



Applications of Chemical and Biological Sciences for a Sustainable Future

Edited by

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Applications of Chemical and Biological Sciences for a Sustainable Future

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Foreword

In an era where sustainable development has become a global priority, the integration of chemical and biological sciences provides a powerful pathway for addressing environmental, technological, and societal challenges. The book *“Application of Chemical and Biological Sciences for Sustainable Future”* edited by, Hari Shankar Biswas, Sandeep Poddar, Ashutosh Ghosh and Amiya Bhaumik, published by Lincoln University College, Malaysia, is a timely and significant contribution that highlights the role of interdisciplinary research in developing innovative and responsible scientific solutions.

This volume presents a diverse collection of research and reviews covering areas such as green chemistry, sustainable catalysis, environmental remediation, nanotechnology, biological conservation, renewable materials, and advanced energy applications. The chapters demonstrate how scientific discoveries can be transformed into practical approaches for protecting ecosystems, improving human well-being, and promoting sustainable technologies.

A notable strength of this book is its emphasis on environmentally conscious methodologies, including green synthesis strategies, waste transformation, sustainable materials development, and innovative approaches for addressing ecological challenges. The contributions give valuable insight into how chemistry and biology can work together to create solutions that balance scientific progress with environmental responsibility.

This book will serve as a valuable resource for researchers, academicians, students, and professionals seeking to understand emerging developments at the intersection of chemical and biological sciences. I commend the editors and contributors for their dedication in bringing together this important collection of knowledge and for inspiring future advancements where science, sustainability, and innovation progress together.

Prof. Dr. Tapas Chakraborty

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Preface

Chemical and biological sciences encompass the study of matter, living organisms, their interactions, life processes, adaptability, and environmental relationships. Green chemistry, biotechnology, bioinformatics, nanotechnology, bioremediation, medicinal chemistry, environmental science, sustainable agriculture, and renewable energy are among the rapidly advancing areas discussed in Application of Chemical and Biological Sciences for Sustainable Future.

The first study explores the diversity and foraging behaviour of insect pollinators visiting *pomelo* (*Citrus maxima*) flowers in North 24 Parganas, West Bengal. It highlights the dominant role of honeybees, the influence of environmental conditions on pollinator activity, and the importance of insect-mediated pollination in enhancing fruit production and ecosystem stability.

Moumita Roy explores the potential of sustainable nickel catalysis as an efficient strategy for water remediation in her chapter. The study highlights a polyaniline-supported nickel catalyst for degrading hazardous organic pollutants, emphasizing its catalytic efficiency, structural characteristics, recyclability, and environmental benefits. The work contributes to developing affordable and reusable systems for cleaner water treatment.

The chapter "Study of accumulation of hazardous metals in edible parts of fishes from Kakmara Rive" examines the accumulation of hazardous metals in commonly consumed fish species from the Kakmara River. The study evaluates metal contamination in river water and edible fish tissues, highlighting potential bioaccumulation and associated consumer health risks. It also emphasizes the need for regular monitoring and conservation of contaminated aquatic environments.

Attreyee Mukherjee's chapter reviews recent advances in green and multicomponent strategies for synthesizing pyrano[2,3-c]pyrazoles, an important class of biologically active fused heterocycles. Emphasizing sustainability, atom economy, recyclable catalysts, alternative energy sources, and eco-friendly reaction conditions, it highlights modern approaches that support efficient, cleaner, and more responsible heterocyclic compound development.

Chandan Kumar's chapter explores the role of task-specific ionic liquids (TSILs) as green reaction media and efficient catalysts in synthetic organic chemistry. Highlighting their low volatility, thermal stability, reusability, and tunable structural features, the chapter presents recent developments in TSIL-based catalysis and emphasizes their growing importance in sustainable and environmentally responsible chemical synthesis.

Priyanka Sanphui and Rupam Mandal's chapter examines the current status, threats, conservation strategies, and human perspectives related to the Tibetan gazelle (*Procapra picticaudata*) in the Indian Trans-Himalayan region. Focusing on Ladakh's Changthang landscape, it highlights restricted distribution, population decline, habitat pressures, and the importance of community awareness, scientific monitoring, and integrated conservation efforts.

The chapter titled "A Review of Fluorescence Spectroscopic Studies Inside Confined Systems" explores the role of fluorescence spectroscopy in understanding photophysical processes within organized molecular assemblies. It highlights confined systems such as micelles, reverse micelles, and vesicles, emphasizing their influence on FRET, PET, solvation dynamics, and molecular behavior in biologically relevant microenvironments.

The chapter "Transformation of Plastic Waste into Carbonaceous Materials for Advanced Energy-Storage Applications" explores the sustainable transformation of plastic waste into valuable carbonaceous materials for advanced energy-storage applications. It highlights emerging upcycling strategies for producing graphene, activated carbon, carbon nanotubes, and porous carbon materials, emphasizing their potential use in batteries and supercapacitors while supporting waste reduction, resource recovery, and greener energy technologies.

Sanchayita Adikari explores the emerging field of green-synthesized trimetallic nanoparticles and their diverse biological applications in her book chapter. It highlights sustainable synthesis

approaches, unique physicochemical properties, and their potential roles in agriculture, healthcare, and environmental sustainability. The discussion reveals how these advanced nanomaterials can contribute to innovative and eco-friendly solutions.

Pritha Mondal's chapter examines the effect of climate change on coral mortality, focusing on ocean warming, acidification, bleaching, and coral–algal symbiosis. It highlights the physiological stress mechanisms, ecological consequences, and increasing severity of mass bleaching events, while emphasizing the urgent need for climate mitigation, conservation strategies, and management of resilient reef ecosystems.

Debasmita Sardar, in his chapter, explores sustainable approaches for synthesizing bimetallic nanoparticles and their growing importance in medicinal and catalytic applications. The review highlights eco-friendly physical and biological synthesis methods, distinctive physicochemical properties, enhanced performance, and the potential of bimetallic nanoparticles as efficient, scalable, and environmentally responsible nanomaterials.

Collectively, these chapters present interdisciplinary advances in chemical and biological sciences, emphasizing sustainability, innovation, and environmental responsibility. The diverse research contributions demonstrate the potential of emerging technologies and scientific approaches to address challenges in healthcare, ecology, agriculture, energy, and environmental protection for a sustainable future.

Hari Shankar Biswas
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Diversity of Insect Pollinators and Their Foraging Behavior on Pomelo (*Citrus maxima*) Flowers in North 24 Parganas, West Bengal, India

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Abstract

Field observations were undertaken between February and March 2025 to examine the diversity of insect pollinators and their foraging behaviour on pomelo (*Citrus maxima*) flowers in North 24 Parganas, West Bengal, India. Observations were carried out on eight medium-sized trees (approximately 10 ft in height) across four study location. In the time of flowering season nearly 120 open flowers from each tree were observed carefully. Altogether, 12 insect species belonging to four different insect orders were written down. Honeybees visited the flowers more frequently than other insects and showed more effective pollination activity during the whole day. Their activity varied according to changes in temperature and humidity. A comparatively higher number of insect forages were found under favourable weather conditions. The findings of the field study suggest that insect pollinators are highly important for successful pollination and better fruit production of pomelo in the study area.

Keywords: Fruit Production; Honeybee; Insect Pollinators; Pollination Activity; Pomelo (*Citrus maxima*)

Introduction

Pomelo is a vast citrus fruit that grows broadly in state of West Bengal. It is known as valuable fruit as well as it has medicinal benefits and has cultural importance among the people in West Bengal specially. Profitably Fruit production in citrus plants largely depends on insect-mediated pollination, which enhances fruit set and improves entire yield (Klein *et al.*, 2007; Corbet, 2000). Citrus fruit attract a wide range of insect visitors including Bees, flies, butterflies, and other nectar feeding species, which engage pollen transfer and fertilization (Rader *et al.*, 2016). Among various pollinators such as bees are considered the most efficient due to their floral constancy, foraging activity and effective pollen dispersal. Their role in increase agricultural production and maintain ecosystem services has been widely accepted (Khalifa *et al.*, 2021).

Recent studies have also declared that animal-mediated pollination significantly increases citrus fruit production and improves nutritional values as well as quality (Monasterolo *et al.*, 2024). Non-bee pollinators like butterflies and other flies also participate to pollination by contributing supplementary services. These insects play complementary roles and help maintain stable pollination under various environmental (Garibaldi *et al.*, 2013). Pollinator behaviour and activity are naturally influenced by environmental factors such as temperature, humidity as well as light intensity (Willmer, 2011).

Beside their ecological and economic importance, message regarding pollinator diversity and foraging behaviour in pomelo cultivation, particularly in the lower Gangetic plains of southern part of West Bengal, remains limited. Therefore, the recent findings aimed to document the diversity of insect pollinators and evaluate their foraging behaviour on pomelo (*Citrus maxima*) flowers in North 24 Parganas, West Bengal, India.

Methodology

Study Area

The field investigation was conducted during February to March 2025, coinciding with the peak flowering season in period of *Citrus maxima*, in the North 24 Parganas district of West Bengal, India. The area is located in the lower Gangetic plains and is characterized by a tropical humid atmosphere.

Field observation was carried out at four selected sites under Habra II Community Development Block: Ashoknagar (22.824° N, 88.642° E), Nurpur (22.903° N, 88.579° E), Sendanga (22.889° N, 88.606° E), and Dighirhat (22.887° N, 88.610° E).

