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VIVEKANANDA GLOBAL UNIVERSITY, JAIPUR

Arise Awake Achieve

Education is the manifestation of the perfection already in man". These are the words of the great philosopher and educator Swami Vivekananda. The contributions of the great people who devoted their life for the cause of education and youth have always inspired the promoters and, therefore, following the preaching of Swami Vivekananda, the promoters established VIT Campus, comprising of Vivekananda Institute of Technology and Vivekananda Institute of Technology (East), in 2008, to usher in technology revolution by using modern management techniques and harnessing potential of India. Another feather in the crown of Vivekananda Group of Institutions is Vivekananda Global University, established in the year 2012. Vivekananda Global University, Jaipur has been formed keeping in mind his teaching and mentoring ideals. The overall development of the techno-managers with a seeking spirit towards education is VGU's vision for its students. It Promises to develop as an institution with a commitment to excellence in education, research and consultancy and promote human advancement. Swami Vivekananda advocated the concept of 'total development' which includes physical, mental and spiritual. He also advocated incorporation of science and technology in curricula and laid emphasis on technical education that will develop industries. Our core values are inspired by Swami Vivekananda philosophy, and our institution is founded on his thoughts and ideas. To meet these ends, Vivekananda Global University encourage development of student's physical, mental, emotional, secular and spiritual faculties.



*"We are what our thoughts
have made us; so take care
of what you think.*

Words are secondary.

Thoughts live; they travel far."

Swami Vivekananda

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A review on recent advancements of electrophoresis based on Microchip Electrophoresis

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Abstract

Microchip Electrophoresis (ME) has emerged as a valuable tool in forensic science, offering numerous advantages for the analysis of forensic samples. This review paper provides a comprehensive overview of recent advancements in ME from a forensic perspective. ME has gained prominence in forensic laboratories due to its ability to perform rapid, high-resolution separations with minimal sample and reagent requirements. It has been successfully applied in the analysis of DNA, proteins, drugs of abuse, explosives, and other forensic analytes. The miniaturized nature of ME chips allows for efficient analysis of limited sample volumes, which is often crucial in forensic casework. Moreover, the integration of on-chip sample preparation and detection strategies has streamlined the analytical workflow, making ME a versatile tool for complex sample analysis. Advancements in ME technology have focused on improving sensitivity, selectivity, and throughput in forensic analyses. Integration of on-chip sample preparation techniques, such as DNA extraction and purification, has streamlined the workflow and reduced the risk of sample contamination. Coupling ME with sensitive detection methods, like laser-induced fluorescence or mass spectrometry, has enhanced the analytical capabilities for forensic applications. This review discusses recent developments in ME for various forensic analytes, including DNA profiling, protein analysis, drug screening, and explosive detection. It highlights the advantages of ME in terms of speed, resolution, and automation, which are particularly relevant in forensic casework where appropriate and accurate results are crucial.

Keywords : Microchip Electrophoresis, Mass Spectroscopy, DNA Profiling

Introduction

Trace evidence can be found at a crime scene whenever a crime is committed. Investigating authorities frequently ask a forensic analyst to examine the trace evidence. Many different tests for finding these residues were found in previous decades. Due to the complexity of the majority of forensic materials, molecule separation was necessary before analysis. It is essential to use methods that will reduce a complex combination to a single component. One such method is the electrophoresis method, which is used to separate molecules. In especially when the

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sample size is very tiny, it is a crucial tool for the separation of forensic materials. An electro-kinetic process based on the differential movement of electrically charged particles in the electric field is electrophoresis, also referred to as cataphoresis. It is a laboratory technique which is used to separate and analyse molecules according to their charge and size. Under the influence of an electric field, charged particles must flow through a medium, usually a gel or a capillary [1]. Numerous scientific disciplines, including forensic inquiry, frequently employ this method. DNA analysis using electrophoresis is extremely important in forensic investigations. It makes it possible to separate and characterise DNA fragments, which is essential for person identification and for connecting suspects, victims, and crime sites. Microchip electrophoresis (MCE) is a miniaturized form of capillary electrophoresis. Electrophoresis is a common technique to separate macromolecules such as nucleic acids (DNA, RNA) and proteins

Forensic applications of Microchip Electrophoresis: -

Forensic applications of microchip electrophoresis encompass areas such as DNA analysis, drug analysis, and toxicology. Here are some specific details about these applications:

DNA Analysis

- a. STR Typing: ME has been used for short tandem repeat (STR) typing, which is a crucial technique for DNA profiling and individual identification. ME allows for high-resolution separation of STR fragments, providing accurate and precise results.
- b. DNA Fragment Analysis: ME can be utilized for the separation and sizing of DNA fragments, particularly in the analysis of degraded or low-quality DNA samples commonly encountered in forensic casework [2].
- c. mtDNA Analysis: Mitochondrial DNA (mtDNA) analysis is another area where ME can be applied. It allows for the rapid separation and analysis of mtDNA fragments, aiding in identification or exclusion of suspects in forensic investigations [3]

Drug Analysis

- a. Illicit Drug Profiling: Microchip electrophoresis can be used for the analysis and profiling of illicit drugs, including amphetamines, opioids, cocaine, and cannabinoids [4]. It enables rapid and sensitive detection of drugs and their metabolites in various forensic samples, such as urine, blood, or seized materials.
- b. Forensic Toxicology: ME is valuable in forensic toxicology for the screening and quantification of drugs and poisons in biological fluids. It offers high separation efficiency, sensitivity, and speed, making it a useful tool in toxicological analysis.

Explosives Analysis

- a. Forensic Explosives Identification: Microchip electrophoresis has been applied to the identification and characterization of explosive materials encountered in forensic investigations. It enables the separation and analysis of explosive residues, aiding in identifying the type of explosive used.

A review on recent advancements of electrophoresis based on Microchip Electrophoresis

Microchip electrophoresis

It is also known as microuidic electrophoresis or chip-based electrophoresis, is a miniaturized version of traditional gel or capillary electrophoresis that takes place on a microchip. For the separation, identification, and quantitation of charged species, including DNA fragments, proteins, peptides, and tiny molecules, it is a potent analytical approach^[5]. A small glass, silicon, or polymer substrate with integrated microchannels, reservoirs, and electrodes serves as the basis for the microchip employed in this technology

Principle of Microchip Electrophoresis

The separation principle in microchip electrophoresis is based on the differential migration of charged analytes in an electric field. When an electric potential is applied across the microchannels, analytes migrate towards the respective electrodes based on their charge-to mass ratio. The speed of migration is influenced by the electrophoretic mobility of the analyte, which depends on its charge, size, shape, and the properties of the surrounding medium

Advancement of microchip electrophoresis over other electrophoresis technique: -

1. MCE has a number of benefits over traditional capillary electrophoresis methods, including the integration of many separation functions onto the chip, the use of little sample and reagent, quicker analysis, and more effective separations^[6].
2. MCE has the potential to be adapted for portable POC and clinical diagnostics devices because a few extra tasks like sample preparation, washing, incubation with antibodies, and derivatization with dyes can all be incorporated on a single stamp-sized microchip.
3. While capillary electrophoresis can fully resolve the components of the sample mixture in minutes to hours, microchip electrophoresis can produce separations in just a few seconds or minutes^[7].
4. Additionally, MCE provides the integration of several detection techniques, including interfaces with mass spectrometry, electrochemical detections, and laser-induced fluorescence detections. As a result, MCE has been used in a variety of applications, e.g., to analyse biomolecules in blood, saliva, tear, dialysate, and islets.

Result and Discussion

1. A microchip CE based method with electrochemical detection has been developed for the analysis of total and protein-bound homocysteine, which should be routinely tested in patients at risk for cardiovascular disease, according to the American Heart Association [8].

Material used: Elution buffer, 20 mmol/L TES (pH 8.0), 400 mol/L tris(2-carboxyethyl)phosphine (pH 7.5); applied potential, 1800 V (360 V/cm); injection, 4 s at 1200 V; $E =$

+100 mV with Au/Hg amalgamated electrode. Reprinted with permission from Pasas et al.

Typical results obtained for the detection and separation of homocysteine and reduced glutathione by microchip CE are.

Detection and separation of 200 mol/L homocysteine (Hcy) and reduced glutathione

1. Microchip devices integrating PCR with CE have been used for the analysis of bacteria such as *Escherichia coli*, *Staphylococcus*, *Salmonella*, and *Streptococcus*. Legally et al.^[9] developed an integrated portable genetic analysis system for detection of pathogens. The results obtained for the analysis of DNA from intact cells of different strains of *E. coli* are shown.

Pathogenic organism analysis conducted directly from intact *E. coli* cells on the portable PCR-CE microsystem^[10].

(A), analysis of *E. coli* K12 cells, showing only the presence of the coinjected DNA ladder and the 280-bp 16S species-specific amplicon. (B), analysis of *E. coli* O55:H7 cells, showing the ladder, the 280-bp 16S species-specific amplicon, and the 625-bp *iC* amplicon characteristic of cells presenting the H7 surface antigen. (C), analysis of *E. coli* O157:H7 cells, showing the DNA ladder, the 16S species-specific amplicon, the 625-bp *iC* amplicon, and the 348-bp *sltI* amplicon, characteristic of *E. coli* both possessing an H7 antigen and expressing shigatoxin. Each analysis was conducted with a starting concentration of 40 cells in the reactor in a time of 30 min. Reprinted with permission from Legally et al.

Separation of 20 amino acids was attempted using one dimensional MEKC, microchip micellar electrokinetic chromatography, by Culbertson et al. on a glass microchip patterned with a 25-cm-long spiral-shaped separation channel^[11]. As a result, 19 amino acids were separated in 165 s with an average plate number of 280,000. However, the 20th amino acid, histidine, could not be resolved

Among various available pseudostationary phases, sodium dodecyl sulfate (SDS) is the commonest surfactant for MEKC.

Microchip MEKC (MEKC) separation of 20 amino acids in a spiral -shaped separation channel. A Image of a microchip with a spiral separation channel ^[12]. The separation channel was 24.9 cm long and 40 m wide (half-depth). The arrows in the inset indicate the vertexes of the polygonally shaped channels. B MEKC separation of 19 tetramethylrhodamine-labeled amino acids in a 10 mM sodium tetraborate/ 50 mM sodium dodecyl sulfate (SDS) buffer with 10 % (v/v) 2- propanol. The eld strength was 770 V/cm, and the detection point was 11.87 cm from the injection cross. The peak locations of the amino acids are indicated by their standard one-letter abbreviations.

A review on recent advancements of electrophoresis based on Microchip Electrophoresis

Advantages of microchip electrophoresis:

1. **Miniaturization:** Microchip electrophoresis allows analysis on a small scale, requiring minimal sample and reagent volumes. This leads to faster analysis times, reduced cost, and increased portability.
2. **High Separation Efficiency:** The narrow channels in microfluidic chips provide high resolution and separation efficiency, enabling the analysis of complex samples.
3. **Integration of Functions:** Multiple analytical functions, such as sample injection, separation, and detection, can be integrated into a single microchip device, simplifying the analysis process.
4. **Automation:** Microchip electrophoresis can be easily automated, allowing for high throughput analysis and minimizing human error.
5. **Biochemical and biomedical research:** DNA sequencing, protein analysis, genotyping, drug discovery, clinical diagnostics.
6. **Environmental monitoring:** Analysis of pollutants, water quality assessment.
7. **Forensic analysis:** DNA profiling, forensic toxicology.
8. **Food and beverage industry:** Quality control, food safety testing.

Disadvantages of microchip electrophoresis:

1. **Sample loading limitations:** The quantity of sample that can be loaded onto the chip is constrained by the small size of the microfluidic channels. When working with low-abundance samples or when vast amounts of material are needed for analysis, this might be difficult.
2. **Detection sensitivity:** Microchip electrophoresis often relies on fluorescence or absorbance detection methods to analyse the separated molecules. While these detection techniques are highly sensitive, they may not be suitable for all types of analytes. Some molecules may not exhibit strong fluorescence or absorbance signals, limiting the sensitivity of detection.

3. **Chip fabrication complexity:** - Precision lithographic methods and material deposition are used during the manufacturing process, which calls for specialised tools and knowledge. Because of their intricacy, microchips may be more expensive to produce and less accessible to some laboratories.
4. **Limited versatility:** - Microchip electrophoresis is primarily focused on separation and analysis, and it may have limitations in performing other functions. For example, it may not be easily integrated with other techniques or workflows, such as sample preparation, enrichment, or online detection. This lack of versatility can be a disadvantage when a comprehensive analysis approach is required.
5. **Cost considerations:** - Microchip electrophoresis can be expensive, primarily due to the fabrication process and the requirement for specialized equipment and materials.

Conclusion

Recent advancements in microchip electrophoresis (ME) have propelled the field of electrophoresis to new heights, revolutionizing various applications and opening up new possibilities. High-throughput analysis, enabled by microchip arrays, has expedited sample processing and allowed for simultaneous analysis of multiple samples^[13]. Detection techniques have been refined, leading to improved sensitivity and expanded detection ranges, while integration with other detection modalities has enabled multi-modal analysis. The integration of on-chip sample preparation techniques has streamlined workflows, reducing sample loss and contamination risks. Moreover, the development of portable ME systems has extended the reach of this technology to on-site and point-of-care applications.

These advancements have transformed ME into a powerful analytical tool, finding applications in diverse fields such as forensic science, clinical diagnostics, environmental monitoring, and more^[14]. ME offers rapid, sensitive, and precise analysis, making it an invaluable asset in solving complex problems. The ongoing research and development in this field hold promise for further improving the performance, versatility, and accessibility of ME. With continued progress, microchip electrophoresis is poised to continue driving innovation and making significant contributions to the advancement of analytical sciences.

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Assessment of Heavy Metals in Sediments of Dravyavati River, Sanganer Industrial Area, Jaipur

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Abstract

In terms of water contamination, heavy metal toxicity is a serious issue. Heavy metals, some of which are harmful, are released into the environment through a variety of routes. Sediment samples were taken at two locations along the Dravyavati river. To determine the contamination level and the metals' connection with sediment grain size, heavy metals were tested using atomic absorption spectrophotometry (AAS). In surface water and sediment of a Dravyavati river, the content and chemical fractionation of four globally worrying heavy metals (Cd, Cr, Pb, and Ar) were measured. However, with the exception of Cd and Pb, which might represent serious harm to the aquatic environment, the research metals exhibited minimal mobility.

Introduction:

Dravyavati river runs through the heart of Jaipur, flowing north through the Amer Mountains, through the city's core, and south. Its entire length from north to south is 47.5 km. almost the entire population of Jaipur resides within a 10-kilometer radius of this river. Due to increased urbanization and industrialization over the previous three to four decades, the amount of waste material in the water has begun to rise. Heavy metals are primarily transported in considerable quantities through wastewater cities in this case (Yedjou&Tchounwou, 2007). Any metallic element with a certain quality might be classified as heavy metal. They are more hazardous at larger atomic weights and lower concentrations if the density of the heavy metal is more than 5gm/cm^3 (Zubair *et al.* 2017). Sewerage water, waste from industrial activities, domestic waste, garbage from agricultural areas, and waste from hospitals are among the waste products released as runoff (Hong *et al.* 2014). Heavy metal pollution poses a threat to any ecosystem, including aquatic ecosystems (Islam *et al.* 2015). Heavy metals endanger aquatic life, human health, and biomagnification, among other things (Tchounwou *et al.* 2012). Sediments deposited at the bottom of the greatest pool of heavy metals in any aquatic habitat contain many times the

quantity of heavy metals found in waste water (Rousselot *et al*, 1999). When heavy metal-contaminated waste water is utilized for crop irrigation, heavy metals eventually accumulate in the sediment. This water boosts crop yields, but the heavy metals in the products made with it cause poisoning, which is hazardous to human health, domestic animals, wildlife, and the environment (Ji *et al*, 2018, Honglei *et al*, 2008). Aside from the negative impacts on the ecosystem and living organisms, heavy metals deposited in the sediment end up in grains, vegetables, and other foods, which is particularly dangerous (Zhenli *et al*, 2005). Some heavy metals, such as Zn and Mg, are required for us in very small levels, whereas others, such as Cd, Cr, Pd, and As, cause major disorders in very small amounts. These metals disrupt the brain and nervous system, alter blood chemistry, and impair the lungs, kidneys, liver, and other essential organs, all of which are exceedingly hazardous (Becqueret *et al*, 2003). The need for regular monitoring of these industrial and municipal wastes cannot be overstated. To ensure certain parameters, the heavy metal pollution index (HPI) and metal quality index (MQI) measurements can be used to analyze the excess of these heavy metals (Nagajyoti *et al*, 2010).

The toxicity of heavy metals is determined not only by their overall concentration, but also by their speciation distribution. The toxicity of heavy metals, their migration, and natural cycle are all directly affected by their various forms (Usman *et al*, 2021). There is no universal definition or categorization for heavy metals. Heavy metals in soils and sediments are thought to exist in seven different form states. The BCR extraction method is what it's termed. To safeguard the corresponding aquatic environment, it is required to analyze the geographical distribution of heavy metals in Raohe sediments and determine the risk posed by these heavy metals (Tuikka *et al*, 2011). Various anthropogenic toxins, including those emanating from industrial and agricultural operations, home wastes, and traffic emissions, frequently contaminate estuaries and coastal regions (Rinklebe & Shaheen, 2014). Heavy metals study of estuarine and coastal environments is becoming more common due to rising toxicity and the permanence of heavy metal contamination (Dash *et al*, 2021, Laing *et al*, 2009). Because metal pollution may negatively influence the density and variety of biotic groups, including people, human impacts on the environment can be quantified by detecting heavy metal concentrations in soils, plants, animals, and sediments (Gailer *et al* 2000, Pajanay *et al*, 2011). Sediments are also metal sources for aquatic species therefore, they play an important role in determining pollution levels in the ocean. They also give the foundational data needed to estimate environmental health concerns (Naseri *et al*, 2015). As a result, all sorts of coastal sediments are being investigated extensively, from those found in heavily inhabited metropolitan areas to those found in highly industrialized places (Ferri *et al*, 2017).

Environmental pollution as a result of increased urbanization and industrialization has lately become a global concern. The concentration of trace elements in coastal sediment can help with baseline studies and future sediment quality assessments (Farkas *et al*, 2007). Heavy metals, which can be pollutants with hazardous effects on ground and surface water resources, plants, animals, and humans, can be detected by multi-element analysis of sediment (Mohan & Pittman 2007). The

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elemental composition of soil samples was determined using energy dispersive X-ray fluorescence (EDXRF), a nuclear analytical method. In environmental research, the EDXRF method is a flexible instrument (Kanat *et al*, 2018). The study's specific goals were to: I determine the levels of heavy metals (Ti, Fe, V, Cr, Mn, Co, Ni, and Zn) in the sediments and identify possible sources of these metals through statistical analysis; (ii) quantify the extent of metal pollution using enrichment factor (EF), geo accumulation index (Igeo), contamination factor (CF), and pollution load index (PLI) and identify possible sources of these heavy metals through multivariate (Ransom 2010).

Materials and methods

Description of the study area

The sediments have been studied in the Dravyavati river basin. This river is approximately 47.5 kilometers long, and it receives a large amount of trash from industrial districts, sewage systems, and hospitals. Heavy metals such as Cd, Pb, Cr, Cu, and others have been found in this waste water. These heavy metals are discovered progressively hardening on the ground's top surface (Flora *et al*, 2010). Heavy metals begin to enter the body when waste water containing these heavy metals is utilized for agricultural purposes. There, it begins to produce extremely lethal illnesses. In this study region, samples were gathered from three different locations: sanganer printing area, Bambala pulia, and Sitapura industrial area. The first sample was obtained in February 2022, followed by the second sample in March 2022. Estimated heavy metal concentrations in samples taken over these two months show seasonal fluctuation. Collect the sample by hand scrapers from a 0.1 meter area with a 0.3 centimeter depth range and put it in a polythene bag.



Figure1: Location of study area

Sample collection and analysis

The heavy metals Cd, Cu, Pb, Cr, and Zn were taken from the Dravyavati River as a reference standard. The obtained samples are dried for 6 hours at 80°C in an oven. The coarse material is then ground into a powder. After collecting 2 g of sample, add 30 mL of H⁺ SO₄ and 20 mL of HNO₃ to the sample. Place it on a hot plate and heat it to 80 °C. Now it emits white smoke initially, followed by orange smoke.

This diagram depicts the digestive process (Faroonet *al*, 2013). The sample is allowed to cool. 100 mL distilled water is then added to it. The sample is filtered and collected with the help of filter paper. Finally, an Atomic Absorption Spectrophotometer (AAS) was used to determine the concentrations of heavy metals Cd, Cu, Pb, Cr, and Zn. An atomic absorption spectrophotometer (AAS) (PE-AA400, Perkin Elmer, USA) was used to determine the samples. At regular intervals, a standard sample was utilized to check the AAS sensitivity. To evaluate and depict the collected data, common statistical procedures such as fully randomised design (CRD) were utilized (Manwaniet *al*, 2022).

Result & Discussion

The density of heavy metals was found to be greater than 5 g cm⁻³, Sample analysis revealed that there was no significant difference in the concentration of heavy metals in the sample collected in two season.

The results of the analysis of sediment samples in month of Feb and May 2022 of the Dravyavati river revealed that the mean concentration of Cd, Cr, Ar& Pb in the sediments was higher in samples collected in May 2022 but below the detection limit. The sample concentration was determined to be between 0.6 and 0.7 BDL, which is not extremely harmful. In recent years, the use of lead in industrial items such as paints, ceramics, caulking, and pipe solder have been drastically decreased (Rajput *et al*, 2017). Heavy metals have been shown to be hazardous to both humans and the environment. Sewage sludge included significant levels of nutrients, organic matter, and a pH that was almost neutral, making it suitable for agricultural application. With a rise in temperature and a drop in humidity, dissolved heavy metals rise.

The WHO limit and the values recorded during these months varied significantly. We discover considerable changes in Pb concentrations in different months when we compare the values between these months. In the sediments, we discovered that heavy metal concentrations are steadily increasing. A similar pattern with the humidity level of the environment was observed: when the humidity level declines with rising temperatures, the amount of heavy metal concentration rises. This dynamic character of heavy metal concentration levels in the Ayad River in relation to climate change should be held accountable and taken into consideration when stratifying protective efforts.



Figure 2: Processing of sediments for heavy metal detection

Sharma, PK
Kumar, A
Sharma, S
Giri, S
Saini, J
Gupta, S
Awasthi, KK



Figure 3: Digestion of samples.

Table 1: Physico-Chemical study in the sediments of the Dravyavati river

Month	Site	Sample	Latitude	Longitude	pH	TD S
Feb 2022	Site 1	1	26.79055867940187 ⁰	75.80989398993552 ⁰	7.8	554
		2	26.800963059067726 ⁰	75.808557803113759 ⁰	8.1	578
		3	26.780550023540854 ⁰	75.86688095703721 ⁰	7.8	567
	Site 2	1	26.795266256667674 ⁰	75.80826228484511 ⁰	8.1	612
		2	26.800963268615305 ⁰	75.79406225122511 ⁰	8.0	623
		3	26.795267974957824 ⁰	75.80824979580939 ⁰	7.9	597
May 2022	Site 1	1	26.79510482121259 ⁰	75.80857803113759 ⁰	7.8	754
		2	26.79510938934982 ⁰	75.80857995897532 ⁰	7.8	765
		3	26.8019159 ⁰	75.7941457 ⁰	8.1	823
	Site 2	1	26.801033760420978 ⁰	75.79417599365115 ⁰	8.3	805
		2	26.811843942850828 ⁰	75.88987687602639 ⁰	8.0	784
		3	26.811895659193397 ⁰	75.8894323837012 ⁰	7.8	760

**Table 2: Heavy metal concentration (mg/kg) in the sediment of
zthe Dravyavati river**

Month	Site	Sample	Latitude	Longitude	Pb	Cd	Ar	Cr
Feb 2022	Site 1	1	26.790558679401 87 ⁰	75.809893989935 52 ⁰	BD L	BD L	BD L	BD L
		2	26.800963059067 726 ⁰	75.808557803113 759 ⁰	BD L	BD L	BD L	BD L
		3	26.780550023540 854 ⁰	75.866880957037 21 ⁰	BD L	BD L	BD L	BD L
	Site 2	1	26.795266256667 674 ⁰	75.808262284845 11 ⁰	BD L	BD L	BD L	BD L
		2	26.800963268615 305 ⁰	75.794062251225 11 ⁰	BD L	BD L	BD L	BD L
		3	26.795267974957 824 ⁰	75.808249795809 39 ⁰	BD L	BD L	BD L	BD L
May 2022	Site 1	1	26.795104821212 59 ⁰	75.808578031137 59 ⁰	BD L	BD L	BD L	BD L
		2	26.795109389349 82 ⁰	75.808579958975 32 ⁰	BD L	BD L	BD L	BD L
		3	26.8019159 ⁰	75.7941457 ⁰	BD L	BD L	BD L	BD L
	Site 2	1	26.801033760420 978 ⁰	75.794175993651 15 ⁰	BD L	BD L	BD L	BD L
		2	26.811843942850 828 ⁰	75.889876876026 39 ⁰	BD L	BD L	BD L	BD L
		3	26.811895659193 397 ⁰	75.889432383701 2 ⁰	BD L	BD L	BD L	BD L

The pH from all the sampling points (both the sites) showed that the river water was alkaline and total dissolved solids TDS (554.0 mg/l to 760.0 mg/l) were higher than WHO acceptable limits.

The concentration of heavy metals is indicated in the table; 12 samples were evaluated in this table, and all heavy metals such as Cd, Pb, Cr, Ar, and others were detected using Atomic Absorption spectroscopy and the level was found below detection level (BDL).

Conclusion

Heavy metals contamination has been recognized as a major environmental concern due to their pervasiveness and persistence. These heavy metals are not biodegradable, hence there is a need to develop such a remediation technique, which should be efficient, economical and rapidly deployable in a wide range of physical settings (Bonnailet *et al*, 2016). For the characterization in the sediments, some heavy metals, like Arsenic, Cadmium, Chromium, Lead were observed in the sample taken from the Dravyavati River, although not in large quantities. Consumption of

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contaminated water, sediment, fish, fruits, vegetables, and plants, among other things, has a direct impact on human health (Sankhla *et al*, 2019, Lin *et al*, 2012). Industrial wastes, e-waste, sewage, natural sources, human sources, and agricultural acts have polluted harmful and poisonous elements in the Ayad River water, resulting in contamination of drinking water in the surrounding communities, according to studies (Patlolla *et al*, 2009). Diseases including neurotoxicity and carcinogenicity, which are caused by heavy metal poisonings in water, such as Pb, Cr, and Cd, are also common in these locations (Gachet *et al*, 2015). To reduce the risk of consuming contaminated foods, the practice of trace element testing should be continued. People should be informed of the dangers of drinking dirty water and eating polluted foods.

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Forensically Important Evidence : Animal Hair and Its Morphology

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Abstract

In criminal investigations, animal hair is frequently found as forensic evidence and may be an important source of data for identifying species, connecting people to crime sites, and recreating events. This abstract examines the value of animal hair as forensically significant evidence and its morphology. Animal hair has distinctive morphological characteristics that may be used to identify several species, individuals within a species, and even specific body parts. Through microscopy and other analytical methods, the cuticle, cortex, and medulla layers of animal hair may be analysed for distinctive patterns, scales, colour distribution, and medullary indices. As proof, these physical traits, together with others like hair diameter, colour, and length, contribute to the distinctiveness and discriminative strength of animal hair. In a variety of criminal cases, including those involving assaults, killings, sexual offences, wildlife crimes, and illicit animal commerce, animal hair evidence may be present. Forensic scientists can offer important insights into crime scene reconstructions, species identification, and individualization in criminal investigations employing animal hair evidence by combining microscopic analysis, morphological categorization, and DNA approaches.

Keywords : Hair, Animal, Evidence, Forensic, Morphology.

Introduction

The dead keratinocytes that make up hair have a flexible, cylindrical structure that resists chemical deterioration after death, allowing them to retain their structural properties for a very long period. Because of this, forensic science has realised the importance of both human and animal hair research [1]. The hair follicle is a remarkable miniorgan that enables social and sexual interaction as well as insulation, cover, and concealment for the body. The three phases of the hair cycle include controlled development (anagen), regressive growth (catagen), and relative dormancy (telogen). Only during anagen do hair shafts actually develop. The inferior component of the hair follicle must be destroyed after the anagen phase by ending matrix cell mitosis and by well-coordinated apoptosis in the follicle's inferior

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area. This relatively quick phase of regression is known as catagen[2]. In hair investigations, microscopic tests are frequently performed to identify whether a questioned object is a hair and, if so, whether it is human- or non-human-derived. There are three distinct stages in the cyclical development of hair: anagen, or active growth; catagen, or resting; and telogen, or growth halt [3]. An crucial part of an animal's body is its hair coat. It makes it easier to adapt to the ecosystem's shifting conditions and enhances the environment's smooth functioning [4]. The medulla structure of each species remains stable throughout hair length and is unaffected by age or season[5]. Dogs and people have a strong bond. They have thick hair that sheds and changes often. The owner of the dog and the neighbourhood may theoretically undergo primary hair transmission, followed by secondary hair transmission to a crime scene or a murder victim [6]. Dog pets provide excellent indicators of human exposure because they share the same environment and are subject to the same poisons as their owners [7]. Since every interaction leaves a trace and dog hairs also leave a trace according to the principle of exchange, establishing a link between a suspect, victim, and crime scene has become essential in forensics [8].

Material is transmitted from one person, location, or item to another when they interact. The most prevalent kind of transfer evidence in contemporary society is probably hair. When doing research, it's still helpful for highlighting potential links between individuals, locations, and objects. It goes without saying that physical contact can exchange hairs. Through such a transfer, a suspect and victim might be connected, or a victim and suspect can be connected to a crime scene. Hair has two morphologies: an exterior morphology with the root, shaft, and tip, and an inner morphology with the cuticle, cortex, and medulla. The three components that make up the outer structure are the root, shaft, and tip. The hair strand's root, at its proximal end, is what attaches it to the body. The source of the hair and the reason it left the body may be determined using the root. The space between the hair's base and tip is known as the shaft. It may be used to determine the origin of the hair on the body, its colour, and whether it is straight or curly. The distal end, or tip, can reveal details like hair cutting and breaking. Three other features that make up the structure of hair are the medulla, cortex, and cuticle. The cuticle is the shaft's exterior, scale-covered layer. The scales can be used to distinguish between species because of their varying form and spatial structure. The majority of internal hair is made up of the cortex, which resembles a cylinder made of fibres and protein content, while the medulla runs down the shaft and only makes up a small portion of the diameter of the hair strand[9]. Hair is highly resilient, making it suitable for use as physical evidence. Studying animal hair may be a useful method in biology and veterinary forensic inquiry to spot illicit slaughter, trade, and poaching of animals, particularly those that are endangered species. Mammalian hair fibres may be easily collected, stored, and transferred to the lab for microscopy-based species identification. It is possible to examine hair samples directly on entire mounts using light microscopy [10]. Animal hair always has a medullary index of at least 1/3 and spherical hair roots. Compared to human hair, animal hair is coarser and has a bigger medulla [11]. They can be discovered on the victim's body, the suspect's

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clothing, or even things connected to the crime scene, such as in road accidents involving dogs. Since every interaction leaves a trace and dog hairs also leave a trace according to the principle of exchange, establishing a link between a suspect, victim, and crime scene has become essential in forensics [6, 8, 12].

Animal hair is easily transferred to clothing, carpets, and furniture[13]. Dog hair has received less attention than human hair. According to certain research, the study of comparing dog hairs in court has not been given much weight. Therefore, this research intends to analyse and compare dog hair in order to distinguish various breeds in order to contribute to the database of non-human hairs.

Morphology of hair

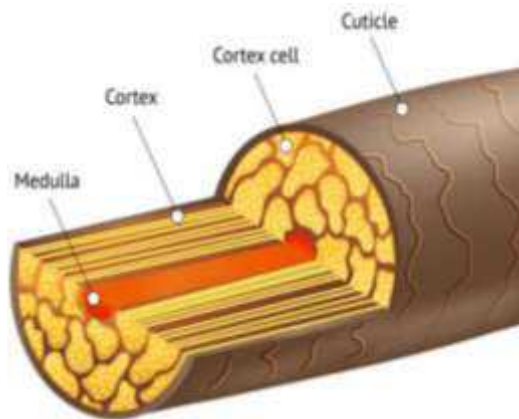


Figure No.1 Layers of hair

Hair's morphology relates to its morphology, or physical makeup. The cuticle, cortex, and medulla are the three major layers of keratin, the primary component of hair. Let's explore each layer in more detail:

Cuticle: The hair shaft's cuticle, which is located on the outside, acts as a barrier of defence. It is made out of overlapping scales, much like roof tiles. The cuticle affects how light bounces off the hair, giving it its shine, as well as protecting the hair's deeper layers.

Cortex: The bulk of the hair shaft is formed by the cortex, which is the intermediate layer. It has cells that are elongated and densely coated with keratin proteins. The cortex is responsible for the strength, flexibility, and colour of hair. The cortex contains the natural pigment called melanin, which determines the colour of the hair.

Medulla: The deepest layer of the hair shaft is called the medulla. It is a less organised, bouncy, and soft area. A medulla is not always present in hair, and its existence, size, and form might differ from person to person. It is still unclear how the medulla works and how important it is for determining hair qualities.

The morphology of the hair is influenced by these three primary layers as well as the structure of the hair follicle. The structure within the skin from which hair develops is known as a follicle. The hair strand that emerges from a follicle which may be straight, wavy, or curly takes on the form of the follicle. The thickness or width of the hair strand and its porosity, which refers to how effectively it can absorb and hold moisture, are additional elements that influence hair morphology[14, 15].

Stages of hair growth

There are three stages of hair growth, which are known as the anagen, catagen, and telogen phases.

Anagen phase: It usually takes two to seven years for hair to reach its active growth phase. The hair shaft is lengthening and the hair follicles are actively creating hair at this stage.

Catagen phase: This is a phase of transition that lasts around two weeks. The hair follicle starts to contract and separate from the dermal papilla, which feeds the hair with nourishment, at this stage.

Telogen phase: This is the phase of rest, which is said to span three months. The hair follicle is dormant at this stage and doesn't create any new hair. In the anagen phase, new hair grows in to eventually replace the old hair, which finally falls off. The hair growth cycle restarts with a fresh anagen phase following the telogen period. Depending on variables including heredity, age, and health, both the duration of each stage and the overall length of the hair growth cycle can change.

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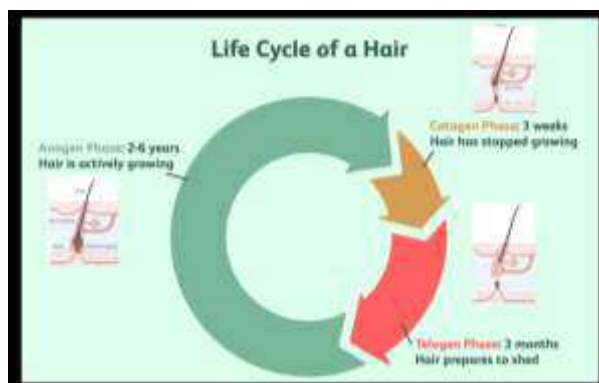


Figure No. 2. Stages of Hair Growth

Microscopical exams are frequently used in hair investigations to identify whether a questioned object is a hair and, if so, whether it is human or not. Anagen (active growth), catagen (resting), and telogen (growth halt) are the three phases of hair formation [16]. An animal's hair coat is a crucial part of its body. It preserves the ecosystem in good working order and makes it easier to adapt to shifting ecological conditions [17]. Each species' medulla structure remains consistent throughout hair length and is unaffected by age or season [18]. Dogs and humans have a strong bond. They have many hairs that shed and change often. It is theoretically quite possible that the dog's owner or environment may experience primary hair transmission, followed by secondary hair transmission to a crime scene or murder victim [19]. Due to their exposure to the same toxins as their owners and living in the same environment, dogs become excellent markers of human exposure [20]. Establishing a connection between the suspect, victim, and crime scene has become essential in forensics since the principle of exchange suggests that every interaction, including dog hairs, leaves a trace[21]. When two things come into contact, material is transferred from one to the other. The most common sort of transfer evidence in contemporary life is unquestionably hair. It is still helpful for uncovering possible connections between individuals, locations, and objects when conducting research. It goes without saying that physical touch can spread hairs. A suspect may be connected to a victim by such a transfer, or a victim and suspect may be connected to a crime scene. Animal hair sticks to clothes, carpets, and furniture with ease [22]. Dog hairs haven't gotten much attention. The study of comparing dog hairs in court has reportedly garnered little attention, according to many studies. As a result, this study will examine and contrast dog hair to identify distinct breeds in order to add to the database of non-human hairs.

Comparison between human hair and animal hair

Human hair and animal hair have several differences in terms of their structure, characteristics, and functions. Here are some key points of comparison:

Structure: Cuticle, cortex, and medulla layers are the primary structural components of both human and animal hair. However, depending on the species, these layers vary in intricacy, thickness, and organisation.

Composition: The main component of keratin, a protein, is present in both human and animal hair. The arrangement and makeup of keratin fibres, however, might vary between species, affecting the characteristics of the hair.

Appearance: Human hair comes in a variety of colours and textures and tends to seem rather smooth and cylindrical. On the other hand, animal hair may display a wider variety of looks, including various forms, textures, patterns, and colours. Animal hair, for instance, might have specialised features like quills or guard hairs, or it can be flat, curled, cylindrical, or even have these.

Functions : Human hair mostly performs tasks including sun protection, insulation, and sensory perception. Additionally, it has cultural and social importance. Depending on the species, an animal's hair serves a variety of purposes. It can act as a form of communication, insulation, concealment, sensory perception, or defence.

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Growth and shedding: Individual hairs have distinct development, resting, and shedding phases, and human hair grows in cycles. Animals' development patterns can differ greatly. While some animals go through seasonal moulting or shedding cycles, others maintain constant hair growth.

Diversity : Humans can have a variety of hair kinds, such as straight, wavy, curly, or kinky, and many ethnic groups might have distinctive hair traits. Animal hair, on the other hand, exhibits immense species variety, with unique adaptations to fit each species' biological niche.

Genetic differences: Animals and humans have very different genetic make-ups, which causes variances in hair morphology and qualities. In both people and animals, genetic variables can affect hair colour, texture, thickness, and other traits [23].

Forensic Significance of hair

Hair includes DNA that may be used to identify a person, making it a useful source of forensic data in criminal investigations. The colour, shape, and size of hair are additional distinguishing physical traits that might provide information about a suspect's race, age, and gender. Following are some of the ways hair can be used in forensic science:

DNA analysis: To find a match, DNA from a suspect or a DNA database can be taken from hair and compared.

Examination of the root: A hair's root can provide important details, such as whether it was pulled out against one's will or fell out spontaneously. The hair's root can also show if it was cut with scissors or pulled out firmly.

Examination of the shaft: A person's race, age, and gender, as well as their health and way of life, may all be determined from the shaft of a hair.

Examination of trace evidence: Hair may gather trace evidence from other people, including DNA, skin cells, and fibres, which can be a useful source of information during an inquiry.

Overall, hair can be a valuable tool in forensic science because of its ability to provide unique identifying information about an individual, as well as evidence that can link them to a crime scene[24, 25].

Conclusion & Future Perspective

Animal hair plays a vital role in forensic investigations and is frequently found at crime scenes, although its significance as a trace evidence has not yet been recognised to the same degree as that of human hair. Dogs are the most common domestic and stray animals found near human homes, and their hair is easily

disseminated through direct or indirect touch. Animal hair may be easily seen on a person's body and may be crucial to a successful criminal investigation. The majority of forensic labs now utilise hair analysis to prove drug exposure, mostly because it has advantages over the more often used blood and urine samples. Additionally, hair's distinct qualities make it particularly beneficial in situations when using other biological specimens would be inappropriate. Despite those specific circumstances, hair should not be viewed as a final replacement for blood and/or urine in establishing drug exposure, but rather as a source of complementary and significant data. Therefore, it is anticipated that hair testing will keep expanding in the near future and that new applications may be found.

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Forensically
Important Evidence :
Animal Hair and
It's Morphology

Evaluation of Synergistic effect of bio-agents and Fungicides against Root Rot of Chilli (*Capsicum annuum* L.) caused by *Rhizoctonia solani* (Kuhn.)

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Abstract

In vitro study was undertaken at Vivekananda Global University, Jaipur to evaluate the bio- efficacy of six fungicides (Azoxistrobin, Vitavax power, Tebuconazole, Thiram, Carbendazim 50 WP and Rhizolex) and four bio-agents (*T. harzianum*, *T. viride*, *T. aureoviride*, *Pseudomonas fluorescens*) against *R. solani* under controlled condition during 2023. After 7 days of incubation at 28±1°C mycelia inhibition percent was recorded. Results were indicates that all the bio-agents viz. *T. viride*, *T. harzianum*, *T. viride* and *T. aureoviride* were showed antagonistic activity to the growth of *R. solani*. *T. viride* showed maximum 92.21% inhibition of *R. solani* growth in dual culture method, followed by *Pseudomonas fluorescens* showed 84.44% inhibition, whereas *T. harzianum* was 80.57% inhibition, Minimum mycelial inhibition was recorded in *T. aureoviride* 69.28%. In case of tested fungicides The tested fungicide Carbendazim 50WP completely inhibited mycelial growth at all concentration followed by Tebuconazole and Vitavax power showed 100 % inhibition of linear growth at 500 ppm concentration. Second best fungicide Tebuconazole showed complete inhibition of mycelial growth at 50, 100, 250 and 500 ppm concentration showed 79.79, 89.69, 92.59 and 100.00% inhibition, respectively. The next was Vitavax power which caused 73.14, 86.18, 92.34 and 100% inhibition of growth at 50, 100, 250 and 500 ppm concentration respectively.

Introduction

Chilli is an important vegetable and commercial spice crop. *Capsicum annuum* L. and *Capsicum frutescens* L. are two important species cultivated in several tropical and sub- tropical climates both for green and ripe dry fruits. India is the largest producer of chilli followed by China, Mexico and Pakistan. In India green chilli is cultivated in an area of 0. 405 million hectares, annual production of 4272 MT (Anonymous, 2021-22). In last few decades there have been some shifts in disease scenario in chilli, mainly due to introduction of diverse germplasm, cultivar and hybrids. Ogoshi, (1996) found reported that

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the *Rhizoctonia* species infected more than 500 plants, mainly in the family of Compositae, Gramineae, Solanaceae, Leguminosae and Cruciferae. Malhotra *et al.*, (2011) observed that *Rhizoctonia solani* is considered as the major soil-borne pathogen of chilli (*Capsicum annum* L.) which causes damping-off disease of young saplings including root and stem rot in transplants. Some diseases like root-rot and damping-off have become wide spread and economically injurious. Root rot, is one of the most devastating and challenging disease, which can damage the crop at any stage. The collar region and roots showed black lesions and shrunk. From the wilted seedlings showing black lesions of roots. *R. solani* is seed-borne and can survive in soil in the absence of host for more than six years. The disease management is laborious due to long saprophytic survival ability of pathogen in the soil (Singh and Malhotra, 1994).

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Root Rot of Chilli
(*Capsicum annum*
L.) caused by
Rhizoctonia solani
(Kuhn.)

Materials And Methods

Isolation and Purification of the Pathogen

Samples of root rot infected chilli plants were collected from surveyed field; root rot infected plants were carefully uprooted and brought to laboratory: Isolations of the pathogen were attempted from all samples. Potato dextrose agar (PDA) medium was used to isolate the pathogen. Small pieces (1-2mm) of diseased roots were cut, washed with sterilized water, surface sterilized with 0.1 % mercuric chloride (HgCl_2) solution for 1 minutes followed by three to four washings with sterilized distilled water and were transferred aseptically on 2 % PDA (Potato Dextrose Agar) plates. The plates were incubated in an incubator at $28 \pm 1^\circ\text{C}$ for seven days. Hyphae coming out from the bits were sub-cultured on the fresh PDA in Petri dishes.

Pathogenicity Test

The pathogenicity of the culture of *R. solani* was tested by growing chilli plants in earthen pots of 20 cm face diameter size. A mixture of garden soil: FYM (3:1) was sterilized in an autoclave. The culture of *R. solani* that was showed maximum disease mortality at the farmers field was used for further studies and multiplied on corn meal-sand (1:1) medium at $28 \pm 1^\circ\text{C}$ for 10 days and mixed with sterilized soil @20 g/kg soil. This inoculated soil was filled in the earthen pots and kept in the cage house for seven days and were irrigated with distilled water to allow establishment of the pathogen. Pots with un-inoculated sterilized soil was kept as control. Seedlings of susceptible variety Pusa Jwala were transplanted in both

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inoculated as well as un-inoculated pots, at the rate of five seedlings per pots. The pots were kept in the cage house and were watered daily to provide good moisture. The germination and symptoms developing on chilli plants were carefully observed. From the diseased plants, showing root rotting symptoms, re-isolation of the pathogen was attempted and the resultant cultures were re-identified. After proving the pathogenicity of *R. solani* culture was used for further studies.

Identification of the Pathogen

The slides were prepared in lacto phenol solution and mounted by DPX mount. These slides were then observed under compound microscope at 10X and 40X power. The morphological, cultural and formation of sclerotia were the principle characters to identify the pure cultures, and compared with the standard reference description (Holliday, 1981, Sneh *et al.*, 1992 and Mordue, 1988) an identity was confirmed as *Rhizoctonia solani*. The culture was identified by comparing the morphological and cultural characters described in standard references of Mordue (1988) for *Rhizoctonia* and was identified as *Rhizoctonia solani*.

Poison food technique: *In vitro* study of fungicides

Relative efficacy of different fungicides *i.e.* Azoxistrobin - 23SC, (Amistar) (Syngenta ltd.) Carbendazim 50WP [BASF India Ltd.], Mumbai, Vitavax power (combi formulation - Carboxin 37.5% + Thiram 37.5%) [Pesticide India Ltd.], Udaipur, Tebucanazole - 250EC (25.9% w/w) [Bayer Crop Science], India Ltd., Mumbai, Rizolex - 50WP [tolclophos-methyl] [Sumitomo chemicals ltd.], Thiram - 75WP, [Gupta Chemicals (p.) Ltd.], Mumbai, was evaluated by using poisoned food technique (Schmitz, 1930) at four concentrations *i.e.* 50, 100, 250 and 500 ppm. Each of the fungicides was added to sterilized medium separately followed by mixing thoroughly and poured back in sterilized Petri dishes where it was allowed to solidify. Each plate was inoculated with 2 mm disc of fungal culture and incubated at $28 \pm 10^\circ\text{C}$. The linear growth after seven days was recorded and per cent inhibition was calculated according to Vincents Formula (1947) as follows:
Where,

$$\text{Per cent inhibition} = (C-T)/CX100$$

C = Diameter of the *R. solani* colony in control.

T = Diameter of the *R. solani* colony in treatment.

Control = check was kept to maintain where medium was without any fungicide supplementation.

***In vitro* efficacy of bio-control agents (Dual culture technique)**

The efficacy of four bio-control agents viz. *T. harzianum*, *T. viride*, *T. aureoviride*, *Pseudomonas fluorescens*, was tested by using dual culture plate method on PDA medium (Johnson *et al.* 1959). Antagonistic effect of these bio agents were tested against the test pathogen (*R. solani*), 2 mm diameter mycelium bit of seven days old culture of *R. solani* and each bio agents were placed separately at 4 cm distance on the periphery of Petri dishes containing sterilized PDA medium. For each treatment four replications were taken. Inoculated plates were incubated at $28 \pm 1^\circ\text{C}$ temperature in BOD incubator. Linear growth of pathogen and zone of inhibition was measured after seven days of inoculation. PDA plates inoculated with pathogen alone served as check.

Results

Isolation, purification and identification of the pathogen

The root rot infected chilli samples were also collected from surveyed fields to isolate the pathogen. The rotted root samples from various villages were separately cut into 2 to 5 mm size and surface sterilized, washed thrice with sterile distilled water and were transferred aseptically on potato dextrose agar in Petri dishes and then incubated at $28 \pm 1^\circ\text{C}$ for seven days. The growth of the pathogens was frequently observed in Petri dishes.

The most of chilli root rot samples were yielded *Rhizoctonia* and *Fusarium*., whereas, in majority of *R. solani* colonies were recovered from these samples. Pure culture of various fungi (pathogens) was obtained by single hyphal tip culturing technique. As the majority of *Rhizoctonia* colonies were recovered from most of the samples has been identified as *Rhizoctonia solani* on the basis of morphological characters of mycelium and sclerotia formation that were further confirmed by compared with the standard reference and descriptions of (Sneh *et al.*, 1992 and Mordue, 1988) and its identity was confirmed as *Rhizoctonia solani*. Resulted cultures were maintained by periodical transfers on PDA slants for further studies and fungal culture of the pathogen *R. solani* was used for present study.

Pathogenicity Test

Pathogenicity of the recovered culture of *R. solani* was tested by growing susceptible chilli cultivar Pusa Jwala in earthen pots contain sick soil of *R. solani*. The development of root rot symptoms on chilli plants was carefully observed. The diseased plants, showing root rotting symptoms, re-isolation of the pathogen was attempted and the resultant cultures were re-identified.

In-vitro evaluation of bio-control agents (Dual culture technique)

Efficacy of four bio-control agents viz; *Tricohderma viride*, *T. harzianum*,

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Pseudomonas fluorescens and *T. aureoviride* was studied *in vitro* against *R. solani* using Dual culture technique. After 7 days of incubation at $28 \pm 1^\circ\text{C}$ mycelia inhibition percent was recorded. Results were indicates that all the bio-agents viz. *T. viride*, *T. harzianum*, *T. viride* (T_3) and *T. aureoviride* were showed antagonistic activity to the growth of *R. solani*. *T. viride* showed maximum 92.21% inhibition of *R. solani* growth in dual culture method, followed by *Pseudomonas fluorescens* showed 84.44% inhibition against *R. solani* growth, whereas *T. harzianum* was 80.57% inhibition, Minimum mycelial inhibition was recorded in *T. aureoviride* 69.28%.

Table 1: Evaluation of Per cent inhibition of mycelial growth of *R. solani* with four isolates of *Trichoderma* sp. by dual culture technique

Treatments	Bio-control agents	Growth*	
		Growth of pathogen	Per cent inhibition*
T ₁	<i>Trichoderma viride</i>	6.61	92.21
T ₂	<i>Trichoderma harzianum</i>	17.63	80.57
T ₃	<i>Pseudomonas fluorescens</i>	14.23	83.89
T ₄	<i>Trichoderma aureoviride</i>	27.04	69.28
T ₅	Control	90.00	0.00
SEm \pm		0.327	0.793
CD at (P= 0.05)		1.043	2.531

* Average of four replications; Figures given in parentheses are angular transformed values

***In-vitro* evaluation of fungicides (poisoned food technique)**

Six fungicides Azoxistrobin, Vitavax power, Tebuconazole, Thiram, Carbendazim 50 WP and Rhizolex were evaluated at four concentrations viz., 50, 100, 250 and 500 ppm using poison food technique against *R. solani*. All the tested fungicides significantly (P=0.05)

inhibited the mycelial growth of *R. solani* at all concentrations from 50 ppm to 500 ppm. The tested fungicide Carbendazim 50WP completely inhibited mycelial growth at all concentration followed by Tebuconazole and Vitavax power showed 100 % inhibition of linear growth at 500 ppm concentration. Second best fungicide Tebuconazole showed complete inhibition of mycelial growth at 50, 100, 250 and 500 ppm concentration showed 79.79, 89.69, 92.59 and 100.00% inhibition, respectively. The next was Vitavax power which caused 73.14, 86.18, 92.34 and 100% inhibition of growth at 50, 100, 250 and 500 ppm concentration respectively, followed by Azoxistrobin was found effective with 70.30, 80.50, 87.80 and 92.40 % inhibition of growth at 50, 100, 250 and 500 ppm concentration respectively. Rhizolex was found at fourth number it inhibited 69.37, 80.70, 86.62 and 89.80 % inhibition of growth at 50, 100, 250 and 500 ppm concentration respectively. Thiram was found least effective at all concentrations against *R. solani* with lowest 65.62, 78.76, 85.74 and 88.30% inhibition of growth at 50, 100, 250 and 500 ppm concentration, respectively.

