); Ca^{++}= Calcium (meq/L);

Mg^{++}= Magnesium (meq/L); Chloride (%); Urea (ppm)

Table 3: Correlation matrix of soil characteristics

	pH	EC	Ca^{++}	Mg^{++}	Cl^{-}	PD	BD	PO_4^{3-}	SO_4^{2-}	NO_2^{-}	NO_3^{2-}	Na^{+}	K^{+}	PO_4^{3-}	OC	AN	Urea	DAP
pH	1																	
EC	-0.093	1																
Ca^{2+}	-0.455	0.489	1															
Mg^{2+}	0.331	-0.024	0.071	1														
Cl^{-}	-0.343	0.294	0.215	-0.727**	1													
PD	-0.488	-0.170	-0.099	-0.200	0.119	1												
BD	-0.232	0.338	0.214	-0.217	0.343	0.111	1											
P	-0.400	-0.230	-0.109	-0.108	0.041	0.938**	-0.197	1										
$S0_4^{2-}$	-0.291	0.398	-0.111	0.042	0.079	0.615*	0.259	0.513	1									
NO_2^{-}	-0.562*	0.214	0.110	-0.071	0.098	-0.139	-0.015	-0.172	0.017	1								
NO_3^{-}	-0.443	0.175	-0.064	-0.118	0.079	-0.204	-0.041	-0.242	0.023	0.977	1							
Na^{+}	-0.102	-0.230	0.163	0.626*	-0.238	-0.136	-0.264	-0.058	0.058	0.109	0.065	1						
K^{+}	0.412	-0.086	0.292	0.551*	-0.550*	-0.471	-0.362	-0.370	-0.331	-0.374	-0.384	0.466	1					
PO_4^{3-}	-0.594*	0.351	0.115	-0.394	0.411	0.024	0.173	-0.062	0.091	0.902**	0.879**	-0.228	-0.637*	1				
OC	0.207	0.215	0.008	-0.545*	0.054	-0.202	0.153	-0.281	-0.186	-0.256	-0.178	-0.731	0.120	-0.067	1			
AN	-0.560*	0.137	0.139	0.002	0.034	-0.118	-0.096	-0.126	-0.049	0.988**	0.947**	0.164	-0.317	0.865**	-0.323	1		
Urea	-0.477	-0.215	-0.225	-0.312	0.108	-0.142	-0.123	-0.170	-0.113	0.817**	0.873**	0.118	-0.347	0.698	-0.108	0.790**	1	
DAP	-0.136	0.396	0.311	-0.169	0.275	-0.569*	0.120	-0.499	-0.326	0.437	0.423	-0.142	-0.197	0.427	0.092	0.388	0.329	1

Where, *= Moderate correlation and **= Strong correlation

Correlation coefficient: Correlation is one among themajor statistical tools which signifies the relationship between two or more different components. Basically, the value shows how a change in one component will influence on another component. Correlation coefficient normally varies from -1 to +1. The value of -1 represents a perfect negative correlation and the value of +1 represents a perfect positive correlation.

In the present study, Sodium and Potassium was moderately correlated with Magnesium. This is due to the moderate presence of Magnesium present in the soil samples. Urea is strongly correlated with Nitrite, Nitrate and with Ammonia nitrogen. This implies that, after the hydrolysis of urea, nitrate is formed as an end product. Since nitrate is negatively charged anion. It will carry all positively charged cations.

Conclusion:

The research on fertilizers is currently focusing on reducing the harmful environmental impacts on both human being and as well as environment. From the present study, it was observed that, all soil samples are acidic in nature. The organic carbon showed the higher percentage. It indicates the fertility of soil in that area is good for cultivation and for better yield. The Calcium, Sodium, Potassium and Phosphate concentrations are within the normal range, which implies that, the soil samples in the study area were not polluted with any inorganic contaminants of salts and acids. But due to the presence of chemical fertilizer residues, it implies that, while application of fertilizer care has to be taken regarding proper dosage application with respect to cropping pattern. So that, it helps to minimize the negative impact on environment which may pose in future.

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INSECT DIVERSITY AND HEALTH RISK IN LANDFILL GARBAGE AND SURROUNDING AREAS

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Abstract:

Organic and inorganic matters are the main constituents of the landfill garbage. More than 50% of thetotal waste materials are organic matter. Improper managementand handling of landfill garbage, causes serious health problems to human directly by the emission of toxic gasses. Moreover, landfill sites are the natural habitat of several microbes and arthropods. The present review enlightens the impact of landfill garbage on insect ecology and human health. Here, the arthropod density, its diversity on garbage landfill is highlighted as well as, more focus is centered onthe population of disease carrying insect vectors, insect scavengers as well as pollinators. It is found that wide variety of insects prefers landfill sites and adjacent areas for breeding and these areas become hotspots for these arthropods. Due to unavailability of resources and population explosion in developing and underdeveloped countries proper management of landfill sites could reduce the population dynamics of various insect pests, and health risks.

Keywords: Insect, Diversity, Garbage, Landfilling

Introduction:

Garbage basically consisting of waste papers, food and other toxic substances, e.g. industrial outlets, paint, batteries, asbestos, healthcare waste, sewage sludge and radioactive material (Samson *et al.,* 2011; Shankar, 2017) and due to these wastes surface environment and groundwater becomes polluted (Taylor and Allen, 2006). However garbage is a decayable waste which comes from animals and vegetables that is produced during handling, preparation, cooking and consumption of food. The waste consisting of organic matter is decomposed by bacteria and fungi (Kunz, 2009).

Garbage is everyday trash material produced from packaging, furniture, clothing, bottles, food scraps and newspapers (Savov, 2011). Garbage could be as edible or non-edible

based on the waste material produced during preparing to serve food and cleaning of food items. The edible part is suitable for animal food such as scrap meat and vegetables, while the non-edible part cannot be used for animal food like bones and eggshells (Walker, 2000). Garbage is categorized into three types, e.g. solid, liquid and gas (Taylor and Allen, 2006).

Gas and liquid wastes pollute their surrounding environment, adversely influence human health as compared to solid. However, some solid wastes which causes environmental pollution decompose very shortly and produce nasty smell and indirectly attract many insect as well as non-insect disease vectors (Zhao *et al.,* 2011; Alam and Ahmad, 2013; Nor Faiza *et al.,* 2019). Most of the dipteran insect pests usually prefer garbage or surroundings for breeding purpose, known as pestiferous or synanthropic in nature and these insect pests cause several health problems to human as well as animals (Battán-Horenstein *et al.,* 2016; De Souza and Von Zuben, 2016; Recinos-Aguilar *et al.,* 2020).

Oviposition as well as the development of various flies takes place in solid garbage and it is a favorite place for most of the insects (Spindola *et al.,* 2017; Kotzé and Tomberlin, 2020). Moreover, natural sights and natural activities destroyed by garbage and produce a bad smell, provide a breeding site to insects, spread disease and pollute the environment.

Landfills are the places used for disposal of waste material to burry in the ground (Carew- Hopkins, 2005). Household and commercial waste is collected and transferred to a site known as a garbage disposal center. A landfill is located at the periphery of the city, is easily accessibleand large enough to accommodate the quantity of waste in the course 10–15 years (Robinson, 2005).

Nowadays landfill is not only a disposal center, butalso it is an engineered area where waste is placed into the land, and it has a system to prevent polluting the groundwater and is operated and monitored to ensure environmentprotection (Boase, 1999; Paoli *et al.,* 2012).

Thelandfills are considered as an unstable environment biologically and do notsupport insect infestation. However, some insect pests could adapt with such dynamic environment, therefor it was found numbers of individual species may reach very high levels, but the diversity remains relativelylow when compared with other habitats (Boase, 1999).Waste material could be used as a raw material to convert into gas as fuel due to the presenceof organic matter (Williams, 2005b) and could be used as food for livestock and source of minerals and vitamins (Walker, 2000). However, there are various bad impacts like it causes birth-abnormalities, genetic problems, various health issues and mortalityin people residingnear landfill areas and causes (Rushton, 2003). It pollutes the environment and release methane gas causes global warming (Williams, 2005a).

Impact of industrial and urban waste on insect and ecosystem:

Waste tipping, compaction and daily cover (soil) are the practices intended to manage the high cost of a landfill site and for environment issue also.Inappropriatewaste management also affects insect population and allows insect pest to come and usethese as breeding medium (Goulson *et al.,* 1999; Heo *et al.,* 2015). Garbage volume is reduced by compaction and this practice reportedly reduces the numbers of fliesinfesting the garbage by making less opportunity for flies to accessand emerge from the waste material, while for compaction only twotimes/week it showed worst fly infestations (Toyama, 1988). To prevent insect pest and other pests such as birds, rodents and other vertebratesfrom accessing the waste materialsdaily cover is another recommended practice in a landfill. It also prevents odors and winddispersing the waste material (Boase, 1999; Paoli *et al.,* 2012).

Soil covering also reduces fly infestation (Toyama, 1988). Population dynamic of the house fly on landfill also affected by age of waste material, as the population density remained up tothe maximum level for three weeks after deposition of garbage inlandfill (Imai, 1984). Therefore, to reduce the odors that attract flies, and ultimately to reduce fly infestation, immediate consolidation, daily compactionand covering minimum twice a week is suggested.

The organism which is most commonly found feeding on feces and garbage are adults and maggots of housefly Afzal*et al.,* 2020) and they also spread different pathogens to other organisms, e.g. human and cats (Bowman *et al.,* 2008). The most common insects collected from landfills were the house flies which were 92% of the total collected insects in (UKLole, 2005) and it is the source of breeding for domestic flies in Hawaii (Siverly and Schoof 1955); Wilton 1961). When comparing garbage dump and other sites, cockroach infestation was highestnear the garbage dumps (Koehler *et al.,* 1987) and Aedesaegypti can be more attracted and breed in water bodies along with garbage (Pérez-Guerra *et al.,* 2009).

A suitable habitat for several insects for breeding is landfill garbage (Nuorteva*et al.,* 1964). For example, multiplication of different flies uses dog excrement as a suitable source (Poorbaugh and Linsdale, 1971). Muscadomesticawas found to be predominant insect in a study in USA on seasonal distributionof flies from fly breeding sites like garbage dumps in the urban areas of (Schoof*et al.,* 1954; Savage and Schoof, 1955). The animal wastes and other garbage materialattract Calliphorids green blowfly and blue blowfly) (Hogsette and Amendt, 2008).

Synanthropic flies have also been collected from urban garbage in Brazil, such as *Phaeniciaeximia, P. sericata, P. cuprina, Chrysomyaputoria, C. albiceps, C. megacephala and M. domestica (*De Souza and Zuben, 2012). Humpbacked flies were alsoobserved on garbage (Suiter *et al.,* 2013). Larvae of black fly (Hydrotaeaaenescens) also called garbage

fly due to affection towards garbage breed in garbage and organic matter and are facultative predators of other fly larvae, suppressing house fly populations (Stafford, 2008). Larvae of black soldier fly (Hermetiaillucens) also helps in the biodegradation of Municipal Organic Waste (Diener *et al.,* 2011). Similarly, Aerial yellow jackets are also found on food and garbage which is thepredator of other insects (Bechinski *et al.,* 2009).

Insect vectors:

Due to availability of organic matter flies and cockroaches live on animal manure and human feces (Bonnefoy *et al.,* 2008) and are vectors of human diseases (Majewska, 1986). Poor sanitation conditions of garbage near residential areas also attract these vectors which cause diseases in such areas with the optimum season due to high population (Greenberg, 1973). Different diseases are spread by these vectors by transferring germs from latrines and garbage dumps to the food materials (Stankus *et al.,* 1990). Similarly, dengue spreading mosquitoes Aedes breed on garbage dump sites (Teng and Singh, 2001). Most of the Calliphorid adults are the vector of various human diseases (Sawabe *et al.,* 2011; Adenusi and Adewoga, 2013; Hemmati *et al.,* 2018), and immature stage cause severe infestation such as myiasis by feeding on the human tissues (Chan *et al.,* 2005; Wang *et al.,* 2012; Ruiz-Zapata *et al.,* 2019) while some of the Calliphorids are known as human parasites (Hemmati *et al.,* 2018). Moreover, in the medical field these flies are forensically important (Wang *et al.,* 2017). Municipality garbage typically contains Calliphorid flies *C. albiceps, C. megacephala, Luciliaeximia, L. cuprina, Cochliomyia macellaria, C. hominivorax, Hemilucilia semidiaphana, H. segmentaria, Chloroprocta idioidea and Paralucilia pseudolycea*) which are vectors of pathogens causing human diseases (Beltran *et al.,* 2012). These flies were highly abundant in the urban locality. A wide range of insect vectors from three insect orders Diptera, Dictyoptera and Coleoptera) were observed in refuse dumps along with most of the families like Blattidae, Serabidae, Muscidae, Fannidae, Culicidae, Calliphoridae and Psychodidae. Among these, dipteran insects were very high, representing more than 80% Population (Ahmed, 2011). And more than 50 species of these flies are responsible for human diseases, out of which 21 species of filth flies reported as causal agents of digestive diseases in human due to synanthropy, communicative behavior and attraction to garbage (Olsen, 1998), and landfill garbage is a major source of breeding for filth flies (Nurita and Hassan, 2013). Similarly, cockroaches are found in hospital garbage and cause different diseases to humans (Olsen, 1998); Pai *et al.,* 2003).

Impact of garbage on insect diversity:

Earlier studies have shown that insect diversity is higher in landfill and garbage area as compared to another habitat (Choudhury and Gupta, 2017), and there were 67 species of Diptera found in landfill, in Czechoslovakia, while in Turkey 17 dipterans species from the Muscidae, Calliphoridae, Sarcophagidae, Otitidae and Syrphidae (Boase, 1999). Decomposition of organic material from waste is carried in composting site which is a kind of landfill and is required to minimize the use of landfill area (Morales and Wolff, 2010). During composting process in a landfill, there involve many insects.In a study the whole collection of insects includesthree orders namely Diptera 98.5%), Coleoptera and Hymenoptera, as well as another species of Arachnidawas observed in the collection (Morales and Wolff, 2010).

Syrphidaefamily of Diptera, was the most abundant during the whole composting process (Morales and Wolff ,2010).One study in Brazil found that the C. megacephala was the dominating among total Calliphoridae captured insects (Dias *et al.,* 2009). *Calliphoridae, Muscidae and Fanniidae* families were collected from compost material having volatile compounds in the garbage which attract insects Laos *et al.,* 2004).Population of flies was found to be higher in summer season as compared to the winter season. *C. megacephala* was most common ~94%) of the complete population of Calliphoridae flies. It is found that 44,688 individuals of Calliphoridae and 1307 individuals of Muscidae were collected, which describe that garbage is a very suitable place for these flies (Dias *et al.,* 2009). Waste material form the urban area is the major hotspot for wide variety of insect and in these waste three orders of insects was collected. Among these diptera was prominent having 98% population with 16 families; Calliphoridae, Drosophilidae, Psychodidae, Fanniidae, Muscidae, Milichiidae, Ulidiidae, Scatopsidae, Sepsidae, Sphaeroceridae, Heleomyzidae, Stratiomyidae, Syrphidae, Phoridae, Tephritidae and Curtonotidae. Similarly hymenopteran insects was also observed (Morales and Wolff, 2010; Hammad *et al.,* 2019).

Biological agents like parasitoids are very important to control insect pests. Garbage heaps and dumps attract some parasitoid species which depends on filth flies. In Thailand, 14 parasitoid species reported from two orders against filth flies in garbage dumps.Of these, 13 species were from Hymenoptera comprising five families; Chalcididae, Diapriidae, Encyrtidae, Pteromalidae and Scelionidae, and one Coleopteran species from Staphylinidae family Apiwathnasorn 2012). Similarly, the high diversity of insect vectors has been observed the landfill waste, including M. domestica, Anopheles spp., Aedes spp. and Periplanetaamericana (Abba *et al.,* 2019; Krystosik *et al.,* 2020).

Conclusion:

Countries in the world found to practice different methods of landfill and these affect the abundance of insect pest population dynamics. Insect vectors for different human as well as animal diseases, scavengers and pollinators mostly attracted towards the landfill garbage. These places are breeding sites and insects get the benefit of such habitat where the Dipterans aredominating among other insect orders such as Hymenoptera andColeoptera. No doubt that the landfill garbage directly affects the human life whereasinsect vectors indirectly cause severe health problem to human being. Practices dealing with landfill such as compacting, covering, composting and insecticide spraying was found to be very effective and make the landfillinappropriatehabitat for the insect pest and ultimately decreases theinsectpest population and decreases the health risk for the surrounding areas.

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EFFECT OF CHEMICALS ON GERMINATION AND GROWTH OF TOMATO (*LYCOPERSICON ESCULENTUM*, MILL.)

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Abstract:

Chemical pesticides are known to pollute the environment. While their intended effects are often short-lived, studies have shown that chemical pesticides remain in the atmosphere, the ground and in our waterways long after the job is over. Chemicals have been used on fields across the world for almost 100 years, creating a build-up of adverse pollution in our environment, which continues to grow with every application. Unfortunately, when pesticides are applied onto a surface, they travel outside their intended area of use by air, soil or water. This is one common way in which chemical pesticides cause collateral damage, beyond their intended use. The aim of this study was to evaluate the effect of chemicals on the germination and growth of tomato plant by treating the seeds externally in pre-sowing condition at different concentration .The percentage of survival seeds were calculated and compared with untreated seeds. The results indicated that the treatment of different chemicals and fungicide (Porpaconezole Ridomil gold HCL, and H_2SO_4) shows significant germination.

Introduction:

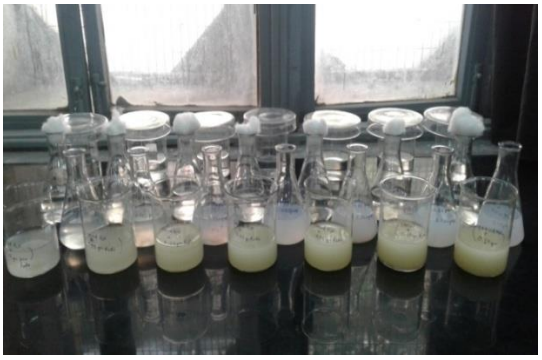
Tomato (*Solanum lycopersicum*) is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. Tomato is widely cultivated crop in India and a good source of proteins fructose and other vitamins. Global demand for tomato production increased tremendously due to its diverse utility in raw, cooked and processed form of food. This necessitates the continued supply of highly nutritious and better yielding improved cultivars to the producers, considering the rapid changing agro climatic condition. They are consumed fresh and are also used to manufacture a wide range of processed products (Madhavi and Salunkhe, 1998).

Tomatoes and tomato products are rich in health-related food components. United States, Turkey, Italy, and Spain are the leading tomato growing countries (Jumah *et al.*, 2004). The advantages of using tomato by-products as food ingredients are noticeable both to reduce environmental pollution and to provide an extra-income for producers (Lavelli and Scarafoni, 2012). Tomato can be consumed as raw or as an ingredient in many dishes, sauces, salads, and drinks. Factors influencing the considerable increase in tomato consumption include consumer awareness of benefits such as preventing cancer and chronic diseases (Lana and Tijskens, 2006). This beneficial effect is due to the action of antioxidant compounds, which reduce oxidative damage in the body (Beecher, 1998). Tomatoes are rich in lycopene (87%) and other carotenoids such as carotene, phytoene, phytofluene, lutein and L-ascorbic acid (Soma, 2013). Lycopene is a carotenoid that can be incorporated into foods with the purposes of conferring both color and functional characteristics (Nunes and Mercadante, 2007).

Lycopene has attracted attention due to its biological and physicochemical properties, especially related to its effects as a natural antioxidant. Lycopene does exhibit a physical quenching rate constant with singlet oxygen almost twice as high as that of beta-carotene (Shi and Le Maguer, 2000). Several food technology studies have been carried out to optimize the processing and storage of the tomato products by preventing the heat and oxidative damage on the antioxidants (Shi *et al.*, 1999). Tomatoes are not as sweet due to its lower sugar content then other edible fruits. Tomatoes are low in calories and a good source of vitamins A and C, the flavor, texture, and cooking characteristics of tomatoes depend on the variety, growing method, local environment, and handling techniques used during and after harvest (Parnell *et al.*, 2004).

Material and Methods:

The three varieties of tomato were purchased from the Kalash seed company Jalna Dist of Maharashtra state. The seeds were brought into the laboratory and treated with different chemicals and fungicide are Ridomil gold (Metalaxyl-M: 4.00% w/w, Mancozeb a.i: 64.00 w/w, Wetting and Dispersing Agent - Sodium lignosulfunate: 2.00% w/w, Dibutyl naphthalene Sulfonic Acid, Sodium Salt: 2.00%w/w, Filler: Precipited silica: q.s), Propaconazol, HCl, H_2SO_4 as follows:

Section 1: Concentration difference

Sr. No.	Time	Concentration			
		HCl	H_2SO_4	Propaconazol	Redomil
1	5 minute	0.2%	0.2%	0.02%	0.02%
2	5 minute	0.4%	0.4%	0.04%	0.04%
3	5 minute	0.6%	0.6%	0.06%	0.06%
4	5 minute	0.8%	0.8%	0.08%	0.08%
5	5 minute	1.0%	1.0%	0.10%	0.10%

Section 2: Time difference

Sr. No.	Time	concentration			
		Hcl	H_2SO_4	Propaconazol	Redomil
1	5 minute	0.1%	0.1%	0.01%	0.01%
2	5 minute	0.5%	0.5%	0.05%	0.05%
3	10 minute	0.1%	0.1%	0.01%	0.01%
4	10 minute	0.5%	0.5%	0.05%	0.05%
5	15 minute	0.1%	0.1%	0.01%	0.01%
6	15 minute	0.5%	0.5%	0.05%	0.05%
7	20 minute	0.1%	0.1%	0.01%	0.01%
8	20 minute	0.5%	0.5%	0.05%	0.05%

Experimental Results:

A) Germination percentage:

1. Variety Jindal:

a)Section 1: Treatment with Concentration difference for germination percentage

Sr. No.	Pot No.	Chemical	Concentration	Treatment Time	Germination Percentage (%) on 3rd day
1	16	HCl	0.2	5 minutes	80
2	28		0.4		100
3	4		0.6		100
4	31		0.8		100
5	5		1.0		100
1	38	H_2SO_4	0.2	5 minutes	80
2	36		0.4		80
3	35		0.6		80
4	39		0.8		60
5	37		1.0		100
1	45	Propaconazol	0.02	5 minutes	100
2	47		0.04		40
3	135		0.06		80
4	46		0.08		40
5	42		0.10		80
1	50	Redomil	0.02	5 minutes	40
2	54		0.04		80
3	52		0.06		40
4	48		0.08		80
5	53		0.10		80

b) Section 2: Treatment with Difference in time for analysis of Germination Percentage

Sr. No.	Pot No.	Chemical	Concentration (M)	Time (Min)	Germination (%)
1	22	HCl	0.1	5	100
2	7		0.5		60
3	3		0.1	10	100
4	6		0.5		100
5	17		0.1	15	100
6	32		0.5		100
7	15		0.1	20	80
8	29		0.5		100
1	40	H_2SO_4	0.1	5	40
2	41		0.5		100
3	2		0.1	10	60
4	30		0.5		100
5	8		0.1	15	60
6	14		0.5		100
7	24		0.1	20	100
8	12		0.5		100
1	44	Propaconazol	0.01	5	80
2	43		0.05		60
3	1		0.01	10	60
4	33		0.05		80
5	10		0.01	15	80
6	11		0.05		60
7	13		0.01	20	100
8	9		0.05		60
1	51	Redomil	0.01	5	100
2	49		0.05		100
3	19		0.01	10	80
4	34		0.05		100
5	18		0.01	15	60
6	20		0.05		100
7	21		0.01	20	100
8	25		0.05		100

