RAYS

Academic Insights of Morning Star

Morning Star Home Science College,
Angamaly

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MORNING STAR HOME SCIENCE COLLEGE
ANGAMALY

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Academic Insights of Morning Star

Second Edition September 2021

Published by
Dr. Sr. Rosily A. V.
Principal,
Morning Star Home Science College
Angamaly, Ernakulam, Kerala

ISBN: 978-93-5408-948-0

Printed at
Read Books, Chentrappinni
ReadBooksPress.com

RAYS

Academic Insights of Morning Star

Editorial Board

Editorial

"Research is turning the unknown into a reality!"

I am elated to contribute a few words of appreciation for the arduous work undertaken to accomplish the second edition of RAYS. Our teachers and students have dedicated their time, effort and skills to make novel accomplishments in the field of scientific research.

I congratulate all the teachers for inspiring and guiding their students through this task, molding the young researchers who are to make greater contributions to the scientific world in the coming years. I also believe this will continue to serve as an arena for teachers and students to bring out their work in print and showcase their discoveries.

May God bless you all!

Best wishes,

Dr. Sr. Rosily A.V
Principal

Contents

1 A Study on the Biodiversity of Gastropod Molluscs of Kuzhuppilly Beach, Kerala
Ardra Surendran and K. Leena Joseph 7

2 Synthesis Characterization and Antibacterial Study of Zinc Oxide Nanoparticles
Anjana A. Sahadevan, Rintu Mary Sebastian, Hana Mariya Joseph, and Teji K. T. 12

3 A Study on Nature Friendly Community With Reference to Moozhikkulam Sala of Parakkadavu, Ernakulam District
Aswathy D. Nair and Teji K. T. 15

4 Comparative Study of the Effect of Organic Fertilizer and Chemical Fertilizers on *Eisenia foetida*
Greeshma A. U. and Dilsha Davis 18

5 A New Era of Crop Improvement: A Review on Genome Editing in Plants
Malavika Ajayakumar 26

6 Computational Chemistry Calculations of CO Stretching Frequencies of Cyclic Ketones, Cyclic Esters and Cyclic Amides
Reeshal Xavier, Prejitha Ajith, Liya Antony, Mily Babu and Sindhu K. S. 36

7 Study of Adsorption Capacity of Some Selected Natural Adsorbents
Rosily A. V., Soubhagya K. Unni, Ann Mary Varghese, Malavika K. G., Akshara R. and Navya Antony 42

List of Figures 48

List of Tables 49

Chapter 1

A Study on the Biodiversity of Gastropod Molluscs of Kuzhuppilly Beach, Kerala

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ABSTRACT

Phylum mollusca is one of the largest and most diverse group in the animal kingdom. The marine molluscan fauna of India includes about 3,370 species, out of which 24 are included in Schedule-I of Indian Wildlife (protection) Act, 1972. The biodiversity of gastropod molluscs of Kuzhuppilly beach were assessed. The samples were collected once in every month for a period of three months (January -March) and species diversity were analysed. A total of 10 species of gastropod molluscs belonging to 7 different orders were identified. They are Neogastropoda (3 species), Trochida (2 Species), Vetigastropoda (1 Species), Littorinimorpha (1 species), Neotaenioglossa (1 Species), Neritimorpha (1 Species), Caenogastropoda (1 species). The present study revealed very less number of Gastropod species which directly indicated the increased exploitation of this fauna and their species diversity. Present information on species diversity of gastropods would be helpful as a baseline data for further monitoring of anthropogenic inputs and natural environmental factors on gastropods.

Introduction

Molluscs are the largest marine phylum, comprising about 23% of all the named marine organisms. They are highly diverse not just in size and anatomical structure but also in behaviour and habitat. Molluscs are extremely diverse in tropical and temperate regions, but can be found at all latitudes (Giribet et.al. 2006). Within the marine ecosystem, Molluscs play an important role in the energy flux and the community structure, due to the fact that many of them work as ecological regulators (Caso M.E, 1994) and as disturbance indicators inside these systems (Villarreal C.G, 1995).

About 80% of all known mollusc species are gastropods. The gastropods are soft-bodied animals commonly known as snails and slugs, belong to a large taxonomic class of invertebrates within the phylum Mollusca. Gastropods have extensive habitats such as river, lakes, swamps, ponds, beaches and even underground aquifers (Boucher P et.al.2008). The structure of the gastropod community was influenced

by many factors such as the way of life, competition, food availability, substrate type, water temperature, and salinity (Mujiono N 2008). Gastropods are characterized by the possession of a single often coiled shell and a body that has undergone torsion so that the pallial cavity faces forwards. The anatomy, behaviour, feeding and reproductive adaptations of gastropods vary significantly from one group to another. Therefore it is difficult to state many generalities for all gastropods.

Gastropods play an important role in the breakdown of dead animal and vegetable matter. Snails can be economic importance carrying parasites that effect both humans (Bathers' itch) and animals (liver fluke). Gastropods are rare in organically polluted or acidic water bodies. Although the class contains a relatively small number of species, so many of its members are eaten by people in large amounts. Gastropods occupy an important role in the commercial shell craft industry in South India.

The interest in studying biodiversity is linked to the lack of knowledge that exists over its magnitude and the constant loss due to human actions of climate change effects. It is important to know and understand the processes that determine the abundance and distribution of biodiversity in the gastropod species, as well as their transformation due to the environment.

Objectives of the Study

- To develop base line information on Gastropod molluscs of Kuzhuppilly beach, Kerala.
- Identification and classification of Gastropod molluscs.
- To study about the species diversity of Gastropod molluscs of Kuzhuppilly beach.

Methodology

Study Area

Study was conducted in Kuzhuppilly beach, Kerala

Study Time

Study was conducted for 3 months from January 2021 to March 2021.

Gastropod molluscs were collected by simple random method from the beach. Monthly observation were made for three months and the samples (Shells & whole specimens) were collected by hand picking. Fresh specimens were preserved in formalin and brought to the laboratory for identification. The Gastropods were sorted out and identified up to species level mainly based on the shell morphology and with the help of available literature (Modayil Joseph Mohan, 2007). The shell characters such as shape, spire length & shape, mouth opening, opercular shape, umbilicus shape and size, colour and ornamentation of the shell were used mainly for the identification of gastropods.

Observations and Results

Name of Order	Name of the Species
Order 1: Neogastropoda	
	1. Murex carbonnieri
	2. Murex virigineus
	3. Conus figulinus
Order 2: Trochida	
	4. Astrea stellate
	5. Turbo petholatus
Order 3. Vetigastopoda	
	6. Trochus tentorium
Order 4. Littorinimorpha	
	7. Phalium areola
Order 5. Caenogastropoda	
	8. Turritella attenuata
Order 6. Neotaenioglossa	
	9. Epitonium scalare
Order 7. Neritimorpha	
	10. Nerita pulchella

Table 1.1: Check List of Gastropod Molluscs Collected and Identified From Kuzhuppilly Beach, Kerala

Sl.no.	Name of OrderS	No. of Species
1	Neogastropoda	3
2	Trochida	2
3	Vetigastopoda	1
4	Littorinimorpha	1
5	Caenogastropoda	1
6	Neotaenioglossa	1
7	Neritimorpha	1

Table 1.2: Table showing number of Gastropod Molluscs of different orders collected from Kuzhuppilly Beach, Kerala

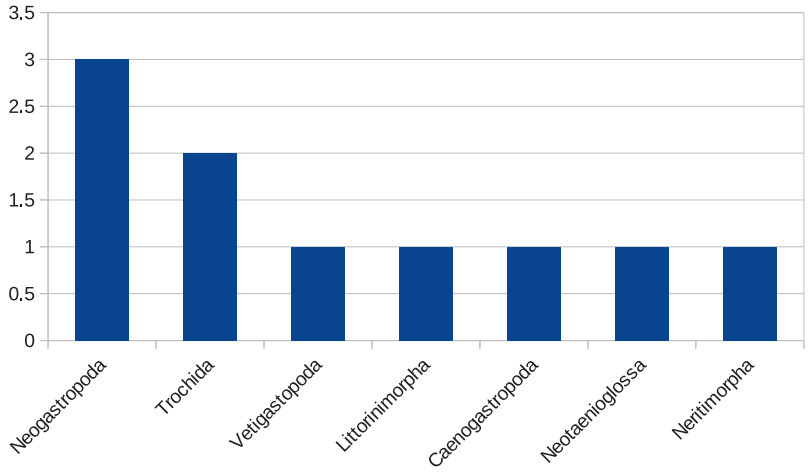

Figure 1.1: Bar diagram showing number of Gastropods of different orders collected from Kuzhuppilly Beach, Kerala

Discussion

Gastropods live in every conceivable habitat on Earth. They are extremely diverse in size, shell morphology and habits and occupy the widest range of ecological niches. In the present study, 10 species of Gastropod molluscs were collected and identified from Kuzhuppilly beach upto Species level. They fall under seven different orders. They are Neogastropoda (3 species), Trochida (2 Species), Vetigastopoda (1 Species), Littorinimorpha (1 species), Neotaenioglossa (1Species), Neritimorpha (1 Species), Caenogastropoda (1 species). Among this, dominant taxon was order Neogastropoda followed by Trochida. Vetigastopoda, Littorinimorpha, Neotaenioglossa, Neritoidea, Caenogastropoda were represented by only one species.

The present study reports very less number of Gastropod species which may directly indicated the increased exploitation of this fauna and their species diversity. People of coastal area is of the opinion that the recent Kerala flood waters washed away all the gastropod molluscs from the beach. Formerly, a lot of species was there and the tides and waves bought so many species towards the beach. Now the diversity and quantity were lowered very much.

The diversity of gastropod molluscs in Vypin–Cherai beach were assessed and total of 35 species of gastropod molluscs belonging to 6 different orders were identified. They are Neogastropoda, Sorbeoconcha, Littorinimorpha, Vetigastopoda, Caenogastropoda and Neritimorpha (Dona Thomas; et al. 2017) The Gastropods are abundantly seen in the Indian coastal waters. The variation in abundance of species could result from anthropogenic activities like overharvesting, habitat loss, disposal of Sewage, wastes and effluents, sedimentation and tourism.

Gastropod molluscs can be considered as a good raw material for the enrichment of handicraft cottage industry in our country. The exploitation and export of these materials is definitely fetch a good revenue for India. Marine gastropod resources are exploited for various purposes and often go unnoticed, as they form only a minor component of marine fishery resources. Despite the potential threat of over exploitation of gastropod resources, there is no comprehensive data available on the distribution, species wise landings, species diversity and the volume of export and import of gastropod resources along the Indian coast. The knowledge on the occurrence, distribution and the utilization of such marine resources are very much needed so that these resources can be properly tapped for foreign exchange earner by the fishing industry of the country and for adopting suitable conservation measures.

Conclusion

An understanding of the diversity of Gastropod molluscs assumes paramount importance in conservation. Although considerable effort has been invested in recording diversity in different habitats, there are a few relevant studies about Gastropod molluscs of Kerala. A perfect understanding of the Gastropod mollusc diversity of the coastal system is an essential prerequisite for implementation of sustainable utilization of gastropod resources and for adopting suitable conservation measures. Sustainable use of marine and coastal living resources cannot be properly established without an adequate knowledge of biodiversity. Study of this kind will be helpful to take remedies against over exploitation and for taking preventive measures for maintaining good species diversity intact.

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Chapter 2

Synthesis Characterization and Antibacterial Study of Zinc Oxide Nanoparticles

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ABSTRACT

Zinc oxide nanoparticles as one of the most important metal oxide nanoparticles and are popularly employed in various fields due to their physical and chemical properties, biocompatibility and low toxicity. Due to the above reasons, it has emerged a promising potential in biomedicine, especially in the fields of anticancer and antibacterial fields. Here the Zinc Oxide nanoparticles where prepared by the coprecipitation method using $ZnSo_4$ and $NaOH$. The crystallite size and lattice parameter of ZnO nanoparticle were determined from the this XRD analysis. Particle of 24.4 nm and the lattice parameter 2.7218 A° where obtained. The Antibacterial behavior of ZnO nanoparticle on *Escherchia coli* and *Staphylococcus* bacteria was also studied and it was found that it showed significant antibacterial behavior on both gram positive and gram-negative bacteria.

Introduction

Nanotechnology deals with the manufacture and application of materials with size up to 100nm. Nano particles are widely used in material science, agriculture, food industry, cosmetics, medical and diagnostic application.[1]

Zinc oxide nanoparticles are essential ingredient of many sun screens, ointments etc.[2] Zinc oxide nanoparticles show remarkable antibacterial activity at very low concentration due to their high surface area to volume ratio and unique chemical and physical features.[3] Zinc is an essential trace element for human system which makes many enzymes like carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase active. While similar elements like cadmium, mercury etc. which belong to the same group are toxic.

[1]Husen A, Siddiqi K S "Photosynthesis of nanoparticles: concept, controversy and application" Nano Res Lett 9:229 (2014).