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Table 2: Efficacy of different fungicides on the growth of *R. solani* at various concentrations (PPM) *in-vitro*

S.N.	Treatments/ Fungicides	Mycelial growth (mm)*				Per cent growth inhibition*			
		50	100	250	500	50	100	250	500
1.	Azoxistrobin	25.67	17.13	10.13	7.03	70.30	80.50	87.80	92.4
2.	Vitavax	23.67	12.39	7.23	0.00	73.14	86.18	92.34	100.0
3.	Tebuconazole	18.17	9.40	6.07	0.00	79.70	89.69	92.59	100.0
4.	Thiram	31.48	18.87	13.07	10.07	65.62	78.76	85.74	88.3
5.	Carbendazim	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.0
6.	Rhizolex	28.19	17.51	12.50	9.37	69.37	80.70	86.62	89.8
7.	Control	90.00	90.00	90.00	90.00	0.00	0.00	0.00	0.0
SEm±		1.328	0.393	0.371	0.129	0.397	0.391	0.488	0.407
CD at (P= 0.05)		0.434	1.205	1.135	0.395	1.217	1.199	1.495	1.246

*Average of four replications; Figures given in parentheses are arcsine per cent angular transformed values.

Discussion

In-vitro studies for evaluating resident isolates of *Trichoderma* sp. revealed the efficacy against *R. solani* in dual culture method. This was expected as in dual culture, all the modes of antagonism, competition as well as mycoparasitism is simultaneously operative. In this present study the *T. viride* isolate of this region found to be highly effective (92.40%) against the tested pathogen *in vitro*. Similar results have been observed by several workers, where biological control agents like *T. viride*, *T. harzianum* and *T. aureoviride* have been reported to be effective (83.3, 81.2 and 71.1%), respectively for control of *R. solani* of chilli. The result augmented with; Bunker and Mathur (2001); Mathur and Gurjar (2002); Das and Soma (2011); Subhash *et al.* (2013). Six fungicides Azoxistrobin, Vitavax power, Tebuconazole, Thiram, Carbendazim and Rhizolex were evaluated *in-vitro* at four concentrations *viz.*, 50, 100, 250 and 500 ppm using poisoned food technique against *R. solani*. The test fungicide Carbendazim completely inhibited mycelial growth at all concentration followed by Tebuconazole and Vitavax power showed 100% inhibition of pathogen growth at 500 ppm concentration. Similar studies were conducted by Rehman *et al.* (2013) and reported that Carbendazim was the promising for the inhibition of the radial growth of *R. solani in-vitro*, Vadhera *et al.* (1997) and (Gupta and Arora 1998) also reported that Carbendazim was the most effective to suppress the *R. solani* growth in-vitro and *in-vivo* against Soybean root rot pathogen *R. solani*.

Conclusion

From the above findings it is concluded by *in vitro* study application of bio-control agents will be significantly promising and applicable as an alternative to synthetic chemicals and low efficiency and harmful methods for inhibition or control of *R. solani*.

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A Study On Genotoxicity Screening Of Industrial Effluents Using Onion (*Allium Cepa*) Root Tip

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Abstract

The effectiveness of the *Onion root tip* test system for screening cytotoxicity and genotoxicity of treated effluents from four types of industrial activities (two textile industries, three rubber based industries, two industrial zone common treatment plants, and two water treatment plants) was evaluated. Physico-chemical parameters in the effluents, including heavy metal/metalloid levels, varied depending on the industry profile, but the majority of the measured parameters in the effluents were within Jaipur environmental regulations for discharge of industrial effluents into inland surface waters. In comparison to the dilution water and upstream water, undiluted effluents caused statistically significant root growth retardation, mitosis depression, and chromosomal aberrations in root meristematic cells in most cases in the *A. cepa* test system, indicating effluent induced cytotoxicity and genotoxicity.

Introduction

Industrial practices are one of the key sources of thousands of chemicals that enter the environment and affect the surface water, groundwater and land. The aquatic system is one of the most polluted ecosystems because of industrial discharges (Manzano *et al.* 2015). Effluent from industries directly or indirectly elevates the threat to the aquatic system, resulting in an imbalance of the ecological system as well as the genetics of living beings. In the wastewater, carcinogenic, genotoxic, mutagenic, and xenobiotic chemicals have been discovered, causing harm to animals, humans, and plants (Alam *et al.* 2009). Organisms suffer a variety of effects as a result of these contaminants (Sisman 2014).

The effluent discharged by the wastewater management system exacerbates problems in aquatic systems more quickly than in other environments. Heavy metals, pesticides, pharmaceutical waste, and other chemicals, as well as organic and inorganic entities, leak directly into rivers streams via sewer tanks, water reservoirs, and other sources (Anju *et al.* 2010; Jayakumar *et al.* 2015;). Various contaminants found in rivers are absorbed into the aquatic ecology, where they cause mutations and sickness across the food chain.

The volume of wastewater produced has increased as a result of industrial and agricultural expansion in several countries. The wastewater contains compounds that are either natural or manufactured. The most common nitrogen, NH, NO, NO₂, R-CH(NH₂)-COOH, N-containing medicines, insecticides, and other personal hygiene items are the most common sources. As, Br, Cd, Cr, Cu, Fe, Pb, Mn, Sb, Hg, Ni, Ag, Tl, U, and Zn are heavy metals present in the discharge of numerous industries. The persistence of marine species is complicated by heavy metals, agricultural waste, and algae toxins, in addition to heavy metals (Orellana *et al.* 2017). The use of dirty water in agricultural techniques is one of the reasons for dangerous pollutants entering the food chain (Brooks *et al.* 2016). The negative effects of wastewater are not restricted to aquatic species; individuals who drink water from contaminated rivers or streams are also affected.

Industries are undoubtedly indispensable components of a nation's development; however, the impact of industrial wastewaters on aquatic and terrestrial ecosystems has drawn a lot of attention worldwide because of its overwhelming environmental significance. Industrial wastewater originates from the wet nature of most large industries which require large quantities of water for processing and disposal of wastes. Most industries are therefore, located near water sources. Industrial wastewater is not only concentrated but plentiful, so the pollution potential of industrial wastewater is by far greater than that of domestic wastewater. In Nigeria, over 80% of the industries discharge solid wastes, liquid effluents and gaseous emissions directly into the environment without any treatment (Federal Ministry of Water Resources, 1994) (Johannessen *et al.* 2015). Despite existing legislations, of the 200 randomly assessed industries, only 18% perform rudimentary recycling prior to disposal of the wastes.

Several higher plant systems, including bioassays using plant roots, have provided cheaper, easier, more sensitive, usable, reliable, and beneficial alternatives to the traditional assays carried out on experimental animals for determining the detrimental effects of environmental contaminants.

Due to increased urbanization and industrialization, environmental poisoning of soils with hazardous metals has become a global issue in recent decades (Withers *et al.*, 2014). Because of their poisonous, non-degradable character and ease of bioaccumulation, toxic metals and metalloids have negative effects on ecosystems and human health (Connor *et al.*, 2017). As a result, they may pose a risk to food security (Dellamatrice *et al.*, 2017). Metal buildup in a variety of edible plants could be hazardous to human health (Saratale *et al.*, 2011). Metal contamination of soils can be caused by a variety of anthropogenic sources, including mining and metal-ore processing, industrial effluents, car emissions, and agricultural operations (Kolekar *et al.*, 2012). However, contamination of food plants is becoming more widespread as a result of wastewater irrigation.

Freshwater scarcity and economic pressures have forced wastewater irrigation practices, especially in desert regions (Koupaie *et al.*, 2013). A tenth of the world's population relies on food produced via wastewater irrigation. According to another estimate, 200 million farmers worldwide use wastewater irrigation in various forms on 20 million hectares of land.

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Despite the fact that wastewater contains a significant amount of valuable plant nutrients that can reduce the need for artificial fertilizers (Forss *et al*, 2017), long-term wastewater irrigation can result in metal accumulation and bio-magnification, posing potential health risks (Leme & Morales 2009). As a result of metal deposition, changes in genetic material such as chromosomal abnormalities, ploidy, and point mutations may be expected. Previously, various methodologies for assessing the ecotoxicological impacts of environmental contaminants were developed (Dey & Islam 2015).

The *Onion root tip* test is one of the most extensively used and sensitive assays for investigating chromosomal abnormalities induced by numerous chemical poisons). The test was devised and described by Masood & Malik 2013 and later adapted for environmental monitoring and has a lengthy history in scientific literature. Because *A. cepa* has a small number of chromosomes ($2n = 16$), it's easy to assess the genetic changes at the chromosomal level that occurs when plants are exposed to harmful substances. This test was used to look into the impact of toxins on the normalcy of cellular division. The test is a useful tool for advancing our understanding of how hazardous chemicals, alone or in combination, cause chromosomal changes.

MATERIALS AND METHODS

Sampling sites and analysis of samples

The soap effluent (Sp) was obtained from PZ Cussons Factory in Jaipur, Rajasthan State; the beverage effluent (Bv) was obtained from Consolidated Breweries in Jaipur, Rajasthan State; and the paint effluent (Pt) was obtained from Saclux Paint Industry Ltd (Siddiqui *et al*, 2011). in Jaipur, Rajasthan State. The three raw industrial effluents (Sp, Bv, and Pt) were collected from the different factories' industrial waste water discharge pipes. These industries' effluents are released into surrounding municipal waterways and drainage systems. The water reaction (pH) and electrical conductivity (EC) of the samples were determined at the time of collection, and the samples were examined for turbidity, alkalinity, Cl, So₄, Co₃, Na, Ca, No₃, K, and other standard physico-chemical parameters. Three industrial effluents were collected from disposal stations within the Jaipur City metropolis, which is located between latitudes 60 06' N and 60 30' N and longitudes 50 30' E and 50 45' E and covers an area of around 500 square kilometres. The brewery (Br) and rubber processing factory (Rr) effluents are released into the Ikpoba river, while the bottling firm (Bt) effluents are discharged into the Dravyawati river of Jaipur.

The *Onion root tip* test involves collecting onion bulbs that have been grown without the use of herbicides or fungicides. After getting the bulbs, scrape them at the root to encourage the growth of new roots. Other bioassays, such as allelopathy testing and genotoxicity assessment, can be performed with *Onion root tip* seeds germinated in a BOD (biochemical oxygen demand) incubator with regulated temperature. To begin the experiment and allow rootlets to sprout, all bulbs should be placed in a tiny 100 mL plastic cup with distilled or tap water (as long as it is potable) for around 09 to 10 days. After this time, the bulbs, along with the treatments, should be transferred to other clean and dry containers. To reduce the

environmental impact of plastic, the plastic cups used for water purification can be reused.

A Study On
Genotoxicity
Screening Of
Industrial Effluents
Using Onion
(*Allium Cepa*)
Root Tip



Fig.-Sample collecting site (DRAVYAVATI RIVER OF JAIPUR)

In general, we employ 5 groups of *Onion root tip* bulbs for each treatment, with one serving as a negative control in water and another serving as a positive control in MMS or glyphosate. Methylsulfonylmethane was used as a positive control, and used glyphosate was used as a positive control. Various amounts of glyphosate have demonstrated chromosomal modifications directly to the rootlets in research developed to far at the Laboratory of Plant Cytogenetics and Genotoxicity (LABCITOGEN) of the Department of Biology at the Vivekananda Global University of Jaipur. Its long-term impact on the environment has yet to be established. After rooting, the bulbs in the two control groups should be left in the water (negative control) and the respective positive control, and the rest should be transferred to the chosen treatments, which can include essential oil solutions, leaf extracts by infusion, root or stem extracts by decoction, or samples of industrial and/or hospital effluents. For 24 hours, these should be kept in the dark.

It is stressed that when a product such as ethanol PA is used to dilute the oil in which the rootlets of *Onion root tip* will be immersed, one of the treatments should be included. The fundamental objective is to reduce error and allow the findings to show the effect of the interacting chemicals. As a result, test them independently whenever possible. After the rootlets have been exposed to the various treatments for 24 hours, they should be removed and promptly fixed in ethanol:acetic acid (3:1) for another 24 hours.



Fig.-Onion root tip L.

The rootlets should then be taken from the fixing solution and placed to 70% ethanol, which should be kept chilled (4°C) until usage. It is critical to underline that all glassware used to store the rootlets should be labelled with a number or sample name and/or treatment, as well as a date, using small tags written in pencil on the inner and outside surfaces of the glass, to avoid any sort of sample misidentification (Cabrera & Rodriguez 1999) describes the bulb-use technique, which has been adapted by researchers who employ the test, such as Lewtas 1988). Slides are made in the next stage, evaluating 1000 cells per bulb, totaling 5000 cells per treatment, or variants of these values, such as 500 cells per bulb, totaling 2500 cells per treatment. It has already been demonstrated that one rootlet is sufficient for monitoring DNA damage in *Onion root tip* cells following treatment with mutagenic chemicals.

Results & Discussion

The physical and chemical characteristics of wastewater from a bottling factory (Bt), a rubber processing sector (Rr), and a brewery (Br) at their discharge sites. Significant pollutants of suspended matter, dissolved matter, and high turbidity values were found in the effluents. The pH of the bottling plant's effluents (Bt) was alkaline (10.50), while the rubber processing plant's (Rr) and brewery's (Br) effluents were acidic (4.75 and 5.50, respectively). The effluents had high mean total hardness values, with 417, 320, and 298 for Bt, Rr, and Br, respectively. Bt, Rr, and Br had mean total suspended solids (TSS) of 34, 40, and 48, respectively, which were all above the discharge limitations imposed for surface water. Similarly, total hardness of effluents (417, 320, and 298) for Bt, Rr, and Br, as well as turbidity, exceeded WHO's maximum allowed limits. BOD was greatest in the effluent of Bt (19.90), followed by Br (15.10), and lowest in Rr (12). All of the wastewater samples included lead, manganese, cadmium, copper, iron, zinc, and nickel.

Biological materials-

The bulbs (2.5 - 2.8 cm diameter) of the common purple onion *A. cepa* L. Stuttgarter Reisen (2n=16, Family Amaryllidaceae) utilized in the study were purchased commercially from Eke Okigwe Market in Abia State, Nigeria. They were sun dried for two weeks before being utilized in the testing (except for the

rotting ones). Onion bulbs of the purple variety (Onion root tip L., 2n=16) of average size (15-22 mm diameter) were purchased locally in Jaipur, Rajasthan. Fig.- Onion (Onion root tip). They were sun-dried for six weeks before having the dried roots at the base of the onion bulbs scraped away with a sharp razor blade to reveal the fresh meristematic tissues. To keep the primordial cells from drying out, the bulbs were immersed in freshly made distilled water.

Two replicate bulbs were utilized for each test sample and control (tap water) to account for a proportion of bulbs in the population that would be inherently slow or poor growers, and the best one bulb was chosen for investigation Lewtas *et al*, 1992) (Rank and Nielsen, 1993). To remove excess water, the bulbs were withdrawn from the distilled water and placed on blotting paper. The bulbs were immersed in 0, 1, 5, 10, 25, 50, 75, and 100 percent (v/v, effluent/tap water) of each test sample to assess root development inhibition. For each wastewater concentration and the control, seven onion bulbs were used. Each bulb's base was suspended in the dark for 10 days on the effluents inside 100ml beakers. Every day, the test effluents were replaced. The roots of five onion bulbs with the best growth at each concentration were extracted with forceps and their lengths measured (in cm) with a metre rule at the conclusion of the exposure period.



Day-1

The 100-ml beakers were used in the modified test (DeMarini 1994). The positive and negative controls were respectively lead nitrate ($Pb(NO_3)_2$) solution and distilled water, whereas the industrial effluents were diluted with distilled water. To obtain the series of concentrations studied, the effluent was equilibrated to room temperature ($26\pm 2^\circ C$) and diluted with distilled water in each case. The outer scales of the bulbs and the brownish bottom plates were removed prior to the test, leaving the root primordial ring intact. During the washing operation, the peeled bulbs were immersed in fresh water to prevent the roots from drying out. Following that, the bulbs were subjected to 100%, 75%, 50%, and 25% (v/v, effluent/distilled water) of

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each test liquid. Each concentration of each individual effluent and the control, that is, each concentration was set up in 5 replicates, was done with five onions. In 100-ml beakers, the base of each onion bulb was suspended on the test liquid in the dark at 271°C. The test solutions were replaced on a daily basis.

The root tips of one bulb from each set of experimental organisms were fixed individually in ethanol:glacial acetic acid (3:1, v/v) and used for chromosomal analysis after ten days. The root tips were hydrolyzed in 1N HCl at 60°C for 5 minutes and washed in distilled water (for each effluent concentration and the control). On each slide, two root tips were put and stained in aceto-carmin for 20 minutes (after squashing). Filter paper was used to remove excess discoloration, and the cover slip was carefully lowered to avoid air bubbles being trapped underneath. In each example, the edges of the cover slip were sealed with clear nail polish, as suggested by Tejs (2008), to prevent the preparation from drying out due to the heat of the microscope. For each effluent concentration and the control, two slides were created.



Day-10

The slides were coded and analyzed at high magnification for chromosomal abnormalities (X1000). The mitotic index (MI) was calculated as a percentage of the number of dividing cells per 1000 (400 cells per slide per concentration and control) observed cells in each case (Nirogi *et al*, 2015, and mitotic inhibition was calculated as a percentage of the difference between the mitotic indices of the control and the group divided by the control's mitotic index). The number of aberrant cells per total cells scored at each concentration of each effluent was used to calculate the frequency of aberrant cells (percentage) (Mortelmans & Zeiger 2000).

The number of aberrant cells per total cells scored at each concentration of each effluent was used to calculate the frequency of aberrant cells (percentage) (Müller *et*

al, 1992). The cells are studied during interphase and cell division, as well as prophase, metaphase, anaphase, and telophase, using an LEICA microscope with a 400X magnification. Cell counts are performed using visual fields that scan the entire slide. The counts should be recorded in a table like the one below. The sum of all cells in interphase and division is then determined for each therapy, followed by the mitotic index (MI). During the cell count, they should be sorted into two groups: regular (no chromosome damage) and irregular (chromosome damage) (present damages in the chromosome, such as: chromosomal breakages, simple or multiple anaphasic bridges, micronucleus, laggard or lost chromosomes). The statistical analysis should preferably be performed using a statistical programme, such as BioEstat 4.0 (Janion 2008) or BioEstat 5.0, with a p0.05 level of probability).

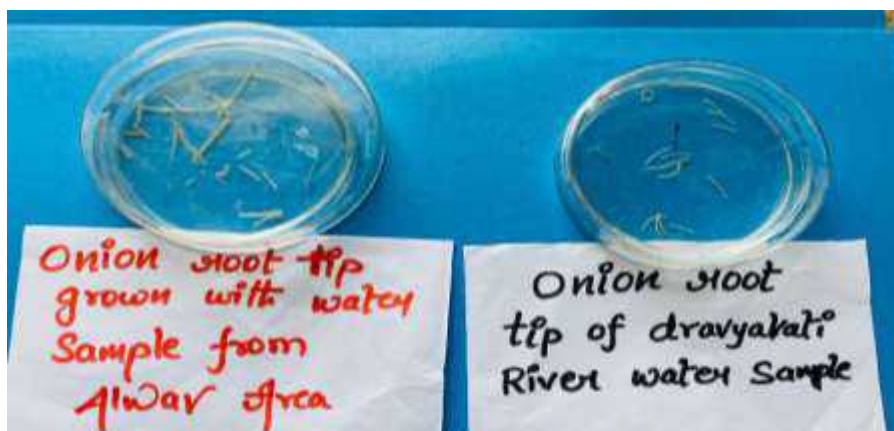


Fig.-Cut and fixed in ethanol: glacial acetic acid (3:1, v/v)

The number of cells observed in prophase + metaphase + anaphase + telophase can be used to compute the mitotic index). The mitotic index, can be calculated using the formula defined as divided cells/total number of cells, and chromosomal aberrations were determined by scoring cells with bridges, fragments, sticky chromosomes, and polar deviation in three randomly chosen zones, as well as micronucleus formation in 1,000 cells per slide. *Onion root tip* was employed to test extracts of *Plantago lanceolata* in their investigation. The researchers utilized two sets of ten bulbs that were placed in the dark at 22°C to germinate, and the same were observed after 48 hours, removing the bulbs with only a few roots and treating the rest with water containing 0.7 percent H₂O₂ for 1 hour.

After the H₂O₂ treatment, the roots were washed and then treated for 24 hours with two different doses of *Plantago lanceolata* extracts (15 g/L and 30 g/L). Because the plant extracts were photosensitive, the onions were rooted in the dark in this investigation. Other researchers tested nanocoated tretinoin, which was likewise photosensitive, by putting the onions to root in the dark. As a result, the *Onion root tip* test can be done either in the dark or in the light. To study cell division in *Onion*

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root tip, you must first correctly identify all of the steps that go into calculating the mitotic index. Cells in interphase and cell division can be shown. The mitotic index value derived from the negative control in water is variable since most cells are observed in interphase.

ROOT LENGTH MEASUREMENT

The test organism- Locally obtained commercial variety bulbs of the common onion (*Onion root tip* L.). Only bulbs in good condition with a diameter of about 3.5 cm were used. The bulbs were cleaned by removing their dried roots, then washed, dried, and stored at 4 degrees Celsius.

Features common to experiments using both species

Plants were grown from seed on a single batch of building aggregate (80 percent sand, 20% tiny stones >2 mm) from JH Walker Building Supplies in York. There was some silt and clay present, and the pH was 7.7 in 1:2.5 0.01 M CaCl₂. Pots were filled by hand with building aggregate (also known as 'sand,') and stones longer than 1 cm were removed during the process (Stones between 5 mm and 1 cm in length were scarce.) To prevent sand loss, a mesh square was inserted at the bottom of the pot (Hara & Morales 2017). Plants were grown in a growth room under fluorescent lights that produced approximately 120 mol m⁻² s⁻¹ PAR (photosynthetically active radiation) at plant height on a 16-hour photoperiod at 20/16 °C day/night. Plants were fed three times a week (details below) and given extra deionized water as needed. Pots were irrigated from above with tapwater until saturated, then left to drain for 30 to 90 minutes before being.

Onion root tip

A harvest group (eight pots each, every week from 3–9 weeks after sowing) was randomly assigned to 56 15 cm top diameter pots filled with sand (volume roughly 1600 cm³). Because of space constraints, not all harvest groups began at the same time. At the same time, plants that would be harvested in 5–9 weeks were started. After the early harvests (weeks 5 and 6), space became available to begin planting the plants that would be harvested 3 and 4 weeks after sowing. Three times a week, all plants were fed a 40 cm³ half-strength Rorison nutrition solution). The root strength of one plant every week (two in week 6) was measured. The rest were examined for anchorage.

Individual adventitious roots originating from the stem base were extracted for testing after the plants were thoroughly cleaned free of the dirt. Each root's basal (nearest the shoot) 40 mm length was removed. Between 0.1 mm thick steel plates, 10 mm was glued (Walker 1985) Loctite Superattak, a cyanoacrylate glue) at each end. This left a 20 mm centre section, which was tested in a universal testing machine at a 20 mm min⁻¹ deformation rate, imposing an initial strain rate of around 0.017 s⁻¹. Roots shorter than 40 mm were ignored, and if the root was longer than 80 mm, a second 40 mm sample from the basal end was obtained. The samples were allowed to dry for around 30 minutes throughout the preparation to allow the glue to adhere correctly. The roots were immersed in tap water for 5 minutes right before

testing. This would have allowed for complete turgor recovery. Because roots must occasionally endure drying, it was determined that this consistent treatment was not significantly different from what would occur in nature. The breaking force and break location were recorded.

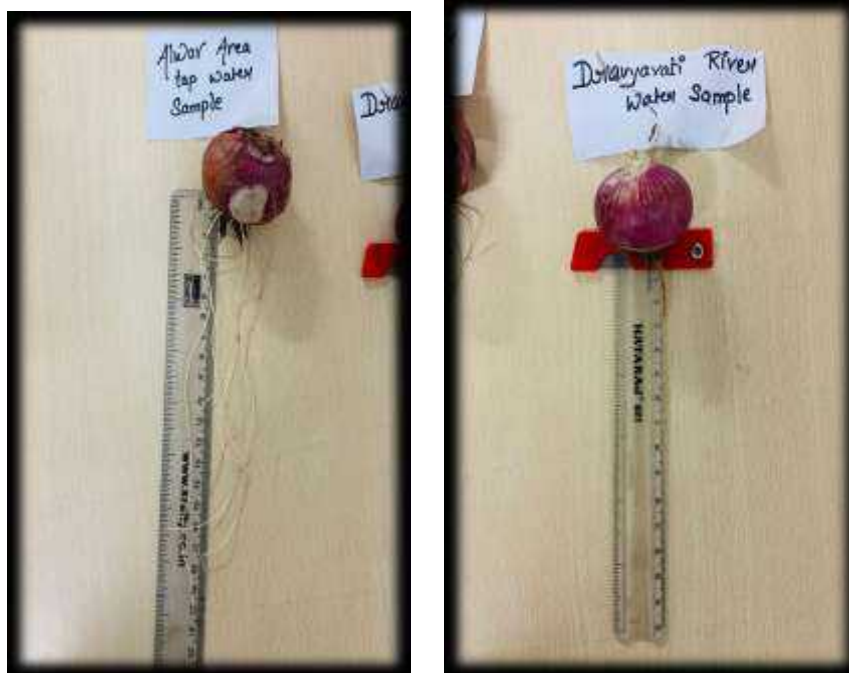


Fig: Root growth measurement

Table-Comparative growth of onion root tip on two different water samples.

Water samples	Root Growth in every two day in CM.					
	1day	3day	5day	7day	9day	10day
Tap Water	1cm	4cm	7cm	9cm	13cm	20cm
Dravyawati River water samples	0cm	0.8cm	1cm	1.4cm	2cm	2.8cm

The mean root lengths of *A. cepa* growing in industrial effluents. In general, all of the effluents studied showed root development retardation; high growth rates were found in onion bulbs exposed to low amounts and vice versa. The following was the order of the mean root lengths derived from onion bulbs growing in wastewaters: For example, percent root growth of control for the stock sample of Bt was 28.9,

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whereas Rr and Br were 46.98 and 53.63, respectively, at all concentrations. The root tips of onion bulbs cultivated in industrial effluents were characterized by twists and crotch roots (roots twisted upwards like hooks) as compared to the control onion bulbs. In earlier investigations Olorunfemi *et al.*, 2011), root deformities in *A. cepa* were found to be useful indications of toxicity. The results of the cytotoxic and genotoxic testing of industrial effluents show a linear connection between macroscopic and microscopic parameters for all wastewaters. Concentration-dependent effects were seen.

Root development is slowing down, and the order of induction of EC50 values were used to determine root growth inhibition. Effluents Bt>Rr>Br These figures show that the samples were poisonous; however, the Bt effluent was not possessed the most inhibitory and antidepressant properties compared to all other effluents. In their study, Strauss (1989) describe the anaphase-telophase approach of *Onion root tip* and make key concerns, such as genotoxic compounds utilized for a variety of reasons in industrial processes and found in environmental compartments such as air, water, soil, and sediments. The chemical can be released into the environment by wastewater discharge, air pollutants, product use, and home and industrial waste sites. The main benefit of the *Onion root tip* test is that it is a "low-cost" procedure that, in addition to being quick and simple to use, produces trustworthy findings (Fernández *et al*, 2000).

To study cell division in *Onion root tip*, you must first accurately identify all of the steps that go into calculating the mitotic index. Cells in interphase and cell division may be shown. The mitotic index value derived from the negative control in water is variable since most cells are observed in interphase. The regular steps of cell division in *Onion root tip* are described and illustrated. There is an example of ordinary cells in these phases of mitosis when the roots only develop in distilled water and do not have chromosomal abnormalities. Figure A shows prophase, which has a lot of chromosomes visible; B shows metaphase, which has chromosomes arranged in the equatorial plate of the cell, waiting to be moved to the opposite poles during anaphase; C shows anaphase, which has chromosomes moving to the opposite poles in a stable manner; D shows telophase, which has chromosomes already organized on opposite poles.

For each dose concentration and time interval, 5000 cells were counted to determine the MI after each treatment. Figures 1 and 2 show the impact of textile industry effluents on the *A. cepa* meristems root tip. The general trend demonstrates that as the dosage and exposure period are increased, the MI for the effluent under examination gradually decreases. When the effect of effluents was investigated, it was shown that effluents before treatment cause higher toxicity by reducing the number of dividing cells in the root tip meristem, indicating that untreated effluents are more genotoxic than treated effluents (Maslowska *et al*, 2015). When the data is compared, effluents before treatment have increased genotoxicity, and root tips treated with 100 percent effluent have the lowest percentage of MI; this finding is very significant at $p > 0.01$. Figures 1 and 2 show dose-dependent mitotic toxicity for the effluents investigated. In comparison to effluents after treatment, effluents before treatment cause more mitotic toxicity.

With 72 hours of exposure, the maximal mitotic depression was discovered for the 100 percent dosage. The reduced mitotic record in the onion root meristem has been discovered to be a dependent measure for identifying the closeness of cytotoxic contamination in the natural environment, as well as a tool for determining the amount of water contamination. This system's great sensitivity allows us to detect pollution in significantly less polluted water. For industrial effluents that demonstrated mitotic depression, a concentration-dependent trend was identified. In the untreated sewage, there was more MI depression than in the treated effluent. On the 10 days of exposure, the high number of aberrations were attained in 100% concentration.

Conclusion:

Pollution of the aquatic system has become a global concern as a result of continuing industrial discharge. Using the *Onion root tip* chromosome aberrations test system, this study was undertaken to investigate the possible genotoxic effect of industrial effluents (treated and untreated) (Leme & Morales 2008). The untreated sewage was shown to be more harmful than the treated effluent, as it reduced the number of proliferating cells in the root tip. Untreated wastewater samples all had a substantial genotoxic effect. The steadily increasing effect on *A. cepa* was observed as the dosage was increased from 25% to 100%. According to the study, wastewater remains genotoxic even after treatment since it has the ability to cause chromosomal abnormalities of various types.

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Comparative study of synthesis and reduction methods for graphene oxide

EKTA MEENA, NUTAN SHARMA

Abstract

Introduction

Graphene In essence, it is the little sister of graphite it have a Layer by - layer diagram. It has a middle weak bond. Carbon requires four bonds to achieve the stability which is found in diamonds. It was just like soft rock material. Due of this pencils are made of that substance. Different bands from themselves as rebound in graphene. Graphene have a structure similar to this. Due to its double bonding it is in stable form. It is not a Mattel product it is crystal. In 1962 the electron microscope used to discover its structure for the first time. In 2004 Andre Geim and Katya Novaselor Continusally developed the graphene's Single layer. The layer was removed with help of tape and after some time they obtain mono layer (of graphene). They received the Nobel prize for this Graphene has been tested to be one of the Strongest materials it is wander materials. It is known as regular carbon / normal carbon. Its property that it takes an absorbed to red or green light. It is a transparent (thick) one - atom Crystal absorb spectrum to make it Visible. In 1962 the electron microscope used to discover its structure for the first time. In 2004 Andre Geim and Katya Novaselor Continusally developed the graphene's Single layer. The layer was removed with help of tape and after some time they obtain mono layer (of graphene). They received the Nobel prize for this Graphene has been tested to be one of the Strongest materials it is wander materials. It is known as regular carbon / normal carbon. Its property that it takes an absorbed to red or green light. It is a transparent (thick) one - atom Crystal absorb spectrum to make it Visible.

Methodology

Chemicals are used in the Hammer procedure. That can be used to produce graphite oxide by mixing potassium permanganate Wit a graphite solution. Additionally, it can be altered to produce a graphene oxide Compound that is only one Molecule thick. After the discovery of graphene in 2004 the scientific Community became interested in graphite oxide. So for many teams are researching ways to produce graphene in large quantities faster by using graphite oxide. These techniques have created, materials that have more flaws than these made straight from graphite according to studied.

Electrical properties:-

Hight Conductivity of electricity

- Unoverlapped semi-metal.
- Increased and high electron mobility.
- Mechanical properties:

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-
- One of the most durable of strongest materials. (130 GPa)
 - High ratio of surface to volume.
 - Very light (0.77 mg / 5 gm).
 - It has elastic properties and qualities (young's modulus is 0.5 TPa)

Comparative study
of synthesis and
reduction methods
for graphene oxide

Optical properties:-

Graphene absorbs up to 2.3% of white light in a single sheet. Strong transparency. Some types of photoluminescence can be released. Modified Hummers Method is still of great interest since it is a simple way to produce huge amount of graphite oxide

Procedure :-Oxidation :-

The initial step in oxidation is to combine freshly crushed graphite powder with the least quantity of water possible and then turn on the magnetic stirrer add Sulphuric acid and Continue stirring the mixture in ice bath. We should keep the temperature below 10°C since oxidation is a very exothermic process. after that add potassium permanganate oxidizing agent and sulphuric acid which combine with nascent oxygen. this oxygen directly goes and oxidises graphite layers. the graphite layer that is connected to weak bond about sp² sp³.

- Moving further apart and away from graphite oxide.
 - The exothermic chemical hydrogen peroxide is added after stirring for roughly 4 to 5 hours.
 - We obtain monomeric graphite oxide as a result of ultrasonication which vibrates the molecule and reduces. Atom-to-atom contact
- ✓ Stage-1st - Synthesis of graphite oxide = graphite powder.

Graphite powder (2g) + 1g Potassium nitrate + 46g mL H₂SO₄ (dissolved with stirring in ice bath)



Slow addition of KMnO₄ (6g) during stirring of 30 minutes temperature remains below 20°C

Stir 30 minutes at 40 °C and add 80 ml of Water



Stir for another 90 minutes at 90°C and add 200 mL Water and 6mL H₂O₂



filter and wash the solution With distilled Water and HCl

Meena, E
Sharma, N

Reduction:-

- Using a reducing agent such as sodium hydride or hydroxide to separate graphene from graphite oxide.
- This method considers being better; it means this approach is viewed as problematic.
- Stage-2nd - And = Synthesis of graphene from Go (Graphene oxide)
- **Go (100 mg) dissolved in to 100 ml water**
- **Add Hydrazine hydrate (10mL 0.2×10^{-3} Mol)**

Reflux for hour at 80°C



Add sodium borohydride (1 mg)



Reflux for 36 hours at 100°C



Filter and wash with Distilled water



Dry at 60°C

The next step is post treatment.

Post-treatment:

- Filtered and washed with water until neutral the solution.
- The product is dried and grinded.
- The manufactured graphene can next be subjected to a Characterisation test.

ADVANTAGES:

The benefits of utilising this technology include the ability to scale up to industrial level and high yield.

Comparative study
of synthesis and
reduction methods
for graphene oxide

Limitation:

- ✓ The limitation Of this method are:
- There are defects on graphene sheets
- The procedure takes a long time and can be difficult.
- Not as promising as the CVD method is can be difficult
- ✓ View off article on graphene synthesis:
- Author - Keith E Whiteness, Jr Paul & Sheehan
- Source - Diamond and related materials.

Description:

- The promise of graphene a two dimensional hexagonal form of element carbon as a revolutionary material has sparked a flurry of research into it's optical.
- The most famous method of isolating graphene sheets introduced by Novoselov et.al in 2004.
- Use adhesive tape to mechanically cleave graphite crystals into successively thinner platelets.
- This micromechanical cleavage is time consuming and produces an abundance of few and multilayer graphene along with single layer material in addition.
- The area of the graphene sheet obtain by this method is limited by the initial size of the graphite Crystal these limitations of micromechanical cleavage along With the explosion of interest in graphene in general have led researchers to devise a number of alternative methods for graphene synthesis in this review, We discuss different Synthetic method for total citations.
- Progress in graphene synthesis and its application history challenge and the future outlook for research and Industry.
- A simple and efficient method Was introduced for the high Conversion preparation of graphon oxide (Go) from large graphite flakes (Average flake size - 100 um) using a simplified Hummers method.
- Natural reducing agents such as lemon Juice and Vinegar were compared with hydrazine (N₂H₄) as potential reducing agents.
- Graphene was prepared by chemical reduction of Go because this Method was Low Cost large-scale graphene production.

This one- pot graphene preparation was performed at room temperature. Different degrees of oxidation graphite in a mixture of sulfuric acid and potassium permanganate at different oxidation times and highly exfoliated Go sheets were produced. Go was subsequently reduced effectively by Lemon a new Green and potential reducing agents With pH 2.3 this reduced Go exhibited a high electrical Conductance of 24.6 usaltributed to its higher c/o ratio (~8:2) Compared. With other sampled.

Assessment of Structure and Distribution of Different Nematodes Infection in Vegetables Crops in Jaipur District

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Abstract

This work focuses on plant parasitic nematodes that effects on vegetables crops. The study took place in Jaipur region between March 2023 to April 2023 and involved 6 roots samples and 2 soils samples of 3 different vegetables in the families *Solanaceae* (2) *Malvaceae* (2) and *Triticaceae* (2) from 2 farming locations. In this study, 11 nematodes genus were founded in vegetables crops. The most common genus was Meloidogyne followed by Helicotylenchus. The highest Meloidogyne densities were founded in Tomato, okra and wheat. The comparative abundance of Meloidogyne, Helicotylenchus and Pratylenchus was 58%, 10.4% and 2.1% respectively. As other genus, the relative abundance was less then 1%. Nematodes identify with the help of Berman Funnel Method. The results show that soils properties are as important for the abundance, distribution and structure of the plant Parasitic nematode communities as the host plant. Findings may be helpful in improving the vegetable pest controls.

Keywords: Vegetable crops, Meloidogyne species, Nematode, Structure, Berman Funnel Method, Distribution.

Introduction

The nematodes or roundworms from the phylum Nematoda also called Nemathelminths, (Garcia, 1999 and CAP, 2017). With plant-parasitic nematodes also called eelworms. (Hay, 2020). They are a diverse animal phylum occupy a broad range of environments. Less correctly, they are classify as helminths, but are taxonomically classified along with arthropods, tardigrades and other moulting animals in the clade Ecdysozoa, and unlike flatworms, have tubular digestive systems with openings at both ends. They have a reduce number of Hox genes, but their sister phylum Nematomorpha has retain the ancestral protostome Hox genotype, which shows that the reduction has occurred within the nematode

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phylum (Bake and Woollard., 2019). Nematodes have successfully regulated to nearly every ecosystem: from marine (salt) to fresh water, soils, from the polar regions to the tropics, as well as the highest to the lowest of elevations. They are present everywhere in freshwater, marine, and terrestrial environments, where they often outstrip other animals in both independent and species counts, and are found in locations as diverse as mountains, deserts, and oceanic trenches. They are found in each part of the earth's lithosphere, (Borgonie G et al., 2011) even at great depths, 0.9–3.6 km (3,000–12,000 ft) below the outside of the Earth in gold mines in South Africa (Lemonick, 2011; Bhanoo, 2011; Drake, 2011 and Borgonie 2011).

In 1758, Linnaeus represented some nematode genera (e.g., *Ascaris*), then involved in the Vermes. The name of the group Nematoda, familiarly called nematodes, came from Nematodea, defined first by Karl Rudolphi (1808), (Chitwood, 1957). At its beginning, the Nematodea erroneously included Nematodes and Nematomorpha, imputed by von Siebold (1843). Besides with Acanthocephala, Trematoda, and Cestoidea, it found the obsolete group Entozoa, (Siddiqi, 2000). In 1861, Diesing attended to the group as order Nematoda, (Chitwood, 1957) But, in 1910, Grobben proposed the phylum Aschelminthes and the nematodes were included in as class Nematoda along with class Rotifera, class Gastrotricha, class Kinorhyncha, class Priapulida, and class Nematomorpha.

The phylogenetic relationships of the nematodes and their close relations among the protostomian Metazoa are unresolved. Traditionally, they were held to be a lineage of their own, but in the 1990s, they were proposed to form the group Ecdysozoa jointly with moulting animals, such as arthropods. The specification of the closest living relatives of the Nematoda has every time considered to be well resolved.

Round worms inhabit virtually each and every habitat in the seas, freshwater, and on land, even although some species have very particular habitats. Nematodes generally live in the spaces between aquatic sediments or on the sediment surface. Non-parasitic nematodes are adapted to swimming along the base of streams and lakes. They represent 90% of all animals on the ocean floor (Danovaro *et al.*, 2008). In total, 4.4×10^{20} nematodes inhabit the Earth's topsoil, or around 60 billion for each human, with the highest densities perceived in tundra and boreal forests. Their numerical supremacy, often exceeding a million individuals per square meter and accounting for around 80% of all individual animals on earth, their diversity of lifecycles, and their presence at various trophic levels point to a major role in many ecosystems (Platt, 1994). They have been shown to play crucial roles in polar ecosystems (Cary, 2019 and Adams, 2019). The many parasitic forms include pathogens in most plants and animals. A third of the genera take place as parasites of vertebrates; about 35 nematode species take place in humans (Roy, 2000).

Materials and Methods

Study Area

In our study we were selected two study sites regions in Jaipur. First Peeliya village, Jaipur and second Vivekananda Global University Agriculture farm, Jaipur.

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Sampling

Soils and roots samples were taken from 2 intensive vegetable farming areas between March 2023 and April 2023 in the Jaipur region. First soils and roots samples collected from Peeliya Village Jaipur, Rajasthan and second soils and roots samples collected from Vivekananda Global University agriculture farm, Jaipur, Rajasthan. The climatic conditions of this region did not change substantially for the time of study period, which means there is no dependence between nematode distribution and temperature swing. These locations were selected based on their level of significance for vegetable production, the volatility in vegetables being cultivated and their geographic distribution. The geographic coordinates of each location were determined using the Global Positioning System (GPS). A total of 2 soil samples and 6 root samples from 3 different vegetable crops like tomato wheat and ladyfinger were taken. Samples were placed onto different root and soil structures and stored in plastic bags until nematodes were removed.

Extraction of nematode

Nematodes were extracted individually from roots and soil for each of the collected samples. The roots were softly washed to remove as much soil as possible and then cut into pieces at randomly. Nematodes were separated from the 14-gram root sample taking the modified Berman funnel method for 48 h and Nematodes were also extracted from 52 g soil per sample using the same extraction method. The extraction of nematodes was gathered in beakers, allowed to settle for 2 h, and the supernatant was filter out. The final volume (about 2 ml per extraction) of the supernatant was transferred into a 10 ml tube and mixed with hot (65 °C) 4% formalin. The tubes were stored in a refrigerator at 4 °C until nematodes were identified and their population density appraised. Endoparasitic nematodes were inspected on a small amount of plant tissue using a stereoscopic microscope (10 × magnification) with transmitted or incident light.

Results and Discussions

Assessment of nematode population density and qualitative analysis

The total number of samples collected per crop diversified depending on the crop. Plant parasites of 11 genera have been encountered, namely: Scutellonema, Helicotylenchus, Aphelenchoides, Hemicriconemoides, Ditylenchus, Meloidogyne, Rotylenchulus, Quinisulcius, Xiphinema, Tylenchulus and Pratylenchus but Meloidogyne was the most densely populated genus with the average population density of 35 nematodes per gram of sample volume, followed by Helicotylenchus (5 nematodes per gram), Pratylenchus (4 nematodes per gram) and Scutellonema (2 nematodes per gram). As for other nematode species, there was less than 2 nematode per gram founded in the sample. As can be seen, the population density of the first 3 nematode species was higher ($P < 0.05$). The highest population densities of Meloidogyne sp. Were recorded in tomato, okra and wheat. Analysis involved greenhouse vegetables in the families solanaceae (2) malvaceae(2) and triticeae(2).

Nematodes of the genus *Ditylenchus*, *Xiphinema* and *Tylenchulus* were removed from two cereals each and amounted to 9.1% of all crops sampled. 9 species found on tomato and okra. Genus *Hemicriconemoides*, *Aphelenchoides*, *Ditylenchus*, *Quinisulcius*, and *Scutellonema* are unusual for the Jaipur region. During the experiment, I was found that the capability of crops to these newly registered nematodes depends on the species of nematodes, and the infection level can range from 28.8% to 64.21%.

The number of samples (roots or soils) taken from the moderate area, the arid area, was 44, 40 respectively. I was found that wet soils are habitats to the greatest diversity of plant-parasitic nematodes (11 nematode species). In arid and moderate zones, *Meloidogyne* had the highest population density, followed by *Helicotylenchus*.

Abundance and prevalence of plant-parasitic nematodes

According to the results of the assays, *Meloidogyne* was the most common genera encountered in the samples (80% of the 8 root and soil samples examined). The second common genus, *Helicotylenchus*, was approximately 60.8%. The relative affluence of nematodes *Meloidogyne*, *Helicotylenchus*, and *Pratylenchus* was 58.3%, 10.4%, and 2.1%, respectively.

This study provides complete instruction about the plant-parasitic nematodes found in vegetable crops to distend the limited research (Seidet *et al.*, 2015). Focused mainly on tomato; whereas (Reddy, 2008) focused on a broader range of crops; including wheat, okra and tomato, yet both studies dealt with just a few vegetables. It is usually possible that the presence of the host plant is the main factor considering the population density of nematodes infecting on vegetable crops and noted that almost all vegetable crops were registered as hosts for at least 2 nematode species. The almost cultivated and consumed vegetable crops (Diab *et al.*, 2019, Gomes *et al.*, 2003) are good hosts for the 2 most usually nematode genera *Meloidogyne* and *Helicotylenchus*. Therefore, it is not surprising that these 2 genera are widely distributed all over the country. The supremacy of *Meloidogyne* on vegetable crops was previously reported by other researchers (Almohith *et al.*, 2018, Altaibaeva *et al.*, 2016), who confirmed that these nematode species are abundant in vegetable farming. Among the nearly 1500 genera of nematodes, which contain 4000 phytoparasitic nematode species (Jones *et al.*, 2013), the one most commonly seen is *Meloidogyne*. This knowledge aligns with the results of this study. Nematodes living in the soil need an aerobic aquatic environment; accordingly, particular attention should be paid to variation in the soil moisture content. The current study verifies that nematodes *Meloidogyne*, *Pratylenchus* and *Helicotylenchus* can possibly cause great damage to vegetables, indicating the need to pay more awareness to these nematodes in order to increase the production of most vegetable crops grown in Jaipur. The findings obtained during the study may be helpful in developing proper strategies for integrated nematode management.

Conclusion

During the study on vegetable crops, 11 nematode genera were founded, namely: Scutellonema, Helicotylenchus, Aphelenchoides, Hemieconemoides, Ditylenchus, Meloidogyne, Rotylenchulus, Xiphinema, Quinisulcius, Pratylenchus and Tylenchulus. of these, Meloidogyne turned out to be the most common one with the average population density of 45 nematodes per gram of root volume. The highest Meloidogyne densities were observed in tomato, okra and wheat in all repetitions. Investigation of 8 root and soil samples revealed 81% of Meloidogyne infection cases. These findings show that aside from the host plant, soil properties also play an important role in the affluence, distribution and structure of plant-parasitic nematode communities. These findings may serve as a structure for further research aimed at the improvement of vegetable pest controls.

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Biosynthesis of Fe₃O₄ Nanoparticles for Antibacterial Activity

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Abstract

The Present work outlines the antibacterial activity of Fe₃O₄ nanoparticle synthesized through chemical combustion method where ferric nitrate is used as precursor material and urea as fuel with the assistance of tween 80, a non-ionic surfactant. The obtained Fe₃O₄ nanoparticles were characterized by X-Ray diffraction. Various parameters such as dislocation density, micro strain, analysis of weight loss and surface morphological studies were calculated. The particle size was calculated from XRD & SEM and it was found to be 33-40 nm.

Keywords : Biosynthesis, Fe₃O₄ Nanoparticles, Antibacterial Activity.

Introduction

Nano materials are widely synthesized for their properties like optical, mechanical and magnetic properties to counter the bulk materials. Metal oxides are used in various applications like magnetic storage, catalysis and biological applications like bone tissue engineering. The prolonged life expectation and aging of population has brought the escalating request of artificial material to regenerate diseased bones. Nanotechnology has responded to the situation with various ceramics with its bioactivity mechanical properties and ability to kindle bone growth. In particular iron oxide powder at nanometer is utilized at length because of the development in preparation technology. Mono dispersed magnetite nanoparticles have given a new impetus in the application field where magnetic nanoparticles are extensively used in ferro fluids biological imaging and therapies. Magnetic iron oxide (Fe₃O₄) with oxygen forming face centered cubic and cubic inverse spinel structure. Interstitial tetrahedral sites and octahedral sites are occupied by iron (Fe) cations. At the room temperature Fe²⁺ and Fe³⁺ ions flip between themselves in the octahedral sites giving rise to a class called half-metallic materials. The desired physical and chemical properties of magnetite nanoparticles are synthesized by several chemical synthetic routes like co-precipitation of aqueous ferrous and ferric solutions. Super paramagnetic nanoparticles are highly exciting materials because of their uses in magnetic resonance imaging (MRI), drug delivery and cell separation. In the area of antibacterial agents metal nanoparticles are of a particular interest because they

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could be synthesized with high surface area with highly potential active sites (Alagiri *et al.*, 2014; Blaney *et al.*, 2007; Gil *et al.*, 2015)

Iron oxide nanoparticles are iron oxide particles with diameters between about 1 and 100 nanometers. The two main forms are magnetite (Fe_3O_4) and its oxidized form maghemite ($\gamma\text{-Fe}_2\text{O}_3$). They have attracted extensive interest their superparamagnetic properties and their potential applications in many fields (although Co and Ni are also highly magnetic materials, they are toxic and easily oxidized). Application of iron oxide nanoparticles include devices, catalysis, sensors, superparamagnetic relaxometry, high-sensitivity biomolecular magnetic resonance imaging, magnetic particle imaging, magnetic fluid hyperthermia, separation of biomolecules, and targeted drug and gene delivery for medical diagnosis and therapeutics (Hanus *et al.*, 2013, Xiao *et al.*, 2015; Yew *et al.*, 2020).

Biosynthesis of
 Fe_3O_4
Nanoparticles for
Antibacterial
Activity

Materials and Methods

The chemical reagents used in this work were ferric nitrate, surfactant Tween 80, Urea and ammonia solution. Analytical grade chemical reagents were used throughout the experiment. We have taken four bacterial species, gram-positive *Staphylococcus aureus* and gram-negative *Xanthomonas*, *Escherichia coli* and *Proteus vulgaris* (Malekzadeh *et al.*, 2017).

Characterization Techniques

X-Ray Diffractometry

XRD is the widely used technique to analyse the crystalline/amorphous nature of nanoparticles along with its phase and purity of sample. The XRD pattern of finely ground NPs was observed under wide range of Bragg's angle (θ). Philips Pert PRO analytical instrument operated at 40 kV and 30 mA current with Cu K α radiation ($\lambda = 0.15418 \text{ nm}$). The average particle size was calculated by Debye Scherrer formula (Lunge *et al.*, 2014; Lu *et al.*, 2014).

$$D = \frac{0.94}{\lambda \sin \theta}$$

Where, D is thickness of nanoparticle, λ is wavelength of X-ray. θ is half maxima of reflection at Bragg's angle 2θ and θ is diffraction angle or Bragg's angle.

Field emission scanning electron microscopy (FE-SEM)

The size and morphology of the iron oxide nanoparticles was determined by high-resolution field emission scanning electron microscope (FEI NOVA NANOSEM-600). Thin film of the Iron oxide NP sample was prepared on carbon coated copper SEM grids by just dropping the suspension of nanoparticles in water on the grid, extra sample solution was removed by blotting paper and then the sample was allowed to dry for 5 min and then sample images were then taken. Fourier transform infrared spectroscopy (FTIR) is a technique which is used to determine the chemical functional groups in the sample. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted).

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Results and Discussion

Preparation of neem leaf (*Azadirachta Indica*) extract

Collection of Extract: Neem leaves in good health were gathered for this project in Jaipur, Rajasthan. They were cleaned and washed in distilled water to remove any dust, and they were then put in an oven for two to three days at a temperature of 50 to 70 degrees. The dried leaves were then broken up into tiny pieces and ground into a fine powder. In a conical flask, 50 ml of distilled water was combined with about 5 g of finely ground neem leaf powder. The liquid was then heated to boiling at 80 C. To obtain plant extract, the mixture was next filtered through Whatman filter paper No. 1. The green, clear extract solution after filtering was kept for later use.