These areas represent mostly semi-urban and rural landscapes abundant by agricultural fields, crop fields homestead gardens, and scattered vegetation, which provide suitable habitats for diverse pollinator gatherings. These areas are presented in Figure 1.

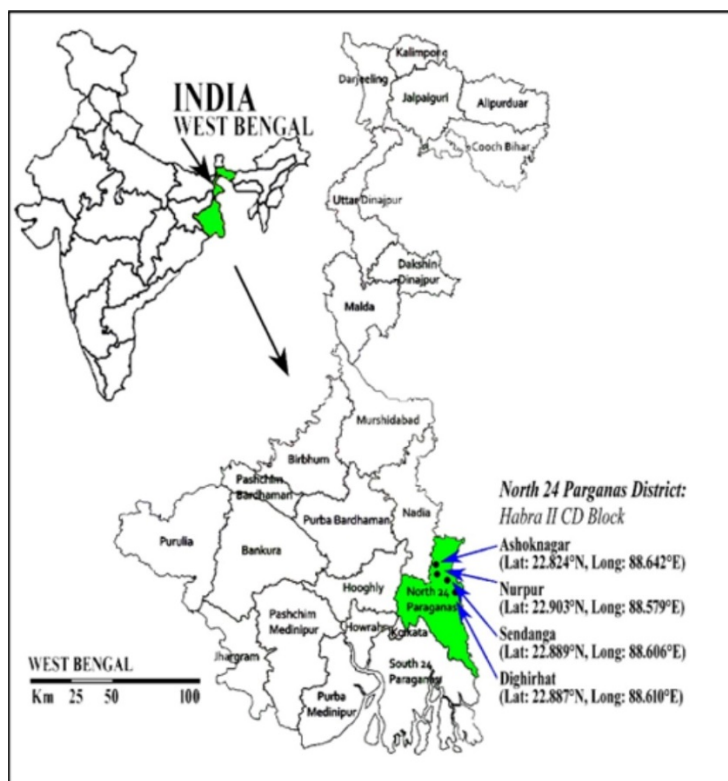


Figure 1: Map Showing the Selected Study Location of Pomelo Trees in N 24 PGS. District, West Bengal, India

Sampling Design and Observation

The whole observations were made across four locations-Ashoknagar, Nurpur, Sendanga, and Dighirhat. Two medium-sized *Citrus maxima* trees of similar growth condition were selected from each site, resulting in a total of eight trees. To stable consistency, observations were conducted on one tree at a time. During the peak flowering phase, approximately 120 flowers in fully open condition were recorded per tree. Regular observations were made to record insect visitation and foraging behavior.

Data Collection and Analysis

Insect visitors were recorded through direct visual observation. The insects were identified based on taxonomic identity, scientific name, common name. Foraging time and relative efficiency were also

recorded. Findings collected from all the trees were pooled to obtain mean values. Observations showed consistent pattern across different sites, indicating similarity in pollinator diversity and activity.

Insect visiting activity and resource gathering behavior were recorded following standard field observation methods commonly used in pollination ecology studies (Monasterolo *et al.*, 2024).

Results

Twelve insect species belonging to four various orders were recorded visiting pomelo (*Citrus maxima*) flowers during the study period. Table 1. Shows the diversity of insect pollinators and their foraging habit on pomelo flowers.

Table 1: Diversity of Insect Pollinators and Their Foraging Behavior on Pomelo Flowers

Sl. No.	Pollinator Type	Order	Scientific Name	Common Name	Foraging Time	Efficiency
1	Insect	Diptera	<i>Stomorphina</i> sp.	Snout fly	Day (Morning-Afternoon)	Low
2	Insect	Diptera	<i>Musca</i> sp.	Housefly	Day	Low
3	Insect	Hymenoptera	<i>Apis cerana indica</i>	Indian Honey Bee	Morning-Noon	High
4	Insect	Hymenoptera	<i>Apis mellifera</i>	European Honey Bee	Morning-Noon	High
5	Insect	Hymenoptera	<i>Euodynerus</i> sp.	Mason wasp	Day	Moderate
6	Insect	Hymenoptera	<i>Paratrechina</i> sp.	Crazy ant	Day	Low
7	Insect	Lepidoptera	<i>Ypthima</i> sp.	Ring butterfly	Sunny Day	Moderate
8	Insect	Lepidoptera	<i>Elymnias</i> sp.	Chocolate pansy	Sunny Day	Moderate
9	Insect	Lepidoptera	<i>Cephrenes</i> sp.	Orange palm dart	Day	Moderate
10	Insect	Lepidoptera	<i>Oriens</i> sp.	Smaller dartlet	Day	Moderate
11	Insect	Lepidoptera	<i>Borbo</i> sp.	Rice swift	Day	Moderate
12	Insect	Neuroptera	<i>Chrysopa</i> sp.	Green lacewing	Day	Low

Among all the groups, the order Lepidoptera showed the highest species diversity followed by Hymenoptera, Diptera and Neuroptera. The order-wise distribution of pollinators is presented in Figure 2. Hymenopteran insects, especially *Apis cerana indica* and *Apis mellifera*, show the maximum foraging efficiency and were mainly active during morning to noon period. Dipteran insects exhibit relatively lower efficiency.

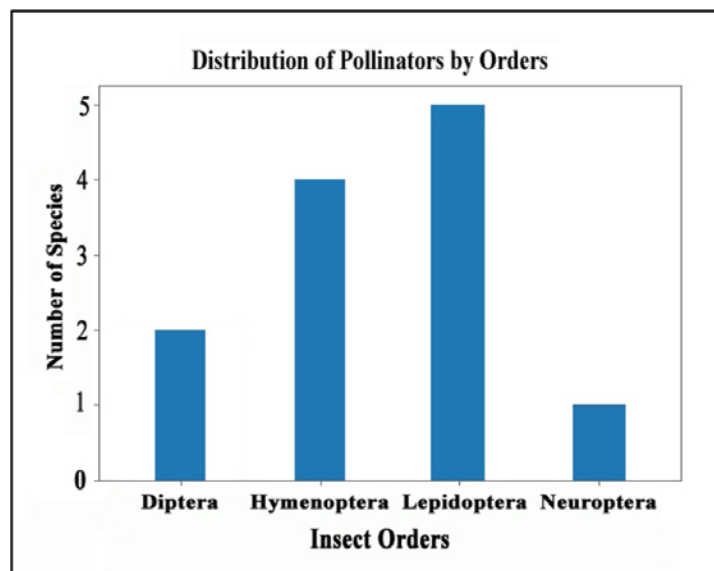


Figure 2: Order-wise Distribution of Insect Pollinators Species Recorded on Flowers of *Citrus maxima* During the Study Period

Based on foraging efficiency, pollinators were categorized into moderate (50%), low (33%) and high (17%) efficiency groups (Figure 3).

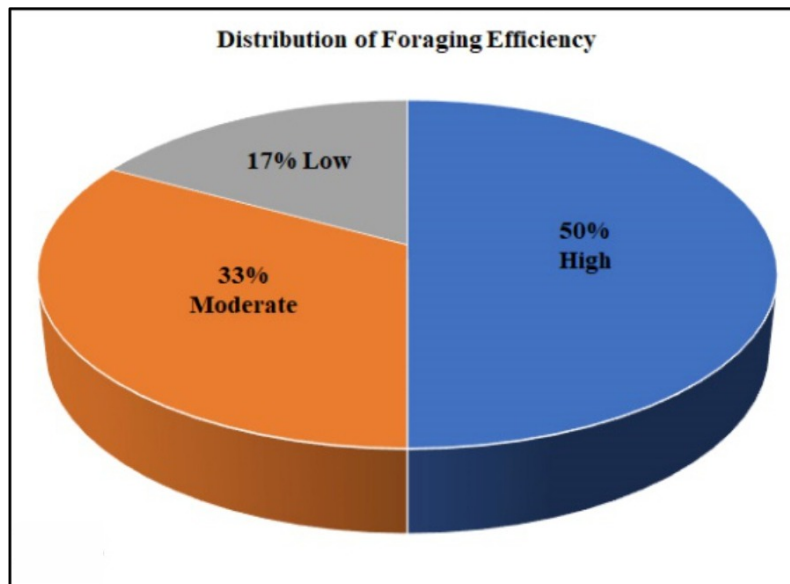


Figure 3: Percentage Distribution of Insect Pollinators Based on Their Relative Foraging Efficiency on Flowers of *Citrus maxima*

Pollinator activity varied during different times of the day. In morning time, the maximum activity observed and gradually declines towards noon, afternoon, and evening (Figure 4)

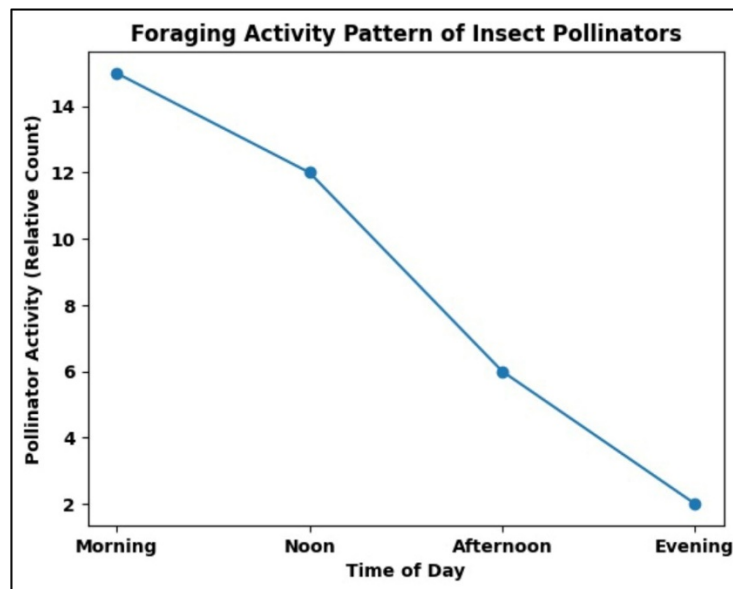


Figure 4: Regular Foraging Pattern of Insect Observed at Different Time Gaps on Pomelo (*Citrus maxima*) Flowers

Environmental factors influence pollinator behavior. The activity of pollinator was highest under moderate temperature and relatively higher humidity conditions. Reduced activity was recorded during high temperature and low humidity (Figure 5).