[2]Khwaja Salahuddin Siddiqi, Aziz ur Rahman, Tajuddin, Azamal Husen "Properties of Zinc Oxide Nanoparticles and their activity against microbes" Nanoscale Research Letters 13:141 (2018).

[3]Rai M, Yadav A, Gade A " Silver nanoparticles as a new generation of antimicrobials" Biotechonol Adv 27: 76 -83 (2009).

In this paper we have prepared zinc oxide using chemical precipitation technique and their effect on the bacterial strains *Escherichia coli* (E. coli) and *Staphylococcus aureus* (S. aureus) are studied.

Experimental techniques

Synthesis of ZnO

35.94g of zinc sulphate is accurately weighed and dissolved in distilled water to prepare 125 ml, 1M zinc sulphate solution. 5g of Sodium hydroxide is accurately weighed and dissolved in distilled water to prepare 125ml, 1M Sodium hydroxide solution. Sodium hydroxide solution is slowly added to zinc sulphate solution drop by drop. The reaction is given uniform and constant stirring using a magnetic stirrer. The reaction time is fixed to 24 hours. The ZnO precipitate is filtered using a filter paper and then washed using distilled water thoroughly. The precipitate is then ground to a fine powder using mortar and dried at temperature of 110°c in a micro wave oven.

Structural Characterization

X Ray Diffraction Technique

The figure shows the XRD pattern obtained from sample. The obtained pattern is compared with the standard JCPDS data card (361-1451) and the sample were found to be crystallized in cubic structure, Reflection corresponding to (100), (002) and (110) Planes are characteristic to ZnO (JCPDS Data card (361-1451) are observed in sample.[4]

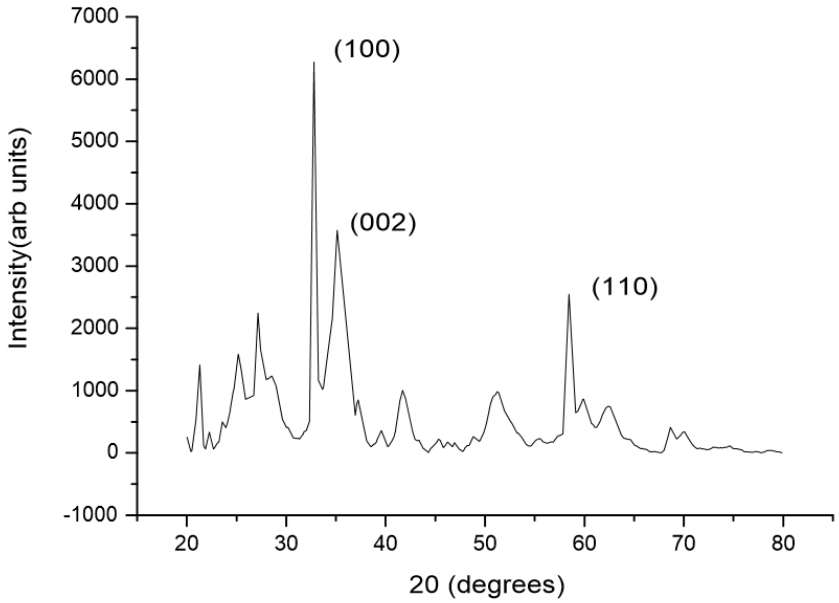

Figure 2.1: XRD pattern obtained from sample

Grain size was calculated using Scherrer formula $D = \frac{K\lambda}{\beta \cos\theta}$ which relates the means crystallite size D, the sample to the broadening, β of its diffraction peaks θ is the Braggs angle. λ is the radiation wavelength and K is a constant which depend on assumption made in theory. (e.g., The peak shape and crystallite habit) but it is anyway closed to unity.

The interplanar distance (d) can be calculated from the Bragg's equation and the equation is;

[4]Hana Mariya Joseph, Poornima N "Synthesis and Characterization of ZnO nanoparticles" Materials Today Proccedings

$$d = \frac{\lambda}{2\sin\theta}$$

The lattice parameter a, b, c was calculated for the most intense peak:

For cubic lattice;

$$\frac{1}{d^2} = \frac{h^2 + k^2 + l^2}{a^2}$$

From the miller indices and interplanar distance, the lattice parameter a can be calculated.

The Crystallite size obtained from the XRD analysis was 24.4 nm. The lattice parameter 'a' obtained was 2.7218 A°.

Antibacterial Sensitivity Of ZnO Nanopaticle

The ZnO nanoparticles is resistant to both gram positive and gram-negative bacteria (*Staphylococcus sp.* and *Escherichia coli bacteria*) and the zone of inhibition is more in gram positive, that means it shows more resistivity towards gram positive bacteria (*Staphylococcus*). The ZnO nanoparticles produced 1.5 cm diameter as inhibition zone in gram positive bacteria where as it produces 0.99 cm inhibition zone in gram negative bacteria.

The ability of the extract from nano particles to inhibit the growth of bacterial strains is an indication of its antibacterial potential that might be utilized in the management of bacterial infections in future, so the nanoparticles may be employed to develop a potential antimicrobial drug. The antimicrobial activity shown by the extract might be due to some antimicrobial substances present in these ZnO nano particles. Here extract prepared from ZnO nanoparticle was found to be resistant to *E. coli* and *Staphylococcus*.

Nanoparticles have great potential as antimicrobial compounds against microorganisms. Thus they can be used in the treatment of infectious diseases caused by resistant microbes. With the rise in disease causing that have become resistant to synthetic drugs, nanoparticles is now gaining importance. The results of the present study confirm the importance of nanoparticles used in indigenous medicine in search of new substances capable of inhibiting *E coli* and *Staphylococcus*.

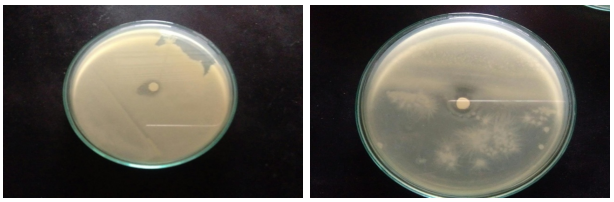

Figure 2.2: Inhibition zone created by Gram positive bacteria and Gram negative bacteria

Conclusion

From the structural Studies pure ZnO samples was prepared without any impurity phases. The crystaline size of the sample prepared was 24.4nm. The antibacterial study revealed the efficacy of nano ZnO particles on both gram positive and gram negative strains.

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Chapter 3

A Study on Nature Friendly Community With Reference to Moozhikkulam Sala of Parakkadavu, Ernakulam District

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ABSTRACT

This work analyse the importance of ecofriendly houses and the community living. The study also helps to understand the ideology of carbon neutral kitchen. We all are aware of the fact that the world is emerged to a global village where pollution, disaster and diseases have spread to a maximum range. The issues have grown to a much greater extent and in contrast with this the concept eco-friendly or going eco-friendly has progressed. Moozhikkulam Sala of Ernakulam district is the unity of a group of persons who are concerned about the mother earth, nature and all the other animate and inanimate things. It is a classic example of community living. Carbon Neutral kitchen is an initiative which reduces the level of CO2 emission. This work will be a benchmark data with respect to Kerala and make people aware of the sustainable development.

Key Words: Moozhikkulam Sala, Community Living, Natue friendly
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Introduction

We are all aware of the literal meaning of the word eco-friendly. The world have evolved into a global village where pollution, disasters, diseases have spread to a maximum range. It is very necessary to preserve the earth and the living things that exist on it (Green homes, Oleg Golubchikov, 2012). As human beings and the dwellers of Mother Nature we have forgotten that we have the responsibility to keep the earth safe and protecting its environment. In order to recognize this very concept we first need to understand that why do we need to go eco-friendly? The reasons can be many but the major ones are (Terry White, 1890) Environmental Pollution and erosion. On the other hand, one might wonder the benefits of going eco-friendly. As a matter of fact, there are many of them. They can be elaborated as

follows: Lower cost, Healthier lifestyle, Quality of life, Development and Sustainability.

An eco-friendly house is always environment friendly. Moozhikkulam Sala Jaiva campus is such a kind of initiative which consists of 51 ecofriendly houses in the banks of Chalakkudy River in Kerala. This organisation cherishes a dream for the contemporary as well as the future generations (Reshmi Radhakrishnan, Mathrubhoomi Daily, 9th October, 2017)

Methodology

Moozhikkulam Sala which is located on the banks of Chalakkudy River in Moozhikkulam village, Parakkadavu Panchayath of Ernakulam district. Moozhikkulam Sala was visited on 26th December 2020, Saturday. Mr. T. R. PremKumar thoroughly explained about Moozhikkulam Sala. He is the author of the book titled "Athijeevanam" which clearly visualizes the life of the people of Moozhikkulam and how Sala is interrelated to them.

Result

- Moozhikkulam Sala is an organization which cherishes a dream for the contemporary as well as the future generations.
- It is the unity of a group of persons who are concerned about the mother earth, human beings, nature and all other animate and inanimate things. Moozhikkulam Sala is the best example of community living.
- This is quite different from the typical villas of cities. Moozhikkulam Sala is featured with ecofriendly houses and species richness.
- This region comprises 23 Naalukettu, each in 5 cents of land with 1080square feet and also 29 small one Bedroom Hall Kitchen (BHK) houses in the river banks. Around 70 people lives here in a secular manner.
- The whole land area consists of 2 acres and 40 cents. No boundary walls between the houses. No one allowed to build upstairs of their house. There is no tarred roads.
- There is a common gate (padippura) which kept open for 24 hours. No security guards, which means anyone who wants to know, study and visit Moozhikkulam sala can come at any time without seeking permission to anyone.
- Walls of the houses are built in order to accommodate more air inside the house. The large windows and nadumuttam also allow sufficient air and light in to the house. So, low energy consumption is required.
- Here raw food is eaten without using gas or firewood. Through this new mode of kitchen even 1 gram of CO_2 is not liberated out. This biocampus addresses the new world through this 'aduppu rahitha adukkala'.
- The authorities strictly restrict any destructive process which is against the natural flora of the river banks. 'Nakshathra vrikshangal', different types of trees, plants can be seen here
- There is a society exclusively selling the natural products needed for the sala inmates at the Moozhikkulam junction. Sala interferes in the socio-political sectors of Kerala and also the environment related activities
- For this enormous effort done by Moozhikkulam Sala in order to protect nature, Aravally visitors center which is an initiative of Arabindo Ashram, Pondicherry awarded biodiversity award on 2018.

Discussion

The environment friendly person moves friendly life with an awareness of how natural resources are used to create and support the life that they live. They recycle, conserve water and fuel, and make other choices that not only reduce their impact on the environment but also support industries that are working towards being more environmentally responsible. Easy Ways to Become More Eco-friendly (Bell, M., Lowe, R and Roberts, P. W., 1996).

Become more aware of resources.

- Practice conservation.

- Plant trees.
- Conserve water.
- Try renewable energy, use rooftop solar.
- Change to LED bulbs.
- Cut down meat on your plate.
- Stop food waste.
- Change your travel habits.
- Use less fossil fuel based products.
- Buy locally grown products.
- Reduce the use of harmful chemicals.
- Use green cleaning products.
- Composting.
- 3R's of waste hierarchy.
- Choose personal hygiene products carefully.
- Buy recycled products.
- Reduce plastic usage.
- Join environment groups.
- Stop littering.
- Protect wildlife.
- Educate others.

Conclusion

This study analyses the importance of eco friendly houses and the community living. The study also helps to understand the ideology of carbon neutral kitchen. Green spaces are a vital part of building a green community and an increased number of community gardens, trees and parks ensures that residents benefit from lower CO_2 emissions, reduced stress levels, increased fitness and overall improved mental health. Moozhikkulam Sala of Ernakulam district is the nearest and classic example of community living. Carbon Neutral kitchen is an initiative which reduces the level of CO_2 emission. This work will be a benchmark data with respect to Kerala and make people aware of the sustainable development.

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Chapter 4

Comparative Study of the Effect of Organic Fertilizer and Chemical Fertilizers on *Eisenia foetida*

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ABSTRACT

Fertilizers are used extensively in modern agriculture in order to improve crop yield. urea is the most popular and widely used dry nitrogen fertilizer. The objective of present study is to characterize the effect of fertilizers on the earthworm. The effect of soil fertilization with inorganic (urea) and organic fertilizer (Crop meal) on earthworm *Eisenia foetida* rearing (Population, biomass, number of cocoons, juveniles, non-clitellate and clitellate) etc. were studied under different doses of the fertilizers for 60 days. When compared, marked changes were observed in the activity of *Eisenia foetida* in both type of fertilizers introduced. The present work indicates towards the deleterious effect of inorganic fertilizers on the survival of earthworm community in soil.