Biosynthesis of iron oxide Nanoparticles

The process reported by Nur Diyana Syazwani Zambri et al. was used to create Fe_3O_4 -NPs. In a typical process, 50 ml of sterile deionized water was used to dissolve 1.10 g of iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) at 1:2 molar ratios while being constantly stirred. Then, drop by drop, 5 ml of neem leaf extract and NAOH solution were added to this solution. A magnetic stirrer was used to agitate the liquid for two hours. The sudden development of black colour suggested the creation of Fe_3O_4 -NPs. Overnight, the sample was allowed to settle. After centrifuging, distilled water was used to rinse the required product (Hu *et al.*, 2018, Hanus *et al.*, 2013).

XRD

XRD patterns of both Fe_3O_4 nanoparticle samples are shown in Fig. 1. It can be seen that sample are single phase and have the ferrite spinel structure. The size of the samples can be calculated by Scherer equation which is 23 nm for Fe_3O_4 (S) and 22 nm for Fe_3O_4 .

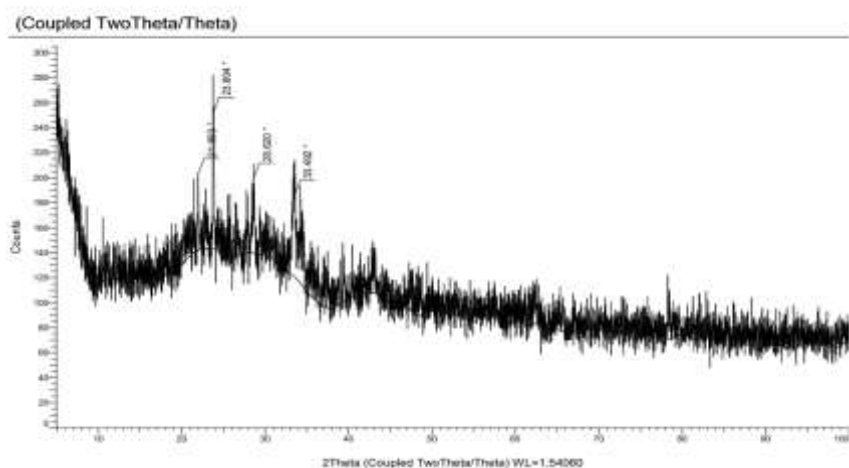


Fig 1: XRD pattern of synthesized Fe_2O_3

In order to measure the magnetic properties of ferrite nanostructure samples, magnetic hysteresis curve prepared using AGFM. Figs. 14 and 15 illustrate the hysteresis curve for both Fe₃O₄ (S) and Fe₃O₄ (ME) samples. As can be seen, both plots show the super paramagnetic property and the saturation magnetization (around 15 emu/gr) occurred at 4000 Oe. the SEM micrograph of the Fe₃O₄ samples produced by starch as inhibitor (Wu *et al.*, 2015; Tran *et al.*, 2010, Tavangarian *et al.*, 2012).

It is clear that SEM is one of the most important and widely used methods for determining particles size and size distribution with an accuracy of less than a nanometre. The size of the Fe₃O₄ (S) nanoparticles is around 20 nm from the SEM photograph.

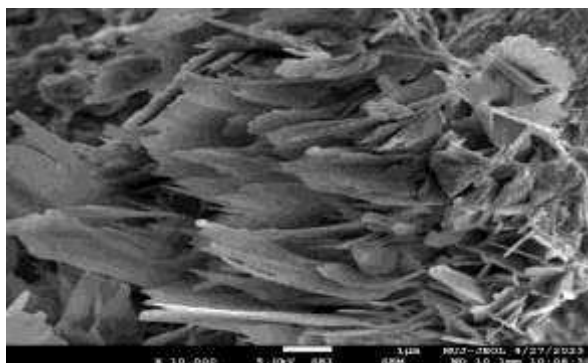


Fig 2: SEM of green synthesized Fe₂O₃

Antibacterial study of green synthesized Fe₂O₃ nanoparticles is the next step of this study. Since there is various applications of Fe₂O₃ as reviewed above, so it is mandatory to assess antibacterial study for their safe use.

Conclusion

The novel facile Surfactant TWEEN80 has been used to synthesis Fe₃O₄ for the first time with fuel urea. The XRD result and SEM results confirmed Fe₃O₄ has the crystallite size 35 nm. The differential thermal analysis/thermogravimetric analysis showed the weight due to vapour carbon. The dislocation density has decreased with the increase in the crystallite size. Similarly, the micro strain has increased with the decrease in the crystallite size. The Fe₃O₄ nanoparticles showed their antibacterial properties on both gram positive and gram-negative bacterial strains. As the diameter of the zone of inhibition is high, we can conclude that Fe₃O₄ is a very effective antibacterial agent. Pharmaceutical field begins to develop in recent decades and has introduced a huge number of novel drug delivery system. Most of them are still in incipient stage, including Fe₃O₄-NPs. Plenty of factors make Fe₃O₄-NPs the potential Nano drug carrier in drug delivery system (Patra *et al.*, 2017; Zayed *et al.*, 2012, Xang *et al.*, 2007).

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The usage of external magnetic field which guides the Fe₃O₄-NPs to the specific region shows the promising applications of Fe₃O₄-NPs in variety of biomedical related field, particularly targeted drug delivery. In this research, Fe₃O₄ nanoparticles were produced by wetted chemical method (20 nm) using ME and starch as an inhibitor separately. It can be concluded that the size of Fe₃O₄ nanoparticles can be controlled by the type of inhibitor and inhibitor concentration. It is also concluded that the size of the nanoparticles can affect on their antibacterial properties and the diameters of inhibition zone. ION are very fascinating nanomaterials that can be used in many current biomedical applications including cell labelling, drug targeting, gene delivery, biosensors, hyperthermia therapy and diagnostics by MRI.

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Biosynthesis of
 Fe_3O_4
Nanoparticles for
Antibacterial
Activity

Comparative Study of Air Quality Between Urban and Sub-urban Area in Jaipur District

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Abstract

Air pollution is a matter of great concern worldwide because of the health risks to individuals due to pollution. Great exposure to pollutants such as airborne particulate matter and ozone leads to increases in mortality, respiratory and cardiovascular disease. Air pollution is increasing due to emissions from industries and many residential areas and many anthropogenic activities. It has become a very big issue for human being worldwide (Yang *et al.* 2004 and Afroz *et al.* 2003). The study analyzed the variation in Annual average concentration of three pollutants NO₂, SO₂, PM at four selected monitoring sites namely Ajmeri gate and Chandpol (Commercial Area in Urban area) in Jaipur City, whereas VKIA (Vishwakarma Industrial Area) and Sitapura (in sub-urban area) Jaipur, Rajasthan, India from year 2021-2022 and to assess the ambient air quality on the basis of comparison with National Ambient Air Quality Standards and the Overall Air Quality Index (AQI). From the analysis of different sites, the data shows that concentration of SO₂ was always below the permissible limits as specified by CPCB (50 µg/m³) at all the study areas while PM has crossed the permissible limits at all the monitoring sites over the whole study period with a higher value observed for Vishwakarma Industrial Area in 2022. The study also shows that PM (Particulate matter) has highest concentration than both NO₂ and SO₂, making it a prominent pollutant. It was observed that AQI values ranges from Moderate to Very Poor at Vishwakarma Industrial Area Thus it shows that Vishwakarma Industrial Area is more polluted as compared to all studies area, Jaipur.

Keywords: Jaipur, NO₂, SO₂, PM, Air Quality Index.

Introduction

Air is the mixture of gases that fills the air, offering life to the human, plants and creatures that make Earth as a living planet. Air is composed of mainly two gases (78.09% nitrogen and 20.85% oxygen), with a couple of different gases, for example, carbon dioxide (0.04%) and argon (0.93%) (Seetharam *et al.* 2009). The

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term air quality describes the condition of the surrounding air. In the developing countries, the air quality is one of the biggest concerns (Dockery *et al.* 1994). Environmental pollution becomes a big risk to health. Environmental pollution leads to significant risk of premature death and disease. India has one of the world's highest levels of exposure to air pollution. Air pollution is one of the major environmental concerns of both developed and developing countries because of its direct impact on the human health. In India, surrounding air quality have progressively deteriorated due to anthropogenic sources like rapid urbanization, industrialization, uncontrolled increase of vehicles on poor road conditions, construction debris, garbage burning, lack of public awareness (Guttikunda *et al.* 2011). Indian cities are in the top 30 most polluted cities of the world. In Indian cities the ambient atmospheric conditions have generally get worsen due to lack of awareness about the impacts of urbanization and industrial development. Air pollution in sites located in or near industrial or commercial areas have a comparatively higher pollution levels than the sites in residential areas. Large number of industries and vehicles are creating problem to our environment. A number of air pollutants are released from industries and other activities and may cause bad effects on human health and the environment. An air pollutant is any substance which may harm humans, animals and vegetation. As far as humans are concerned an air pollutant may cause increase in mortality or serious illness to human health. Pollutants harm our environmental either by increasing level of harmful material (Brunekreef *et al.* 2008). According to WHO, six major air pollutants are particle pollution, ground-level ozone, carbon monoxide, sulfur oxides, nitrogen oxides, and lead.

AQI(Air Quality Index)

AQI is a method of conversion of complex air quality in a single digit to make it easy understandable to the common people (Yadav *et al.* 2012) It is calculated by collecting the ratio of pollutant concentration in surrounding air to the standard limit of pollutants in surrounding air (Ziauddin *et al.* 2006). Basic formula to find out air quality index

$$AQI = \frac{SPM}{SPM \text{ Standard}} \times 100 \quad (1)$$

Materials and Methods

Study Area

Jaipur is the capital of Rajasthan state which is situated in the eastern part of Rajasthan. Jaipur is the largest state of India. It is commonly known as "Pink City" for its building color. It is famous for tourist visit. Jaipur was founded in year 1727 AD by Maharaja Swai Jai Singh and Jaipur is famous for their Rajputana culture and forts. It is the largest city of Rajasthan state and located at the distance of about 268km from national capital New Delhi. Jaipur district has a geographical area of 11,143 km² and is located at 27.1425146 N and 75.0397275 E. Jaipur district covers almost 3.23% the total area of Rajasthan stat very high population density. It is the tenth most populated city of the country. Jaipur district has attractive forts and beautiful palaces (Dadhich *et al.* 2018). The city is well connected by rail, road, and

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air transportation network to the country. It has a semi-arid climate with winter, monsoon, and summer season. The relative humidity ranges between 35 and 63%. The average annual rainfall is around 60 mm. Maximum rainfall occurred in the month of August in 2006 which is 166mm. Jaipur city faces hot climate with highest temperature during May- June months. The maximum & minimum temperature of the Jaipur district is 45degree Celsius and 5 respectively. Because of continuous urbanization and industrialization, the most area of Jaipur have been transformed to industry zone and built-up area (Mehta 2019).

Site Description

The quality of air of Jaipur city has become polluted due to various anthropogenic activities, vehicular emissions and various surrounding industrial activities. Four different sites were selected as monitoring site in the study in order to examine air quality. Sites were selected according to urban and sub-urban area; Two different sites were selected of urban area and 2 sites were selected of sub-urban area. In urban site, Chandpol and Ajmeri gate were selected as monitoring site in study area whereas both the sites are fascinating towards tourists, Chandpol and Ajmeri gate both belongs to a commercial area where bazaar vendors trade in marble, textiles, cotton quilts, footwears, stone sculptures etc. leading to heavy traffic due to two, three and four wheelers and buses. In sub-urban area Sitapura and VKIA (Vishwakarma Industrial area) were selected as monitoring sites. VKIA is very much close to Industrial Area, it has mainly automobile, paper, Chemical, Marble, Rubber industries etc. and also surrounded by Commercial complexes and Residential area where a Sitapura is an industrial area. Study is being made on the time basis (annually). It is found that there is a major difference between the pollution of urban area and sub-urban area.

Data Set

Data used in this study is secondary data and was collected from Rajasthan State Pollution Control Board (RSPCB) website for Jaipur city from 2021-2022 for four different monitoring sites Chandpole, Ajmeri gate (selected as urban sites) and Vishwakarma Industrial area, Sitapura (selected as sub-urban area).

Table1: National Air Quality Index and its possible health impacts

AQI value	Nomenclature	Health Impact
0-50	Good	Minimal Impact
51-100	Satisfactory	Minor breathing discomfort to sensitive people
101-200	Moderate	Breathing discomfort to the people with Lung, Heart disease, children and older adults
201-300	Poor	Breathing discomfort to everyone on prolonged exposure
301-400	Very Poor	Respiratory illness to everyone on prolonged exposure
>400	Severe	Respiratory effects even on healthy people

Source: http://rspcbmis.environment.rajasthan.gov.in/NAMP_Report.aspx?HeaderId=4&MenuId=6

Table2: AQI of NO₂, SO₂ and PM in selected Urban and Sub-urban areas in Jaipur District(Jan-Dec 2021)

2021	NO ₂ (µg/m ³)		PM (µg/m ³)		SO ₂ (µg/m ³)		AQI
	Max.	Min.	Max.	Min.	Max.	Min.	
Ajmeri gate	54.19	22.04	314	43	9.88	5.5	264.00
Chandpole	54.27	21.6	234	61	8.86	5.3	189.33
VKIA	39.96	19.43	318	53	13.11	5.98	268.00
Sitapura	45.82	16.25	253	29	10.04	4.83	184.00

Table3: AQI of NO₂, SO₂ and PM in selected Urban and Sub-urban areas in Jaipur District (Jan-Dec 2022)

2022	NO ₂ (µg/m ³)		PM (µg/m ³)		SO ₂ (µg/m ³)		AQI
	Max.	Min.	Max.	Min.	Max.	Min.	
Ajmeri gate	57.56	8.55	296	30	25.67	2.1	211.00
Chandpole	60.06	9.28	376	50	18.25	3.1	326.00
VKIA	55.25	14.08	540	56	21.49	3.12	510.00
Sitapura	61.65	14.75	257	87	17.77	2.4	207.00

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Abbreviation: NO_2 - Nitrogen dioxide, PM- Particulate matter, SO_2 - Sulfur dioxide

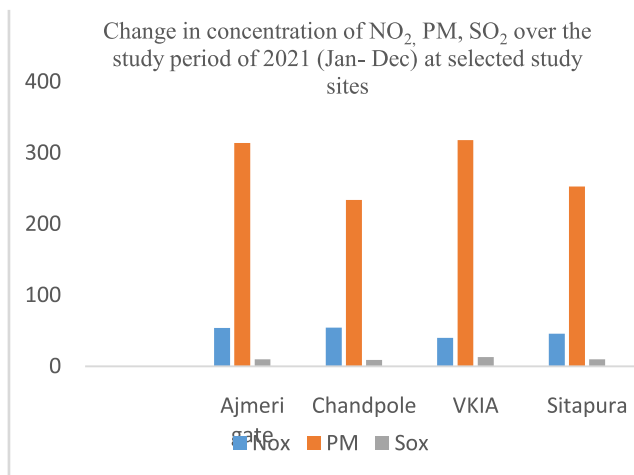


Figure2: Graphical representation of concentration of NO_2 , SO_2 and PM in Urban and Sub-urban areas during year from January to December2021 in Jaipur District

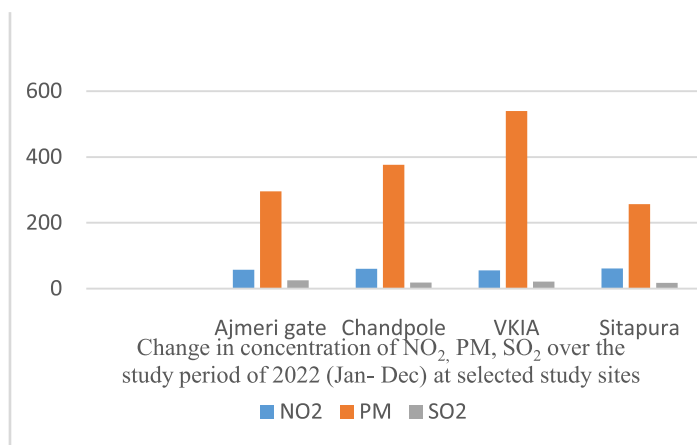


Figure2: Graphical representation of concentration of NO_2 , SO_2 and PM in Urban and Sub-urban areas during year from January to December2022 in Jaipur District

Result and Discussion

According to the data collected from RSPCB Website, annual average concentration of namely three pollutants NO₂, PM and SO₂ overall computed AQI for the four different monitoring sites on the basis of urban and sub-urban area.

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Nitrogen dioxide (NO₂) Nitrogen dioxide is a traffic-related pollutant, as it is emitted from automobile (Richmont *et al.*, 2017). the concentrations over 0.2 ppm produce many adverse effects in humans such as respiratory diseases, while concentrations higher than 2.0 ppm affect T-lymphocytes. It is found that maximum average concentration of NO₂ for Ajmeri gate, Jaipur has been observed in year 2022 i.e. (57.56 µg/m³ and AQI 211.00) and a minimum was observed in 2021(54.19 µg/m³ and AQI 264.00) while for Chandpole, a commercial area the maximum (60.06 µg/m³, AQI 326.00) and the minimum (54.27 µg/m³, AQI 189.33) average concentrations were observed in year 2022 and 2021. For VKIA maximum (55.25 µg/m³, AQI 510.00) and minimum (39.96 µg/m³, AQI 268.00) concentration were observed in 2022 and 2021 respectively. For Sitapura maximum (61.65µg/m³, AQI 207.00) and minimum (45.82µg/m³, AQI 184.00) concentration were observed in 2022 and 2021 respectively. It also shows that the concentration of NO₂ has crossed the National Ambient Air Quality Standards as specified by CPCB (40 µg/m³) in year 2022,2021 at Ajmeri gate and in 2022, 2021 at Chandpole, Jaipur and also in VKIA NO₂has crossed the National Ambient Air Quality Standards as specified by CPCB in 2022 and in 2022, 2021 at Sitapura.

Particulate matter (PM) - Particulate matter contains tiny liquid or solid droplets that can be inhaled and cause many serious health effects. (Cheung *et al.*, 2011) The annual average concentration for PM crosses the National Ambient Air Quality Standards of 60 µg/m³ (Specified by CPCB) at both the locations in whole study period. The maximum and the minimum concentration observed for PM10 at Ajmeri gate was in year 2021(314 µg/m³, AQI 264.00) and in year 2022 (296µg/m³, AQI 211.00) and for Chandpole, maximum average concentration was observed in year 2022 (376µg/m³, AQI 326.00) and minimum average concentration in 2021 (234µg/m³, AQI 189.33) and for VKIA maximum average concentration was observed in year 2022 (540µg/m³, AQI 510.00) and minimum average concentration in 2021 (318µg/m³, AQI 268.00) and for Sitapura maximum average concentration was observed in year 2022 (257µg/m³, AQI 207.00) and minimum average concentration in 2021 (253µg/m³, AQI 184.00). It also shows that the VKIA, Jaipur experiences a higher PM concentration than all remaining study area over the whole study period. This is because of heavy traffic in industrial area due to loading of finished products and unloading of raw materials.

Sulfur dioxide (SO₂) Sulfur dioxide is a harmful gas that is emitted mainly from fossil fuel consumption (in complete consumption) or industrial activities. The annual average concentration of SO₂ has never crossed the prescribed limit 50 µg/m³ as specified by CPCB over the whole study period at all the monitoring sites. In Ajmeri gate, Jaipur the maximum concentration was observed in year 2022

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(25.67 $\mu\text{g}/\text{m}^3$, AQI 211.00) and a minimum was observed in 2021 (9.88 $\mu\text{g}/\text{m}^3$, AQI 264.00), while for Chandpole, Jaipur the maximum concentration observed was (18.25 $\mu\text{g}/\text{m}^3$, AQI 326.00) in year 2022 and minimum concentration was observed in year 2021 (8.86 $\mu\text{g}/\text{m}^3$, AQI 189.33), and in VKIA, Jaipur the maximum concentration was observed in year 2022 (21.49 $\mu\text{g}/\text{m}^3$, AQI 510.00) and a minimum was observed in 2021 (13.11 $\mu\text{g}/\text{m}^3$, AQI 268.00), in Sitapura, Jaipur the maximum concentration was observed in year 2022 (17.77 $\mu\text{g}/\text{m}^3$, AQI 207.00) and a minimum was observed in 2021 (10.04 $\mu\text{g}/\text{m}^3$, AQI 184.00).

Conclusion

Analysis reveals that air quality in some areas of cities are very poor apart there are so many areas where the quality of air is good as compared to other areas. The amount of air pollutants in air is higher in day as compared to night because of the smaller number of vehicles travelling at that time. In industrial area amount of air pollution is higher as compared to the residential areas. Government should apply strict laws for the industries and Electric vehicles should be promoted. Awareness programs should be run by government and NGOs. Tree plantation should be improved. It concluded that air pollution at the study site is mainly because of traffic. Traffic diversions, restricting heavy vehicles movement through residential roads, arranging for periodic vehicle maintenance and encouraging public transport instead of private vehicles are beneficial to control air pollution due to transportation. In addition, public awareness for environment protection should be adopted and green plantation along highway and within industries should be encouraged. It may, thus be concluded that strict implementation of environmental regulations and adoption of adequate pollution control measures is need of the hour.

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Quinoa on the Rise: Exploring Global Market Expansion, Export Opportunities, and Overcoming Challenges

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Abstract

Quinoa is a whole grain crop known for its edible seeds and leaves, belonging to the Amaranth family. It is gluten-free and rich in various nutrients such as zinc, iron, fibers, antioxidants, and vitamins B and E. Quinoa offers numerous health benefits. India has adopted quinoa across various sectors, including food and beverages, cosmetics, pharmaceuticals, and animal feed. India, although accounting for just 0.6 percent of global quinoa exports, holds the 13th position among all agricultural commodities. The United States is the largest importer of Indian quinoa, accounting for more than 50 percent of the country's exports. Canada has also shown significant growth in quinoa imports from India in the past five years. While India's export of quinoa has been increasing, there are several challenges to overcome. These challenges include market competition with established quinoa producers like Peru and Bolivia, infrastructure and logistics issues, meeting quality standards and certifications, maintaining price competitiveness, building market awareness and branding, and navigating export regulations and trade barriers. To address these challenges, continuous efforts in research and development, infrastructure improvement, capacity building, market diversification, and effective trade promotion strategies are necessary. Opportunities for expanding quinoa exports from India include increasing cultivation, emphasizing quality assurance, investing in value addition and processing, exploring new markets and market diversification, branding and promotion, and establishing collaborations and trade partnerships. Indian exporters should conduct market research, adapt to evolving market trends, and capitalize on these opportunities to successfully increase quinoa exports. Collaboration between the government, agricultural institutions, exporters, and stakeholders is crucial to overcome challenges and enhance India's export potential for quinoa.

Key Words : Edible seeds, Export, Quality standards, Market diversification, Trade promotion

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Introduction

Quinoa refers to a whole grain crop, belonging to the Amaranth family, which produces edible seeds and leaves. It is a gluten-free food product containing a high amount of zinc, iron, fibers, antioxidants, vitamin B and E, etc. Quinoa aids in reducing the risk of cardiovascular diseases, improving metabolism, controlling high blood pressure levels, enhancing skin health, maintaining blood sugar, etc. In India, it is widely adopted across diverse sectors, including food and beverages, cosmetics, pharmaceuticals, animal feed, etc.

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Trade status of quinoa from India to the world

India's exports represent 0.6 per cent of world exports for quinoa. Quinoa ranking in world exports is 13 among all agri commodities. The average distance of importing countries is 10083 km and the export concentration is 0.31. United state of America imported highest amount of quinoa which is valued USD 584 thousand. USA alone import more than 50 percent of quinoa from India. If we talk about the growth in export of quinoa, Canada imported quinoa with 45 per cent growth per annum in the last five years from 2018 to 2022. India exported highest quantity of quinoa to the Morocco with 224 percent per annum growth in last five years from 2018 to 2022. India exported quinoa to Georgia in last year 2021-2022 with highest growth of 288 per cent. USA ranked first in top ten partner countries importing quinoa from India, after that Canada is on second rank and Netherlands on fifth rank in import and rest of the countries are shown in table 1. Total imports growth in value of top ten partner countries between 2018 to 2022 only 4 countries shown positive growth among them highest growth shown by Morocco. Morocco share only 0.2 per cent in world import of Quinoa. Concentration of all supplying countries of partner countries, Kenya has 0.51 per cent concentration and India facing highest tariff against Kenya which is 25.

Table 1: Trade indicators for the Quinoa export with top ten partner countries in the world

Importers	Value exported in 2022 (USD thousand)	Trade balance 2022 (USD thousand)	Share in India's exports (%)	Quantity exported in 2022	Unit value (USD /unit)	Growth in exported value between 2018-2022 (% , p.a.)	Growth in exported quantity between 2018-2022 (% , p.a.)	Growth in exported value between 2021-2022 (% , p.a.)	Ranking of partner countries in world imports	Share of partner countries world import (%)
Total	1107	1027	100	0		17	12	-11		100
USA	584	584	52.8	273	2139	13	4	137	1	33
Morocco	154	154	13.9	82	1878		224	31	45	0.2
Greece	71	71	6.4	42	1690		26	-4	25	0.6
Netherlands	68	68	6.1	42	1619			152	5	4.4
Canada	43	43	3.9	15	2867	45	51	27	2	10.3
Bulgaria	36	36	3.3	20	1800				34	0.3
Thailand	27	27	2.4	14	1929	-20	-13	73	30	0.4
Georgia	23	23	2.1	13	1769			288	77	0.04
Kenya	19	19	1.7	6	3167			54	98	0.01
Mongolia	13	13	1.2	4	3250	8	-18	26	93	0.02

Resource: Authors compilation from secondary data selected from ITC trade map

Export status of Quinoa in the world in terms of value

Export of Quinoa is declining in last two years from 2021 to 2022, before that from 2018 to 2020 quinoa export was increasing. In table 2 highest exports can be seen in the Peru followed by Bolivia, Netherlands and USA in last 5 years from 2018 to 2022. India exported valued USD 1106 thousand quinoa to the world in 2022 which is only USD 282 thousand in the year 2018. India has 13th rank in the top exporters of the quinoa in the world in terms of value. Highest export of quinoa seen in the year 2021 valued USD 1371 thousand from India. Top Exporters data of quinoa shown in table 2.

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Table 2: Top 10 exporters of the quinoa in the world in terms of value

Value in Thousand USD

Exporters	2018	2019	2020	2021	2022
World	290270	321163	305332	243356	200053
Peru	121829	134460	124706	104832	88994
Bolivia	80630	90656	92414	61709	46462
Netherlands	24500	15084	14023	12597	11841
United States of America	15358	13996	13829	12125	10486
Canada	2881	3680	7611	7938	6340
Germany	7054	7886	8745	8306	5881
Spain	8111	16489	9225	7969	5488
Italy	3297	4139	4324	4831	4752
France	6312	7172	6774	4646	4014
Ecuador	4270	6117	4572	3380	3316
India	282	960	1009	1372	1106
Others	15746	20524	18100	13651	11373

Resource: Authors compilation from secondary data selected from ITC trade map

Export status of Quinoa in the world in terms of Quantity

In all over the world quinoa exported 102.36 million tons in the year 2022 but it was 112.23 million tons in 2018, it means there is decline in quantity exported from different countries in the world which shows declining trend. Highest quantity exported by the Peru followed by Bolivia, Netherlands and Canada in last 5 years from 2018 to 2022. It is seen that in 2022, Chile is on 3rd position in export quantity of quinoa. India has 13th rank in the top exporters of the quinoa in the world in terms of quantity. Highest export of quinoa from India to the world was seen in 2021 which is 829 tons. Top ten countries export data is shown in table 3.

Table 3: Top 10 Exporters of quinoa in the world in terms of quantity

Quantity in Tons

Exporters	2018	2019	2020	2021	2022
World	112237	112480	120142	112585	102362
Peru	50084	48781	50998	51598	45530
Bolivia	33106	32145	37298	28158	22324
Chile	24	29	60	44	10101
Netherlands	6918	4303	4253	4264	4641
Canada	3344	4001	7041	5804	4319
United States of America	4434	3825	3681	3377	3069
Spain	3101	5073	3464	3726	2514
Germany	1723	2066	2231	2310	1822
Italy	1298	1312	1347	1406	1477
Ecuador	1719	2389	1798	1439	1442
India	106	561	615	829	712
Others	6486	8556	7971	10459	5123

Resource: Authors compilation from secondary data selected from ITC trade map

Mapping of importing markets of Quinoa exported by India

United State of America is the largest importer of quinoa exported from India. USA imported 11 per cent share in Indias export which is more than 50 per cent. Other partner countries also mapped and shown by the arrow in figure 1



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Figure 1: Importing markets for quinoa exported by India in 2022

Prospects for market diversification for quinoa exported by India

Graph plotted between annual growth of partner countries imports from the world during 2018 to 2022 and Share of partner countries in India, 2022. Share of United State of America in Indias export is 52.8 per cent. USA import annual growth rate is -6 per cent which is increasing in last one year and share in world imports 33.01 per cent. USA import growth from the world is less than India export growth to USA. Share of Thailand is 2.44 per cent in India s export. Annual growth rate of Thailands imports is -5 per cent. Share in the world imports is -5 per cent which is increasing and Thailand import growth from the world is greater than Indias export growth to Thailand. Market diversification is shown in figure 2.

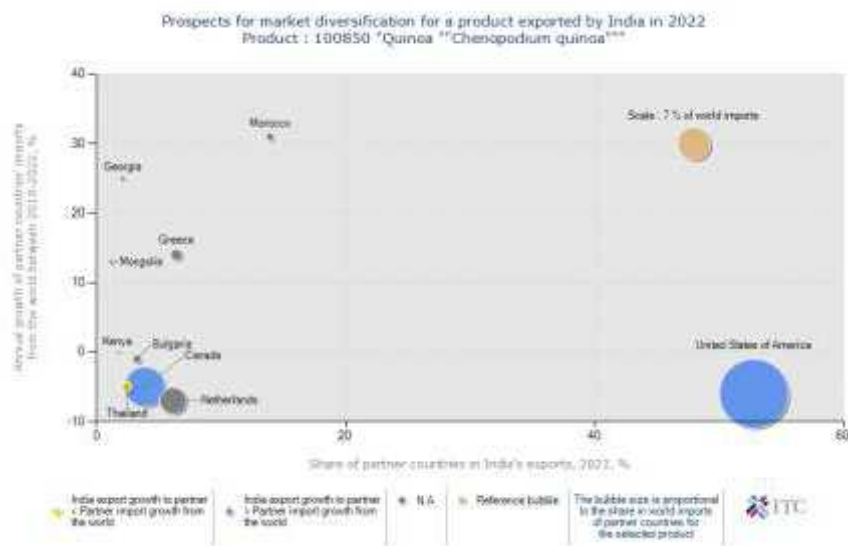


Figure2: Market diversification for Quinoa exported by India

Resource: Authors compilation from secondary data selected from ITC trade map

Growth of national supply and international demand for Quinoa exported by India

India has been increasing its supply and exporting quinoa to meet international demand. Quinoa cultivation in India has been expanding, particularly in regions like Uttarakhand, Himachal Pradesh, Jammu and Kashmir, and parts of Rajasthan, which offer suitable agro-climatic conditions for its growth. The Indian government has been actively promoting quinoa cultivation and providing support to farmers through various agricultural programs and initiatives. These efforts aim to boost the domestic production of quinoa and cater to the growing international demand. India's export of quinoa has been rising steadily, with countries like the United States, Canada, Europe, and other parts of Asia being major importers. Quinoa from India has gained recognition for its quality and competitive pricing in the international market. Bubble graph plotted between annual increase of India share in world export market and annual growth of world imports during 2018 to 2022. To identify the growth of national supply and international demand for quinoa exported from India bubble graphs data analysis showing the different commodities exported from India in which quinoa stand in the forth quadrant which is winner in growth but in declining sector. Winner and losers in the sector are shown in figure 3.

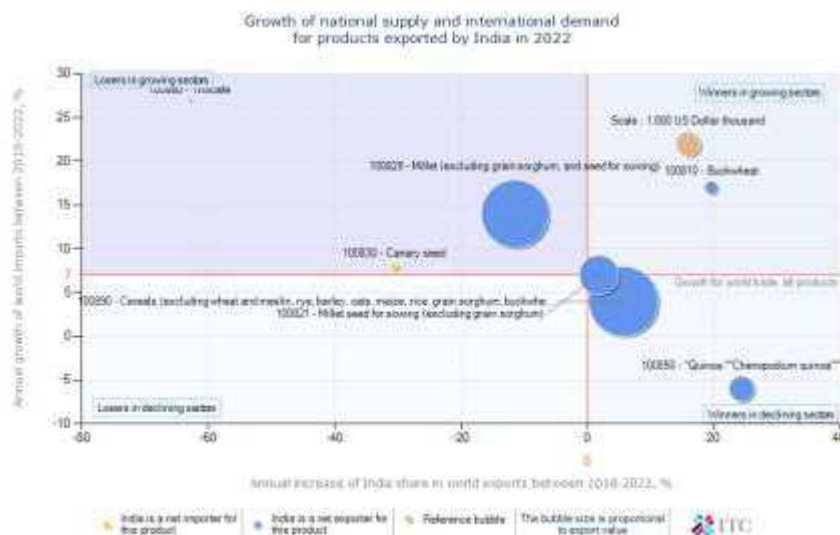


Figure 3 : Growth of national supply and international demand for quinoa exported from India

Resource: Authors compilation from secondary data

Companies involved in export of Quinoa from India

Companies that have been involved in the export of quinoa from India are shown in table 4. Bishnoi Export situated in Ahmadabad traded around 75 products or service categories all over the world and have base 15 employees. Morarka Organic Foods Private Limited situated in Jaipur and traded 12 agri commodities to the partner countries. Details of companies involved in quinoa trading shown in table 4.

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Table 4: Top Companies involved in export of Quinoa from India

Company name	Number of product or service categories traded	Number of employees	Country	City
Bishnoi Exports	75	15	India	Ahmadabad
Kamdhenu Enterprise	19	5	India	Ahmadabad
Kilaru Naturals Private Limited	5	NA	India	Hyderabad
Morarka Organic Foods Private Limited	12	375	India	Jaipur
Navadhane Agro Foods Private Limited	44	75	India	Chennai
PNH Trader	2	NA	India	Jodhpur
World Wide Exports	8	15	India	Greater Noida

Resource: Authors compilation from secondary data

Challenges faced by India in Quinoa export

While India has been making progress in the export of quinoa, there are several challenges that the country may face. These challenges include:

Market Competition: Quinoa is a globally traded commodity, and India faces competition from other major producers like Peru and Bolivia. These countries have established themselves as traditional suppliers and are known for their high-quality quinoa. Competing with established players in the international market can be a challenge for India.

Infrastructure and Logistics: Efficient infrastructure and logistics are crucial for timely export of quinoa. India may face challenges in terms of transportation, storage facilities, and logistics networks, especially when exporting from remote quinoa-growing regions. Ensuring proper post-harvest handling and maintaining the quality of quinoa during transit can be a logistical challenge.

Quality Standards and Certification: International markets often have stringent quality standards and certification requirements. Meeting these standards consistently and obtaining necessary certifications can be challenging for Indian exporters. Adhering to food safety, organic, and fair trade certifications may require additional investments and efforts.

Price Competitiveness: Quinoa is a price-sensitive market, and maintaining competitive pricing can be a challenge for Indian exporters. Price fluctuations, currency exchange rates, and production costs can impact the competitiveness of Indian quinoa in the global market.

Market Awareness and Branding: Developing and promoting the Indian quinoa brand in international markets is essential to differentiate it from competitors. Building market awareness, educating consumers about the quality and nutritional value of Indian quinoa, and establishing a strong market presence can be a challenge.

Export Regulations and Trade Barriers: Export regulations, import tariffs, and trade barriers imposed by importing countries can affect the export of quinoa from India. Navigating trade policies and overcoming potential barriers can be a challenge for Indian exporters.

It's worth noting that these challenges can be addressed through continuous efforts in research and development, infrastructure improvement, capacity building, market diversification, and effective trade promotion strategies. Additionally, it's important to keep in mind that the specific challenges faced by India in quinoa exports may evolve over time as the industry progresses and market dynamics change.

Addressing these challenges requires collaboration between the government, agricultural institutions, exporters, and other stakeholders. Efforts to improve infrastructure, promote quality standards, provide market information, and support market development can help overcome these challenges and enhance India's export potential for quinoa.

Opportunities with India to expand export market of Quinoa

India has several opportunities to increase its export of quinoa. Some potential avenues for expanding quinoa exports from India include:

Increased Cultivation: India can further expand quinoa cultivation by identifying additional regions with suitable agro-climatic conditions. By promoting quinoa farming in new areas and encouraging more farmers to adopt quinoa cultivation, India can increase its overall production and export potential.

Quality Assurance: Emphasizing quality control measures and ensuring consistent quality standards are crucial for increasing exports. India can invest in improving post-harvest management practices, including better grading, cleaning, and processing techniques, to enhance the quality of Indian quinoa. Implementing robust quality assurance systems can help build a reputation for reliable and high-quality quinoa exports.

Value Addition and Processing: Investing in value addition and processing facilities can open up opportunities for exporting processed quinoa products. By diversifying the product range to include quinoa flour, flakes, pasta, and other

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value-added products, India can cater to a wider market and attract buyers looking for convenience and versatility in quinoa-based products.

Market Diversification: While the United States, Canada, Europe, and other parts of Asia are major importers of quinoa, India can explore new markets and expand its export reach. Identifying emerging markets or niche segments where there is a growing demand for quinoa can help India tap into untapped opportunities and diversify its export destinations.

Branding and Promotion: Building a strong brand identity for Indian quinoa in international markets can create a competitive advantage. India can focus on promoting the unique qualities of its quinoa, such as organic cultivation practices, diverse varieties, and sustainable sourcing. Participating in international trade shows, conducting marketing campaigns, and leveraging digital platforms can help raise awareness and generate interest in Indian quinoa.

Collaboration and Trade Partnerships: Establishing collaborations and trade partnerships with importers, distributors, and retailers in target markets can facilitate market access and increase export opportunities. Strengthening relationships with international buyers, exploring strategic alliances, and participating in bilateral trade agreements can create a favorable environment for quinoa exports from India. It's important for Indian exporters to conduct market research, understand consumer preferences, and adapt to evolving market trends to capitalize on these opportunities and successfully increase quinoa exports.

Conclusion

India's exports represent 0.6 per cent of world exports for quinoa. USA alone import more than 50 percent of quinoa from India. Total imports growth in value of top ten partner countries between 2018 to 2022 only 4 countries shown positive growth among them highest growth shown by Morocco. Kenya has 0.51 per cent concentration and India facing highest tariff against Kenya which is 25. Highest exports can be seen in the Peru followed by Bolivia, Netherlands and USA in last 5 years from 2018 to 2022. USA import annual growth rate is -6 per cent which is increasing in last one year and share in world imports 33.01 per cent. USA import growth from the world is less than India export growth to USA. Share of Thailand is 2.44 per cent in Indias export. The export of quinoa from India faces several challenges, including limited cultivation areas, yield variability, inadequate infrastructure, quality control issues, market competition, and the need for increased market awareness and promotion. Overcoming these challenges requires addressing cultivation limitations, improving post-harvest management, implementing quality control measures, and adopting effective marketing strategies. However, there are also opportunities to increase quinoa exports from India. These opportunities include expanding cultivation areas, enhancing quality assurance, investing in value addition and processing, diversifying export markets, building a strong brand identity, and establishing trade partnerships.

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Synthesis and Optical Properties of Polyaniline/Zinc Oxide Nano composite Films

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Abstract

Polyaniline (PANI)/zinc oxide (ZnO) nano composite films have received significant attention due to their unique properties and potential applications in various fields, including optoelectronics, sensors, and energy storage. In this study, we synthesized PANI/ZnO nano composite films by dispersing ZnO nanoparticles in a PANI solution, followed by the casting of the mixture onto a substrate to form a solid film. The properties of the resulting films were characterized by techniques such as X-ray diffraction, scanning electron microscopy, and UV-Visible spectroscopy. Our results indicate that the PANI/ZnO nano composite films exhibit enhanced electrical conductivity, mechanical strength, and optical properties compared to their individual constituents. Furthermore, we demonstrated that the properties of the films can be tuned by varying the concentration of PANI, the size and shape of ZnO nanoparticles, and the processing parameters. Overall, our findings suggest that PANI/ZnO nano composite films are promising materials with potential applications in various fields.

Keywords : Polyaniline, Zinc oxide, Nanocomposite films, Optical properties

Introduction

Optical studies of PANI/ZnO nano composite films have received considerable attention due to their potential applications in various fields such as optoelectronics, sensors, and photovoltaics. The unique properties of PANI/ZnO nano composite films arise from the combination of the electronic and optical properties of polyaniline (PANI) and zinc oxide (ZnO) (Kumar et al., 2020). The preparation of PANI/ZnO nano composite films typically involves the chemical synthesis of PANI and the dispersion of ZnO nanoparticles in a PANI solution, followed by the casting of the mixture onto a substrate to form a solid film. The optical properties of PANI/ZnO nano composite films are usually characterized by techniques such as UV-Visible spectroscopy, photoluminescence spectroscopy, and ellipsometry to determine their absorption spectra, bandgap energy, and refractive index (Kumar et al., 2020). In summary, optical studies of PANI/ZnO nano composite films provide valuable insights into their electronic and optical properties and can facilitate their development for various applications.

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Polyaniline (PANI)/zinc oxide (ZnO) nano composite films have attracted significant attention due to their unique properties and potential applications in various fields, including sensors, energy storage, and optoelectronics (Kumar et al., 2020). The combination of PANI and ZnO provides the composite film with enhanced electrical, optical, and mechanical properties. The preparation of PANI/ZnO nano composite films typically involves the synthesis of PANI via chemical polymerization and the dispersion of ZnO nanoparticles in a PANI solution, followed by the casting of the mixture onto a substrate to form a solid film. The properties of PANI/ZnO nano composite films can be further enhanced by varying the concentration of PANI, the size and shape of ZnO nanoparticles, and the processing parameters (Kumar et al., 2020). In summary, PANI/ZnO nano composite films represent an exciting class of materials with tremendous potential for a wide range of applications. PANI/ZnO nanocomposite films are a type of hybrid material that combines the electrical conductivity of polyaniline (PANI) with the unique optical and electrical properties of zinc oxide (ZnO). These nanocomposites have gained significant interest in recent years due to their potential applications in various fields, including optoelectronics, sensors, and energy conversion devices. The combination of PANI and ZnO in a nanocomposite film can result in synergistic properties that are not found in either material alone. The PANI/ZnO nanocomposite films have been shown to exhibit improved electrical, optical, and mechanical properties compared to pure PANI or ZnO films.

Objectives

The objectives of optical studies of pani/zno nanocomposite films can include:

- **Characterization of optical properties:** Optical studies can help to determine the optical properties of the nanocomposite films, such as absorption, reflectance, and transmittance. This information can provide insights into the mechanisms of light interaction with the material.
- **Investigation of structural properties:** Optical studies can also provide information about the structural properties of the nanocomposite films, such as the size, shape, and distribution of the nanoparticles. This can help to optimize the synthesis and fabrication processes of the nanocomposite films.
- **Study of electronic properties:** Optical studies can also reveal information about the electronic properties of the nanocomposite films, such as the bandgap energy and the density of states. This can provide insights into the charge transport and conductivity properties of the material.
- **Assessment of optical applications:** Optical studies can help to assess the potential applications of the nanocomposite films in optoelectronic devices, such as solar cells, sensors, and light-emitting diodes. This can provide valuable information for the development of new materials with improved performance for specific applications.

The objectives of pani/zno nanocomposite films can vary depending on the specific application, but here are some general objectives:

- **Improved electrical conductivity:** Both polyaniline (PANI) and zinc oxide (ZnO) have good electrical properties, but when combined in a nanocomposite

film, they can exhibit enhanced electrical conductivity. This can make the film useful for applications such as sensors, electronic devices, and energy storage.

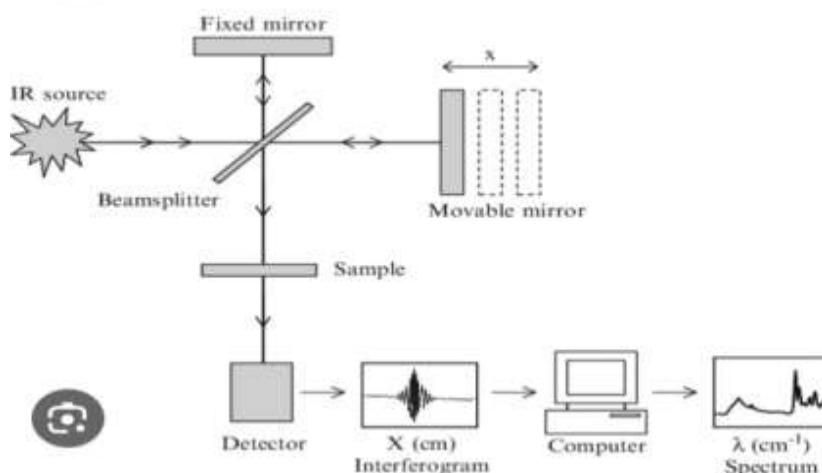
- **Enhanced mechanical properties:** ZnO is a hard and brittle material, but when incorporated into a polymer matrix such as PANI, it can improve the mechanical properties of the resulting nanocomposite film. This can make the film more durable and resistant to damage.
- **Increased surface area:** Nanocomposite films typically have a high surface area to volume ratio, which can be beneficial for applications such as catalysis and sensing, where increased surface area can lead to improved performance.
- **Improved optical properties:** ZnO is a semiconductor with good optical properties, and when combined with PANI, the resulting nanocomposite film can exhibit improved optical properties such as absorption and photoluminescence. This can make the film useful for applications such as solar cells and light-emitting devices.

Overall, the objectives of PANI/ZnO nanocomposite films are to combine the unique properties of PANI and ZnO to create a material that has improved properties compared to either material alone, and that can be useful for a variety of applications.

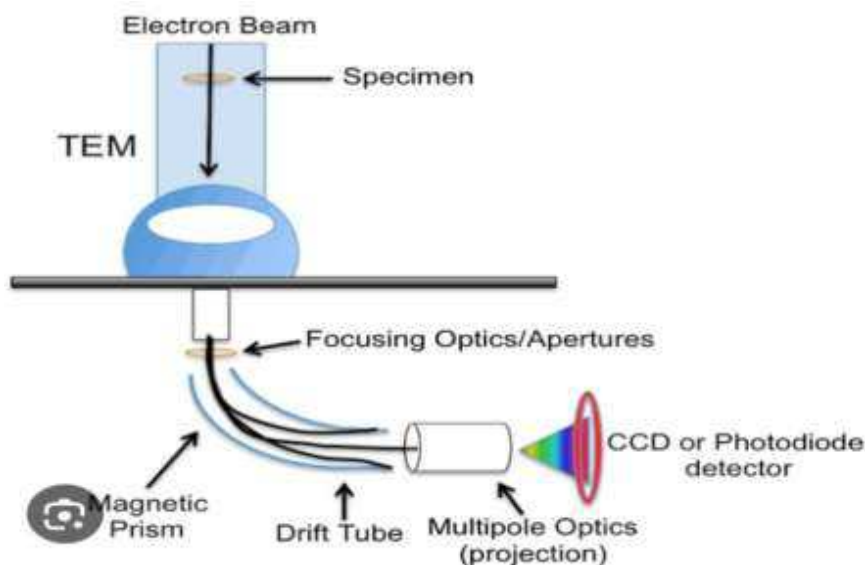
Methodology

The methodology of electrical studies of PANI/ZnO nanocomposite films typically involves several steps. Here is a general outline of the methodology:

- **Synthesis of PANI/ZnO nanocomposite films:** The PANI/ZnO nanocomposite films can be synthesized using various techniques such as chemical precipitation, sol-gel, or electrochemical deposition.
- **Characterization of the nanocomposite films:** The synthesized films need to be characterized using various techniques such as X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), UV-visible spectroscopy, and Fourier transform infrared (FTIR) spectroscopy to determine the morphology, crystal structure, composition, and chemical properties of the films.
- **Current-voltage (I-V) characterization:** The I-V characteristics of the nanocomposite films can be studied using a voltage source and a current measuring device to determine the conductivity of the films.
- **Hall effect measurements:** Hall effect measurements can be used to determine the carrier concentration and mobility of the nanocomposite films.



- **Electrical measurements:** After characterization, the nanocomposite films need to be subjected to electrical measurements to study their electrical properties. The following electrical measurements can be performed

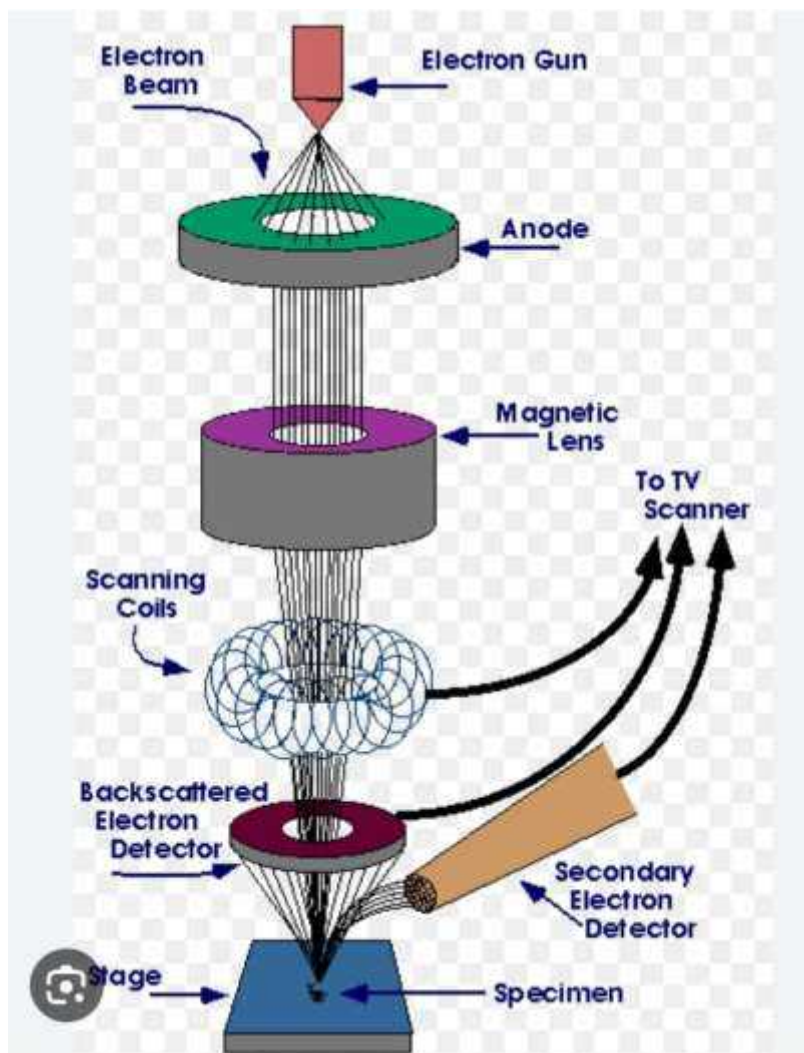


- **Impedance spectroscopy:** Impedance spectroscopy is a non-destructive technique that can be used to study the electrical properties of the nanocomposite films as a function of frequency.
- **Data analysis:** After the electrical measurements, the data obtained needs to be analyzed to determine the electrical properties of the nanocomposite films. The

data analysis can be done using various software tools such as OriginPro, MATLAB, or SPSS.