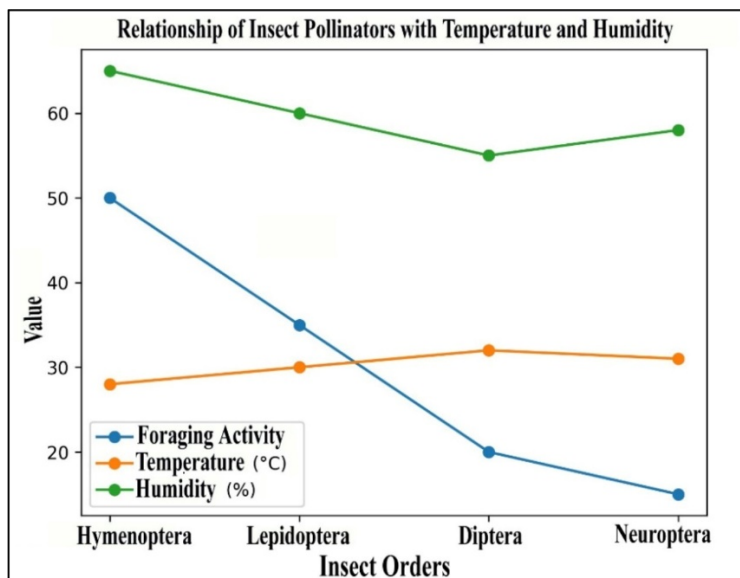


Figure 5: Influence of Environmental Factors (Temperature and Relative Humidity) on Foraging Activity of Insect Species on Pomelo (*Citrus maxima*) Flowers

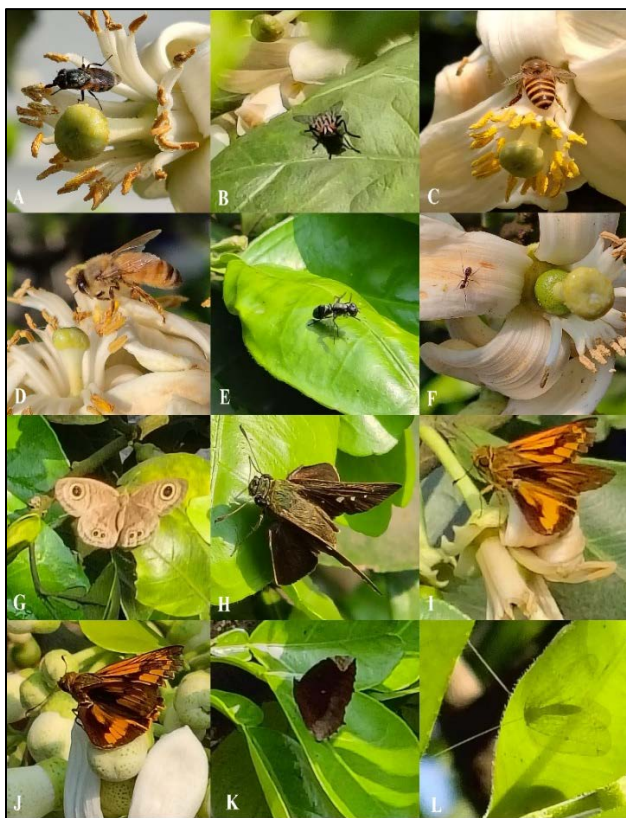


Figure 6: Photographic Representation of Insect Pollinators Visiting Pomelo (*Citrus maxima*) Flowers Recorded During the Study Period: A. *Stomorphina* sp. B. *Musca* sp. C. *Apis cerana indica* D. *Apis mellifera* E. *Euodynerus* sp. F. *Paratrechina* sp. G. *Ypthima* sp. H. *Elymnias* sp. I. *Cephrenes* sp. J. *Orens* sp. K. *Borbo* sp. L. *Chrysopa* sp.

Discussion

Findings from the present field observations indicate that flowers of *Citrus maxima* support a diverse assemblage of insect pollinator community in N 24 PGS. district. A total of 12 species belonging to four insect orders were recorded. Although Lepidoptera showed the highest species richness, hymenopteran insects, particularly *Apis cerana indica* and *Apis mellifera* were indicated as most efficient pollinators. Frequent visits by the insects and much more transfer of pollen played a major activity in successful pollination (Villa-Galaviz *et al.*, 2023).

The results of present study agree with previous findings that explore the importance of apes in flower pollination and improved fruit production (Potts *et al.*, 2010; Garibaldi *et al.*, 2013). Lepidopterans showed moderate pollination efficiency and were mostly active in diurnal conditions. In comparison, Dipteran and neuropteran insects were less effective, although they also assist in complementary pollination (Nurdiansyah *et al.*, 2004).

The presence of a variety of pollinators reflects functional diversity and promotes balanced pollination systems. Environmental factors like temperature and humidity greatly affect the behavior and foraging efficiency of pollinators. The findings of the study may help improve pollinator-friendly practices in citrus farming and promote sustainable fruit production (Pradhan *et al.*, 2025).

The present findings emphasize the ecological value of maintaining diverse pollinator communities in citrus cultivation area. While honeybees have shown the most efficient foraging activity, the contribution of non-paid pollinators deserves attention. Butterflies, flies, ants, and other flower-visiting insects may provide pollination assistance and support maintaining reliable pollination in response to environmental changes. Recent studies have shown that diverse pollinator communities enhance pollination efficiency, fruit production, and environmental resilience (Monasterolo *et al.*, 2024; Fijen *et al.*, 2025).

The present study showed that environmental conditions also affected the activity of pollinators. The Higher number of pollinator visits observed during the morning hours may be associated with favorable temperature and humidity conditions that support vigorous foraging activity. Same thing happened in recent pollination studies, where environmental factors significantly affected the movement, visitation frequency, and efficiency of pollinating insects (Kratz *et al.*, 2025; Balfour & Ratnieks, 2025).

The observation of different insect species on pomelo flowers suggests that active pollination depends on the diverse contribution of various pollinators rather than one species. Such factors can contribute to sustainable fruit production by ensuring effective and viable pollen transfer across varying environmental conditions. Recent research suggests that protecting pollinator diversity plays a key role in improving fruit yield, pollination efficiency, and long-term agricultural sustainability (Cholis *et al.*, 2020).

Conclusion

The present study highlights the importance of insect pollinators in pomelo cultivation in North 24 Parganas, West Bengal. Twelve insect species from four different insect orders were agree with visiting pomelo flowers during the flowering season.

Among the observed pollinators, apes were found to be the most active and efficient flower visitors. Pollinator activity was greater during morning hours and varied with environmental conditions such as temperature and humidity.

The study indicates that conserving a variety of pollinators is essential for better fruit production in pomelo. The findings may also serve as useful reference material for future research related to pollinator ecology and citrus cultivation.

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Sustainable Nickel Catalysis for Water Remediation

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Abstract

Clean water is the need of the hour. Sustainable water remediation requires affordable, reusable and environmentally friendly catalytic system. In this direction, a nickel-incorporated polyaniline (PANI–Ni) catalyst was synthesized and tested for the reduction of selected organic pollutants in aqueous media. The catalyst showed effective degradation of model dyes such as methylene blue, rhodamine B, and p-nitrophenol under mild conditions. The chapter elaborates the characterization of the catalyst using Fourier-transform infrared spectroscopy (FTIR), Scanning Electron Microscopy-Energy Dispersive X-Ray (SEM-EDX), Powder X-ray diffraction (XRD) as well as optimization of catalyst loading and pH of the reaction medium. Moreover, it shades light on the recovery and recyclability of the PANI–Ni.

Keywords: Heterogeneous Catalysis; Organic Dye Degradation; Polyaniline-supported Nickel Catalyst; Wastewater Remediation

Introduction

Catalytic reduction has emerged as a promising strategy for transforming hazardous pollutants into less harmful products. In particular, heterogeneous catalysts are advantageous due to their ease of separation and recyclability. Nickel-based systems have gained attention owing to their affordability and catalytic activity. However, issues related to aggregation and stability often limit their performance.

To address these challenges, conducting polymers such as polyaniline provide an effective support matrix. Their unique electronic properties and structural versatility enable strong interactions with metal species, enhancing dispersion and catalytic efficiency. In this study, a PANI-supported nickel catalyst is developed and evaluated for wastewater remediation applications.