Introduction

Earthworms are known as farmer's best friends, because of multitude of services they provide that improve soil health and consequently plant health. They are terrestrial invertebrates, belong to the Order Haplotaxia and Class Oligochaeta of the Phylum Annelida. They are long cylindrical and also are hermaphrodites. We studied about the effects of fertilizers on earthworm named *Eisenia foetida*. *Eisenia foetida* worms are used for vermicomposting of both domestic and industrial organic waste. *Eisenia foetida* worms are used for vermicomposting of both domestic and industrial organic waste. They are native to Europe, but have been introduced (both intentionally and unintentionally) to every other continent except Antarctica. It has world wide distribution and can colonize organic substrates naturally. These worms are capable of tolerating a wide temperature and moisture range. They are resilient and can be readily handled. It is distinguished by the striped or banded appearance due to the pale colour of intersegmental groove. Adult worm has 27-130 mm length and 1.5-2.5mm width. The life span of these epigeic earthworm ranges between 3-5 years.

Several studies demonstrated that the application

of chemical fertilizers as pulverization or powder can have disastrous effect on earthworm populations. Fertilizers with nitrogen create acidific conditions in soil, which is fatal for earthworms. Nithi Rai *et al*.,2014 studied on the comparative study of the effect of chemical fertilizers and organic fertilizers on *Eisenia foetida* and the result shows that treatment with inorganic fertilizer urea is very harmful for *Eisenia foetida* whereas the organic fertilizer Kala Sona was formed to have favorable effect all over. Toxicity is associated with accumulation of chlorpyrifos in excess amounts and inhibition of AChE, which proves to be injurious to earthworms (J. Venkateswara Rao *et al*., 2003). Also Brian L Roberts and H Wyman Dorough, 1984 studied on the relative toxicities of chemicals to the earthworm *Eisenia foetida* and reported that several chemicals, considered only moderately or relatively nontoxic to mammals, were extremely or very toxic to earthworms; among these compounds were carbaryl, malathion, cypermethrin and benomyl and some study refers to the toxic effect of urea on earthworm determined by a simple paper contact method and reported that as the neural degradation and the body part deformation are occurred, the filter paper bed was absorbed with the body fluid and serum of the earthworms. Hence, the lethal effect of the regularly used chemical fertilizer urea is killing the friend of farmer in a drastic way (Dash *et al*., 2015).

Materials and Methods

Study Area

Earthworm *Eisenia foetida* were collected from and the study was conducted in the vermicomposting unit of Morning Star Home Science College Angamaly, Kerala. Worms used in this experiment were approximately same body weight and body length.

- Average Length of one Earthworm ~ 7.375cm
- Average Weight of one Earthworm ~ 0.17g

Chemicals used

Urea: The inorganic fertilizer used in the experiment was urea. Once applied to the soil urea is converted to ammonia which reacts with water to form ammonium ions within 2-3 days (faster under warm condition.)

Crop meal: It is a mixture of bone meal, leather meal and veppinppinnakku. Crop meal is a best organic fertilizer which is easily available in the local market.

Preparation of soil bed and experimental set up

Four buckets were used for this experiment. In each bucket a hole was made for the easy going of stagnant water to prevent the damage of vermicompost and also for the collection of vermiwash. Small pebbles were added first to each bucket followed by the addition of coconut fiber, 250 g cow dung, 1kg soil. Then water was added to moisten the soil. 20 live *Eisenia foetida* worms were added, after that peel of banana tree and dry leaves were introduced to avoid starvation.

Addition of Urea

The urea dose being practically applied in the local agricultural lands for the kharif crop was found to be 174Kg/hectare of land area. In this experimental set up, the soil bed contained 1Kg soil and 250g cow dung. Therefore, the calculated value of urea for the soil bed was 3.48 g/Kg of soil. In addition to the dose being practiced by the farmers i.e, 3.4g/Kg, two more doses of urea were set viz, 1.5g/kg and 2.25g/Kg of urea.

Addition of Crop meal

In this experimental set up, we used 2.2g of Crop meal. 20 mature *Eisenia foetida* worms were added to each bucket. The bucket were covered with wet jute sac. Thus one control set and 4 experimental set up were prepared. After 15, 30, 45, and 60 days the changes were observed in activity, morphology, growth of earthworm as well as the number of cocoons, juvenile, non-clitellate and clitellates.

Result and Discussion

Urea is found to be fatal for the earthworm population when the dose is above 1.5g/Kg in soil. A parallel control experimental setup was also set to compare the morphological and behavioral changes. The morphological changes observed in all the experimental set are discussed below.

DAY	PARAMETER	CONTROL	1.5g/Kg Urea	2.25g/Kg Urea	3.48g/Kg Urea	2.2g/Kg Crop meal
Day 1	Number	20	20	20	20	20
	Biomass of alive Earthworms(g)	3.4	3.42	3.42	3.42	3.43
	Biomass/Individual (g)	0.17	0.171	0.171	0.171	0.171
Day 15	Number	20	20	18	18	20
	Biomass of alive Earthworms(g)	6.52	6.61	6.73	3.52	6.61
	Biomass/Individual (g)	0.226	0.33	0.257	0.195	0.236
	Cocoons	17	20	18	15	18
Day 30	Number	20	20	16	13	20
	Biomass of alive Earthworms(g)	7.61	7.55	4.52	3	7.81
	Biomass/Individual (g)	0.33	0.377	0.282	0.23	0.375
	Cocoons	63	58	42	11	60
	Juveniles	15	13	7	-	21
Day 45	Number	20	20	14	10	20
	Biomass of alive Earthworms(g)	8.21	8.12	4.21	2.81	8.52
	Biomass/Individual (g)	0.41	0.4	0.21	0.281	0.426
	Cocoons	109	106	56	9	115
	Juveniles	58	46	8	4	69
	Non clitellates	16	12	3	-	19
Day 60	Number	20	20	13	7	20
	Biomass of alive Earthworms(g)	9	8.08	4.12	1.9	9.54
	Biomass/Individual (g)	0.45	0.417	0.316	0.271	0.47
	Cocoons	122	116	75	12	131
	Juveniles	64	55	13	2	72
	Non clitellates	21	17	6	-	25
	Clitellates	8	4	1	-	10

Figure 4.1: The table showing the result of morphological and physiological changes happened during the period of experimental study in given doses of organic fertilizer and chemical fertilizers.

The initial number of worms in all the set along with the control set was 20. At the end of the 60th day, all the 20 worms were alive in both the control setup and in the organic fertilizer soil bed. In addition, under the dose of 1.5g/Kg urea, the earthworms were also safe. But as the dose increased from 1.5g/Kg to 2.25g/Kg and 3.48g/Kg, mortality among the test animal was seen.

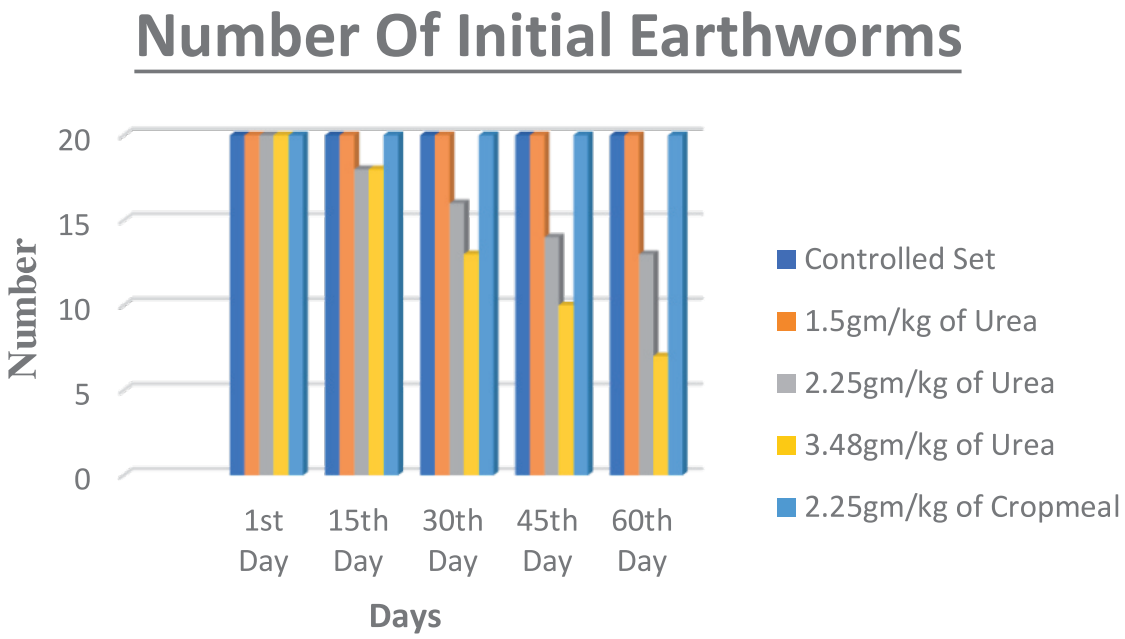

Figure 4.2: Graphical representation of variation in the number of earthworms under the controlled and experimental set up during 60 days.

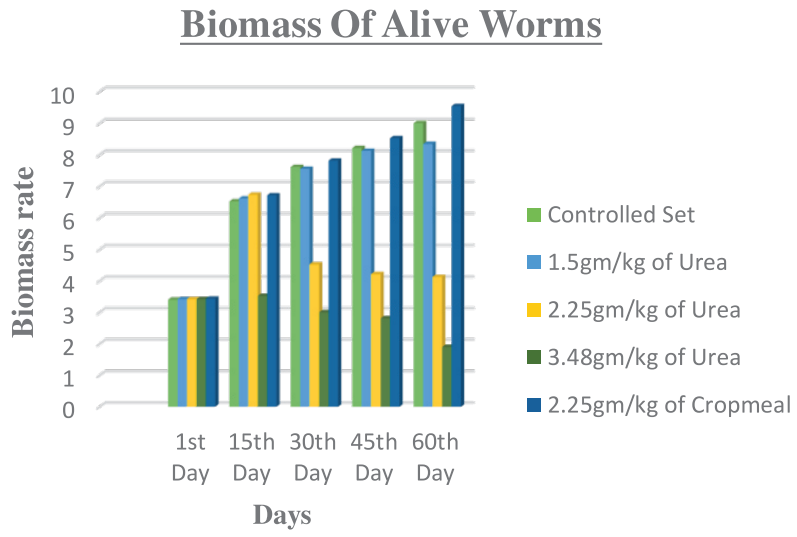

Figure 4.3: Graphical representation of variation in the biomass of earthworms under the controlled and experimental set up during 60 days.

Apart from the observed mortality among the test animal, the changes in weight was noticed in the worms which were able to survive throughout the study period under the higher doses of urea than 1.5g/Kg. The weight of earthworm was found to be increasing in the starting days of the experiment but after one month a steady pattern of weight loss was observed in the urea doses of 2.25g/Kg and 3.48g/Kg. Also, the earthworms in these set was seen rather weakened in the late days. On the other hand, there was appreciable weight gain under the organic fertilizer set during the whole study than the control set.

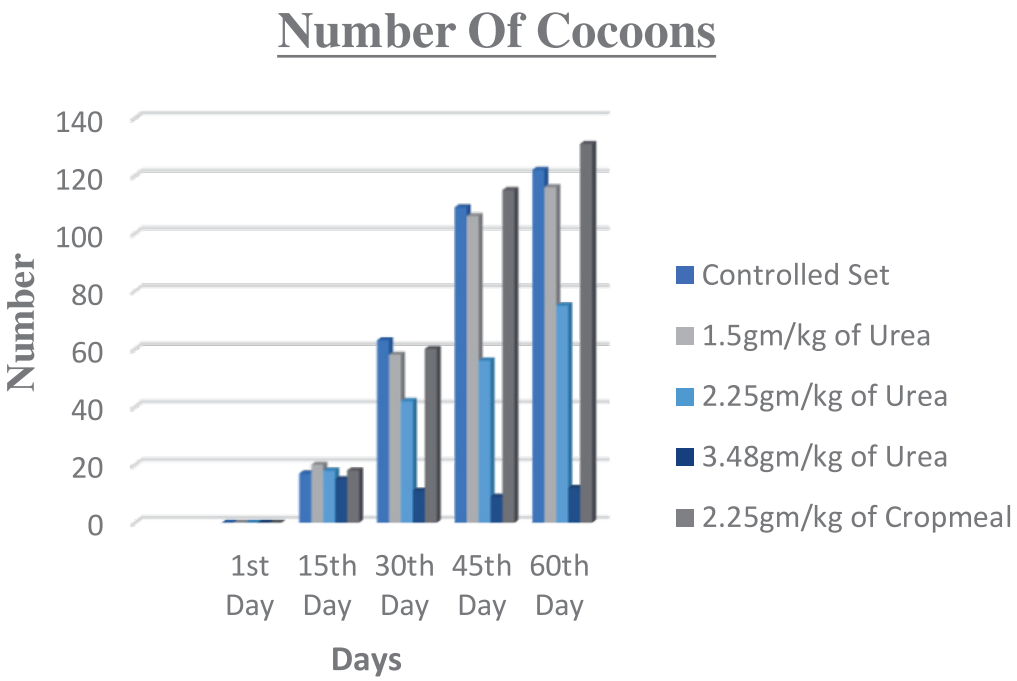

Figure 4.4: Graphical representation of variation in the number of cocoons under the controlled and experimental set up during 60 days.