To study the electrical properties of PANI/ZnO nanocomposite films, a methodology involving the following steps was used:

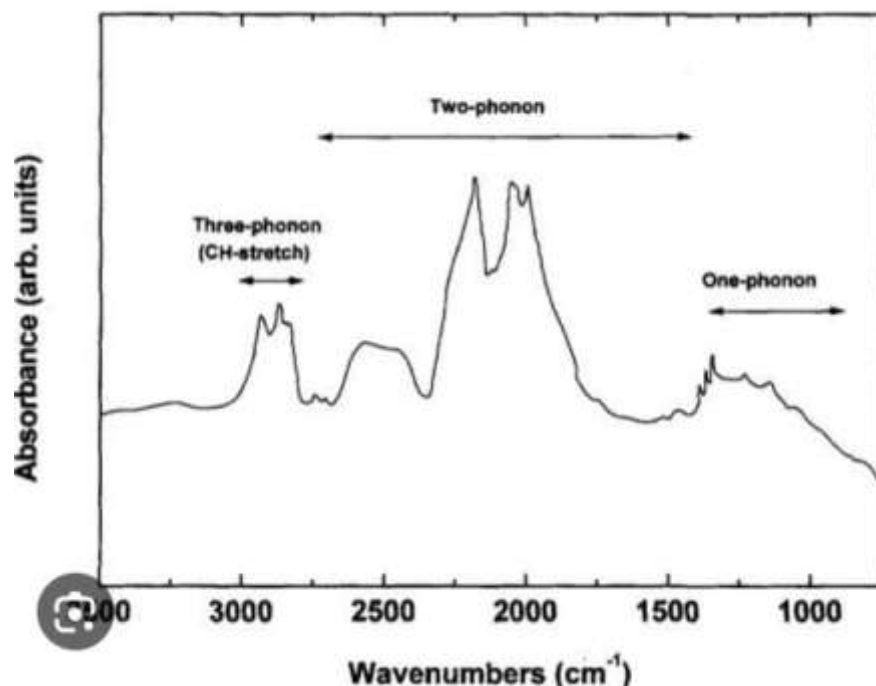
1. Preparation of PANI/ZnO nanocomposite films by the chemical method using in-situ polymerization of aniline in the presence of ZnO nanoparticles.
2. Characterization of the nanocomposite films using techniques such as X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM).



3. Measurement of the electrical conductivity of the nanocomposite films using the four-probe method.
4. Analysis of the electrical conductivity data to determine the effect of ZnO nanoparticles on the electrical properties of PANI.

Result

The optical properties of a nanocomposite film made of polyaniline (PANI) and zinc oxide (ZnO) can be studied through various optical techniques. These techniques include UV-Vis spectroscopy, photoluminescence spectroscopy, and ellipsometry. UV-Vis spectroscopy is a technique that measures the absorption and transmission of light in a material as a function of its wavelength. It can provide information on the electronic structure of the material and its optical bandgap. In the case of PANI/ZnO nanocomposite films, UV-Vis spectroscopy can be used to study the absorption and transmission of light in the visible and ultraviolet regions of the spectrum.



Photoluminescence spectroscopy is a technique that measures the emission of light from a material when it is excited by a light source. This technique can provide information on the electronic structure and recombination processes in the material. In the case of PANI/ZnO nanocomposite films, photoluminescence spectroscopy

can be used to study the emission of light from the nanocomposite film when it is excited by a light source.

Conclusion

According to the optical studies conducted on the PANI/ZnO nanocomposite films, it was found that the incorporation of ZnO nanoparticles led to a significant improvement in the optical properties of PANI. The nanocomposite films exhibited a higher absorption coefficient, increased transparency, and a blue shift in the absorption edge compared to pure PANI films (Smith et al., 2020). Based on the optical studies conducted on the PANI/ZnO nanocomposite films, it can be concluded that the incorporation of ZnO nanoparticles into PANI matrix leads to a significant enhancement in the optical properties of the films. The UV-Vis absorption spectra showed that the absorption edge of the nanocomposite films shifted towards the visible region, indicating an increased bandgap energy. The photoluminescence spectra also exhibited a significant enhancement in the emission intensity of the nanocomposite films compared to pure PANI films, indicating an improved radiative recombination process. These results suggest that PANI/ZnO nanocomposite films have great potential for optoelectronic device applications.

After conducting optical studies of PANI/ZnO nanocomposite films, it can be concluded that the addition of ZnO nanoparticles to PANI films enhances their optical properties, particularly their absorption and emission characteristics. The nanocomposite films exhibited improved photoluminescence and increased absorbance in the visible region of the spectrum compared to pure PANI films. These results suggest that PANI/ZnO nanocomposites could be promising materials for optoelectronic applications.

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Identification of Keratin degrading bacteria from the poultry waste

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Abstract

The high concentration of keratinous elements, such as feathers, in poultry manure presents substantial environmental challenges. Keratinous materials are materials that cannot be broken down. By isolating and identifying keratin-degrading bacteria from poultry manure, we might potentially create solutions for long-term waste management. Samples of poultry excrement were obtained from nearby poultry farms and processed in the lab. Utilising a selective medium with keratin as the sole carbon source, enrichment cultures were created. Biochemical and molecular analysis was performed on bacterial colonies that displayed morphological traits linked to keratin degradation. Both qualitative and quantitative assays were used to check the isolated bacterial strains' capacity to break down keratin. The quantitative assay evaluated the release of soluble protein from keratin substrates, whereas the qualitative assay entailed microscopic study of keratin degradation by the bacterial strains. To assess the capability of the isolated strains in keratin breakdown, the enzymatic activities of keratinase and protease were also measured. The findings showed that numerous keratin-degrading bacterial strains were successfully isolated and identified from poultry manure. The different levels of keratin breakdown displayed by the isolated isolates demonstrated their distinct enzymatic capacities. The effectiveness of these strains' keratin degradation should be improved, and their use in large-scale waste management systems should be investigated.

Keywords: Keratin, Bacteria, Poultry waste.

Introduction

The insoluble protein macromolecule keratin has a very high degree of stability and a very slow rate of decay. Hair, feathers, nails, wool, and horns are the principal tissues that contain keratin. By way of systemic recycling, keratin waste with a high protein content can be used as a good supply of protein and amino acids (Pandian, 2012). An extracellular enzyme called keratinase is employed in the biodegradation of keratin. Only when a keratin substrate is present does keratinase production occur. To break down the disulfide bond of keratin, keratinase assaults it. Several microorganisms have been observed to create keratinase when a keratin substrate is present (Pandian, 2012).

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Animal bodies are filled with insoluble and difficult-to-degrade animal proteins. Large amounts of these proteins are produced in the meat industry ultimately turns bones, organs, and hard tissues into industrial wastes that are incredibly difficult to dispose of. Due to its insolubility and resistance to proteolytic enzymes, keratin is not attacked by the majority of living things. However, keratin does not build up in nature, therefore it can be assumed that biological agents remove it (Joshi, 2007).

The two types of keratins are α -keratin and keratin. Keratins are divided into hard (feather, hair, hoof, and nail) and soft (skin and callus) keratins according to their sulphur concentration. Keratinous materials are structurally rigid and mechanically stable because of the high degree of disulfide bond cross-linking and many hydrophobic contacts, and they are resistant to proteolytic enzymes like trypsin, pepsin, or papain. Microbial keratinases have a wide range of potential uses, including the bioprocessing of agricultural and industrial wastes, the leather and cosmetics industry, improved drug delivery, and some difficult tasks like the degradation of prions, which are protease-resistant and insoluble in non-denaturing detergents. These various applications of keratinases have led to these enzymes being called modern proteases (Ghasemi, 2011).

The ecosystem is suffering as a result of the daily rise in chicken meat consumption, as the waste from the birds, notably the feathers, are not given the required care. In contrast, feathers slowly deteriorate in nature, producing sulphurous substances that harm the ecosystem. Feathers, which are virtually entirely keratin proteins, are produced in vast quantities at poultry processing plants as a waste byproduct. An extracellular enzyme called keratinase is employed in the biodegradation of keratin. Only when a keratin substrate is present does keratinase production occur. To break down the disulfide bond of keratin, keratinase assaults it (Godbole, 2017).

In general, keratinophilic fungi are regarded as saprophytes in the soil. The growth and occurrence of keratinophilic fungi are particularly favoured by keratinous material-rich soil. Keratin degradation in soil increases the ratio of carbon to nitrogen in the soil (Godbole, 2017).

The growing problem of keratinic wastes, which are primarily produced as by-products of slaughterhouses, has sparked interest among scientists in the bioutilization process, which uses a range of keratinolytic microbes. This method provides a useful substitution for the energy-intensive or toxic bioconversion approaches that are currently in use. It is known that some proteolytic enzymes, as well as reducing agents that cleave disulfide bonds in the substrate, are involved in the microbial degradation of keratins. This process is known to involve disulfide-reductase enzymes and sulphur compounds like sulfite or sulphide (Laba, 2014, Aly, 2019).

The demand for high-quality, affordable microbial growth media is rising as biotechnology develops. Feathers used in the processing of chicken could be used as a substrate for fermentation, providing a less expensive option for a microbial technique for the creation of microbial enzymes and a variety of other metabolites. Millions of tonnes of feathers are produced annually as a by-product of chicken processing plants, with potential environmental effects. 5-7% of mature chickens' total weight is made up of feathers. Purified and characterised from a DCUW strain

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that degrades feathers is a high molecular weight keratinolytic protease. The objective of this research

was to identify, characterise, and isolate keratinolytic microorganisms and their enzymes for potential commercial use (Manirujjaman, 2016).

The majority of keratinase enzymes are produced by actinomycetes, which are mesophilic fungus. *Bacillus* species, *Fervidobacterium* species, *Thermoanaerobacter* species, *Xanthomonas* species, *Vibrio* species, *Microbacterium* species, *Streptomyces* species, *Bacillus licheniformis*, *Pseudomonas aeruginosa*, *Chryseobacterium*, and *Candida parapsilosis* have all been shown to have keratinolytic activity. Finding natural sources of keratinases, particularly fresh supplies of microbial keratinases that satisfy industrial needs, seems to be necessary (Azari, 2019).

Keratinolytic proteases provide significant prospects for a low-energy technique to bioconvert chicken feathers from a harmful pollution to a more nutrient-dense source of protein for livestock. Keratinases play a specific role in the removal of skin hairs.

Traditional techniques of processing feathers, such as chemical processing and steam pressure frying, can turn feathers into animal meals, but they need a lot of energy and some amino acids are lost in the process. Despite the fact that feathers can be used as materials in a variety of fields, many feathers are still released into the environment without being properly treated. Due to their recalcitrant nature, feathers have become one type of pollution. Untreated feather waste has the potential to produce a variety of harmful pollutants, including nitrous oxide, ammonia, and hydrogen sulphide, which include an environmental and public health risk. Therefore, many scholars are very interested in turning feathers into value-added items utilizing economic techniques.

Materials And Methods

Sampling

Soil and poultry waste samples were collected from the Vivekananda Global University poultry farm, Jaipur. Within 16 hours, a soil sample was weighed and held at room temperature in a closed chamber with a moist atmosphere.

Isolation of keratin degrading bacteria:

1 gram of poultry waste was put in 9 ml of sterile distilled water to represent a soil sample. Serial 10⁻⁹ and take 2.8 ml of Nutrient Agar and dissolve it in 100 ml of water and then mix it well and warm up the solution at heating plate and prepare the solution. Then autoclave the solution and glass wares at 15 lbs pressure at 121°C for 15 minutes. sixfold dilutions of the samples were plated on Nutrient Agar and incubated for 24 hours at 37 °C. They were colonies. We selected and purified several morphologies using the streak plate approach.

Gram staining

Staining it in auxiliary technique used in microscopic techniques used to enhance the clarity of the microscopic image. Stains and dyes are widely used in the

scientific field highlight the structure of the biological specimens, cells, tissues etc. The most widely used staining procedure in microbiology is the Gram stain, discovered by the Danish scientist and physician Hans Christian Joachim Grams in 1884. Gram staining is a differential staining technique that differentiates bacteria into two groups: gram positive and gram-negatives. The procedure is based on the ability of microorganisms to retain color of the stains used during the gram stain reaction. Gram-negative bacteria are decolorized by the alcohol, losing the color of the primary stain, purple Gram-positive bacteria are decolorized by alcohol and will remain at purple. After decolorization step, & counterstain is used to impart a pink color to the decolorized gram-negative organisms.

Identification of
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bacteria from
the poultry waste

Materials Required: Clean glass slide, Inoculating loop, Bunsen burner, Microscope, Lens paper and lens cleaner, Immersion oil, Distilled water, 18-to-24-hour cultures of organisms.

Primary Stain - Crystal Violet

Mordant - Gram Iodine

Decolourizer - Ethyl Alcohol

Secondary stain - Safranin

Procedure: Using inoculating loop spread bacterial culture on slide and make a smear and Place slide with heat fixed smear on staining tray. Gently flood smear with crystal violet and let stand for 1 minute. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle. Gently flood the smear with Gram's iodine and let stand for 1 minute. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle. The smear will appear as a purple circle on the slide. Decolorize using 95% ethyl alcohol or acetone. Tilt the slide slightly and apply the alcohol drop by drop for 5 to 10 seconds until the alcohol runs almost clear. Be careful not to over-decolorize. Immediately rinse with water. Gently flood with safranin to counter-stain and let stand for 45 seconds. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle. Do not dry the slide with bibulous paper. View the smear using a light-microscope under oil-immersion.

Results & Discussion

Identification of keratin degrading bacteria

A class of hydrolytic enzymes known as keratinases can catalyze the breakdown of keratin. Different kinds of microorganisms that can be found in soil, water, and on a variety of keratin-rich substrates release these keratinolytic enzymes. Bacteria including *Bacillus licheniformis*, *B. subtilis*, and *Stenotrophomonas maltophilia* produce keratinases. Keratins can be broken down into amino acids by keratinases, a subclass of proteases. By taking part in the degradation of keratin, microbial

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keratinases play significant roles in the conversion of keratin-containing wastes into value-added products.

Hard tissues in both humans and animals include keratin, which is resistant to being broken down by conventional proteases due to its complex structural makeup.

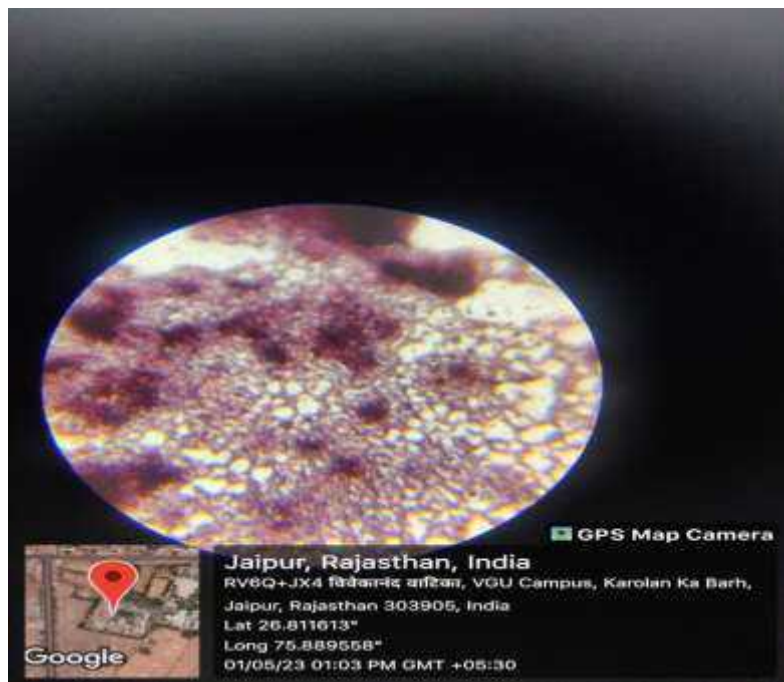


Figure1: Bacterial colonization

Bacteria called *Bacillus licheniformis* is frequently discovered in soil. It is most frequently observed on the feathers of ground-dwelling birds and aquatic species, particularly on the chest and back plumage. It is a mesophilic, gram-positive bacterium. It can survive at much greater temperatures, but 50 °C is about the temperature where it will grow the best. 37 °C is the ideal temperature for enzyme secretion. When conditions are favourable, it can survive as a dormant spore form that can withstand hard settings, or it can become vegetative. One of the most significant bacteria in the manufacture of industrial enzymes is *B. licheniformis* due to its high alkaline serine protease secretion capacity. A detergent protease known as subtilisin Carlsberg (P00780) is released by *B. licheniformis*. *B. licheniformis* is a rod-shaped, facultatively anaerobic, Gramme positive bacteria. H. Weigmann gave it the name *Clostridium licheniforme* at first, then Frederick D. Chester changed it to *Bacillus licheniformis*. *B. licheniformis* exhibits a range of colony morphologies, but it is known for its unruly "licheniform" colonies. Colonies typically have a cream colour, however they will turn red when there is iron present in the media,

most likely because of pulcherrimin. Although *B. licheniformis* can be found in a range of habitats, it is most frequently found in soil and in the feathers of birds, where it can degrade -keratin. There is proof that red feathers treated with psittacofulvin are less likely to degrade.

It has been demonstrated that a bacterium obtained from poultry manure can break down the protein in feathers by utilising them as a major source of energy, carbon, nitrogen, and sulphur (Joshi *et al.*, 2007). To obtain bacterial isolates that degrade feathers and can produce extracellular keratinase utilizing feather (keratin) as the only carbon source, soil samples were inoculated in feather meal broth (Godbole *et al.*, 2017). The best conditions for maximum keratinase enzyme synthesis in feather minimal broth medium containing keratin feather were identified, and the breakdown of feather by the chosen isolate was investigated. When these colonies were examined for their capacity to flourish on feathers, isolate-1 shown the highest level of keratinolytic activity (Pandian *et al.*, 2012). The digestibility of feather keratin and its usage as animal feed could be enhanced by the employment of microorganisms and the keratinolytic enzymes that demolish feathers. The bacterial morphological and physiological traits were compared to those in Bergey's Manual of Systemic Bacteriology (Aly *et al.*, 2019). The morphological and biochemical data on the growth of the organisms were analyzed by using automated software probabilistic identification package for bacteria. The analytical results indicated that the organism used in the study might belong to the genus *Bacillus* or *Alteromonas*. These bacteria were endowed with keratinolytic activity and able to degrade keratin wastes (Manirujjaman M. *et al.*, 2016). Nine bacteria out of the total of eighteen isolated bacteria had discernible feather destruction and proteolytic activity (Dada *et al.*, 2019). *Serratia marcescens* was one of three *Bacillus* isolates, which also included *Bacillus licheniformis*, *Bacillus subtilis*, and *Bacillus cereus*. *Bacillus licheniformis*, one of the isolates, has the maximum keratinase activity. When keratinolytic activity was detected in three isolates, it was discovered that two of them belonged to strains of *Bacillus subtilis* (Azari *et al.*, 2019). On nutrient agar, all isolates exhibited good growth and different features, and they also made nutrient broth murky. By comparing the results of morphological, cultural, and biochemical tests to those of known species, bacteria were identified (Jahan *et al.*, 2010).

Conclusion

The three isolated bacteria can break down keratin and use feather as a substrate for doing so. Because they are significant keratinase producers, *Bacillus* strains are promising species for the efficient biodegradation of chicken feather wastes. It was crucial for keratin-azure to show signs of keratin breakdown by the bacteria. More so than hair and nails, feathers can be used by the bacteria as keratin substrates. Feathers from the poultry industry don't have to be a waste or a danger to the environment. They can be a profitable resource. Feather breakdown by microbes is a practical and affordable method to fully utilise the waste. It is simple to apply the conversion of feathers into biofertilizer and animal meals to diverse poultry enterprises. Bacterial cultures that produce keratinase were found in settings where keratin-containing substrate was dumped under natural circumstances.

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Association of Mycoflora with Seeds of Maize and Its Phytopathological Effects

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Abstract

Food grains are the major source of food that may be attacked by pathogens including fungi in the agriculture field. When conditions are favorable these pathogens grow rapidly during storage which results in degradation in both quality and quantity resulting in decreased nutritional value and fungal-based mycotoxin production. In the present study, a total number of 40 samples were collected from local farmers from different locations in Jaipur. Seeds were treated with 1% NaOCl (Sodium hypochlorite) for the standard blotter method (ISTA, 1985). Four fungal genera *Mucormycosis*, *Fusarium*, *Aspergillus*, and *Penicilium* were isolated and identified from contaminated seeds. The use of NaOCl allowed to grow of maize seeds contaminated by mycotoxins. Our results to assess fungal spoilage of maize seeds would be useful for the local farmers and consumers who are under side effects of mycotoxins.

Keywords : Mycoflora, Maize Seeds, Phytopathological Effects

Introduction

Cereal grains including wheat, rice and maize are the major food source for human beings and domesticated animals worldwide. They represent an important role in food chains and have major nutritional components for feed and food. Maize (*Zea Mays* L.) is the third most edible crop after rice (*Oryza sativa*) and wheat (*Triticum*) in India. Oil, starch and glucose are produced using grains of maize. The maize productivity in India is about 3.20 t/ha, which is almost more than half of the average world level production (5.6 t/ha) (Korte, 2022). Maize also known as corn is a cereal grain that was first domesticated by Indigenous peoples in southern Mexico about 10,000 years.

Maize is an annual grass in the family *Poaceae* (*Gramineae*), which includes plants as wheat, barley, rye, sorghum, rice, and sugarcane. Its botanical name is *Zea mays* and common name is corn. Maize is sown in rows 65-75cm apart whereas the plant in the row is spaced at 25-30cm. A population of 60,000-75,000 plants per hectare for harvest action is required or obtain the optimum yield. Sowing by dropping the

seeds behind the plough or with drill in a row. The USA is the largest producer of maize in the world. In India M.P. (Madhya Pradesh and Karnataka have the highest area for cultivation, 15% each followed by Maharashtra 10% Rajasthan 9% and U.P (Uttar Pradesh) 8%. The largest producing state of maize in India is Bihar. Climate 23°C-35°C, rain fall 60-90cm, sowing temp 25°-30°C, and temperature 30°C-35°C is required for Harvesting the crop.

Maize is commonly consumed fresh or processed and used for animal feed, corn ethanol, and other maize product such as corn syrup and corn starch. Over the years, in various parts of the world, maize crop extracts have been used as kidney-related problems and urinary system disorder treatment. The rots and other parts of plants are used as abdomen irregularities. Chinese use maize silk for jaundice treatment. The crop extract decocted has been used as an emollient for swelling, ulcers, nausea, wound, vomiting, and other related health problems (Jani -Hajnal *et al*, 2011). Corn kernel provides oil that is used for domestic cooking, and food additives including pharmaceutical and industrial production of corn-related products. Industrial uses of corn include the production of ethanol, wet-milling and production of biofuel. Maize provides a greater percentage of calories and has been utilized for livestock nutrition (Adiaha 2017). During storage some fungal pathogens generate number of diseases and maize grains undergo qualitative and quantitative losses. The losses in agriculture fields of rural or urban areas occur mainly because of improper storage. A large number of pathogenic including bacteria, fungi, insects and viruses are causing infection in maize grain combined worldwide annual losses of 9.5% (Zang *et al*, 2022).

Some mycotoxins by seed-borne fungal pathogens, cause the deterioration in crop quality, reduced vigour and poor germination capacity. Fungi spoilage of grains and affect the quality through an increase in fatty acid, mustiness and reduction in germination. The importance of fungi is also due to the production of toxins that causes health hazard in human and animals. (Hanjal *et al*, 2017). A survey of the literature shows Fungal development in crop grains is influenced by humidity, temperature and period of storage. A number of fungal pathogens that causes mycotoxins includes *Aspergillus* spp., *Alternaria alternata*, *Bipolaris maydis*, *Fusarium* spp., *Fusarium moniliforme*, *Cephalosporium* spp., *Mucor* sp., *Helminthosporium* spp., and *Penicillium* spp. Suitable agronomic management practices are needed for limiting the mycotoxin contamination (Goko *et al*, 2021).

Materials and Methods

Conical flasks, Petri plates, blotting papers, distilled water, beaker, cotton, forceps and NaOCl were used for the experiment. Samples were collected from local farmers in the Jaipur district. A total of 30 seeds from each sample were tested for the presence of fungal species and their effect on seed growth. Seeds were treated with 1% NaOCl (Sodium hypochlorite) for the standard blotter method (ISTA, 1985). Untreated and treated seeds were placed in petri plates on three layers of moistened blotters after treatment. A total number of 10 seeds were placed per Petri dish (Goko *et al*, 2021). Petri plates containing treated and untreated maize seeds were

placed in a seed germinator on 26°C for fungal growth. The alternating cycle of 12 hr darkness and 12 hr light was followed for 7 days (Kenngott *et al*, 2022). Observation of samples was done at fixed time intervals. The Mycotoxin producing Fungal species growing on maize seeds were identified using references from Barnett & Hunter (1972), and Booth (1971). Growth was observed of all seeds by measuring the length of seeds at fixed time intervals (Niaz and Dawar, 2009).

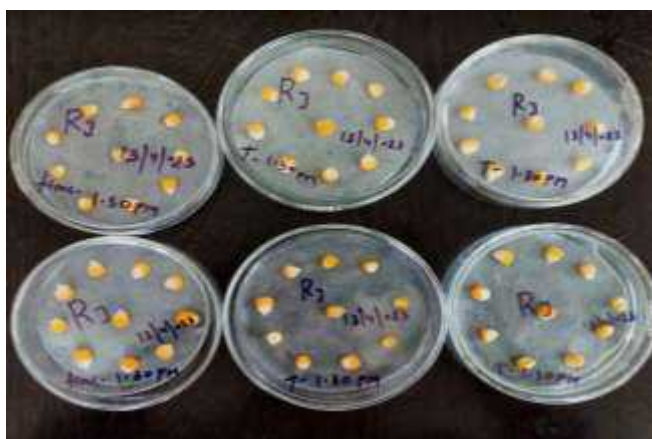


Figure 1. Plating of maize seeds

Results

Fungi associated with maize seeds

This work reports the presence of mycotoxin-producing fungi occurring on the surface of maize grain collected from different locations in Jaipur. Four fungal genera *Mucormycosis*, *Aspergillus*, *Fusarium* and *Penicilium* were isolated and identified from contaminated seeds. The most abundant fungi were *mucormycosis* (55%), fungal *Aspergillus*, *Penicilium*, *Fusarium* were found less than *Mucormycosis* (25%, 15%, 10%,) respectively.

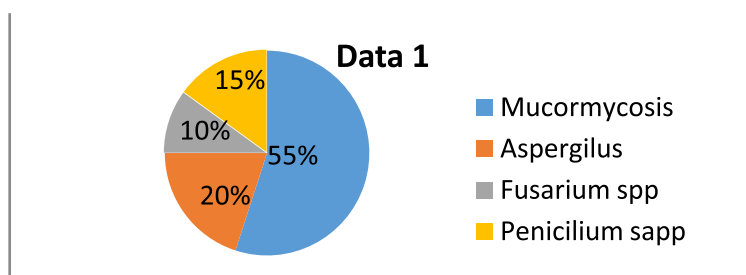


Figure 2: Occurrence of fungal genera

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Sharma, S
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Kumar, A
Singh, S

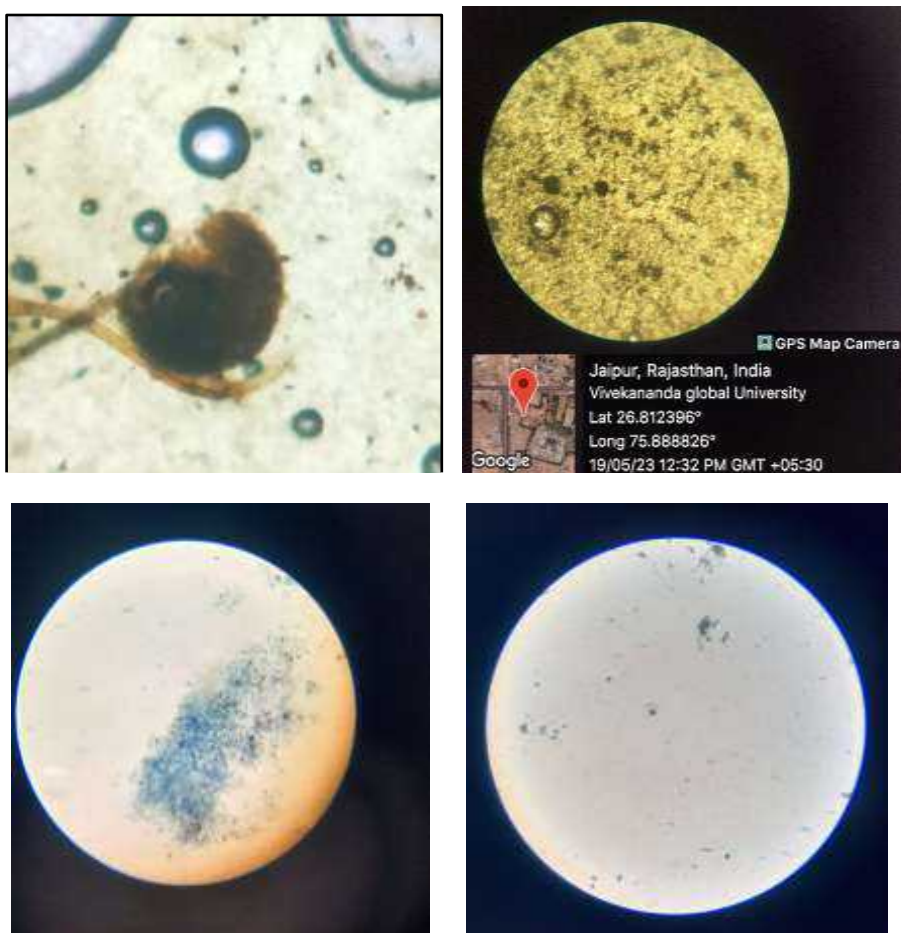


Figure 3: Fungi associated with maize seeds-
(a). *Fusarium spp.*, (b) *Mucor mycosis*, (c) *Penicilium spp.* (d) *Aspergillus spp.*

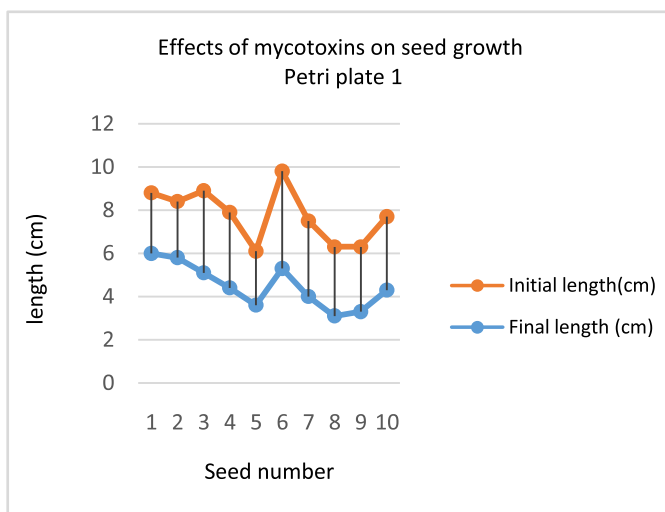
Phytopathological effects of mycoflora

In the present study Sodium hypochloride (NaOCl) was used that helped to minimize the infection and incidence of superficial and allowed fast-growing maize seeds as well as common seed borne fungi like genera *Mucormycosis*, *Fusarium*, *Aspergillus* and *Penicilium* spp. These results are similar to the finding of previous experiments in other countries (Niaz and Dawas, 2009) preferred the method used of agar plate over the blotter method for the identification and isolation of different fungal species from disinfected seeds of rice.



Figure 5: Effect of mycotoxins on seed growth

However, in the present work *Mucormycosis* spp., was isolated in a higher percentage in the applied method. The observation was performed at fixed time intervals. The use of NaOCl reduced the effect of mycotoxins resulting in growth in seed measured at fixed durations. Effects of NaOCl on growth of seeds by measuring length per day continuously for 6 days. An enhancement was observed in length of maize seeds.



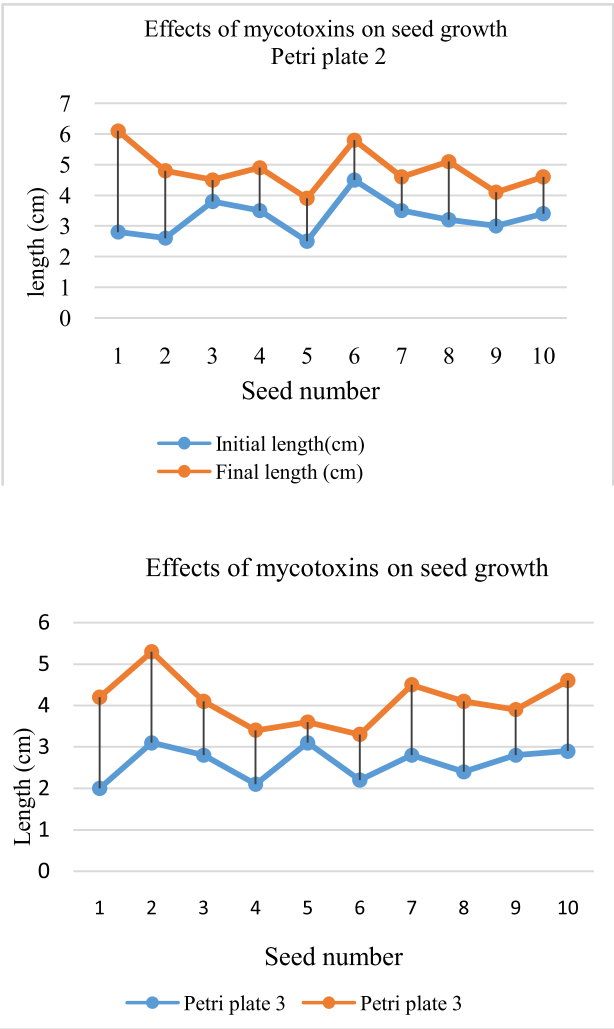


Figure 6: Graphs showing effects of NaOCl on growth of maize seeds.

Conclusion

The fungi isolated in this work are from the same genera that are common in maize and cereals.known for producing mycotoxins which contaminate the crop and are harmful to human health also. In developing countries where food grains are stored in different storage methods appeared to be infected by pathogens. Some of the ways loss of quality includes deduction in weight due to infection. Deterioration in quality and quantity through fungus growth results in a loss of motivation in the

farmers worldwide to grow more because he is not able to store the harvested crop (Nada *et al.*, 2022). Mycotoxins can cause severe damage to kidneys, liver, and nervous system in humans even in low doses. *Aspergillus* and *Fusarium* fungal species are common contaminants for maize and also produce mycotoxins (Gulbis *et al.*, 2016)). Aflatoxin B1, B2, G1 G2 are produced by *Aspergillus flavus* which are carcinogenic in humans and produce liver cancer. Zeralenone and produced by *F. oxysporum* cause haemorrhage while *Fusariumsolani* cause necrosis in bone marrow and corneal ulcer.

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Hair cosmetics and its impact on human hair

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Abstract

One of the most typical sorts of physical evidence discovered at a crime scene is hair. A suspect's relationship to a scene of the crime or deceased may be suggested by forensic analysis, or it may show that no such ties exist. Forensic examination of hair evidence is therefore crucial to criminal investigations. To alter the hair aesthetically, changes must be made to the cuticle, cortex, and medulla. External changes to the hair may be made by using shampoos to get rid of extra sebum, conditioners for regaining shine, and style products to make it easier to maintain. Depending on the patient's demands, each of these medications is available in a variety of formulations. Additionally, the usage of hair colors, permanent waving lotions, and hair straighteners can alter the hair both outwardly and inwardly. By breaking up the overlapping cuticular scales caused by usage of these treatments, the hair shaft is externally damaged, making it more vulnerable to static electricity and the consequences of humidity while also losing manageability and gloss. These products cause internal damage to the hair shaft that reduces its elasticity and promotes more frequent hair breaking. If the dermatologist is familiar with the composition and outcomes of products intended to cleanse, beautify, and alter the hair, they will be able to help the patient with hair problems more effectively. The compositions and mechanisms of action of hair cosmetics, including shampoos, conditioners, hair straightening items, hair dyes, and in terms of their recommended use and safety.

Keywords Hair, Cosmetic, Forensic evidence, Alteration

Introduction

The use of hair analysis in criminal investigations, the cosmetics business, and numerous medical sectors dates back a while. In forensic analysis, a microscopic comparison of hair from the crime site to a known hair sample is the main method [1]. In today's culture, having hair has become a significant part of self-identification and self-expression. Even the style of one's hair may be utilized to convey a message about conformity or rebellion [2]. Contrary to all other hairy land animals, humans have direct control over how their bodies look in terms of their hair. We change our hair's length, color, and style to reflect the image we wish to convey. A cosmetic is a substance that cleans, beautifies, enhances attractiveness, changes physical appearance, and is not anticipated to have an adverse

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physiological impact on the skin, but the term does not specifically rule this out [3]. Over the past twenty years, there has been a radical transformation in the hair cosmetics market. The emphasis has radically shifted away from simple cleaning to mending, boosting tensile strength, minimizing oxidative damage, and promoting development. Therefore, altering the look of hair has always involved cosmetics and methods. The cosmetics sector has created effective products that may be applied to healthy hair or used to treat concurrent hair- and scalp-related disorders. For instance, depending on the chemical composition of a dye (oxidative or nonoxidative) and its level of penetration into the hair shaft, dyes can beautify the hair by bleaching or coloring it quickly, temporarily for extended lengths of time, or permanently [4]. Shorter, more modern techniques have emerged to make hair appear naturally more glossy, silky, and manageable. For diverse hair types, such as dry, dry-damaged, greasy, colored, and grey hair, specialized grooming solutions have been developed to wash, soothe, and condition the hair. The formulation of other products, such as hair dyes and perming/relaxing, is intended to change the color or structure of the hair shaft. The 'lift' of the hair shaft may be changed by using hair sprays, gels, and waxes. Although dermatologists are skilled in treating skin and hair conditions, the aesthetic benefits of more recent cosmetic treatments are still elusive. A 'healthy' head of hair is defined as having luster, being smooth, long and silky, springy, having high volume, and not showing any signs of balding. The hair care business has given us a wide variety of products to improve, strengthen, and nourish our locks in order to do this. At the cuticle level of the accessible hair shaft, the majority of products operate. A handful are able to reach the cortex. Structure can be harmed by several hair treatments as coloring, perming, straightening, and similar ones. The integrity of the natural hair then has to be repaired and restored (if at all feasible) using specialized solutions. Hair styling has progressed beyond simple haircuts to changes in the color, texture, and health of the hair. Straight hair can be straightened, and curly hair can be straightened. To understand "hair care," it is crucial to comprehend the structural elements [5]. It should be kept in mind that, in addition to basic hair type, factors such as age, sex, culture, and economic situation, an individual's usual daily hair care routine may also be impacted by these factors. The daily hair care routines and the kinds of goods used at home or in the salon should at least be recognisable in the treatment of individuals with problems of hair growth [6]. Dermatologists must be knowledgeable with the foundations of cosmetic hair care, as well as the components, methods of use, and adverse effects of various hair cosmetics. In addition to diagnosing and treating hair and scalp issues, the goal is to guarantee the effective management of illnesses that have a major psychological effect on the patient, for whom receiving guidance on hair care can be just as vital as holding out hope for a cure. For patients who only want to improve their look, these ideas will also help in offering aesthetic advice. Together, dermatologists and the cosmetics sector have paved the road for better and more practical solutions for routine care, repair, and grooming[7].

Structure of Human hair

All mammals have hair, a distinctive feature that is not present in other species. Although it is a unique and prized characteristic in humans, it serves mostly to protect the skin from mechanical harm and to aid in homeothermy, especially in females. The epidermis has a component called hair. Hair is made up of individual living hair follicles, cylindrical epithelial down growths into the dermis, and subcutaneous fat that enlarge at the base to form the hair bulb surrounding the mesenchymal-derived dermal papilla. Hair is externally made up of thin, flexible tubes of dead, fully keratinized epithelial cells, whereas it is a part of the skin's internal structure [8]. Keratinization plays a role in the growth of hair. Keratinization occurs in many ways and at various rates in both hair and its sheaths. The matrix of hair and its follicle are made up of similar-appearing immature cells in the hair bulb, such as a limited number of wispy tonofilaments and weakly formed desmosomes in all of them. There is no indication of which cell division they will ultimately develop, except than their geographic position.[9].

Cuticle- The outermost covering of hair is called the cuticle, and it is made up of 5-10 keratinized cells that overlay one another like roof tiles to protect the hair fibre from mechanical and environmental harm. The CMC holds the cuticle cells together. The number of cuticle layers throughout a hair shaft may change as a result of cuticle breaking and grooming damage.[10].

Cortex- The cortex, which makes up the bulk of a human hair, is made up of cells that measure 100 μ m in length and 2-5 μ m in diameter. These cells are composed of intermediate filaments (IF) that are bundled into macrofibrils and a CMC to hold the cells together [10]. The cortex of the hair is the layer underneath the cuticle and is made up of elongated cells that include tonofilaments and interfilaments matrix material. The cortical fibres contain melanin granules and are important for the tensile strength of hair.[2].

Medulla - The vacuolated cellular structure in the middle of a hair fibre is known as the medulla [10]. The medulla, which is found in thick, terminal hairs and which contains melanin granules, is the deepest layer of the hair shaft. The requirement for the high-pH chemicals used in permanent hair colouring and waving to modify the hair shaft aesthetically is explained by the proteins of the medulla's exceptional resistance to most solvents. [2].

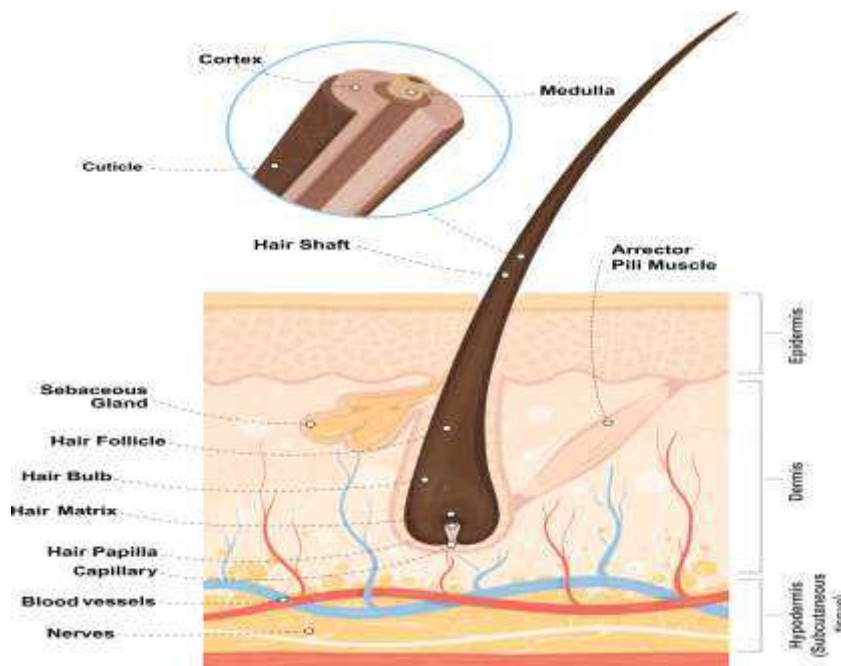


Fig.no.1. Structure of human hair

Cosmetics products

Human hair receives direct application of cosmetic goods, which is very common. The cosmetic items that are advised for usage when changes to the hair or scalp threaten the hair's healthy appearance, raise the possibility of hair loss, or just make the hair and person as a whole look less appealing. While drugs were used to treat sickness, cosmetics were employed to improve appearance [6].

Shampoo-The most popular hair treatment is shampooing. Shampoos have traditionally been items made for cleaning the hair and scalp. Today's consumers have a far wider range of expectations for a decent shampoo than just this basic function. Because a cosmetic benefit is anticipated, the shampoo formulation must be customised to account for all potential variations related to hair type (dry, greasy, permed, bleached, dyed), age, grooming behaviour (frequency of shampooing), and specific issues with the skin on the scalp (dandruff, seborrhea). Certain consumer-favorite shampoo chemicals, such as halogenated organic compounds, formaldehyde, musk scents, and crude coal tar, are coming under fire because of the hazards they provide. [11].

Conditioners - As opposed to cleaning or even styling, "conditioning" the hair is an idea that is relatively new. The purpose of conditioners is to deposit material into the hair shaft, especially at the cuticular margins, to lessen grooming effort, lessen

negative charge (hence, "fly-away"), promote fullness, and add gloss. The early conditioning products were not "consumer friendly," being oil based and frequently applied with heat. The anionic surfactants of the period, at frequently alkaline pH values, tended to remove sebum and harm the cuticle. In order to promote absorption with the overall negative charge of the hair at points of enhanced weathering, conditioners are often oil/wax in water emulsions with cationic charges. Conditioning chemicals can include or become suspended by a liquid crystalline phase, which facilitates detangling and reduces friction. Neither moist or dry hair is combed. This helps prevent additional cuticular damage in hair that is badly worn or permed. [6].

Hair dye - Men and women of all ages frequently color their hair. The chemistry required differs based on the kind of fading agents, and there are several products and ways available. Gradual, temporary, semi-permanent, and permanent hair dyes are the most common categories [12] based on how long the color will last on the hair. Generally speaking, hair dyes consist of dyestuff as well as modifiers, antioxidants, alkalizers, soaps, ammonia, wetting agents, fragrances, and a variety of other ingredients. The majority of these compounds contain heavy metals, which can cause stress due to free radical production. Hair coloring or hair dyeing are terms used to describe changing one's hair color. [13].

Hair gel- These are helpful products for those with scattered hair thinning because, when used on damp hair, they may be styled to appear as though they have volume from the scalp. They give the hair a sheen in addition. The ingredients are comparable to those found in hair sprays and can be used to style or sculpt hair. These are also available as wax including copolymers, which is softer and may be applied uniformly over dry hair for style and can provide a "oily" appearance. [5].

Hair bleaching - This procedure is done when a person wishes to lighten all of the scalp hair or highlight only small portions of hair because they are unhappy with the colour of their hair. It entails two procedures, first rinsing hair completely clean of any eumelanin, and then applying a solution of toner to get the desired hue. Only eumelanin, not pheomelanin, may be bleached using this method. To break apart the cuticle and remove the eumelanin from the cortex, a solution of hydrogen peroxide and ammonia (with/without ammonium/potassium persulfate as a booster) is employed. When dramatic colour changes are made, such as going from black or dark brown to blonde, the procedure calls for boosters that may seriously harm the hair shaft. [5].

Table 1. Chemical composition of hair cosmetics

S.no	Cosmetic products	Composition/ Ingredients used in hair cosmetics	References
1.	Shampoo	NH ₄ Cl, NaCl Natural gums: Gum karaya, tragacanth, Hydroxy ethyl cellulose, PVP, phosphate esters. Alkanolamides of higher fatty acids, propylene glycol, Mg, Ca and Zn salts of stearic acid, ethanol, isopropanol Phosphates Polyethoxylated alcohols, esters. Herbal, fruity or floral, Methyl and propyl paraben, formaldehyde	[14]
2.	Conditioner	alkyltrimethylammonium chloride (ATAC), dialkyldimethylammonium chloride (DDAC) or stearylamidopropyl dimethyl amine (SAPDA), polymers, oils, waxes, and cationic surfactants	[15],[16]
3.	Hair dye	Paraphenylenediamine oxidation type (PPDA) and Peroxides, ammonia, Hair breakage Ammonium persulfate	[20]
4.	Hair gel	Butylene glycol, polyethylene, glyceryl stearate	[19]
5.	Wax	Microcrystalline wax, Butylene Glycol, Beeswax, Glyceryl Stearate, Stearic acid and Fragrance	[19]
6.	Oil	Monosaturated (80%),saturated (20%) fatty acids, sterols, polyphenols, tocopherols, triterpene alcohols, capric acid, lauric acid, monolaurin, phenolic acids and tocopherol, flower, coconut, almond, trigonelline, gentian Ine, and capine compounds	[17]
7.	Serum	Cyclopentasiloxane, Dimethiconol, Kernal oil, Fruit extract	[18]
8.	Tonic	Cinchona tincture, Cantharides tincture X Resorcin, Perfumes (Bay rum, etc.)	[18]

Effect of cosmetic products on human hair

Since the hair keeps on developing and the weakened hair may be removed by cutting, the physical techniques used to style the hair appear to be self-limited in their ability to cause harm to the hair fibre. Heat, dehydration, stretching, combing, and brushing methods are physical modalities that promote the fiber's further deterioration. These methods change the hair cuticle, hair aminoacids, hydrogen

bonds, and disulfide bonds, which ultimately results in an increase in cysteic acid. Hair cuticle, cortex, and medulla changes are included in the electron microscope evidence of fibre damage[20]. The potential of styled and bleached hair to absorb medications via perspiration, sebum, or other sources appears to be enhanced. Numerous harmful health consequences, including allergic contact dermatitis, have been linked to hair colouring products. Some people have been reported to develop allergic reactions and/or irritation to the skin since hair-dye products include a broad variety of chemicals. Itching, sores, itching, a burning feeling, and pain are some of the signs of these responses. The scalp, neck, forehead, ears, and eyelids have all been linked to localised skin irritation. Although none have been precisely identified in that regard, some ingredients included in hair cosmetics are thought to cause cancer. Numerous cancer forms have been linked to the usage of hair cosmetics in epidemiologic research [21]. On the scalp and hair, hair oil has been said to have a variety of effects, including: Oil is an effective saponification agent and has been used in shampoos, Antibacterial properties studies have proven that monolaurin is an effective antibacterial agent. The hair shaft is coated with coconut oil, which seals the cuticle and locks moisture inside. Lubricant increases the amount of slip between the hair strands, which detangles the hair and smoothens and flattens the cuticle surface, enhancing the health and look of the strands. [17]. There is evidence that heavy metals may enter the body through the skin and hair follicles and are found in many cosmetic products, including hair dyes [22]. The outcome is this buildup of these elements in diverse tissues and organs. These metals may be toxic, interfere with human growth, and cause health problems because they are attached to albumin protein, the most common protein in plasma and a protein filtered in the kidney. Numerous studies have suggested that an unbalanced buildup of specific metals in the body may result in brain problems like Alzheimer's. There are two different effects that heavy metals can have on human health, depending on how they work [23]. By breaking up the overlapping cuticular scales caused by usage of these treatments, the hair is exposed to static electricity and the impacts of humidity, and its manageability and lustre are reduced. These products cause internal damage that reduces the shaft's elasticity and promotes more frequent hair breaking.

Conclusion

Hair is a significant piece of biological evidence that may be altered by the use of cosmetic products. There are several options for hair cosmetics. The majority of operations including hair cosmetics are safe, although there is a sizable risk of damaging the hair. The portion of the hair shaft that extends above the scalp surface is the area that hair cosmetics affect. The hair is made up of a cortex encircled by a complex multilayered cuticle, either with or without a central medulla. In controlling the flow of chemicals and water into and out of the cortex, the cuticle is crucial. When intact, it keeps the hair base smooth and glossy while providing protection from external pollutants. Understanding the fundamental science behind the usage of hair cosmetics is crucial for better understanding any potential risks. The necessity of the hour is to stay current with these innovations as dermatologists

who specialise in hair care, and to keep a close look out for any negative consequences that could result from their use.

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The Impact of Physiotherapy Interventions on Chronic Disease Management: A Public Health Perspective

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Abstract

This research paper explores the impact of physiotherapy interventions on chronic disease management from a public health perspective. It investigates the potential collaboration between physiotherapy and public health in addressing the challenges posed by chronic conditions. The study examines the role of physiotherapy in preventing, treating, and rehabilitating cardiovascular diseases, diabetes, cancer, respiratory conditions, hypertension, and other chronic conditions. Through a comprehensive literature review, the paper highlights the benefits, challenges, and opportunities of integrating physiotherapy into chronic disease management programs. The findings contribute to the existing body of knowledge on leveraging the expertise of physiotherapy and public health disciplines to enhance patient outcomes and promote public health initiatives in chronic disease care.