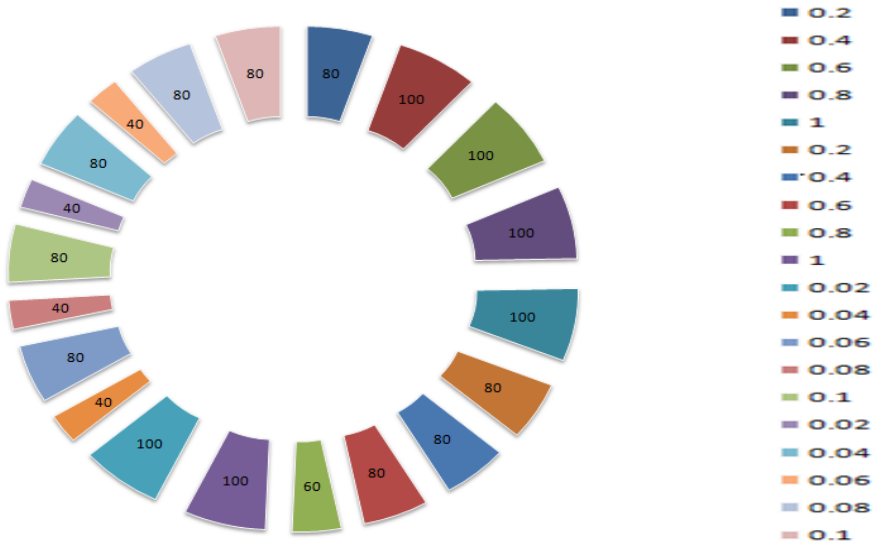


Figure 1: Treatment with concentration difference for germination percentage

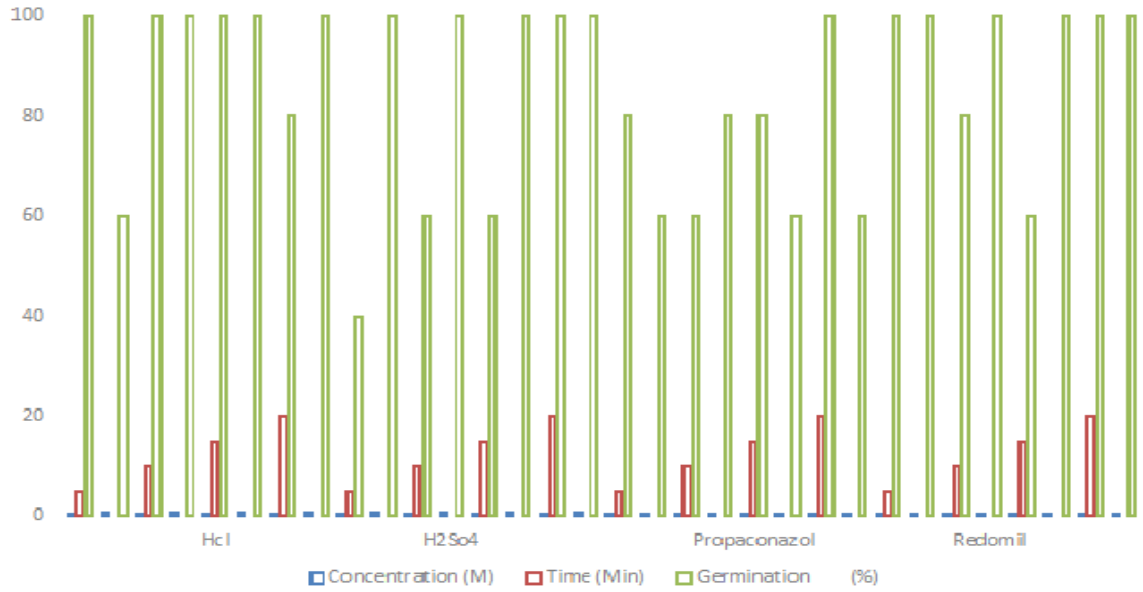


Figure 2: Treatment with difference in time for analysis of germination percentage

Result:

The seed were treated with 0.2, 0.4, 0.6, 0.8 and 1.0 M solution of Redomil Propaconezol, HCl and H_2SO_4. In Case of Propaconezole at 0.02 Conc. of germination is 100% and 0.08 M conc. solution germination was 40%. As concentration of fungicide increases the germination deceases and in case of 0.02 M conc. of Redomil the germination was 40% and in case of 0.1m solution germination was 80%.

Conclusion:

Tomatoes are the most valuable and are the most commonly used crop in many food dishes. They are very much beneficial to our health due to their antioxidant properties. The effect of germination and growth rate was calculated for the germination percentage and disease free induction of the plant. Redomil, Hcl and H_2SO_4 in germination percentage and growth of plant shows very well results and disease free plant as compared to untreated plant .This will help to agriculturalist, researchers and scientist to make the disease free initiation of tomato plant.

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TECHNIQUES OF RESEARCH IN ECOLOGY

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Introduction:

The word ecology comes from two Greek words (Oikos = household or home or place to live or habitation of organism or dwelling place of organism and Logos = to discourse or to study). The word ecology was coined by Hans Reiter (1868). The term ecology was applied by Ernst Haeckel in 1869 to depict the relation of animals with organic as well as inorganic environment. It can be called as place of organism where they reside. The ecology deals with interrelationships between the biotic and abiotic components of an ecosystem. The modern ecology is a multidisciplinary science constituted of number of disciplines such as physics, chemistry, mathematics, meteorology, climatology, geology, geography, economics, forestry, agricultural science, physiology, genetics, evolution, ethology, paleoecology, genecology, system ecology etc. The ecology can be divided into two basic types, The Autecology deals with the study of individual organism or species, while Synecology deals with the study of groups of organisms or species which are associated together as a unit. The ecology consisted of aquatic and terrestrial ecosystems. The term ecosystem was first coined by A. G. Tansley (1935).

The ecosystem can be defined as a community of organisms interacting with one another and with environment where they live. The terrestrial ecosystems are existed on the land; they are crop ecosystem, grassland ecosystem, desert ecosystem, forest ecosystem, Tundra ecosystem etc. The aquatic ecosystems are consisted of water. The water ecosystems are divided into freshwater and marine water. The freshwater ecosystem is again divided into river ecosystem, pond ecosystem, Lake Ecosystem. The marine water ecosystem is again divided into sea ecosystem, ocean ecosystem and estuarine ecosystem. The ecosystem constitutes of two components or factors such as biotic factors and abiotic factors . The biotic factors are the living factors or components of ecosystem. They are again divided into autotrophic and heterotrophic components. The autotrophic factors are the producers or plants. The heterotrophic components are called as consumers. The consumers are again sub divided into Macro consumers (Primary consumers, secondary consumers,

tertiary consumers and quaternary consumers). The microconsumers are the decomposers in soil. The abiotic factors are the non living factors of environment**.** They include soil, water, air, light, temperature, altitude, moisture, rainfall, fire, wind, tide etc.

The environment consists of both biotic and abiotic factors. The word environment is derived from French word (Environner = to encircle or surround). Every living organism is surrounded by materials and forces that constitutes environment. The environment is the sum of all social, economical, biological, physical and chemical factors which forms the surroundings of organism. The biosphere includes all biotic parts of hydrosphere, lithosphere and atmosphere.

Sample Collection:

Well-designed sampling methods are necessary to carry out research in ecology. It is not possible to count all the animals or plants in any ecosystem. The design of sample collection is a science and skill. The habitat, the size, the number, the distribution and the time of sampling are the most important things in ecological research. The sampling in the air is done with the help of Suction traps that are used to collect sample of flying insects, pollen grains and airborne spores. The sampling from herbage and trees are made with the help of suction sampler. The spiders and insects are collected by chemical knock-down. The Phytoplankton and zooplanktons are usually sampled with the help of plankton net. The bottom of lakes, deeper rivers and marine habitats are sampled by dredges, trawls and grabs. A removal trapping technique is commonly used to estimate fish densities by electric fishing. A reach of stream is netted off and fished three or more times. This technique can be used for estimation of ground beetle numbers by pitfall trapping. The Point and line survey techniques are widely used for estimating the density of birds, butterflies and whales. The structure of a plant community can be studied quantitatively through vegetation analysis by making small sample units which are dispersed widely. The technique of quadrat is used for collection of sample from the vegetation. The samples collected by quadrat method are used to determine frequency, abundance and density of different plant species in a community.

The ecological research depends upon the collection of samples and analysis of data by applying statistical principles and parameters. The samples from terrestrial ecosystems are collected through frequent field visits and survey of particular habitat and ecosystems on land .The samples from terrestrial ecosystems may be collected individually or in groups .In aquatic ecosystem the samples of plants and animals are collected from the seafloor and its sediments. The data obtained through sampling is used to understand the marine

habitat and marine environment of plants and animals. The samples from marine environment are collected with the help of boat. Before collection of sample from marine environment, presence of any hazardous substances is checked through recognized environmental concern (rec) ecologist. The information gathered through rec is used to monitor the seafloor for any negative impacts.

Tools of sample collection:

There are some important tools used in collection of samples for ecological research. They are as below

1. Haman Grab

The Haman Grab is a rectangular frame supporting bucket of a sample. It is slowly lowered to the seafloor from the boat to collect sea animals and seafloor sediments. The sample is taken into a container and photographs are taken. A little amount of sample is taken to measure the size of sediments while remaining sample is sieved and sorted to remove the animals. The analysis of sample for the size of sediments and identification of animals are carried out.

2. Beam trawl

The beam trawl is used for collection of larger and mobile sea animals from the seafloor. In this method a net is attached to a heavy steel beam. This device is deployed from the back of the vessel and is towed with it. The location of these trawl runs coincides with the locations of hamon grab samples and video camera runs. The samples are poured into containers on the ship board .The photographs are then taken. The animals are identified and counted. The data is configured and analysed statistically to arrive at certain conclusion.

3. Video Sledge

It is used for collecting the images of sea animals from the seafloor. A camera is set on a sledge and towed behind the boat at different locations .The camera film takes still images of the seafloor. The computer is used to record the geographical position of the sample collected from the seafloor.

4. Water Curtain Camera

It is used where there is lot of sediment particles in the sea water, which are difficult to see the bottom. In this technique a camera is mounted vertically in a square steel frame and it looks vertically down through a freshwater tank with a perspex base, which makes it easier to see the sea bed and images.

5. Ham-Cam

It is a miniature camera attached to the hamon grab. If the water is turbulent then ham-cam is towed to take video shooting or film. These videos or film is used for research purpose.

6. Geophysical images

The images are useful to see the patterns of sand waves, rocky area and biogenic reefs on the seafloor.

Population study:

The relative population can be studied with different tools such as Traps, Radar and Sonar. The traps are used to study the population of animals. The light traps are usually used for study of night-flying insects, moths and larval fishes. The pitfall traps are used for the study of population of spiders and beetles. The traps and tangle nets are used for study of fish population. Radar is another means of population study which is used to monitor birds migration and trace the flight of large insects like bees. The sonar survey is widely used to study fish population and its distribution in river and lake ecosystem. The distinctive signal is produced by the swim bladder of the fish which is used to detect locality of fish (South wood and Henderson, 2000). The presence of animals can be detected through observing their footprints, faeces, hair, nests, burrows or cast skins etc. The signs of insect damage to plants are also used by economic entomologists to study the insect population. The life tables and key factor analysis are important for understanding dynamics of species population. The construction of life table is useful to show the pattern of birth and death of organisms with time. The key factor analysis is used to identify particular stages in the life for determining the size of population. The growth rings are used to determine the age of woody plants. The age of fish is measured on the basis of seasonal growth checks visible on the scales on body. In molluscs growth bands are seen on the shell used to determine age. The damage and wear are used to determine the age of insects. The development of wear on teeth is used to determine age of mammals.

Research methods:

i) Field survey and observations

The experiments always requires observations. The ecologists must observe the environment with species of plants and animals along with their interactions. It is necessary to observe population, their growth, behaviour and reproduction of species with respect to effect of abiotic factors of environment. The ecology researcher must develop

keen interest to observe nature. The field visits should be arranged frequently to go in nature and observe different events from time to time and season to season. There are many tools for observations and sampling. The observation and sample collection should be in a random fashion to avoid bias. A field work often starts with non quantitative observations. The observational data can be collected through the use of sensors or research towers. The observation of animals may be made through capture and release of animals. They can be observed for feeding habit, health, reproduction and behaviour. The radio collaring technique is generally used to track movements and behaviour. In terms of direct survey a researcher can **o**bserve plants and animals in their natural habitat and environment. The help of binoculars, photographing or filming of events can be undertaken. In indirect survey a researchers look for any signs of animals like their skin , hairs, foot prints ,faces, remains left of the prey , horns etc. The data of observations are collected, analysed and predictions are made for their existence in habitat. The sampling methods may be used to record images of habitat with the help of video sledges, water curtain cameras and Ham-Cams. The Ham-Cams is also used for survey of animal population. In Hamon Grab method of survey**,** a sample of sediments is collected from the seafloor and animals are sorted through photograph and are identified in a laboratory. The beam trawl is used to obtain larger sea animals. The samples of plants and animals are brought on board of boat and they are photographed and counted in number and data is collected.

ii) Ecological experiments

The field data is an important step to understand ecosystems. To obtain quality data the experiments must be carefully designed and planned. The hypothesis of research is the first step of experimental design .The field work experiments include the size and shape of an area need to be sampled. The field site can range from small to very large depending on type of study. The herbaceous plants and small mammals require field sites of up to 30 square meters. The trees and birds may require hectares of field area .For the study of mobile animals such as deer, bears, tiger, lion etc. need several hectares of field area. In some field experiments only one site can be useful for study. The **t**ools used for experimental field sites includes quadrates, line transects, the transect-intercept, belt transect etc. The researchers should not be biased with replication and randomization of experimental design. In manipulative experiments a researcher alters a factor to see how it affects an ecosystem. This provides interference in a controlled manner. The change of variables is difficult in such experiments. The natural experiments are manipulations of an ecosystem caused by nature itself e.g. natural disaster, climate change, invasive

species introduction. The ecosystem itself is a natural experiment. The observational experiments require adequate replications for high-quality data. The efforts are required to collect data during weather condition and other disturbances. In such experiments randomization before experimentation is necessary, the randomization strengthens data collection because it reduces bias and prejudices regarding experimentation.

iii) Modelling

Modelling is an important tool useful for the study of ecosystems. It helps to make predictions about the ecosystems. The range of expansion can be predicted with the help of models .They are also useful to assess the risk factors .The ecological research methods depends on statistical analysis and mathematical models. The mathematical models predict the consequences in ecosystem with respect to environmental conditions .They can be used in place of field work. The models provides streamline information effectively .The models may be in the form of equations, simulations, graphs and statistical analyses. They are useful to produce informative maps and allow for calculation of data .The computer model help for rapid and comparative analysis of data. The simulation model enables the description of systems because traditional calculus is difficult and complex. The model allows scientists to study co-existence, population dynamics and many other aspects of ecology. The pattern of climatic changes can be predicted through mathematical models. The energy flow models are useful for understanding of energy transfer in different ecosystems .There is unidirectional flow of energy from sun to the producers (plants) and then to consumers (animals) and decomposers. The energy flow models are based upon the two laws of thermodynamics. The first law states that energy can neither be created nor be destroyed but can transformed from one form to another .The second law states that the energy transformation involves degradation or dissipation of energy from a concentrated to a dispersed form. According to E.P. Odum (1963) the flow of energy involving three trophic levels with universal energy flow model .The flow of energy takes place and there is gradual loss of energy at every trophic level thereby resulting in less energy available at next trophic level.

Lindeman (1942) put forth a model regarding unidirectional energy flow in fresh water ecosystem. Out of total energy of solar radiation, only little amount of energy is utilized at each trophic level.In terms of animals after analysis of biotypes the maps are prepared to show distribution pattern of animals in marine environment .It is impossible to sample every bit of the seafloor, so producing a map is necessary and this map serve as a model. These modelling makes estimation about, which biotope is likely to belong to the sampled and unsampled areas. In preparation of map a wide range of data is analysed and

used to calculate the presence of most likely biotopes in habitat. The modelling maps of an area are used for easily understanding of habitat and their biotypes.

iv) Statistical analysis

The discipline of statistics is related with mathematical techniques utilized for obtaining and analyzing numerical data of observations in order to draw a scientific conclusion with respect to research work .In statistical data analysis various statistical parameters are used to draw a conclusion. The parameters are mean, mode, median, dispersion, variance, standard deviation, coefficient of variation, correlation analysis etc. The samples collected through experimentation and observations are tested for their significance. The data of samples are tested by different test of significance such as t - test or F-test, Chi square test etc. The correlation and regression analysis is also a technique of statistical analysis of data. The data obtained from sampling or through observations can be either qualitative or quantitative. The qualitative data is *descriptive; it is* collected by taking many observations. It includes colour, shape, taste, odour and external appearance. The quantitative data in the form of numbers or numerical values .It can **be** measured in the form of number. The quantitative data includes pH of soil and water , the number of mice in a field site, the number of trees , salinity levels, temperature, moisture, height of plants , height of animals, weight of human , weight of plants and animals, rate of production, number of individuals. The quantitative data is considered as more reliable in ecological research. The synthesis of research data is utmost important to come to a conclusion about research findings .The data is synthesized from field observations, experiments and models or charts. Different types of programmes are designed for synthesis of fruitful information and knowledge .The National Ecological Observatory Network (NEON) is a new network of locations where a huge range of data can be collected. The experimental and observational data is used to build large scale statistical and mathematical models useful for predictions about different ecological aspects such as threats to invasive species, pattern of climate changes, disturbance in ecosystems, interactions between abiotic and biotic factors, energy transfer, nutrient transfer, food chain, food web,carrying capacities etc.

Advancement in research:

In recent years the ecological research is advanced due to technological enrichments. The technology is useful for collection of sample and produce maximum of plants and animals for study. The expanding technologies are useful for getting high resolution information. The samples can be collected at very micro level up to even centimetre. The small unmanned aircraft can map large landscapes with sub-centimetre resolution

(Anderson and Gaston, 2013) .The temperature, humidity and light sensors can be densely deployed to record even very micro-climatic variations (Keller *et al.* 2008). Thus the advancement in technology helped to gather information of natural environment.

There are some tools used in ecology research as follows

i) Bio-Loggers

Bio-logging technology is incorporating the sensors like heart rate loggers,VHF and GPS .Accelerometers are used to record fine-scale animal movement in real time by direct observation (Shamoun-Baranes *et al.,* 2012).It is also used to examine the movements of cryptic animals such as birds (Aldoumani *et al.,* 2016) and whales (Lopez *et al.,* 2016) .The study regarding their movement and way of giving response to the external stimuli are studied.

Table 1: The significance of different tools required for ecological research

Sr. No.	Name of the Tools	Significance
1	Accelerometers	It measures animal movement .
2	Automated Sensors	They are used to measure and log environmental variables.
3	Autonomous Vehicles	It collects ecological data automatically and remotely.
4	Camera Traps	It is used to record presence of wildlife and their behaviour.
5	Cup Anemometer	It measures the direction and speed of wind.
6	Fortin's Barometer	It measures atmospheric pressure.
7	GPS Tracking	It is a satellite tracking system with frequency, accuracy and precision .
8	Gyroscopes and Magnet	It tracks three-dimensional movement of animals.
9	Hygrometer	It measures relative humidity.
10	ICARUS	It observes global migratory movements of animals through satellite.
11	Infrared Camera Traps	Used to sense animal movement in dark and take images without a visible flash.
12	Land Sat Imagery	It is useful in land-remote sensing data.
13	Li DAR	These are remote sensors used in measurement of distance by illuminating a target with a laser and analyzing the refracted light.

14	Li DAR -3D	It is used in accurate measurement of 3D ecosystem structure
15	Lux Meter	It is used to measure light intensity
16	Mainframe Computers	Statistical analysis of large data sets.
17	Multi Spectral Land Sat	It is satellite imagery used for measurement through water and vegetation.
18	Plankton net	It help to collect Phytoplankton and zooplanktons from water bodies
19	Psychrometer	It is used to measure humidity
20	Satellite	It transfers data from central hubs in the environment to researchers.
21	Side Scan Sonar	It is used to create an image of large areas of the sea floor.
22	Solar Power	Transfer data to autonomous vehicles for easy data retrieval.
23	Sonar	It is used to locate and record schools of fish.
24	Thematic Land Sat	This scanner measure global warming and climate change.
25	Thermal Bio-Loggers	These are devices used to measure temperature of animal body.
26	Tracking - 3D	Along with GPS it help to create real-time animal movement tracks .
27	VHF tracking	It is the radio tracking system useful for remotely monitor wild animals.
28	Video Traps	It determines animal behaviour.

ii) Bio-Batteries

Bio-batteries are fuel cells, which convert chemical energy into electricity generation using low-cost biocatalyst enzymes. It is used for research. The capabilities of bio-batteries combined with low-power radio communication devices could revolutionize field-based data acquisition. The bio batteries are used to run field equipments by plugging it into the tree.

iii) Low-power and Long-range telemetry

The ecological data collection may be required from difficult and hazardous locations. The data retrieval often influences the sensor placement and limiting the data collection. In such situation use of sensors in remote areas becomes necessary. The difficult location may be dense forests, high mountain ranges, swamps etc.The sensor devices becomes useful to transmit information to a base station which will result in faster data collection and more convenient data retrieval. The long range telemetry would help to transmitting data from the field to the laboratory.

iv) IoT

The IoT is called as Internet of Things. Today it is now possible to wirelessly connect devices to one another so that they can share information automatically. Due to internet, variety of things or objects can connect to each other, they interact and co-operate with each other closely (Gershenfeld et al. 2004). Each device is capable of acting independently and it can communicate to gain additional information. IoT could be used to set up peer-to-peer networking to transfer data from one device to the next until reaching a location.

vi) Swarm Theory

A Swarm theory is related to complimentary nature of technology with ecology. It works collectively to accomplish certain research target or goal. It is used to study the organization of ant colonies, flocking behaviour of birds and insects. It is useful for faster data acquisition and communication over large geographic area and dynamic ecological survey. This theory is directly applicable to the collection of remotely sensed data by multiple unmanned vehicles. The Unmanned aerial vehicles (UAVs) are used for landscape mapping and wildlife identification (Anderson and Gaston 2013, Humle *et al.,* 2014, Lucieer *et al.,* 2014).

vi) 3-D Printing

This is a technology in which 3 -dimensional printing is used in building organic small molecules and mimicking the production of molecules in nature. The researchers are able to print specialized platforms for sensor equipment like GPS collars. The flowers of intricate and exact shape and colour could be printed with heating elements embedded more easily and realistically.

Conclusion:

The success of ecological research depends on different sophisticated tools and techniques used for collection of samples and analysis of statistical data. The computer aided models, maps and charts are useful for prediction about ecological habitats and animal behaviour. The data analysed gives insights into study of interactions and functions of ecosystem. The mathematical programming models are utilized for study on applied aspects of ecology. The sophisticated techniques and methods are utilized for management of natural resources and solve agricultural and habitat problems of organisms in an ecosystem. The biotelemetry and electronic tracking equipments are used to study the movements and behaviour of animals. The Radioisotopes are used for tracing the pathways of nutrients and energy transfer through different trophic levels in ecosystems. The technological developments offer a great potential for ecological research and

environmental applications. The mathematical algorithms are being used to interpret neural coding and brain behaviour of animals to determine the intent to move and to understand how animals make decisions with respect to surrounding environment. The information gathered is used to study species niches, dispersal, trophic interactions. The new technique of optical resolution and image processing display images at a sub-cellular level. The technologies, both current and emerging have the capacity to do ecological research at very micro level. The techno ecology is focused on the employment of physical technology to acquire new volumes of ecological data. The success of technology in ecological research depends on how the ecologist and researchers are adapting themselves with new technological innovations pertaining to ecological study. The anthropological impact upon the environment is increasing day by day; therefore it becomes crucial to use different methods of ecological research to find ways to mitigate ecological issues

Future perspective:

The ecological research can be carried out with the help of highly sophisticated tools and techniques. The physical and chemical means of mechanical apparatus may be used to carry ecological research on different aspects of environment. The advanced experimental designs and bio statistical methods with improved sampling technique would be a gift for researchers in the field of ecology and environmental science. The ecology constitutes terrestrial and aquatic ecosystems .The ecosystems on earth are complex and dynamic. The ecologists often rely on technology to quantify ecological phenomena. The technological advancements have often been the catalyst for understanding of ecosystem functions and dynamics which aids in proper management of environment. The inception of very high frequency telemetry is used to track animals. It allows to researchers to remotely monitor the behaviour, physiology, movement, resource selection, and demographics of wild animals. The advancements in geographical positioning system and satellite communications technology have largely supplemented the tracking. The GPS has the ability to log locations, greater accuracy and understanding of species habitat use and their interactions with other organisms. The ability of a swarm to locate and track individuals of different species in real time may be helpful to revolutionize understanding of ecological phenomena such as dispersal, migration, competition and predation. The drone's technology would be beneficial to detect the species or individuals of interest. On the basis of geographic information detection and tracking would be possible. The computer graphics would be beneficial to interpret about the ecological status of plants and animals in nature. The data analysis can be very fast and accurate by using technological analysis

tools. The ecological research through satellites would be upcoming technology in the field of ecology research. The miniature satellites will be helpful in gathering and transformation of ecological research data. The satellites with sensers can be used for ecological study of earth from the space. The satellite information would be accurate, authentic and predictable in relation to ecological and environmental point of view and interest. Bioinformatics would play vital role in the use of next-generation ecological data. It is required to develop methods for sampling, sorting, analyzing, categorizing large data of research. The multidisciplinary approach would be great boon to ecological research. Increasingly complex research and global challenges in terms of climate change and biodiversity loss are the driving forces of rapid development, refinement and use of new technology. These technologies would revolutionize ecological research.