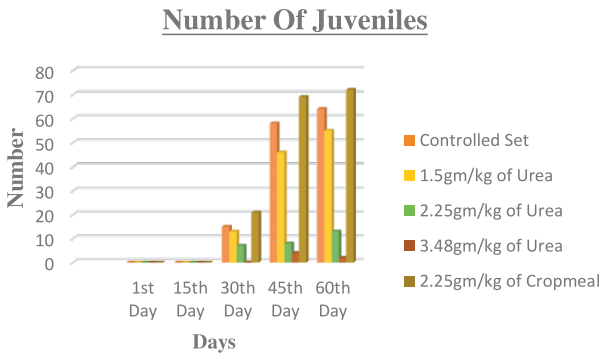

Figure 4.5: Graphical representation of variation in the number of juveniles under the controlled and experimental set up during 60 days.

When the weight gain by the earthworm on the 60th day was taken into consideration, highest weight gain was noted at the end of the experiment in the organic fertilizer set and the least in 3.48g/Kg urea set.

The number of cocoons was highest in the organic fertilizer set and least in urea dose 3.48g/Kg. Organic fertilizer set has the highest number of juveniles compared to the rest of the experimental setup. At the end of the study period, in the urea dose of 3.48g/Kg the number of juveniles were only 2, which was the least among all the setup, this can be attributed to the least number of cocoons in this set of urea dose.

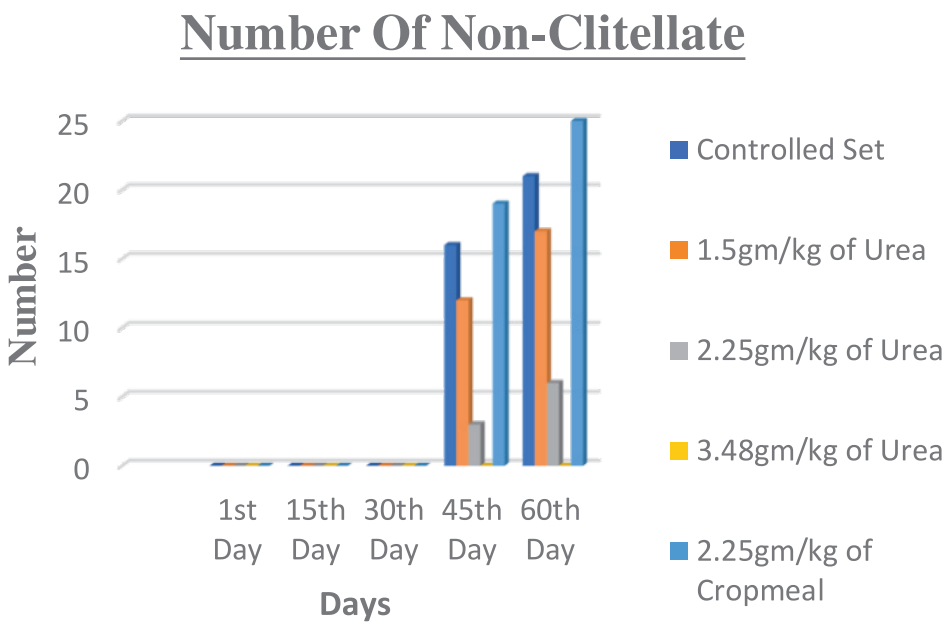

Figure 4.6: Graphical representation of variation in the number of non-clitellates under the controlled and experimental set up during 60 days.

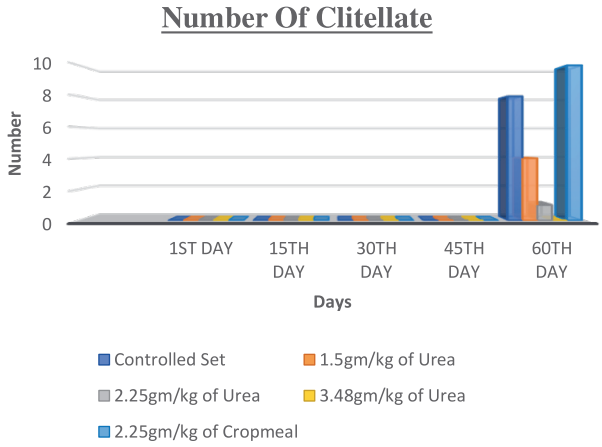

Figure 4.7: Graphical representation of variation in the number of clitellates under the controlled and experimental set up during 60 days.

In the urea dose of 3.48g/kg soil, shrinking of earthworm body and rupturing of the epidermis and cuticle with the secretion of yellowish fluid was observed. Least number of earthworms i.e. 7 were left in the soil bed treated with urea dose of 3.48g/kg at the end of experiment. Although, these number of worms were alive in this experimental set up but the worm's body was found weakened with less body weight. The worms in this set were found trying to escape out from the tub.

At the end of our experiment, the healthiest earthworms were seen in the experimental set up with the organic fertilizer and the weakened were the chemical fertilizer urea treated worms. Healthy numbers of cocoons, juveniles, non-clitellates and clitellates were counted in the organic fertilizer treated set.

Discussion

This investigation revealed many interesting facts. The chemical fertilizer urea was found to be quite toxic to the earthworms. Different doses of urea was administered to the soil and simultaneously one organic fertilizer was also used to conduct a perfect comparison of the two types fertilizer on the earthworm activity. A control set up was run parallel to the experimental set up. There were significant changes in the mortality and weight of tested earthworms after exposure to urea. There was a positive correlation between earthworm mortality

and the concentration of urea added to soil. The high mortality was showed when the dose of urea reached 3.48g/kg which Is the actual dose being practiced by the farmers in the agricultural land. The weight of earthworms exposed to urea decreased steadily with the increase in the dose of urea. The loss in body weight changed with increased exposure time. The sharp decrease in weight of earthworms revealed that the high concentration of urea was very toxic to the worms or it could be lethal for the total population. However, at the low concentration of urea there was no significant change in morphology except reduction in body weight. The Juvenile and immature worms were also found to be influenced by urea application.

Other researchers found similar or different results on this topic. Healthy earthworms in the organic fertilizer set up can be attributed to the fact that the organic fertilizers probably provide food directly for the earthworms and this might be the reason for the higher earthworm populations in the pots treated with organic matter (E. K. Bunnemann, G.D. Schwenke, and L.V. Zwieten, 2017). Studies showed that inorganic fertilizer particularly urea can be toxic to earthworms when contacted directly (Abbiramy K. S. and Ross P. R. 2013), (Dash, Anshurekha & Shuvendu Shekar Mohapatra, 2018). It was reported that various inorganic fertilizers have varying effects on earthworm. It can vary from site to site and depth to depth as well. Epigeic species are most affected because of their low drilling power. The study suggests that if it is necessary to use these fertilizers, it is advisable to use them in deep soil depth. Also recommend to use organic fertilizers along with inorganic fertilizers (Yahyaabadi, M., Hamidian, A. H., & Ashrafi, S. 2018). While some Studies showed that the applications of fertilizers with nitrogen and phosphorous caused significant increases in earthworm number and bio-mass (M. Iordache, and I. Borz, 2010). Similar results were obtained by Tiwari S. C. 1993.

Conclusion

This work was done to examine the effects of organic fertilizer, Crop meal and inorganic fertilizer, urea on earthworm *Eisenia foetida*. The application of environmentally levelheaded doses of urea exposed the possible harmful effects on earthworms when comes in contact directly. The results of the present investigation clearly demonstrate that treatment with inorganic fertilizer urea is very harmful for *Eisenia foetida*. Whereas, the organic fertilizer crop meal was found to have a favorable effect all over. This study may be useful to evaluate ecological risk from agricultural activities such as the application of agrochemicals, and to avoid ecological damage from inappropriate application of chemical fertilizers. Therefore, it is advised that the use of nitrogenous fertilizer like urea should be within ecologically safe limits. The necessary precautions and regulations should be implemented for the usage of the chemical fertilizers like urea on the agricultural lands. The social awareness is most needed for this serious issue about the soil health.

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Chapter 5

A New Era of Crop Improvement: A Review on Genome Editing in Plants

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ABSTRACT

The advent of genome editing by the discovery of sequence specific nucleases (SSNs) have revolutionized biology. Genome editing can occur naturally via random mutagenesis. In genome editing technology, it is possible to make various modifications like knockouts, insertions and oligonucleotide directed mutagenesis (ODM) by using SSNs. Major tools used in genome editing are mega nucleases, zinc finger nucleases (ZFNs), transcription activator like effector proteins (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR associated protein 9 (Cas9). In this review, tools used for genome editing, achievements, advantages, impact assessment of genome editing and its future prospects are discussed.

Introduction

The study of gene mutants is the main aspect to study the function of genes in plants and for the genetic improvement of crops. Many studies done in the past years in this field had helped to identify the mutants and lead to the construction of mutant libraries corresponding to thousands of genes in plants under study. But these studies used various physical, chemical and biological mutagenesis method for the same which can result in random mutagenesis resulting in unwanted and undesirable mutations resulting in large scale screening of mutants, which is costly and tedious process. Also, random mutagenesis may result in repression of specific genes associated with those under study. Therefore outputs from these methods may not be reliable.

Here comes the importance of new technologies, which creates specific and targeted mutagenesis, thus facilitating study of gene and its function [11]. Cells have inherent mechanisms for double stranded DNA breaks repair which are actually the core approaches for targeted genome editing or modification, which introduce precise breaks at the targeted sites. Earlier, approaches for modifying genome relied on Watson crick base pairing, like induced self-splicing introns, homing endonucleases encoded by introns, etc. In 1994, Rouet and Smith, for the first time performed experiments with rare cutting mega nuclease I Sec 1, which showed that targeted double stranded cuts

(DSB) formed using mega nuclease can result in localised mutagenesis along with incorporation of homologous donor sequences at the target genomic site. This was the first milestone in the field of genome editing [22].

The use of programmable sequence specific nucleases (SSNs) for genome editing began with use of zinc finger nucleases (ZFN) in the year 2002, which are the first truly target specific reagents ever used. ZFNs DNA binding domain recognizes three base pairs at the target site and being a dimer, two ZFN DNA binding domains are attached to Fok 1 monomer, which will attach to the site with a spacer region of 5-6 base pairs, resulting in function Fok 1 activity and creation of DSB. In 1996, Kim et al. had already established ZFN as a restriction enzyme but its use as SSN stated only after 2002 [2].From then, ZFN found use in various studies conducted in plants like Arabidopsis, tobacco and maize. Then came transcription activator –like effector nucleases (TALENs), developed from bacteria Xanthomonas and first applied in plants [11]. TALENs recognize one nucleotide instead of three as in ZFN, making it highly specific and more desirable than ZFN [22]. Although easy to use, it required complicated tandem repeat domains like the TAL proteins [11].

Recently, a new genome editing tool was developed from type 2 CRISPR adaptive immunity system found in bacteria Streptococcus pyogenes, which help bacteria and archea against invading phages, named clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein (Cas9). They can induce site directed specific mutations efficiently when compared to first generation editing tools like ZFN and TALENs by base pairing to target DNA site using engineered single guide RNA (sg RNA), which makes its use much easier. The presence of such a CRISPR in bacteria was identified in 1987, but information on their function remained a mystery till 2005 and it was found out in 2007 about the association of CRISPR along with Cas protein in providing immunity to bacteria.

Doudna and associates in 2012, firstly performed genome editing using CRISPR/cas9 in a cell free system and afterwards, genome editing was done in animal system by five independent research groups. It was only in 2013 that genome editing using CRISPR/cas9 was done in model plants namely Arabidopsis and Nicotiana nebthamina and rice [22].

This review paper mainly focuses on the newly developed discipline of genome editing, its tools and methods, potential application, their benefits, achievements, impact assessment and also its future prospects.

Genome Editing Tools

Modern biotechnology has provided not only deeper understandings about dynamics of genome structure and function, also it has provide tools which can be used for controlled alteration of DNA sequences within plant genome otherwise known as genome editing. They are reagents which are customizable, called sequence specific nucleases (SSNs), which function by introducing double stranded breaks in target loci. The cell in turn recognizes this DNA damage and the enzymatic repair machinery will be recruited to the site. If the repair is done by homologous recombination (HR), the information is copied from a DNA template during the process of repair, where the template DNA can either be homologous chromosome, a sister chromatid or a user supplied DNA which is homologous to the break site. On the other hand, if the repair is done by NHEJ, the chromosomes are joined precisely but small deletions or insertions can occur at the site of break. If the joining is not precise, frame shift mutations can occur which can knockout gene function. Also, mutations like removal of a few amino acids within coding sequence without alteration in reading frame, insertion or deletion in promoter can occur which can disrupt key regulatory sequences and alter gene function.