Industrial effluents containing synthetic dyes and nitroaromatic compounds are among the most persistent environmental pollutants (Ismail *et al.* 2019). These substances are widely used in textile, pharmaceutical, and chemical industries, and their discharge into water bodies poses serious ecological and health risks. A variety of techniques, such as reduction, oxidation, coagulation, adsorption, photochemical processes, and electrochemical methods, have been applied for the treatment of wastewater (Lanjwani *et al.*, 2024). Reductive degradation of organic dyes in presence of metal catalyst is one of the most sought-after techniques for water remediation (Arif, 2025).

Literature is abundant with palladium catalyzed reductive degradation of organic dyes (Bhat *et al.* 2020). Other metals such as Cobalt, platinum, gold, silver, nickel are also reported to be effective (Haleem *et al.* 2026).

Nickel being one of the cheapest and catalytically efficient metal, attracted attention from several groups all over the world for the development of cost effective heterogenous catalytic system for the reduction of nitrophenols (NP)/organic dyes as outlined in Table-1.

Table 1: Heterogeneous Nickel Catalysts for Reduction of Nitrophenol

Catalyst	Remark	Reference
PVDF-[C ₆ (mpy) ₂][NiCl ₄] ²⁻ Ionic liquid-based catalyst	NP: 90% Yield in 5 min, Recycled 4 times	Chinnappan & Kim, 2013
Ni-PAMAM-PVAm/SBA-15 Mesoporous silica-polyamidoamine-polyvinylamine supported Ni nanoparticles catalyst	NP: 98% Yield immediately Recycled 10 times	Kalbasi & Zamani 2014
L-lysine derived nickel nanoparticles	MB: 100% reduction in 160 s No recyclability reported	Khaskheli <i>et al.</i> , 2016
Tetrabutyl ammonium bromide stabilized Nickel nanoparticles	Methyl orange: Complete reduction Recyclable	Mondal <i>et al.</i> , 2018
Ni/CPM (carbon porous materials)	MB: Complete reduction in 13 min Recyclable 6 times	Veerakumar <i>et al.</i> , 2015
Polyaniline-NiO	MB: Complete reduction 23 min No recyclability reported	Jamil <i>et al.</i> , 2021
NiO-CMK-3 Nickel oxide nanoparticles dispersed in mesoporous carbon	MB: Complete reduction 3 min Recyclable 5 times	Younus <i>et al.</i> , 2024

To develop a sustainable catalytic approach for the removal of organic dyes, polyaniline (PANI) was chosen as supporting backbone for its chemical stability, low cost and easy to prepare and most importantly availability of metal chelation sites and nickel as the catalytically active metal. The following section presents the synthesis, characterization, and catalytic performance of polyaniline-anchored nickel (PANI–Ni) in the reduction of various organic dyes.

Methodology

Materials

Ni (OAc)₂·4H₂O, ammonium persulfate, MB, RhB, p-NP and all other chemicals were of commercial grades and used as such without further purification. Prior to its use, aniline was purified via distillation.

Preparation of Polyaniline Base (PANI)

Polyaniline was prepared via oxidative polymerization of aniline in acidic medium using ammonium persulfate as the oxidant (Roy *et al.* 2019). The resulting product was filtered, washed repeatedly to remove residual impurities, and dried to obtain the PANI salt which was further treated with sodium hydroxide to afford the PANI base.

Preparation of PANI- Ni

A suspension of PANI (1000 mg) in an aqueous solution (50 mL) of nickel acetate tetrahydrate (250 mg) was sonicated for 1 h. The resulting material was recovered by filtration, washed sequentially with water and acetone, and dried under vacuum at 40 °C for 8 h to yield PANI–Ni (1100 mg). The nickel loading, determined by ICP–AES, was 0.41 mmol g^{−1} (Roy *et al.* 2025).

Catalytic Reduction Procedure

Degradation of p-NP

In a representative experiment, PANI–Ni catalyst (5.0 mg) was introduced into an aqueous solution of p-nitrophenol (10 mL, 1 × 10^{−6} M) in a reaction flask. Subsequently, an aqueous solution of sodium borohydride (10 mL, 2 × 10^{−5} M) was added under continuous stirring. The reaction progress was monitored at ambient conditions using UV–visible spectroscopy. The pH of the reaction medium was maintained at 9.3. The concentration of p-nitrophenol was determined from the absorbance at λ_{max} = 400 nm.

Degradation of MB and RhB

In a typical experiment, a measured amount of PANI–Ni catalyst and an aqueous sodium borohydride solution (10 mL, 1.5×10^{-5} M) were sequentially added to an aqueous solution of methylene blue (MB) (10 mL, 1×10^{-6} M). The reaction mixture was monitored at room temperature using UV–visible spectroscopy. The concentration of MB was determined from its absorbance at $\lambda_{\text{max}} = 664$ nm.

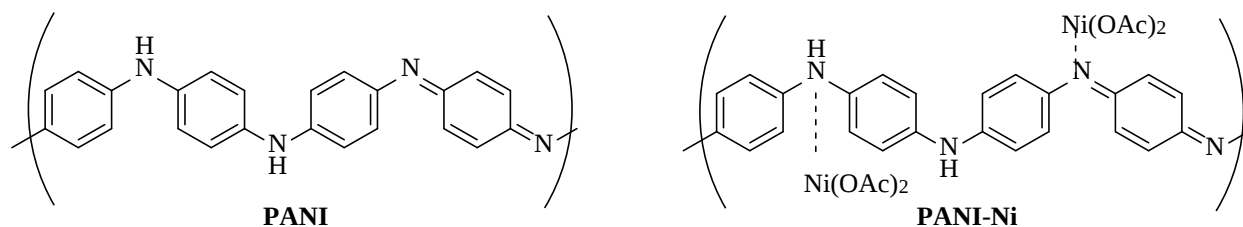
A similar procedure was followed for rhodamine B (RhB), with the reaction progress monitored at $\lambda_{\text{max}} = 554$ nm.

Results and Discussion

Characterization of PANI–Ni

Structural Characterization

The FTIR spectra of polyaniline (PANI) and polyaniline–nickel (PANI–Ni) (Figure 1) show no significant changes in the characteristic absorption bands after complexation with $\text{Ni}(\text{OAc})_2$. The key peaks corresponding to the quinoid ring (~ 1590 cm^{-1}), benzenoid ring (~ 1509 cm^{-1}), C–N stretching of secondary aromatic amine (~ 1295 cm^{-1}), N=Q=N and/or aromatic C–H in-plane deformation (~ 1170 and 1140 cm^{-1}), and C–H out-of-plane bending (~ 824 cm^{-1}) remain largely unchanged which is in agreement with similar study (Huerta *et al.* 2021). The probable structures of PANI and PANI–Ni are illustrated in Scheme 1.



Scheme 1: (A) Proposed Structure of Polyaniline (PANI) and (B) Polyaniline-Supported Nickel (PANI–Ni)

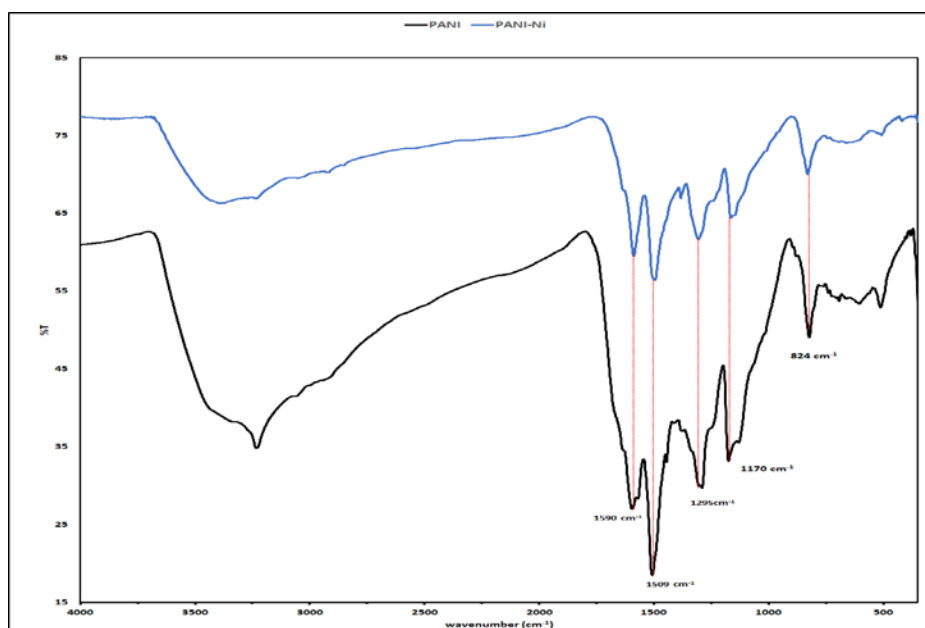


Figure 1: FTIR Spectra of Polyaniline (PANI) and Nickel-Modified Polyaniline (PANI–Ni)

Morphological and Elemental Analysis

The morphology of PANI–Ni, as examined by SEM–EDX (Figure 2), shows a rough and porous aggregated structure, with a nickel content of approximately 3.4%.