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ENVIRONMENT AND ECOSYSTEM DISTURBANCES

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Introduction:

Environment is defined as all conditions influences that have the impact on development and life style of organisms. The ecosystem is capable of self maintenance. However the equilibrium is very sensitive to external stimuli, such as human activities promoted by socioeconomic goals. The socioeconomic system of msn in contrast to natural ecosystem is based on material. Increasing demands from human society which creates rising demands for goods and services. In humankinds present day economic system only money oriented. It leads overproduction of wastes which causes threat to survival of mankind. We are surrounded with desertification, floods, droughts, urbanization, pollutions, and threat to many plants and animals. The population growth is not in control hence demand for food, shelter, clothing, energy and basic needs etc. Environmental management is interdisciplinary approach to conservation and recycling resources by hu8manbeing. The main theme of environmental management is to reduce or minimize the impact of human activities on the environment

The environment gradually degrades by the processes of waste dispersal, nutrient cycling and spread of crop pests. The quality of air, water and soil deteriorates as a result of human activity. Environmental disruption may be due to discharges of wastes in to the air, water and land production of heat, noise, and removal of natural vegetations and fauna. The cost of which society and mankind have to pay for the above damage include death, diseases, hunger, starvation and adverse effects on property caused by pollution. Pollution exerts some direct effects on human health and property as well as some indirect effects on the ecosystems that make the life possible on this planet.

Environment:

Everything which influences life process of the organism is called as the environment.The environment is nothing but surrounding of the organisms.Each and every organism has its specific surrounding to which it continuously interacts and remains fully adapted.Thus an environment is a collective term for all the surrounding conditions in

which an organism lives.In oter words the environment is the sum total of abiotic (physical) and biotic conditions influencing responces of the organisms.(S.C.Kendeigh, 1974).Abiotic or physical conditions and the biotic conditions interacting with each other are called as environmental factors or ecological factors.

Environmental factors:

The environmental factors are grouped in to two groups such as

1. Group of non-living or abiotic or physical environmental factors.
2. Group of living or biotic environmental factors.

The biotic factors and the abiotic factors together form overall ecosystem (structural and functional unit of the nature). The abiotic factors include nonliving things like matter and energy where as the biotic factors include the influence of nonliving things and living organisms upon the vegetation.A marked effect of interaction between the living organism and the physical conditions (matter and energy) upon any vegetation in any is called as biotic effect.

The environment is nothing but the surrounding in which the organism live. The environment is everything which influences life of the organism. The life is nothing but form, functioning and development of the organisms. The study of distribution, structure, various aspects of life of the organisms and their interaction with the environment is called ecology. The environment is composed of many living and non living things which affect of influence directly or indirectly life prossess of the organisms. The environment is everything which influences life of the organisms. Any non-living or living conditions or constituents of the environment which affect life of the organisms are called as the environmental factors or ecological factor. Thus there are many non-living and living environmental factors. The environmental factors are interrelated and intricately mixed with each other. They act and react through one another and affect life of the organisms. All living organisms including human beings live in some sort of non-living (abiotic) components of the environment that contains matter and energy. The ecological factors affect the existence, organization and metabolism of the living organisms.

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and the physical conditions (matter and energy) upon any vegetation in any is called as biotic effect.

Abiotic ecological factors

Any non-living conditions or constituents of the environment which affect life of the organisms are called as the abiotic environmental or ecological factors. The abiotic ecological factors are also called as the physical or non living factors. The obiotic factors include matter and energy. They are of four types such as

1. Climatic factors
2. Physiographic factors
3. Edaphic factors
4. Catastrophic factors

Climatic factors:

Any non-living conditions or constituents of the aerial environment of the organisms are called as the climatic factors. The climatic factors are also called as the aerial factors. There are many climatic factors some of which are such as light, temperature, atmosphere (gases and wind), atmospheric humidity, water, air and rainfall (Precipitation).

Light (Sunlight):

It is a climatic factor of great physiological importance. It affects structure, growth and activities of the organisms. Light is the main source of energy for all organisms. It is essential for photosynthesis. Photosynthesis is a process in which green plants synthesise their food on which rest of the living world depends. Development of photosynthetic pigments, pigments for floral colour, red, far red, absorbing phytochrome pigaments (Which regulate morphogenetic process), induction and regulation of many enzymes are the light regulated processes. Photoperiod is an important factor in the flowering plants. The light affects the life particularly the palnt life in different ways such as chorophyll production, heating action, effect on transpiration rate, stomatal movement, distribution of plants, overall vegetative development of plant parts, photoperiodism and succession. On the basis of requirement and effect of light on overall vegetative development, the palnts are classified into four ecological groups such as

1. **Heliophytes**: Plants growing best in full sunlight.
2. **Sciophytes**: Plants growing best at low light intiensities.
3. **Facultative heliophytes**: Plants growing best at lower light intensities but growing well in full sunlight.
4. **Facultative sciophytes**: Plants growing best in full sunlight but growing well under shade.

The light affects photoperiodism in plants. The total length of daily light period to which plants are exposed is called as photoperiodism or photoperiod. The photoperiod has pronounced effect on vegetative growth as well as flowering of the plants. On the basis of photoperiod, the plants are classified into three groups such as

1. **Short day plants (SDP):** The plants which show best vegetative growth and produce flowers only when they exposed to day light for less than twelve hours per day are called as the SDP.
2. **Long day plants (LDP):** The plants which need day light for more than twelve hours followed successively by shorter period of darkness before flowering are called as the LDP.
3. **Day neutral plants (DNP):** The plants without definite photoperiod are called flowers regardless of length of day light or darkness exposure per day are called as the DNP.

Temperature:

It is a climatic factor of great physiological importance. Most of the living organisms can survive only in a narrow range of temperature (5^0C to 35^0C). The term temperature includes the temperature of water for aquatic plants animals and temperature of air for terrestrial plants and animals. The temperature affects life of plants in different ways. It affects metabolism, reproduction, growth and development of plants and animals. The plants are classified into four groups on the basis of temperature requirement such as

1. **Megatherms:** The plants of warm habitat which require high degree of heat throughout the year are called as the megatherms. These plants commonly occure in desert habitats of the tropical climate.
2. **Mesotherms:** The plants of tropical and subtropical habitats which require neither very hot nor very cold temperature are called as the mesotherms. These plants cannot withstand at extemely high or low temperature.
3. **Microtherms:** The plants of cold or temperate habitat which require low temerature for their growth are called as the microtherms. These plants also occur in tropical and subtropical areas at high elevations where temperature falls at low degree.
4. **Hekostotherms**: The plants of cold and alpine regions are called as the hekostotherms. These plants do not grow well where the temperature is very hot and they can withstand long and at very severe winter.

Temperature varies in various quarters of the earth according to latitude and altitude. It is influenced by plant cover, atmospheric humidity, water reservoirs, air current

and snow. Various vegetation zones have been recognised according to changes in the temperature with increase in the latitude.

Atmosphere:

A gaseous layer in which the earth is enveloped is called as the atmosphere. The atmosphere extends into outer space some 1000 km or so above the earth surface. It interacts with all major types of the enviroment of the earth and greatly affects their ability to support life on the earth. It is the reservoir of several elements essential to life. It serves many functions including the filtration of the radiant energy coming from the sun, insulation from heat less at the earth surface and stabilization of weather and climate owing to heat capacity of the air. There are five concentric layers of atmosphere on the basis of temperature such as

1. **Troposphere**: The lowest layer of atmosphere in which man and other living organisms live is called as the Teoposphere. The temperature of this layer is generally low and it decreases up to 60^0C. It is just about 18-20 km above the earth surface. This layer is composed of water vapours, clouds, dust and precipitation.
2. **Stratosphere**: The layer of atmosphere about 50 km above the earth surface and about 30 km above the tropopause (The upper layer of troposphere with thin ozone layer) is called the stratosphere. The tropopause gradually merges with the stratosphere. The temperature of this layer increases up to 90^0C.
3. **Mesosphere**: It is the layer of atmosphere about 40 km in height above the stratosphere.
4. **Thermosphere**: Next to mesosphere is the thermosphere. It extends upto 500 km above the earth surface.
5. **Exosphere**: The rest of the region of the atmosphere above the thermosphere is called as the exosphere or outer space. This extends upto 32,190 km from the earth.

Atmospheric humidity:

Humidity is one of the different forms of the water in nature. The moisture in th form of invisible vapours in the atmosphere is known as the atmospheric humidity. The humidity of air is expressed in terms of relative humidity value. The atmospheric humidity plays an important role in plants and animals life. The processes like tranpiration and absorption of water are highly influenced by the atmospheric humidity.

Atmospheric humidity affects the life of plants in various ways. Effects of moist air on plants are more or less similar to those of reduced light intensity. Some plants like Orchids, Lichens and Mosses make direct use of atmospheric moisture or humidity. In fungi and other microbes, it plays an important role in germination of spores and subsequent

stages in the life cycle. Many plants respond to changes in humidity. Transpiration depends in the gradient of moisture and the overlying air as well as the moisture in the soil. The lower the atmospheric humidity, greater is the transpiration rate.

Water:

Water is an essential requirement of life. Life cannot exist without water. The protoplasm of the cell contains 80-90% of the water. The requirement of water varies from organism to organism. The distribution of organisum depends upon the special adaptations in them for the conservation of water. The plants of dry area are called as xerophytes. The xerophytes develop modifications to increase water absorption. Reduce transpiration and store absorbed water. Plants of aquatic habitats are called as hydrophytes. The hydrophytes possess aerenchyma (air containing parenchyma) to support themselves in water. The depth, salt content, clarity and water currents determine growth and distribution of plants in the water.

Air:

Air currents determine the weather conditions and also affect living organisms. It increases transpiration, which may lead to wilting of many plants. Wind helps in pollination, dispersal of fruits and seeds of many plants. Strong wind uproots the plants and cause loading (flattening of plants on the ground) of many crops. Areas frequented by unidirectional winds develop flag trees which have branches on one side only. Persistent strong winds restrict height of the plants due to excessive loss of water by transpiration. The plants of such areas usually possess strong spreading roots and strong flexible shoots.

Physiographic factors:

The abiotic factors introduced by the structure, conformity and behavior of the earth surface by elevation and slopes, silting, erosion, mountain hills, valleys which affect the plant and animal life are called as the physiographic factors. The physiographic factors tend to produce marked climates or microclimates.

Edaphic factors:

The abiotic factors related to soil conditions which affect the plant and animal life are called as the edaphic factors.

Soil:

The earth is a solid, spherical, cooled planet of the solar system. It spins on its own axis and revolves around the sun at a certain constant distance. The earth is made up of two components such as solid component and the liquid component. The solid component of earth is called as lithosphere and the liquid component as the hydrosphere. The lithosphere of the earth is made up of three layers such as crust, mantle and outer and inner core.

The core is the central fluid or vaporized layer of the lithosphere. It has diameter of about 25,00 km from the centre. It is possibly composed of Nickel and Iron in molten or vapourized state. The mantle extends about 2,900 km above the core. Generally it is in molten state.

The crust is outermost layer of the lithosphere just above the mantle. It is about 8 to 40 km. above the mantle layer. The crust is very complex in composition.

The outer surface of the the crust which is made up of mixture of loose, frible, unconsolidated weathered rocks and organic matter in which plants can grow is called as soil.

Ecosystem:

The ecosystem is the functional unit of ecology and the study of ecology is in fact, the study of ecosystems. The different animal, populations living in a particular habitat constitute as the animal community. The different plant population in a particular habitat is known as the plant community. The animals and plants together make up the biotic community. The biotic or living community and its abiotic or non-living environment (habitat) together make up the ecosystem. The biotic community forms the organic components of the ecosystem where as the abiotic (non-living) enviroment (Habitat) supports the biotic community and forms the inorganic components of the ecosystem. The ecosystem is the fundamental unit of ecology because it includes living organisms and their enviroment. The term biogeocenosis used by Soviet ecologists is synonymous with the ecosystem. The sum total of the ecosystems is called as the biosphere. The ecosystem is relatively stable in time. Solar energy, water, nuterients and atmospheric gases enter into the ecosystem. Heat, oxygen, carbondioxide, humix compounds and living material are carried away from the ecosystem by water and other media.

The biotic community and the abiotic environment thus interact with each other and an exchange of material between them takes place. In other words, living organisms and their non-living environment are inetrdependent and together form an intergrated unit in which each and every component of it functions like a part of a machine. All the components of this integrated unit are well coordinated for the well-being of the entire unit. Such a natural unit of living community and non-living environment interacting and exchanging material between themselves is called as an ecosystem or ecological system. The scientific term ecosystem is the equivalent of 'nature'. The term was used first by Tansley (1935) from England.

Structure of an Ecosystem:

An ecosystem is made up of biotic or living community and its abiotic or non-living environment. The entire living community including plants and animals of all types is called as biotic component of a ecosystem. The non-living environment including all types of elements, compounds and physical forces of the environment is called as abiotic component of an ecosystem.

Abiotic Component of an Ecosystem:

The abiotic component of an ecosystem is composed of

1. Inorganic substances
2. Organic compounds
3. Climatic regime

Inorganic substances:

The water, soil, the elements like Calcium, Potassium, Magnesium, Iron, Phosphorus, the componds like Calcium carbonates, Phosphates; the gases like oxygen, carbondioxide and nitrogen are the inorganic substances of the abiotic component of a ecosystem. These substances are called as the nutrients or raw materials for green plants. The amount of all of these inorganic substances that are present at any given time is known as their standing state or standing quality.

Organic compounds:

The organic compounds are proteins, lipids, carbohydrates etc. of thedead plants and animals. The intermediate and the end products like humus and urea produced by the decomposition of plants and animals are also called the organic compounds. The organic compounds are ultimately derived from the organisms. The remains of plants and animals contain many minerals and salts in bound form. These minerals and salts in bound form are converted into inorganic form by the activity of saprophytic bacteria and fungi. These inorganic forms of minerals and salts are reutilized by the green plants. The organic compounds thus maintain a like between biotic and biotic components of the ecosystem. The amount of organic compunds that are present at any given time is known their standing state or standing quality.

Climate regime:

The physical part of the environment including light, temperature, solar energy, moisture etc is called as the climate regime. The radient or solar energy of the sun is the only significant energy sorce for any ecosystem. The solar energy is trapped by the plasid pigments of green plants in photosysthesis and it is stored there in the form of chemical

energy of organic molecules. It is this energy that flows through the entire biotic community and makes life possible on the earth.

Biotic component of ecosystem:

The biotic component of an ecosystem is made up of all living organisms present in the environmental system. From nutritional point of view the biotic component is of three types such as

1. Producers
2. Consumers
3. Decomposers and Transformer

Producers:

All green plants which fix the solar energy of sun and manufacture food from inorganic substances are called the producers. As they prepare food material for their own, they are also called as Autotrophs.

The green plants are the producers. They bring about the photosynthesis regularly. They use solar or light energy of sun, carbon dioxidefrom the atmosphere and water from the soil and synthesize energy rich carbon compounds like carbohydrates during photosysnthesis. Here in this process the light energy is converted into chemical energy which is actually locked up in the carbon compounds. Oxygen is evolved as the byproduct in the photosynthesis. This is used in respiration by aall living organisms. Algae and other plants in water, grasses and trees in fields and forests are the example of producers.

The term producer is misleading one because, in an energy context, producers produce carbohydrates and not the energy. They convert or transduce the light energy into chemical form. Therefore they are actually not the energy producers but are the energy converters or transducers. Because of wide use only, the term producer is still retained.

Consumers:

All living members of an ecosystem which consume the food synthesized by the producers are called the consumers. Consumers are the hetertrophic components of the ecosystem. All non-green plants and animals which take food from autotrophs are called the consumers. There are four types of consumers such as

1. Primary consumers
2. Secondary consumers
3. Tertiary consumers
4. Parasites, Scavengers and Saprobes.

Primary Consumers:

The purely herbivorous animals which depend on producers or green plants for food are called the primary consumers. Insects, Rodents, Rabiits, Deer, Cow, Buffalow, Goat and Elephent etc. are some common terrestrial primary consumers. Small Crustaceans, Molluscs etc. are the aquatic primary consumers. The herbivores or primary consumers are named as "Key industry animals". They serve as the chief food source for carnivores.

Secondary consumers:

The purely carnivores and omnivores are called as the secondary consumers. They depend on primary consumers for food. The strictly flesh eating animals are called as Carnivores and the animals which consume herbivores as well as plants as their food are called as the Omnivores. Fox, Wolves, Dogs, Cats, Snakes, Sparrow, Cow etc. are the examples of secondary consumers.

Tertiary consumers:

The top carnivores which prey upon other carnivores, omnivores and herbivores are called the tertiary consumers. Lions, Tiger, Hawks, Vulture etc. are considered as the tertiary or top consumers.

Parasites, Scavengers and Saprobes:

The plants and animals which utilize living tissues of different plants and animals are called as parasites and are included in the consumers. The organisms which utilize dead remains of animals and plants as their food are called as scavengers and saprobes and are included in the consumers.

Decomposers and Transformers:

The organisms which break down organic material of plant or animals origin into simple compounds are called the decomposers and transformers. They are the living components of the ecosystem. Decomposers attack the dead remains of producers and consumers and degrade the complex organic matter into simpler compounds.

The simple organic matters are then attacked by another kind of bacteria called the transformers which change these simple organic matters into inorganic forms which are suitable or reuse by producers or green plants. Thus the decomposers and transformers play an important role in maintaining the dynamic nature of ecosystem.

Indirect effects of pollution (ecosystem disruption):

Human activities modify ecosystems in diverse ways. Agriculture is the most obvious example. Forest clearing, dam building, fires and application of defoliants to jungles are some other noteworthy instances.

Mechanical assaults on ecosystems interact with pollution and resources overexploitation, threatening species with extinction.

Deforestation and "Jhum" Cultivation:

Forest provides timber, wood and good recreational resources. They regulate climate, hydrology, erosion, nutrient cycling, and exert a cleansing effect on the air and water in nearby streams. Tropical forests contain a wealth of diverse species of plants and animals which are of direct and indirect benefit for society. Deforestation destroys the habitats of several animals and plants, and leads to serious nutrient losses from the soil. The released nutrients diffuse into streams and lakes, causing eutrophication. Deforestation causes flooding of rivers and silting of dams. It also tends to make the climate more arid.

In North-East India (Meghalaya), non-mechanzied farming system called "Jhum" cultivation has been practiced since long. A clearing is cut in a forest during the dry months; trees are felled, allowed to dry and then burnt. The fire kills weeds, seeds and insects and provides the mineral rich ash. With the onset of rains, the farmer plants a crop. The grain is harvested and the rest of the plants are burnt. When this system is practiced for 2 or 3 years, the soil fertility progressively decreases as there is less and less of ash every year (as compared to the amount in the first year when the trees were burnt). When it is no longer profitable to cultivate that part, the farmer moves on to another forest and repeats the same cycle. Serious loss of soil fertility is an inevitable consequence of increased population density among jhum framers. Increasing pressure of population compels the farmers to revisit an already exploited forest clearing before it has had time to re-establish as a forest.

Another serious consequence of human activities (including that of the jhum framers) in the tropics concerns the formation of lateritic soils. Deforestation interrupts the otherwise continous cycling of butrients. Heavy rains leach off the soil nutrients, the last to be washed off being the oxides of iron and aluninum. Certain iron-rich lateritic soils underlie upto about 10% of tropical forests. These soils tend to erode with their exposure to sun and oxygen, and the resulting chemical changes lead to the formation of a rocky brick like substance called laterite. Laterization is thus equivalent to heavy nutrient losses from soil, and is a direct index of environmental disruption in the tropics.

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FOREST ECOLOGY OF NORTHERN SATPURA MOUNTAIN REGION IN NANDURBAR DISTRICT USING GIS AND REMOTE SENSING APPLICATION

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Abstract:

The present study aims to study the forest ecology change detection in northern Satpura mountain region (Nandurbar District) from 1990 –2015. Normalized deference vegetation index (NDVI) was used to measure the change detection of Northern Satpura mountain region in Nandurbar District. The images of two different time periods 1990 (TM) and 2015 (OLI) have been used to measure the change detection in the study area. Spatial and radiometric enhancement has been carried out to improve the image quality. The resulted values between -1 and +1 were classified as no vegetation, sparse vegetation, moderate vegetation and dense vegetation. The result of vegetation changes in the study area demonstrates that there has been drastic decline in dense vegetation cover 278 km^2 from 1990–2015. The vegetation cover has been classified into four categories no vegetation -1-0, less dense vegetation 0 – 0.33, moderate dense vegetation 0.3–0.66, dense healthy vegetation >0.66-1. Dense healthy vegetation has reduced by 278 km^2 followed by unhealthy vegetation (less dense vegetation) 356 km^2 while as dead vegetation (no vegetation) has increased by 0.5 km^2. The forest change detection reveals that major changes was observed from dense very healthy vegetation to unhealthy vegetation (less dense vegetation) 51.4 km^2 and from moderately healthy vegetation to dead vegetation (no vegetation) 6.6 km^2.

Keywords: Ecology, NDVI, change detection, GIS, forest cover, deforestation.

Introduction:

Where geographical condition permits vegetation to take the form of trees, the forest is one of the major forms of the natural landscape. The forest resources are valuable as an

integral part of the ecology, from the commercial point of view and as providers of shelter to wildlife. Today forest provides raw material for over 5000 products worth about 23 million dollars. They support industry which employs 1.3 million people. In fact forests are still the natural habitat of several species of plant and animal as well as of several tribal groups of Northern Satpura mountain region in Nandurbar district. The history of the exploitation of forest is as old as men himself but during earlier times it was balanced through a natural growth process because at that time forest cutting was done for personal or community use only. .Most of the presentday agricultural land was once forested and then cleared for the use of agriculture. But now it has reached the stage where further clearance will be dangerous for the entire ecology. There aretribals in some part of Northern Satpura mountain region in Nandurbar district where shifting cultivation is still a part of their system of land procurement.

Forests are the most widely distributed ecosystem on the earth, affecting the lives of most humans daily, either as an economic good or an environmental regulator. As forests are a complex and widely distributed ecosystem, remote sensing provides a valuable means of monitoring them. Remote-sensing instruments allow for the collection of digital data through a range of scales in a synoptic and timely manner. Accordingly, a variety of image-processing techniques have been developed for the estimation of forest inventory and biophysical parameters from remotely sensed images. The use of remotely sensed images allows for the mapping of large areas efficiently and in a digital manner that allows for accuracy assessment and integration with geographic information systems.Current advancements in remote-sensor technology are increasing the information content of remotely sensed data and resulting in a need for new analysis techniques.