In order to create targeted mutations, SSNs should be able to recognize a specific DNA sequence in a genome. For this, currently four classes of SSNs can be used: Zinc finger nuclease (ZFN), Transcription activator – like effector nuclease (TALEN), engineered homing nucleases or Mega nuclease, and Clustered regularly interspaced short palindromic repeats (CRISPR) and associated nuclease (Cas9). Also, oligonucleotide directed mutagenesis (ODM) are used to provide custom made SNPs (used as molecular markers) used for detecting naturally

occurring variation in genetic composition in a population of species [21].

Meganuclease

Meganucleases are naturally occurring endonucleases with large recognition sites. They are also known as homing endonucleases and many was discovered after its initial reporting. They were first reported from yeast were two proteins, HO and I-SceI which were associated with yeast mobile genetic elements, in the year 1980. Since these nucleases identified the largest sequence till date, they were named mega nucleases [20].

Meganuclease recognize DNA sequence elements of 12 to 40 bp and cut both strands of DNA in a site-specific manner. Among MNs, the I-CreI protein has been reported to be effective in maize [25].

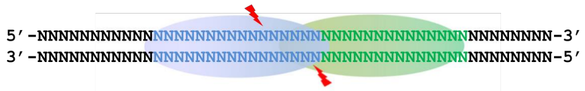

Figure 5.1: Meganuclease - recognize a DNA sequence element, cut both strands at specific sites, forming sticky double-stranded breaks (DSBs) [25].

Rare occurrence of recognizable sites limits the ability of meaganuclease. To broaden the application researchers have used mutagenesis or combinatorial assembly to produce Meganuclease variants that target the desired DNA sequence [25]. And this can be controlled by engineered natural mega nucleases to be used in HR based editing [20]. The overlapping recognition and catalytic domains of modified Meganucleases cause difficulties and often compromise their catalytic activity. For these reasons, they have not been widely used by plant scientists [25].

Zinc Finger Nucleases (ZFN)

The research for possible linking of the non - specific Fok 1 endonuclease domain to specific DNA binding domains, which aimed at the development of methods for precise DNA recognition and restriction, began a decade ago and eventually zinc finger domains were selected because of the fact that they serve as the most abundant DNA binding motif [7]. Two subsequent studies in 2003 marked the beginning of genome editing by utilising ZFNs in Drosophila and in human cell line [6]. ZFNs consist of zinc finger protein domain, fused to a nuclease domain for sequence specific binding and cleavage of DNA respectively. ZFN bind to specific site as a result of an array of 3-6 zinc finger protein domains that individually recognizes approximately 3 bp of DNA. There are considerable interactions with neighbouring domains which result in specific binding but the challenging part is that of its design [18]. Each zinc fingers are 30 amino acids in length which are arranged in a $\beta\beta\alpha$ configuration and the amino acids from α helix interact with the DNA and provide the binding specificity. Key amino acid residues from -1 to +6 relative to the α-helix of the zinc fingers control the specificity and different specificities can be obtained by altering these residues and by keeping the backbone residues constant. Synthetic ZFN contain 3-6 repeats of such zinc fingers which are connected to each other by connector sequences and this assembly allows the recognition of 9-18 DNA bases which can be engineered for sequence specificity. The Fok 1 domain of ZFN requires dimerization for DNA cleavage but the interface is not strong, therefore in order for two fused Fok1 domains to overlap, the zinc finger domains should be aligned in an opposite orientations and recognition should happen between two separate neighbouring sequences. Also, Fok 1 variants requiring heterodimerization were developed, with enhanced sequence specificity and reducing off-site cleavage [6]. ZFNs are designed so as to bind and cleave a specific gene locus, which would result in NHEJ – mediated sequence alteration. In case of presence of DNA sequence homologous to those flanking regions of the DSB, the HR uses it as template for strand annealing whereas if the homologous sequences are present in donor DNA, the design of a DSB can be read out by the repair template composition [18].

Transcription Activator – Like Effector Nucleases (TALEN)

Transcription activator – like (TALE) proteins were discovered at first in the year 2007 in Xanthomonas campestris pv. Vesicatoria, a plant pathogen. TAL are basically virulence factors, which are produced as result of host parasite interaction, whose function is to silence transcription of defence – related response genes by binding to specific sequences such as promoters[6] TALENs are also chimeric proteins with two major subunits in which there is a domain which recognize target sequence and other Fok 1 nuclease domain which can bind to the other Fok1 nuclease domain of the TALEN bound to the opposite DNA strand, which would result in a DSB in the target site in between the TALENs. Repair of the strand is either by NHEJ or HR in presence of supplied homologous DNA strand. The DNA recognition domain of TALENs derived from TALEs (transcription activatorlike effectors) produced by plant pathogenic bacteria (Xanthomonas sp.) has a domain which bind to target sequence, known as recognition domain, has 12 – 27 identical repeat units (tandem repeats) of 33 - 35 amino acids sequence motif [23].but the last repeat has only 20 amino acids, thus known as half repeat). The 12th and 13th amino acid positions are variable within each unit. These two variable amino acids are known as repeat- variable diresidues (RVDs) which is in direct contact with one nucleotide base of the target DNA site. There are sets of codes which indicate which RVDs will pair with particular base at the target sequence (Weeks et al, 2016). According to one of the codes widely used, an asparagine – isoleucine RVD recognize adenosine, NG recognize Thymine, HD recognize cysteine and NN recognize guanine or adenine [3]NK and NH tend to be more guanine specific than NN [6]and this code greatly helps in engineering TALENs that can recognize any DNA sequence in any gene. Nuclear localisation sequences (NLSs) and Fok 1 nuclease domains are added to N terminal of the TAL repeats resulted in a pair of TALENs for targeted gene sequence cleavage. TALE binds to target DNA in a right handed super helix manner where each repeat unit forms a left handed two helix bundle where an RVD containing loop is present to the DNA major groove [3].

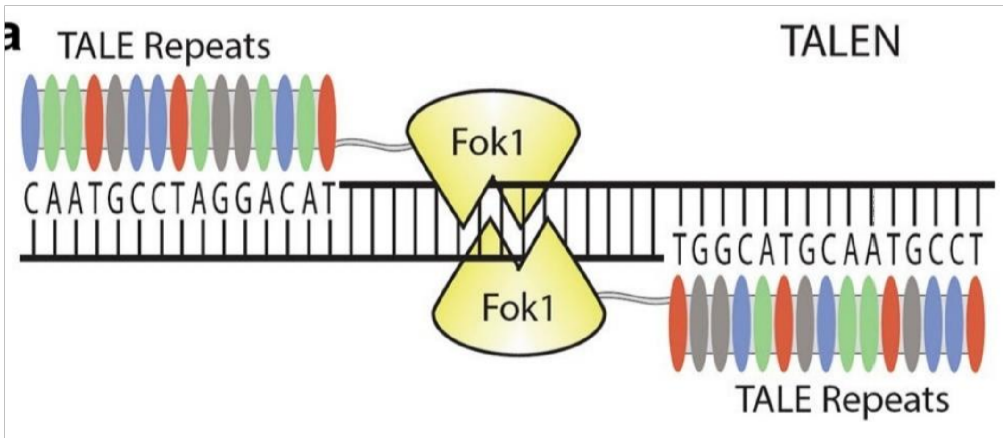

Figure 5.3: Structure of TALEN. Each repeat variable residue recognize single specific nucleotide [12].

Oligonucleotide Directed Mutagenesis (ODM)

The field of crop breeding revolutionized by the advancement of SNPs (single nucleotide polymorphisms) being used as markers, which occur naturally in the genome. One method which can be used for providing custom made SNPs are oligonucleotide directed mutagenesis. When ODM was studied in eukaryotic cell systems, the oligonucleotide which contain sequences homologous to the target sequence is delivered along with a mismatch and is

introduced into the cell which gets incorporated with the nuclear DNA target sequence. The mismatch in the homologous oligonucleotide trigger repair mechanism in cells which eventually transform the required change in the target site [21].

Clustered Regularly Interspaced Palindromic Repeats and Associated Proteins (CRISPR/Cas9)

CRISPR-Cas system was discovered in 1987 by a group of scientist when working on iap enzyme in E.coli and is basically an immunity system present in bacteria. They spotted 29 nucleotide repeats downstream iap gene. By 2005, it was clearly evident that the spacer regions separating the individual direct repeats had extra chromosomal and phage-related origin. It was then named CRISPR is clustered regularly interspaced palindromic repeats and Cas are CRISPR associated proteins (CRISPR-Cas). It was then hypothesized that, it is a part of bacterial immunity and it resisted the infection of those phages whose genome parts are still carried by the bacteria. By 2011, CRISPR was well understood and efforts to use them in genome editing were taken [16].). It has unique ability to introduce non host DNA sequences within the CRISPRs. These sequences are known as spacers which are introduced at specific locations within CRISPR [17].

They form non-coding RNAs on transcription and are able to form complexes with CAS proteins. CAS protein in turn function by binding to non-host DNA or RNA sequence if homologous which lead to its degradation [6].

Basically CRISPR system are of three types: Type I-Cas 3, Type II –Cas 9, Type III-Cas 10.Type II contains only one endonuclease namely Cas9. This makes it easy to use and this is why type II is used widely in genome editing [17]. In bacterial cell, this system has following regions:

- Cr RNA (CRISPR RNA)
- Tracr RNA (Trans CRISPR RNA)
- Cas 9 nuclease [16]

The newly transcribed RNA from spacers is known as pre-cr RNA. It has two regions -Spacer region (derived from non-genomic sequence or from phages) and Protospacer adjacent motif (PAM) (highly conserved near the spacer) [17] Pre-cr RNA by the action of endoribonuclease cleave into smaller cr RNA and when complexed with Cas 9 and bind to any sequence initiate cleavage. Genome editing in eukaryotes can be achieved by introducing Cas (nuclease and a guide RNA known as gRNA or sg RNA [6]. This sg RNA is formed by fusion of cr RNA and tracer RNA [16] and would be complementary to the target site and tracr RNA and is a chimera of cr RNA. Therefore it serve as a connecting link between cr RNA and Cas9.

Cas9 is an RNA guided DNA nuclease. It consists of two twinned domains which are catalytically active and functional as nuclease-NHN and ruvC. Cas9 is linked to sgRNA by a complex scaffold structure. SgRNA spacer region then can bind to complementary DNA sequence with Cas9 and create a double stranded break (DSB). For this to happen the target sequence neighbours a PAM at the 3' end of target site. Cas9 orthologs only differ each other from the requirements of sg RNA scaffold structures and PAM sequences in order to make a DSB. The Cas9 is derived from Streptococcus pyogenes and require NGG (N-nucleotide any, G –guanine) PAM sequence. The DSB s are repaired by repair pathways namely NHEJ and HR. NHEJ or non – homologous end joining can be error prone, resulting in insertions or deletions. HR or homologous recombination or homology directed repair may result in introduction of new sequences a homologous template is provided in the medium. [17].

The first CRISPR/Cas 9 mediated genome editing was accomplished by in vitro in 2012, which was followed by a multiplex genome editing in mammalian cells in 2013[17]. One of the main problem to deal with when applying CRISPR system is that off -targeting activity. Concentration of sg RNA and Cas9 is very crucial and any alteration in this would lead to off -targeting. Another factor is the complementarity between sg RNA and target DNA. There should be match between the seed sequence (last 8-12 bases of sgRNA) and region near to PAM sequence in target DNA sequence. Promoters also

play an important editing tool. A change of a 20-bp spacer sequence in the gRNA can easily reprogram Cas9 to target a different DNA site. By providing multiple grants, the system also enables multiplex genome editing at high efficiencies.

Multiple sgRNAs are of utmost importance because the innate ability of Cas9 to edit multiple loci simultaneously in the same individual find many potential applications in both basic and applied research. The area of applications are mutation of multiple members of gene families or functionally related genes that control complex traits. Multiplex genome editing requires efficient assemblage of multiple sgRNA expression cassettes into a single binary CRISPR/Cas9 vector and this can be achieved by either of the two methods, like Golden Gate ligation or Gibson Assembly [14].