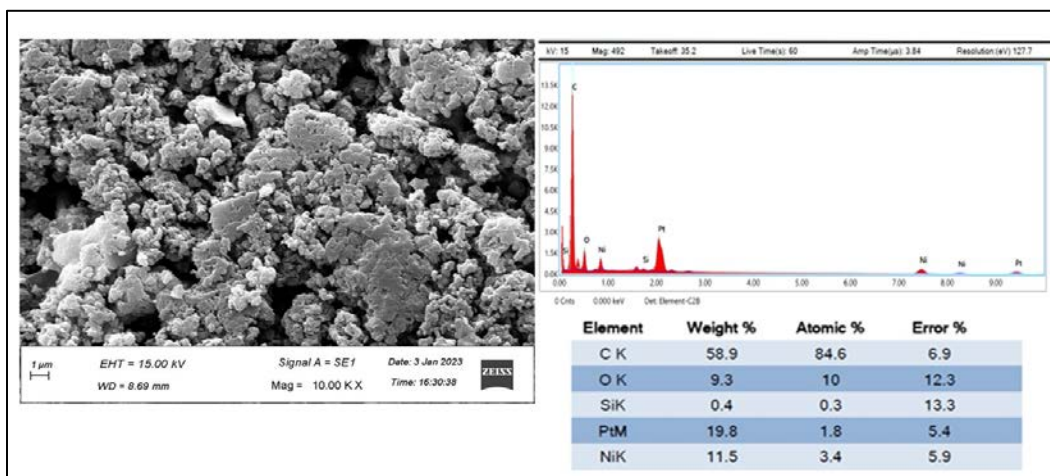


Figure 2: SEM Micrograph and Corresponding EDX Analysis of PANI–Ni

XRD pattern of PANI–Ni

X-ray diffraction (XRD) patterns of PANI and PANI–Ni were recorded over a 2θ range of $5\text{--}50^\circ$ with a step size of 0.02° , using $\text{Cu K}\alpha_1$ radiation ($\lambda = 1.54 \text{ \AA}$). PANI exhibited broad diffraction peaks at approximately $2\theta = 20^\circ$ and 26.3° , indicative of its predominantly amorphous nature (Figure 3). A similar diffraction profile was observed for PANI–Ni, suggesting that incorporation of nickel does not significantly alter the polymer backbone. NiO showed distinct peaks in XRD around $2\theta \sim 37.5^\circ$ (111), 43.3° (200), 62.9° (220), and 75.4° (311) (Hamzah *et al* 2025) whereas Ni (0) particles exhibited characteristic peaks around 44.5° (111), 51.8° (200), and 76.4° (220) (Zhu *et al* 2025). As no distinct peaks corresponding to metallic Ni or NiO were detected in PANI–Ni, this indicates the absence of reduction or hydrolysis of $\text{Ni}(\text{OAc})_2$.

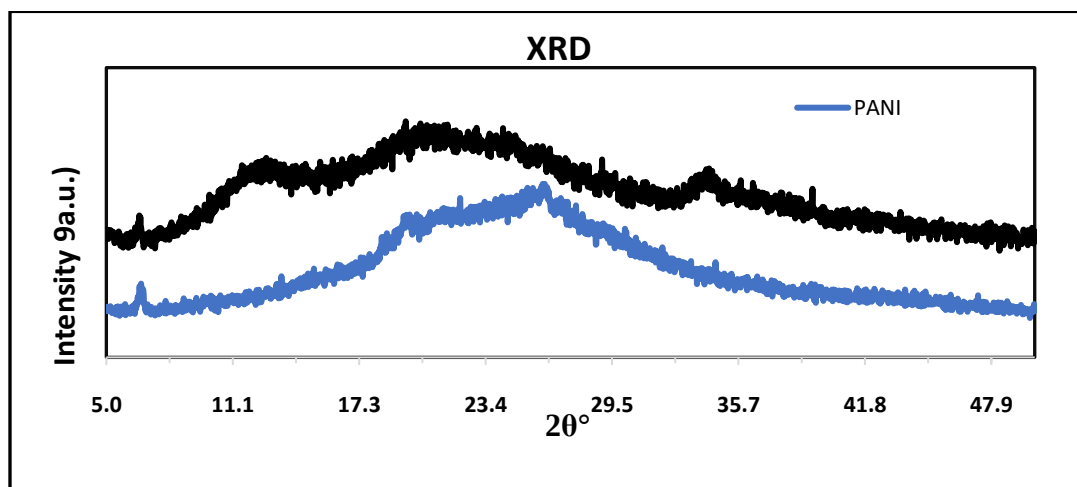


Figure 3: Powder XRD Pattern of the PANI and PANI–Ni Catalyst

Catalytic degradation of p-NP

The catalytic reduction of p-nitrophenol (p-NP) using PANI–Ni (5 mg) in the presence of sodium borohydride was monitored by UV–visible spectroscopy at $\lambda = 400 \text{ nm}$ (Figure 4A). Complete conversion was achieved

within 12 min, accompanied by the emergence of a characteristic absorption band of p-aminophenol at $\lambda = 290$ nm.

Increasing the loading of PANI-Ni catalyst showed much faster reduction of the p-NP as shown in Figure-4B. The observation is in line with expectation as the increased amount of catalysts provided a higher active site for the reduction (Roy *et al.* 2019). Recently $\text{Ni}_3\text{S}_2@\text{NF}$ (Nickel Foam treated with sulfide) reported similar trend in reduction of p-NP in presence of NaBH_4 (Cao *et al.* 2026). Moreover, the catalyst afforded 93.35% of p-NP within 25 min.

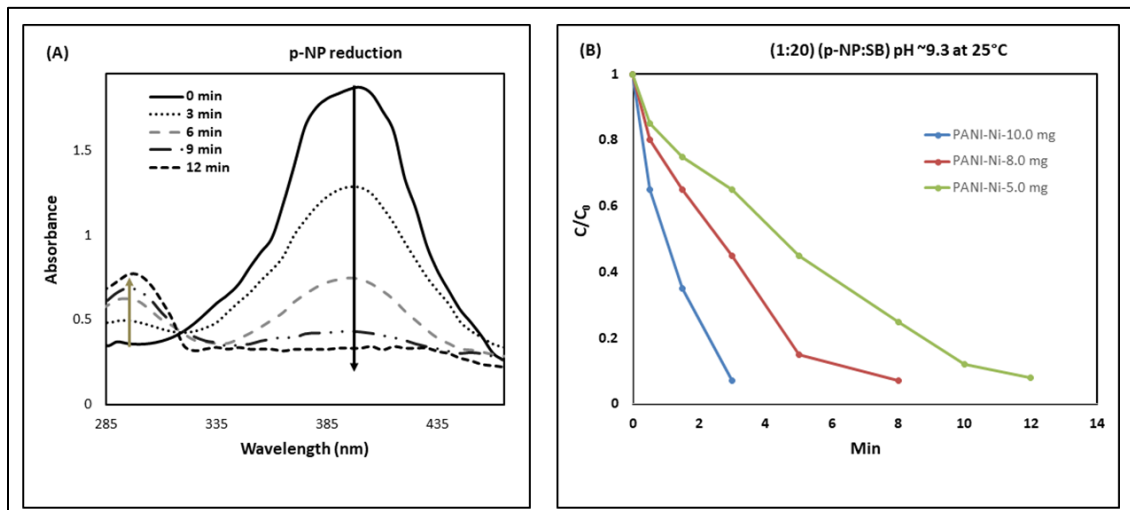


Figure 4: (A) Catalytic Reduction of p-nitrophenol (p-NP) in the Presence of PANI-Ni (5 mg); (B) Dependence of p-NP Degradation on PANI-Ni Catalyst Loading

Catalytic reduction of Methylene Blue (MB)

The reduction of methylene blue (MB) was investigated in the presence of sodium borohydride (1.5×10^{-5} M) using different loadings of PANI-Ni catalyst. A decrease in catalyst amount from 10.0 mg to 3.0 mg led to a corresponding increase in the time required for complete reduction, from 5 min to 25 min (Figure 5).

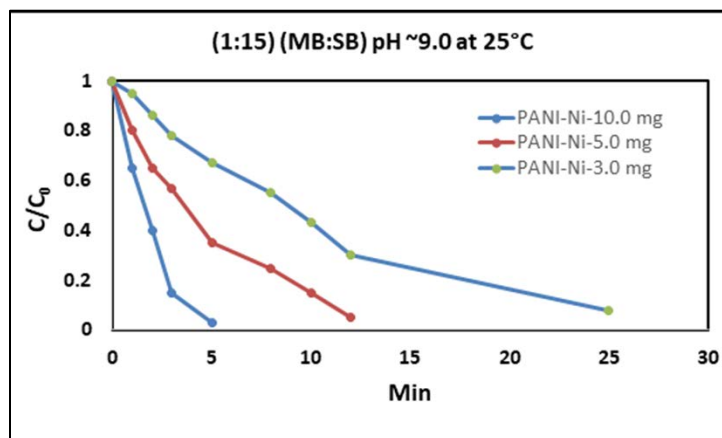


Figure 5: Dependence of Methylene Blue (MB) Degradation on PANI-Ni Catalyst Loading

A pH-dependent study (pH 8.5–11.0) was conducted using 10.0 mg of PANI-Ni catalyst. The reaction proceeded rapidly at pH 8.5, reaching near-complete conversion within 4 min, while at pH 11 only partial reduction was observed even after 50 min (Figure 6).