Study Area:

Satpura Mountain is located in northern part of Nandurbar district in Maharashtra. Satpura region is bounded to the west and side by Gujarat state to the eastjhabhua district of Madhya Pradesh. Narmada river forms the boundary about 70 k.ms of the northern border of the Satpura mountain region. To the north of Nandurbar district is a very remote and dense mountainous region.

This mountainous region is spread over thebasin of Tapi and Narmada river.The study region lies between 21º 30' north and 22º 00' north latitudes and 73º 50' east to 74º 30' east longitudes. The total area of study region is 2315sq.k.ms. The Satpura hilly region of Nandurbar district stretches horizontally from Akalkuwa taluka to Shahadatahsilfromeast

to west Satpura mountain range is 74.4 km long from west to east. From Dhadgaon taluka to Kothar village in Taloda taluka, north-south length is 36.7km.

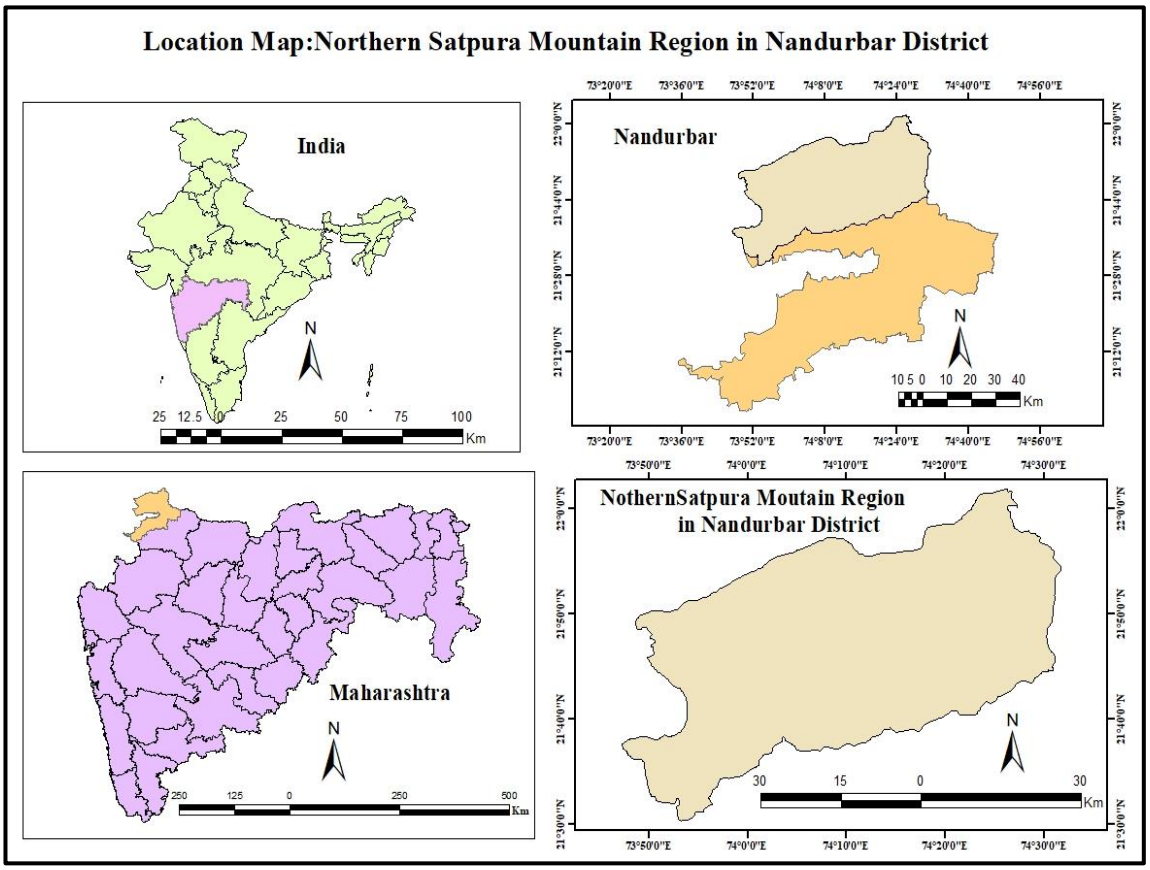

Plate 1: Location Map of Northern Satpura mountain region in Nadurbar district

Objective:

1. To classified the forest cover of Northern Satpura mountain region in Nandurbar district.
2. To generate NDVI index of study area from Landsat satellite data of 1990 and 2015.
3. To analyse change detection of study area generated NDVI indices.

Data base and research methodology:

The present research paper uses Landsat 5 and 8 satellite imagery data.The images of two different time periods 1990 (TM) and 2015 (OLI) have been used to show the forest cover change detection in the study area using Normalized Deference Vegetation Index. The calculation of vegetation has been carried out through the manipulation of reflected energy of an object in the red, infra-red and mid-infrared regions.

The obtained satellite images have been analyzed with Google maps topographical map prepared by the Survey of India sheet number F43O01, F43O02, F43O05, F43O06, F43O07 and F43O09 for the improvement of accuracy. The collected satellite imageries have been verified to assess the quality of data for the study. After the determination of data quality, the atmospheric, radiometric and geometric correction of imagery has been carried out. The satellite imageries with 60 meter spatial resolution (TM satellite) were reclassified into 30 meter to match its spatial resolution with the 2015 (OLI). Arc GIS 10.5 and QGIS 3.10 software are used for calcification of Landsat satellite image.

The vegetation cover has been classified into four categories using the NDVI values with less than -0-0 dead vegetation or inanimate object are named as unhealthy vegetation 0 - 0.33, moderate healthy vegetation 0.33- 0.66, very healthy vegetation 0.66-1. After the classification of vegetation classes the ground truth verification were carried out and the errors in it have been corrected to prepare the accurate vegetation cover maps.

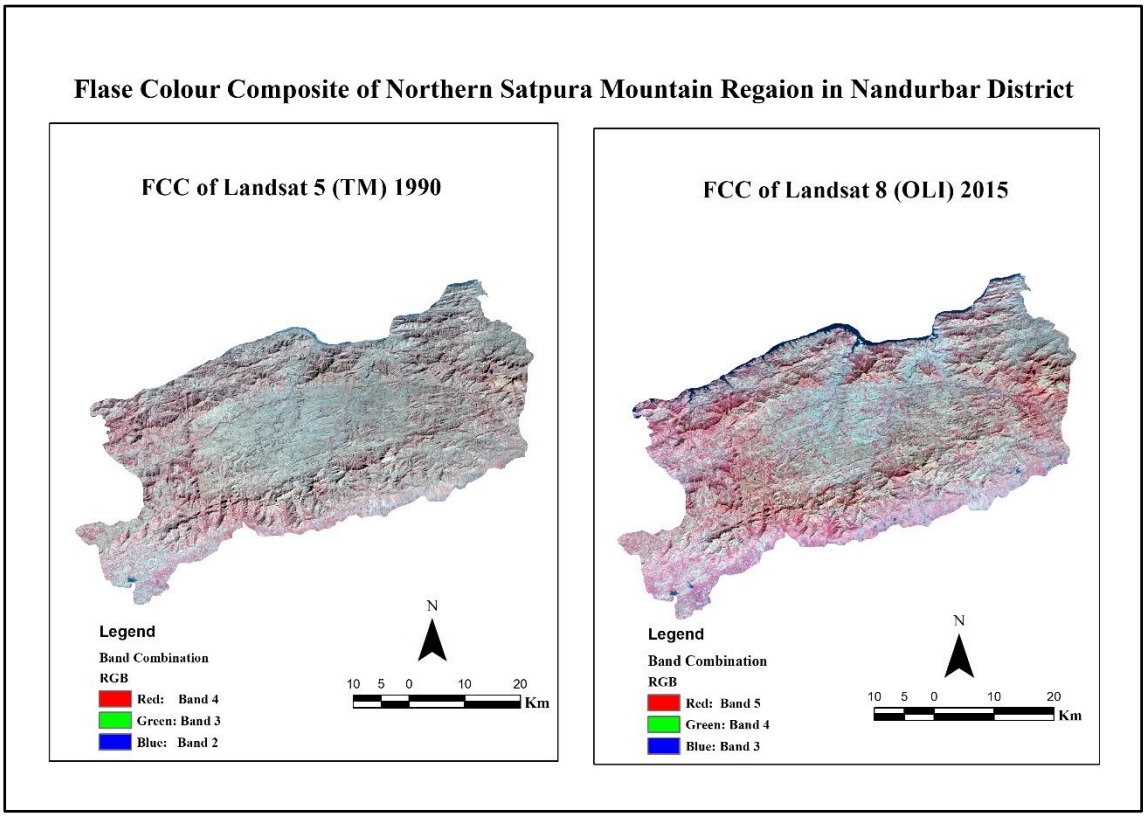

Plate 2: Flase colour composite of Northern Satpura mountain region in Nadurbar district

The overall methodology of this study is briefly presented below:

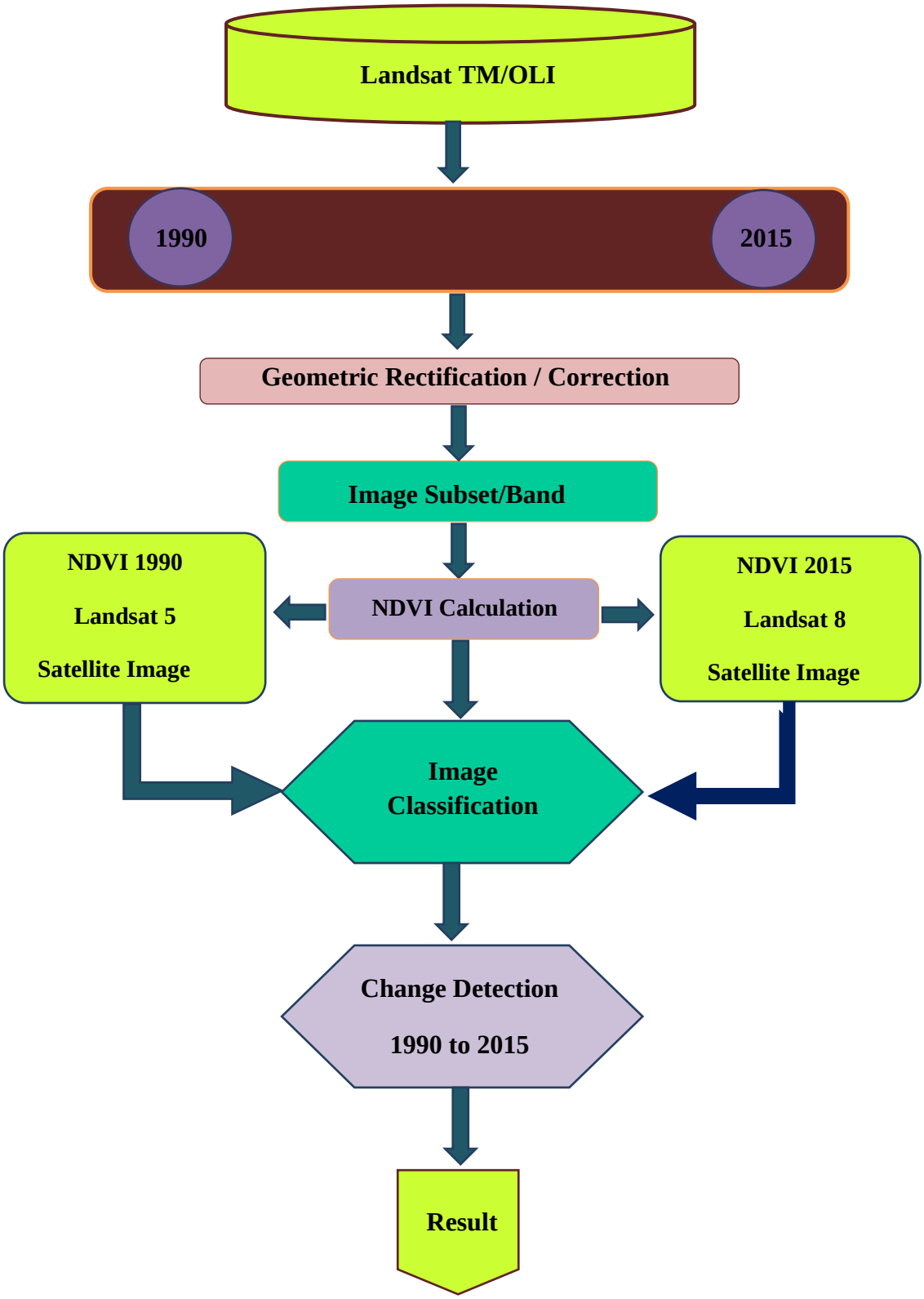

Figure 1: Flow diagram of methodology

Result and Discussion:

The present research paper uses remote sensing techniques to classify landsate satellite images (1990-2015) to study the forest ecology of the northern Satpuda mountainous region of Nandurbar district. The method of NDVI, change detection and forest density in remote sensing techniques has been used for accurate classification of forests ecology of Satpura mountain region.

1. Normalized Deference Vegetation Index (NDVI) Calculation:

The NDVI, as mentioned earlier, is a function of two bands: the red band and near-infrared spectralband. It is calculated for both images using the following relationship.

$$(NDVI) = (NIR-RED) / (NIR+RED)$$

Where: NIR= near-infrared band

RED = red band

Table 1: Forest cover in study Area (NDVI Result)

Sr. No.	NDVI Class	Area (Km^2)	Area (Km^2)	Change Area (Km^2)
		1990	**2015**	
1	Dead Vegetation	43	38	05
2	Unhealthy Vegetation	997	1353	356
3	Moderately Healthy Vegetation	989	916	73
4	Very (Dense) Healthy Vegetation	286	8	278
Total Area=		**2315**	**2315**	**712**
Sources: Landsate Satellite 5and8 imagery classified in Arc GIS 10.5 and QGIS 3.10 Software				

The detailed areal extent of each class is shown in table 1. First the individual year assessment have been calculated, from which it has been found that, total 286 km^2 area were having very dense healthy vegetation in 1990 and 08 km^2 in 2015. The unhealthy vegetation has an area of 997 km^2 in 1990 and 1335 km^2 in 2015 and the moderately healthy vegetation having an area of 989 km^2 in 1990 and 916 km^2 in 2015. The dead vegetation has an area of 43 km^2 in 1990 and 38km^2 in 2015.

Table 1 shows the graphical representation of forest cover of Northern Satpura mountain region in Nandurbar district, where it shows the declining trend in dense, moderate and dead vegetation and increasing trend in unhealthy vegetation in respective

periods. The result of vegetation changes in the study area demonstrates that there has been decline in dense vegetation cover change over the 25 years period of 1990 - 2015 has shown a dramatic change 278 km^2 of dense vegetation.

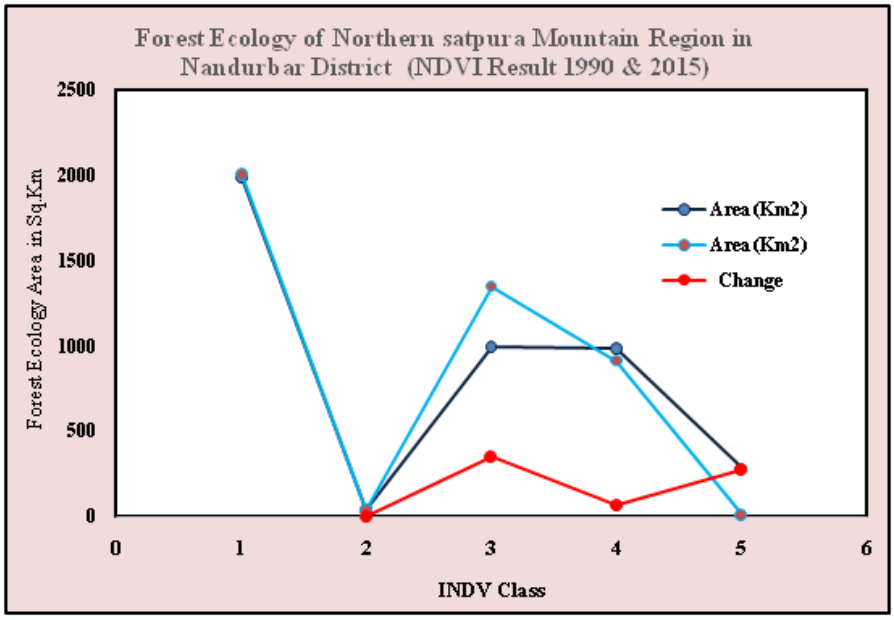

Figure 2: Forest Ecology of Northern satpura Mountain Region in Nandurbar District

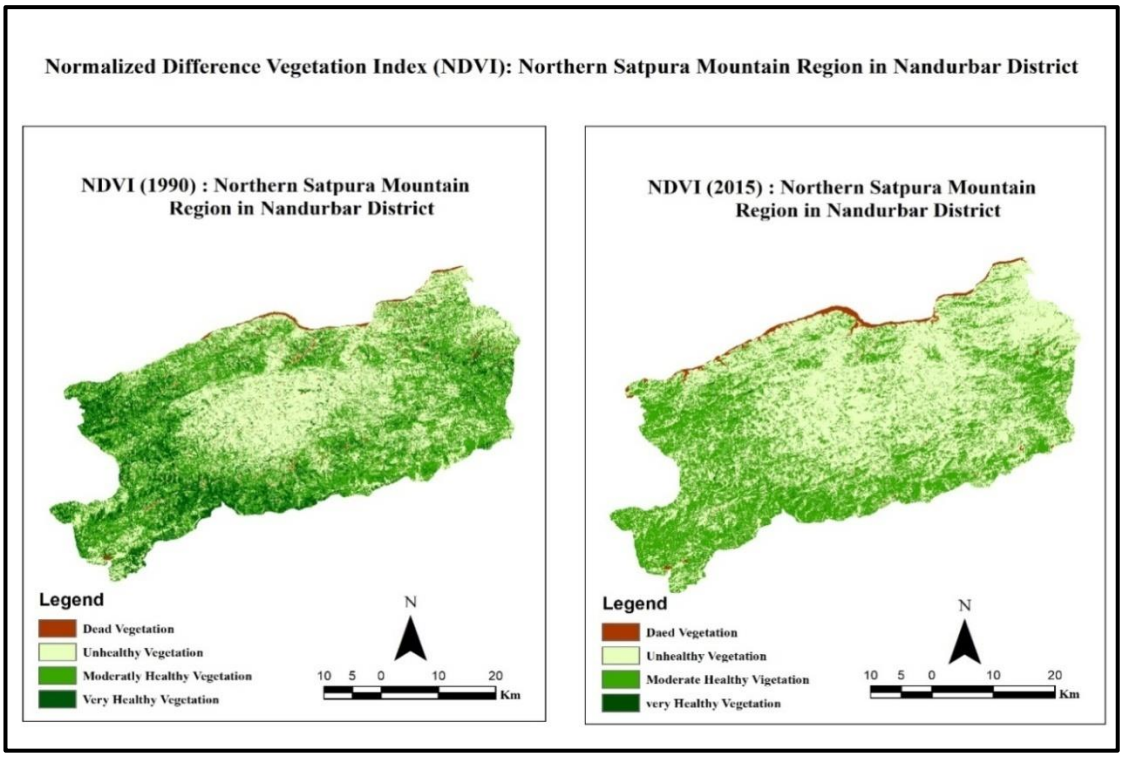

Figure 3: Normalized deference vegetation index (NDVI): Northern Satpura Mountain Region in Nandurbar District

Moderate healthy vegetation in the study area also has shown as decrease of 73 km^2 during the same period. In case of unhealthy vegetation there has been observed an increase of 356 km^2 over the period of 25 years. Analyzing the data of dead vegetation class where decreasing trend has been observed like 05 km^2 from 1990–2015.

2) Change Detection:

The change detection statistics routine is easy to use and identifies not only where changes occurredbut also the class into which the pixels changed. This routine was applied in this study to compile a detailedtabulation of changes between two classification images. This routine differs significantly from a simpledifferencing of the two images. While the statistics report does include a class-for-class image difference, theanalysis focuses primarily on the Initial State classification changes-that is, for each Initial State class, theanalysis identifies the classes into which those pixels changed in the Final State image. Changes can be reportedas pixel counts, percentages, and areas.

Once individual selected years land use and land cover have been detected the overlay analysis has been performed in Arc GIS 10.5and QGIS 3.10 to find out the changes between the selected years. The analysis has been performed to find out the changes between 1990 and 2015. The detailed discussion of changes between the mentioned time periods is following below. Figure 1 shows the forest conversion of Northern satpura mountain region in Nandurbar District between 1990 and 2015.

Table 2 shows a summary of the Normalize differences vegetation cover conversions that have been taken place from 1990 to 2015 within the Northern satpura mountain region, while Figure 2 shows its corresponding change map. From the table, total 14.9 km^2 of dead Vegetation were remained as dead vegetation use, while 1.1 km^2 were converted to moderately dense vegetation to dead vegetation, 164.9 km^2unhealthy to moderate forest, 0.9 km^2 to unhealthy and very dense healthy vegetation.

From the total area of moderate healthy vegetation 449.5 km^2 of area were changed into unhealthy vegetation, 6.6 $km^2$to moderate healthy vegetation to dead vegetation, 520.8 km^2 moderate heathy vegetation to moderate heathy vegetation, 1.5 km^2 moderate heathy vegetation to very dense healthy forest. The total very healthy dense vegetation area was 4.7 km^2 during the year 1990 and this were remained as same till the year 2015without any changes, while 1.3 km^2 area were converted to dead vegetation , 51.4 km^2 area were converted to moderate healthy vegetation. 229 km^2 area were converted to very healthy vegetation.

Table 2: Forest Ecology of Change Detection of Northern Satpura Mountain Region in Nandurbar District (1990 to 2015)

Sr. No.	NDVI Class of Change Detection	Forest Change
1	Dead Vegetation - Dead Vegetation	14.9
2	Dead Vegetation - Unhealthy Vegetation	26.5
3	Dead Vegetation - Moderately Healthy Vegetation	1.1
4	Dead Vegetation - Very Healthy Vegetation	0.2
5	Unhealthy Vegetation - Dead Vegetation	15.8
6	Unhealthy Vegetation - Unhealthy Vegetation	815.9
7	Unhealthy Vegetation - Moderately Healthy Vegetation	164.9
8	Unhealthy Vegetation - Very Healthy Vegetation	0.9
9	Moderately Healthy Vegetation - Dead Vegetation	6.6
10	Moderately Healthy Vegetation - Unhealthy Vegetation	459.5
11	Moderately Healthy Vegetation - Moderately Healthy vegetation	520.8
12	Moderately Healthy Vegetation - Very Healthy Vegetation	1.5
13	Very Healthy Vegetation - Dead Vegetation	1.3
14	Very Healthy Vegetation - Unhealthy Vegetation	51.4
15	Very Healthy Vegetation - Moderately Healthy Vegetation	229
16	Very Healthy Vegetation - Very Healthy Vegetation	4.7
	Total Area =	**2315**

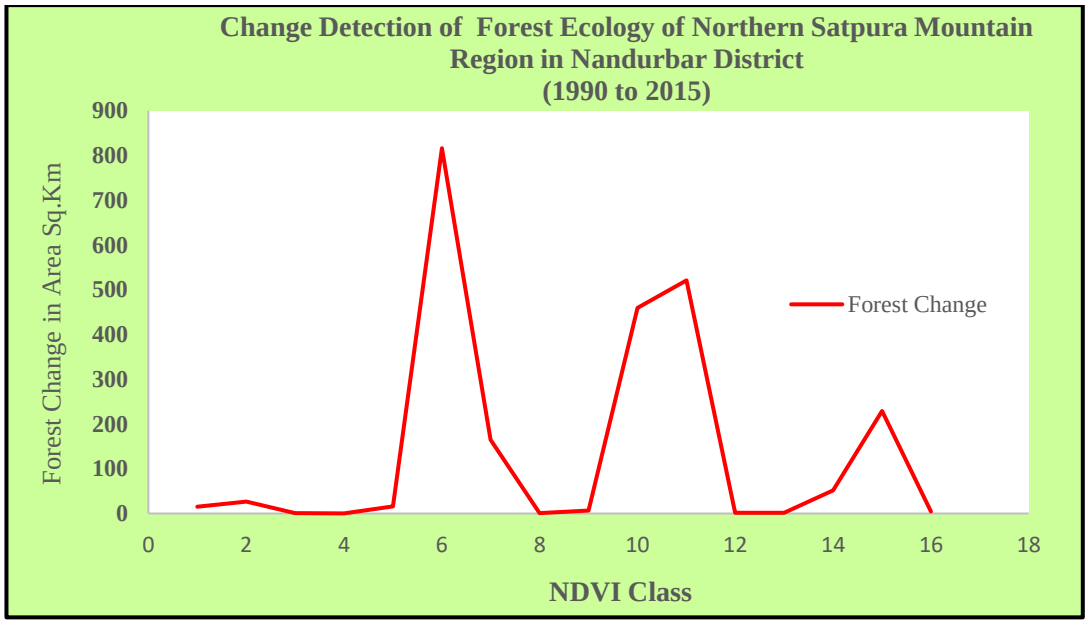

Figure 4: Change Detection of Forest Ecology of Northern Satpura Mountain Region in Nandurbar District

In the case of unhealthy vegetation total 14.9 km^2 were not changed into any other classes while 26.5 km^2 of dead vegetation were converted into unhealthy vegetation , 0.2 km^2 were converted to very dense healthy vegetation , 1.1 km^2 were converted to moderate heathy vegetation.