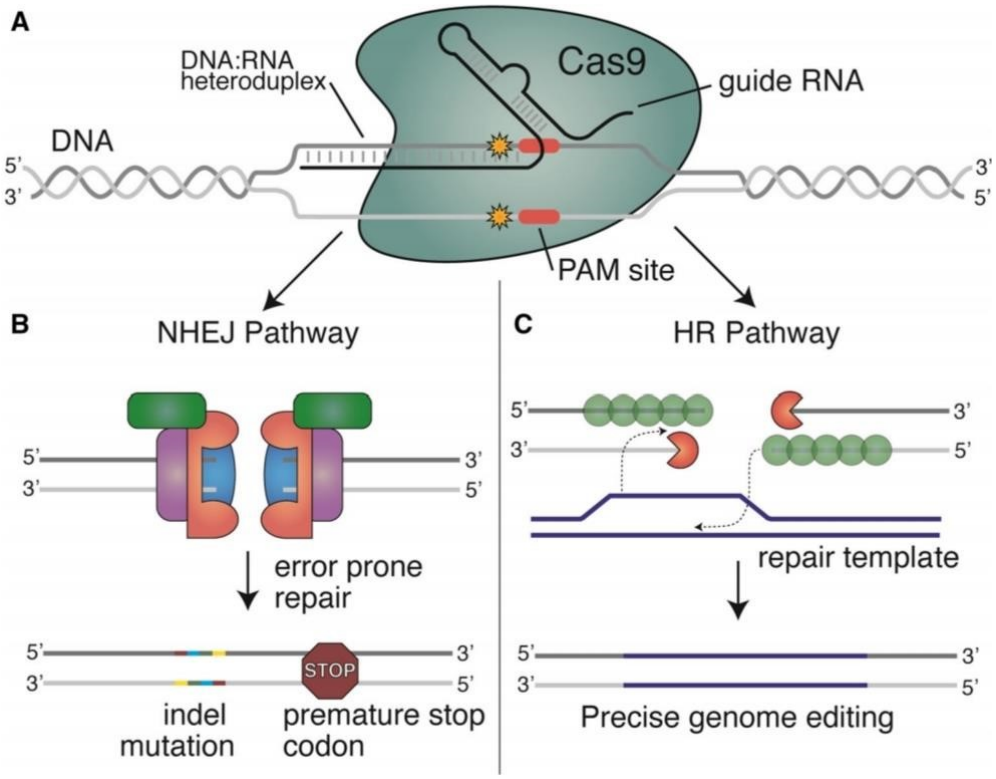

Figure 5.4: CRISPR/Cas9 mediated genome editing [17].

Achievements

In order to meet the global growing demand for food, feed and fuel, it is necessary to enhance genetic and phenotypic variation in crops that are relied upon. And creation of novel variations can be achieved through genome editing. There has been tremendous work done for improvement of crops in withstanding both biotic and abiotic stresses.

There are various improved crop varieties that we use, which were genome edited for enhanced and new characters. Some of the major crops and plants that have been genetically edited are Oryza sativa, Triticum aestivum, Solanum lycopersicum, Zea mays, Gossypium, Solanum tuberosum, Saccharum officinarum, Glycine max, Cocos nucifera, Nicotiana tobacum, Theobroma cacao, etc. and various fruit crops like Citrus, Persea Americana, Vitis, Phoenix dactylifera, Carica papaya, Musa, etc. The characters imparted include disease resistance to specific pestsenhanced yield and productivity, higher shelf life of fruits and vegetables, resistance to various

abiotic factors, etc. [19, 15, 27, 8, and 9].

Impact Assessment

Being a novel and latest advancements in science related to agriculture and crop improvement, there are various concerns over the output of genome editing technology and its direct and indirect impacts on humans and nature itself as a whole. The below discussed are some finding:

1. Off - target effects: To reduce the off-target effects or modifications target sites of double stranded breaks (DSB) having repeated sequences and homology in high degree with other regions are to be avoided.

2. Regulation of genome edited plants (GE plants): Issue comes in the process by which genome edited plants were created. They should be assessed on the quality of their products only. Therefore, a new set of regulations for the products should come up for products made by genome [23].

3. Gene drives: This can be a point of concern as they may enable biased inheritance of a specific trait with a chance of more than 50% in the offspring population. Creation of a gene drive can be either purposeful or inadvertent, but the outcome is the same—gene expression is monoallelic as continued target gene edits in subsequent generations.

4. Genome Characterization: Molecular characterization can be used in comparing both intended and unintended effects as a result of genome editing. Complete DNA sequence data about transcriptomic, metabolomic, epigenomic, and phenomic effects can be useful to downstream efficacy and safety attributes of the plant. This would help in subsequent risk assessment of potential effects of genome edited crops and their derived products.

5. Omics Characterization: Omics refers to the collective characterization and quantification of pools of biological molecules that make up the structure, function, and dynamics of an organism or organisms. It combines different - techniques such as transcriptomics and proteomics. These methodologies for plant characterization have not shown unambiguous changes for the specific genome edited crop, therefore do not raise any safety concerns.

6. Bioinformatics and Computational Characterization: Lack of knowledge regarding the reliability of these techniques make regulatory assessments difficult Improved and validated predictive algorithms are required and are essential [5].

Advantages

There exist many beneficial elements regarding genome editing when compared to conventional methods in plant breeding and genome modification (GM) crops. The advantages largely reflect in terms of agronomic performance, like disease resistance, drought tolerance, high yields etc., final product quality, like nutrition, shelf life, etc., climate change resilience, and global food security.These advantages as derived from the accuracy and precision of genome-editing technology, which is believed to save years of development time and lower the production cost of certain traits in crops. Also, it would aid in creation of varieties which would reduce agro-food waste and lead to enhancement of biodiversity [10].

Following are the advantages or benefits associated with genome editing in plants:

- Targeted mutagenesis
- Multiplex genome editing
- Gene regulation
- Epigenetic modification and post transcriptional modifications lead to alteration in chromatin structure and regulation of gene expression patterns.
- Gene replacement and gene knock-in [13].

The below described factors aids in creating crops with the following enhanced characters:

- Improved resistance to biotic stresses
- Increased abiotic stress tolerance
- Improved crop yield and quality
- Improved nutritional, functional quality [1, 10].

Future Prospects

CRISPR/Cas based genome editing was materialized as a crusader recently due to its immeasurable potential to make targeted changes in genome and its multifaceted characteristic purposes [22]. Therefore, it will surely lead to advancements in field of plant biology and crop breeding. It would result in simultaneous modifications to be done in multiple loci in crops, which would lead to crop improvement as well as would lead to enhanced global food security [25].However, there are some prerequisites for effective genome editing, which is the complete understanding of genome sequence and gene function which in turn can be provided to an extent by genome editing itself, which would lead to the probable use of CRISPR in annotating genome structure and function in plants [25]. Public awareness regarding the difference between genome modified crops and genome edited crops is very essential. Also, there should be clear cut laws in countries for the utilization of genetically modified and edited, crops, the market would be trouble free and can provide agriculture with a sustainable future [22].

The ongoing developments and discovery in genome editing would boost crop improvement and have allowed the studies and further research on various crops other than conventional model plants, therefore would play an assured role in addressing the growing global food demands [1]. Genome editing which ease quickly assemble of multiple traits, will result in efficient improvement of complex agronomic traits of crops which places the probability of more edited crop products to be out in market in very near future [25].

There are various concerns still surrounding genome editing which need to be looked into which includes establishment of unified delivery method, increasing efficiency of homologous recombination thereby reducing off target editing which can be achieved by further studies in modification of tools and perception cell repair pathways [25, 22]. Moreover, the issues of climate change should be looked into which would demand for greater flexibility and innovation in crop production and durability. Also, governmental regulations and consumer acceptance for the use of these new breeding approaches must be considered. This would also lead to the production of more food with a less impact on the environment which would lower the cost of crop breeding and leads to accelerated production of new high-yield, stress-tolerant, more nutritious crops with higher nutrient use efficiency.

Due to the salient features and characters possessed by genome editing, it got settled as a robust restructuring tool in plant biology similar to the changes made by molecular cloning and PCR techniques. Therefore unwanted probe about genome edited crops would lead to reduction in utilization of its benefits in feeding the fast enlarging global population [1].

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Chapter 6

Computational Chemistry Calculations of CO Stretching Frequencies of Cyclic Ketones, Cyclic Esters and Cyclic Amides

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ABSTRACT

The presence of a Carbonyl group as part of a ring is usually contribute ring strain and it affects CO stretching frequency of IR spectra of the compounds like cyclic ketones, cyclic esters and cyclic amides. Due to the availability of variety of conformations for large rings, ring strain is found to be relieved in these case. But in case of smaller rings, ring strain is not relieved. The current study focuses on trends observed in carbonyl stretching frequency in cyclic ketones, lactones and lactams. This is done with the help of computational chemistry techniques using DFT method with B3LYP, 6-31G (d,p) and optimized using Avogadro. It is found that the frequency of C=O group in cyclic ketones increases with decreasing the ring size. The ring strain shift the absorption values to a higher frequency. Sometimes variations are found in CO stretching frequency calculations which may be due to the dependence of stereochemistry rather than the electronic structure.

Introduction

Infrared radiation refers to that region of the electromagnetic spectrum which lies between visible and microwave region ranging from 3×10^{12} to $3 \times 10^{14} Hz$. IR spectroscopy is the measurement of the interaction of infrared radiation with matter by absorption, emission or reflection. It is used to study and identify chemical substances or functional groups in solid, liquid or gaseous forms. When an analytical chemists speaks of infrared spectroscopy, he usually means the range from 2.5 to 25μ or 4000 to 400 wave numbers (wave per cm). This range gives him the important information about the vibrations of molecules and about the structure of molecules. IR spectroscopy is one of the most powerful analytical technique that offers the possibilities of chemical identification. This technique can be coupled with intensity measurements for quantitative analysis. One of the most important advantage of IR spectroscopy over the other usual methods of structural analysis (X-ray diffraction, Electron Spin

Resonance (ESR) etc) is that it provides valuable information about the structures of molecule quickly without many evaluation methods.

This technique is based on the simple fact that a chemical substance shows marked selective absorption in the infrared region. After absorption of IR radiations, the molecules of a chemical substance vibrate at many rates of vibrations; giving rise to close packed absorption bands called an IR absorption spectrum, which may extend over wide wavelength range. Various bands present in IR spectrum corresponds to the characteristic functional groups and bonds present in a chemical substance. Thus an IR spectrum of a chemical substance is the Finger print for its identification.

CO Stretching Frequency

For simple aldehydes and ketones we obtain carbonyl stretching vibrations at 1710 to 1740cm^{-1}. The dipole moment increases on stretching (single bond character is greater) and it results in a strong absorption. Alkyl substituent's stabilize the carbocation character of the ionic contributor, ketone carbonyls have slightly lower stretching frequencies, 1715 ± 7 cm^{-1}, compared with aldehydes, 1730 ± 7 cm^{-1}. The values mentioned are for pure liquid or CCl4 solution spectra. Hydrogen bonding solvents will lower these frequencies by 15 to 20 cm^{-1}.

Cycloalkanes are cyclic hydrocarbons in which carbons of the molecule are arranged in the form of a ring. In cycloalkanes all carbon atoms in the ring are connected through single bond (no double or triple bonds). Two or more cycloalkanes are joined together to form multiple rings, called polycyclic alkanes.

We can't make stable cycloalkanes with any carbon chain length. Carbon adopts the sp^3 tetrahedral geometry with bond angle 109.5°. For the formation of some cycloalkanes there occur deviation from the ideal angle, this type of an effect is known as angle strain. In addition to this some hydrogen atoms closer to each other than desirable (become eclipsed), this effect is called torsion strain. These angle strain and torsional strain are destabilizing effects and are together known as ring strain. The smaller cycloalkanes like cyclopropane and cyclobutane have particularly high ring strains due to the deviation of bond angles from 109.5° and eclipsed hydrogen's. With small amount of ring strain cyclopentane is more stable molecule than cyclobutane and cyclopropane. Cyclohexane adopt the perfect geometry of cycloalkane i.e, all bond angles are 109.5° and no hydrogen's are eclipsed as a result it has no ring strain.

Cycloalkanes tend to give off a very high and non-favorable energy, and the spatial orientation of the atoms is called the ring strain. When atoms are close together, their proximity is highly unfavorable and causes steric hindrance. The reason we do not want ring and steric hindrance is because heat will be released due to an increase in energy; therefore a lot of that energy is stored in the bonds and molecules, causing the ring to be unstable and reactive. Another reason to avoid ring strain is that it will affect the structures and the conformational function of the smaller cycloalkanes. By using heat of combustion we can determine the presence of ring strain. By comparing the heat of combustion with the values measured for the straight chain molecule, we can determine the stability of the ring. There are two types of strain, eclipsing/torsion strain and bond angle strain. Bond angle strain causes ring to have poor overlap between the atoms, resulting in weak reactive C-C bonds. An eclipsed spatial arrangement of the atoms on the cycloalkanes results in high energy.

Computational chemistry is valuable for studying the CO stretching frequencies of organic compounds especially which are difficult to prepare by conventional methods. It is fairly cheap and fast compared to experiment and it is environmentally safe. It doesn't replace experiment, which remains the final definition of truth about nature. To know the trends of CO stretching frequencies of cyclic ketones, cyclic esters and cyclic amides which are very difficult to prepare in pure forms and time consuming, we need to go into lab, but computation made this process easy. Hence many scientists employed it before embarking on experimental project. The current work focuses on trends observed in carbonyl stretching frequency in cyclic ketones, lactones and lactams. This is done by using B3LYP, 6-31G (d,p) in Facio and optimized using Avogadro.