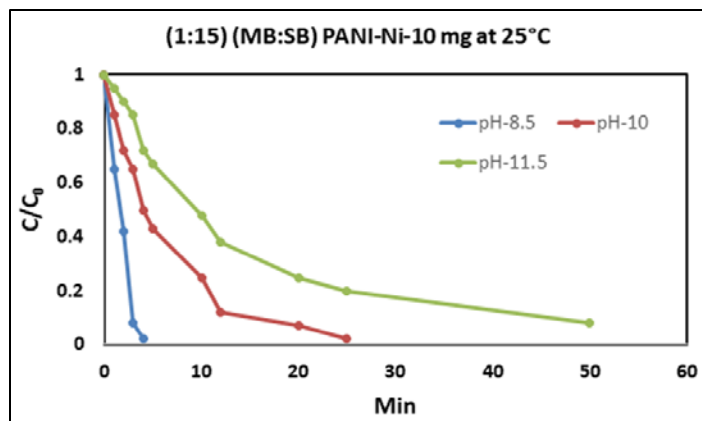


Figure 6: Influence of pH on the Reduction of Methylene Blue (MB) in the Presence of PANI–Ni and Sodium Borohydride

Table-2 shows a comparison of PANI-Ni with recently reported Ni catalyzed reduction of MB.

Table 2: Reduction of MB using different Ni catalysts

Catalyst	MB Reduction	Reference
PANI-Ni	10 mg catalyst afforded complete reduction within 5 min	Current Study
Cellulose Acetate Composite Membrane Fabricated with Nickel Nanoparticles (CA/Ni)	20 mg of CA/Ni afforded 88% reduction in 28 min	Bawazeer 2025
NiO nanoparticles-loaded mesoporous carbon (NiO/CMK-3)	100 mg of NiO/CMK-3 afforded complete reduction within 3 min	Younus <i>et al.</i> 2024
gellan gum-co-polymethacrylic acid hydrogel supported Ni-Cu bimetallic composite (GG-co-PMAA)@Ni-Cu	(GG-co-PMAA)@Ni-Cu afforded Complete reduction within 4 min	Afsheen <i>et al.</i> 2026

Catalytic reduction of Rhodamine B (RhB)

The reduction of rhodamine B (RhB), a xanthene dye, was carried out using sodium borohydride (10 mL, 2×10^{-5} M) and PANI–Ni (10.0 mg), achieving complete conversion within 15 min (Figure 7). A systematic increase in catalyst loading from 5.0 mg to 15.0 mg led to a noticeable enhancement in the reaction rate (Figure 8).

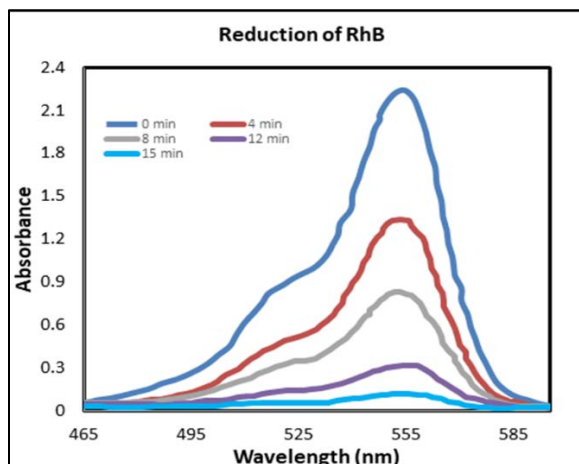


Figure 7: Catalytic Reduction of Rhodamine B (RhB) using PANI–Ni (10 mg) with Sodium Borohydride at pH 8.7

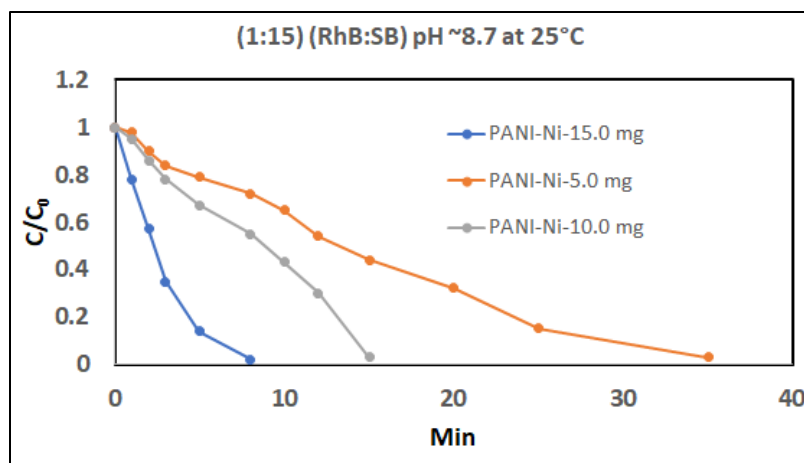


Figure 8: Influence of PANI-Ni Catalyst Loading on the Degradation of Rhodamine B (RhB)

Recently a $\text{NiFe}_2\text{O}_4@\text{C}/\text{PPy}$ Catalyst reported for the degradation of RhB in presence of SB affording ~80% reduction of RhB under optimal condition within 100 min (Thomas *et al.* 2025).

Recyclability Studies

For any sustainable catalytic system, recyclability is one of the most important criteria. Herein the PANI-Ni catalyst was treated with MB and recovered after filtration and reused in the next cycle (Figure-9). PANI-Ni showed excellent reduction of MB till 6th cycle. Slight activity loss was observed during the course of this study. To understand the loss in activity, Ni content of the fresh and recovered PANI-Ni was compared. PANI-Ni fresh showed 0.41 mmol g^{-1} Ni vs 0.35 mmol g^{-1} Ni after the 6th cycle. PANI-Ni recovered was further analyzed by XPS and it showed presence of Ni (0) and Ni(II) (Figure-10). A TEM analysis of the recovered PANI-Ni revealed presence of nickel nanoparticles of > 25 nm (Figure-11). The activity loss is a combined effect of nickel leaching, reduction followed by agglomeration of nickel particles.

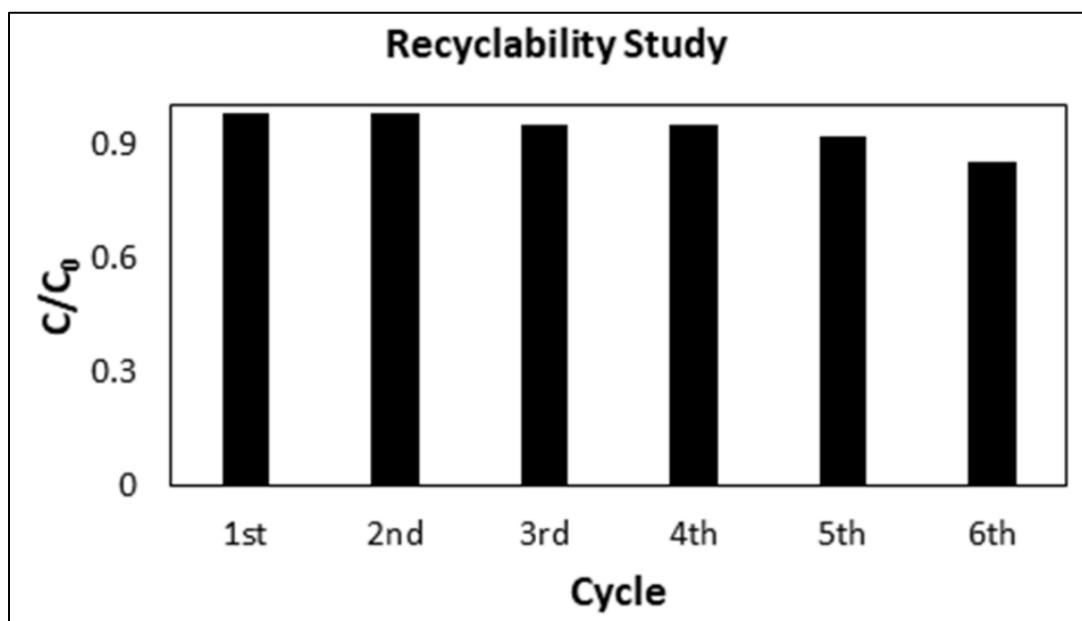


Figure 9: Recyclability Study of PANI-Ni for the Reduction of Methylene Blue (MB) in the Presence of Sodium Borohydride