Conclusion:

The study has analysed the ecological forest density of two time periods (1990-2015). The study showed the significant change in the forest density were found from dense to moderate, moderate to unhealthy forest and unhealthy forest to dead forest (no forest) area. Majority of the change were observed in dense forest cover followed by unhealthy forest which has showed the declining change. While as the moderate, dead forest (no, forest) and non-forest coverage have shown a positive change over the period of 25 years.

Heterogeneous human disturbance during the residential expansion process has attributed to the varying numbers of patch in forest landscape over the years. Among the dead forest (no, forest) and unhealthy forest, unhealthy forest has increased dramatically then moderate forest. This reveals the dense and moderate forest has converted to the unhealthy forest.

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MECHANISM OF FEMALE REPRODUCTIVE SYSTEM

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Reproduction:

The term "reproduction" depicts to a biological process by which a living organism resuscitate a contemporary life with genetical similitude to themselves. In sexual reproduction, two morphologically disjunct cells called gamete which involves a female's ovum and a male's sperm blend together to form zygote [Chodasewicz, 2014]. In this process of fertilization, sperm encounters the ovum in the fallopian tube which is positioned on the upper pelvic cavity. The offspring of placental mammals are delivered with complete sex organs which are not reproductively functional when juvenile but after a period of time the sex organs further progress to become sexually nubile [Bouniol *et al.,* 1995]. Reproduction is not anincessant process and occurs in cyclic patterns. The female reproductive system is well systematized to produce ova, consign the ripen oocyte to the area of fertilization in fallopian tube, nourishes the embryo and extricate the foetus at pertinent time. Among all the mammals, humans have the exorbitant level of sexual differentiation [Wilhelm *et al.,* 2007].

Female reproductive system:

Female reproductive organs perform a prime responsibility in passing the genetic information from one generation to further generation. The female reproductive organs encompass of external genitalia (vulva) which includes mons pubis, labia minora, labia majora, vestibule, clitoris, hymen and internal genitalia comprising of cervix, vagina, ovaries, uterusand fallopian tubes [Hennekam *et al.,* 2013]. The internal genital organs are contrived to carry multiple functions like production of ovum, perpetuate the hormonal

balance for secondary sexual character, transportation of ova to place of fertilization, providing appropriate environment for embryo maturation and parturition of baby [Fonolla, 1998].

Fallopian tubes:

Fallopian tube is the junction where the sperm and ovum approach each other and hence termed as site of fertilization. There are two fallopian tubes, each bestowed at the upper corner of uterus measuring about 10 cm and stretch outwards near ovary. Fallopian tube is also called as uterine tubes or oviduct. These fallopian tubes are accommodated in between the ligaments of uterus [Eddy and Pauerstein, 1980]. Fallopian tube comprises of four parts, interstitial portion within uterus, ampulla outspread segment, isthmus narrow section connecting with uterus and infundibulum is the distal part of fallopian tube. Infundibulum is a funnel sculptured body secured with ciliated epithelium, and unfolds into the peritoneal cavity as finger-like processes termed fimbriae. These fimbriae cumulates the ripen ovum from the ovary into the lumen of uterus. The disgorged sperm decamp the uterine pH and reaches the ova in the fallopian tube [King *et al.,* 2011].

Ovary:

Ovary is a petite, dynamic, paired organ positioned at the distal periphery of uterine horns (less protuberant in human female). Ovary produces the female gamete "Ovum", and steroid hormones like estrogen, progesterone and testosterone through the procedure of steroidogenesis [Edson *et al.,* 2009]. Fully-grown human ovary weighs around 10 g - 20 g with length of 2 u– 5 cm, width of 1.5 – 3 cm. Ovary encompass of three regions, surface of the ovary is called tunica albuginea enveloped by germinal epithelial cells with follicles forming the inner part of the cortex. The oocytes are ingrained in the follicles. The central medulla is made up of stroma, and a hilum enclosing the area of mesovarium to the ovary [Silvestris *et al.,* 2015].

Development of follicle:

Folliculogenesis is a mechanism by which the immature follicles transfigure to preovulatory follicles through a concatenation of intermediary follicles.The primordial germ cells are differentiated from the embryonic somatic genealogy and cognized during the third week of gestation. These germ cells are diagnosed on the endodermal caudal region of the yolk sac during embryonic development and later they relocate to the gonadal ridge [Motta *et al.,* 1994]. The newly emerged female gonad proliferates and enters into meiotic division.

After the primordial germ cells outreach the foetal ovary, a continual proliferative mitotic division takes place to produce 6 -7 million oogonia at the end of 20th week of gestation [Shifren *et al.,* 1993]. During the menarche the germ cells diminish by a process called atresia to dwindle the oocyte population. The primordial follicle remains in dormancy until recruited by the maturing follicular pool. The ovarian follicle proliferates and differentiates, during which the oocytes amplify in size. Gonadotropins are a prerequisite for the maturation of follicles and fibronectin assists in the migration of the germ cells into the yolk sac [Hung *et al.,* 1989]. Most of the follicles are in the non-growing (primordial) phase, later after the recruitment of the primordial follicle, the oocyte dramatically enlarge in size forming the growing phase. The growing follicles are classified into primary, secondary, tertiary and Graafian follicle. The first three stages of follicle development is regulated by intraovarian mechanisms and gonadotrophins independent [Dunlop and Anderson, 2014].

Primordial follicle:

The emergence of primordial follicle is to establish the ovarian follicle reservoir. The primordial follicles are the preliminary stage of follicular development. This follicle are ingrained into the ovarian cortex beneath tunica albuginea and contains immature oocyte which are surrounded by single layer of flat squamous pre-granulosa cells. The basal lamina discrete primordial follicles from the stroma [Bortolussi *et al.,* 1989]. A single oocyte is enfolded in a layer of spindle-shaped protoplasmic cells for the supply of nutrients and arrested in the diplotene stage of the first meiotic division [Baker and Franchi, 1967].

Primary follicle:

The flat granulosa cells of primordial follicles become cuboidal, and follicle differentiates into a single layer of columnar cells, marking the genesis of the primary follicle. These cuboidal granulosa cells undergo repeated mitotic division to form monolayer of zona granulosa or stratum granulosa. During this stage the primitive paracrine signaling pathways between the oocyte and follicle become well established and the oocyte genome is being initiated and the genes gets transcribed [Johnson, 2003]. The mRNA level of *zona pellucida* proteins (ZP-1, ZP-2, ZP-3), Bone Morphogenic Protein – 15 (BMP-15), Growth Differentiation Factor-9 (GDF-9) are escalated in oocyte during this stage. GDF-9 aids in the enrolment of primordial follicle. The oocyte and follicle magnifies in size reaching upto 0.1 mm in diameter [Di Pasquale *et al.,* 2004]. The oocyte emanates zona pellucida which is glycoprotein containing polymer mucous substance which detaches it from the surrounding granulosa cells. The primary follicles display receptors to follicle stimulating hormone

(FSH), but they are gonadotropin independent until the tertiary follicle stage [Dunlop and Anderson, 2014].

Secondary follicle:

In the secondary follicle stage, monolayer of zona granulosa proliferates into multilayer of granulosa cells around the oocyte. The stromal cells exterior to the basal lamina extricate in a concentric layer of perifollicular cells called as theca cells. The part of theca cell neighboring the basal lamina is called as theca interna and the portion of theca cell which integrated with surrounding stroma is termed as theca externa. A network of capillary vessels is formed between the two thecal layers to circulate blood for follicle [Kacinskis *et al.,* 2005].

Tertiary follicle:

Tertiary follicular stage is a gonadotropin dependent stage [Dunlop and Anderson, 2014]. During the tertiary follicle stage numerous fluid filled sacs are formed within zona granulosa cells called as antrum, due to which the granulosa cell and thecal cell establish gap junctions. These gap junctions help in cell-cell communication, and allow tiny molecules to progress through it [Fair *et al.,* 1997]. The primary oocyte is arrested within the tertiary follicle and piled up by granulosa cells called as cumulus oophorus. The tertiary follicle are differentiated into three distinct regions, namely corona radiata bordering the zona pellucida, membrana which is internal to basal lamina, periantral region neighbouring the antrum and cumulus oophorous[Westergaard *et al.,* 2007]. The hypertrophy of theca is transpired with the distinct zone of theca interna and theca externa visible. The Luteinizing Hormone (LH) receptors are evidenced in theca cells. LH persuades the theca cell to produce androgens, especially androstendione. The granulosa cells express *follicle stimulating hormone (FSH)* receptors, and each cell type in granulosa behave differently in response to FSH. The androstendione produced in thecal cells are aromatized by granulosa cells to synthesize estradiol [Gougeon, 1996]. The antrum contains the secretory product of granulosa cells, one of which is estrogen whose concentration is excessive than that of peripheral blood [Mais *et al.,* 1986].

Graafian follicle (Preovulatory follicle):

During the Graafian follicle stage, the antral fluid surged in volume and the granulosa cells deteriorates causing the primary oocyte to float. The mature Graafian follicle liberates the ovum by the process of ovulation. Once the dominant follicle is selected,

the aromatase appears in the granulosa cells, which is differentiated as preovulatory stage. This enables the granulosa cells to aromatize the androstenedione of thecal cell into estradiol [Gougeon, 1996].In response to the escalated level of FSH, the antral follicles begin to discharge estrogen and inhibin, which has a negative feedback effect to inhibit FSH. Due to this negative feedback effect, the FSH-receptors will not mature further, and exhibits retardation of their growth rate and becomes atretic, hence only one follicle develop as a preovulatory or dominant follicle [Scaramuzzi *et al.,* 1999].

Ovarian steroidogenesis:

The process of ovarian steroidogenesis can be well elucidated by two-cell, two-gonadotropin theory. The major endocrine responsibility of the dominant follicle is to produce estradiol, which communicates with the hypothalamus and pituitary creating a hypothalamo – pituitary – ovarian axis [Hinshelwood *et al.,* 2003]. The ovulatory surge of LH during the midcycle is synchronized by the estradiol concentrations, which acts through the pituitary by negative feedback mechanism. The cholesterol acts as the precursor for steroidogenic process. The theca cells comprise of LH receptors, which is the key steroidogenic enzyme requisite for the biosynthesis of androgen from cholesterol [Scaramuzzi *et al.,* 1999]. The key factor that regulate theca cell differentiation are insulin-like growth factor (IGF), GDF9 [Palomino *et al.,* 2014], KITLG (KIT-Ligand), whereas IGF1 persuade the expression of luteinizing hormone/ choriogonadotropin receptor (LHCGR). In rat thecal cell, the KITLG gene stimulates StAR protein and CYP17A1 expression [Mais *et al.,* 1986]. The thecal layer formation is dwindled in mice which lacks *GDF9* gene [Tuck *et al.,* 2015]. The thecal cells exhibit various steroidogenic genes, which includes steroidogenic acute regulatory protein (StAR), LH receptor, side-chain cleavage enzyme (*CYP11A1*), 3-β hydroxysteroid dehydrogenase (3-β HSD), 17-hydroxylase (17, 20-lyase) (*CYP17A1*). The granulosa cells express aromatase (CYP19), which is necessitating for the conversion of androstenedione to estradiol [Weck and Mayo, 2006]. LH receptor and 17-hydroxylase (*CYP17)* converts pregnenolone to dehydroepiandrosterone (DHEA), and progesterone to androstenedione, while the aromatase (*CYP19*) converts androstenedione to estradiol. Both thecal and granulosa cells have side-chain cleavage enzyme (*CYP11A1*), 3-β hydroxysteroid dehydrogenase (3-β HSD) in common, hence granulosa cells can synthesize pregnenolone and progesterone only in minimal concentration from cholesterol, due to vascularization and luteinization of the granulosa cells by gonadotropins, they are in a scanty exposure to cholesterol and oxygen supply. The granulosa cells lack *CYP17* and cannot metabolize progestin to androgens, hence depend upon thecal cells for androgen production [Hillier*et*

al., 1994]. Since progesterone is mandatory for the ovulation and oocyte maturation, a minimal amount of progesterone is produced by the ovarian granulosa cells. The steroid hormone synthesis is mainly modulated by hormones from the anterior pituitary such as FSH and LH, and negative feedback signals by elevated levels of circulating steroids hormones suppress the production of tropic hormones by acting on anterior pituitary. FSH is essential for the normal ovarian follicle development, whereas LH surge at the mid-menstrual surge is required for ovulation and corpus luteum formation. The corpus luteum or luteal cells secrete progesterone [Svechnikov and Soder, 2008].

Ovulation:

Ovulation is a process by which the mature oocyte from a Graafian follicle is ruptured out into the peritoneal cavity. Along with oocyte a small amount of follicular fluid, blood, and cumulus mass is also discharged. The release of oocyte is initiated by the LH surge [50]. This LH surge takes place during the second half of the follicular phase, about 24–48 hours after plasma estradiol level elevates up. The positive feedback mechanism of estradiol at the level of pituitary and hypothalamus results in the hypothalamic gonadotropin-releasing hormone (GnRH) surge and then initiation of LH surge [Quirk *et al.,* 2006]. The midcycle preovulatory LH surge occurs around the 14^{th} day of a menstrual cycle. Ovulation occurs within 36 hours after the LH surge. FSH and progesterone level remains low before ovulation but slightly raises during LH surge. After the ovulation, there is a decline in LH level owing to the loss of positive feedback mechanism by estrogen, and the increasing inhibitory feedback effect of progesterone.The progesterone downregulate the GnRH receptors in pituitary and inhibit LH secretion. Increased level of prostaglandin and proteolytic enzymes like collagenases play a role in banishment of the oocyte [Bauminger and Lindner, 1975]. During the LH surge, the arrested meiotic I stage in oocyte, vanquish the oocyte maturation inhibitor (OMI) and progress to second meiotic metaphase division. This OMI increases the concentration of cAMP in the cumulus cells during the early folliculogenesis period, but after the LH surge, the cAMP level gets diminished when LH obstructs OMI, which progress to the meiotic division [Kawamura *et al.,* 2011].

Luteinization:

Luteinization is a process by which the granulosa and theca cells gets transformed into corpus luteum. LH surge causes morphological changes in the follicle which becomes corpus luteum with a life span of 14 days [Maia *et al.,* 1980]. Due to these changes the

granulosa cell acquires the capacity for the *de novo* synthesis of progesterone and estrogen. Once the oocyte is expelled the granulosa cell amplify in size and becomes vacuolated, to accumulate lutein, which is a yellow eosinophilic pigment. The lutein cell demonstrates side-chain cleavage enzyme and 3-β HSD to produce progesterone and estrogen [Spitschak and Vanselow, 2012]. The capillaries invade into the granulosa cell to secrete vascular endothelial growth factor (VEGF) by the granulosa and thecal cells and provides with low-density lipoproteins (LDL), which act as source for cholesterol [Frederick *et al.,* 1984]. The basal lamina of the follicle gets disintegrated in the surrounding environment. When the ovum is fertilized the progesterone mainly prepares the endometrium for the implantation of the fertilized ovum [Houserman *et al.,* 1989]. After 14 days, at the end of luteal phase the corpus luteum ceases its function and disintegrates by a process called luteolysis [Maia *et al.,* 1980]. Estrogen and prostaglandin F2α (PGF2α) acts as luteolytic factors, which synthesis endothelin-1 (ET-1) [Patwardhan and Lanthier, 1974]. This ET-1 inhibits steroidogenesis and liberates cytokine, tumor necrosis factor-α (TNF-α), which causes apoptosis. The human chorionic gonadotropin (hCG) produced during pregnancy halts the luteolysis process. If pregnancy does not occur the corpus luteum loses its endocrine function, and replaced by scar like tissue called as corpus albicans. The vascularization is reduced and the secretory granules shrink with elevated amount of lipid droplets and cytoplasmic vacuoles [Basini *et al.,* 2002].

Structural development of human uterus:

The uterus is a predominant reproductive organ where the fetus develops during gestational period. Uterus is comprised of different types of muscles like luminal, epithelial, glandular, stromal and smooth muscle. This heterogeneous muscle population undergoes continuous proliferation and differentiation in response to estrogen and progesterone. Uterus is the place for implantation of embryo, nourishment for the fetus, and at the end of gestation period, departs the fetus out [Harris, 2010].The uterus are being originatedfrom the intermediary mesoderm layer. A pair of Mullerian duct (paramesonephric ducts) are formed from coelomic epithelial cells, surrounded by mesenchymal cells and extends beside the mesonephric ducts (Wolffian duct). In female, the Wolffian duct atrophy occurs and the fusion of the Mullerian duct forming uterus. Due to invagination in paramesonephric ducts, the abdominalostium of the fallopian tube emerges and the uterine cavity is formed in the 12th week [Pietryga and Wozniak, 1992]. The Mullerian duct forms the epithelial lining of uterus, uterine tube and upper vaginal canal. At the 9th-20th week of gestation the paramesonephric ducts and urogenital sinus condense to form sinusal tubercle, then

becomes vaginal plate, which becomes hollow forming sinovaginal bulb, finally developing vagina.Uterine horn is developed during the 20th week of gestation. The uterine glands involving glandular and luminal epithelium penetrates the uterine stroma, and the outer muscles of uterine increases in thickness due to genesis. The adult uterus measures approximately 6.25 cm from orifice to the fundus [Soriano *et al.,* 1999]. Uterine adenogenesis continues postnatally and is highly susceptible to hormones and cyclic changes which occur during puberty.In human it weighs about 60 grams and measures approximately 7.6 cm long, 4.5 cm broad and 3.0 cm thick. The uterus is located inside the pelvic girdle, ventrally to rectum and dorsally to urinary bladder. The human uterus is a pear-shaped organ with three distinct regions namely, outer perimetrium, middle myometrium, and inner endometrium [Beddington and Robertson, 1999].

Perimetrium:

The perimetrium is the outer most serous coat around the uterus, which is a layer of loosely packed connective tissue. This is derived from visceral peritoneum, containing enormous number of lymphatic vessels [Abd-Elnaeim *et al.,* 2001].

Myometrium:

The myometrium forms the middle layer of the uterus, made up of smooth muscle cells, which is called as uterine myocytes. The myometrium is located in between perimetrium or serosa and endometrium and composed of stromal and vascular tissue.Primary function of myometrium is uterine contraction. Myometrium is composed of three distinct regions. Outer derived from subperimetrial mesenchyme, made of longitudinal smooth muscles, which assists in uteri contraction during parturition. Middle layer is comprised of muscle fibers and crisscross nerve fibers, and contains large blood vessels, which prevents blood loss during various cyclic events [Metaxa-Mariatou *et al.,* 2002]. The inner layer of myometrium originated from Mullerian duct, helps in peristaltic and anti-peristaltic activity and the inner circular layer is an intermediate layer of ductal mesenchymal cells. The myosin and actin are the predominant proteins expressed in smooth muscles of myometrium, which helps in uterine contractions, called “Ferguson reflex” during the menstrual cycle. The muscular tissues degenerates during pregnancy and the GAP-junction becomes more prominent [Morgan, 2014].

Endometrium:

The endometrium is the inner most mucous layer of the uterus, made up of columnar epithelium with secretory cells, which is derived from ductal mesenchymal tissue beneath the stromal connective tissue. Endometrium is considered as the implantation window of uterus. Numerous spiral arteries, carrying blood penetrates the endometrial surface from the basal stroma [Evans and Salamonsen, 2014]. Uterus undergoes numerous proliferations and differentiation during menstrual cycle, theseinterchanges is induced by estrogen and progesterone. The endometrium consists of two layer stratum functionalis and stratum basalis. The stratum basalis (stratum compactum) is lined with luminal epithelium, which is a densely organized stromal zone. Basalis is inert to ovarian hormones, and forms new stratum functionalis after every menstrual cycle [Morris *et al.,* 1985].Stratum functionalis (stratum spongiosum) is a non-systematic stromal zone, which is a superficial layer, with immune cells and reciprocate to numerous cyclic changes created by gonadotropins, ovarian hormones and sheds off during every menstrual cycle [Gellersen and Brosens, 2014].The endometrium consists of tunica mucosa, tunica submucosa, and tunica propria underlying the smooth muscular region with numerous neutrophils to prevent infection. Uterine glands are present in tunica propria, which extends to the entire stromal length. Caruncles are the region that connects the uterus with other reproductive membranes [Takacs *et al.,* 2005].

Blood supply to female reproductive system:

The uterine artery provides blood supply to the uterus, which arises above ureter at a distance of 2 cm from uterus. This uterine artery anastomoses with ovarian artery to supply blood to ureter, cervix and vagina [Lampmann *et al.,* 2004]. The ovarian artery arises from the aorta just below the renal artery and branches with uterine artery. Vaginal artery is a branch of uterine artery and supplies blood to it.The middle rectal artery and the pudendal artery leaves the pelvic cavity through the sciatic foramen, enters the ischiorectal fossa, and gives rise to the inferior rectal artery, then terminates into branches that supply the perineal and vulvar structures [Takasaki *et al.,* 2010]. The hypogastric artery bifurcates from iliac artery and diverge into anterior and posterior artery, which supply all pelvic viscera. The superior rectal artery is a continuation of mesenteric artery, which branches into rectum [Reich *et al.,* 1965].

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BIODIVERSITY: CONCEPT, THREATS AND CONSERVATION

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Abstract:

Biodiversity is the variety of different forms of life on earth, including the different plants, animals, micro-organisms, the genes they contain and the ecosystem they form. It refers to genetic variation, ecosystem variation, species variation (number of species) within an area, biome or planet. Relative to the range of habitats, biotic communities and ecological processes in the biosphere, biodiversity is vital in a number of ways including promoting the aesthetic value of the natural environment, contribution to our material well-being through utilitarian values by providing food, fodder, fuel, timber and medicine. Biodiversity is the life support system. Organisms depend on it for the air to breathe, the food to eat, and the water to drink. Wetlands filter pollutants from water, trees and plants reduce global warming by absorbing carbon, and bacteria and fungi break down organic material and fertilize the soil. It has been empirically shown that native species richness is linked to the health of ecosystems, as is the quality of life for humans. The ecosystem services of biodiversity is maintained through formation and protection of soil, conservation and purification of water, maintaining hydrological cycles, regulation of biochemical cycles, absorption and breakdown of pollutants and waste materials through decomposition, determination and regulation of the natural world climate. Despite the benefits from biodiversity, today's threats to species and ecosystems are increasing day by day with alarming rate and virtually all of them are caused by human mismanagement of biological resources often stimulated by imprudent economic policies, pollution and faulty institutions in-addition to climate change. To ensure intra and intergenerational equity, it is important to conserve biodiversity. Some of the existing measures of biodiversity conservation include; reforestation, zoological gardens, botanical gardens, national parks, biosphere reserves, germplasm banks and adoption of breeding techniques, tissue culture techniques, social forestry to minimize stress on the exploitation of forest resources.