Method

In order to find the CO stretching frequency, first the molecules are visualized through Avogadro software. After visualization, the geometry of the molecules are optimized. Then the optimized geometries are incorporated GAMESS, a free software and calculated with B3LYP, 6-31G(d,p) basis set in gas phase on singlet, neutral charge. Then it is saved in firefly with a file name (for eg: acetone). Then the geometry is loaded in the facio software, with Cartesian coordinates and the frequency is calculated and wait till terminated normally in facio worksheet.

After this, normal mode of vibrations are found out, and animation frames for each normal mode are calculated and the frequency for CO stretching and normal mode of vibration. This procedure is used for the calculation of CO stretching frequency of cyclic ketones, acid residues, lactones, lactams etc. The CO stretching frequency usually falls in the range of 1600 to 1900cm-1. The frequency of different compounds are calculated, tabulated and compared with experimental values.

Results and Discussion

In this section we discussed the results of computational calculations outlined above. The CO stretching frequency of different cyclic ketones, lactones and lactams are noted, thus the trend of stretching frequency in IR spectrum can be evaluated. The result is compared with experimental values.

1. Cyclic Ketones

Acetone

Using avogadro, the structure of acetone was sketched and later optimized the geometry. Further we calculated equilibrium geometry using the basis sets. Next, we have drawn all the CO vibrations and calculated the co stretching frequency of acetone. The value is then compared with experimental values. The value of co stretching frequency of acetone using computational chemistry is 1756.48896cm^{-1}. There is a slight variation from the experimental value.

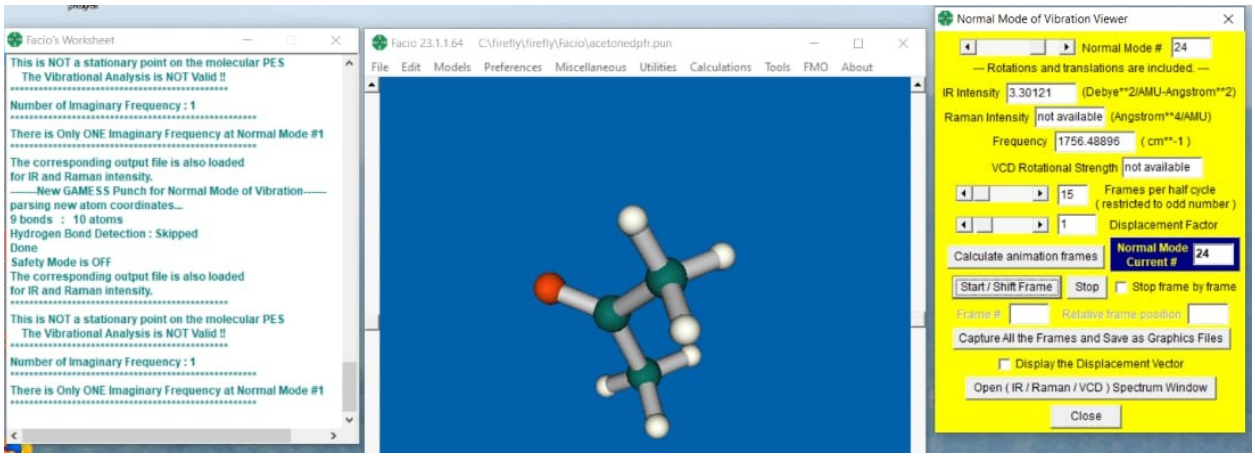

Figure 6.1: Acetone

Cyclopropanone

Similarly, we sketch the structure of cyclopropenone and optimize it. Next, we draw all the vibrations of cyclopropanone and calculate CO stretching frequency. Both experimental values and obtained values are calculated. The obtained value is 1869.34291cm^{-1}.

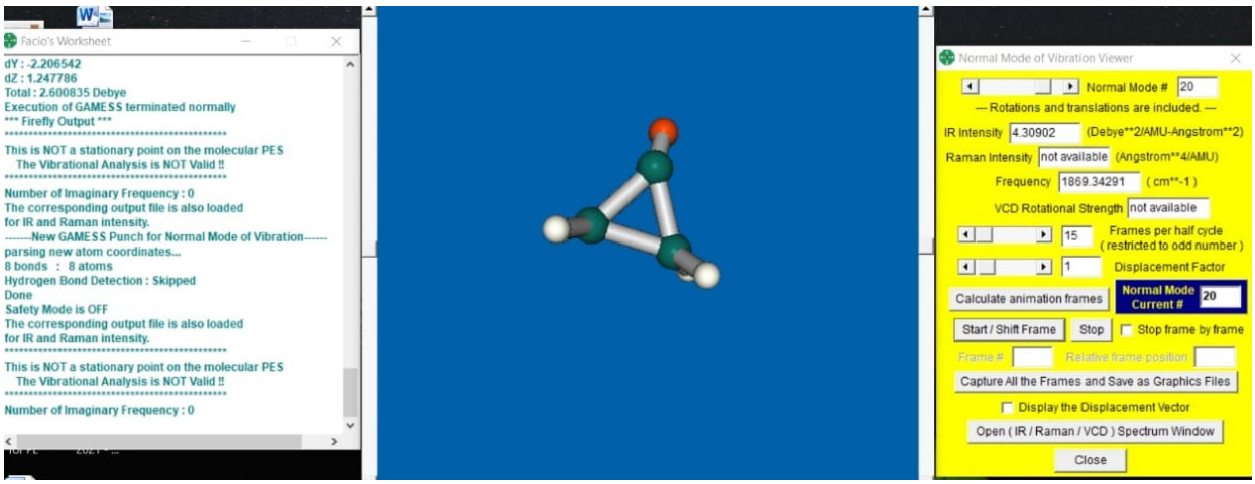

Figure 6.2: Cyclopropanone

Cyclobutanone

Similarly, we first sketch the geometry of cyclobutanone. Further we optimize the geometry using Avogadro. In the next step we draw all the vibrations of cyclobutanone and find the CO stretching frequency of it. The observed value of cyclobutanone is 1839.21606cm^{-1}.

Cyclopentanone

In the case of cyclopentanone, we first draw the structure and optimize it using Avogadro. Next draw all the vibrations and CO stretching frequency of cyclopentanone is calculated.

The observed value is 1785.79323cm^{-1}.

Cyclohexanone

Similarly, we first draw the optimized structure of cyclohexanone. Later draw all the vibrations and find the CO stretching frequency of cyclohexanone.

The observed value is 1741.72371cm^{-1}.

Cycloheptanone

In the same way we first draw optimized geometry of cycloheptanone and draw all the vibrations of it. Next, we find the CO stretching frequency of cycloheptanone.

The observed value is 1736.09531cm^{-1}.

Observations

Name of the compound	CO stretching frequency from calculation cm^{-1}	Observed CO stretching frequency cm^{-1}
Acetone	1756.48896	1740
Cyclopropanone	1869.34291	1815
Cyclobutanone	1839.21606	1810
Cyclopentanone	1785.79323	1774
Cyclohexanone	1741.72371	1715
Cycloheptanone	1736.09531	1709

Table 6.1: CO Stretching Frequencies of Cyclic Ketons

From the above observations the trend in CO stretching frequencies of various cyclic ketones can be analyzed. The results give the idea that cyclopropanone has higher CO stretching frequency compared to other cyclic ketones. The reason behind the high CO stretching frequency is due to ring strain present in it. The angle between the ring is small compared to actual angle that is 120 degree in cyclopropanone. As the angle changes, the CO bond strengthens and increase the energy requires to stretch it. The ring strain in cyclopropanone is high. As ring strain increases CO stretching frequency increases. Cyclopropanone has shorter, stiffer and stronger bond.

Thus the order for the co stretching frequencies is;

Cyclohexanone < Cyclopentanone < Cyclobutanone < Cyclopropanone

2. Cyclic Esters

In cyclic esters (lactones), C=O stretching is shifted to higher frequency with decreasing ring size. The unstrained six membered cyclic ester, δ - valerolactone, absorbs at the same value as a non-cyclic ester. Because of ring strain, β - propiolactone and γ - butyrolactone and show C=O stretching at 1864 cm-1 and 1801 cm-1, respectively. With increase in ring size normal mode of vibration increases. With increase in ring strain (ring size decreases) CO stretching frequency increases.

Order of CO stretching frequency;
δ—Valerolactone < γ—¿butyrolactone < β-propiolactone < α-acetolactone

Name of the compound	CO stretching frequency from calculation cm^{-1}	Observed CO stretching frequency cm^{-1}
α-Acetolactone	1888.09869	1860
β-Propiolactone	1864.47747	1840
γ- Butyrolactone	1801.13445	1775
δ-Valerolactone	1762.00678	1740

Table 6.2: CO Stretching Frequencies of Cyclic Ketons

3. Cyclic Amides

Like lactones, cyclic amides (lactams) also follows the trend of decreasing co stretching frequency with increase in ring size. With increase in ring strain (ring size decreases) CO stretching frequency increases in lactams, whereas normal mode of vibrations increase with ring size.

Name of the compound	CO stretching frequency from calculation cm^{-1}	Observed CO stretching frequency cm^{-1}
β-Lactam	1926.11769	1745
γ-Lactam	1846.8343	1700
δ-Lactam	1783.90977	1668
ε-Lactam	1794.99427	1671

Table 6.3: CO Stretching Frequencies of Cyclic Amides

β-Lactam > γ-Lactam > δ-Lactam > ε-Lactam

Conclusion

We conclude that the carbonyl frequency is determined by the hybridization or bond angle at the carbonyl group. As ring strain increases, CO stretching frequency increases. The practical implementation of the project is performed using computational electronic structure calculations using the DFT method in conjugation with 6-31G (d, p) basis set. All computational chemistry calculations were performed using Avogadro and Facio, free software. We obtained accurate results for most calculations in comparison with experimental data. From the results the general conclusion is, sometimes variations are found in CO stretching frequency calculations. The haziness of ring strain energy values in computational method may be mainly due to the dependence of stereochemistry rather than the electronic structure. The small imprecision in CO stretching frequency is due to the complexity in electronic structure of compounds due to the delocalization of electrons. The use of recently developed basis sets may account this complexity in the electronic structure of the molecules and further improve the accuracy of computational calculations. Moreover, computational chemistry methods can be utilized by researchers in the calculation of chemical properties such as resonance energy, ring strain energy, ionization energy and electron affinity before doing or using sophisticated experimental methods.

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Chapter 7

Study of Adsorption Capacity of Some Selected Natural Adsorbents

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ABSTRACT

Water resources are sources of potential use to living and non-living things as in the way of agricultural, industrial and daily life uses. But because of rapid growth in industrialization and improper waste management water resources are getting polluted especially in developing countries. A vast research is going on in this field to find out the effective ways of purifying the waste water. The heavy metal ion contaminated wastewater will cause threat to ecosystem as they are non-biodegradable and carcinogenic. Different techniques are employed in recent years for water purification out of which the most simplest and cheaper is adsorption with natural adsorbents. In this study we have used some plant based biomaterials as natural adsorbents and evaluated the effect of surface area on the rate of adsorption using colorimetric analysis. The study showed that rate of adsorption increases with increase in surface area of adsorbent.

Introduction

Water is a significant natural resource for humans as well as all living creatures in this world. However environmental pollution is one of the severe problems across the globe. Contamination of soil and aquatic environment by heavy metals is one of the major threats to the water resources of the world today [1] This is mainly due to the non-degradability nature of metal ion pollutants which is hazardous when discharged into the water body. They will remain persistent in the environment and enter the food chain of living organisms. Moreover heavy metals also pose a great threat to human health which can lead to severe health abnormalities such as high blood pressure, kidney damage, sterility in male, cancer etc. [2]. Wastewater effluents generated in industries are major contributors to a variety of water pollution problems.

The heavy metals are present in the environment

in air, water, and soil. For example, factory chimneys release metal oxides into the air, thus transmitting heavy metal pollution to humans, animals and plants. In addition, car exhausts release lead oxides, resulting from the combustion of tetraethyl lead, into the atmosphere and this is one of the most widespread routes to leading contamination of marine organisms with metals, and the transit of these contaminants via sea fishing to humans and animals. Furthermore, agricultural soil is one of the most important sources of food polluted by heavy metals, which arises through the irrigation of crops with polluted water or the use of pesticides. In this situation, the metals are transmitted through the vascular system of plants and fruits. Therefore, field crops irrigated with drainage water contaminated by heavy metal ions are one of the most important and most dangerous sources for the entry of poisonous heavy metals into the human body[3].