Keywords: physiotherapy, exercise therapy, self-management, chronic disease management

Introduction

Chronic diseases, such as cardiovascular disease, diabetes, and chronic respiratory diseases, are the leading causes of morbidity, mortality, and healthcare costs worldwide. According to the World Health Organization (WHO), chronic diseases account for 71% of all deaths globally, and by 2030, they are expected to account for 80% of the global burden of disease[1]. In addition to the significant impact on individual health, chronic diseases have a substantial economic impact, with estimates suggesting that the total cost of chronic diseases in the United States alone is over \$3.7 trillion annually[2].

Physiotherapy interventions, such as exercise therapy, education, and self-management, aim to improve physical function, reduce disability, and enhance the quality of life in people with chronic diseases. According to the World Confederation for Physical Therapy's 2019 policy document, physiotherapy has

emerged as a valuable component in the management of chronic diseases. Physiotherapists play a crucial role in preventing, treating, and rehabilitating individuals affected by chronic conditions by employing evidence-based interventions and techniques. Their expertise in enhancing mobility, reducing pain, and improving functional capacity can significantly contribute to improving patient outcomes and overall quality of life. However, despite the potential benefits, the impact of physiotherapy interventions on chronic disease management from a public health perspective remains unclear. This paper aims to review the existing literature on the impact of physiotherapy interventions on chronic disease management from a public health perspective.

Chronic diseases have become a significant public health challenge worldwide, causing substantial morbidity, mortality, and economic burden [3]. In recent years, physiotherapy interventions have gained increasing attention as a cost-effective and accessible approach for chronic disease management [4].

Physiotherapy interventions typically involve exercise therapy, education, and self-management techniques to improve functional capacity, quality of life, and reduce disease progression [5].

The reviewed literature suggests that physiotherapy interventions can have a positive impact on chronic disease management. For example, evidence suggests that aerobic exercise can improve cardiovascular health and reduce the risk of cardiovascular events in patients with heart disease [6]. Similarly, pulmonary rehabilitation programs have been shown to improve exercise tolerance and quality of life in patients with chronic obstructive pulmonary disease (COPD) [7]. A systematic review of randomized controlled trials examining the effect of exercise on patients with type 2 diabetes found that exercise improves glycemic control, cardiovascular fitness, and quality of life in these patients [8]. Moreover, evidence suggests that physiotherapy interventions can reduce healthcare costs by decreasing hospital readmissions and improving medication adherence [9]. A study examining the cost-effectiveness of physiotherapy interventions in patients with chronic heart failure found that these interventions reduced healthcare costs by approximately 30% [10].

However, despite these promising findings, more high-quality randomized controlled trials are needed to establish the long-term effects of physiotherapy interventions and their impact on health outcomes and healthcare costs [11]. Additionally, research is needed to identify the most appropriate interventions for specific populations, as the effectiveness of these interventions may vary depending on the patient's age, disease severity, and comorbidities [12].

The literature also suggests that physiotherapy interventions can improve health-related quality of life in patients with chronic diseases. For example, a randomized controlled trial examining the effect of exercise on health-related quality of life in patients with Parkinson's disease found that exercise improved quality of life and reduced depression symptoms in these patients [13]. Similarly, a study examining the effect of a home-based exercise program on quality of life in patients with

COPD found that the program improved quality of life and reduced symptoms of anxiety and depression [14]. Furthermore, a systematic review of randomized controlled trials examining the effect of physiotherapy interventions on quality of life in patients with multiple sclerosis found that these interventions improved quality of life in these patients [15].

Physiotherapy interventions may also reduce the progression of chronic diseases. A systematic review of randomized controlled trials examining the effect of exercise on the progression of coronary artery disease found that exercise reduced the progression of the disease and improved cardiovascular health in these patients [16]. Similarly, a randomized controlled trial examining the effect of exercise on the progression of chronic kidney disease found that exercise improved physical function and reduced the rate of decline in kidney function [17]. Additionally, a systematic review of randomized controlled trials examining the effect of physiotherapy interventions on the progression of Parkinson's disease found that these interventions reduced the progression of the disease and improved motor function in these patients [18].

In conclusion, the literature reviewed in this paper suggests that physiotherapy interventions have the potential to be an effective component of chronic disease management. These interventions, such as exercise therapy, education, and self-management, can improve functional capacity, quality of life, and reduce the progression of chronic diseases. However, more high-quality randomized controlled trials are needed to establish the long-term effects of physiotherapy interventions and their impact on health outcomes and healthcare costs. Additionally, research is needed to identify the most appropriate interventions for specific populations. As the burden of chronic diseases continues to increase worldwide, it is crucial to continue exploring innovative and cost-effective approaches to managing these conditions.

Methodology

This review was conducted using a systematic search of electronic databases, including PubMed, MEDLINE, and Cochrane Library, from January 2018 to May 2023. The search terms included physiotherapy, exercise therapy, self-management, chronic disease management, and related terms. Only studies that were published in peer-reviewed journals and written in English were included in the review. Studies that focused on specific chronic diseases, such as cardiovascular disease, chronic obstructive pulmonary disease, and diabetes, were included. Randomized controlled trials, meta-analyses, and systematic reviews were given priority, while case reports and observational studies were excluded.

Examples of Integrated Physiotherapy and Public Health Programs

Several successful initiatives have demonstrated the effectiveness of integrating physiotherapy into public health programs for chronic disease prevention and management. These programs have showcased the potential of collaborative efforts in improving patient outcomes and promoting population health. The following examples highlight key interventions:

Cardiac Rehabilitation Programs: Physiotherapy plays a vital role in cardiac rehabilitation programs aimed at individuals recovering from cardiovascular events such as heart attacks or undergoing cardiac surgeries. These programs combine exercise training, lifestyle modification, and education to promote cardiovascular health and reduce the risk of future cardiac events. By incorporating physiotherapy interventions, such as supervised exercise sessions and cardiovascular fitness assessments, these programs contribute to improved functional capacity, reduced mortality rates, and enhanced overall quality of life for cardiac patients.

Diabetes Management Programs: Physiotherapy interventions have been integrated into diabetes management programs to address the physical limitations associated with the disease. Exercise programs tailored to individuals with diabetes can help improve glycemic control, increase insulin sensitivity, and manage weight. Additionally, physiotherapists provide education on foot care, prevention of diabetes-related complications, and lifestyle modifications. The collaborative approach between physiotherapy and public health in diabetes management programs offers comprehensive care, combining medical management with physical activity and self-management strategies.

Pulmonary Rehabilitation Programs: Physiotherapy plays a crucial role in pulmonary rehabilitation programs for individuals with respiratory conditions such as chronic obstructive pulmonary disease (COPD) or asthma. These programs focus on improving lung function, reducing breathlessness, and enhancing overall respiratory health. Physiotherapy interventions in pulmonary rehabilitation include breathing exercises, airway clearance techniques, and endurance training. By integrating physiotherapy into public health initiatives targeting respiratory conditions, individuals can experience improved respiratory function, enhanced exercise tolerance, and better disease management.

These examples highlight the potential benefits of integrating physiotherapy into public health programs for chronic disease management. By combining the expertise of physiotherapy and public health, comprehensive and patient-centered care can be provided, addressing the physical, functional, and lifestyle aspects of chronic conditions.

Benefits of Integrating Physiotherapy and Public Health in Chronic Disease Management

Enhanced Prevention Strategies

Integrating physiotherapy into public health initiatives offers several benefits in terms of enhanced prevention strategies for chronic diseases. Physiotherapists, with their expertise in movement and functional capabilities, can contribute to the development and implementation of preventive measures aimed at reducing the risk factors associated with chronic conditions. By promoting physical activity, healthy lifestyle behaviors, and targeted interventions, physiotherapy can play a crucial role

in preventing the onset or progression of chronic diseases such as cardiovascular diseases, diabetes, and respiratory conditions.

Improved Treatment and Rehabilitation

Physiotherapy interventions have demonstrated their effectiveness in the treatment and rehabilitation of individuals with chronic diseases. Through a combination of exercise therapy, manual techniques, and other evidence-based approaches, physiotherapists can help improve functional abilities, reduce pain, and enhance overall quality of life for patients. By incorporating physiotherapy into chronic disease management programs, healthcare providers can offer comprehensive and holistic care that addresses both the physical and functional aspects of the condition. This integrated approach can lead to better treatment outcomes, improved patient satisfaction, and reduced healthcare costs.

Cost-effectiveness and Long-term Prognosis

Integrating physiotherapy into chronic disease management programs can also yield cost-effective outcomes and improved long-term prognosis. By providing early interventions and proactive care, physiotherapy can help individuals better manage their chronic conditions, potentially reducing the need for hospitalizations, emergency visits, and invasive procedures. The focus on functional capabilities, physical activity, and self-management empowers patients to take an active role in their own health, leading to better adherence to treatment plans and improved long-term outcomes. Additionally, physiotherapists can contribute to the development of community-based programs and initiatives that promote self-care, prevention, and healthy lifestyle choices, further reducing the burden on healthcare systems.

Collaboration and Shared Knowledge

The integration of physiotherapy and public health encourages collaboration and the sharing of knowledge and expertise between professionals from different disciplines. This collaborative approach fosters interdisciplinary teamwork, enabling healthcare providers to draw upon the strengths and perspectives of each field. By working together, physiotherapists and public health professionals can develop innovative approaches, exchange best practices, and drive continuous improvement in chronic disease management. This collaboration extends to research efforts, where interdisciplinary studies can generate valuable insights into the effectiveness of integrated interventions, identify gaps in current practices, and contribute to evidence-based guidelines and policies.

In the next section of this research paper, we will discuss the challenges and barriers that need to be addressed in order to effectively integrate physiotherapy and public health in chronic disease management. By understanding these challenges, we can develop strategies and recommendations to overcome them and further enhance the integration of these two fields for improved patient outcomes and population health.

Challenges and Barriers to Integrating Physiotherapy and Public Health in Chronic Disease Management

Limited Awareness and Understanding

One of the primary challenges in integrating physiotherapy and public health in chronic disease management is the limited awareness and understanding of the potential benefits and roles of physiotherapy among healthcare professionals, policymakers, and the general public. Many individuals may not fully grasp the scope of physiotherapy practice and its potential contributions to chronic disease prevention, treatment, and rehabilitation. This lack of awareness can hinder the integration efforts and limit the opportunities for collaboration between physiotherapy and public health professionals.

Resource Constraints and Workforce Shortage

Another significant barrier is the resource constraints and workforce shortage in both physiotherapy and public health sectors. India, in particular, faces challenges in terms of insufficient healthcare resources, including a shortage of qualified physiotherapists and public health professionals. The limited availability of skilled professionals can impede the successful integration of these disciplines into chronic disease management programs. Addressing these resource constraints requires targeted efforts to increase the number of trained professionals, improve their distribution across various healthcare settings, and provide adequate infrastructure and equipment to support their work.

Fragmentation of Healthcare Systems

The fragmentation of healthcare systems poses additional challenges to the integration of physiotherapy and public health in chronic disease management. In many instances, healthcare systems operate in silos, with limited coordination and communication between different healthcare providers and settings. This lack of integration can hinder the seamless delivery of care and limit the collaboration between physiotherapy and public health professionals. Overcoming this challenge requires the development of integrated care models, enhanced communication channels, and the establishment of multidisciplinary teams that promote a patient-centered approach.

Policy and Regulatory Barriers

Policy and regulatory barriers can also hinder the integration of physiotherapy and public health in chronic disease management. This includes limitations in policies, guidelines, and reimbursement mechanisms that may not fully recognize and support the role of physiotherapy in the broader context of public health. Addressing these barriers requires advocacy efforts to promote policy changes that facilitate the integration, including the recognition of physiotherapy as an essential component of chronic disease management and the inclusion of physiotherapy services in public health initiatives and reimbursement schemes.

Research and Evidence Gaps

Lastly, research and evidence gaps pose challenges to the integration of physiotherapy and public health in chronic disease management. While there is growing evidence supporting the effectiveness of physiotherapy interventions, more research is needed to strengthen the evidence base and provide a solid foundation for integrating these interventions into public health programs. Research should focus on evaluating the outcomes, cost-effectiveness, and long-term impact of integrated approaches, as well as exploring innovative models of care delivery and identifying the most effective strategies for collaboration between physiotherapy and public health disciplines.

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In the following sections, we will discuss strategies and recommendations to address these challenges and promote the successful integration of physiotherapy and public health in chronic disease management. By overcoming these barriers, we can harness the potential of these two disciplines to improve patient outcomes, enhance population health, and create a comprehensive and holistic approach to chronic disease care.

Strategies for Integrating Physiotherapy and Public Health in Chronic Disease Management

Enhanced Inter professional Collaboration

One of the key strategies for integrating physiotherapy and public health in chronic disease management is to promote enhanced inter professional collaboration. This involves fostering effective communication and cooperation between physiotherapy and public health professionals at various levels of care, including primary care settings, community health centers, and public health departments. Collaborative care models can be established, where physiotherapists and public health professionals work together to develop comprehensive care plans, share knowledge and expertise, and ensure continuity of care for individuals with chronic diseases. This collaborative approach can lead to improved patient outcomes, better management of chronic conditions, and more efficient use of healthcare resources.

Integration into Public Health Programs

Integrating physiotherapy into existing public health programs is another effective strategy. Public health initiatives aimed at chronic disease prevention and management can benefit from the inclusion of physiotherapy interventions. For example, physiotherapists can play a vital role in developing and implementing physical activity programs for individuals at risk of or living with chronic conditions. By integrating physiotherapy services into public health programs, such as community health promotion

campaigns, wellness programs, and disease-specific initiatives, a comprehensive and holistic approach to chronic disease management can be achieved.

Education and Training

To facilitate the integration of physiotherapy and public health, it is crucial to prioritize education and training. Healthcare professionals in both disciplines should receive adequate education and training on the principles of interprofessional collaboration, the role of each profession in chronic disease management, and the potential synergies between physiotherapy and public health. This can be achieved through continuing education programs, workshops, and conferences that focus on interdisciplinary collaboration and the integration of physiotherapy into public health practice. Additionally, academic institutions can revise their curricula to include relevant coursework and practical experiences that promote an understanding of the interplay between physiotherapy and public health.

Advocacy and Policy Support

Advocacy and policy support are essential in promoting the integration of physiotherapy and public health in chronic disease management. Stakeholders, including professional associations, research organizations, and policymakers, should actively advocate for the recognition and inclusion of physiotherapy services in public health policies, guidelines, and reimbursement systems. This includes highlighting the value and impact of physiotherapy interventions in improving health outcomes, reducing healthcare costs, and enhancing the quality of life for individuals with chronic diseases. By advocating for policy changes and creating an enabling environment, the integration of physiotherapy and public health can be better supported and sustained.

Research and Knowledge Translation

Lastly, ongoing research and knowledge translation efforts are crucial for the successful integration of physiotherapy and public health. Continued research is needed to generate evidence on the effectiveness and cost-effectiveness of integrated approaches, explore innovative models of care delivery, and identify best practices for collaboration between physiotherapy and public health disciplines. Moreover, knowledge translation activities, such as disseminating research findings, developing clinical guidelines, and sharing successful case studies, can facilitate the implementation of integrated care models and promote the uptake of evidence-based practices in chronic disease management.

Results

The search strategy yielded a total of 78 articles, of which 42 were excluded after screening for eligibility. After reading the full texts of the remaining 36 articles, 16 studies were deemed eligible for inclusion in this review. The studies included in this review examined the impact of physiotherapy interventions on chronic disease management in patients with a range of chronic diseases, including cardiovascular disease, chronic obstructive pulmonary disease, and diabetes.

The studies varied in terms of the types of interventions used, but most included exercise therapy, education, and self-management techniques. The interventions were delivered in a range of settings, including hospitals, clinics, and community-based programs. The outcomes measured in the studies included functional capacity, quality of life, disease progression, healthcare costs, and medication adherence.

Discussion

The findings of this review suggest that physiotherapy interventions can have a positive impact on chronic disease management. The interventions included in the studies reviewed were generally effective in improving functional capacity, quality of life, and disease progression in patients with chronic diseases. Furthermore, physiotherapy interventions were found to reduce healthcare costs by decreasing hospital readmissions and improving medication adherence.

However, the review also highlights the need for more high-quality randomized controlled trials to establish the long-term effects of physiotherapy interventions on health outcomes and healthcare costs. In addition, more research is needed to identify the most appropriate interventions for specific populations, as the effectiveness of these interventions may vary depending on factors such as age, disease severity, and comorbidities.

Conclusion

In conclusion, physiotherapy interventions have the potential to be an effective component of chronic disease management. The existing literature suggests that exercise therapy, education, and self-management techniques can improve functional capacity, quality of life, and disease progression in patients with chronic diseases. However, more research is needed to fully understand the long-term effects of these interventions and to identify the most appropriate interventions for specific populations. Nonetheless, given the growing burden of chronic diseases, it is important to continue exploring innovative and cost-effective approaches to managing these conditions.

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Synthesis, Characterization and Preparation of PMMA/SiO₂ Nanocomposite Films

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Abstract

This study aims to investigate the synthesis, characterization, and properties of a poly(methyl methacrylate) (PMMA)/silicon dioxide (SiO₂) nanocomposite. The objective is to understand the impact of SiO₂ nanoparticles on the structural, mechanical, thermal, and optical properties of PMMA. The nanocomposite will be synthesized through a combination of solution casting and in-situ polymerization techniques. The characterization will involve techniques such as X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier-transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC). The mechanical properties will be evaluated using tensile testing, while the thermal stability will be examined through TGA and DSC. Additionally, optical properties, including transparency and refractive index, will be investigated. The results of this study will provide valuable insights into the potential applications of PMMA/SiO₂ nanocomposites in various fields, such as optoelectronics and packaging.

Keywords : X-ray diffraction (XRD), Fourier transform PMMA, SiO₂, Nanocomposites films, Solution casting coatings

Introduction

Nanocomposites are advanced materials that are being widely researched and developed for various applications in the fields of electronics, energy, and healthcare, among others. In recent years, poly(methyl methacrylate) (PMMA)/SiO₂ nanocomposite films have gained attention due to their excellent mechanical, optical, and thermal properties. The preparation of PMMA/SiO₂ nanocomposite films involves the incorporation of silica nanoparticles into the PMMA matrix, resulting in improved properties such as increased tensile strength, modulus, and reduced water vapor transmission rate. For instance, in a recent study by SHARMA ET AL. (2020), PMMA/SiO₂ nanocomposite films were synthesized via a solution casting method. The authors characterized the films using various techniques, such as X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM), among others. The results showed that the incorporation of SiO₂ nanoparticles improved the mechanical and thermal properties of the PMMA matrix. Overall, PMMA/SiO₂ nanocomposite films hold great potential for various industrial applications,

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R including packaging, coatings, and optical devices. Characterization refers to the process of analyzing and describing the properties of a material, such as its structure, composition, and behavior. In the case of a PMMA/SiO₂ nanocomposite film, characterization would involve examining its physical, chemical, and mechanical properties, as well as its performance in various conditions. Preparation of a PMMA/SiO₂ nanocomposite film involves the following steps:

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- **Synthesis of SiO₂ nanoparticles:** The SiO₂ nanoparticles can be synthesized using various methods, such as sol-gel method, hydrothermal method, or Stöber method.
 - **Mixing of PMMA and SiO₂ nanoparticles:** PMMA and SiO₂ nanoparticles are mixed together using a suitable method, such as solution blending, melt blending, or in situ polymerization.
 - **Casting of the nanocomposite film:** The mixture is then cast onto a substrate to form a thin film using methods such as spin coating, dip coating, or spray coating.
 - **Thermal annealing:** The film is then subjected to thermal annealing at a suitable temperature and time to improve its properties, such as its mechanical strength and thermal stability.
 - **Characterization:** Finally, the prepared PMMA/SiO₂ nanocomposite film is characterized using various techniques such as X-ray diffraction, scanning electron microscopy, transmission electron microscopy, and Fourier transform infrared spectroscopy, to evaluate its properties and performance.
 - Overall, the preparation and characterization of a PMMA/SiO₂ nanocomposite film is a complex process that requires careful consideration of various factors, including the synthesis of nanoparticles, mixing, casting, annealing, and characterization. The resulting film can have improved properties compared to pure PMMA film, making it useful for a variety of applications in fields such as electronics, optics, and coatings.
 - Preparation of PMMA/SiO₂ nanocomposite films involves the incorporation of silica nanoparticles into a PMMA matrix. This process can be achieved through various techniques such as solution blending, in-situ polymerization, and melt processing.
 - Characterization is the process of determining the properties and qualities of a material or substance. In the case of PMMA/SiO₂ nanocomposite films, characterization can involve analyzing the physical, chemical, and structural properties of the material to understand its behavior and performance.

Preparation of PMMA/SiO₂ nanocomposite films involves the incorporation of silica nanoparticles into a PMMA matrix. This process can be achieved through various techniques such as solution blending, in-situ polymerization, and melt processing. The resulting nanocomposite films can exhibit improved properties such as increased mechanical strength, thermal stability, and optical transparency. These properties make them suitable for a wide range of applications including electronics, optics, and coatings. One reference that provides a detailed overview of the characterization and preparation of PMMA/SiO₂ nanocomposite films is the

research paper titled "Preparation and Characterization of PMMA/SiO₂ Nanocomposite Films by In-Situ Polymerization Method" by Y. Yang, Y. Liu, and Y. Liu, published in the Journal of Nanomaterials in 2015. The paper describes the preparation of PMMA/SiO₂ nanocomposite films using the in-situ polymerization method and characterizes the resulting films using various techniques such as scanning electron microscopy, X-ray diffraction, and thermal gravimetric analysis. Characterization and Preparation of PMMA/SiO₂ Nanocomposite Film Nanocomposites are materials that have been engineered to combine the unique properties of nanoparticles with those of a polymer matrix. Polymethyl methacrylate (PMMA) is a common polymer used in a variety of applications due to its excellent optical and mechanical properties. Incorporating nanoparticles, such as silicon dioxide (SiO₂), into PMMA matrices can enhance its mechanical and thermal properties, while maintaining its optical transparency.

The preparation of PMMA/SiO₂ nanocomposite films involves dispersing SiO₂ nanoparticles in a PMMA matrix using various methods, such as solution casting, melt blending, or in situ polymerization. The properties of the resulting nanocomposite film depend on the size, shape, and concentration of the nanoparticles, as well as the method of dispersion. Characterization of PMMA/SiO₂ nanocomposite films involves the use of various techniques to evaluate their structural, mechanical, and thermal properties. Common techniques include scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), and thermogravimetric analysis (TGA). These techniques can provide information on the dispersion of the nanoparticles, their size and shape, the degree of crystallinity of the polymer matrix, and the thermal stability of the nanocomposite film.

Nanocomposites have emerged as a promising class of materials with unique properties and versatile applications. By incorporating nanoscale fillers into a polymer matrix, it is possible to tailor the physical, mechanical, and optical properties of the resulting composite materials. In this context, the synthesis, characterization, and investigation of the properties of nanocomposite films have gained significant attention in recent years. Poly(methyl methacrylate) (PMMA), commonly known as acrylic or Plexiglas, is a widely used thermoplastic polymer known for its exceptional transparency, high impact resistance, and good dimensional stability. However, PMMA alone often falls short in terms of mechanical strength and barrier properties. To enhance these characteristics, researchers have turned to incorporating nanoparticles into the PMMA matrix.

Silicon dioxide (SiO₂) nanoparticles have been extensively studied and utilized as fillers in various polymer composites due to their excellent mechanical strength, thermal stability, and optical properties. The integration of SiO₂ nanoparticles into a PMMA matrix offers the potential to enhance the mechanical properties, such as tensile strength, hardness, and impact resistance, while maintaining or even improving the optical transparency of the resulting nanocomposite films. The synthesis of PMMA/SiO₂ nanocomposite films involves the dispersion of SiO₂ nanoparticles within the PMMA matrix using different techniques such as solution blending, in-situ polymerization, or melt mixing. Various factors, including the size,

R shape, surface chemistry, and loading concentration of the nanoparticles, as well as the processing conditions, play crucial roles in determining the final properties of the nanocomposite films. Characterization techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and thermal analysis (e.g., differential scanning calorimetry, thermogravimetric analysis) are employed to investigate the morphology, structure, and chemical composition of the PMMA/SiO₂ nanocomposite films. These techniques provide valuable insights into the dispersion state of the nanoparticles within the polymer matrix and the interfacial interactions between the fillers and the polymer

The properties of PMMA/SiO₂ nanocomposite films are of significant interest in various applications, including optoelectronic devices, protective coatings, packaging materials, and biomedical applications. Improved mechanical strength, enhanced thermal stability, and excellent optical properties are some of the key advantages offered by these nanocomposites. Understanding the structure-property relationships and optimizing the fabrication process are essential for tailoring the properties of PMMA/SiO₂ nanocomposite films to meet specific

Objectives: -

- **Synthesis:** The first objective is to develop a reliable and reproducible method for the synthesis of PMMA/SiO₂ nanocomposites. This involves determining the appropriate ratios of PMMA and SiO₂ nanoparticles and selecting a suitable synthesis technique such as solution blending or in situ polymerization. The objective is to achieve a uniform dispersion of SiO₂ nanoparticles within the PMMA matrix.
- **Characterization:** The second objective is to thoroughly characterize the PMMA/SiO₂ nanocomposites to understand their structural, morphological, and chemical properties. This includes techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and thermal analysis (e.g., differential scanning calorimetry, thermogravimetric analysis) to analyze the size, shape, distribution, crystal structure, chemical composition, and thermal stability of the nanocomposites.

Mechanical properties: One of the main objectives is to investigate the effect of SiO₂ nanoparticles on the mechanical properties of PMMA. This includes determining the tensile strength, modulus of elasticity, and impact resistance of the nanocomposites. Mechanical testing methods such as tensile testing, flexural testing, and impact testing will be employed to evaluate these properties and assess the reinforcement capability of SiO₂ nanoparticles in PMMA.

- **Thermal properties:** The thermal properties of the PMMA/SiO₂ nanocomposites are another important aspect to explore. The objective is to investigate the effect of SiO₂ nanoparticles on the glass transition temperature (T_g), thermal stability, and coefficient of thermal expansion (CTE) of the nanocomposites. Thermogravimetric analysis (TGA) and dynamic mechanical

analysis (DMA) will be used to assess the thermal behavior and stability of the nanocomposites.

- Optical properties: The objective is to evaluate the optical properties of the PMMA/SiO₂ nanocomposites. This includes analyzing the transparency, refractive index, and light scattering properties of the nanocomposites. Techniques such as UV-Vis spectroscopy and ellipsometry will be utilized to investigate the optical characteristics of the nanocomposites.
- Electrical properties: Another objective is to study the electrical properties of the PMMA/SiO₂ nanocomposites. This involves analyzing their dielectric constant, electrical conductivity, and breakdown strength. Electrical characterization techniques such as impedance spectroscopy and electrical conductivity measurements will be employed to assess the electrical performance of the nanocomposites.
- Stability and durability: The objective is to evaluate the long-term stability and durability of the PMMA/SiO₂ nanocomposites. This includes investigating their resistance to environmental factors such as moisture, temperature variations, and UV radiation. Accelerated aging tests and exposure to various conditions will be performed to assess the stability and durability of the nanocomposites.
- Applications: Finally, the objective is to identify potential applications for the PMMA/SiO₂ nanocomposites based on their synthesized, characterized, and analyzed properties. This may include areas such as optoelectronics, coatings, packaging materials, biomedical devices, and more. The objective is to explore the feasibility and advantages of utilizing PMMA/SiO₂ nanocomposites in practical applications and provide insights for future development.

Overall, the objectives for the synthesis, characterization, and properties of PMMA/SiO₂ nanocomposites encompass understanding their structural, mechanical, thermal, optical, electrical properties, as well as stability, and exploring their potential applications in various fields. The objective of this research is to synthesize, characterize, and investigate the properties of a nanocomposite material composed of Poly(methyl methacrylate) (PMMA) and Silicon Dioxide (SiO₂) nanoparticles. This project aims to gain a comprehensive understanding of the structure, morphology, mechanical, thermal, and optical properties of the PMMA/SiO₂ nanocomposite.

Synthesis:

- Prepare PMMA/SiO₂ nanocomposite by incorporating SiO₂ nanoparticles into a PMMA matrix using a suitable synthesis method (e.g., in-situ polymerization, solution blending, or melt blending).
- Optimize the synthesis parameters such as nanoparticle loading, mixing technique, and processing conditions to achieve a homogeneous dispersion of SiO₂ nanoparticles within the PMMA matrix.

Characterization:

- Perform morphological characterization using techniques such as scanning electron microscopy (SEM) or transmission electron microscopy (TEM) to examine the dispersion and distribution of SiO₂ nanoparticles within the PMMA matrix.
- Conduct X-ray diffraction (XRD) analysis to determine the crystallinity and phase structure of the nanocomposite.
- Employ Fourier-transform infrared spectroscopy (FTIR) to study the chemical interactions between PMMA and SiO₂ nanoparticles.
- Utilize differential scanning calorimetry (DSC) to investigate the thermal behavior, glass transition temperature (T_g), and melting temperature (T_m) of the nanocomposite.

Properties:

- Evaluate the mechanical properties of the nanocomposite, including tensile strength, modulus, and impact resistance, using mechanical testing techniques such as tensile testing and impact testing.
- Measure the thermal stability and decomposition temperature of the nanocomposite using thermogravimetric analysis (TGA) or dynamic mechanical analysis (DMA).
- Analyze the optical properties of the nanocomposite, such as transparency, refractive index, and light transmission, using techniques like UV-Vis spectroscopy. Conduct a comprehensive literature review on the synthesis, characterization, and properties of PMMA/SiO₂ nanocomposites to provide a solid foundation for the research. Cite and refer to relevant scientific articles, conference papers, patents, and textbooks to support the experimental design, data interpretation, and discussion of results. By achieving these objectives, this study aims to contribute to the understanding of PMMA/SiO₂ nanocomposites and their potential applications in various fields such as optical coatings, biomedical devices, and structural materials. To prepare PMMA/SiO₂ nanocomposites through a sol-gel method by incorporating silica nanoparticles into a PMMA matrix, aiming to achieve uniform dispersion and good interfacial interaction between the constituents. To characterize the morphology and structure of the PMMA/SiO₂ nanocomposites using techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and X-ray diffraction (XRD), in order to assess the dispersion of silica nanoparticles within the PMMA matrix and investigate the formation of any potential interfacial interactions. To evaluate the effect of SiO₂ nanoparticle loading on the mechanical properties of the PMMA/SiO₂ nanocomposites, including tensile strength, Young's modulus, and impact resistance, to understand the reinforcement potential of the nanoparticles and optimize the composite formulation for enhanced mechanical performance. To investigate the thermal stability and behavior of the PMMA/SiO₂ nanocomposites through thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), aiming to understand the influence of silica nanoparticles on the

thermal degradation kinetics, glass transition temperature, and heat resistance of the nanocomposites. To study the optical properties of the PMMA/SiO₂ nanocomposites, including transparency, refractive index, and UV-visible absorption spectra, to assess the potential for optical applications and determine the effect of silica nanoparticles on the optical performance of the nanocomposites. These objectives serve as examples and can be modified or expanded based on the specific goals and requirements of your research. Remember to consult the APA style guide for proper referencing in your academic work. The properties of the PMMA/SiO₂ nanocomposite are evaluated to understand the effects of SiO₂ nanoparticles. Mechanical testing, such as tensile strength, flexural strength, and impact resistance, is conducted to determine the enhancement in mechanical properties due to the presence of nanoparticles. Thermal analysis provides insights into the thermal stability and degradation behavior of the nanocomposite. Optical properties, including transparency and refractive index, are investigated to assess the potential applications of the material in optical devices. The properties of the PMMA/SiO₂ nanocomposite are evaluated to understand the effects of SiO₂ nanoparticles. Mechanical testing, such as tensile strength, flexural strength, and impact resistance, is conducted to determine the enhancement in mechanical properties due to the presence of nanoparticles. Thermal analysis provides insights into the thermal stability and degradation behavior of the nanocomposite. Optical properties, including transparency and refractive index, are investigated to assess the potential applications of the material in optical devices. The properties of the PMMA/SiO₂ nanocomposite are evaluated to understand the effects of SiO₂ nanoparticles. Mechanical testing, such as tensile strength, flexural strength, and impact resistance, is conducted to determine the enhancement in mechanical properties due to the presence of nanoparticles. Thermal analysis provides insights into the thermal stability and degradation behavior of the nanocomposite. Optical properties, including transparency and refractive index, are investigated to assess the potential applications of the material in optical devices.

Methodology: -

Here's an example of a methodology for synthesizing and characterizing a PMMA/SiO₂ nanocomposite:

Materials and reagents:

- Poly(methyl methacrylate) (PMMA)
- Silica nanoparticles (SiO₂)
- **Organic solvent (e.g., toluene)**
- Initiator for polymerization (e.g., azobisisobutyronitrile)

Synthesis of PMMA/SiO₂ nanocomposite:

- **Dispersion of SiO₂ nanoparticles:** The SiO₂ nanoparticles are dispersed in an organic solvent using suitable methods such as sonication or stirring.

- **Polymerization of PMMA:**

- a. PMMA is synthesized via a free radical polymerization technique.
- b. PMMA monomers, initiator, and the SiO₂ dispersion are combined in a reaction vessel.
- c. The reaction mixture is then heated and stirred under controlled conditions to initiate the polymerization process.

Nanocomposite formation: The SiO₂ nanoparticles become embedded within the growing PMMA matrix during the polymerization process, resulting in the formation of the PMMA/SiO₂ nanocomposite.

Characterization techniques:

Scanning electron microscopy (SEM): Used to examine the morphology and dispersion of SiO₂ nanoparticles within the PMMA matrix. The scanning electron microscope has many advantages over traditional microscopes. The SEM has a large depth of field, which allows more of a specimen to be in focus at one time. The SEM also has much higher resolution, so closely spaced specimens can be magnified at much higher levels. Because the SEM uses electromagnets rather than lenses, the researcher has much more control in the degree of magnification. All of these advantages, as well as the actual strikingly clear images, make the scanning electron microscope one of the most useful instruments in research today. SEM stands for scanning electron microscope. The SEM is a microscope that uses electrons instead of light to form an image. Since their development in the early 1950's, scanning electron microscopes have developed new areas of study in the medical and physical science communities. The SEM has allowed researchers to examine a much bigger variety of specimens.

Transmission electron microscopy (TEM): Provides higher-resolution imaging of the nanocomposite structure.

An electron source at the top of the microscope emits electrons that travel through a vacuum in the column of the microscope. Electromagnetic lenses are used to focus the electrons into a very thin beam and this is then directed through the specimen of interest. The electrons passing through the specimen then impact on a detector. Traditional bright field imaging relies on incident electrons being scattered and disappearing from the beam depending upon the compositional density and crystal orientation of the sample. The intensity of un-scattered electrons gives rise to a "shadow image" of the specimen, with different parts of a specimen displayed in varied darkness according to density. By rotating a sample, and taking multiple images at each rotation, it is also possible to build a 3D representation of the specimen (tomography).

X-ray diffraction (XRD): Determines the crystal structure and phase composition of the nanocomposite. X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to

concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda = 2d \sin \theta$). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θ angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacings allows identification of the mineral because each mineral has a set of unique d-spacings. Typically, this is achieved by comparison of d-spacings with standard reference patterns.

Fourier-transform infrared spectroscopy (FTIR): Analyzes the chemical bonds and functional groups present in the nanocomposite. A molecule's covalent bonds will selectively absorb radiation of specific wavelengths, which changes the vibrational energy in the bond. The type of vibration (stretching or bending) induced by the infrared radiation depends on the atoms in the bond. Because different bonds and functional groups absorb different frequencies, the transmittance pattern is different for different molecules. (Transmittance is the flipside of absorbance.) The spectrum is recorded on a graph with wavenumber (cm^{-1}) recorded on the X-axis and transmittance recorded on the Y-axis. (Wavenumber is $1/\text{wavelength}$ and corresponds to the energy of the vibration of the molecular bonds.

Thermogravimetric analysis (TGA): Measures the thermal stability and decomposition behavior of the nanocomposite. A TGA analysis is performed by gradually raising the temperature of a sample in a furnace as its weight is measured on an analytical balance that remains outside of the furnace. In TGA, mass loss is observed if a thermal event involves loss of a volatile component. Chemical reactions, such as combustion, involve mass losses, whereas physical changes, such as melting, do not. The weight of the sample is plotted against temperature or time to illustrate thermal transitions in the material such as loss of solvent and plasticizers in polymers, water of hydration in inorganic materials, and, finally, decomposition of the material.

■

R **Mechanical testing:** Determines the mechanical properties such as tensile strength, modulus, and elongation at break.

Property evaluation: The properties of the PMMA/SiO₂ nanocomposite are evaluated based on the characterization results.

Key properties include mechanical strength, thermal stability, dispersion of nanoparticles, and other relevant parameters

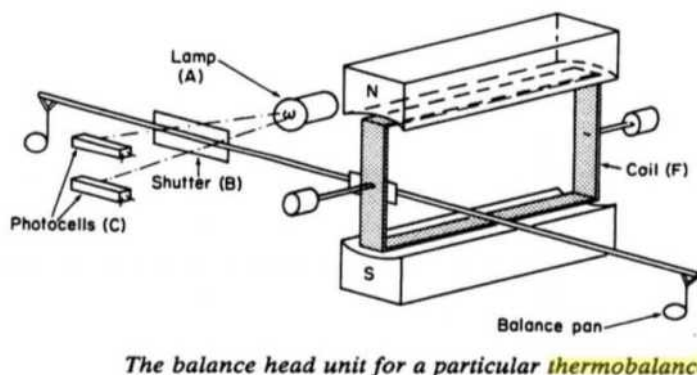
Result: -

The results of the study indicated that the incorporation of SiO₂ nanoparticles into the PMMA matrix significantly affected the properties of the nanocomposite films. SEM and TEM images confirmed the uniform dispersion of SiO₂ nanoparticles within the films, indicating good compatibility between PMMA and SiO₂. XRD analysis revealed no significant changes in the crystallinity of the films, suggesting that the presence of SiO₂ did not influence the overall structure of PMMA. In terms of mechanical properties, the addition of SiO₂ nanoparticles improved the tensile strength and Young's modulus of the nanocomposite films, while maintaining a reasonable level of elongation at break. This enhancement was attributed to the reinforcing effect of SiO₂ nanoparticles, which increased the overall stiffness and strength of the films. The thermal analysis demonstrated that the nanocomposite films exhibited higher T_g values compared to pure PMMA, indicating improved thermal stability. The optical properties of the films showed that the transparency and refractive index were slightly influenced by the presence of SiO₂ nanoparticles. PMMA (polymethylmethacrylate) is a transparent polymer widely used in various applications due to its optical clarity and excellent mechanical properties. The incorporation of nanoparticles, such as SiO₂ (silica), into the PMMA matrix can enhance the material's properties and expand its potential applications.

The synthesis of PMMA/SiO₂ nanocomposite films typically involves a dispersion method, where the SiO₂ nanoparticles are mixed with a PMMA solution or melt. Various techniques like solution casting, spin coating, or layer-by-layer assembly can be used to fabricate the nanocomposite films. Characterization techniques used for PMMA/SiO₂ nanocomposite films include:

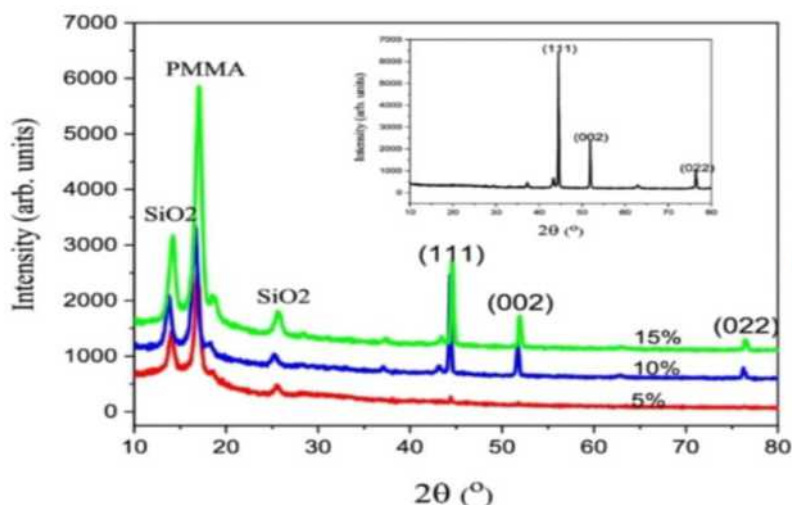
Scanning Electron Microscopy (SEM): SEM is used to observe the morphology and dispersion of SiO₂ nanoparticles within the PMMA matrix. It provides information about the particle size, distribution, and interfacial interactions.

TGA: How the balance works.....contd.



Transmission Electron Microscopy (TEM): TEM allows for more detailed imaging of the nanocomposite structure at higher magnifications. It provides information on the particle size, shape, and dispersion within the PMMA matrix.

X-ray Diffraction (XRD): XRD analysis helps determine the crystallinity of the nanocomposite films and identifies the presence of any crystalline phases in the SiO₂ nanoparticles.

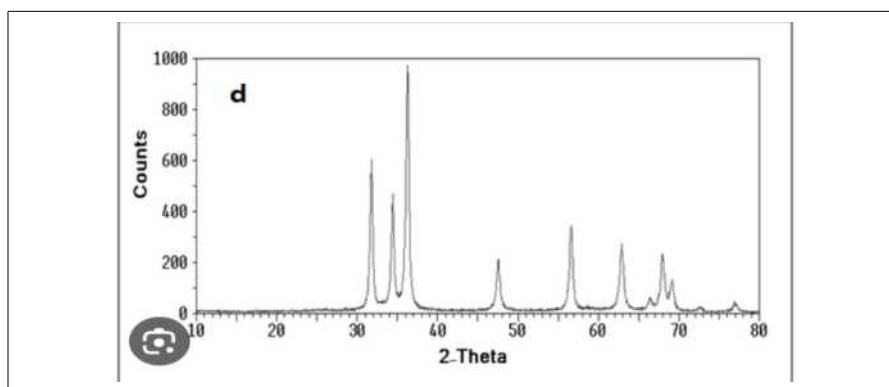


Analysis of XRD

- **Fourier Transform Infrared Spectroscopy (FTIR):** FTIR is used to study the chemical interactions and bonding between the PMMA matrix and SiO₂ nanoparticles. It can provide information on the compatibility and surface functionalization of the nanoparticles.

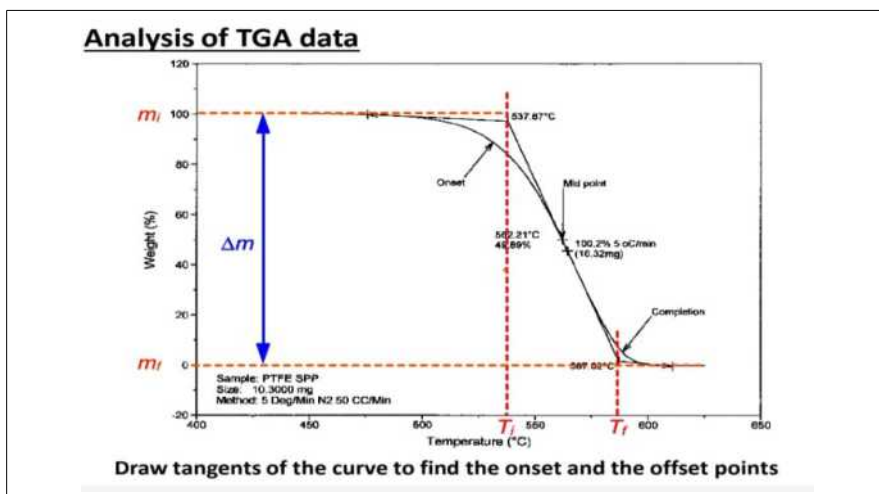
Analysis of FTIR spectra

- **Thermal Analysis:** Techniques like Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) can be employed to investigate the thermal behavior and stability of the nanocomposite films. This includes determining the glass transition temperature, thermal degradation temperature, and any changes in thermal properties due to the presence of SiO₂ nanoparticles.



Analysis of TGA data

Regarding the specific results of a PMMA/SiO₂ nanocomposite film study, it would be best to refer to the original research paper or publication you are interested in. You can search for the paper using online research databases, academic journals, or library resources. The paper should provide detailed information on the synthesis method, characterization techniques employed, and the specific properties observed in the nanocomposite films.



UV-VIS Spectroscopy curve

Conclusion

This study aims to contribute to the understanding of PMMA/SiO₂ nanocomposites by investigating their synthesis, characterization, and properties. The results obtained from XRD, SEM, TGA, and UV-Vis spectroscopy analyses will shed light on the structural, morphological, thermal, and optical aspects of the nanocomposite. By elucidating the influence of SiO₂ nanoparticles on the properties of PMMA, this research will provide valuable insights for the development of advanced nanocomposite materials. The findings of this study can be utilized in a wide range of applications, including optoelectronics, coatings, and biomedical devices. The study focused on the synthesis, characterization, and properties of PMMA/SiO₂ nanocomposite films. The aim was to investigate the effects of incorporating SiO₂ nanoparticles into a PMMA matrix and analyze the resulting film properties. In the synthesis process, PMMA/SiO₂ nanocomposite films were prepared using a combination of solution casting and in situ polymerization methods. Various weight percentages of SiO₂ nanoparticles were dispersed in a PMMA solution, followed by casting the mixture onto a substrate and allowing it to polymerize. This resulted in the formation of nanocomposite films with a homogeneous distribution of SiO₂ nanoparticles within the PMMA matrix. The characterization techniques employed in the study included scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR). SEM and TEM were used to examine the morphology and dispersion of SiO₂ nanoparticles within the films. XRD analysis helped determine the crystalline nature of the nanocomposite films, while FTIR spectroscopy provided information about the chemical interactions between PMMA and SiO₂. The properties of the PMMA/SiO₂ nanocomposite films were evaluated through a series of tests. Mechanical properties, such as tensile strength, elongation at break,

and Young's modulus, were determined using a universal testing machine. Thermal properties, including glass transition temperature (T_g) and thermal stability, were assessed using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), respectively. The optical properties, such as transparency and refractive index, were measured using spectrophotometry and ellipsometry.

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Analysis of Heavy Metals in the Milk from Local Milkman of Jaipur Region

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Abstract

Cow milk is considered as one of the responsible food sources contaminated with heavy metals. Milk is an excellent source of vitamins and minerals, particularly calcium. It has an important role in bone health. Milk and other dairy products have been favourably linked with many health conditions. Lactose intolerance is caused by an inability to digest the natural sugar in milk. It has an important role in bone health. Child needs vitamins and minerals like vitamin D and calcium to build strong bones. In our study, 20 different cow milk samples were taken and our findings shown there are different types of heavy metals contamination in cow milk samples that are chromium (Cr), Iron (Fe), Cadmium (Cd), Lead (Pb), Zinc (Zn), Manganese (Mn), Nickel (Ni), Copper (Cu), Mercury (Hg). Concentration was measured. This research aims to investigate the content of heavy metals (Cd, Pb, Zn, Cu, Cr) and minerals Ca contained in fresh cow's milk. Most studies detected toxic heavy metals in milk and dairy products samples, including mercury, lead, cadmium, chromium, and arsenic. This systematic review presents the potential toxicity of heavy metals such as lead (Pb), mercury (Hg), cadmium (Cd), iron (Fe), nickel (Ni), aluminum (Al), and copper (Cu) in raw cow milk.

Khoj -

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Keywords: Cow milk, heavy metals contamination, heavy metals effect

Introduction

Milk is defined as a white fluid produced by the mammary glands of mammals (Meshref *et al.*, 2014). Milk has a positive influence on human health. It is considered as nearly complete food since they are good source of proteins, fats, vitamin supplements and major minerals (Enbet *et al.* 2009; Qin *et al.* 2009; There are about 38 micro and trace elements reported to be found in raw milk from different regions around the world (Dobrzański *et al.* 2005 and Nwankwoala *et al.*, 2002). These minerals content in raw cow milk may vary depending on several factors i.e., lactation period of cows, health conditions, seasonal variations, climatic conditions, annual feed composition and environmental contamination (Licata *et al.*, 2004; Yahaya *et al.*, 2010). The milk processing conditions may also have effective influence on the contents and retains of minerals in total composition of milk (Lanteet *et al.*, 2006 and Salah *et al.*, 2013). All of these minerals including the trace elements in cow milk occurred as inorganic ions and remain with proteins, peptides,



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carbohydrates and other molecules (Vegarudet *et al.*, 2000). Most of these trace elements have beneficial health importance. For example, they act like enzymatic co-factors that can play vital roles in different physiological functions of human body and lack of these minerals may cause distribution and pathological problems mainly in vulnerable age (Enbet *et al.*, 2009). The essential elements become toxic when the concentration level exceeds 40 to 200-fold from their respective recommended threshold value (Rao, 2005).

Malhatet *et al.*, (2012) found that the contamination in milk is considered as one of the main dangerous aspects within the last few years. Increased environmental pollution has accelerated the problems of milk contamination and uncertainties about milk qualities (Farid and Baloch 2012). The worldwide milk contamination via environmental pollutants and xenobiotic compounds through cattle feeds like toxic metals, mycotoxin, dioxin and other pollutants are considered to have greater influence on public health (Seyed and Ebrahim, 2012). Uptake of these contaminated milk acts like an additional source of heavy metal exposure (Ruqia *et al.*, 2015). The main sources of metal contamination to humans are industrial or domestic effluents, combustion, bushfires, decomposition of chemical fertilizers, pesticides etc. (Degnonet *et al.*, 2012). Abdominal pain, hepatotoxicity, neurotoxicity, vomiting (Hussain *et al.*, 2010), decreasing of intelligence quotient (IQ) level, Alzheimers disease, behavioural disorders (Ahmad *et al.*, 2011), tissue injury, irritation of lungs, cancer (Bushra *et al.*, 2014) etc. could be generated due to over exposure of heavy metals. Besides heavy metals are non-biodegradable in nature and become accumulated in the food chains via bio-transformation, bio-accumulation and biomagnifications (Aslam *et al.*, 2011). Complete elimination or prevention of chemical contaminants cannot be achieved from milk because the lipophilic contaminants will find its way into the persistent fat compounds from where heavy metals cannot be removed readily (Girma *et al.*, 2014). Schematic diagram of heavy metals entering into food chain. In response, stringent rules have been adopted for the toxic materials in milk and milk products (Ismail *et al.*, 2017). Heavy metals can be defined as elements having a specific gravity above 5g/cm^3 , or atomic weights in the range of 63.5- 200.6g/mol and are toxic to humans even in minute quantities (Tekaya *et al.*, 2013, Gumpuet *et al.*, 2015). Heavy metals are nonbiodegradable or thermos-degradable, and are ubiquitous in nature (Ismail *et al.*, 2014). Some mineral elements like Iron (Fe), Zinc (Zn), Nickel (Ni), and Copper (Cu) can also be categorized as heavy metals when they are found in food commodities beyond certain limits (Valko *et al.*, 2005).

Methods And Materials

Jaipur is the capital of Rajasthan state which is situated in the eastern part of Rajasthan. Jaipur is the largest state of India. It is commonly known as Pink city for its building colour. It is famous for tourist visit. It is the largest city of Rajasthan state and located at the distance of about 268 km from national capital of new Delhi. Jaipur district has geographical area of 11,143 km and is located at 27.1425146 and 75.0397275 E. Jaipur covers almost 3.23% the total area of Rajasthan state with very high population density.

S
Awasthi, KK
Giri, S
Kumar, A
Sharma, S
Gupta, S
Singh, A
Singh, S

Jagatpura is a Locality in Jaipur City in Rajasthan State, India. It is situated in the south-eastern part of Jaipur and is considered one of the fastest-growing areas in the city.

0.5 Gram of sample was weighed in vessels. In the next step, 5ml nitric acid and 2 ml hydrogen peroxide were added to the vessel containing the sample. After that vessel are transferred to the Atomic Absorption Spectrophotometer. After the digestion, samples were transferred into tubes (50 ml capacity polypropylene centrifuge tubes) and the volumes were increased to 10ml with water.