Keywords: Biodiversity, conservation, ecosystem services

Introduction:

Biodiversity is made up of two words bio means life diversity means verity, it means verity among living organism is called as biodiversity. Living organisms found on the earth are bacteria, fungi, pteridophytes, bryophytes, reptiles, birds Mammals are the basics of Biodiversity. Biodiversity occur in small aquarium or in large ocean, it may be present in small region or it occur in large ecosystem like Terrestrial ecosystem, aquatic ecosystem, desert ecosystem or grassland ecosystem. Everywhere we can see biodiversity.

So far, about 2.1 million species have been identified, mostly small creatures such as insects. Scientists believe that there are actually about 13 million species, though as per UNEP estimates there are 9.0 to 52 million species exist on earth (Mora *et al.*, 2011). Biodiversity also includes genetic differences within each species - for example, between varieties of crops and breeds of livestock. Chromosomes, genes, and DNA the building blocks of life-determine the uniqueness of each individual and each species. In each ecosystem, living creatures including human form a community, interacting with one another and with the air, water, and soil around them.

Biodiversity is thus considered at 3 major levels: Genetic diversity: This is the variety of genetic information contained in all of the individual plants, animals and microorganisms occurring within populations of species. Simply it is the variation of genes within species and populations. Species diversity: This is the variety of species or the living organisms. It is measured in terms of- Species Richness - This refers to the total count of species in a defined area. Species Abundance - This refers to the relative numbers among species. In nature, not all species of a community are equally different. It is possible to classify species on the basis of their functions) Functional types: Functional types are those species, which perform different ecological functions. b) Functional analogues: Functional analogues represent distinct taxa performing the same or very similar ecological functions.

Benefits of biodiversity:

Utilitarian benefits Biodiversity contribute to our material well-being. We obtained various productive materials from biodiversity e.g. Many species of plants are consumed by man Near about 90% off the food crops have been domesticated from wild tropical plants .Although there are about1,000 species of cereals out of them200 species have been domesticated as food crop less than 20 crops are cultivated to produce 85 percent of World's food. Many species of algae and mushrooms are also consumed by man. Many wild plants also have medicinal values. Value of biodiversity may be direct or indirect. Biodiversity may

be direct for consumptive purpose such as food, medicine and fuel. Indirect value of biodiversity is that ecosystem service value, ethical value and social service value etc.

Many still do, but many do not.

Following are some important benefits of Biodiversity.

- Increase ecosystem productivity; each species in an ecosystem has a specific niche—a role to play.
- Support a larger number of plant species and, therefore, a greater variety of crops.
- Protect freshwater resources.
- Promote soils formation and protection.
- Provide for nutrient storage and recycling.
- Aid in breaking down pollutants.
- Contribute to climate stability.
- Speed recovery from natural disasters.
- Provide more food resources.
- Provide more medicinal resources and pharmaceutical drugs.
- Offer environments for recreation and tourism.

Causes of the loss of biodiversity:

The main cause of the loss of biodiversity can be attributed to the influence of human beings on the world's ecosystem, In fact human beings have deeply altered the environment, and have modified the territory, exploiting the species directly, for example by fishing and hunting, changing the biogeochemical cycles and transferring species from one area to another of the Planet. The threats to biodiversity can be summarized in the following main points:

- Alteration and loss of the habitats: the transformation of the natural areas determines not only the loss of the vegetable species, but also a decrease in the animal species associated to them.
- Introduction of exotic species and genetically modified organisms: species originating from a particular area, introduced into new natural environments can lead to different forms of imbalance in the ecological equilibrium. Refer to, "Introduction of exotic species and genetically modified organisms".
- Pollution: human activity influences the natural environment producing negative, direct or indirect, effects that alter the flow of energy, the chemical and physical constitution of the environment and abundance of the species;

- Climate change: for example, heating of the Earth's surface affects biodiversity because it endangers all the species that adapted to the cold due to the latitude (the Polar species) or the altitude (mountain species).
- Overexploitation of resources: when the activities connected with capturing and harvesting (hunting, fishing, farming) a renewable natural resource in a particular area is excessively intense, the resource itself may become exhausted. Sardine Cod and Tuna are the fishes which are consumed by man .Liver oil of such fishes are extracted .Cod liver oil is the rich source of Vitamin A and Vitamin D. Man captured them largely and their species become exhausted.

Biodiversity and its conservation methods:

Biodiversity is the huge varieties of the living organisms on the earth. It can be conserved in the following ways:

- *In-situ* Conservation
- *Ex-situ* Conservation

In-situ Conservation:

National park and Wildlife Sanctuaries are some of the example of in- situ conservation.

The in-situ conservation has several advantages. Following are the important advantages of in-situ conservation:

1. It is a cost-effective and a convenient method of conserving biodiversity.
2. A large number of living organisms can be conserved simultaneously.
3. Since the organisms are in a natural ecosystem, they can evolve better and can easily adjust to different environmental conditions.

National parks:

These are small reserves maintained by the government. National parks often allows protected species to flourish. eg., Kanha National Park, Bandipur National Park.

Wildlife sanctuaries:

Wild life sanctuaries is an area where animal habitat and protected from any type of hazards like killing, capturing, poaching etc. Human activities such as timber harvesting, cultivation, collection of woods and other forest products are allowed here as long as they do not interfere with the conservation project. Also, tourists visit these places for recreation.

Biosphere reserves:

Biosphere reserves are unique representative ecosystemof coastal and terrestrial area which are internationally recognized. Tourist and research activities are permitted here.

Ex-situ Conservation:

Ex-situ conservation is the process of protecting an endanger species. Ex-situ conservation of biodiversity involves the breeding and maintenance of endangered species in artificial ecosystems such as zoos, nurseries, botanical gardens, gene banks, etc.

Ex-situ conservation has the following advantages:

1. The animals are provided with a longer time and breeding activity.
2. The species bred in captivity can be reintroduced in the wild.
3. Genetic techniques can be used for the preservation of endangered species.

Strategies for Biodiversity Conservation

Following are the important strategies for biodiversity conservation:

1. All the varieties of food, timber plants, livestock, microbes and agricultural animals should be conserved.
2. All the economically important organisms should be identified and conserved.
3. Unique ecosystems should be preserved first.
4. The resources should be utilized efficiently.
5. Poaching and hunting of wild animals should be prevented.
6. The reserves and protected areas should be developed carefully.
7. The levels of pollutants should be reduced in the environment.
8. Deforestation should be strictly prohibited.
9. Environmental laws should be followed strictly.
10. The useful and endangered species of plants and animals should be conserved in their nature as well as artificial habitats.
11. Public awareness should be created regarding biodiversity conservation and its importance.

Why should you conserve Biodiversity?

We should conserve biodiversity so as to maintain the food chain because any disturbances in the food chain may affect the whole ecosystem.

Protected forests are not safe for wild animal because people live near the forest utilize all the resources from the forest to fulfill their own requirement we can further claim the necessity of biodiversity by considering our degree of dependency on the environment. Similarly, we depend on various species of animals and microbes for different reasons.

Biodiversity loss includes extinction of plan or animal species or loss of species in any habitat, resulting in a loss of biodiversity. Major factors for loss of Biological diversity are:

1. Habitat loss

2. Over –Exploitation
3. Climate change
4. Human activity

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CONTROL OF WATER POLLUTION : A NEED OF TOMORROW

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Introduction:

Water is a substance composed of chemical elements hydrogen and oxygen and existing in gaseous, liquid and solid states. It is one of the most abundant and essential compounds. A tasteless and odourless liquid at room temperature, it has the important ability to dissolve many other substances; therefore water is also called as universal solvent. Water occurs as a liquid on the surface of Earth under normal conditions, which makes it invaluable for transportation, for recreation, and as a habitat for a myriad of plants and animals. The fact that water is readily changed to a vapour (gas) allows it to be transported through the atmosphere from the oceans to inland areas where it condenses and, as rain, nourishes plant and animal life (Zumdahl, 2021).

Water is essential for life. From the time that primeval species ventured from the oceans to live on land, a major key to survival has been prevention of dehydration. The critical adaptations cross an array of species, including man. Without water, humans can survive only for days. Water comprises from 75% body weight in infants to 55% in elderly and is essential for cellular homeostasis and life. Ecosystems – such as forests, wetlands and grasslands – are a critical part of the global water cycle. All freshwater ultimately depends on the continued healthy functioning of ecosystems, and recognizing the water cycle as a biophysical process is essential to achieving sustainable water management.

One of the most important challenges facing humanity today is to conserve and sustain water resources (either surface water or groundwater). This challenge became more pronounced with the increase of urban, agricultural, and industrial activities that discharge a considerable amount of wastewater (Breida *et al.*, 2019). Water pollution can be defined as 'the contamination of a stream, river, lake, ocean or any other stretch of water, depleting water quality and making it toxic for the environment and humans'. Many chemical substances such

as cleaners, dyes, paints, pesticides and solvents, which are poured down drains, are a substantial and dangerous form of pollution emitted into the environment from anthropogenic sources poses a threat to the functioning of aquatic ecosystems and to the use of water for various purposes. The need for strengthened measures to prevent and to control the release of these substances into the aquatic environment has led many countries to develop and to implement water management policies and strategies based on, amongst others, water quality criteria and objectives.

Causes of water pollution:

Water pollution occurs when harmful substances—often chemicals or microorganisms - contaminate a stream, river, lake, ocean, aquifer, or other body of water, degrading water quality and rendering it toxic to humans or the environment.Due to population growth, accelerated urbanization and economic development, the quantity of wastewater generated and its overall pollution load are increasing globally. Some of the causes are explained below:

(1) Runoff from Agricultural operations:

Not only is the agricultural sector the biggest consumer of global freshwater resources, with farming and livestock production using about 70 percent of the Earth's surface water supplies, but it's also a serious water polluter. Around the world, agriculture is the leading cause of water degradation. Agriculture has an impact on water pollution due to the use of chemicals such as fertilizers, pesticides, fungicides, herbicides or insecticides running off in the water, as well as livestock's excrement, manure and methane (greenhouse effect). Each year approximately 70,000 kinds of organic chemicals are placed on the market that ultimately makes their way through environment to the water. Regarding aquaculture, pollution is directly in the water, as excess food and fertilizers are causing dystrophication.

(2) Leakage from underground storage and piping:

Groundwater gets polluted when contaminants—from pesticides and fertilizers to waste leached from landfills and septic systems—make their way into an aquifer, rendering it unsafe for human use. A tank or piping network that has at least 10 percent of its volume underground is known as an underground storage tank (UST). They often store substances such as petroleum, that are harmful to the surrounding environment should it become contaminated. Many UST's constructed before 1980 are made from steel pipes that are directly exposed to the environment. Over time the steel corrodes and causes leakages,

affecting surrounding soil and groundwater. Poorly operated or abandoned mine sites are often significant sources of water contamination; contaminants of particular health concern from these sources include heavy metals, and mineral-processing chemicals, such as cyanide and can adversely affect the health of nearby communities that rely on this source for drinking-water or agriculture.

(3) Leakage sewers:

Swage is the term use for wastewater that often contains faeces, urine and laundry waste. Mainly in low-income areas of cities and towns within developing countries, a large proportion of wastewater is discharged directly into the closest surface water drain or informal drainage channel, sometime without or with very little treatment. In addition to household effluent and human waste, urban-based hospitals and industries such as small-scale mining and motor garages, often dump highly toxic chemicals and medical waste into the wastewater system. Inadequate sewage collection and treatment are sources of water pollution. According to United Nations, more than 8% of the worldwide wastewater goes back in the environment without being treated or reused.

(4) Vehicle emission:

Automobiles include cars, trucks, motorcycles and boats (anything that burns gas). Vehicle-related particulates in highway runoff come mostly from tire and pavement wear (~ 1/3 each), engine and brake wear (~ 20%), and exhaust (~ 8%) (EPA, 1996). Each year, approximately 185 million gallons of improperly discharged used motor oil pollute streams, lakes, and coastal areas (EPA, 1999b). They leave oil, antifreeze, grease and metals on streets and driveways. vehicle emissions not only cause harmful health impacts for people near them but they also affect the quality of the water near urban areas by adding nitrogen dioxides to the water off the coast which promotes algae growth, starving the fish of oxygen.

5) Industrial activities:

Industry is a huge source of water pollution, it produces pollutants like asbestos, lead, mercury, nitrates, phosphates, sulphur, petrochemicals that are extremely harmful to people and the environment. This can stop water plants receiving enough light for photosynthesis. It is also harmful for fish and marine birds. The industries that cause pollution are printing, electroplating, soap manufacture, food products, rubber and plastics, chemicals, textiles, steel, sugar factories, glass manufacture, etc. if industrial waste are not released directly into water bodies, they can also percolate through the soil and pollute the

groundwater. Industrial wastes impair water quality in diverse ways. Wastes may contain bacteria or viruses harmful to human health. Besides obvious aesthetic considerations, the decomposition of organic wastes robs water of dissolved oxygen essential to support the life processes of aquatic creatures. Salts, acids, phenols, alkali and other compounds present in industrial waste waters degrade water for a wide range of uses, while the various organic or inorganic chemicals industry discharges into water disrupt the delicate food chains of lower levels of animal and vegetable life and ultimately may prove toxic to people.

(6) Hazardous waste:

Big spills may dominate headlines, but consumers account for the vast majority of oil pollution in our seas, including oil and gasoline that drips from millions of cars and trucks every day. Moreover, nearly half of the estimated 1 million tons of oil that makes its way into marine environments each year comes not from tanker spills but from land-based sources such as factories, farms, and cities. At sea, tanker spills account for about 10 percent of the oil in waters around the world, while regular operations of the shipping industry—through both legal and illegal discharges—contribute about one-third. Radioactive pollutants include wastewater dischargesfrom factories, hospitals and uranium mines. These pollutants can also come from natural isotopes, such as radon.

Effects:

(1) Destruction of aquatic flora and fauna:

Marine debris—particularly plastic—is blown in by the wind or washed in via storm drains and sewers. Our seas are also sometimes spoiled by oil spills and leaks—big and small—and are consistently soaking up carbon pollution from the air. The ocean absorbs as much as a quarter of man-made carbon emissions. Within aquatic ecosystems a complex interaction of physical and biochemical cycles exists. Anthropogenic stresses, particularly the introduction of chemicals into water, may adversely affect many species of aquatic flora and fauna that are dependent on both abiotic and biotic conditions. . If there is a large supply of organic matter in the water, oxygen-consuming (aerobic bacteria multiply quickly, consume all available oxygen, and kill all aquatic life.

When biodegradable waste enters a water supply, the waste provides an energy source (organic carbon) for bacteria. Organic carbon is converted to carbon dioxide and water, which can cause atmospheric pollution and acid rain; this form of pollution is far more widespread and problematic than other forms of pollutants, such as radioactive waste. Many radioactive isotopes escape to water reservoirs, rivers, and seas from nuclear power

reactors; they enter food chain in ecosystem. These wastes may accumulate in the bodies of aquatic animals like fishes causing harm to them as well as animals which eat them. At high enough concentrations it can kill; in lower concentrations it can cause cancers and illness.

(2) Agriculture and Economic downturns:

Poor quality water may affect irrigated crops by causing accumulation of salts in the root zone, by causing loss of permeability of the soil due to excess sodium or calcium leaching, or by containing pathogens or contaminants which are directly toxic to plants or to those consuming them. Contaminants in irrigation water may accumulate in the soil and, after a period of years, render the soil unfit for agriculture.The general public also bears a portion of the cost in the form of opportunity loss resulting from the diminished range of desired uses which can be made of the polluted water. As a member of the water-using public, industry may accrue a proportionate share of the indirect cost of pollution through loss in property value or inability to attract a high quality work force to the area; but generally, these costs are so hidden that they are either not perceived or are regarded as inconsequential in comparison to the cost of instituting and maintaining an adequate waste management program (Hines, 1968).

Controllingmeasures of water pollution:

Adaptation of monitoring programmes, surveillance systems and laboratory practices are necessary in the implementation of water quality objectives. In recent years there has been a remarkable consensus on market-friendly and environment-friendly policies for managing water resources and for delivering water and sanitation services on an efficient, equitable and sustainable basis. At the heart of this consensus are three closely related guiding principles expressed at the 1992 Dublin International Conference on Water and the Environment, namely (1) The ecosystem principle: Planners and policy makers at all levels should take a holistic approach linking social and economic management with protection of natural systems. (2) The institutional principle: Water development and management should be based on a participatory approach, involving user, planners and policy makers at all levels, with decisions taken at the lowest appropriate level. (3)The instrument principle: Water has an economic value in all its competing uses and should be recognised as an economic good (Helmer and Hespanhol, 1997).

Education is important for control of water pollution by educating family, friends and even society as through joint campaign and advocacy. Educative topics can include

avoid throwing of oil, paints, chemicals and medicines in sink drain or toilet can as well contribute to reduce the dangers of water pollution. Waste consumer products and utilities such as drugs, battery cells can be disposed at designated disposal where the relevant authorities can collect and dispose them effectively;use of more environmentally friendly consumer products at home and in public places.

Tomorrow's need:

Water pollution is jeopardizing our health. Unsafe water kills more people each year than war and all other forms of violence combined. Meanwhile, our drinkable water sources are finite: Less than 1 percent of the Earth's freshwater are actually accessible to us. Without action, the challenges will only increase by 2050, when global demand for freshwater is expected to be one-third greater than it is now. According to United Nations, "Good water quality is essential to human health, social and economic development, and the ecosystem". However, as populations grow and natural environments become degraded, ensuring there are sufficient and safe water supplies for everyone is becoming increasingly challenging. Without water conservation, we won't go very far. It is central in making sure the world has better access to clean water. It means being aware that water is a scarce resource, taking care of it accordingly, and managing it responsibly. A major part of the solution is to produce less pollution and improve the way we manage wastewater. Water must be carefully managed during every part of the water cycle: from fresh water abstraction, pre-treatment, distribution, use, collection and post-treatment, to the use of treated wastewater and its ultimate return to the environment, ready to be abstracted to start the cycle again".

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STUDY OF PHYSICOCHEMICAL PARAMETERS OF GODAVARI RIVER, PANCHAVATI NASHIK MAHARASHTRA

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Abstract:

The study of Physico-chemical parameters of Godavari River was carried out. This investigation was carried to study Physico-chemical parameters of water such as color, Temperature, Turbidity, pH, Odor, Total hardness, Sulphate (SO4), Total dissolved solids (TDS), Dissolved oxygen (O2), Phosphate (PO4) such parameters were examined from sampling station. Variation in physical and chemical parameters of water sample from sampling station was observed affected the quality and quantity of water, aquatic ecosystem there due to the human activities are seen during the study.

Introduction:

Water is most important thing to all living beings. Most of our demand for water is fulfilled by rainwater, which gets deposited in surface and ground water resources. Due to the increase in industrialization, urbanization and increase in human activities near the water bodies, the natural water bodies are getting affected. Due to this pollution the physical and chemical parameters are also affected. Study of physicochemical parameters help in the identification of pollution. Heavy pollution is seen in the rivers which flow through cities, industrial areas, power plants etc.

Material and Method:

The water sample from Godavari River was collected from three different stations in a plastic bottle regularly after 20 days for three months. The water samples were immediately brought to laboratory for the estimation of various physico – chemical parameters, like water temperature and pH were recorded at the time of sample collection by using thermometer and pH paper. Remaining parameters like colour, odor, turbidity,

total hardness, sulphate content, DO, TDS, Phosphate content were estimated in the laboratory by using standard protocol.

Study Area:

The area under investigation was Godavari River. It starts from Brahmagiri Mountain Triambakeshwar Nashik. It starts from western state of Maharashtra and flows through the southern state of Andhra Pradesh before reaching the Bay of Bengal. It forms one of the largest river basins in India with a length of 1,465 kms.The sample were collected from the Panchavati Nashik to study physico – chemical parameters. Nasik is an ancient city and even Godavari River has its own religious importance. Activities like washing clothes, vehicles, raising markets add on to the pollution. Religious activities like 'Kumbh-Mela', Ganesh festival, Holi, 'Chhat-puja', Funerals and cremations done in the river and on river banks heavily add various wastes and in turn lead to pollution of Godavari.

Results and Discussion:

The results of the study are summarized in table 1.

Table 1: Physicochemical parameters of selected study site

Sr. No.	**Parameter**	**Study Site**
Physical		
1.	Color	Light Green
2.	Odor	Odorless
3.	Temperature ◦ C	21.5◦ C
4.	Turbidity NTU	59 NTU
5.	pH	7.50
Chemical		
6.	Total Hardness	130.3 mg/l
7.	Sulphate Content	16.2 mg/l
8.	Total Dissolved Solids	48 mg/l
9.	Dissolved Oxygen	3.2 mg/l
10.	Phosphate Content	0.50 mg/l

Climate:

The area under the project is in wet and dry zone. Summers, which last from March to mid-June, are hot, with maximum temperatures reaching 36 °C, but are extremely dry. They are followed by the monsoon season, which continues till early October. Temperatures rise slightly in October, but this is followed by the cool season from November to February. The cool season sees warm temperatures of around 28 °C during the day, but cool nights, with lows averaging 13 °C, and extremely dry air.

Colour:

Samples collected from study site are respectively light green colour. Some of algae are observed by necked eye but when the colour of sample is compared with the white tile no colour is seen. Around the study site different human activities are seen.

Temperature:

The temperature 21.5^{o}C was recorded at study site. Water temperature plays an important factor which influences the chemical, bio-chemical characteristics of water body.

Odor:

The sample collected from study site was odorless, but presence of industrial treated or untreated waste, human activities, various type of chemicals are disposed.

Turbidity:

The Turbidity of water is 59 NTU. It may be due to human activities, decrease in the water level and presence of suspended particulate matter.

pH:

pH value is 7.50. The factors like air temperature bring about changes in the pH of water. The change in pH values suggests that carbon dioxide, carbonate-bicarbonate equilibrium is not maintained.

Total Hardness:

The value of hardness was 130.3 mg/l. The water sample is hard so cannot be used for drinking purpose directly. High value of hardness can be resulted to decrease in water volume and increase of rate of evaporation of water.

Sulphate Content:

The value of Sulphate was 16.2 mg/l. It is due to the pollutants introduced in river water through the waste from human activities, city sewage etc.

Total Dissolved Solids:

The total dissolved solid were 48 mg/l. some amount of organic matter, human waste, dumped matter, inorganic salts are present in sample.

Dissolved Oxygen:

The value of DO is 3.2 mg/l. The high value of dissolved oxygen was observed due to direct sunlight. It accelerates the photosynthesis by phytoplankton, utilizing carbon dioxide and giving off oxygen.

Phosphate Content:

The value of Phosphate was 0.50 mg/l. The high value of phosphate content is mainly due to some surface water runoff, washer man activity could have also contributed to the increase of phosphate content.

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A COMPARATIVE STUDY OF FLORA OF CAMPUS AREA OF MAHATMA FULE ARTS, COMMERCE AND SITARAMJI CHOUDHARI SCIENCE COLLEGE, WARUD, DIST-AMRAVATI

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Abstract:

The current piece of work is a focus on the flora of our college-campus at Warud, Dist Amravati. The word "flora" refers to the plants occurring within a given region as well as to the publication of scientific descriptions of those plants. A Flora may contain anything from a simple list of the plants occurring in an area to a very detailed account of those plants. Warud Tahsil is situated along Satpuda mountain ranges. Our college campus has various trees, some are wild, some are forest herbs, some are flowering, some are aromatic some are shrubs.The plants belongs to different families like Annonaceae, Myrtaceae Moringaceae, Ulmaceae, Meliceae, Tecomaceae, Nyctaginaceae, Annonaceae, Rutaceae, Caesalpiniaceae,Fabaceae, Rutaceae, Palmae, Rubiaceae, Lythraceae, Caesalpiniaceae, Euphorbiaceae, Moraceae, Oleaceae, Cupressaceae, Cycadaceae, Cactaceae, Amaryllidaceae, Nymphaeaceae Lauraceae, Umbelliferae, and **Labiatae** families. **Aromatic plants** are a special kind of **plants** used for their aroma and flavor. *Ocimum americanum, Latana camara, Hyptis* plants in our college campus, are wild and they are well-known for their aromatic smell and are also used for medicinal purposes. **Aromatic** compounds are present in these plants i.e. in the root, wood, bark, foliage; flower, fruit, and seed etc. Many of them are also used for medicinal purposes. Aromatic plants are from a numerically large group of economically important plants. Some aromatic plants in our college campus like *Ocimum, Latana, Hyptis* are highly aromatic plants.