Improper waste management in the industries will result in disposal of harmful chemicals to waterbodies. Thus main focus of environmental research is to get rid of these pollutants and save the waterbodies and environment [4]. Several technologies have been proposed to treat wastewater contaminated with metal species Adsorption is considered to be one of the most promising and easily applied technique for wastewater treatment over the last decades in removing hazardous metal ion pollutants from water bodies. The use of low cost materials for adsorption commonly termed as green adsorption is the focus of interests for many researches associated with removal of metal ion contaminants from aquatic reservoirs.

Iron is a lustrous silvery-gray metal in the first transition series from group VIII of the periodic table with a melting point at 1538°C, boiling point at 2862°C and density of 7.87 g/cm at 200C. It is the fourth most common element in the Earth's crust. Iron is the most widely used metal for metal-processing industries such as the construction of machinery and machine tools, automobiles, the hulls of large ships, and structural components for buildings. Iron is a necessary trace element for all living organisms. But, excessive iron can be damaging to the gastrointestinal system. Over time, iron can accumulate in the organs, and cause fatal damage to the liver or brain. In healthy people, taking high doses of iron supplements (especially on an empty stomach) can cause an upset stomach, constipation, nausea, abdominal pain, vomiting, and fainting.

In this work Natural products usually considered such as corn silk, water lettuce, charcoal, banana peel, plantain stem, corn husk, Drumstick peel etc are employed as adsorbents to extract metal ions from water. Techniques such as mass spectroscopy, emission spectroscopy etc are complicated and time consuming. Colorimetric methods have emerged as alternative spectroscopic methods because of the convenience in operation, low cost and quick responses.

In this work we have compared the metal ion adsorption capacity of some selected natural adsorbents. The approximate ferrous ion content in the selected natural adsorbents were evaluated by colorimetric analysis.

Materials and Methods

Ferric Ammonium Sulphate Solution $(FeNH_4(SO_4)_2{\bullet}12H_2O)$, Potassium Dichromate $(K_2Cr_2O_7)$, $6N$ Sulphuric Acid, 20% Ammonium Thiocyanate, $5N$ Hydrochloric Acid, Diphenyl Carbazide, 250 ml Beaker, 250ml conical flask, Funnel, Filter paper, Burette, Distilled water, Colorimeter, Cuvette.

Preparation of Iron Solution

About 8.64g of Ammonium iron Sulphate is weighed out into a 1000ml Standard Flask. Then, 100ml concentrated HCl is added and made up with distilled water. This solution is labelled as solution *A*. Take 100 ml of this solution (solution *A*) and it is transferred into a 1000ml standard flask and it is made up to 1000ml using distilled water carefully. This solution is labelled as solution *B*.

Preparation of Sample for Estimation of Iron

About 1g of each sample (crushed) is taken in 2 beakers. Mark the beakers as *day—1* and *day—2*

respectively. Add 50ml of prepared Iron solution (solution *B*) into the sample and it is covered with watch glass. The sample which is labelled as *day—1* is kept for 24 hours and the sample which is labelled as *day—2* is kept for 48 hours. Then, filter the sample using funnel and filter paper. And 8ml of the filtrate is transferred into a 100ml standard flask and the reagents such as 5ml $5NHCl$ and 5ml 20% ammonium thiocyanate are added. And it is made up to 100ml using distilled water. Then, colorimetric estimation is done using a colorimeter. Similarly, experiment is repeated in Day-2 and the procedure is repeated for non crushed samples.

Figure 7.1: Adsorption Day 1

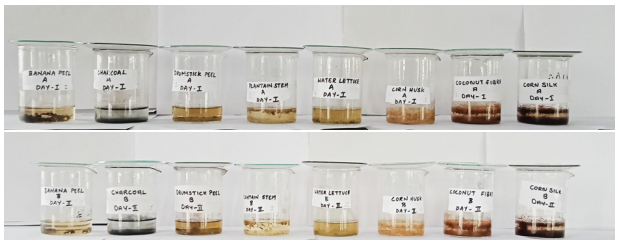

Figure 7.2: Adsorption Day 2

Result and Discussion

In order to evaluate the adsorption of iron the samples taken are crushed and non crushed samples of charcoal, banana peel, coconut fibre, drumstick peel, corn husk, water lettuce and plantain stem respectively. The adsorption was analysed based on colorimetric analysis.

Colorimetry is based on the principle of the Beer-Lambert law which states that the quantity of light absorbed by a substance is directly proportional to the concentration of the substance and the path length of the light through the solution.

$$A = \log(\frac{Io}{It}) = \varepsilon cl$$

where,

A is the absorbance or optical density

ε is the molar absorptivity or molar extinction coefficient (which depends on the nature of the chemical and the wavelength of the light used)

l is the length of the path light must travel in the solution in centimetres

c is the concentration of a given solution

The crushed and non-crushed samples showed difference in rate of adsorption. The effect of increased surface area might have caused increased adsorption rate in crushed samples when compared to non-crushed ones. The standard calibration curve was first generated using standard iron solution. After that a fixed concentration of iron solution was selected for adsorption studies. The adsorption capacity of each natural adsorbents were compared after a fixed time duration of contact between metal ion solution and adsorbents. Each adsorbents differ in their texture, cellulose content and other phytocomponents. The variety of functional groups including carboxyl, amino, hydroxyl, phosphate, thiol, etc. on the surface of adsorbent materials affect the rate of the adsorption. This interaction between pollutants and the surface of the adsorbents can occur through electrostatic interaction, complexation, ion exchange, oxidation,reduction etc. The solution pH will get changed depending on the surface charge density located on the adsorbent surface. Hence more analysis need to be done on the basis of effect of pH, time, temperature, and concentration of adsorbent on the adsorption rate of selected adsorbents. But as a preliminary study we adopted a fixed concentration of adsorbent for our analysis. The experimental observation were in accordance with the results published by other researchers [5-8].

Concentration of Iron	Absorbance (Au)
2%	0.19
4%	0.39
6%	0.60
8%	0.79
10%	0.99

Table 7.1: Calibration Curve data for Estimation of Iron.

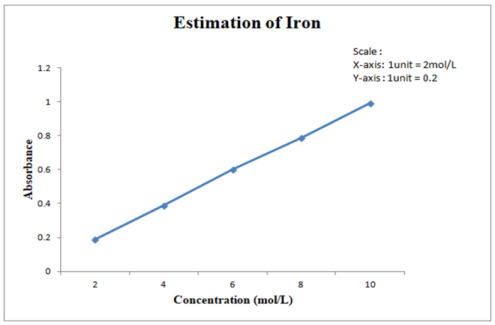

Figure 7.3: Plot between Concentration of Iron and Absorbance.

Figure 7.4: Standard iron solution prepared for calibration curve.

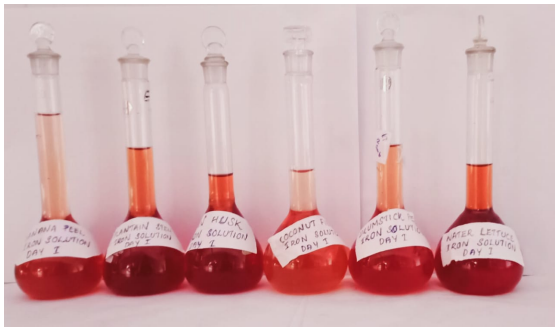

Figure 7.5: Colorimetric analysis of iron solution on day I after adsorption using natural adsorbents.

Figure 7.6: Colorimetric analysis of iron solution on day 2 after adsorption using natural adsorbents.

Samples	Day 1 Absorbance (Au)	Day 2 Absorbance (Au)
Coconut fibre	0.18	0.11
Charcoal	0.23	0.46
Banana Peel	0.35	0.13
Water Lettuce	0.77	0.65
Corn Husk	0.69	0.64
Drumstick Peel	0.44	0.38
Plantain Stem	0.64	0.51

Table 7.2: i. Estimation of Iron — Day 1 (non-crushed sample), ii. Day 2 (non-crushed sample)

On day 1 the filtrate from the sample mixture was collected separately and definite amount corresponding to that to initial concentration were prepared. It was then complexed with Ammonium thiocyanate and amount of iron present in the filterate after adsorption was measured by evaluating the absorbance value obtained from colorimetric analysis. This provided the adsorption data after 24hrs of contact of adsorbent with metal ion solution. The experimental observation was taken for the sample after 48 hrs in day 2.The rate of adsorption was evaluated based on non-crushed and crushed samples. The absorbance values showed a proportional increase which might be due to desorption of metal ion from adsorbent surface that occurred after 48 hours of contact. This can be attributed to leaching because of the multilayer adsorption that occurred due to weak electrostatic interaction between adsorbent and the adsorbate.

Samples	Day 1 Absorbance (Au)(i)	Day 2 Absorbance (Au)(ii)
Coconut fibre	0.09	0.09
Corn Silk	0.10	0.12
Banana Peel	0.25	0.36
Charcoal	0.23	0.46
Water Lettuce	0.35	0.55
Corn Husk	0.35	0.62
Drumstick Peel	0.25	0.31
Plantain Stem	0.21	0.22

Table 7.3: i. Estimation of Iron — Day 1 (crushed sample), ii. Day 2 (crushed sample)

Conclusion

In this study we conducted colorimetric estimation of Iron in the prepared samples before and after adsorption by natural adsorbents. The results were evaluated based on colorimetric analysis. In order to determine the adsorption capacity of various samples, we conducted the experiment with both crushed and non crushed samples. We took samples such as banana peel, plantain stem, charcoal, coconut fibre, drumstick peel, water lettuce, corn husk etc. In the case of adsorption of Iron on after 24 hrs on Day 1 and after 48 hrs on Day 2 on crushed and non crushed samples it was observed that charcoal, coconut fibre, plantain stem are good adsorbents of iron from the given solution. And these materials can be promising materials instead of chemical adsorbents for the purification of water.

From these experimental observations we can also conclude that adsorption increases with increase in surface area, area of contact and it also depends upon several factors such as pH, temperature, etc. As we conduct the colorimetric estimation of Iron with both crushed and non crushed samples, adsorption capacity is more for crushes samples than the non crushed samples.

So from all our experimental observations it was concluded that all the samples used in this experiment differ in their adsorption capacity. The adsorption rate increases with increase in surface area of adsorbents. Among the different adsorbents selected charcoal and coconut fibre exhibited under specified experimental conditions better adsorption capacity and can be considered as good adsorbents. And surely both these adsorbents can be used in the purification of water. But further studies based on effect of pH, time, temperature and concentration of adsorption need to be carried out to effectively select the adsorbents for waste water purification.

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List of Figures

1.1 Bar diagram showing number of Gastropods of different orders collected from Kuzhuppilly Beach, Kerala 10

2.1 XRD pattern obtained from sample 13
2.2 Inhibition zone created by Gram positive bacteria and Gram negative bacteria 14

4.1 The table showing the result of morphological and physiological changes happened during the period of experimental study in given doses of organic fertilizer and chemical fertilizers. 20
4.2 Graphical representation of variation in the number of earthworms under the controlled and experimental set up during 60 days. 21
4.3 Graphical representation of variation in the biomass of earthworms under the controlled and experimental set up during 60 days. 21
4.4 Graphical representation of variation in the number of cocoons under the controlled and experimental set up during 60 days. 22
4.5 Graphical representation of variation in the number of juveniles under the controlled and experimental set up during 60 days. 22
4.6 Graphical representation of variation in the number of non-clitellates under the controlled and experimental set up during 60 days. 23
4.7 Graphical representation of variation in the number of clitellates under the controlled and experimental set up during 60 days. 23

5.1 Meganuclease - recognize a DNA sequence element, cut both strands at specific sites, forming sticky double-stranded breaks (DSBs) 28
5.3 Structure of TALEN. Each repeat variable residue recognize single specific nucleotide 29
5.4 CRISPR/Cas9 mediated genome editing 31

6.1 Acetone 38
6.2 Cyclopropanone 39

7.1 Adsorption Day 1 44
7.2 Adsorption Day 2 44
7.3 Plot between Concentration of Iron and Absorbance. 45
7.4 Standard iron solution prepared for calibration curve. 45
7.5 Colorimetric analysis of iron solution on day I after adsorption using natural adsorbents. 45
7.6 Colorimetric analysis of iron solution on day 2 after adsorption using natural adsorbents. . . . 45

List of Tables

1.1 Check List of Gastropod Molluscs Collected and Identified From Kuzhuppilly Beach, Kerala . 9
1.2 Table showing number of Gastropod Molluscs of different orders collected from Kuzhuppilly Beach, Kerala 9

6.1 CO Stretching Frequencies of Cyclic Ketons 39
6.2 CO Stretching Frequencies of Cyclic Ketons 40
6.3 CO Stretching Frequencies of Cyclic Amides 40

7.1 Calibration Curve data for Estimation of Iron. 45
7.2 i. Estimation of Iron — Day 1 (non-crushed sample), ii. Day 2 (non-crushed sample) 45
7.3 i. Estimation of Iron — Day 1 (crushed sample), ii. Day 2 (crushed sample) 46

RAYS

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ISBN : 978-93-5408-948-0

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