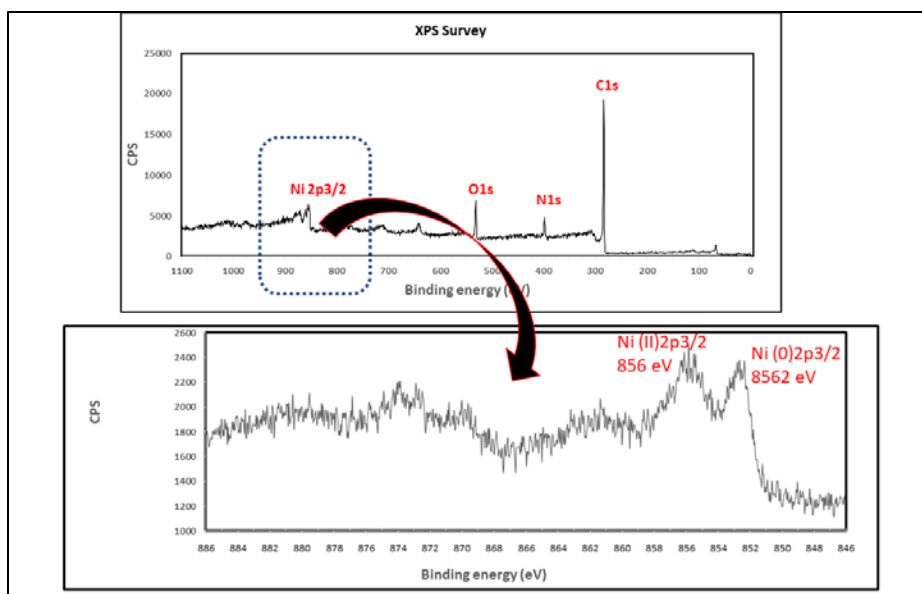


Figure 10: XPS Study of the Recovered PANI-Ni Catalyst

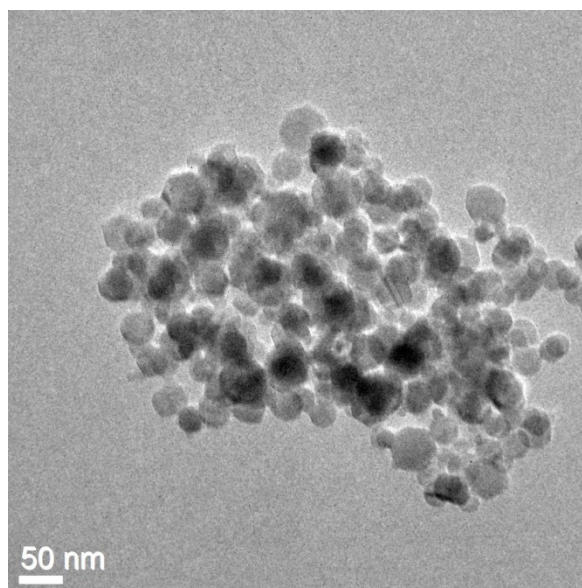


Figure 11: TEM Characterization of the Recovered PANI-Ni Catalyst

Table 3 presents a comparative study of recyclability of the PANI-Ni and recently reported Ni catalyst in degradation of dyes.

Table 3: Recyclability of Ni Catalysts in Dye Degradation

Catalyst	Dye	Remark	Reference
PANI-Ni	MB	Activity dropped after 6 cycle and agglomeration of Ni particle observed	Current Study
NiO/CMK-3	MB	Recycled for 5 times. Very low level of Ni leaching and structural integrity of the catalyst established	Younus <i>et al.</i> 2024
GG-co-PMAA]@Ni-Cu	MB	Recycled for 5 consecutive cycles with 87% catalytic activity	Afsheen <i>et al.</i> 2026
Ni ₃ S ₂ @NF	p-NP	Retained 90% activity after 15 cycles	Cao <i>et al.</i> 2026

Conclusion

In this work, a polyaniline-supported nickel catalyst (PANI–Ni) was prepared and applied for the reduction of organic pollutants such as p-nitrophenol, methylene blue, and rhodamine B. The catalyst showed good activity under mild conditions, enabling rapid conversion of these substrates.

The material could be readily recovered after the reaction and reused over several cycles with only a small decrease in activity. Characterization of the recovered catalyst by XPS, TEM, and metal analysis indicates that the loss in performance is likely due to a combination of nickel leaching, changes in the oxidation state of the active sites, and aggregation of nickel species within the polymer framework.

These observations help to better understand the stability of polymer-supported nickel systems and the factors that influence their long-term performance. The results suggest that PANI–Ni can serve as a useful catalyst for wastewater treatment, and that further improvements in catalyst design could enhance its durability and practical applicability.

Acknowledgement

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Study of Accumulation of Hazardous Metals in Edible Parts of Fishes from Kakmara River

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Abstract

The Kakmara River is rich in ichthyofaunal diversity, and it is one of the most important breeding grounds of many fresh water and estuarine fishes along the riparian zone of the Mridangabhangha River. The present study was conducted for four consecutive years at five study sites: Kuemuri, Kuemuri Bamboo Bridge (Lakshmijanardanpur), Herombogopalpur, Purbo Dwarikapur (1, 2), fish muscle tissue was collected and preserved, along with bones and intestines from each site for metal study. River water metal content was also studied from all study sites. The potential consumers' health risks are caused by six heavy metals (Cd, Zn, Co, Pb, Ni, and Cu) found in four most common fish species (*Mystus tengara*; *Amblypharyngodon mola*; *Puntius ticto*, and *Chela labuca*) which are less priced and easily available from the River Kakmara. The fish species were collected at an interval of 15 days from each site along with river water. This study reveals that at Kuemuri there was a significant effect of raised zinc metal concentration in water (highest 2 mg/L) that might have raised zinc value in fish *Chela labuca* muscle tissue. The study determined that the zinc absorption value in water at Purbadwarkapur 1 was 1.09 mg/l and at Kuemuri Bamboo bridge site 1.85 mg/l, where fish *Chela labuca* muscle tissue also showed a higher amount of absorption. The study recommended that those sites where the absorption limit in river water is significantly affecting the fish tissue (muscle) should be taken under proper conservation.

Keywords: Acceptable Limit; Edible Muscle Tissue; Hazardous Metals

Introduction

In the last few decades, rapid progression of industrialization and urbanization has caused immense environmental deterioration and as trail deforestation, damming, mining, and urbanization shot up (Islam *et al.*, 2025). Most of the water bodies became dumping grounds of wastes. The toxic metals from domestic, industrial, farmland and mining areas are deposited in the water bodies, which are dumped directly into the water bodies, causing damage to the aquatic life. The key impact rivers have is reduced flow and a slowdown effect caused by heavy siltation along with the waste deposition. Toxic metals, which are non-biodegradable, always harm aquatic life and the effect of deposition ultimately flows to the consumers who consume those contaminated edible parts. Not only industrial waste but also agricultural development and use of fertilizers and pesticides also cause immense damage to society. Examination of the water metal content and accumulation of metals in the edible parts of the aquatic organisms may indicate the risk of metal toxicity for the consumers. Fish is a highly preferred food, being a good source of protein and healthy polyunsaturated omega-3 fatty acids. The edible parts of the fish that accumulated toxic metals when exposed to contaminated water might create health hazards and sometimes be lethal to the consumers (Shaltout, 2024). The present study aimed to investigate the anthropogenic intrusion in the river contamination of toxic wastes in water, which simultaneously percolates to the aquatic life. The study intended to determine the amount of toxic metals accumulated in the tissue of fishes from a contaminated environment in which fishes are exposed. On the other hand, the value of bioaccumulation of those toxic metals in the fish (food) that passes ultimately to the consumer (human). The study was done throughout the stretch of the Kakmara River (Latitude 21°53'10.9"N, Longitude 88°26'25.3"E). The Kakmara River is a tributary of the Mridangabhangha River, which, on the other hand, is a major

tributary of the Ganga River. The Kakmara River has a total length of approximately 6.8 km. Emerging from River Mridangabhanga, South 24 Pargana, it flows through villages named Kuemuri, Herambogopalpur, PurbaDwarkapur. The river is flushed by estuarine water in every high-tide session. Moreover, the riparian zone becomes flooded during every monsoon as well. The study was conducted during four consecutive years, July 2022-July 2025, at five study sites on the river 1) kuemuri, 2) kuemuri Bamboo bridge 3) Herombogopalpur, 4) Purbo Dwarikapur-1 and 5) Purbo Dwarikapur-2 covering the total length of the river. During this study the adjacent ponds and ditches, which get drowned during each high tide, were also studied to integrate comprehensive sampling. Four fish species (*Mystus tengara*, *Amblypharyngodon mola*, *Puntius ticto*, and *Chela labuca*) were collected fortnightly from five study sites to observe the toxic metal absorption limit of muscle tissue and water parameters were also studied during every study. These four fish species had a rich population in this river and a definite breeding ground (Sarkar, 2025). The present work aimed to study the metal absorption limit in river water and four fish species in three seasons to determine the bioaccumulation values of hazardous metals that definitely harm the consumers and results will provide to the local people the firsthand knowledge of toxic metals (Cu, Pb, Ni, Zn, Co, Cd) absorbed in fish edible parts and water and also their effects when consumed. Majumder *et al.* (2024) opined that toxic chemicals that are absorbed in the edible tissue of aquatic organisms might be hazardous to the consumers. During each study, water parameters (Dissolved Oxygen, Free Carbon dioxide, Ph, Alkalinity, Turbidity and Temperature were also studied in all study sites).

Methodology

Fish samples of *Mystus tengara*, *Amblypharyngodon mola*, *Puntius ticto*, and *Chela labuca* were caught by engaging fishermen in all five study sites, including those ponds and ditches on the bank that were flooded by the river water during every high tide, fortnightly, over four consecutive years. The sample fishes were thoroughly washed, managing to remove slime from the body, and then muscle from the dorsal side, ventral side, and caudal muscle was collected. Muscle tissue was dipped and fixed in HNO_3 at the collection site immediately for future study. Digestion was done in the laboratory with HNO_3 , H_2SO_4 , and H_3PO_4 following Loon (1980). The filtrate was collected after digestion of tissue and stored in a glass-stoppered bottle before analysis. Analysis of metals was done in AAS (Varian AA 0.575). Water parameters (Dissolved Oxygen, Free Carbon dioxide, Alkalinity, Ph, salinity, Temperature of Water and Air) were studied by water testing kit.