Key words: Warud, urban green, scrape, floristic, mahatma Fule College, checklist

Introduction:

The current piece of work is a focus on flora of our college campus at Warud, dist Amravati. The word "flora" refers to the plants occurring within a given region as well as to the publication of scientific descriptions of those plants. A Flora may contain anything from a simple list of the plants occurring in an area to a very detailed account of those plants. Warud Tahsil is situated along the Satpuda mountain ranges. Our college campus has various trees, some of them are wild, some forest herbs, some flowering, some aromatic and some are shrubs. The plants that produce aromatic substances are used in making perfumes, in pharmaceutical and liquor industries. These plants belongs to different families such as, Annonaceae, Myrtaceae Moringaceae, Ulmaceae, Meliceae, Tecomaceae, Nyctaginaceae, Annonaceae, Rutaceae, Caesalpiniaceae,Fabaceae, Rutaceae, Palmae, Rubiaceae, Lythraceae, Caesalpiniaceae, Euphorbiaceae, Moraceae, Oleaceae, Cupressaceae, Cycadaceae, Cactaceae, Amaryllidaceae, Nymphaeaceae Lauraceae, Umbelliferae and Labiatae. Aromatic plants are special kind of **plants** used for their aroma and flavor. The plants like, *Ocimum americanum, Latana camara, Hyptis* in our college campus are wild and are well known for their aromatic smell and are also used for medicinal purposes. **Aromatic plants** are from a numerically large group of economically important **plants**. **Aromatic** compounds are found in plants i.e. in the root, wood, bark, foliage; flower, fruit, and seed etc. Many of them are also used for medicinal purposes. We have around hundred type different plants in our college Campus. They belong to difeerent groups like, dicotyledon, monocotyledon and aquatic plant. With the increase in urbanization, studies focusing on urban ecology have developed rapidly in recent years (Celesti-Grapow, 2006). Within urban ecosystems, themes like the flora in and around human settlements have been in lime light in recent decades (Pyšek, 1998; Aronson *et al.,* 2014). Floristically, cities have been observed to be richer than adjoining areas owing to high habitat heterogeneity as well as the presence of exotic species (Pyšek, 1998; Chocholoušková, 2003). Urban green spaces are of great importance in cities, because of the multiple ecosystem services they provide and may exist in the form of domestic, public or botanical gardens, unused fields. Thus, the aim of the present study was to understand the changes in the flora over more than five decades since the publication of the first study. For this, we assessed the total current specie's richness in the campus and compared it with the results. Also, a detailed unified inventory of all the vascular plants that are recorded till date in the campus is provided with notes about historical status, rarity, and ecological remarks.

Study area:

The study area is Mahatma Fule Arts, Commerce and Sitaramji Choudhari Science Mahavidhyalya, Warud (Run by the Shree Shivaji Education Society). This college was founded in 1960, which only comprised the main building at that time. The study area was isolated from the main city and sustained stunted scrub vegetation at that time, which is evident from archival photographs and literature. Mahatma Fule Arts, Commerce and Sitaramji Chaudhari Science Mahavidhyalya, is located in Warud City, Dist.Amravati, in Maharashtra, India (with a 3-acre area (Fig.1). The campus can be divided into three sections: the main campus which consists of main building and is surrounded by tall trees having maximum age upto seven to eight years. The second section is Office that lies towards the north side of the main campus. The original vegetation type of Warud is dry and deciduous. The type of soil here is black soil having some bolders in the plains.

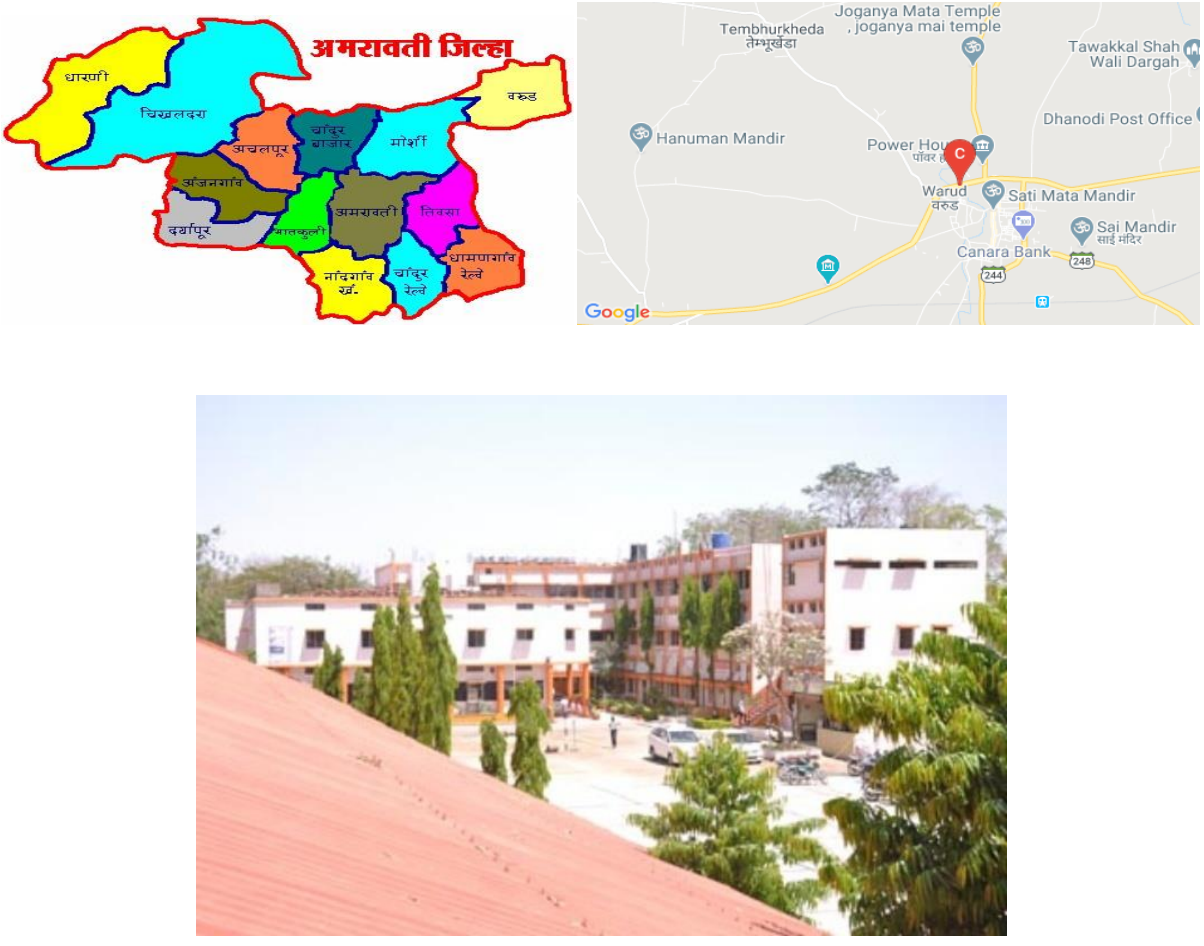

Figure 1: Map of Stduy area (Mahatma Fule College Campus, Warud)

Methodology:

The entire work was undertaken from September 2019 to February 2021. Floristic studies were carried out in the Mahatma Fule College campus during 2019-2021 A detailed survey of study area was done and information of plant species was recorded. All plants were identified under the expert in taxonomy. All habitats of the study area were surveyed carefully. Plant collection was carried out by standard method (Jain and Rao, 1977).; Shah, 1978; Duthi,1960; Gamble, 1915; Hains, 1921-1924; Cook, 1903; Hooker,1872-1897; Naik, 1998) and according to other taxonomic literature. A comprehensive checklist was drafted on uniting the data from all the aforementioned sources, which include current as well as past status of occurrence for each species. This assessment was done for all vascular plants including gymnosperms as well as Pteridophytes. Lower cryptogams including algae and fungi were not assessed, but a brief literature review is presented here for reference.

Result and Discussion:

An extensive plant survey was carried out in the Mahatma Fule Arts, Commerce and Sitaramji Chaudhari Science Mahavidyalaya, Warud, in 2019-2021. During this survey, more than 150 plants were recorded. 104 plants among them were identified and it was found that there are 100 Angiosperm plants having 54 species-54 genera and 30 families belonging to dicotyledonous, while 20 species-20 genera and 4 families belonging to monocotyledons (Table-1). Due to various factors such as changing environmental Conditions, biotic factors, destruction of habitat, biotic factors, destruction of habitat some plant species are facing threats for their existence. Conservation of the flora is one of the vital segments in natural resource management. The study area shows rich Floristic diversity in respect to the distribution of species, genera and families of both dicotyledonous and monocotyledons. Table-2 indicates a list of flowering plants which were found in campus area. Before few decades, campus area was floristically very rich with diverse habitat. But due to various factors, the vegetation of the campus has faced rapid destructions of habitat of the plants. It was found that Lamiaceae, Leguminosae and Poaceae are the dominating dicotyledonous and monocotyledonous families respectively and an inventory of all the species recorded is provided here. A comparative species composition account of the analysis of plants recorded in this study was done according to method suggested by Vartak (1958) and it is provided in Fig.4. However, the results may not be comparable in the true sense as the methodology followed by the earlier researchers might not be exactly replicated and the present findings are rather baseline broader-level indicative changes and minor intricacies might need to be amended in the near future.




























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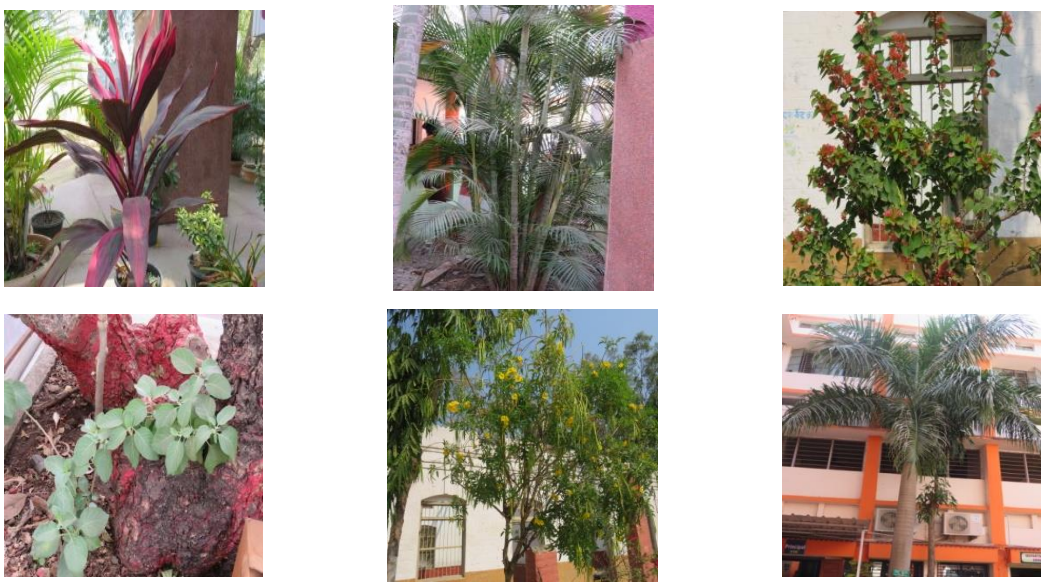

Figure 2: Plants of study Area

Table 1: Distribution of Angiospermic Plants

Angiosperm	Class	Species	Genera	Families
	Polypetalae	54	54	30
Dicotyledons	Gamopetalae	20	20	14
	Monoclamaydeae	10	10	05
	Total	83	83	49
Monocotyledons		20	20	04
	Grand total	104	104	53

Table 2: List of Flowering plants at Study area

Sr.No.	Family	Genera	Botanical Name	Common Name
1	Annonaceae	*Annona*	*Annona squamosa*	Sitafal
2	Annonaceae	*Polyalthia*	*Polyalthia longifolia*	Ashoka
3	Myrtaceae	*Eucalyptus*	*Eucalyptus globulus*	Nilgiri
4	Ulmaceae	*Holoptelea*	*Holoptelea integrifolia*	Papad
5	Meliceae	*Azadirachta*	*Azadirachta indica*	Kaduneem
6	Moringaceae	*Moringa*	*Moringa concanensis*	Shevga
7	Tecomaceae	*Tecoma*	*Tecoma stans*	Tecoma
8	Myrtaceae	*Calystemon*	*Calystemon lanceolatus*	Bottle brush
9	Nyctaginaceae	*Nyctanthus*	*Nyctanthus arbor-*	Parijatak

			tristis	
10	Annonaceae	*Annona*	*Annona squamosa*	Sitafal
11	Myrtaceae	*Psidium*	*Psidium guajava*	Peru
12	Apocynaceae	*Alstonia*	*Alstonia scholaris*	Saptparni
13	Rutaceae	*Citrus*	*Citrus reticulata*	Orange
14	Caesalpiniaceae	*Peltophorum*	*Peltophorum pterocarpum*	Sonmohar
15	Rubiaceae	*Murraya*	*Murraya paniculata*	Madhumalati
16	Rutaceae	*Murraya*	*Murraya koenigii*	Godneem
17	Rutaceae	*Dypsis*	*Dypsis lutescens*	Bambupalm
18	Rubiaceae	*Ixora*	*Ixora coccinea*	jungle geranium
20	Rubiaceae	*Delonix*	*Delonix regia*	Gulmohar
22	Lythraceae	*Ficus*	*Ficus benjamina*	weeping fig
23	Caesalpiniaceae	*Jasminum*	*Jasminum sambac*	Mogara
24	Euphorbiaceae	*Diphenbekia .*	*Diphenbekia sp.*	Mother-in-law's tongue
25	Moraceae	*Thuja*	*Thuja occidentalis*	Vidya
27	Oleaceae	*Plumeria*	*Plumeria alba*	Pandhara chafa
28	------	*Pongamia*	*Pongamia pinnata*	Kadubadam
29	Cupressaceae	*Bougainvelia*	*Bougainvelia spectabilis*	Boganvel
30	Apocynaceae	*Tabornaemontana*	*Tabornaemontana divericata*	Swastik
31	Fabaceae	*Ehretia*	*Ehretia laevis*	Khanduchaka
32	Nyctaginaceae	*Nerium*	*Nerium oleander*	Kanher
33	Rosaceae	*Cycus*	*Cycus revoluta*	Cycas
34	Apocyanaceae	*Roystonia*	*Roystonia regia plam*	Royal palm
35	Ehretiaceae	*Beaucarnea*	*Beaucarnea recurvata*	Elephant foot
37	Euphorbiaceae	*Lantana*	*Lantana camara*	Raymoni
39	Apocynaceae	*Duranta*	*Duranta erecta*	Nilkanta
40	Cycadaceae	*Ficus*	*Ficus hispida*	Katumbar
41	Cactaceae	*Bryophyllum*	*Bryophyllum pinnatum*	Pattharchata
44	Amaryllidaceae	*Ailanthus*	*Ailanthus excelsa*	Maharukh

45	Lamiaceae	*Ocimum*	*Ocimum sanctum*	Tulas
46	Casuarinaceae	*Casuarina*	*Casuarina equisetifolia*	Todojodo
47	Moraceae	*Morus*	*Morus alba*	Mulbery
48	*Bombacaceae*	*Cieba*	*Cieba pentandra*	Hirvi sawar
49	Arecaceae	*Cocos*	*Cocos nucifera*	Naral
50	Asclepiadaceae	*Calotropis*	*Calotropis procera*	Rui
51	Asclepiadaceae	*Calotropis*	*Calotropis gigantea*	Mothi Rui
52	Malvaceae	*Hibiscus rasa-*	*Hibiscus rasa-sinensis*	Jaswand
53	Asparagaceae	*Dracaena*	*Dracaena sp*	Song of India
54	Apocynaceae	*Catharanthus*	*Catharanthus roseus*	Sadafuli

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ECOLOGICAL SOLUTION TO ENVIRONMENTAL POLLUTION – BIOREMEDIATION

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Environmental pollution is the world's greatest problem facing humanity. Man's activities through urbanization, industrialization, mining, and exploration are at the forefront of global environmental pollution. Both developed and developing nations share this burden together, though awareness and stricter laws have contributed to a larger extent in protecting their environment.

Environmental pollution has been on the rise in the past few decades due to increased human activities. The main reasons for the current situition are population explosion, unsafe agricultural practices, unplanned urbanization, deforestation, rapid industrialization and non-judicious use of energy reservoirs and other anthropogenic activities.

Despite the global attention towards pollution, the impact is still being felt due to its severe long-term consequences. Thousands of hazardous waste sites are identified and many more are going to be added in the coming decades. Release of pollutants into the environment comes from illegal dumping by chemical companies and industries. Many of the techniques utilized for site clean-up in the past, such as digging up the contaminated soil and hauling it away to be land filled or incinerated have proved expensive and do not provide permanent solution and prohibited by law due to its hazardous effect on environment.

In context of the above phenomenon "Bioremediation is an attractive and successful cleaning technique to remove toxic waste from polluted environment. Bioremediation proves the efficient and safe waste management technique that includes the use of living organisms to eradicate or neutralize pollutants from a contaminated site."

Microorganisms grow in the widest range of habitats in the earth's biosphere. Their absolute numbers and their appetite for a wide range of chemicals make microorganisms the perfect candidate for acting as our environmental caretakers

Definition of bioremediation:

Bioremediation is a process where biological organisms are used to remove or neutralize an environmental pollutant by metabolic process. The "biological" organisms include microscopic organisms, such as fungi, algae and bacteria, and the "remediation"—treating the situation.

"Bioremediation is a 'treatment techniques' that uses naturally occurring organisms to break down harmful materials into less toxic or non-toxic materials" *Sharma* (2020)

Bioremediation is highly involved in degradation, eradication, immobilization, or detoxification diverse chemical wastes and physical hazardous materials from the surrounding through the all-inclusive and action of microorganisms. The main principle is degrading and converting pollutants to less toxic forms. Bioremediation can be carried out ex-situ and in-situ, depending on several factors, which include site characteristics, type, and concentration of pollutants. Hence, appropriate bioremediation technique is selected. Additionally, the major methodologies to develop bioremediation are biostimulation, bioaugmentation, bioventing, biopiles, and bioattenuation provided the environmental factors that decide the completion of bioremediation. Bioremediation is the most effective, economical, eco-friendly management tool to manage the polluted environment. All bioremediation techniques have its own advantage and disadvantage because it has its own specific applications.

Bioremediation technique can be carried out ex-situ and in-situ site of application . Pollutant nature, depth and amount of pollution, type of environment,location, cost, and environmental policies are the selection standards that are considered for selecting any bioremediation technique. Performance based on oxygen and nutrient concentrations, temperature, pH, and other abiotic factors that determine the success of bioremediation processes .Frutos et al.(2012)

Ex-situ bioremediation techniques involve digging pollutants from polluted sites and successively transporting them to another site for treatment. Ex-situ bioremediation techniques are regularly considered based on the depth of pollution, type of pollutant, degree of pollution, cost of treatment and geographical location of the polluted site. Performance standards also regulate the choice of ex-situ bioremediation techniques.

Microorganisms used in bioremediation:

Microorganisms play an important role on nutritional chains that are important part of the biological balance in life. Bioremediation involves the removal of the contaminated materials with the help of bacteria, fungi, algae and yeast. Microbes can grow at below zero temperature as well as extreme heat in the presence of hazardous compounds or any waste stream. The adaptability of microbes and the biological system made them suitable for remediation process. Carbon is the main requirement for microbial activity. Bioremediation process was carried out by microbial consortium in different environments

Factors affecting microbial bioremediation:

Microorganisms are act on the pollutants only when they have contact to the compounds which help them to generate energy and nutrients to multiply cells. The effectiveness of bioremediation depends on many factors; including, the chemical nature and concentration of pollutants, the physicochemical characteristics of the environment, and their accessibility to existing microorganisms.

The factors are mainly the microbial population for degrading the pollutants, the accessibility of contaminants to the microbial population and environment factors like type of soils, pH, temperature, oxygen and nutrients.

Biotic or biological factors:

Biotic factors are helpful for the degradation of organic compounds by microorganisms with insufficient carbon sources, antagonistic interactions among microorganisms or the protozoa and bacteriophages. The rate of contaminant degradation is frequently dependent on the concentration of the contaminant and the amount of catalyst present in biochemical reaction. The major biological factors are included enzyme activity, interaction (competition, succession, and predation), mutation, horizontal gene transfer, its growth for biomass production, population size and its composition.

Abiotic or environmental factors:

The successful interaction between the microbes and pollutant depends on the environmental situations. Microbial growth and activity are depended on temperature, pH, moisture, soil structure, water solubility, nutrients, site conditions, oxygen content and redox potential, deficiency of resources and physico-chemical bioavailability of pollutants, concentration, chemical structure, type, solubility and toxicity. These are above factors control the degradation kinetics.

Biodegradation of pollutant can occur under range of pH (6.5–8.5) is generally optimal for biodegradation in most aquatic and terrestrial environment. Moisture affects the metabolism of contaminant because it depends on the kind and amount of soluble constituents that are accessible as well as the pH and osmotic pressure of terrestrial and aquatic systems are other abiotic factors that determine the success of bioremediation processes.

Solid-phase bioremediation is an **ex-situ** technology in which the contaminated soil is excavated and placed into piles. It is also includes organic waste like leaves, animal manures and agriculture wastes, domestic, industrial wastes and municipal wastes. Bacterial growth is moved through pipes that are distributed throughout the piles. Air pulling through the pipes is necessary for ventilation and microbial respiration. Solid-phase system required huge amount of space and cleanups require more time to complete as compared to slurry-phase processes. Solid-phase treatment processes include biopiles, windrows, land farming, composting, etc. (Kulshreshta *et al.,* 2014)

There are far more than nine types of bioremediation, but the following are the most common ways in which it is used.

1. Land filling:

Land farming is the simplest, outstanding bioremediation techniques due to its low cost and less equipment requirement for operation. It is mostly observed in ex-situ bioremediation, while in some cases of in-situ bioremediation technique. This consideration is due to the site of treatment. Pollutant depth is important in land farming which can be carried out ex-situ or in-situ. Generally, excavated polluted soils are carefully applied on a fixed layer support above the ground surface to allow aerobic biodegradation of pollutant by autochthonous i.e. the species of microorganisms which are specifically found at the site. Over all, land farming bioremediation technique is very simple to design and implement, requires low capital input and can be used to treat large volume of polluted soil with minimal environmental impact and energy requirement.

2. Windrows:

Windrows is bioremediation techniques depends on periodic rotating the piled polluted soil to improve bioremediation by increasing microbial degradation activities of native and transient hydrocarbonoclastic i.e. oil degrading bacteria species present in polluted soil. The periodic turning of polluted soil increase in aeration with addition of water, uniform distribution of nutrients, pollutants and microbial degradation activities, accordingly increase the rate of bioremediation, which can be proficient through

acclimatization, biotransformation and mineralization. Windrow treatment as compared to biopile treatment, showed higher rate of hydrocarbon removal. However, can't be implementing in bioremediation of soil polluted with toxic volatile compounds. The use of windrow treatment has been associated in greenhouse gas (CH_4) release due to formation of anaerobic zone inside piled polluted soil, which frequently reduced aeration.