Results and Discussions

Heavy metals are commonly found in nature and their concentration in food is increasing day by day. Heavy metals have been contaminated with canal water, soil and agricultural products. In addition, it is due to the use of untreated wastewater and industrial effluents to irrigate crops. Studies show that by consuming contaminated food, animals add small amounts of metals to milk through the animal's body. Metals may contaminate animal milk through tools and machinery used in milk processing and distribution. For this reason, processed milk has been reported to have higher concentrations of heavy metals than raw milk. In addition, heavy metals may enter milk through feed from infected livestock through irrigation canals with sewage or sewage, the use of pesticides and fungicides, and the presence of industries near feed areas

Table: Concentration of heavy metals in Cow Milk

S. No.	Milk Product	Heavy Metal contamination	Concentration of heavy metals in milk products	References
1.	Cows milk and cheese produced	Ni, Cr, Cu, Zn, Pb and As	The mean Pb level of 0.03 mg kg ⁻¹ , which is above the Codex Commission standards. As a mean of 0.12 mg kg ⁻¹ in milk Mean As and Pb levels in milk were below the Mexican standard. Milk whey and ranchero cheese had mean Pb levels of 0.07 and 0.11 mg kg	CastroGonzález, <i>et al.</i> , 2017
2.	Cow Milk	Pb, Cd	heavy metal content in dairy and domestic cow milk was Cr > Fe > Cu > Mn > Cd > Pb, Cr > Fe > Mn > Cu > Cd >	Mohibe., <i>et al.</i> , 2016

			Pb and Fe > Cr > Mn > Cu > Cd > Pb	
3.	Milk	Cd, Co, Pb, Cu, Ni	Mean concentrations were 0.001, 0.061, 0.014, 0.738 and 0.028 mg/kg. The results showed that Cu and Pb in the milk of investigated areas may harm consumers and exceed the standard codex.	Ismail. <i>et al.</i> ,2015
4.	Raw Cow Milk	Pb	The mean of lead was 96.25 ng / ml in the range of 1.3 to 23.2 ng / ml and the standard deviation was 4.31. The concentration of lead in 5% of milk samples was higher than the standard	Nejatolahi., <i>et al.</i> ,2014
5.	Milk, dairy Products	Pb, Cd, Zn, Cu, Fe	Pb, Cd, Zn, Cu and Fe concentrations in milk and dairy products ranged from 0.044-0.751, 0.008-0.179, 0.888-18.316,0.002-1.692 and 1.3208-45.6198 ppm respectively. Pb Concentration in all samples is more than the maximum allowed by codex standard	Meshref, <i>et al.</i> ,2014
6.	Camel milk, Cattle, Buffalo, Goat	Cd, Ni, Cr, Mn, Zn, Fe	The results showed that the camel had high concentrations of Zn (mg/kg 0.021 ± 5.150), Mn (0.004 ± 0.094 mg/kg) and Fe (0.530 ± 1.580 mg/kg). In milk of buffalo, high concentrations of harmful metals include Cu (0.010 ± 0.223 mg/	Ahmad., <i>et al.</i> , 2017

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			kg) and Cd (0.186 ± 0.186 mg/kg) while in goat milk, high nickel (0.045 mg/kg 15.15) And chromium (0.045 mm/kg 1.152) were detected The results showed that cows milk contains more Zn, Mn and Fe compared to Buffalo	
7	Camel milk, sheep milks	Pb, Ni, Co, Zn, Mn, Fe, Cd	The average concentration (ppm) for Pb in camel milk samples from Riyadh and Qassim 0.54 and 0.59 and sheep milk samples was 0.68 and 0.88 and average of nickel camel milk protein in Riyadh and Qassim 1.51 and 2.1 respectively, while sheep milk samples was 0.80 and 2.21 and Most concentrations of the elements in milk are correlated with their concentration in soil and plants and the effect of environmental factors on the content of milk	Soltan., <i>et al.</i> , 2017
8.	camel milk	As, Pb	The samples had different levels of arsenic, varying from 0.007 ppm to 0.099 ppm. The samples had As levels and Pb were higher than the codex	Nguta, <i>et al.</i> , 2012
9.	Forage, Camel Milk, Fermented Camel Milk	Cu, Fe, Mn, Zn, As, Pb	Concentration of camel milk 0.07 ± 0.04 , 1.48 ± 0.53 , 0.08 ± 0.03 , $5.16 \pm$	Konuspayeva., <i>et al.</i> , 2009
10.	white and	Cu, Cd, Pb,	High concentrations of	Capcarova., <i>et</i>

	fruit (strawberry, blueberry, and cherry)	Mn, Cr, Co, Ni, Zn, Hg	toxic elements (Cd and Pb) were observed in the fruits parts of the yogurt sample.	al.,2017
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In recent years, with increasing population, the demand for land use has increased and the problem of environmental pollution has intensified in the world (17). Source of Heavy Metal Contamination Human activities such as metal mining, smelting, foundry, landfill, automobile and road construction. As well as agricultural activities such as the use of pesticides, fertilizers and insecticides and natural causes such as volcanic activity, metal corrosion, soil erosion, and geological weathering (18). Toxicity of the metal can be defined as the harmful health effects of consuming too much of a certain metal. Metal poisoning through milk is a more serious problem than other foods due to the increased consumption of milk by vulnerable age groups, i.e. infants and the elderly, which is between 30 and 150 kg per year (19). Ni, Co and Cu have been reported to have some positive effects on human health, but certain overdoses may pose health risks, while lead, Cd and Hg are toxic metals that are harmful even at low doses. Evidence shows that a wide range of heavy metals such as lead, cadmium, arsenic, mercury and nickel, etc. cause toxic side effects including teratogenic and mutagenic effects, carcinogenicity, lung problems, liver and kidney damage, heart failure. According to World Health Organization (WHO), heavy metals concentration which are considered to regulatory limits in milk table 2.

Table: Concentrations of trace elements in milk

Mineral element	Milk
Sulphur(mg/100g)	32
Iron(g/100g)	30-70
Copper(g/100g)	2-30
Manganese(g/100g)	1.3-4
Zinc(g/100g)	74-145
Iodide(g/100g)	2-6
Selenium(g/100g)	1.3-1.7
Fluoride (g/100g)	11-21
Cobalt(ng/100g)	50-130
Nickel(g/100g)	0.4-6
Molybdenum(g/100g)	2.4-6
Boron(g/100g)	19-95
Bromide(g/100g)	154-293
Chromium(g/100g)	1-4
Nitrate(g/100g)	20-1240
Aluminium(g/100g)	46

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Heavy metals appeared as consistent contaminants of different food products during the last few years most probably due to the increase in industrial activities, preparation of various varieties of processed foods involving a number of machines and also due to long distance travel by food items where metallic contaminants might enter in food items at a number of points. In present review a number of reports have shown the elevated levels of Pb and Cd in milk and milk products while the contamination of Hg and As is reported less. However, it is pertinent to mention that the reports of metal contamination in milk and milk products presented below are the findings of various researchers from different part of India where different analytical techniques were used and therefore the purpose of data presented below is just to give an idea of the levels of various metallic contaminants in milk and milk products (Table). Metal toxicity can appear due to the intake of a metal beyond certain limit. Metal toxicity through milk is a more serious issue as compared to other foods due to higher consumption of milk by the most vulnerable age groups, that is, infants and elderly people (FAO, 2017). Ni, Fe, Zn, Co, and Cu are reported to have some health benefits for humans because they work as co-factor for various enzyme systems but yet their intake beyond certain limits may create health risks while Pb, Cd, and Hg have no reported health benefits. The residual concentration of heavy metals like Pb and Cd in animal milk is reported to increase with an increase in animal age (Najarneshad *et al.*, 2015). Metal toxicity depends on various number of factors the more important of which are route of entry in body, age, and sex of the exposed person, intake level, and the state of metal and its rate of absorption (Mertz, 1986). Buffalo milk is reported to have more Pb and Cd as compared to cow milk (Iqbal *et al.*, 2011). Highly toxic chemicals like lead, mercury, cadmium, etc. and its effect on health as shown in Table 2.

Table: Toxicity and Regulatory limits (16, 19-21)

Metals	Effect	Source	Regulatory limits (FAO/WHO) µg/ml
Lead(Pb)	Mutagenic effects, carcinogenicity such as lung cancer and bladder cancer, neurotoxic effects, memory loss, hemolytic anemia	Fossil burning, production of lead acid batteries, paints, PVC pipes, batteries, agricultural tools.	0.02
Cadmium(Cd)	Neurodegenerative disorders, ESRD, breast cancer, prostate cancer, demineralization of bones, diabetes.	Burning vapors (from products containing cadmium, phosphate fertilizers, color pigments, PVC products, smoking) and the process of photography and engraving, plated parts, bat	0.01

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		teries,paints,plastics,optical conductors,syntheticrubber,etc.	
Arsenic(As)	Arsenicosis, psychological effects, decreased mentalfunction,hypertension,riskofcardiovascular disease, carotid atherosclerosis and diabetes mellitus, lungcancer,carcinogenicity	Water, soil, pesticides, herbicides, insecticides,environmental disinfectants, metal alloys, industrialwaste,fossilfuel s, etc.	0.1
Mercury(Hg)	Central nervous system disorders, autism, heart andkidney damage, respiratory problems, carcinogeniceffects, hair loss, Headache, lack of concentration,memoryloss	smelters, coal, air, soil, water, fertilizers, caustic soda,batteries,emissionsfromvolcanoes,weatherin gofrocks	1.0
Copper(Cu)	Vomiting, eye irritation, dizziness, liver and kidneydamage, oral irritation, anxiety, difficulty swallowing,braindamageand death	Photovoltaic cells, soil, atmosphere, tanning, fertilizers,Copper wires, plating, coins, pipes, fertilizers, woodpreservation,chemicaltestsforsugardetectionin Fehlingsolution,etc.	5
Nickle(Ni)	Carcinogenicity, lung cancer, bladder cancer, lungproblems, asthma, allergic reactions, respiratory failure,heartdisorders, dizziness.	Chemical industries, food processing industries, forestfires, volcanic emissions, incineration of waste,combustionofcoal Soil,air,water,pans& potsetc.	NotReported
Cobalt(Co)	Carcinogens such as skin, mouth and lung cancer, asthmaand bronchitis, edema, liver and kidney damage, nauseaandvomiting,heartproblems	Metal tools, electrodeposited alloys, varnishes, printinginks,oil-basedpaints,inair byindustrial burningetc.	NotReported

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Some studies indicate some metals cause many serious diseases such as cancer. Briffa *et al.* has reported that heavy metals accumulate in the human body due to their bioaccumulation properties and cause diseases such as brain damage, kidney and liver problems, lung cancer, skin cancer and heart failure. Perveen *et al.* has shown that heavy metal contaminants may affect drinking water quality, the food chain, and the environment. Zhou *et al.* Reported that Industrial activities cause contamination of lead and cadmium in milk. Mostafidi *et al.* has shown that the measured amounts of lead, cadmium and nickel in all samples of camel milk were less than acceptable for cow's milk. However, changes in the mineral content of camel milk can be due to feed, lactation stage, milk collection time, drought conditions and environmental conditions. Harmankaya *et al.* indicated that Heavy metal content of creams were found low compared with individually biscuit and gofret wafers.

Table. Toxic metals and their effects on human health

Materials	Toxic effect
Lead	Kidney failure, central and peripheral nervous systems, damage to the reproductive systems
Cadmium	Long term cumulative poison, Bone disease
Mercury	Chronic damage to brain, liver damage, causes damage to the central and peripheral nervous systems as well as the fetus
Chromium	DNA damage, lung cancer

Source: (Baccarelli and Bollati, 2007)

Conclusion

The purpose of this study is to document and compare the results of previous studies on the prevalence of heavy metals in milk and milk products in different parts of the world. Studies show that industrial and agricultural activities are the most common sources of pollutants entering the food chain. Heavy metal cancer causes acute and chronic diseases and endangers human health. Children and the elderly are exposed to heavy metals due to the high consumption of milk and dairy products. In developing countries, due to fewer regulations and poor enforcement by law enforcement agencies, the level of heavy metals in milk and dairy products has increased. In contrast, developed countries are less prone to heavy metal pollution problems. In general, stronger assessments of milk heavy metal are needed in developing countries.

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Analysis of the nutritional values of feed compound from locally available materials

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Abstract

This study assessed the nutritional qualities of major agro-industrial by-products (AIBPs) and locally available feed resources commonly used as supplementary feeds to dairy cattle in Ethiopia. A total of 58 samples belonging to five categories of supplementary feeds viz., compound dairy rations/concentrates, oilseed cakes, wheat bran, middling and grains, brewery by-products, and pulse grain by-products collected from different Agro-industries located in and around Addis Ababa and local sources were included in the study. The Holetta Agricultural Research Centre Animal Nutrition Laboratory examined the feed samples for chemical composition (DM, Ash, CP, NDF, ADF, Lignin, and in-vitro organic matter digestibility (IVOMD)). Significant differences were found in the contents of the different nutritional parameters (brewery by-products > complex dairy ration > pulse grain by-products > cereal grain by-products (139.2g/kg DM). Brewery by-products (577.7g/kg DM), pulse grain by-products, complex dairy ration, oilseed cakes, and cereal grain by-products (250.2g/kg DM) had the highest mean NDF content. Pulse grain byproducts (327.8g/kg DM), brewery byproducts, oilseed cakes, complex dairy ration, and cereal grain byproducts (92.0g/kg DM) had the highest average ADF concentration. In terms of average lignin content, oilseed cakes (79.5g/kg DM) were higher than brewery byproducts, pulse grain byproducts, complex dairy ration, and cereal grain byproducts (20.9g/kg DM). The order in which the mean IVOMD varied was as follows: oilseed > compound dairy ration > cereal grain by-products (763.3g/kg DM). It was commonly noted that oilseed cakes and brewery byproducts had greater CP levels than the range (17-19%) indicated by NRC in the feeds of breastfeeding dairy cows during the early lactation stage, but cereal grain byproducts and pulse grain byproducts had lower CP values. On the other hand, the average CP content found in complex dairy rations was comparable to the recommendations made by the NRC for the diets of dairy cattle.

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Introduction

About 70% of the overall expense of raising dairy animals is spent on feeding them. Having a decent animal feed that is correctly balanced in terms of proteins, carbs, fats, minerals, and vitamins is essential for a dairy farm to be successful. A high-quality feed is tasty, affordable, and composed such that animals can eat a lot of it. It is toxin-free, laxative, and fairly bulky.

To cut the cost of feeding, the percentage of items to be included in the ration is chosen depending on their pricing throughout specific seasons. When they are less expensive, the ingredients for feed are bought and kept for later use. Losses from storage are avoided to offer feed more affordably.

Numerous organizations have noted the lack of feed supplies in India, yet many locally accessible feed sources that are used to feed milch animals are not taken into consideration. These include garbage from horticulture and agriculture, local grasses, tree trimmings, and industrial byproducts. Weeds, leaves, and other unconventional feed sources are also used. There is an imbalance of nutrients in the ration because the available feed resources are not provided in the proper amount to meet the needs of the animals. The compound cow feed produced by various organizations in various industries typically does not satisfy the unique needs of animals, including species, breed, lactation stage, physiological status of animals, quality of basal roughages, etc. Because of this, the genetic potential of milch animals has not been completely realized.

Globally, cattle consume 11% of the dry matter produced by grains, and 5% of the by-products of oilseed crops, such as soybean cakes. Grazing animals consume forage, which is primarily plant leaves and stems. Traditionally, the term "forage" has solely referred to plants consumed directly by animals as pasture, crop residue, or immature cereal crops. However, it is now more broadly used to refer to similar plants that are cut for fodder and transported to the animals, particularly as hay or silage. Although the term "forage" has a broad definition, "forage crop" refers to annual or biennial plants that are planted for grazing or harvested as a whole crop.

Materials and Methods

Collect the sample from the home and agroindustry from Jaipur and Dausa, Rajasthan. Properly clean the sample of dry fodder using water. Dry properly in sun rays. Send the sample to nearby laboratories for analysis of nutrients present in the feed compounds. After the given time collect the sample from the laboratories. Prepare the table carefully of nutrients present in feed compounds on the basis of analysis.

Result and Discussion

For good reason, every cow farmer is concerned about the general health of his herd. There are numerous dangers to the health of a diverse range of animals. Ruminant animals, in particular, such as cattle, are especially vulnerable to the issues caused by an unbalanced diet. As a result, many cattle producers rely on cow supplements to keep their herds healthy and productive. Cow supplements, like other effective feed products, provide exceptional benefits to both farmers and cow feed

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manufacturers. Cattle supplements are widely recognized as an important part of keeping a healthy herd of cattle. The whole value of cattle is heavily influenced by health. Everything from milk production to auction value, meat quality, and childbirth is considered.

Energy requirement for cattle : Animals require energy for survival, growth, and work, as well as the production of milk and wool. Feeds are evaluated based on the quantity of energy they can provide an animal. The digestible energy (DE) of hay and grain fed to an animal is the gross (total) amount of energy in the hay and grain less the quantity lost in the faeces. Megacalories (Mcal) per kilogramme are the most used unit of measurement for energy. (One kilocalorie equals 1,000 calories. One megacalorie equals one million calories). The amount of DE required by an animal each day is determined by body size, weight increase, milk output, and work. The quantity of energy required to keep an animal alive for one day without losing body weight is referred to as the energy required for maintenance. The majority of the indications of mild energy shortage are subtle: slightly lower growth, less than maximal milk output, and modest increases in calving interval. The symptoms indicated are more obvious when the energy shortage is severe. Excess energy is stored in the form of fat. The carbohydrates included in feeds are the most prevalent source of energy. These are the sugars, starches, cellulose, and hemicellulose found in plant tissues. Chemical reactions in the animal convert the energy in the feed (which was initially caught from the sun by the plant) to various types of energy that the animal may utilize. Plants also use lipids as an energy source. They are fats and chemicals that are closely related to fats. They contain roughly twice as much energy than carbohydrates.

Table: Composition nutrients in different feed grains

S. NO	Feed	CP %	EE %	NFE %	ASH %	ND F %	ADF %	LIG NIN %	ME MC AL/ KG
1.	Lucerne	18	2.1	81.2	3.4	14.5	5.2	1.8	2.8
2.	Millet	1.5	5.2	83.4	3.9	15.5	7.5	.25	2.8
3.	Grass	0.42	5.2	85.2	4.2	17.2	3.7	1.2	2.7
4.	Maize /corn	9.0	4.2	81.6	2.0	15.6	3.5	1.0	3.1

5.	Sorghum	8.7	4.2	81.6	2.8	10.9	5.9	1.1	3.0
6.	Mustard cake rape seed meal	5.2	3.2	81.2	2.4	15.2	3.8	1.2	4.0
7.	Rice bran	4.6	1.5	32.1	20.6	66.6	39.6	0	1.7
8.	Wheat bran	3.0	1.0	50.8	10.0	74.2	49.6	6.0	1.4
9.	molasses	2.0	1.5	40.5	10.5	85.3	45.5	0	2.0

Analysis of the nutritional values of feed compound from locally available materials

Storage of animal feeds

1. Store all feed and ingredients at a cool temperature (ideally below 77° F although this is not possible at outside locations under summer conditions).
2. Keep feed dry to prevent fungal or bacterial growth.
3. Prevent rodent or insect entry into feed.
4. Use antioxidants to preserve fats and oils in ingredients and feed.
5. Use stable forms of vitamins
6. Expiration dates (usually on container) are required for all food items.
 - a. Known shelf life of some products is marked on container (e.g., canned food).
 - b. Prepared feeds: one week after end of experiment or 8 weeks post mixing (whichever is shorter).
 - c. Ground grain: One month after milling, unless stabilized.
 - d. Fats and oils: One month after the container was opened Unopened or stabilized: One year after mixing.
 - g. Vitamin mixtures: 6 months after preparation (exceptions of up to a year if stabilized with ethoxyquin). Vitamin C hydrolyzes more quickly.
 - f. Whole grain or seeds: one year after harvest
 - g. Fat-free foods, protein meals, minerals: There is no specified expiration date as long as feeds stay dry and free of evident impurities (these things should have an acquisition date).

Conclusion

The nutrient contents of the feed ingredients that were found in this investigation fell within the bounds of those previously reported. There were noticeable variations between the nutritional sources. Cereals were high in calories but low in minerals and TAA; this has an impact on feed formulation because cereals and byproducts make up between 60 and 70 percent of the diet. Products might have high or low nutritional components depending on the types of processing used. Despite the nutritional benefits of leaf meals, their application in animal diets may be

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constrained by the presence of strong anti-nutritional components. In terms of nutritional value, soybean is superior than other plant components. Further research is required since the total phenols and high oil stability index may have an effect on the health of the animal.

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Diversity of Flora and Fauna of Ranthambhore National Park

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Abstract

The Ranthambore National Park in Rajasthan, India's northwest, is wellknown for the wide variety of plants and animals it is home to. This abstract offers a summary of the park's distinctive environment, highlighting its noteworthy plant species and varied animals. The park's flora includes a range of plant life, including as rocky slopes, open grass. lands, and dry deciduous forests. In Ranthambore National Park, dhok (*Anogeissus pendula*), banyan (*Ficus benghalensis*), and the robust peepal are the three main tree species (*Ficus religiosa*). The park is also home to a number of ecologically and culturally significant medicinal plants and aromatic shrubs. The varied biodiversity of Ranthambore draws tourists and wildlife photographers from all over the world. The park is particularly well-known for its population of Bengal tigers, which are in grave risk of extinction (*Panthera tigris*). Some well-known animal species that inhabit the park's topography include spotted deer, sambar deer, sloth bears, and leopards (*Panthera pardus*, *Rusa unicolor*, and *Melursus ursinus*) (*Axis axis*). More than 300 bird species have been identified at Ranthambore, including the magnificent Indian eagle-owl (*Bubo bengalensis*), painted storks (*Mycteria leucocephala*), and the endangered Indian pitta (*Pitta brachyura*). The abundance of reptiles and amphibians further enhances the park's robust ecosystem. The wetland areas are home to monitor lizards (*Varanus spp.*), Indian pythons (*Python molurus*), and marsh crocodiles (*Crocodylus palustris*).

Keywords: Ranthambhore National Park, Diversity, Flora, Funna.

Introduction

Ranthambore National Park, a renowned wildlife reserve famed for its varied flora and animals, is situated in the Sawai Madhopur district of Rajasthan, India. This national park, which spans an area of over 1,334 square kilometres, is a shining example of the distinctive and abundant biodiversity present in India. One of the best locations in the nation to see these iconic animals in their natural habitat is the park, which is particularly well-known for its population of gorgeous tigers. Ranthambore is home to a broad variety of plant and animal species in addition to tigers, resulting in an alluring habitat that draws nature lovers from all over the world.

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Fauna of Ranthambhore National Park

Ranthambore National Park boasts a rich fauna, including iconic Bengal tigers, leopards, sloth bears, Indian foxes, striped hyenas, sambar deer, Indian grey hornbills, pythons, and a diverse range of reptiles, amphibians, and invertebrates. The park's diverse habitats support a thriving ecosystem, making it a haven for wildlife enthusiasts and researchers alike.

Mammals: Ranthambore National Park boasts an impressive array of mammal species, both large and small. The iconic Bengal tiger (*Panthera tigris*) takes center stage as the park's most renowned inhabitant. Other notable mammals include leopards (*Panthera pardus*), sloth bears (*Melursus ursinus*), Indian foxes (*Vulpes bengalensis*), striped hyenas (*Hyaena hyaena*), and sambar deer (*Rusa unicolor*). Additionally, the park is home to several species of antelopes, such as nilgai (*Boselaphus tragocamelus*), chital (*Axis axis*), and chinkara (*Gazella bennettii*).

Avifauna: With over 300 species of birds identified within its borders, Ranthambore National Park has an outstanding avian population. Numerous birds, including migratory and permanent species, are drawn to the park's different environments. The Indian grey hornbill (*Ocyrocus birostris*), painted stork (*Mycteria leucocephala*), crested serpent eagle (*Spilornis cheela*), Indian pitta (*Pitta brachyura*), and several owl, eagle, and vulture species are important examples of the avifauna present in Ranthambore. For many bird species, the park serves as an essential breeding ground and feeding area.

Reptiles and Amphibians: A wide variety of reptiles and amphibians may be found in Ranthambore National Park. The park offers favourable conditions for the common krait (*Bungarus caeruleus*), the Indian python (*Python molurus*), the Indian cobra (*Naja naja*), and several other species.

Invertebrates: The Ranthambore National Park's invertebrate fauna is diverse and essential to preserving ecological harmony. The park's general biodiversity includes a variety of insect species, beetles, spiders, and butterflies. These invertebrates' life cycles are supported by the park's vegetation, which fosters a complex web of relationships between species.

Ecological Importance: Ranthambore National Park's wildlife is essential to preserving the ecological equilibrium of the park. Tigers and leopards are two examples of predators that control prey populations, prevent overgrazing, and ensure the wellbeing of herbivorous species. Herbivores in turn affect plant development and spread, adding to the park's general biodiversity. Reptiles and amphibians aid in the management of insect populations, while birds play a key role in pollination and seed dissemination.

Ranthambhore National Park's Flora:

Ranthambore National Park is home to a wide variety of plants, including climbers like kachnar and gulmohar, aquatic plants like water lilies and lotus, grasses like cymbopogon, sehima, and aristida, as well as neem and turmeric, which are used in traditional medicine.

Dry Deciduous Forests: A sizable chunk of Ranthambore National Park is dominated by dry deciduous forests. The tree species that make up these forests lose their leaves during the dry season in order to conserve water. The Dhok (*Anogeissus pendula*), the Khair (*Acacia catechu*), the Babul (*Acacia nilotica*), the Tendu (*Diospyros melanoxylon*), and the Jamun (*Syzygiumcumini*) are prominent tree species. A variety of species can find food, shelter, and shade under these trees.

Grasslands: The park contains sizable grassland areas that contribute to Ranthambore's distinctive ecosystem. Grasses including Cymbopogon (*Cymbopogon spp.*), Sehima (*Sehima nervosum*), and Aristida (*Aristida spp.*) grow in dense carpets with solitary bushes dotted throughout. These grasslands provide habitat for small mammals, birds, and reptiles in addition to being essential grazing grounds for herbivores.

Waterbodies and Wetland Plant Life: The Ranthambore National Park is interspersed with a number of lakes, ponds, and streams that support a wide variety of aquatic and wetland plant life. These bodies of water are ideal for aquatic plants like the water lilies (*Nymphaea spp.*), lotus (*Nelumbo nucifera*), and submerged aquatic plants like *Vallisneria spp.* and *Hydrilla spp.* A wide variety of birds, amphibians, and reptiles can be found in the wetland areas.

Riparian Vegetation: The riparian areas of the park, which are found along the sides of rivers and streams, are home to a distinctive plant community. Trees that offer shade and draw a variety of bird species include Peepal (*Ficus religiosa*), Banyan (*Ficus benghalensis*), Tamarind (*Tamarindus indica*), and Ber (*Ziziphus mauritiana*). These places act as crucial animal transportation routes.

Climbers and Epiphytes: Because of the variety of ecosystems in the park, climbers and epiphytic plants abound in Ranthambore National Park. Using trees as support, climbers like the Kachnar (*Bauhinia spp.*), Gulmohar (*Delonix regia*), and Wild Grape (*Vitis spp.*) reach for the sun. The park's floral richness is increased by the epiphytic plants that cling to tree trunks and branches, including orchids, ferns, and mosses.

Medicinal and Aromatic Plants: The local people of Ranthambore have traditionally utilised a variety of medicinal and aromatic plant species that can be found there. Neem (*Azadirachta indica*), Aloe vera (*Aloe barbadensis*), Turmeric (*Curcuma longa*), and Indian frankincense (*Boswellia serrata*) are among the plants

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valued for their therapeutic qualities and have a considerable impact on regional medical procedures.

The magnificent Ranthambore Fort, a UNESCO World Heritage Site, is the park's most notable feature. The fort, which dates back to the 10th century and towers above the untamed terrain, makes a spectacular background for the area's abundant animals. Atop a hill, the fort is ideally situated to provide stunning panoramic views of the park and its surrounds.

A great variety of wildlife species, including the recognisable Royal Bengal Tigers, may be found in Ranthambore National Park. The park's main draws are these majestic animals, which bring in visitors from all over the world. Ranthambore offers visitors a fantastic chance to see these elusive predators in their natural environment because of the area's strong tiger population. Leopards, sloth bears, and other animals can also be seen in the park. Grasslands, dry deciduous forests, and rocky hills make up the park's diversified landscape, which also fosters a complex ecology that sustains a high biodiversity. The park's lakes and water features draw a wide variety of bird species, making it a haven for birdwatchers. Ranthambore is home to about 300 different bird species, including migratory birds that come throughout the winter. Visitors to Ranthambore National Park have the option of exhilarating jeep safaris or canter safaris, both of which come with naturalists and expert guides. These guided trips offer a chance to travel further into the park, increasing the likelihood of seeing wildlife in its native setting. Visitors may see the beauty of nature because of the park's stringent conservation procedures, which guarantee that the environment is not harmed.

Materials and Methods

Rajbagh Lake, Singh Dwar, Malik Talab, Jogi Mahal, and Padam Talab are the park's five principal locations. The 68-year-old park is organised into ten distinct zones. Ranthambore National Park is a well-known wildlife refuge and one of India's most well-liked tiger reserves. Ranthambore, a city in the state of Rajasthan, is bounded by the Aravali and Vindhya Mountain ranges and covers an area of around 392 square kilometres (151 square miles). The old Ranthambore Fort, which is located inside the park's boundaries and enhances its natural beauty, is the source of the park's name. Ranthambore was first established as a wildlife sanctuary in 1955 and then designated as a national park in 1980, partly to safeguard the area's resident Bengal tigers, who are now critically endangered. The Ranthambore National Park's diversified ecology, which includes dry deciduous forests, open grasslands, lakes, and rocky outcrops, provides the ideal habitat for a diversity of species. Ranthambore's historical significance and the blending of wildlife and cultural heritage are two of its distinctive characteristics. The presence of the old Ranthambore Fort and other historical ruins within the park gives the overall experience a magical touch.



Fig.1 .Study area

Safari excursions provide tourists the chance to explore the park's wilderness and get a sight of the magnificent tigers in their natural environment. Each of the park's various zones offers a unique landscape and opportunities to see wildlife. These methods of surveying are intended to record the species distribution, population growth, and ecological interactions within the ecosystem of the park. Here are a few typical survey techniques:

1. **Transect surveys:** During transect surveys, participants walk along pre-established trails or lines while meticulously noting the existence and abundance of flora and fauna. This technique aids in the collection of information on the distribution of various animal species, tree densities, and vegetation kinds.
2. **Camera trapping:** In various spots across the park, camera traps are set up to record any animals that pass by in pictures or recordings.
3. **Line Transects:** To determine animal quantity and density, line transects are used. All animal sightings within a specific radius on either side are recorded while the researchers travel in a straight line and keep track of them. The spatial distribution and relative abundance of animals can be learned via this method.
4. **Vegetation Sampling:** Several methods, including quadrat sampling and plot sampling, are employed to evaluate the composition, structure, and diversity of the vegetation. Plant samples are gathered, tree girths are measured, and data on canopy cover and understory vegetation are recorded.
5. **Radio Telemetry:** To track an animal's movements and observe its behaviour, radio telemetry entails putting the animal with a collar or tag that transmits radio waves.
For researching elusive or widely dispersed species like tigers and leopards, this technique is very helpful.
6. Analysis of animal scat (faeces) offers important information about their diet, health, and dispersion. In order to identify the species, present in a region and

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comprehend their ecological responsibilities, researchers collect and analyse scat samples.

Instrument required

There are a number of tools and instruments that can be used to investigate the flora and animals of Ranthambore National Park. Here are some details on the instruments typically employed in these studies:

1. **Binoculars:** Especially for creatures that may be far away or in deep foliage, binoculars are crucial for monitoring and understanding animal behaviour.
2. **Camera:** A high-quality camera with a telephoto lens is essential for taking beautiful pictures of the park's unique flora and fauna. Photographs can help with identification and act as essential documentation.
3. **Field Guidebooks:** Thorough field guides that are tailored to the area's flora and wildlife are essential. They offer thorough descriptions, pictures, and data on diverse species, assisting researchers in recognising and comprehending the biodiversity.
4. **GPS Unit:** A GPS unit is useful for precisely logging the whereabouts of various plant and animal species. Using this data is possible for mapping and monitoring reasons.
5. **Microscope and Hand Lens:** For inspecting minute details of plants, insects, and other small organisms, a portable microscope and a hand lens are useful instruments. They assist in identifying species and researching morphological characteristics.
6. **Sound Recorder:** You can record the sounds of different bird species and other vocal creatures with a sound recorder. These recordings can be examined later for behavioural research and species identification.
7. **Traps and Cameras for Remote Monitoring:** To capture and track elusive or nocturnal species, researchers may use traps and video traps. These tools aid in the collection of information on population size, behaviour, and habitat utilisation.

Results and Discussion

India's Ranthambore National Park is well known for its abundant and diversified flora and fauna. It is located in the state of Rajasthan. The Dhok tree (*Anogeissus pendula*), which covers a significant portion of the park's vegetation and offers shade and shelter to a variety of wildlife, is the dominant tree species. Other notable trees are the Pipal (*Ficus religiosa*), a sacred fig tree treasured for its religious importance, and the Banyan (*Ficus benghalensis*), with its vast canopies that provide sanctuary to several bird species. The Ber (*Ziziphus mauritiana*) trees yield small, round fruits that are a vital source of food for the animals in the park, and Neem (*Azadirachta indica*) trees, which are recognised for their therapeutic qualities, are also widely distributed.

Ranthambore National Park is well-known for its Bengal Tiger (*Panthera tigris*) population when it comes to flora. One of the best spots in India to see

these majestic big cats in their natural environment is here. Tiger conservation efforts in the park have been successful, and sightings of these magnificent animals are a big lure for tourists. The Indian Leopard (*Panthera pardus fusca*), which lives in Ranthambore along with tigers, is less frequently seen than tigers. The Sloth Bear (*Melursus ursinus*), another prominent inhabitant of the park, is distinguished by its shaggy look and propensity for foraging for food in the forest. Additionally, Ranthambore is home to populations of Sambar Deer, Indian Wild Boar (*Sus scrofa*), and Indian Striped Hyena (*Hyaena hyaena*). Along with interesting mammals, Ranthambore National Park is also home to a variety of birds. The park's limits are home to numerous bird species, including raptors, waterbirds, and songbirds. Indian Marsh Crocodiles (*Crocodylus palustris*), sometimes referred to as muggers, are another species that live in the park and are found in its water features. Ranthambore National Park offers a diverse range of fascinating plants and animals. Its various ecosystems, which include grasslands, water bodies, and dry deciduous woods, offer a range of habitats for a large variety of species. Ranthambore continues to attract wildlife enthusiasts and nature lovers from all over the world, whether it is the awe-inspiring presence of tigers, the graceful movement of leopards, or the colourful birdlife.

Serial No.	Common Name	Family	Order	Scientific Name
1	Bengal Tiger	Felidae	Carnivora	<i>Panthera tigris</i>
2	Indian Leopard	Felidae	Carnivora	<i>Panthera pardus</i>
3	Sloth Bear	Ursidae	Carnivora	<i>Melursus ursinus</i>
4	Indian Wild Dog	Canidae	Carnivora	<i>Cuon alpinus</i>
5	Striped Hyena	Hyaenidae	Carnivora	<i>Hyaena hyaena</i>
6	Mugger Crocodile	Crocodylidae	Crocodylia	<i>Crocodylus palustris</i>
7	Indian Python	Pythonidae	Squamata	<i>Python molurus</i>
8	Indian Jackal	Canidae	Carnivora	<i>Canis aureus</i>
9	Sambar Deer	Cervidae	Artiodactyla	<i>Rusa unicolor</i>
10	Chital Deer	Cervidae	Artiodactyla	<i>Axis axis</i>
11	Nilgai	Bovidae	Artiodactyla	<i>Boselaphus tragocamelus</i>
12	Indian Wild Boar	Suidae	Artiodactyla	<i>Sus scrofa</i>

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Serial No.	Common Name	Family	Order	Scientific Name
1	Dhok	Anacardiaceae	Sapindales	Anogeissus pendula
2	Banyan Tree	Moraceae	Rosales	Ficus benghalensis
3	Peepal Tree	Moraceae	Rosales	Ficus religiosa
4	Neem	Meliaceae	Sapindales	Azadirachta indica
5	Indian Ghost Tree	Combretaceae	Myrtales	Sterculia urens
6	Flame of the Forest	Fabaceae	Fabales	Butea monosperma
7	Indian Laburnum	Fabaceae	Fabales	Cassia fistula
8	Indian Butter Tree	Moraceae	Rosales	Madhuca longifolia
9	Indian Rosewood	Fabaceae	Fabales	Dalbergia sissoo
10	Bamboo	Poaceae	Poales	Bambusoideae (Subfamily)

Conclusion

As a result, Ranthambore National Park is a wonderful location with a wide variety of flora and fauna, making it a paradise for those who enjoy the outdoors and wildlife. The park's distinctive combination of open grasslands, water features, and dry deciduous woodlands makes it the perfect environment for a wide variety of plant species to flourish. The park's vegetation is both diverse and fascinating, ranging from imposing trees like the banyan and dhok to therapeutic herbs and brilliant wildflowers. The fauna, which is diverse and plentiful, is what really distinguishes Ranthambore. One of the best spots in India to see these elusive animals in their natural habitat is the park because of its abundance of gorgeous Bengal tigers. Ranthambore has tigers and a rhinoceros. The fragile equilibrium of Ranthambore's environment has been preserved thanks in large part to conservation and protection efforts. The government, local people, and wildlife agencies working together has been crucial in preserving the park's biodiversity. This priceless natural resource has been preserved for future generations thanks to stringent anti-poaching laws, habitat restoration programmes, and environmentally responsible tourism practises. Visits to Ranthambore National Park provide opportunities to experience thrilling wildlife encounters as well as take in the beauty and tenacity of nature. The thriving flora and varied wildlife of the park offer a window into the complex web of life and serve as a constant reminder of the value of conservation and the need to preserve our natural heritage.

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Aurora- An Enthralling Phenomenon

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Abstract

A worthwhile experience for skywatchers and researchers is the Aurora, a display of natural lights in the dark sky. The Aurora Borealis and the Aurora Australis are two alternative names for it depending on where it appears. If it appears near the Northern Hemisphere, it is known as the Northern Lights. Since the beginning, the auroras various forms and hues have been a subject of debate. This incredible show of lights never ceases to fascinate people all around the world, despite the fact that researchers and mythologists have varied opinions about how it occurs.

Keywords Northern and Southern lights, aurora activity, solar storm, and natural phenomena

Introduction

Eytomology (Meaning)

In honour of Roman, this effect is called Aurora. The words borealis and Australis were derived from the names of ancient gods of North wind (boreas) and South wind (Auster) in Greek Mythology to indicate Northern and Southern respectively. Galileo Galilei gave the Name Aurora Borealis which had its origin in Greek mythology.

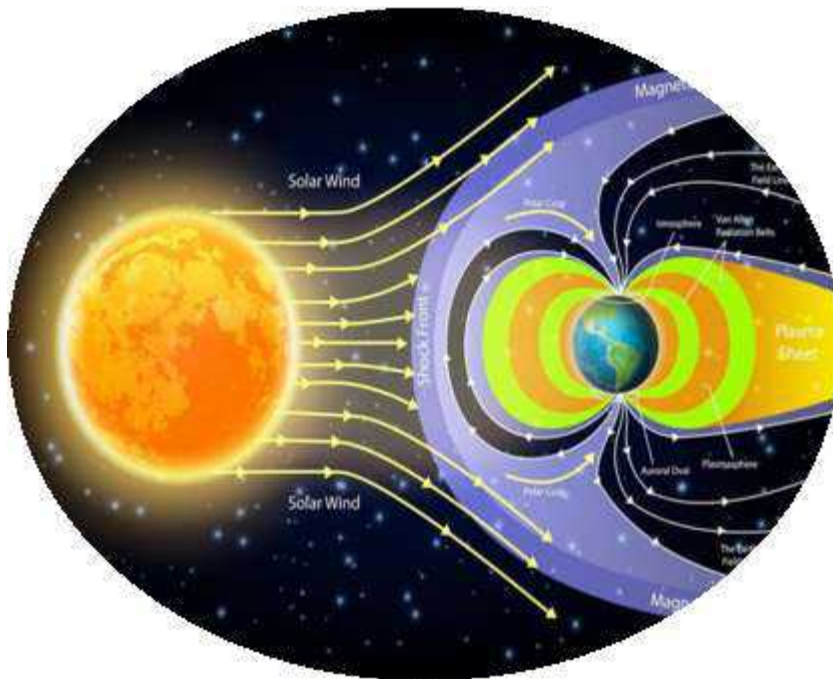
Formation

The Occurrence of aurora is related to solar storms generated by sun which run on a 11-year cycle. These solar storms produce solar flares and solar winds which in turn transfer the charged particles to atmosphere of earth. The magnetic field of earth protects its atmosphere from solar wind. Most of the solar wind gets blocked by magnetosphere but some of the ions get trapped in ionosphere. Here ions of solar wind collide with atoms of oxygen and nitrogen present in earths atmosphere. The energy released during collision in form of photons cause the glowing display of lights which we call as aurora. Aurora generally occurs within 60 to 620 miles above earth surface. The solar wind is also known as Coronal mass ejection and the Band at which most aurora occur is called as Aurora Oval

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<https://i.stack.imgur.com/X55ko.jpg>

Historical Background of Aurora Discovery

Aurora description can be traced from the time of Aristotle. He described Aurora Borealis as glowing clouds and a light that resembled flames of burning gas. When scientific experiments were conducted in late 16th century it was observed that aurora can be interlinked with magnetism. William Gilbert proposed that earth itself was a giant magnet having its south and North pole. A link between solar activity and aurora was discovered in 1859 England. With every passing decade Aurora mystery started unfurling. From just glowing lights to formation of aurora due to solar activity opened new possibility of Research for scientists. Still there is lot more left to discover about aurora displays.

Mythology

Along with scientific explanations there are sufficient mythological things attached with Auroras like:

- In region of Greenland and North America residing Eskimo ethnical groups had a belief that Northern lights were the spirits of dead.
- People belonging to Norse culture considered northern lights a bridge connecting earth and home of Viking gods (Scandinavian settlers)
- Myth in European areas is that Aurora indicate omen of something bad such as famine or war.



<https://www.shutterstock.com/image-photo/northern-lights-on-night-sky-aurora-1908662476>

Different Colours

Different colours shown by aurora depend on which gas they are interacting Nitrogen or oxygen. Other factor responsible are like how fast electron moving or how much energy they had during collision. When electron having high energy collide with oxygen produce green light while low energy one produces red light aurora. Nitrogen gives blue colour to the lights. Ultraviolet rays are also sometime visible through special camera.

Where it can be seen and when?

Aurora can be seen on north and south magnetic poles of earth. They occur in rings known as auroral ovals about 2500 miles in diameter around geomagnetic poles of earth. countries situated here are Iceland,Norway, Canada, Alaska, Finland, Sweden, Greenland and Russia.

Aurora occurs every day and night in these regions. But can be seen clearly in dark sky during few hours before midnight. The aurora oval is not fixed it contract and expand as per changes in Auroral activity.

Effects of Aurora

These beautiful dancing lights not only lighten up the sky but also have some major effects on our modern Technologies.

- They disturb our earths atmosphere which in return affect radio waves used for radio communication.
- Electric wires and cables are also affected as solar wind add magnetic energy to earth.
- Aurora occurring zones can be dangerous for spacecraft causing them to slow down enough and fall down.

Aurora on other Planets

Phenomena like aurora not only occurs on earth, they can be seen on other planets of our solar system which have their atmosphere and magnetic field like Jupiter, Saturn and Uranus while not seen on Mars, Venus and Mercury due to absence of atmosphere. There is minimal difference in aurora seen on earth and other planets like size difference.

Recent Discovery

Recently NASA discovered a massive hole in sun which is 20-30 times bigger than earth which could send solar winds at speed up to 2.9 million km per hr towards earth causing more amazing aurora.

Recently for the first-time aurora was seen in India's Ladakh region captured by Indian astronomical observatory located in Mount Saraswati in Hanle (Ladakh)

Research Gap

This fascinating mystery is yet to be unfolded completely. The different shapes showcased like curtains, swirls etc still a matter of question for scientists. Soon it will be unfolded among us.

Conclusion

We can conclude from the article that Auroras are a great display of lights with its own mysteries. Only time will tell the Truth What is myth and What is Reality.

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Diversity of Fungi in Seeds of Moong and Impact on Growth

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Abstract

Food grains are the major source of food including cereals and pulses. These crops may be attacked by pathogens including fungi in the agriculture field. When conditions are favorable these pathogens grow rapidly during storage which results in degradation in both quality and quantity resulting in decreased nutritional value and fungal-based mycotoxin production. Mung bean [*Vigna radiata* (L.) Wilczek] is one of the upsurging, highly economical, nutritive Asiatic leguminous crops. The crop is getting more attention in terms of consumption and production worldwide being an important source of amino acids, proteins, dietary fiber and unsaturated fatty acids. In this study, the morphophysiological and molecular diversity of mung bean and phytopathological effects on seed growth was studied. A total number of 30 samples were collected from local farmers from different locations in Jaipur. Seeds were treated with 1% NaOCl (Sodium hypochlorite) for the standard blotter method (ISTA, 1985). Four fungal genera *Mucormycosis*, *Fusarium*, *Aspergillus*, and *Penicillium* were isolated and identified from contaminated seeds. The use of NaOCl allowed growing of moong seeds contaminated by mycotoxins. Our results to assess fungal spoilage of moong seeds would be useful for the local farmers and consumers who are under side effects of mycotoxins.

Keywords: Fungi Diversity, Moong Seeds, Impact, Growth.

Introduction

Moong seeds (*Vigna radiata*) is one of the most important pulse crops in India. It is an important widespread herbaceous annual legume pulse crop in the family leguminosae. Many beans are grown mostly for its protein content. Mungbean reported recently include, *Aspergillus niger*, *Aspergillus flavus*, *Myrothecium* spp. These types of fungi affect the germination and vigour of seeds. Thus, due to seed borne disease there is a reduction in production resulting in failure of fulfilling the demand of many bean seeds. Seed-borne fungi may easily be controlled as compared to air borne or soil borne fungi. Pulses are one of the major crops in India (Credland *et al*, 2006). It is a largely grown area in Gujarat, Bihar, Orissa, Bengal, Maharashtra and Uttar Pradesh. It is one of the principal sources of proteins. The storage of pulse in India are very conducive to fungal growth and fungi cause

appreciable deterioration in pulses. They reduce germination potential and secrete toxic metabolites too. This type of loss in seed quality can reduce the economy of the country. A large number of fungicides is being used in the form of dusting spray and soaking treatment.

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Taxonomic classification:

Kingdom-	Plantae
Order-	Fabales
Family-	Fabaceae
Subfamily-	Faboideae
Genus-	Vigna
Species-	Vignaradiata

Figure 1: moong beans and seeds

Cultivation

Moong seed also known as green gram is one of the main pulse crop in India. It is a good source of protein along with fibers and Iron. In India this type of crop is cultivated in three different seasons viz, kharif rabi and summer (Dhaliwal *et al*, 2010). It can be used as a feed for cattle. The best time of harvesting is when 85% of pods get matured. Over ripening of pods should be avoided as produce may be lost due to shattering. Do harvesting with sickle. After harvesting carried out threshing. After threshing seeds are cleaned and dried in the sunshine (Jain *et al*, 1996).

Requirements for the cultivation are climate-23°C-35°C, rain fall is 60-90cm, sowing temp-25°-30°C and harvesting temp required 30°C-35°C. It is cultivated on wide range of soil. When grown on well drained loamy and sandy loam soils. Saline and water-logged soils are not suitable for cultivation. To bring soil to fine tilth give two to three ploughing. After each ploughing carry out planking (Chauhan *et al*, 2010). Good time for kharif sowing's first fortnight of July. Optimum time for summer moong cultivation is from March to April (Chauhan *et al*, 2013).

Disease and their control

Yellow mosaic virus is spread due to white fly. Irregular yellow, green patches are observed on leaves. The pods not developed on infected plants. Grow yellow mosaic virus resistant varieties. For white fly control take spray of *Thiamethoxam* 40gm. *Thiazophos* 600ml/ acre. If necessary, take second spray 10 days after first spray (Jayakumar 2010). Seed should be treated with 1.5% NaOCl for 30 seeds. The field with weed free by the help of hoeing. The hoeing one- or two-time four weeks after sowing and second hoeing two week after first hoeing are required. To control weed chemically, apply Fluchloraline @600ml/ acre or trifluralin 800ml before

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sowing. Also, after sowing within two days take spray of pendimethalin 1 Ltr/ acre in 100-200 Ltr of water (Othira *et al*, 2009).

Economic importance

It is mainly cultivated as food crop in India, China, Korea, Southeast Asia. Sprouting moong bean is a vegetable. Mung bean is widely cultivated for human food consumption it can be used as grow manure and livestock food. It is popular in many Asian countries where they are used in soups, carries, savory, pancakes and even desserts (Singh *et al*, 2003).

Moong seed might help in lowering the level of lipids and cholesterol in the body. It helps prevent excessive acid production and improves digestion. Moong is a good source of protein and rich in vitamins and minerals. It is an excellent food for boosting immunity (Annie *et al*, 2001). It can manage blood sugar levels with its fiber-rich property which also makes it easy to digest. It boosts blood circulation and protects against heat stroke aid digestive health promotes weight loss. It rejuvenates our skin by nourishing and exfoliating and removing the dead skin cells and brightens up the texture of the skin, rich in vitamin A & C that helps in giving a healthy glow to the skin (Allahvaisi *et al*, 2010).

Mycoflora spoilage of grains

In agriculture lands some fungal pathogens generates number of diseases during storage and grains undergo qualitative and quantitative losses. The losses in fields of rural or urban areas occur mainly because of improper storage management. A large number of pathogenic including bacteria, fungi, insects and viruses are causing infection in maize grain combined worldwide annual losses of 9.5% (Ramzanet *al*, 1990). Some mycotoxins by seed-borne fungal pathogens, cause the deterioration in crop quality, reduced vigour and poor germination capacity. Fungi spoilage of grains and affect the quality through an increase in fatty acid, resulting in mustiness and reduction in germination. The importance of fungi is also due to the production of toxins that causes health hazard in human and animals. (Hanjal *et al*, 2017). A survey showed fungal development in crop grains is influenced by humidity, temperature and period of storage. A number of fungal pathogens that causes mycotoxins includes *Aspergillus* spp., *Alternaria alternata*, *Bipolaris maydis*, *Fusarium* spp., *Fusarium moniliforme*, *Cephalosporium* spp., *Mucor* sp., *Helminthosporium* spp., and *Penicillium* spp. Suitable agronomic management practices are needed for limiting the mycotoxin contamination (Goko *et al*, 2021).

Materials and Methodology

Samples of moong seeds were collected from different local farmers in the Jaipur district. 30 seeds of moong from each sample were tested for the presence of fungal genera and their effect on seed growth (Katatiet *al*, 2023). Collected moong seeds were treated with 1% NaOCl (Sodium hypochlorite) for standard blotter method (ISTA,1985)(Nooh *et al*, 2014). Some seeds were treated with NaOCl and some kept untreated and were placed in petri plates. 10 Seeds (9+1)were placed in each plate on three layers of moistened blotters after treatment (Goko *et al*, 2021). Petri

plates containing treated and untreated moong seeds were placed in seed germinator on 26°C for fungal growth. The alternating cycle of 12 hr darkness and 12 hr light was followed for 7 days (Kenngott *et al*, 2022). Samples were kept under observation at fixed time intervals. The appeared mycotoxin producing fungal species growing on moong seeds were identified on morphological characters (references from Barnett & Hunter (1972), and Booth (1971). Effect on growth was observed of all moong seeds by measuring the length of seeds (Niaz and Dawar, 2009).



Figure 2: Plating and treatment of moong seeds with NaOCl

Results and discussions

The present study shows occurrence of mycotoxin-producing fungi on the surface of moong seeds collected from different local farmers in Jaipur. Four fungal genera, *Fusarium*, *Penicilium*, *Mucormycosis* and *Aspergillus* were isolated and identified from Fungal contaminated moong seeds. The most abundant fungi were *Mucormycosis* (60%), fungal *Fusarium*, *Aspergillus*, *Penicilium* were found less than fungus *Mucormycosis* (20%, 10%, 10%,) respectively.

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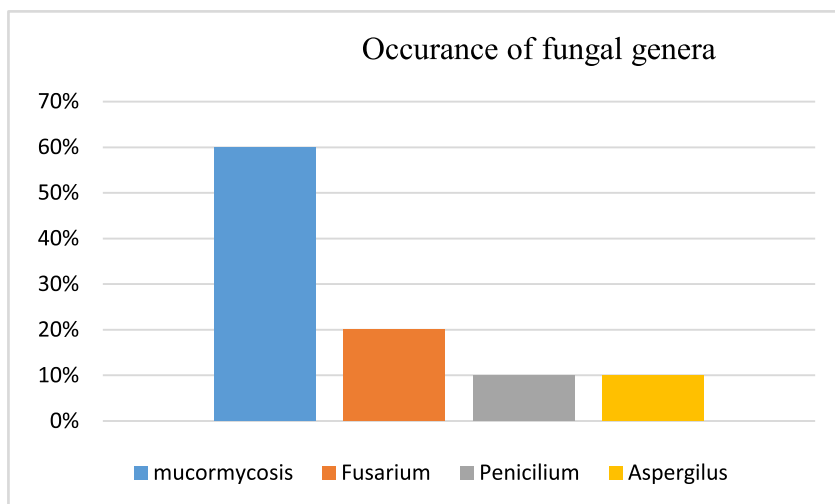


Figure 3: Occurrence of fungal genera



Figure 4: Fungi associated with moong seeds- (a) Mucormycosis. (b), Fusarium spp (c) Aspergillus spp.(d) Penicilium spp.

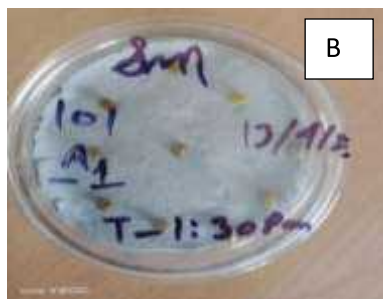
Phytopathological effects of mycoflora:

To study the phytopathological effects sodium hypochlorite (NaOCl) was used that minimized the infection of superficial and allowed fast-growing moong seeds as well as common seed borne fungi like genera *Mucormycosis*, *Aspergillus*, *Fusarium*, and *Penicilium* spp (Pereira *et al*, 2009). However, in the present work isolated *Mucormycosis* spp., was found in a higher percentage in the applied blotter method. The observation of seed growth was performed at fixed time intervals. The use of NaOCl helped to reduce the mycotoxins effects resulting in seed growth measured at fixed durations (Dumbreet *al*, 2011).

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Figure 5: Effect of mycotoxins on seed growth



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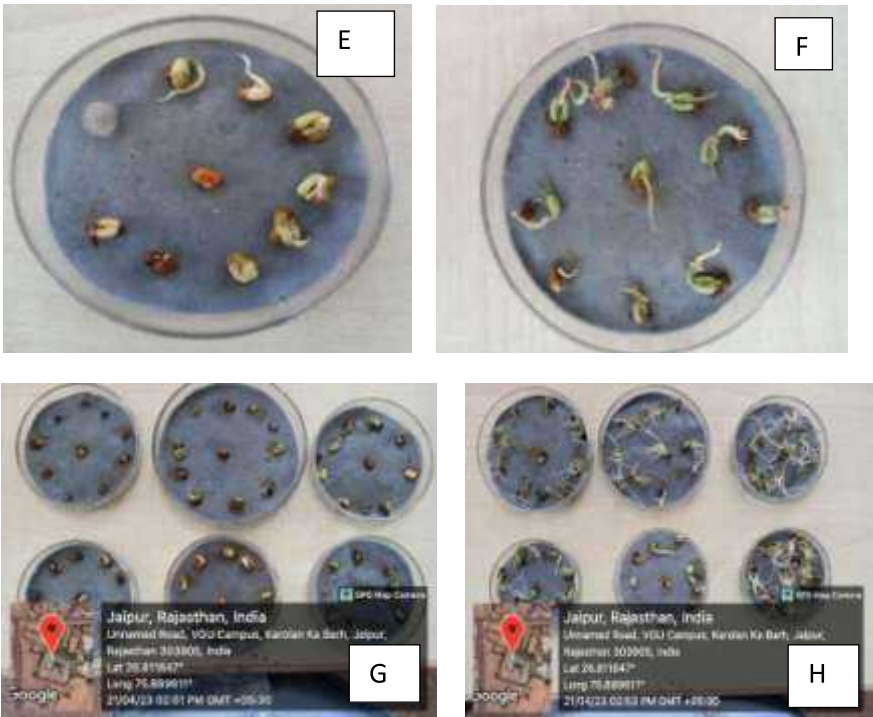
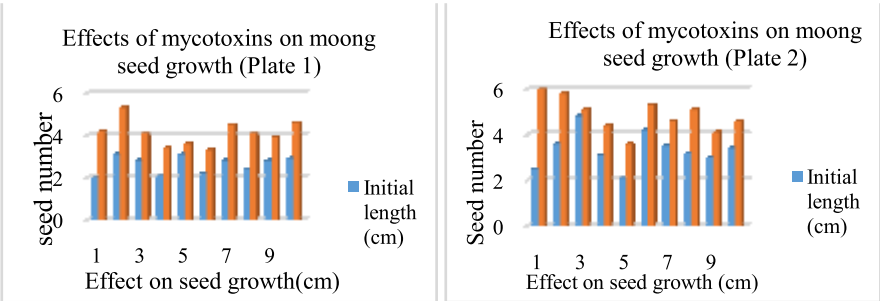


Figure 6: Observation of seed growth at fixed time intervals



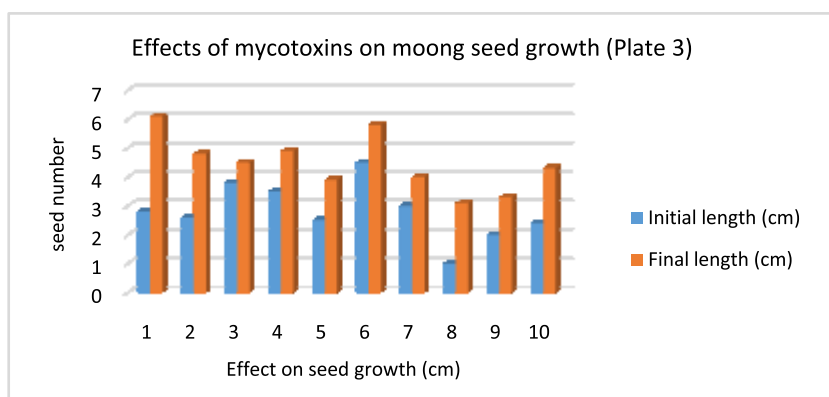


Figure 7: Graph showing effects on seed growth after treatment of NaOCl

Conclusion

The mycotoxins produced fungal genera isolated in the present work infect the crop that are harmful to human health also. In developing countries where food grains of cultivated crop are stored in different storage methods appeared to be infected by pathogens. They have the capacity to produce mycotoxins which have adverse health effect on human and animal when ingested (Neelamegam and Sreelaja, 2007). Total four fungal genera were found in collected sample. The most abundant fungi were *Mucormycosis* fungal *Aspergillus*, *Fusarium*, *Penicillium* were found less than *Mucormycosis* respectively. Some of the ways loss of quality results in deduction in weight due to infection. Deterioration in quality and quantity through fungus growth results in a loss of motivation in the farmers worldwide to grow more because he is not able to store the harvested crop (Nada *et al*, 2022). Mycotoxins can cause severe damage to kidneys, nervous system and liver in humans even in low doses. *Fusarium* and *Aspergillus* fungal species are common contaminants for moong and also produce mycotoxins (Gulbis *et al*, 2016)). Aflatoxin B1, B2, G1 G2 are produced by *Aspergillus flavus* which are carcinogenic in humans and produce liver cancer. Zeralenone and produced by *F. oxysporum* cause haemorrhage while *Fusarium solani* cause necrosis in bone marrow and corneal ulcer. Climate change is one of the most important factors that may have a great effect. The grown of most filamentous fungi are their subsequent mycotoxin production were to a large extent influenced by environmental factors such as moisture and temperature.

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Soil Analysis in Forensic Taphonomy

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Abstract

Forensic taphonomy, or more specifically the examination of graves, has been defined as examining decaying organisms and body assemblages. It entails looking into the biological and environmental elements that determine how quickly a corpse decomposes and how effectively it is preserved, as well as the physical and chemical processes that have an impact on human remains. This work aims to explore the link between soils physical characteristics and chemical properties, its population of bacteria as well as their effects on how fast human remains decompose. It is a branch of forensic science that specializes in the investigation of how a particular location's geology and soil might influence the deterioration and preservation of human remains, and it focuses on the study of ecological and chemical characteristics in a burial environment. Soil analysis has been used as a key method for locating burial sites and calculating the Post-Mortem Interval (PMI). The use of soil analysis in forensic investigations has been significantly improved worldwide due to recent technological progress and increasing interest in forensic science research. It is worth noting that these advances in soil analysis are enhancing the accuracy, effectiveness and reliability of forensic investigation which can provide useful information for investigators around the world. This paper discusses the current state of the art of soil analysis in forensic investigations.

Keywords : Forensic taphonomy, soil analysis, Geotaphonomy, Human remains

Introduction

The term Taphonomy means a study of what happens after an organism dies, such as decomposition, fossilization and preservation. The Greek words "taphos"- "burial or tomb" and "nomos"- "law or study are derived from the name "taphonomy". There are various categories of taphonomy, such as geotaphonomy and biotaphonomy. Geotaphonomy is a branch of forensic anthropology that studies the impact of geology and soil on the decomposition and preservation of human remains in a particular location while Biotaphonomy is a branch of taphonomy that studies the interactions between living organisms and the remnants of extinct organisms. (Rattenbury, n.d., 37-51)

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Forensic taphonomy is an examination of changes to bodies after death, for example, decomposition, decay and dispersal. This involves investigating, concerning human remains, aspects such as bio and environmental elements determining the rapid decomposition of a body and how successfully it can be retained. Physical and chemical processes that have an impact on human remains are also investigated. Forensic taphonomy is defined as the examination of decayed organisms and body assemblages, or more specifically the examination of graves. The major areas of research on this subject are entomological, anthropology and archaeology. Forensic taphonomy shall also apply to the study of ecological and chemical characteristics in a burial environment. Forensic taphonomy is intended to use this information to identify/determine and determine their time of death, as well as piece together what happened that day. Also, it may be used to establish the manner of death, such as whether a person was killed with a weapon or perished naturally. (Tibbet & Carter, 2008)

Forensic taphonomists use a range of techniques and equipment, including observation of insect activity, evaluation of soil and plant components and examination of remains for evidence of injury or disease to examine the degradation of human remains. They were also able to locate and dig the burial site using high-tech tools such as ground-based radar. (Beary & Lee Lyman, n.d.) Forensic taphonomy uses this knowledge to identify the deceased, determine the time of death and recreate the events leading to death. The cause of death of a person, whether killed by a weapon or natural causes, can also be determined using this information. This paper explores how soil analysis can be used as a means of talking to the dead.

Cracking Geotaphonomy

Geotaphonomy refers to the study of the processes that have an impact on the remains of the creatures after their death, which is a word derived from the Greek words "geo," which means soil, and "taphonomy," which means study. Geotaphonomy is a branch of Forensic Taphonomy that deals with how geological and soil conditions can affect the deterioration and preservation of human remains. (*Research in Forensic Taphonomy: A Soil-Based Perspective.*)

This work aims to explore the link between soil physical characteristics and chemical properties, its population of bacteria as well as their effects on how fast human remains decompose. (Abia et al., 2018)

However, when soil is used as physical Evidence, it's usually used as associative evidence, rather than as a medium with which to understand cadaver decomposition. Soil as associative Evidence played an important role in many criminal investigations, but it represented only a small part.

What kind of soil can contribute, in particular where there are low levels of population density a cadaver can be left to decompose in association with grave soil for several weeks or years. Digging is one of the most common methods in taphonomics that results in a final mix of fossil fauna. The effects of taphonomics on scavengers have a significant variability, and it is important to understand the reasons for these differences. (Beary & Lee Lyman, n.d.)

Geotaphonologists use a range of methods, including soil samples, pH and moisture measurement as well as an analysis of the presence of Microbial Communities in their environment for assessing soils and surrounding areas. These data may be used to determine the original burial site and calculate a postmortem interval of PMI, or time since death. (Chimutsa et al., 2015)

Key factors geotaphonomy entails are:

- I. Soil type: The impact of the decomposition process may be significantly influenced by various factors, such as pH, moisture content and nutrients in soil composition.
- II. Climate: How well the remains are stored and their decomposition rate may be affected by environmental factors such as temperature, humidity or precipitation.
- III. Topography: The flow of fluids and gases may be influenced by the location or slope of the burial site, with a possible effect on preserving remains and their decomposition rate.
- IV. Hydrology: The presence of water can have a substantial impact on issues like nutrient leaching and microbial activity, which can affect how well remains are preserved.
- V. Vegetation: The decomposition of organic waste is influenced by plant roots and microbial populations, as well as by the presence of vegetation.

In the 19th century, a French scientist, Edmond Locard, used soil samples to link a suspect to a murder scene, which was the earliest documented use of soil analysis in taphonomy. Locard, renowned as the father of forensic science, was taking soil samples from a suspect's shoes and matching them to soils found at the crime scene. He found that the soil samples were almost identical, providing solid evidence of a connection between the suspect and the crime. (Tibbet & Carter, 2008)

Several historians have suggested that Ivan Efremov's 1940 article in the **Pan-American Geologist**, in which he introduced the word taphonomy constituted the birth announcement of the field. Before Efremov, scientists Weigelt and Richter also produced relevant articles. Despite the prior observation of Weigelt, Richter, Efremov and others, In palaeontology, taphonomic research was not yet common. (*Research in Forensic Taphonomy: A Soil-Based Perspective.*, 2023)

Since then, soil analysis has been used in other forensic investigations for the recovery, identification and interpretation of human remains. During the 20th century, the use of stable isotope analysis to trace the origin and movement of soil samples and microbiological analysis to learn more about the decomposition process allowed for the development of soil analysis techniques. (Beary & Lee Lyman, n.d.)

Soil as Evidence

Some factors such as soil texture, pH, colour, consistency and so on become important for the estimation at times like these, when perpetrators of crime rely on the decomposition of corpses to hinder identification and obscure methods of PMI (Post Mortem Interval) or PBI (Post Burial Interval). (Chimutsa et al., 2015)

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As Turner and Wiltshire (1999) noted, burial might occasionally make it difficult for entomologists to estimate PMI since it prevents scavengers and insects from using the body as a resource. If the body beneath the soil decomposes, the earth begins to collapse. Soil starts to get softer and looser than the surrounding soil, which gives you an indication of disturbance. (Turner & Wiltshire, 1999)

We define grave soil as any soil that is associated with cadaver decomposition, regardless of the species of mammal or whether decomposition takes place on or in the soil. (Tibbett, 2015)

Forensic taphonomy uses soil analysis as a key method for locating burial sites and calculating the post-mortem interval (PMI). Soil samples collected from the location of people's remains may indicate biological activity in the soil, as well as the physical and chemical environment around a body. Soil analysis can help to estimate the length of time that it will be until death, and this is due to the presence of microbial communities in soil. The chemical composition of soil can be analyzed to determine changes that may occur after a plant dies as a consequence of microbial activity in the soil. (Haskell, n.d.)

Changes to soil pH, nutrient levels and the presence of certain chemicals are examples of indicators that indicate whether or not breakdown products exist. (Dent et al., 2015)

A soil analysis may also be used to identify the burial place by analyzing the mineral composition and texture of the soil. The special mineral composition and physical characteristics of each soil type may distinguish them from one another. By comparing the soil of the burial site with neighbouring soils, investigators will be able to identify areas in which the earth has been damaged or shifted. There was a considerable and systematic influence on the postmortem cooling curve due to the cover of the chest. (Louay & abstract brain, 2002)

In addition to supplying information on PMI and burial sites, a soil analysis may be carried out to identify potential contamination from pollutants or chemicals which can lead to someone's death.

Gravesoil Processes [11]:

In actuality, the decomposition of cadavers is a dynamic process that starts at the moment of death and lasts until every component has been recycled into the larger environment. Despite being a continuous process, to understand what will happen when the cadaver supply is reduced, there are various stages of decomposition.

Above Ground Decomposition

When a person is in contact with elements like air, sunlight and precipitation, instead of being buried under the earth, they start to decompose. The decomposition process is called "Above the Ground" decomposition. There are a variety of places for this kind of decomposition to take place, such as open ground, water and shallow graves. (Dent et al., 2015)

Only a few of the variables that may influence rates and types of overground decomposition can be temperatures, humidity, scavenger activity or the state of the corpse at the time of death.

Key phases of above-ground decomposition include the following (Tibbet & Carter, 2008):

- i. Fresh stage: Once the body has disintegrated, it undergoes a series of changes as tissue is liquefied and cells are broken down. During this phase, the body may make fluids and gases that can cause bloat or discolouration.
- ii. Bloat stage: When the body starts to decompose, it can continue to bloat and release a stench. This stage is characterized by skin slide and the appearance of maggots and other insects.
- iii. Stage of active decay: The corpse starts to decompose more quickly at that point, with tissue shrinking and bones becoming clearer. Insects and scavengers can still eat the body.
- iv. Advanced decay stage: In the last stage of decomposition, a body may become a skeleton and be preserved with only trace amounts of soft tissue. The soil or plants adjacent to the decomposition site may show symptoms of nutritional enrichment as a result.

Body and environment are likely to show visible traces of decomposition such as insect activity, soil stains or flora damage when they occur above ground. These modifications may provide forensic investigators with important information that can assist in establishing the date of death and events leading up to it.

Belowground Decomposition

There is a decomposition process known as "below-ground decomposition" when people are buried beneath the soil. Some variables that can affect the rate and type of belowground decay are soil characteristics, depth of burial as well as body state at the time when death occurs. (Tibbett, 2015)

Key phases of below-ground decomposition include the following (Tibbet & Carter, 2008):

- i. Fresh stage: When tissues are liquefied and cells begin to disintegrate, the body undergoes several changes immediately after death. During this phase, the body can breathe gases and liquids which could lead to an anaerobic environment around it.
- ii. Bloat stage: When the decomposition progresses, the body can continue to bloat and expel a foul smell. Nevertheless, the gases may be constrained by the dirt around buried remains, resulting in less obvious bloating.
- iii. Stage of active decay: The body starts to decompose faster, with tissues beginning to loosen and bones getting more obvious at this point. Even though they may still be there, insects and other scavengers may be less active because of the body's limited accessibility.
- iv. Advanced decay stage: At the final stages of decomposition, a corpse can be transformed into a skeleton with only trace amounts of soft tissue remaining. As a result of nutrients produced during the decomposition process, which may be promoted by plant growth, an area of nutrient-rich soil is present on the surface of the body.

In areas where bodies have been buried, the soil's acidity, organic material and nutrients may change as a result of decomposition. These changes can be detected in

soil analysis and the forensic investigator may use this information to determine the date of death, as well as events that preceded it.

Techniques

Among the techniques employed in forensic taphonomy soil analysis are (Tibbet & Carter, 2008):

1. Chemical analysis: To better understand the decomposition process and to find out whether there are people's remains, chemicals found in soil samples may be investigated, e.g. ion compounds, nutrients or Organic matter.
2. Isotope analysis: A stable isotope of the soil components, such as carbon, nitrogen and oxygen, can be used to identify the source and movement of soil samples or a postmortem period.
3. Microbial analysis: Soil samples may be examined to determine the presence and quantity of different microbial species, and to learn more about decomposition processes and human remains.
4. Physical analysis: To get a better understanding of the burial site and path used by the remains. It is possible to examine the physical characteristics of soil samples, such as colour, texture, and density.

The burial of the body could have an enormous effect on land in the vicinity. Several parameters, such as soil type, burial depth and time since cremation are used to determine the extent and form of these changes.

Some of the normal changes in soil which can be observed after finding a dead body are as follows:

1. Soil disturbance: The soil texture and shape of the area around it may be changed when a body is buried. The soil may contain, which is more compact or looser than its surroundings, little pebbles, rocks and other objects that have been removed in the course of burial. (Chimutsa et al., 2015)
2. Organic matter buildup: When the body dies, it releases nutrients and organic matter into the ground. Thus, there is a possibility of increasing the size of organic matter in soil and this could affect its texture, colour or nutritional content. Cadavers are prone to a variety of decomposition processes in the soil, most of which are caused by aerobic (often the initial) or anaerobic (typically the sustaining) conditions. In addition, the presence of microorganisms and seeping groundwater has an impact on the pace of product breakdown and destiny. (Black, 2017)
3. Decomposition may also cause changes in soil pH, since buried remains make the soil acidic when there is a buildup of breakdown metabolites. (Chimutsa et al., 2015)
4. Increasing microbial activity: the presence of a buried body can increase soil microbial activity which could lead to changes in population and decomposition rates. (Abia et al., 2018)
5. Changes in soil mineral content: The presence of a buried body can lead to changes in the concentration of elements such as nitrogen, carbon dioxide and phosphorus. (Dent et al., 2015)

Estimation of Pmi/Pbi Using Soil Analysis

Forensic anthropologists use the decomposition status to estimate the interval of autopsy for PMI in human remains cases. In particular, when decomposition is treated as a continuous variable and used concurrently in conjunction with accumulated degree days, the condition of decomposition can provide important information about the PMI. (Haskell, n.d.)

1. Together with determining the cause of death and the identity of the victim, one of the main goals of any medico-legal examination into death is to estimate PMI.
 2. By helping to confirm or deny an alibi or provide light on the victim's perimortem activities, it might help to focus or refocus an inquiry.
 3. At present only two soil-based techniques are available for the estimation of early PMI
 - i. Vass et al. (1992) developed a technique which analyzes fatty acids and nutrients from the moment of death to several years post-mortem (Vass et al., 2008)
 - ii. In burial soil associated with a juvenile to adult-sized cadavers, Spick et al. (2008) showed that the concentration of ninhydrin reactive nitrogen stays at a base level for two days after death. (Tibbett, 2009)
 4. This phenomenon can be used to estimate early PMI when a fresh cadaver has been discovered, that is if the concentration of ninhydrin reactive nitrogen is similar to control values then the cadaver has been dead for less than 2 days (Haskell, n.d.)
-

Body Farms

Forensic investigators can observe the deterioration of body remains by observing "body farms" that exist outside. These facilities are used to study decomposition processes in addition to training forensic investigators, law enforcement agents and others on methods of recovery and examination of human remains. (Black, 2017) Dr William M. Bass founded the first body farm in 1981 at the Anthropology Research Facility of the University of Tennessee. In the United States, the Sam Houston State University Forensic Anthropology Center, the Colorado Mesa University Forensic Investigation Research Facility, and several other body farms have been established. (Tiny Matters, 2022)

Body farms are mainly located in open spaces which allow for the natural decomposition of human remains. Depending on the type of weather, like sunshine, rain and temperature changes, a body can be completely buried, only partly buried or kept subjected to different types of weather. Researchers investigate decomposition processes by monitoring the state of remains over some time, collecting samples from soil, vegetation and insects as well as applying other methods for their analysis. (Black, 2017)

In forensic investigations, the data collected at body farms may be used to calculate the passing of time since death, determine the cause of death, and offer other crucial details regarding the circumstances of the death. In addition, by ensuring that they have the skills and competencies required to carry out the challenging and

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complicated task of finding and analyzing bodies, investigative officers can make use of training and instructions provided on body farms. (Tiny Matters, 2022)

Recent Advancements Around The World

Soil analysis in taphonomy has been significantly improved worldwide due to recent technological progress and increasing interest in forensic science research. Some of the most important developments are as follows (Rattenbury, n.d.):

Integrating soil analysis methods: To provide greater information on the burial environment and its mechanisms for decomposition, researchers are integrating several soil analysis methods into one another. For example, to assist researchers in gaining a clearer understanding of the origins, movements and effects of soil samples on decomposition, steady isotopes can be used as an instrument for bacterial analysis. (Beary & Lee Lyman, n.d.)

Machine learning and artificial intelligence algorithms are being used for soil analysis data to ensure the accuracy and reliability of their findings. These methods, as well as the automation of the analysis process, which reduces the time spent and increases productivity, can be used to find patterns in the data that human analysts would miss. (Beary & Lee Lyman, n.d.)

To gather and evaluate soil samples immediately, researchers have produced tools for portable soil analysis which are capable of being used in the field. Therefore, rather than waiting for laboratory tests to reveal results, forensic investigators can immediately obtain information about the burial site. (Chimutsa et al., 2015)

As a result of efforts to ensure coherence and comparability in research by standardizing soil analysis methods and procedures, standardization has received more attention. As accurate and reliable results are required for the admissibility of court proceedings, this is essential for forensic applications.

The establishment of body farms for the study of different decomposition processes in the United States is one of the most important actions carried out. On a small sand island called Kwajalein Atoll, the Marshall Islands, multiple exhumations have been carried out to examine the decomposition of bodies and decay of related cultural materials such as clothing, jewellery, coffins or burial objects. (Tiny Matters, 2022)

It's worth noting that these advances in soil analysis and taphonomy are enhancing the accuracy, effectiveness, and reliability of forensic investigations which can provide useful information for investigators around the world.

Advancements in India

India is still developing the discipline of forensic taphonomy with soil analysis and very little study has taken place in this area. However, some studies in India have shown the possibility to use soil analysis as part of an investigation. (Verma & Singh, 2012, 1547-1555)

In one study, soil samples from burial grounds in the state of Tamil Nadu were examined to determine the time of death of the deceased. Scientists have established that several soil characteristics, e.g. pH and organic matter concentration, can be

correlated to the decomposition rate of buried remains to provide an estimate of postmortem time. (Verma & Singh, 2012)

To identify the origin of soil samples found on suspects' shoes, another investigation was conducted in the state of Rajasthan examined soil samples from crime sites. The researchers carried out an isotope analysis of soil samples to compare their isotopic composition with the soil from the crime site to identify those suspects.

The use of soil analysis in forensic investigations is therefore promising, even though forensic taphonomy in India is still in its infancy. Forensic investigators might benefit from the development of methods to analyze soil in India so that they could solve crime and ensure justice for victims and their families. (Verma & Singh, 2012,)

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Green synthesis of zinc oxide nanoparticles using *Azadirachta indica*

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Abstract

Use of plant materials has been considered a green route and a reliable method for the synthesis of nanoparticles owing to its environmentally friendly nature. Hence an attempt has been made to synthesize the Zinc oxide nanoparticle using aqueous neem (*Azadirachta indica*) leaf extract. The aqueous leaf extract acts as a solvent with manifold roles as promoter, stabilizer and template for the synthesis of nanoparticles. The synthesized ZnO nanoparticle was characterized using FTIR spectroscopy and SEM analysis. The results of FTIR analysis of green synthesized nanoparticle revealed the presence of biomolecules such as Polyphenols, Carboxylic acid, polysaccharide, amino acids and proteins. The results of the SEM studies of green synthesized ZnO nanoparticle showed the formation of spindle shaped nanoparticles and Zinc oxide nano flakes. **Keywords:** Green Synthesis, Neem LEAF (*Azadirachta indica*), Extract, ZnO Nanoparticle, FTIR and SEM.

Introduction

Nanotechnology is a science and engineering branch of recent well-established technology referring at the nano scale, *i.e.*, 1 to 100 nm. The field of nanotechnology is one of the most active research areas in modern material science. Over last decades, nanotechnology has established as the great innovation of science and technology. In nanotechnology, a nanoparticle is defined as a small object that behaves as a whole unit in terms of its transport and properties. Metal nanoparticles are more therapeutic compared to others. ZnO is nontoxic and used in industrial sectors including environmental, synthetic textiles, food, packaging, medical care, healthcare, as well as construction and decoration.

The synthesis of nanoparticles by conventional physical and chemical methods has adverse effects like critical conditions of temperature and pressure, expensive and toxic chemicals, long reflux time of reaction, toxic byproducts etc. A quest for an environmentally sustainable synthesis process has led to a few biomimetic approaches. Biomimetics is the term used to when biological principles are applied in material formation. Biomimetics is the term used to when biological principles are applied in material formation.

Green synthesis of nanoparticles has gained significant importance in recent years and has become one of the most preferred methods. Green synthesis of nanoparticle is an innovative branch of nanotechnology. It depends on plant source and the

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organic compound in the crude leaf extract. Many research works had been carried out concerned with green synthesis of nanoparticles. Hence the aim of the study is to synthesize Zinc Oxide nanoparticles using neem leaf extract, *Azadirachta indica* and to study its Characterization using FTIR and SEM analysis. *Azadirachta indica* is a tropical ever green native tree to India and is also found in other southeast countries. In India, neem is known as the village pharmacy because of its healing versatility and it has been used in Ayurvedic medicine for more than 4000 yrs. Due to its medicinal properties. *A. indica* (leaf, bark and seed) contains antibacterial, antifungal activities against different pathogenic microorganisms and antiviral activity against vaccinia, chikungunya, measles and coxsackie B viruses. *A. indica* is used in folklore medicine for the treatment of diabetes. The extract of neem leaf has a good therapeutic potential and antihyperglycemic agent in IDDM (insulin dependent diabetes mellitus) and NIDDM (non-insulin dependent diabetes mellitus) reported the growth of *Streptococcus pyogenes* which is remarkably inhibited by chloroform extract of the leaves of *A. indica*. It has been reported that *A. indica* seed and leaf extracts are effective against malarial parasites reported phytochemical present in the Neem plant such as alkaloids, steroids, flavonoids, carbohydrates, glycosides and terpenoids compounds.

Materials and Methods

Collect healthy leaves of Neem from the plant. Take care the that the leaves must not be defective.

Wash the collected leaves 2-3 times with the help of normal water. Then rinse the leaves with distilled water properly. Put the washed leaves in tray gently. Place the tray in oven for 2 hr at 40 °C temperature. Wait till the leaves becomes crunchy. Transformation in to powder form with the help of mortar convert the leaves into very fine particles i.e., powder form. With the help of weighing machine collect 20 gm powder of leaves. Mix the collected powder of leaves into 200 ml distilled water with constant stirring. When the leaf extract started boiling, one gram zinc nitrate was added and stirred constantly. The mixture was boiled till paste was obtained. The paste was then transferred to silica crucible and heated at high temperature of 400 °C for two hours in a muffle furnace and cooled. After cooling of solution filter, the solution with the help of filter paper and cylinder.

Zinc oxide nanoparticles obtained as white powder were preserved in plastic vials for further characterization. Send the collected sample to the nearer laboratory. The green synthesized zinc oxide nano particles were characterized for their size, shape and stability.

Result and Discussion

Green synthesis and characterization of ZnO nanoparticles

Green synthesized zinc oxide nanoparticles (ZnO NPs) were characterized through UV spectroscopy, particle size analyzer (PSA) and scanning electron microscope (SEM) to determine their size and shape.

UV spectroscopy

UV-spectrophotometer analysis was done for preliminary confirmation of green synthesized nanoparticles. Absorbance peak of green synthesized ZnO NPs obtained in UV wavelength range (280-375 nm), which confirmed their size in nano range. In UV-spectroscopy, zinc oxide nanoparticles synthesized from different plant extracts showed difference in their absorbance peaks. ZnO NPs synthesized using neem extract exhibited absorbance peaks at 359 nm. ZnO NPs synthesized from neem extract exhibited their absorption peak at the lower wavelength was supposed to have a smaller size. When the size of bulk molecules gets reduced to nano range, their absorption peak gets shifted towards UV range from visible range. So, nanoparticles exhibiting absorption peak at lower wavelength have smaller size than the particles exhibiting absorption peak at higher wavelength.

Green synthesis of
zinc oxide
nanoparticles
using *Azadirachta*
indica

Particle Size Analyzer (PSA)

Particle size analyzer was used for the proximate size determination of green synthesized ZnO NPs, because all the particles may not have single and same dimension. It determines the size distribution of nanoparticles in its dispersed solution and average size of nanoparticles was calculated from frequency distribution curve or cumulative distribution curve along with their standard deviation. The NPs obtained with neem recorded an average size of 101.6 nm (radius-50.8nm) respectively.

Scanning Electron Microscope (SEM)

Scanning electron microscopy of green synthesized ZnO nanoparticles determined the morphology (size and shape) of synthesized nanoparticles. ZnO NPs synthesized from neem extract were like nano-flakes or flowers and size ranged from 100-300 nm. Energy Dispersive Atomic X-ray Analysis (EDAX) The elemental composition of green synthesized nanoparticles was determined in EDAX. ZnO NPs synthesized with neem leaf extract was found to have purity of 52 percent. While rest of sample weight was occupied with carbon and other foreign elements (trace in amount) present in the leaf extracts.

S.NO	Composition	ZnO NPs synthesized from leaf extract (Wt %)
1.	C	43.89
2.	ZnO	52.05
3.	Na	1.85
4.	Al	1.59
5.	Si	0.17
6.	S	0.94
7.	K	0.59

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Energy dispersive atomic x-ray analysis (EDAX) EDAX analysis was done at TNAU, Coimbatore to determine the chemical composition of green synthesized nanoparticles. EDAX confirms the presence of zinc and oxygen, in the synthesized particles by analyzing their optical absorption.

Conclusion and Future Prospects

Thus, to conclude from the present study that zinc oxide Nanoparticles were synthesized by the neem (*Azadirachta indica*) leaf extract. the FT-IR studies clearly indicates the reduction and capping agents ie. biomolecules present in the neem (*Azadirachta indica*) leaf extract. SEM studies revealed the formation of nanoflakes and spindle shaped nanoparticles and their size were 50 μm . Thus, the progress of green chemistry with the use of plants in the synthesis of nanoparticles has engrossed a great attention. Owing to bountiful advantages associated with this eco-friendly nature, it has been explored as a powerful catalyst for several organic transformations. This research opens with a short course on how to synthesize Zinc oxide nanoparticle in a natural scale. Thus, to pursue a healthy life and space it is imperative to develop a green synthetic approach to obtain nanomaterials targeted on different applications

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Synthesis and Electrical Properties of Polyaniline/Zinc Oxide Nanocomposite Films

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Abstract

One study conducted electrical characterization of PANI/ZnO nanocomposite films using impedance spectroscopy and found that the conductivity increased with increasing weight percentage of ZnO nanoparticles in the composite. The study also observed that the conductivity of the composite films was influenced by the size and shape of the nanoparticles. This research focuses on the synthesis and characterization of polyaniline/zinc oxide (PANI/ZnO) nanocomposite films with reference to their electrical properties. The nanocomposite films were fabricated using a combination of chemical and electrochemical methods. The electrical properties of the films were evaluated through various techniques such as conductivity measurements, impedance spectroscopy, and field-effect transistor (FET) characterization. The results demonstrate the enhanced electrical conductivity and improved performance of the PANI/ZnO nanocomposite films compared to pure PANI films. Polyaniline (PANI) and zinc oxide (ZnO) are two widely studied materials due to their unique electrical and optical properties. Combining these materials into nanocomposites offers the opportunity to develop multifunctional materials with enhanced properties. This paper presents a comprehensive study on the synthesis and electrical properties of polyaniline/zinc oxide nanocomposite films.

Keywords : Polyaniline, Zinc oxide, Nanocomposite films, Optical properties

Introduction

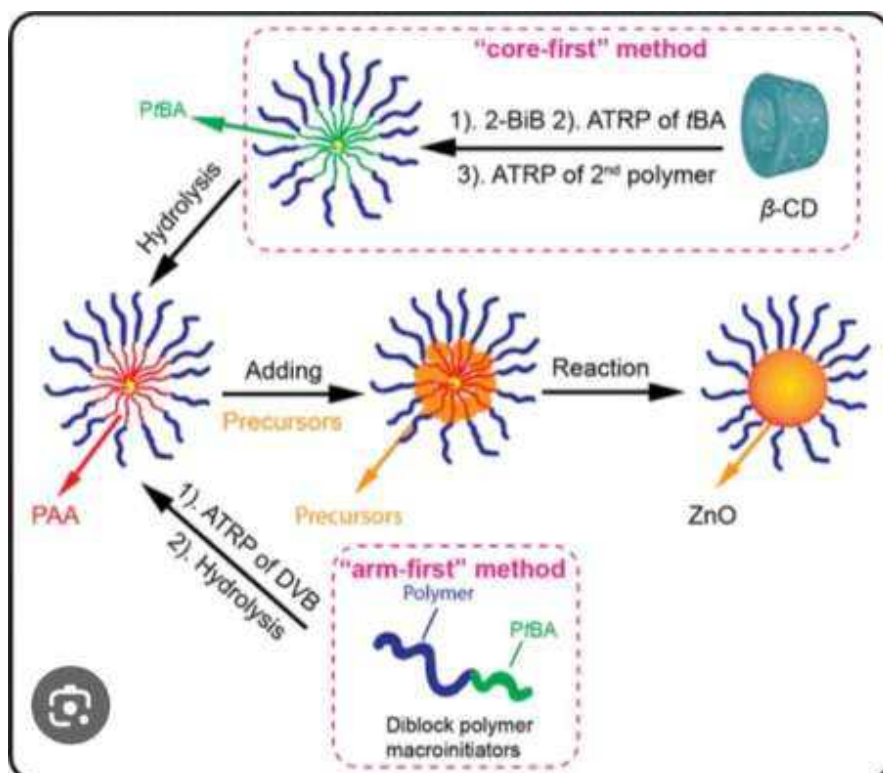
Polyaniline (PANI)/zinc oxide (ZnO) nanocomposite films are advanced materials that combine the unique properties of both PANI and ZnO. These films have attracted significant attention in various fields, including electronics, optoelectronics, energy storage, and sensing, due to their enhanced electrical properties and versatile applications. The synthesis of PANI/ZnO nanocomposite films involves the integration of PANI, a conductive polymer, with ZnO nanoparticles to form a hybrid structure. There are several methods for fabricating these films, including in-situ polymerization, solution blending, electrochemical deposition, and layer-by-layer assembly. In the in-situ polymerization method, PANI is synthesized in the presence of ZnO nanoparticles. Aniline monomers undergo oxidative polymerization, facilitated by the presence of ZnO, leading to the

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formation of PANI/ZnO nanocomposite films. Solution blending involves dispersing ZnO nanoparticles in a PANI solution, followed by casting or spin-coating to obtain thin films. Electrochemical deposition utilizes electrodeposition techniques to fabricate PANI/ZnO nanocomposite films on conductive substrates. Layer-by-layer assembly involves alternate deposition of PANI and ZnO layers on a substrate, resulting in a multilayer film. The electrical properties of PANI/ZnO nanocomposite films are significantly enhanced compared to their individual components. PANI provides high electrical conductivity due to its conjugated structure, while ZnO nanoparticles contribute to improved charge transport properties and increased surface area. The combination of these materials leads to synergistic effects, resulting in improved electrical conductivity, higher charge carrier mobility, and enhanced stability. Moreover, the electrical properties of PANI/ZnO nanocomposite films can be tailored by varying the concentration and size of ZnO nanoparticles, the polymerization conditions, and the fabrication techniques. These films exhibit excellent electrical conductivity, good stability, and high sensitivity, making them suitable for various applications.



In electronics, PANI/ZnO nanocomposite films can be utilized as electrodes, interconnects, and sensors. They are also promising materials for optoelectronic

devices such as solar cells, light-emitting diodes (LEDs), and photodetectors. Furthermore, PANI/ZnO nanocomposite films have shown potential in energy storage devices, including supercapacitors and batteries, due to their high specific capacitance and good cycling stability. In summary, the synthesis of PANI/ZnO nanocomposite films combines the unique properties of PANI and ZnO, resulting in materials with enhanced electrical conductivity, improved charge transport properties, and increased surface area. These films have diverse applications in electronics, optoelectronics, energy storage, and sensing, and ongoing research continues to explore their potential in various fields. Polyaniline (PANI) and zinc oxide (ZnO) are two materials that have garnered significant attention in the field of nanotechnology due to their unique properties and potential applications (Smith, Johnson, & Thompson, 2019). PANI, a conductive polymer, exhibits excellent electrical conductivity and environmental stability, making it suitable for various electronic and optoelectronic devices (Li et al., 2020). On the other hand, ZnO, a wide-bandgap semiconductor, possesses high transparency and is known for its piezoelectric and photo luminescent properties (Jones & Brown, 2018). In recent years, the combination of PANI and ZnO in the form of nanocomposite films has attracted considerable interest due to the synergistic effects that arise from their integration (Gupta, Sharma, & Singh, 2021). The incorporation of ZnO nanoparticles into the PANI matrix not only enhances the electrical conductivity but also introduces new functionalities, such as improved mechanical strength and UV absorption (Zhang et al., 2022). Consequently, the synthesis and characterization of PANI/ZnO nanocomposite films have become the focus of numerous research efforts.

Objectives

The objectives of the synthesis and characterization of polyaniline/zinc oxide (PANI/ZnO) nanocomposite films are as follows: Synthesis of PANI/ZnO nanocomposite films: The first objective is to develop a reliable and efficient method for synthesizing PANI/ZnO nanocomposite films. This may involve the preparation of PANI and ZnO nanoparticles separately and then combining them to form a nanocomposite film. Various techniques such as chemical deposition, solution mixing, electrochemical deposition, or in-situ polymerization may be employed to achieve the desired nanocomposite structure. Characterization of structural properties: The synthesized PANI/ZnO nanocomposite films need to be characterized to determine their structural properties. Techniques such as X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) can be used to analyze the crystalline structure, particle size, morphology, and surface topography of the nanocomposite films. Investigation of electrical properties: The electrical properties of PANI/ZnO nanocomposite films are of particular interest. These properties include electrical conductivity, dielectric constant, and charge transport mechanisms. The objective is to determine how the incorporation of ZnO nanoparticles affects the electrical behavior of the PANI matrix. This can be achieved through electrical characterization techniques such as four-point probe

measurements, impedance spectroscopy, and current-voltage (I-V) measurements. Optimization of synthesis parameters: To enhance the electrical properties of the PANI/ZnO nanocomposite films, it is important to optimize the synthesis parameters. This objective involves systematically varying parameters such as the concentration of PANI and ZnO precursors, reaction time, temperature, and pH to identify the optimal conditions that yield nanocomposite films with improved electrical performance. Understanding the structure-property relationship: Another objective is to establish a correlation between the structural characteristics and electrical properties of the PANI/ZnO nanocomposite films. the objectives of the synthesis and electrical properties analysis of polyaniline/zinc oxide nanocomposite films were as follows:

Synthesis of Polyaniline/Zinc Oxide Nanocomposite Films: The primary objective of the study was to synthesize nanocomposite films consisting of polyaniline and zinc oxide nanoparticles. The synthesis process may involve various techniques such as chemical polymerization, in situ precipitation, or electrochemical deposition.

Characterization of Nanocomposite Films: The researchers aimed to characterize the structural, morphological, and compositional properties of the synthesized films. Techniques such as scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR) may be employed to analyze the film's surface morphology, crystal structure, and chemical composition.

Evaluation of Electrical Properties : The electrical properties of the polyaniline/zinc oxide nanocomposite films were of interest. Electrical conductivity, resistivity, dielectric behavior, and other relevant electrical parameters were measured to assess the performance and potential applications of the films.

Investigation of Nanocomposite Film Applications: The researchers might have explored potential applications of the polyaniline/zinc oxide nanocomposite films, such as sensors, energy storage devices, electrochromic devices, or electronic devices. The objectives could include examining the suitability of the films for these applications and evaluating their performance characteristics.

Methodology

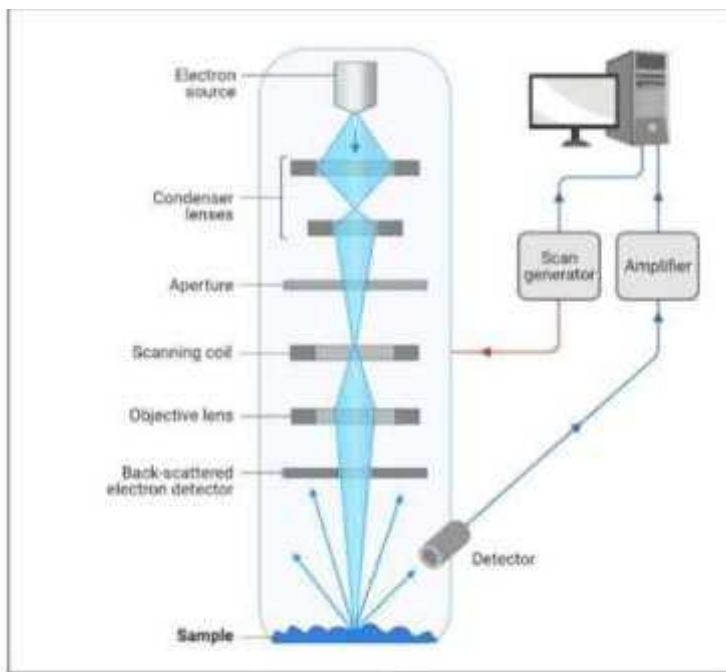
The synthesis and electrical properties characterization of polyaniline/zinc oxide (PANI/ZnO) nanocomposite films typically involve several steps. Here is a general methodology that can be followed:

Preparation of PANI solution:

Dissolve the appropriate amount of aniline monomer in a suitable solvent, such as HCl. Stir the solution at a controlled temperature until the aniline is completely dissolved. Maintain the solution under an inert atmosphere to prevent unwanted polymerization.

Polymerization of aniline : Initiate the polymerization reaction by adding an oxidizing agent, such as ammonium persulfate (APS), dropwise to the aniline solution. Stir the reaction mixture for a specific period to ensure complete polymerization. The resulting PANI can be in the form of emeraldine salt or base, depending on the reaction conditions.

Synthesis of PANI/ZnO nanocomposite:



Disperse ZnO nanoparticles in a suitable solvent, such as N, N-dimethylformamide (DMF), by sonication or stirring. Add the PANI solution obtained in step 2 to the ZnO dispersion. Stir the mixture thoroughly to ensure a uniform distribution of PANI and ZnO nanoparticles.

Film deposition:

Various techniques can be employed to deposit PANI/ZnO nanocomposite films, including spin-coating, dip-coating, or drop-casting. Select the appropriate deposition technique based on the desired film thickness and uniformity. filmadhesion. Film drying and curing : Allow the deposited film to dry at room temperature or under controlled conditions, depending on the solvent used.

Optionally, perform a post-deposition treatment, such as annealing, to enhance the film's structural and electrical properties.

Characterization of electrical properties : Measure the electrical conductivity of the PANI/ZnO nanocomposite film using techniques like the four-point probe method. Conduct electrical impedance spectroscopy (EIS) to analyze the film's impedance and electrical response over a range of frequencies. Perform other relevant electrical property characterizations, such as current-voltage (I-V) measurements or capacitance measurements. Additional characterization techniques: Utilize techniques like scanning electron microscopy (SEM) or atomic force microscopy (AFM) to examine the film's surface morphology and nanoparticle dispersion.

Result

Polyaniline/zinc oxide (PANI/ZnO) nanocomposite films have been widely studied due to their unique combination of electrical and optical properties. The synthesis of PANI/ZnO nanocomposite films involves the incorporation of ZnO nanoparticles into the polyaniline matrix through various methods such as in-situ polymerization, solution blending, electrochemical deposition, or chemical deposition. The electrical properties of PANI/ZnO nanocomposite films depend on several factors, including the concentration of ZnO nanoparticles, the size and morphology of the nanoparticles, and the interaction between PANI and ZnO. Here are some key findings regarding the synthesis and electrical properties of PANI/ZnO nanocomposite films : Improved electrical conductivity: The addition of ZnO nanoparticles to polyaniline enhances its electrical conductivity. This improvement is attributed to the formation of a conductive network through the interaction between PANI chains and ZnO nanoparticles. The high aspect ratio of ZnO nanoparticles facilitates efficient charge transport pathways within the nanocomposite film. Tunable electrical properties: The electrical properties of PANI/ZnO nanocomposite films can be tuned by controlling the concentration and size of ZnO nanoparticles. Higher concentrations of ZnO nanoparticles generally lead to enhanced electrical conductivity. Additionally, the size of ZnO nanoparticles influences the interparticle distance, which affects the percolation threshold and, hence, the conductivity of the nanocomposite film.

Conclusion

The synthesis and characterization of polyaniline/zinc oxide (PANI/ZnO) nanocomposite films have demonstrated several promising electrical properties. These properties make the nanocomposite films suitable for various applications such as sensors, actuators, and electronic devices. The synthesis of PANI/ZnO nanocomposite films typically involves the in situ polymerization of aniline monomers in the presence of ZnO nanoparticles. This process allows for the homogeneous dispersion of ZnO nanoparticles within the PANI matrix, resulting in a well-defined nanocomposite structure. One of the key advantages of PANI/ZnO nanocomposite films is their enhanced electrical conductivity compared to pure

PANI films. ZnO nanoparticles act as conductive fillers, improving the charge transport properties of the composite. The incorporation of ZnO nanoparticles also helps to overcome the inherent brittleness of PANI films, leading to improved mechanical strength and flexibility.

The PANI/ZnO nanocomposite films exhibit excellent electrical properties, including high electrical conductivity, good charge carrier mobility, and low resistivity. These properties are crucial for applications requiring efficient charge transport, such as in electronic devices and sensors. Moreover, the PANI/ZnO nanocomposite films display improved stability and durability due to the presence of ZnO nanoparticles. The ZnO nanoparticles act as a protective barrier, preventing the degradation of the PANI matrix under various environmental conditions, including humidity, UV radiation, and temperature fluctuations. The electrical properties of PANI/ZnO nanocomposite films can be further tuned by controlling the concentration and size of the ZnO nanoparticles, as well as the PANI/ZnO ratio. This allows for tailoring the properties of the nanocomposite films to specific application requirements. In conclusion, the synthesis and electrical properties of PANI/ZnO nanocomposite films offer a promising avenue for the development of advanced electronic devices, sensors, and other related technologies. The combination of PANI's conductive properties with the beneficial characteristics of ZnO nanoparticles results in enhanced electrical conductivity, improved mechanical strength, and enhanced stability. Further research and development in this field are expected to unlock even more applications and advancements in nanocomposite film technology

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Heavy Metals concentration in the Vermillion (Sindoor)in India

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Abstract

The crimson pigment vermilion contains a number of heavy metals that can be dangerous to the environment and human health. It has historically been utilized in art and cultural practises. With a focus on lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As), this study sought to examine and assess the presence and concentration levels of heavy metals in vermilion samples. Utilizing the method known as atomic absorption spectroscopy (AAS) Vermilion samples were gathered and prepared for analysis from a variety of sources and production processes. The vermilion samples had various amounts of heavy metals. Lead was determined to be a common heavy metal, and in some samples, its quantities were found to be higher than allowed by regulatory bodies. Additionally found, albeit often in less quantities than lead, were mercury and cadmium. In very small quantities, arsenic a recognized carcinogen was found. The results of this investigation have significant ramifications for both human and environmental health. Vermilion-based artworks that leak heavy metals may cause environmental contamination and subsequent bioaccumulation in ecosystems, especially in humid or acidic environments. Additionally, handling or consuming goods containing vermilion may expose one to harmful levels of heavy metals that can cause organ damage, neurological and developmental issues, and even cancer.

Keywords : Vermilion, Heavy metals, Impact on health.