3. Biopile:

Biopile techneque includes above-ground piling of dug polluted soil, followed by aeration and nutrient amendment to improve bioremediation by microbial metabolic activities. This technique comprises aeration, irrigation, nutrients, leachate collection and treatment bed systems. This specific ex-situ technique is progressively being measured due to its useful features with cost effectiveness, which allows operative biodegradation conditions includes pH, nutrient, temperature and aeration are effectively controlled. The biopile method is in use to treat volatile low molecular weight pollutants; it can also be used effectively to remediate polluted very cold extreme environments . The flexibility of biopile allows remediation time to be shortened as heating system can be integrated into biopile design to increase microbial activities and contaminant availability thus increasing the rate of biodegradation.

4. Bioreactor:

Bioreactor is a vessel in which raw materials are converted to specific product(s) following series of biological reactions. There are different operational modes of bioreactors, which include: batch, fed-batch, sequencing batch, continuous and multistage. Bioreactor provides optimal growth conditions for bioremediation. Bioreactor filled with polluted samples for remediation process. The bioreactor based treatment of polluted soil has several advantages as compared to ex-situ bioremediation procedures. Bioreactor-based bioremediation process having excellent control of pH, temperature, agitation and aeration, substrate and inoculum concentrations efficiently reduces bioremediation time. The ability to control and manipulate process parameters in a bioreactor implies the biological reactions. The flexible nature of bioreactor designs allows maximum biological degradation while minimizing abiotic losses (Sharma, 2020)

Slurry-phase bioremediation:

Slurry-phase bioremediation is a relative more rapid process compared to the other treatment processes. Contaminated soil is combined with water, nutrient and oxygen in the bioreactor to create the optimum environment for the microorganisms to degrade the contaminants which are present in soil. This processing involves the separation of stones

and rubbles from the contaminated soil. The added water concentration depends on the concentration of pollutants, the biodegradation process rate and the physicochemical properties of the soil. After completion of this process the soil is removed and dried up by using vacuum filters, pressure filters and centrifuges. The subsequent procedure is soil disposition and advance treatment of the resultant fluids. So this ***Ex-situ*** bioremediation is suitable for a wide range of contaminants.

***In-situ* Bioremediation techniques:**

These techniques comprise treating polluted substances at the pollution site. It does not need any excavation and by little or no disturbance in soil construction.

Perfectly, these techniques should to be cost effective compared to ex-situ bioremediation techniques. Some in-situ bioremediation techniques like **bioventing, biosparging and phytoremediation** may be enhanced, while others may progress without any form of improvement such as intrinsic bioremediation or natural attenuation. *In-situ* bioremediation techniques have been effectively used to treat chlorinated solvents, heavy metals, dyes, and hydrocarbons polluted sites.

Types of *in-situ* bioremediation:

In-situ bioremediation is two types; these are intrinsic and engineered bioremediation.

1. Intrinsic bioremediation:

Intrinsic bioremediation also known as natural reduction is an in-situ bioremediation technique, which involves passive remediation of polluted sites, without any external human intervention. This process deals with stimulation of indigenous or naturally occurring microbial population. The process based on both microbial aerobic and anaerobic processes to biodegrade polluting constituents containing those that are recalcitrant. The absence of external force implies that the technique is less expensive compared to other in-situ techniques

2. Engineered in-situ bioremediation:

The second approach involves the introduction of certain microorganism to the site of contamination. Genetically Engineered microorganisms used in the in-situ bioremediation accelerate the degradation process by enhancing the physicochemical conditions to encourage the growth of microorganisms.

1. Biovening:

Bioventing techniques involve controlled stimulation of airflow by delivering oxygen to unsaturated zone in order to increase activities of indigenous microbes for

bioremediation. In bioventing, amendments are made by adding nutrients and moisture to increase bioremediation. That will achieve microbial transformation of pollutants to a harmless state. This technique has gained popularity among other **in-situ** bioremediation techniques (Hohenerp *et al.,* 2014)

2. Biosparging:

This technique is similar to bioventing in this air is injected into soil subsurface to improve microbial activities which stimulate pollutant removal from polluted sites The efficiency of biosparging depends on two major factors specifically soil permeability and pollutant biodegradability, it stimulates biodegradation. Biosparging has been generally used in treating aquifers contaminated with diesel and kerosene.

3. Phytoremediation:

Phytoremediation is depolluting the contaminated soils, based on plant interactions in contaminated sites to diminish the toxic properties of pollutants. Which is depending on pollutant amount and nature, there are several mechanisms such as extraction, degradation, filtration, accumulation, stabilization and volatilization involved in phytoremediation. Organic pollutants hydrocarbons and chlorinated compounds are mostly removed by degradation, rhizoremediation, stabilization and volatilization, with mineralization being possible when some plants such as willow and alfalfa are used. In phytoremediation removal of pollutant includes uptake, translocation from roots to shoots. Further, translocation and accumulation depends on transpiration and partitioning. Mostly the plants growing in any polluted site are good phytoremediators. Therefore, the success of any phytoremediation method mainly depends on improving the remediation potentials of native plants growing in polluted sites. One of the major advantages of using plants to remediate polluted site is that some precious metals can bioaccumulate in some plants and recovered after remediation, a process known as phytomining.

4. Permeable reactive barrier (PRB):

This technique is commonly observed as a physical method for remediating contaminated groundwater. However, biological mechanisms are precipitation degradation and sorption of pollutant removal used in PRB method. In general, PRB is an in-situ technique used for remediating heavy metals and chlorinated compounds in groundwater pollution (Thiruvenkatachari *et al.,* 2007).

Nutrient availability, low population or absence of microbes with degradative capabilities, and pollutant bioavailability may delay the achievement of bioremediation. Since bioremediation depends on microbial process, biostimulation and bioaugmentation approaches speed up microbial activities in polluted sites. Biostimulation increase microbial

activities by the addition of nutrients to a polluted sampleThis activity due to metabolic diversities of individual isolates, which potency create from their isolation source, adaptation process, pollutant composition, and synergistic effects, which may lead to complete and rapid degradation of pollutants when such isolates are mixed together. Additional so, both bioaugmentation and biostimulation were effective in removing pollutant such as polyaromatic hydrocarbons (PAHs) from heavily polluted sample.

Further, derivative pathway of genetically engineering microorganisms with a target polluted compound using biological approach could increase bioremediation efficiency. Nanomaterials decline the toxicity of pollutant to microorganisms because nanomaterials having increase surface area and lower activation energy, which reduce time and cost of bioremediation.

Bioremediation applications:

Bioremediation must be considered as appropriate methods that can applied to all states of matter in the environment.

- Solids (soils, sediment and sludge),
- Liquids (ground water, surface water and industrial waste water),
- Gases (industrial air emissions),and
- Sub-surface environments (saturated and vadose zones).

Bioremediation technologies came into extensive usage and continue growing today at an exponential rate. Remediation of polluted sites using microbial process (bioremediation) has proven effective and reliable due to its eco-friendly features. In the past two decades, there have been recent developments in bioremediation techniques with the decisive goal being to successfully restore polluted environments in an economic, eco-friendly approach. Researchers have developed different bioremediation techniques that restore polluted environments.

All bioremediation techniques have its own advantage and disadvantage;

The advantage of bioremediation:

- Environmentally friendly and cost effective are among the major advantages of bioremediation compared to both chemical and physical methods of remediation.
- It is a natural process; it takes a little time, as an adequate waste treatment process for contaminated material such as soil.
- Microbes able to degrade the contaminant, the biodegradative populations become reduced. The treatment products are commonly harmless including cell biomass, water and carbon dioxide.

- It needs a very less effort and can commonly carry out on site, regularly without disturbing normal microbial activities.
- This also eradicates the transport amount of waste off site and the possible threats to human health and the environment.
- It is functional in a cost effective process and supports in complete degradation of the pollutants; many of the toxic hazardous compounds can be transformed to less harmful products and disposal of contaminated material.
- It does not use any dangerous chemicals.
- Nutrients especially fertilizers added to make active and fast microbial growth. Because of bioremediation change harmful chemicals into water and harmless gases, the harmful chemicals are completely destroyed.
- Simple, less labor intensive and cheap due to their natural role in the environment.
- Contaminants are destroyed, not simply transferred to different environmental. Nonintrusive, possibly allowing for continued site use.
- Current way of remediating environment from large contaminates and acts as ecofriendly sustainable opportunities.

Disadvantages of Bioremediation:

- Biological processes are highly specific.
- Important site factors mandatory for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.
- It is difficult to scale up bioremediation process from batch and pilot scale studies applicable to large scale field operations.
- More research is required to develop modern engineered bioremediation technologies that are suitable for sites with composite combinations of contaminants that are not equally distributed in the environment. It may be present as solids, liquids and gases forms.
- Bioremediation takes longer time compare to other treatment options, such as excavation and removal of soil from contaminated site.
- The performance evaluation of bioremediation is difficult, and there is no acceptable endpoint for bioremediation treatment.

Conclusion:

Despite its short-comings, its pertinence in this world is unquestionable in the light of present day environmental hazard 'Bioremediation' provides a technique

for cleaning up pollution by enhancing the same biodegradation processes that occur in nature. So by developing an understanding of microbial communities and their response to the natural environment and pollutants, expanding the knowledge of the genetics of the microbes to increase capabilities to degrade pollutants, conducting field studies of new bioremediation techniques which are cost effective , these opportunities offer potential for significant advances in Bioremediation technique. There is no doubt that bioremediation is in the process of paving a way to ecologically and economically viable solution to environmental pollution.

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EFFECT OF ANESTHESIA WITH CLOVE OIL ON THE *POECILIA RETICULATA*, (GUPPY): REVIEW

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Abstract:

Anesthetics are drugs which cause anesthesia and are widely used in many branches of biology. Clove oil is an effective, local and natural anesthetic. Many hatcheries and research studies use clove oil to immobilize fish for handling, sorting, tagging, artificial reproduction procedures and surgery and to suppress sensory systems during invasive procedures. Clove oil may be more appropriate for use in commercial aquaculture situations. Improper clove oil use can decrease fish viability, distort physiological data or result in mortalities. Because animals may be anaesthetized by unskilled labourers and released in natural water bodies, training in the proper use of clove oil may decrease variability in recovery and experimental results and increase fish survival.

The present study reveals the clove oil anesthetizes guppy for 3-4 minutes by which the necessary investigations can be done. Clove oil is safe anesthetic agent which does not leave any physiological by-products in the fish. The fish recover from anesthesia within 5-10 minutes and it is safe for the handler also. Clove oil has become a popular fish anesthetic for invasive fisheries research procedures, but few studies have examined the use of low concentration of clove oil to achieve sedation for aquaculture procedures such as fish handling and transport.

Keywords: Ataxia, Clove oil, Anesthesia, Guppy fish.

Introduction:

An anesthetic is a drug that causes anesthesia i.e. reversible loss of sensation. Anesthetic are generally some sort of drugs which are administered to facilitate surgery. A wide variety of drugs are used in modern anesthetic practice. Anesthetics are categorized into two classes – general anesthetics which cause a reversible loss of consciousness and

local anesthetics which cause reversible loss of sensation for a limited region of the body while maintaining consciousness.

Anaesthesia is a biological state with the partial or complete loss of sensation or loss of voluntary neuromotor control induced by chemical or nonchemical means (Summerfelt and Smith, 1990). Anaesthesia abolishes pain in fish and induces a calming effect followed by loss of equilibrium, mobility and consciousness (Summerfelt and Smith, 1990). Anaesthetics in fish farms are used to minimize motility during handling and transport. This may reduce susceptibility to pathogens and infection (Woody *et al.,* 2002; Matin *et al.,* 2009). Anaesthetics are also used in fish during artificial spawning, weighing, tagging, grading, blood sampling, surgery and surgical procedures (Matin *et al.,* 2009; Anderson *et al.,* 1997). When choosing an anaesthetic, a number of considerations are important, such as efficacy, cost, availability and ease of use, as well as toxicity to fish, humans and the environment (Soto and Burhanuddin, 1995; Akbulut *et al.,* 2010), and the choice may also depend on the nature of the experiment and species of fish (Summerfelt and Smith, 1990; Munday and Wilson, 1997). Anaesthesia in fish may be produced by different agents, mainly tricaine methane sulphonate (MS-222), quinaldine sulphate, benzocaine and phenoxyethanol. Clove oil is considered to be a potential fish anaesthetic (Woody *et al.,* 2002).

The use of anti-stress agents is a common practice in modern aquaculture. Such substances are used to induce anesthesia during handling and sorting, tagging, artificial reproduction or surgery, thus reducing stress induced problems such as decrease in feeding and immune functions (Ross and Ross, 1984, 1999). The anesthetics most commonly used in aquaculture are MS-222, benzocaine, quinaldine sulphate, metomidate, clove oil and 2-phenoxyethanol (Waterstrat, 1999), with anesthesia being usually induced by immersing the fish in an anesthetic solution. Several workers such as McFarland (1960), Soto and Burhanuddin (1995), Teo and Chen (1993), Velisek *et al.* (2005a, 2005b) and Waterstrat (1999) studied the effect of anesthetics on fish. Sedation in fish depends on a number of factors, including the concentration of anesthetic, water temperature, fish size and species.

How do anesthetic agents work?

Anesthetic agents are inhaled through the gills and rapidly enter the blood stream. From there they are transported to the central nervous system and excreted via the gills upon the fish return to freshwater. They work by inducing a calming effect followed by a successive loss of equilibrium, mobility, consciousness and reflex actions.

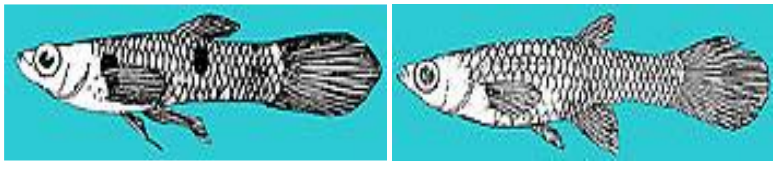
Respiratory and cardiac failure follows overdose. Before sedation or general anesthesia, fish should be fasted for at least 24 hrs. or until one can ensure that the stomach is empty of food to prevent regurgitation. It is important that anesthesia and recover (without anesthetic agent) tanks are prepared in advance.

The main purpose of this paper is to inform fisheries researchers and practitioners on the use of clove oil for anaesthetizing fish. This review should also be useful for others working on other sizes or species of fish.

Material and Methods:

Clove oil is a natural product obtained by distillation of the flowers, stems and leaves of the clove tree *Syzygium aromaticum* (i.e. *Eugenia aromaticum* or *Eugenia caryophyllata*). It is a dark brown liquid with a rich, aromatic odour and flavour. It has been used as a mild topical anaesthetic since antiquity and to help with toothache, headaches and joint pains. Clove is relatively inexpensive (Ross and Ross 2008) and is more potent than other anaesthetics used in fish and also has a long history as a local anaesthetic for humans (Woody *et al.,* 2002; Soto and Burhanuddin 1995). Its active ingredients are eugenol (4-allyl-2-methoxyphenol) and iso-eugenol (4-propenyl-2-methoxyphenol), which comprise 90–95 % of clove oil by weight, but raw clove oil also contains acetyl eugenol and a very wide range of turpenoid compounds, which impart their characteristic odour and flavour. It has also been found to have potentially beneficial antiviral (Siddiqui 1996), antimicrobial (Stecchini *et al.,* 1993) and antifungal (Karapinar 1990) properties. Clove oil is a known irritant when applied topically to laboratory rodents, rabbits and dogs, and it causes inflammation and local cellular necrosis (Sladky *et al.,* 2001). The useful features of clove oil prompted the development of a new anaesthetic compound for fish, named AQUI-S, at the Seafood Research Laboratory in New Zealand (Ross and Ross 2008). Aqui-S is reported to contain 50 % isoeugenol (2-methoxy-4-propenylphenol) and 50 % polysorbate 80. These materials are classified as GRAS by the FDA (Ross and Ross 2008; Sharp *et al.,* 2004; FDA (U.S. Food and Drug Administration) 1997).

***Poecilia reticulata* (GUPPY)**



Guppy – Male **Guppy - Female**

Like Gambusia, Guppy is also an exotic fish introduced in India in 1910. It is easy to care for, and it reproduces quickly and prolifically. It is now widely distributed in India and is an important larvivorous fish. Habitat- It is a very hardy fish and survives in all types of water bodies. It tolerates high degree of pollution with organic matter. The temperature range suitable for breeding is from 24 ^{0}C to 34 ^{0}C. It can survive in water with pH ranging from 6.5 to 9.0 However, it can not survive in cold water (often below 100 ^{O}C) and stock may need replenishment if the temperature fall below 100 ^{O}C. A single fish eats about 80 to 100 mosquito larvae in 24 hours. It is a surface feeder. Guppy - Male ,Guppy - Female : Size and Longevity : The male is 3 cm long, where as the female is up to 6 cm in length. The Guppy lives for 4 + 1 years.

Clove oil:

Clove oil has traditionally been used for a variety of purposes, including: as an antimicrobial, to help kill bacteria, as a pain reliever for conditions such as toothache and muscle pain, for digestive upset, to relieve respiratory conditions like cough and asthma. While many different chemicals have been identified in clove oil, a compound called eugenol is one of the primary components. Like many essential oils, researchers have been working to evaluate the potential health benefits of clove oil and its components. Let's take a deeper dive into what some of the research says so far.

It is derived from the stems, leaves and buds of the Eugenia caryophyllata tree. It is a commonly used anesthetic for commercial (non-food) fish but should not be used in fish destined for human consumption. The use of clove oil is characterized by rapid induction, prolonged recovery and the narrow margin of safety. It is also known as Eugenol, 4-allyl-2-2-methoxyphenol and AQUI-S. The active ingredient is eugenol, a phenolic compound which is not completely soluble in water (at low temperatures i.e. <150C). It is necessary to dilute the product in 1:10 in 95% ethanol to yield a working stock solution of 1100 mg/ml. Many claims have been made for this product ranging from antioxidant, antifungal agent, antibacterial agent, analgesic and local anesthetic. Concentrations between 25 and 50 mg/L are effective in freshwater and marine species. Recovery may be prolonged.

The Food and Drug Administration of the US has certified eugenol as being safe for humans when used at levels not exceeding 1500 ppm.

Each ml of clove oil contains 1 gm of eugenol and is not completely soluble in water and hence was dissolved in ethanol and diluted in water.

The experiment was conducted in sets of 3 i.e. each set contained three fish. The water temperature was between 23-28 ^{0}C and the water quality was not maintained.

Selected fish were exposed to varying concentrations of clove oil. A total of 3 fish were kept in each set in 500 ml beakers. Concentration of clove oil in different sets was 0.5 ml/100 ml, 1 ml/ 100 ml, 3 ml/ 100ml. The duration of exposure was 90 seconds, 120 seconds, 150 seconds, 180 seconds and 210 seconds. After exposure, the fish were transferred to freshwater with minimum handling and agitation. The aim of the study was to investigate the acute toxicity of clove oil to guppy fish and using the values of exposure time in anesthetic solution, time at which the fish gets anesthetized and the time of recovery were studied to assess the effects of exposure to clove oil on the fish.

Results and Discussion:

Table 1: Experimantal sets

	Set 1	**Set 2**	**Set 3**
Size of fish	5-10 mm	5-10 mm	5-10 mm
Sex of fish	Unsexed	Unsexed	Unsexed
Conc. of clove oil soln.	1/100 ml	1/100 ml	1/100 ml
Exposure time in anesthetic solution	90 seconds	90 seconds	120 seconds
Volume of water	100 ml	100 ml	100 ml
Stages of anesthesia	Description of stages	Description of stages	Description of stages
0-60 seconds	Loss of equilibrium	Reduced movement	Ataxia
60-90 seconds	Reduced movement	Immobilized fins but movement in tail	Loss of gross movement of body
90-120 seconds			Totally immobilized
	Transferred to recovery tank	Transferred to recovery tank	Transferred to recovery tank
Stages of recovery	Description of stages	Description of stages	Description of stages
0-120 seconds	Immobile	Immobile	Immobile
2-4 minutes	Body immobile but faint movement started in tail	Body immobile but faint movement started in tail	Body immobile but faint movement started in tail
4-5 minutes	Movement in tail increased	Movement in tail increased	Movement in tail increased
5-7 minutes	Gross body movement begins	Gross body movement begins	Gross body movement begins
7-10 minutes	Started to regain equilibrium	Started to regain equilibrium	Started to regain equilibrium
10-15 minutes	Equilibrium regained and normal movement resumed	Equilibrium regained and normal movement resumed	Equilibrium regained and normal movement resumed

Table 2: Experimatnal sets

	Set 1	**Set 2**	**Set 3**	**Set 4**	**Set 5**
Size of fish	18-22 mm	18-22 mm	18-22 mm	18-22 mm	18-22 mm
Sex of fish	Unsexed	Unsexed	Unsexed	Unsexed	Unsexed
Conc. of clove oil soln.	1/200 ml	2/200 ml	3/200 ml	3/200 ml	3/200 ml
Exposure time in anesthetic solution	180 seconds	180 seconds	150 seconds	210 seconds	210 seconds
Volume of water	200 ml	200 ml	200 ml	200 ml	200 ml
Stages of anesthesia	Description of stages	Description of stages	Description of stages	Description of stages	Description of stages
0-60 seconds	Ataxia	Ataxia	Ataxia	Ataxia	Ataxia
60-120 seconds	Ataxia continues	Ataxia continues	Fins immobile, tail mobile	Jerky movement	Fast jerky movement
120-150 seconds	--	--	Totally immobilized	--	--
150-180 seconds	Ataxia continues	Slow jerky movement	--	Gross loss in jerky movement	Gross loss in jerky movement
180-210 seconds	--	--	--	Totally immobilized	Totally immobilized
	Transferred to recovery tank	Transferred to recovery tank	Transferred to recovery tank	Transferred to recovery tank	Transferred to recovery tank
Stages of recovery	Description of stages	Description of stages	Description of stages	Description of stages	Description of stages
0-60 seconds	Ataxia	Ataxia	Immobile	Immobile	Immobile
1-2 minutes	Ataxia continues	Ataxia with jerks in fins and tail	Body immobile but slow movement started in tail	Immobile	Immobile
2-3 minutes	Ataxia continues	Ataxia with jerks in fins and tail	Movement in tail increased	Immobile	Body immobile but slow movement started in tail

3-4 minutes	Movement reduced than earlier	Ataxia and jerky movement continued	Movement started in fins and movement in tail increased	Body immobile but slow movement started in tail	Movement in tail increased
4-5 minutes	Movement reduced than earlier	Ataxia continues but jerks stopped	Gross body movement started	Movement in tail increased	Gross body movement started
5-6 minutes	Movement still restless	--	Movement of body increased slowly	Gross body movement started	Movement of body increased slowly
6-8 minutes	Started to regain equilibrium	Movement reduced than earlier	Started to regain equilibrium	Movement of body increased slowly	Movement of body increased slowly
8-10 minutes	Started to regain equilibrium but sudden jerks in movement	Movement of tail started to become normal	Equilibrium regained	Movement of body increased slowly	Started to regain equilibrium
10-12 minutes	Started to regain equilibrium but sudden jerks in movement	Started to regain equilibrium	Equilibrium regained completely, normal movement resumed	Started to regain equilibrium	
12-15 minutes	Equilibrium regained	Equilibrium regained	--	Equilibrium regained	Equilibrium regained completely, normal movement resumed
15-17 minutes	Equilibrium regained completely, normal movement resumed	Equilibrium regained completely, normal movement resumed	--	Equilibrium regained completely, normal movement resumed	--

Discussion:

In the present study, guppy, *Poecilia reticulata*, is treated with clove oil. Clove oil procured from Loba-Chemie is used in different concentrations for different durations of time. The effect of clove oil exposure is recorded and presented in tables above.

With increase in clove oil concentration and duration of exposure, the duration of fish remaining immobile also increases. On an average, the fish were totally anesthetized in 3 minutes. Upon placing the fish in freshwater, they started recovery after 4 minutes and fully recovered to normal state of movement in about 15 minutes.

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