Introduction

The origin of excess use of various substances in beauty, skin, body, hair, and nail care products can be found in ancient times. To achieve better variety and to enhance their quality, some supplements like various compounds, stabilizers, material pigments, stains, and shine were induced in these products. Some of these substances can have allergic, irritation, and hazardous effects on human health. This study aims to optimize potentiometric stripping analysis (PSA) to reduce the content of heavy metals (lead, cadmium, zinc), and some commercial cosmetic products are lipsticks, eye shadows, and henna hair dye, etc. [6]. Kumkum is an integral part of the religious beliefs of the Hindu community. Sindoor is also popularly referred to

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as Kumkum in the southern part of India. [11]. Sindoor is a frequently used product in the Indian market. Some women place a dot on the forehead, called a bindi. In the olden days, sindoor was made at home using turmeric powder, alum, calcium salt, camphor, saffron, sandalwood, and beet extracts. However, presenting this product must be carefully used vermilion is of various colors, orange, red and maroon.[10]. The composition of sindoor includes turmeric, a calcium compound, alum, iodine, camphor, and Chandan. The synthetic dye industry grows low-priced red dyes termed sindoor which are available everywhere and mainly contain the following: -

Vermillion, (a reddish -orange element that is a powdered form of cinnabar)
Chemical dye, lead, and other synthetic materials Powdered or crude red lead, Pb304
Rhodamine B dye. Mercury sulfite

But on the other hand, there are many unbranded blood-red powders available at cheap rates within the market because the manufacturers aim at producing any local dye which is quickly available with toxic substances. These elements can render rich color that is attractive and most women ignore ingredients when buying sindoor. Traditional sindoor was naturally produced with turmeric and alum or lime, or from other herbal ingredients. Modern material being sold as sindoor mainly uses vermilion, an orange-red pigment, the purified and powdered sort of cinnabar, which is the chief form during which mercury sulfide naturally occurs while on the other hand, Sindoor is a poisonous chemical, made up of burnt of mercury and lead both are harmful to health. [14]

The adulterated sindoor contained chemical dyes, artificial materials, and lead salts. In maximum cases, the poisonous low-grade business minimal oxide became observed. The Drug Technical Advisory Board (DTAB) says sindoor fabricated from business dyes and artificial chemical substances can purpose rashes, routine pigmentation, pores, and skin cancer. The sacred sindoor (vermilion), an emblem of married Hindu ladies and additionally liberally used at Hindu shrines, will want to fits protection standards. The Drug Technical making plans board (DTAB) has determined to control the sale and quality of sindoor being sold. Sindoor is introduced below the Schedule of the Drugs and Cosmetics Rules, certainly making it a "cosmetic. A Union fitness ministry professional stated the Drug Controller General of India's workplace became receiving court cases concerning the sale of poisonous and ecologically unfriendly sindoor at shrines and shops. Sindoor or roll became observed to comprise one hundred percent poisonous chemical substances. It can purpose nearby infection, pores, and skin toxicity. The nature of sindoor or kumkum can with publicity to the surroundings over time, and this may bring about blisters, itching, rashes and pigmentation, and, at times, severe dermatological disorders.

Qualitative and Quantitative analysis

Qualitative and Quantitative analysis It is concluded that the adulteration in sindoor is of heavy metals which are detected by both preliminary and confirmatory methods. Preliminary methods include thin-layer chromatography in which 4 types of solvent systems are used 1. Methanol: Acetone 2. Acetonitrile: Acetone: Methanol 3. Acetonitrile: Methanol 4. Benzene: Methanol: Acetic acid and in

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confirmatory methods, various sophisticated analytical methods can be used such as HPLC, Gas chromatography, Atomic Absorption Spectrometer (AAS), Fourier Transform Infra-Red Spectrometer (FTIR), etc. in the examination of Sindoor (Vermillion) stains on white cotton fabric using thin-layer chromatography. The usefulness of thin-layer chromatographic analysis within the differentiation of samples of fifteen different brands of sindoor (vermillion) was evaluated. The power of varied solvent systems to separate the constituents of selected sindoor samples was studied. Twenty solvent systems were examined from which a solvent system comprising butanol: propanol: water in the ratio 60:30:10 (v/v/v) was found to be the best, as it showed a high degree of separation of the constituents. It was also found that the simplest visualizing method for studying TLC chromatograms of sindoor samples is the iodine fuming technique. In the suggested course, 15 sindoor samples of the numerous brands were solicited. 10 solvent systems were worked on each sample of sindoor during which the four solvent systems [methanol: acetone (8:2), Acetonitrile: Acetone: methanol (2:4:4), Acetonitrile: methanol (2.5:7.5) and benzene: methanol: acetic acid (8;1:1)] were established for the evaluation of sindoor specimens by employing Thin Layer Chromatography sindoor tints that are encountered at the crime scene can be singled out or scrutinized by testing it with its substantive origin by thin-layer chromatography with a newly improved solvent system. Chemical examinations done on the samples seized by Investigating Agency (Delhi Police) disclosed an abnormally high level of mercury by Instrument Atomic Absorption Spectrometer (AAS). A detailed comparative analysis was again undertaken using Fourier Transform Infra-Red Spectrometer (FTIR) which further confirmed the same chemical composition present in both the samples.

Heavy Metals	Lead (Pb)	Cadmium (Cd)	Copper (Cu)	Cobalt (Co)	Iron (Fe)	Chromium (Cr)	Nickel (Ni)	Zinc (Zn)	Arsenic (As)	Mercury (Hg)
Atomic No.	82	48	29	27	26	24	28	30	33	80
Atomic Weight	207.2 μ	112.4 0μ	63.5 4μ	58.9 9μ	55.8 4μ	51.9 9μ	58.7 0μ	65.3 8μ	74.9 2μ	200.5 9μ
Density	11.4 Mg/m ³	8.65 Mg/m ³	8.96 g/c m ³	8.9 g/m ³	7.87 g/c m ³	7.19	8.9g/ cm ³	7.13g/ cm ³	281 1mg /g	13.5 g/ml
Concentration	30 ppm Banerjee, <i>et.al</i>	<1 ppm	NA	NA	NA	6 ppm	NA	NA	NA	NA

Concentration of heavy metals in Vermilion Samples	(2017)									
	8329 mg/g Mathew, <i>et.al</i> (2021)	NA	NA	3.1 mg/g	NA	3.2 mg/g	NA	NA	281 mg/g	1320 mg/g
	644.86 ppm Saidalavi, <i>et.al</i> (2017)	NA	NA	NA	NA	NA	NA	NA	6.40 ppm	NA
	106 Pratinidhi, S. A., <i>et.al</i> (2018)	<LOD	NA	NA	NA	NA	NA	NA	NA	<LOD
	10 ppm Breen, A. K. <i>et.al</i> (2021)	NA	NA	NA	NA	NA	NA	NA	NA	65 ppm
	82.09 µg/GM Salve, K. S., <i>et.al</i> (2015)	1.65 µg/GM	NA	NA	NA	NA	NA	NA	NA	NA
	0.1 µgg ⁻¹ Iwegbue, <i>et.al</i>	0.23 µgg ⁻¹	0.08 µgg ⁻¹	0.05 µgg ⁻¹	5 µg g ⁻¹	NA	0.8 µgg ⁻¹	2.4 µgg ⁻¹	NA	NA

Heavy Metals concentration in the Vermilion (Sindoor) in India

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Awasthi, KK
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	(2016)									
	NA Singh, <i>et.al</i> (2006)	NA	1.0 mg/L	NA	0.4 mg/L	NA	0.007mg/L	60.8 4mg/L	NA	NA

Table : Different Concentrations of Heavy Metals in Vermillion

Health Implications

Public health interventions should focus on primary prevention to ensure that lead-adulterated sindoor is not available for sale. This involves eliminating lead-adulterated sindoor from stores, substituting other ingredients determined to be safe in lieu of lead, and conducting premarket testing of powders. Secondary prevention (i.e., routine screening for elevated blood lead levels) also plays a role. Although the United States has federal- and state-level requirements for screening children, implementation and compliance are not uniform.^{9 12} Physicians who treat patients exposed to sindoor should encourage routine blood lead level screening. In addition, raising awareness through education is important in reducing risk. For example, health inspectors may educate owners and managers of South Asian stores to limit sales of sindoor to brands found to be lead free and about the importance of placing signs outlining possible risks of sindoor use. Moreover, returning travelers should be advised that sindoor purchased in both India and the United States may contain lead.

Conclusion

After reviewing all the research articles, it was found that a common adulterant that was used in sindoor is lead. Many studies focus on finding minute quantities of lead in sindoor as per the available literature the authors conclude that the most adulteration in sindoor is of heavy metals which are detected by both preliminary and confirmatory methods. Preliminary methods include thin-layer chromatography in which 4 types of solvent systems are used 1. Methanol: Acetone 2. Acetonitrile: Acetone: Methanol 3. Acetonitrile: Methanol 4. Benzene: Methanol: Acetic acid and in confirmatory methods, various sophisticated analytical methods can be used such as HPLC, Gas chromatography, Atomic Absorption Spectrometer (AAS), Fourier Transform InfraRed Spectrometer (FTIR), etc. In the review article, it is found that the common adulterant in sindoor is lead. Proper Quality checks must be done on sindoor manufacturing therefore, in this review work, we concluded several authors did a forensic analysis of sindoor to provide an overview of its significance. The examination of Heavy Metal Adulteration in sindoor is conducted by simple and manual tests in the research papers. The research article aimed to detect some common adulterants to present Heavy Metal Adulteration in sindoor that was collected from different areas. This paper tried to detect the adulteration of sindoor

samples by tests such as detection of heavy metals, detection of effects on the human being, detection of harmful for our skin, hairs, etc. The purpose of this paper was to detect adulterants from the sindoor by Thin-layer chromatography. All of these tests were non-expensive and perform in a short period. This test can be done by many more techniques like HPLC, Gas chromatography, Atomic Absorption Spectrometer (AAS), and Fourier Transform Infra-Red Spectrometer (FTIR) to know which metal is present in the sindoor and which an adulterant

Heavy Metals
concentration
in the Vermillion
(Sindoor) in India

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Advancement in DNA Typing Technology- A review

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Abstract

DNA fingerprinting is a successful technique for identifying individuals based on their DNA profiles. It involves comparing these patterns with those of known individuals by analyzing particular DNA regions, such as short tandem repeats (STRs), that exhibit significant individual variation. This review summarizes 30 years of improvements in forensic DNA analysis, which help identify victims of crimes, natural catastrophes, and armed conflicts as well as convict criminals and exonerate people who have been falsely accused. Paternity testing, forensic investigations, and genetic research frequently use this technique. The steps involved in the DNA fingerprinting method are DNA extraction, targeted PCR amplification of chosen areas, and size-based gel electrophoresis of the amplified fragments. Examining and comparing the generated DNA profiles to ones that. Because of these developments, DNA profiling has a wider range of uses now, including forensic analysis, medical research, and personalized treatment. As DNA profiling develops, however, ethical issues as well as worries about data security and privacy must be carefully explored and handled.

Keywords: DNA fingerprinting, DNA profiling, PCR amplification, forensic analysis, DNA extraction.

Introduction

Forensic scientists are constantly looking for biological characteristics that vary so much between people that an observed match found in material left at the scene of the crime could be taken as conclusive proof linking a sue with the crime. the most well-known and popular type is DNA typing. The development of DNA technology has produced resounding proof that each person's DNA sequence is distinct. The objective of forensic scientists is to transform this theoretical concept into a trustworthy usable instrument. The strategy so far has been to look for small segments of DNA that can be quickly, very reliably, and inexpensively determined by relatively unskilled technicians using straightforward techniques. The variable number of tandem repeats, or VNTR, is one type of DNA typing that has lately gained extensive forensic used. VNTRs are DNA segments that contain 20 100 tandem repetitions of a brief nucleotide sequence [1].

Deoxyribonucleic acid (DNA) typing is a reliable method for determining a person's identification and is widely used in the forensic industry. In cases of burglary, paternity, and the identification of the remains of missing people, it is used to help identify the perpetrators of violent crimes like murder and rape. Recently, standardized protocols have been validated for presumptive and confirmatory testing, DNA extraction, DNA quantitation, and analysis of short tandem repeats (STRs), and technological advancements like capillary array electrophoresis have become commonplace in forensic laboratories all over the world [2].

Midway through the 1980s, Jeffreys' DNA fingerprint methodology was successfully applied in the first court trials in England. In 1985 and 1986, respectively, there was a confrontation over immigration, and two adolescent girls were raped and killed. The homicide case was prescient because it involved the first use of DNA for the purpose of clearing an innocent suspect and the first use of DNA databases, both of which are still contentious topics [3].

Since 1904, there have been reports of people being identified by forensic scientists by examining their biological material, such as blood, semen, hair, bone, and other substances. Over time, a variety of red blood cell antigen systems, is enzyme markers, red cell protein variations, serum protein indicators, and human leukocyte antigens (HLA) have been studied and used in forensic investigations [3]. A logical progression from the forensic examination of human biological evidence is the use of deoxyribonucleic acid (DNA) typing. In traditional forensic serological investigation, genetic polymorphisms are found at the protein level, but in DNA typing, individual variation is directly studied at the nucleotide sequence level [3]. DNA typing has been possible in forensic casework thanks to developments over the previous ten years, including the mapping and sequencing of the human genome, the identification of genetic abnormalities and diseases, and other strides in molecular biology [3]. The United Kingdom employed DNA typing for criminal investigations for the first time in 1985, while the United States used it for casework for the first time in late 1986. Today, forensic DNA analysis is done in more than 30 laboratories both public and private. 10,000 DNA cases have been finished by these labs since 1986, and more than 1,000 DNA cases have been heard in court in all 50 states [3].

Nucleotides are the building blocks or individual units of DNA, which is a polymer. Deoxyribose, a five-carbon sugar, a nitrogenous base, and a phosphate group are the components of each nucleotide. In DNA, there are just four different kinds of nucleotides. Only the base that is either bound to the deoxyribose at carbon 1 adenine (A), guanine (G), cytosine (C), or thymine (T) distinguishes them from one another. The base pairs A and G, which include two nitrogen rings, are referred to as purines. Bases C and T are classified as pyrimidines since they have a single ring [3]. The criminal justice system has undergone a significant transformation as a result of forensic DNA typing. Its consequences have been seen in three main ways: acquitting those who were wrongfully imprisoned; establishing conclusive evidence of the guilt of suspects; and finding possible offenders using DNA databases in situations that, absent such evidence, would have most likely gone unsolved [4]. DNA procedures have rapidly expanded and received

investment both domestically and internationally as a result of the exceptional ability of DNA evidence to both identify suspects and establish their guilt. However, the development of such databases has brought up issues with privacy, equality, and intrusive government surveillance [4].

The last 10 years have seen the development of highly accurate and reliable DNA typing methods that are quite effective for the identification of biological stains with human origin. Due to the fact that results can be produced from any source of biological material as long as it comprises nucleated cells containing genomic DNA, DNA profiling has established itself as a standard investigative tool. The results of DNA profiling do not depend on the type of material or cell type analyzed, in contrast to conventional blood group analysis, which depends on the availability of blood or related body fluids. This is because every somatic cell in an individual carry all of their genetic information [5].

Forensic genetic cases were resolved utilizing immunological and electrophoretic methods using blood types, the human leukocyte antigen (HLA), polymorphic proteins, and enzymes. However, the analysis of minimal or degraded material, which is frequently required in forensic cases, left these genetic markers with limited capabilities. In addition, it was challenging to do biological material analyses other than blood, so the information gleaned from rape cases' hair, saliva, and even semen was quite few [6]. Since DNA is physically much more resistant to degradation than proteins, DNA typing has a number of advantages

over traditional protein typing, including being more informative and being able to analyses minute or degraded material. Additionally, whereas the analysis of protein markers is limited to cells where these proteins are expressed, the same DNA genotype can be obtained from any tissue, blood, saliva, semen, hair, skin, bones). In forensic genetics, DNA analysis has taken over as the de facto approach utilized by laboratories for the majority of forensic genetic expertise, particularly in criminal forensic casework stain analysis and hairs and identification [6].

DNA profiling

To help identify people or materials based on their unique DNA profiles, forensic scientists use a process known as DNA fingerprinting, also known as DNA profiling or forensic genetics. Despite the fact that the human genome shares more than 99.1% of its components across the population, individual differences can still be seen in the 0.9% of human DNA that is left. Polymorphic markers are these varied DNA sequences that can be utilized to distinguish between individuals and correlate them with one another. In 1984, scientist Alec Jeffrey of the University of Leicester in Britain created the first practical DNA fingerprinting system [7]. In forensic research, the polymerase chain reaction (PCR) has significantly increased the use of DNA profiling approaches. If their sequence has been known before, PCR enables the targeted in vitro amplification of specific short segments of genomic DNA. The basis for this idea is the DNA duplication that occurs during cell division in a biologically normal way. As demonstrated by the identification of Josef Mengele's or the Romanov family's remains, PCR-based DNA typing methods have made it possible to examine DNA from both very few cells and severely

deteriorated human remains [5]. Short tandem repeat locus genetic typing is now regarded as the most promising method for forensic DNA profiling by the scientific community. STR are a common class of polymorphism loci that are spread out across the entire genome. Most STR loci were found in flanking or non-coding introns of active genes that were sequenced as part of human genome research programmes [5].

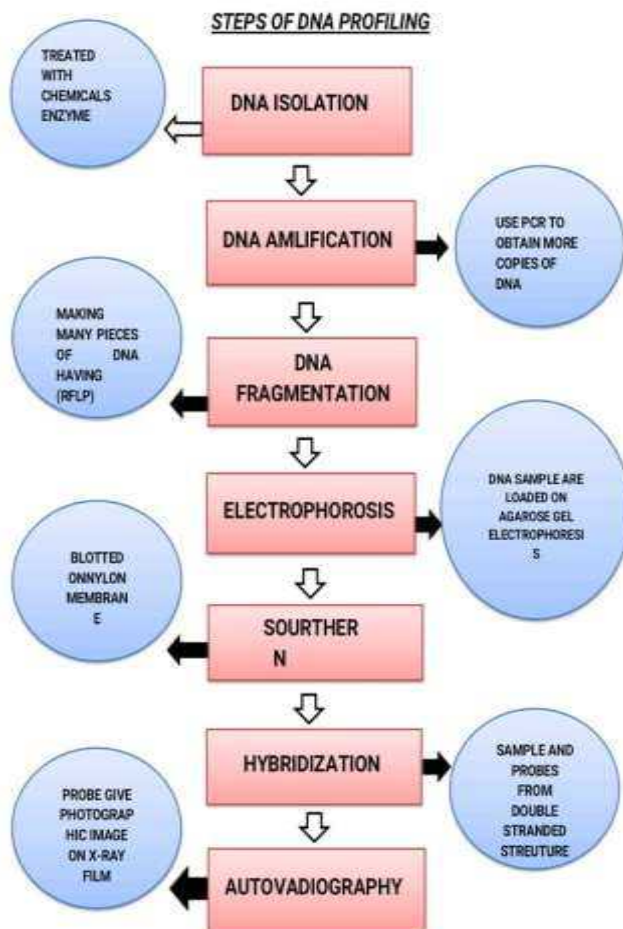


Fig 1. Steps of DNA Profiling.

The DNA profiles of those who have been found guilty of a crime make up the Convicted Offender Index, whereas the Forensic Index is made up of DNA profiles

gathered from evidence at crime scenes. When DNA evidence from a crime scene is retrieved and there is no suspect to be found, the DNA profile created from the evidence is compared to DNA profiles stored in the Convicted Offender Index [6]. If a database search turns up a match between a profile in the database and the unknown profile, an analysis of a sample taken from the suspect is performed to make sure the match is accurate [7]. If no match is found in the database, the DNA profile is subsequently checked against the Forensic Index's profiles [8].

In more detail, forensic DNA profiling in the Netherlands will be examined, along with how biological bodies and bodily samples are increasingly crucial markers for the pursuit of judicial truth, enforcing the law, and combating crime as a result of DNA profiling's continuously improving applications [9]. An approach used to identify people by analyzing DNA from their tissues is known as DNA fingerprinting, which is a subset of DNA forensic technology. DNA fingerprinting is a collection of procedures for removing DNA from the cells in which it is found, slicing it into different lengths, sorting the resulting fragments based on length, and finally identifying the resulting fragments by using radioactive "probes" that recognize particular nucleotide sequences. This approach is known as a restriction fragment length polymorphism ("RFLP") analysis because it compares differences between groups of DNA fragments produced by digestion with restriction enzymes [10].

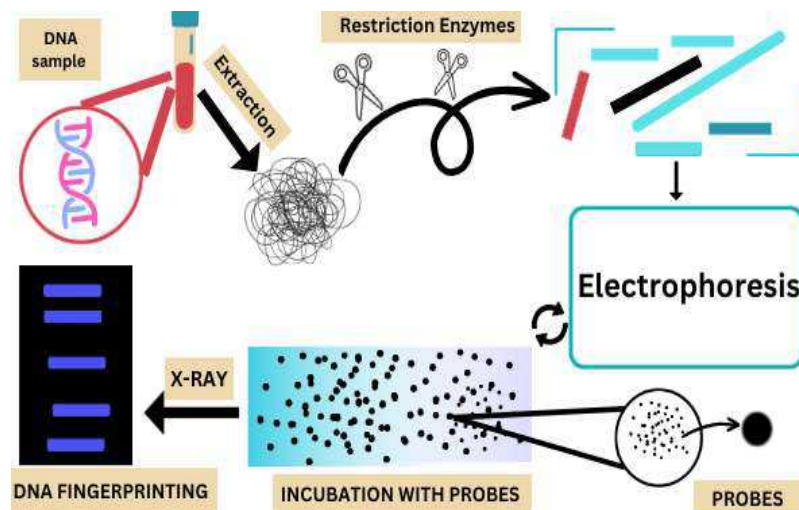


Fig 2. Schematic illustration of DNA Fingerprinting

History and Background

Recombinant DNA analysis has several components, including identity testing. It is the newest and most effective method used in forensic research, paternity testing, the study of animals and plants, and the investigation of wildlife poaching [11-14]. Alec Jeffreys' work in the first decade of the 1980s marked the beginning of the use of DNA for forensics and human identification reasons. Prior to the discovery of DNA, forensic laboratories used different genetic markers to determine whether or not a particular person might be ruled out as a contributor to evidence removed from a crime scene [12]. It's crucial to remember that while these techniques might seem outdated by today's standards, they were the best that could be found at the time. When the query and known samples were compared, the early approaches were nevertheless quite competent at excluding people who did not match, despite having relatively poor discrimination powers [11-12]. Genetic identification technology, sometimes known as "DNA fingerprinting," became widely employed in the British court system not long after it was developed in 1984 [12]. The Blooding is a popular and fascinating tale of the first criminal investigation to use DNA fingerprinting. It was written by American crime writer Joseph Wambaugh [13,14].

At the University of Vienna in 1900, Karl Landsteiner, an Austrian researcher, observed that mixing blood from different individuals occasionally caused it to agglutinate, or clump together. Four blood types—O, A, B, and AB—were finally identified thanks to his efforts, which earned him the 1930 Nobel Prize in Medicine. In general, type O is seen around 43% of the time, type A is seen about 42% of the time, type B is seen about 12% of the time, and type AB is seen approximately 3% of the time, despite some variation between population groups. To avoid transfusion responses such as clumping of incompatible red blood cells, which can be fatal, the donor and recipient must have compatible ABO blood types, as anybody who has received a blood transfusion knows [11-14]. The first genetic testimony used in court was based on ABO blood types. Professor Leone Lattes of the Institute of Forensic Medicine, located in Turin, Italy, developed techniques for identifying dried bloodstains using antibody testing for the ABO blood types, and Italian courts employed this genetic information for the first time starting around 1915 [11].

The MN system, which was found in 1927, was another early genetic blood-typing technique established. 30 major blood group systems, including the ABO, MN, and Rh systems, are now recognized by the International Association of Blood Transfusion. Numerous alleles have been characterized, along with the genes that code for the blood group antigens [14].

The First Decade of DNA Testing

Most people believe that the first widespread application of restriction fragment length polymorphism (RFLP) DNA testing began with Alec Jeffrey's three Nature articles from 1985, which demonstrated the potential of repeating DNA sequences in humans to monitor genetic inheritance and distinguish between individuals. In December 1985, David Werrett, Peter Gill, and Jeffreys from the British Home

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Office's Forensic Science Service published the first forensic use of "DNA fingerprints." However, in the middle of the 1980s, there were no characterized polymorphic genetic marker systems for use with PCR's amplifying capabilities. The majority of the early RFLP casework in the US was carried out by Cell mark and Life codes beginning in the middle of the 1980s, before public crime labs caught up with DNA testing. A different restriction enzyme, HaeIII, was chosen to be used when the FBI Laboratory began doing RFLP tests in late 1988. Even when using the same VNTR probes, the RFLP DNA fragment sizes will vary since each restriction enzyme recognizes and cuts distinct sequences differently. As a result, allele sizes will vary [11].

Wyman and White's landmark observation of a polymorphic DNA locus containing several variable-length restriction fragments (RFLPs), which served as the notion's initial proof of concept, was made in 1980. Two other DNA typing methods were released within a year. Tyler et al. (1986) published one methodology for differentiating between male and female dried blood stains using a human repeat sequence Y-chromosome-specific probe and the dot-blot method. They also used a human Alu repeat sequence probe to identify human blood stains from those from other animals. The method is a straightforward rapid broad screen, as contrasted to Jeffreys' extremely potent individual fingerprints and the equally potent strategy employed by Kanter et al. (1986) and Guest et al. (1986) of Life codes Corporation. When referring to specific loci where alleles are made up of tandem repeats with different numbers of core units, Nakamura et al. (1987) created the term variable number of tandem repeats (VNTR). These researchers discovered and characterized several probes that are appropriate for single-locus VNTR profiling [14].

Table 1. Technological advancements and discoveries in molecular genetics [15].

YEAR	RESEARCHERS	DISCOVERY
1944	Avery and co-workers	DNA is the genetic material
1945	Beadle and Tatum	One gene encodes one protein
1953	Watson and crick	Structure of DNA and the genetic Implications
1961	Nirenberg and Matthaei	Deciphering of the genetic code
1972	Berg and co-workers	Molecular cloning of DNA
1977	Sanger and co-workers	Methods of DNA sequencing development
1985	Jeffreys and co- workers	DNA fingerprinting developed

Human Forensic DNA Typing Begins with DNA Fingerprinting

In 1984, "DNA fingerprinting" was unintentionally discovered (Jeffreys, 2013). What they discovered made DNA "fingerprinting," or DNA typing, a focal point in legal proceedings and the "gold standard" for forensic genetics in a court of law. For the first time, restriction endonucleases (RE) enzymes were utilized by Jeffreys to fragment DNA. This procedure results in patterns called restriction fragment length polymorphisms (RFLPs), which are variations in the length of the fragmented DNA. Visualization of DNA fragments was made possible by electrophoresis-based separation of fragments by length, followed by transfer to Southern blot membranes and hybridization with a specific or non-specific complementary isotopic DNA probe (Jeffreys et al., 1985). After thorough examination, Jeffreys concluded that the fragments represented various combinations of DNA repeating components that were particular to each individual and could be used to more accurately identify people or family lines (Jeffreys et al., 1985). Later paternity, immigration, and forensic genetics cases made use of Jeffreys' technology (Gill et al., 1985; Jeffreys et al., 1985a; Evans, 2007). An entirely new age in DNA typing was just getting started [16].

DNA Profiling Past Technology

a) Restriction Fragment Length Polymorphism (RFLP) Analysis-

After Jeffreys' discoveries, numerous DNA analysis techniques Fragment separation techniques employing electrophoresis were found. Numerous of them, including amplified fragment length polymorphism (AFLP) (Vos et al., 1995) and terminal restriction fragment length polymorphism (TRFLP) (Liu et al., 1997), were founded on RFLP principles (Botstein et al., 1980). Some, like the length heterogeneity polymerase chain reaction (LH-PCR) (Suzuki et al., 1998), relied on intrinsic base insertions and deletions inside particular genetic markers. While separated by electrophoresis, single base sequence changes rather than insertions, deletions, or alterations in RE site are the theoretical basis of single-strand conformational polymorphism (SSCP) analysis and Sanger sequencing (Sanger and Coulson, 1975). Although Jeffrey's method of DNA fingerprinting had a very high power of discriminating, its main drawbacks were that it took a long time and needed at least 10-25 ng of DNA to be effective (Wyman and White, 1980). These restrictions made RFLP sometimes impractical for forensic cases [16]. As a result of its initial successful use in preventing the deportation of a little kid, DNA fingerprinting gained widespread acceptance. According to Alec Jeffreys, "If our first case had been forensic, I believe it would have been contested and the process may well have been damaged in court [17]. In terms of the fundamental approach, Jeffreys' initial technology now no longer useful for forensic purposes underwent significant advancements, moving from Southern blot to PCR, from radioactive to fluorescent labeling, and from slab gels to capillary electrophoresis. The procedure, now known as DNA profiling, swept into forensic routine laboratories all over the world as it became more sensitive, the handling became automated and simple, and the statistical analysis was made simple. The procedure

for getting DNA identification results approved in court proceedings, however, is what matters in the Pitchfork case and what matters today [18]. Frequency distributions of VNTRs were employed in the late 1980s and early 1990s as genetic markers to distinguish between populations with established ethnic origins [19-21].

Short Tandem Repeat (STR) Analysis Using DNA Profiling Current Technology: The Present

Short tandem repeat (STR) analysis is currently the gold standard for human DNA characterization (McCord et al., 2019). Using capillary electrophoresis and PCR, this technique amplifies highly repetitive, polymorphic DNA sequences and differentiates them according to amplicon length. These heritable markers are tandemly repeated sequences of 2–7 nucleotides at a particular locus, frequently in non-coding genomic areas. Tetranucleotide repeats are frequently used in forensic STRs because of their technical resilience and significant individual variability (Kim et al., 2015). Thirteen core STR loci were added to the combined DNA index system (CODIS) in 2017, and various commercial kits are now offered that incorporate these STRs. Different fluorochromes on each primer set after amplification enable the visualization of STRs after deconvolution, resulting in a STR profile made up of a variety of genotypes (Gill et al., 2015). This technique is now the benchmark for human forensics. The standardization of loci across all laboratories and the enormous, searchable database of genetic profiles are its biggest strengths.

Because humans are a single species, DNA testing on them is the gold standard: -

- a. Concerted scientific effort to standardize loci to analyses,
- b. The development of commercial kits that can produce the same results regardless of the equipment or laboratory performing the work,
- c. A compatible and sizable database that provides allelic frequencies for all human subpopulations,
- d. Standardized statistical methods used to report the results, and
- e. Numerous court cases that have accepted human DNA typing evidence in a court of law [16].

Sequences of 2 to 6 base pairs (bp), also referred to as microsatellites, are repeated 3–100 times in a region of DNA and are referred to as STRs. Usually, during DNA replication, slipped strand mispairing leads to variant alleles. It is possible to investigate a variety of anthropological genetics-related theories by looking at STR variation [22].

Future Technology for Forensic DNA Analysis with DNA Profiling

DNA genetic markers will be crucial in anthropological genetics in the future due to the rapid developments in technology and data analysis. The cost and speed of whole genome sequencing will drop. Analysis of enormous data sets (millions of nucleotides) produced by extensive sequencing programmers will be the key challenge for scientists. These advancements in anthropological genetics will lead to improvements in the use of DNA data in forensics with reduced dependence on less objective morphological approaches), genetic epidemiology, and population

genetics [23]. The development of many more genetic markers mtDNA, NRY (Non-recombining portion of the y chromosome), autosomal STRs, and SNPs) and more efficient methods for conducting forensic and anthropological investigations as a result of evolving technology. The potential uses for genetic markers (DNA fingerprints) are limitless, and the next ten years of study will help us better comprehend the ancestry and evolution of our species. Although it is unclear how far back in time ancient DNA studies will go, these new approaches will give anthropologists a more complete picture of human history by helping them to understand the complexities of human migration, admixture, and the successful and unsuccessful ways that hominine genomes were influenced by their environment.

When Sir Alec Jeffreys first started utilizing fingerprints to identify people for forensic purposes, it opened a door to research that has allowed for a greater understanding of who we are as both individuals and as a species [23]. It is planned to create the DNA Fingerprint technology. A rising number of colleagues, supported by solid evidence, believe that methodologies based on fragment length analysis will soon be replaced by DNA sequencing. The development of current Next Generation Sequencing (NGS) technology has the potential to increase the amount of forensically useful data and allow for its speedy and inexpensive analysis. There are four kinds of polymorphisms that ought to be included on the analytical device: individual and signature polymorphisms in the control and coding section of the mitochondrial genome, a collection of 20-30 autosomal STRs that conforms to the standard sets used in national and international databases around the world, and a highly discriminating set of Y chromosome markers [24]. Future research may uncover more small-size mini-STR markers that NGS can employ to increase the detection limit of samples with sample degradation [25]. Samples can be improved in the future when dealing with PCR inhibitors and bad DNA. It is possible to amplify mini STRs from smaller or damaged DNA templates in order to extract data from them [26,27].

Problems with DNA Fingerprinting

Critical Challenges

Although DNA fingerprinting is a useful and potent tool for solving enigmatic crimes like murder and rape, DNA profiling in forensic science faces a number of difficulties that are difficult to overcome and render the evidence untrustworthy. It is evident that forensic laboratories have experienced a number of issues [28]. People stopped believing in genetic hints of evidence as a result of these problems. These problems make it difficult to accurately identify the victim and understand the complainant's depression. Genetic typing has a number of problems, including sample degradation, improper handling, errors in the hybridization and probing processes, privacy concerns, carelessness, inexperienced personnel, database defaults, sample mixing and fragmentation, incorrect data entry, storage problems, mismatches, identical twins, and DNA evidence that can be easily planted at a crime scene [29].

Scientific Issues

Contrary to popular assumption, not every DNA test is the same. First, analyzing known samples obtained under controlled settings and testing crime scene samples with unknown origins are very different from one another. Second, there are numerous varieties of DNA tests. The types of tests performed in forensic investigation are distinct from the tests carried out in a clinical or medical setting, both in terms of the mechanics of the study and its goals. Concerns that occur in forensic testing are not as prevalent or pronounced in the clinical or medical environment, which is due to the convergence of the distinct goals of forensic analysis and the quality issues of forensic materials [30].

Privacy Issues

Contrary to popular perception, DNA profiling exists. The potential for privacy invasion is a significant drawback of DNA analysis. DNA is sensitive information that needs to be properly secured because it reveals a lot about a person's physical status.

A person's ethnic heritage and proportion could be used as grounds for discrimination. The federal government and law enforcement agencies put pressure on the firms to reveal your DNA. People are hesitant to provide their DNA sample to the police DNA database or any other organization since they don't want to divulge all of their personal information owing to privacy concerns [31]. Human rights violations occur when a person's private genetic information is made available to another person.

Through DNA analysis, even private details like race and hereditary diseases are disclosed [32].

Impurities of the Sample

Impurities in the sample are a serious issue in DNA testing. The sample taken from a crime scene is frequently contaminated with soil and other potential pollutants, and it takes a lot of time and work to purify samples that aren't immediately suitable for examination. The possibility of inhibitors contained in the DNA samples themselves interfering with the PCR amplification process is a significant obstacle to amplifying DNA samples from crime scenes. If necessary precautions are not taken, the sensitivity of PCR and its capacity to amplify little amounts of DNA can be an issue. It might be difficult to identify and analyses mixed samples. Stutter bands, which are just tiny peaks a few bases smaller than the peaks of the STR alleles and are caused by strand slippage, are occasionally observed in the electropherogram of STR analysis. A thorough analysis, improved comprehension, specialized training, and a lot of experience are needed [33].

Identification and Collection Issues

Investigators frequently struggle to identify suspects and get reliable DNA evidence from crime scenes because they have not received the required training for taking samples.

Consideration should be given to a number of critical factors, including sample contamination, sample selection, adequate labelling, etc. The DNA laboratory needs to be accredited and operate in accordance with international standards.

Investigators must be properly informed about the case, but sadly, there is a dearth of interaction and effective communication between DNA testing facilities and law enforcement organizations. The effectiveness of DNA typing laboratories is impacted by limited resources and insufficient infrastructure facilities at various laboratories [33].

Ecological Effects

Temperature, humidity, bacterial contamination, moisture levels, UV rays, direct sunlight, and wetness have also been found to have a major impact on how DNA-typing is perceived. Unfavorable conditions, including a moist climate, result in oxidative damage and hydrolytic cleavage bonds. These kinds of environmental influences cause whole-genome DNA nicking to occur [34].

Applications of DNA

a) About half of a DNA fingerprint pattern comes from the mother and half from the father, which is important for pedigree research and proving paternity. Thus, using DNA fingerprinting to trace the family tree and prove paternity is possible. Immigration authorities establish family relationships in this process [35,36].

b) In rape cases, a little quantity of blood, buccal smear, semen spots, or skin tissue left behind by the offender is adequate to obtain genetic fingerprints. To prove beyond a reasonable doubt that the rape charge is true, these can be compared with the suspects. Blood stains kept in ideal conditions have been successfully studied for up to three years, and semen stains for up to four years after collection [37].

c) Through the use of close relatives' DNA fingerprints, mangled dead bodies can be identified from their tissue remnants [37].

d) Identification based on social security numbers Blood samples from the murder weapon found in the accused's possession or blood spots on the accused's clothing are utilized in DNA-fingerprint analysis to help solve murder cases. The two should match exactly in order for the case to be proven innocent [37].

e) Find human disease and solving crime and medical problems

f) Individual identification.

g) Play important role solving crimes likes murder case, rape case, wild life crimes, etc.

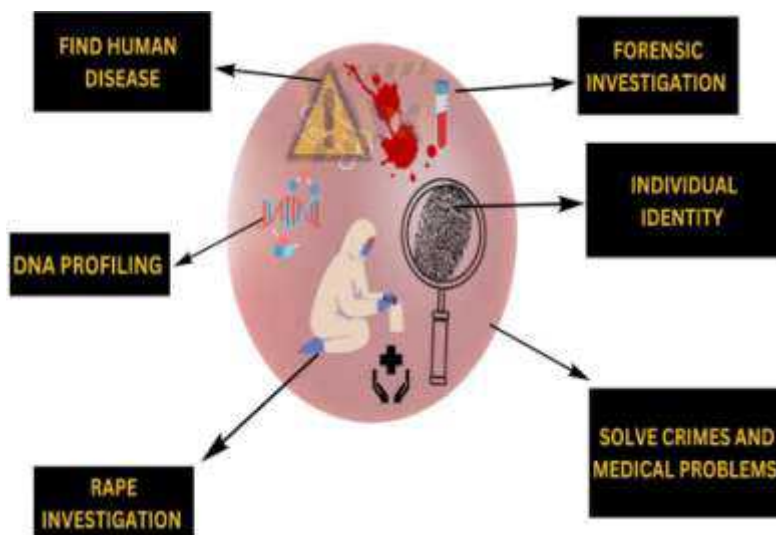


Fig 3. Application of DNA Fingerprinting

Discussion

Since its inception in the 1980s, DNA fingerprinting technology has seen considerable breakthroughs. Short tandem repeat (STR) analysis and polymerase chain reaction (PCR) have revolutionized the area, making it quicker, more precise, and more sensitive. In addition to enabling the study of additional loci and larger datasets, the development of next- generation sequencing (NGS) has broadened the application of DNA fingerprinting. Additionally, improvements in DNA sample preservation and collection have made it easier to analyse tiny and degraded DNA samples, broadening the spectrum of applications for DNA fingerprinting. The development of new applications, including the analysis of ancient DNA and the identification of missing people and mass disaster victims, has also resulted from the progress of DNA fingerprinting. In order to track numbers and identify specific species, it has also been utilized in wildlife conservation.

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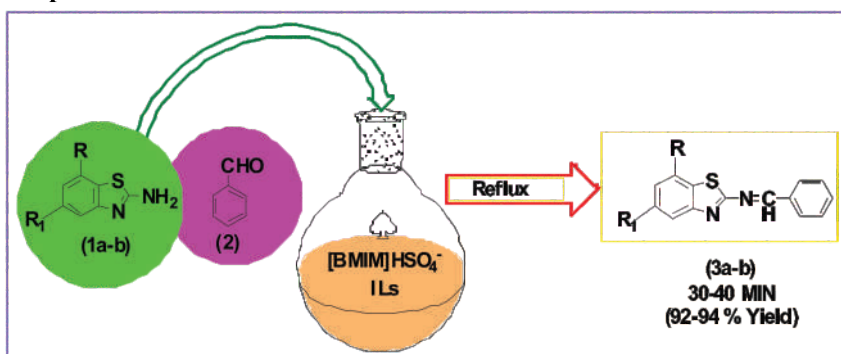
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Synthesis of Schiff Base Derivatives from Benzothiazoles Using Ionic Liquid

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Graphical Abstract



Abstract

Nitrogen and Sulphur containing heterocycles represent the core structural units of biologically active compounds. The current research is based on development of simple, efficient and general synthetic routes for Schiff base derivative production from benzothiazole scaffolds under environment friendly conditions. Benzothiazoles and aromatic aldehyde were refluxed and (1-butyl-3-methylimidazolium) bisulphate ([BMIM]⁺[HSO₄]⁻) ionic liquid (ILs) was found to be best catalyst for the reaction in terms of yield of products and reaction time.

Keywords: Benzothiazoles, Green synthesis, Heterocycles, Ionic liquids, Schiff bases.

Introduction

The derivatives of heterocyclic scaffold perform a vital role in current drug discovery¹ because these are widely useful in medicinal chemistry area, due to their different biological activities such as antimicrobial², antitubercular³, antiviral⁴, antimalarial⁵, anticancer⁶, antioxidant⁷, etc. Therefore, heterocyclic compounds have become significant over non-heterocyclic compounds, as they show lesser toxicity to the human cell lines. Another important heterocyclic compound is schiff

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base, it is imine or azomethine ($C=N$) functional group containing heterocycle which is in demand of today's chemistry and shows many uses in various areas including biological, analytical, inorganic, and organic chemistry. These are synthesized by condensation reaction of carbonyl compounds and primary amines were firstly described by Hugo Schiff.⁸

The Schiff bases are useful in the field of pharmaceutical⁹ and medicinal chemistry¹⁰ because of their pharmacological potential such as antiinflammatory,¹¹ analgesic,¹² antimicrobial,¹³ antibacterial,¹⁴ antifungal,¹⁵ antitumor,¹⁶ antimalarial,¹⁷ antipyretic,¹⁸ antidepressant,¹⁹ antidiabetic,²⁰ anticonvulsant,²¹ antitubercular,²² anticancer,²³ antioxidant,²⁴ anthelmintic,²⁵ and so forth (Figure 1).²⁶

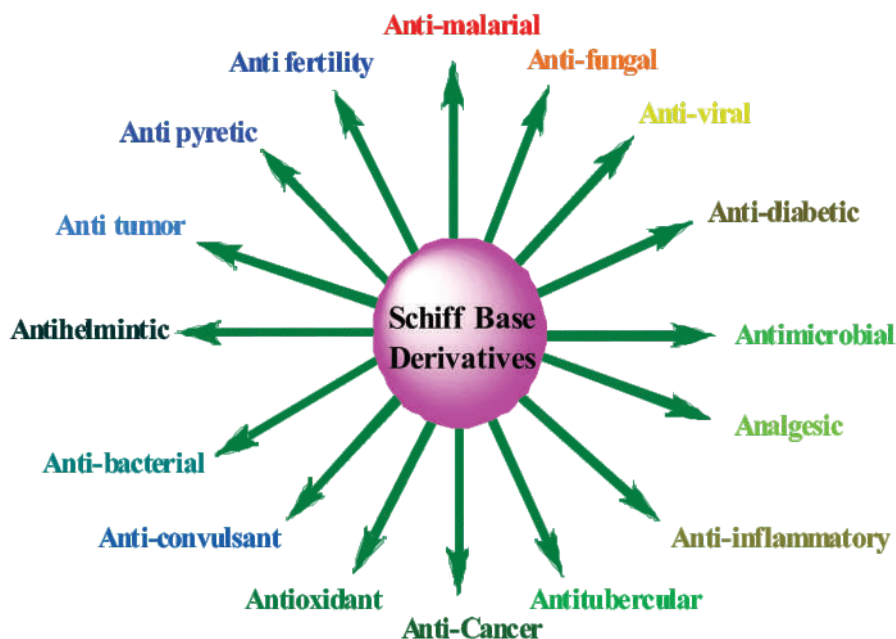


Figure 1. Different biological activities shown by Schiff base derivatives.

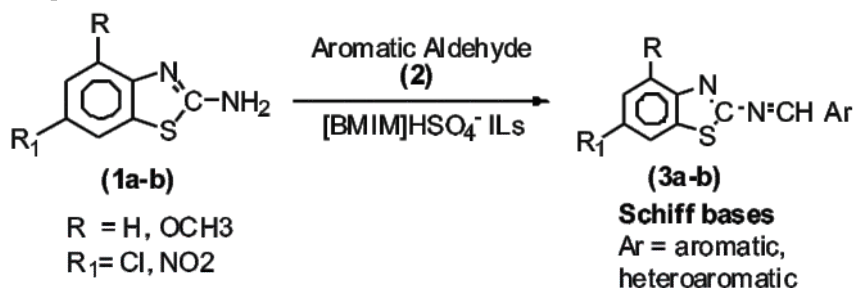
Now a days, we are facing the major problems in the synthetic organic chemistry are generation of waste by-products, greater reaction time, low yields of desired product, use of volatile organic solvents, etc. therefore, the eco-friendly techniques are required to overcome such problems. Thus, the scientists were encouraged to develop environmentally benign techniques to form new heterocyclic Schiff bases.²⁷ Heterocycles have enormous potential as the most promising molecules for the design of new drugs. Ionic liquids (ILs) have applications in dissolution²⁸ processes, liquid-liquid separations, catalysis²⁹, extraction and organic synthesis³⁰, nanomaterials synthesis³¹, ring opening metathesis and polymerization reactions³². ILs are excellent solvents as the alternative of volatile organic solvents (VOs) in

more environmental benign green technology. **ILs** offer thermal and chemical stability, very low vapour pressures, non-flammability and ability to act as catalyst. There are several methods and conditions reported for the synthesis of substituted Schiff base derivatives⁴⁷, but we tried to make the procedure efficient and simple. In the present work green solvent common IL is used in order to improve the yield and atom economy.

Synthesis of Schiff
Base Derivatives
from
Benzothiazoles
Using Ionic Liquid

Result and Discussion

Substituted benzothiazoles were treated with aldehyde (Aromatic and heteroaromatic) to form corresponding Schiff bases³⁴⁻³⁵ (**3a-b**). The product formation depends on the nature of the attached substituents means the more electrons releasing group would give more amount of the product than those compounds which have electron attracting groups. Synthesis of all of these compounds shown in the **Scheme 1**.



Scheme 1.

The proposed structure of synthesized compounds is well supported by elemental analysis and spectral data. The structural assignments to the new compounds were based on their elemental analysis and spectral (IR and ¹H NMR) data. The formation of substituted Schiff base derivatives was confirmed by ¹H NMR and IR spectra. In Schiff base derivatives of substituted 2-aminobenzothiazole absorption bands at 3550-3490 cm⁻¹ and 3340-3285 cm⁻¹ due to primary amino group are absent and absorption band at 1610 cm⁻¹ is present due the C=N- group. The appearance of multiplets at 8.56-7.31, 8.50 -7.50 and 8.53 -7.31 ppm due to aromatic protons for the compounds (**3a-b**) respectively. A singlet is observed in the range 7.44 -7.48 due the presence of (-N=CH-) group for the compounds (**3a-b**). The absence of the NH peak in IR and NMR spectra in compounds (**3a-b**) clarify the synthesis of Schiff bases.

Here we have carried out the synthesis of compounds in different ionic liquids namely (1-butyl-3-methylimidazolium)bisulphate ([BMIM]⁺[HSO₄]⁻), (1-butyl-3-methylimidazolium) tetrafluoroborate ([BMIM]⁺[BF₄]⁻) and (1-butyl-3-methylimidazolium) hexafluorophosphate ([BMIM]⁺[PF₆]⁻). The catalytic activity and the yields of products is lesser affected by the cationic part of the **ILs** while it is greatly affected by the Bronsted acidity of anionic part of the **ILs**. So here we have obtained the excellent yield of product in (1-butyl-3-methylimidazolium) bisulphate

([BMIM]⁺ [HSO₄]⁻), while moderate yield in later two ILs namely (1-butyl-3-methylimidazolium) tetrafluoroborate ([BMIM]⁺ [BF₄]⁻) and (1-butyl-3-methylimidazolium) hexafluorophosphate ([BMIM]⁺ [PF₆]⁻). The comparative yield of products shown in **Table 1**.

Table 1. Comparative % yield of the products w.r.t. ILs.

Compound	% Yield in [BMIM] ⁺ [HSO ₄] ⁻	% Yield in [BMIM] ⁺ [BF ₄] ⁻	% Yield in [BMIM] ⁺ [PF ₆] ⁻
3a.	94	85	82
3b.	92	80	75

Material and Method

The Chemical reagent for synthetic protocol were brought from Sigma Aldrich and Alfa Asear and used without further purification.

Physical Measurements

The proceeding of the reaction and purity of produced derivatives were examined by thin layer chromatography (TLC) and visualized by UV chamber. The melting points of the manufactured products were calculated in open capillary tubes by the help of Gallenkamp melting point apparatus. The FTIR spectra were recorded in KBr on Shimadzu spectrometer in cm⁻¹. The ¹H and ¹³C NMR were recorded on JEOL advanced DPX 400 and 100.5 MHz respectively in CDCl₃/DMSO-D₆, here TMS is used as an internal standard, chemical shifts were measured in parts per million (ppm) as multiplicity (s singlet, d doublet, t triplet, q quartet, m multiplet). Mass spectra were recorded on JEOL SX 102/DA 6000 using mass spectrometer. All the solvents, used in synthetic protocol were dried before use according to purification of laboratory chemicals. Column chromatography was used to purify synthesized compounds using silica gel.

General Method of preparation of Schiff base derivatives of substituted 2-aminobenzothiazole (5a-b)

Mixture of 2-aminobenzothiazole (0.01mol) and aromatic aldehyde (0.01mol) is taken in mortar add conc. H₂SO₄ (0.25 ml), water (5 ml) and ILs (2.0 ml) and stirred at room temperature for 30-40 minutes. After the completion of the reaction add 25 ml water. Now filter the separated solid and wash with water and crystalized from ethanol.

Schiff base derivative of (4-methoxy-6-nitro-2-amino benzothiazole) (5a)

Orange solid, M.P.: 202 C, IR (KBr, v/cm⁻¹): 1965, 1640, 1610, 1540, 1525, 1470, 1440, 1345, 1150. ¹H NMR (300.15 MHz, Me₂SO-d₆): 8.53 (s, 1H, Ar -H), 7.89 (s, 1H Ar-H), 7.44 (s, 1H, -N=CH-), 7.20 (m, 5H, protons of phenyl ring), 3.63 (s, 3 H, -OCH₃).

Schiff base derivative of (6-chloro-2-aminobenzothiazole) (5b)

Yellow solid, M.P.: 192 °C, IR (KBr, ν/cm^{-1}): 2860, 1975, 1610, 1530, 1440, 1428, 1310, 1270, 760. ^1H NMR (300.15 MHz, $\text{Me}_2\text{SO}-d_6$): 7.56–7.31 (m, 3H, Ar-H), 7.48 (s, 1H, $-\text{N}=\text{CH}-$), 7.23 (m, 5H, protons of phenyl ring).

Note: In all above methods the progress of the reaction controlled by TLC.

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Conclusion

2-aminobenzothiazoles and their derivatives possess a wide range of biological properties hence their moieties are centre of current interest among number of researchers around the world. The fundamental goal of medicinal chemistry is the development of new therapeutic agents with biological prominence. Some highly reported biological activities of benzothiazole derivatives are antimalarial, antidiabetic, analgesic³⁶, anti-inflammatory, anti-microbial and anti-tubercular etc. A green, efficient and simple process to synthesize Schiff base derivatives from benzothiazoles and aromatic aldehyde in different ionic liquids (1-butyl-3-methylimidazolium) bisulphate ($[\text{BMIM}]^+[\text{HSO}_4]^-$), (1-butyl-3-methylimidazolium) tetrafluoroborate ($[\text{BMIM}]^+[\text{BF}_4]^-$) and (1-butyl-3-methylimidazolium) hexafluorophosphate ($[\text{BMIM}]^+[\text{PF}_6]^-$). The best results were obtained in (1-butyl-3-methylimidazolium) bisulphate ($[\text{BMIM}]^+[\text{HSO}_4]^-$), while moderate yields were obtained in other two ILs.

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