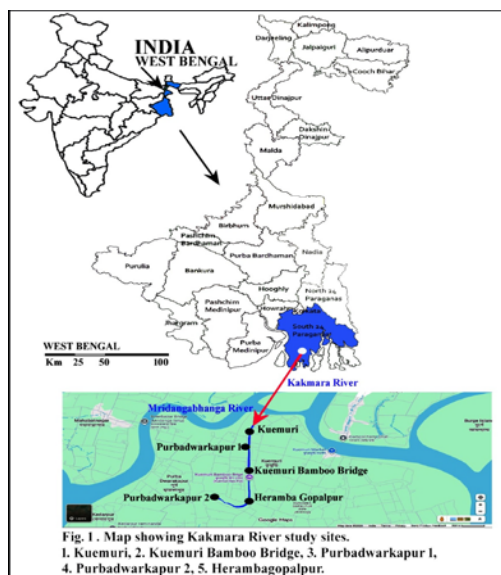


Figure 1: Map Showing Study Sites at Kakmara River

Results

Results of the study of toxic metals in fish muscle tissue and river water

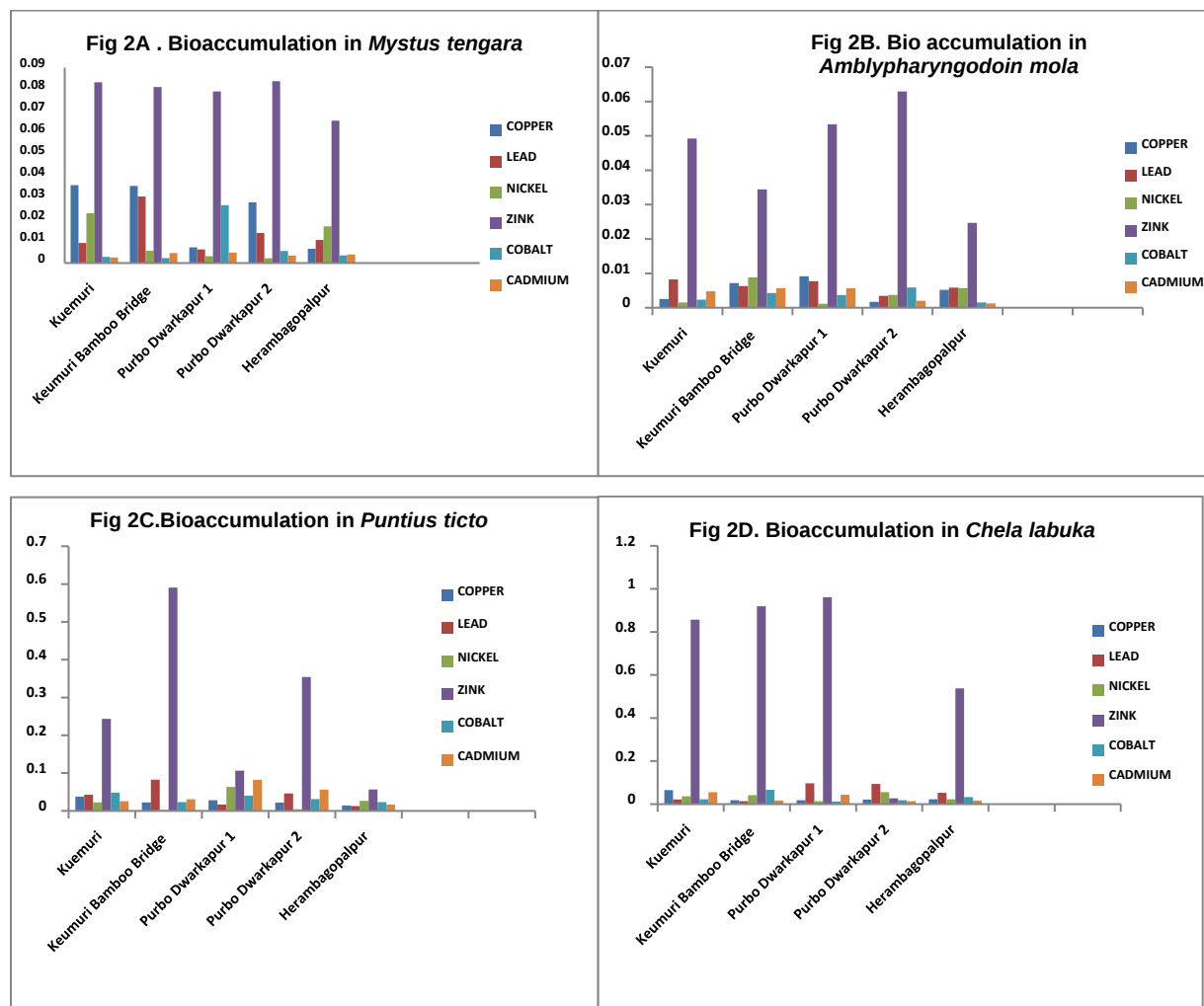


Figure 2: Bioaccumulation of Toxic Metals in Fishes (*Mystus tengara*; *Amblypharyngodon mola*; *Puntius ticto*, and *Chela labuka*) Muscle Tissue of Kakmara River

The statistical analysis of bioaccumulation of metals in fish muscle tissue (Fig. 2.A-D) indicated that Zn in muscle of *Mystus tengara* showed the highest value, $0.83 \pm 0.08 \mu\text{g/g}$, during the premonsoon season, and the lowest value was determined during the monsoon, $0.002 \pm 0.02 \mu\text{g/g}$, at the Purbadwarkapur 1 site; in *Amblypharyngodon mola*, the highest bioaccumulation value observed was $0.062 \pm 0.06 \mu\text{g/g}$, which remained within the permissible limit. This study reveals that at Kuemuri there was a significant effect of raised zinc metal concentration in water (highest 9 mg/l) that might have raised zinc value in fish *Mystus tengara* $0.098 \pm 0.09 \mu\text{g/g}$ in muscle tissue. The study determined that the zinc absorption value in water at Purbadwarkapur 1 was 1.09 mg/l and at the Kuemuri Bamboo bridge site 1.85 mg/l, where the muscle tissue of fish *Chela labuka* also showed a higher $0.091 \pm 0.09 \mu\text{g/g}$ value of absorption. The findings of Islam *et al.* (2025) indicated a higher Pb, Cu and Zn content in muscle tissue in *Mystus tengara* collected from a river in Bangladesh, but Murugan *et al.* (2008) observed lower zinc absorbed in muscle tissue of fish, though higher in water. During the present study, bioaccumulation of zinc in muscle tissue of all four fishes was found within permissible limits that corroborates with Murugan *et al.* (2008). Madhusudan *et al.* (2003) opined that the metal Zn percolates through gills and integument from the

environment and is then ultimately absorbed in muscle tissue and transferred to the other organs as well. Murugan *et al.* (2008), while working with *Channa punctata* in confined water, revealed that Zn metal absorption limit in tissue of fish was higher when it remained higher in the aquatic medium, as the fishes absorbed it more from the medium. The study indicated that Zn. Bandpe *et al.* (2016) opined that carp species that accumulated higher Zn in their muscle tissue might contaminate consumers, but the present study determined that the fishes from all five study sites in four consecutive years, all the fishes in all seasons in all years, showed a higher concentration of Zn in muscle tissue.

The metal nickel is corrosive to lung function; it damages kidneys in addition when deposited in the human body. The studied Ni bioaccumulation value (Fig. 2.A-D) in *Puntius ticto* was $0.063 \pm 0.06 \mu\text{g/g}$, indicating the highest among all other fish station-wise at Purbo Dwarkapur 1, which is within the permissible limit.

Copper is an essential metal for the human body, but when deposited in excess amounts, it is lethal to mankind. Rahman *et al.* (2012) in the Bangsi River in Bangladesh and Ahmad *et al.* (2015) in Kabul River found much higher levels of copper in fish muscle tissue and in river water as well. The bioaccumulation value of copper observed in the present study was highest at Kuemuri ($0.0652 \pm 0.06 \mu\text{g/g}$), which indicates the highest value during this study, which is within the permissible limit. The present study observed that determined copper in Kakmara River water is within the permissible limit at all study sites and the bioaccumulation value of Cu (Fig. 2.A-D) in muscle tissue in all four fish was also within the permissible limit.

Tuzen and Soylak (2007) opined that cobalt metal is essential for fish metabolism but causes damage when present in excess in the habitat. The analysis of river water in Kakmara River indicated that the highest cobalt concentration in river water was 0.07 mg/l at Herombogopalpur, but the bioaccumulation value (Fig. 2.A-D) results are within the permissible limit.

Majumder *et al.* (2024) and Tabrez *et al.* (2020) opined that the metal cadmium is toxic in smaller amounts also; when present in an aquatic body, it harms GI tract and kidneys when absorbed in the human body as well as fishes. Cadmium concentration in water in all five study sites through Kakmara River does not exceed beyond permissible limit (FAO/WHO, 1972) bioaccumulation value (Fig. 2.A-D) also was within permissible limit in edible muscle tissue in four fishes *Amblypharyngodon mola*, *Mystus tengara*, *Puntius ticto*, and *Chela labuca*.

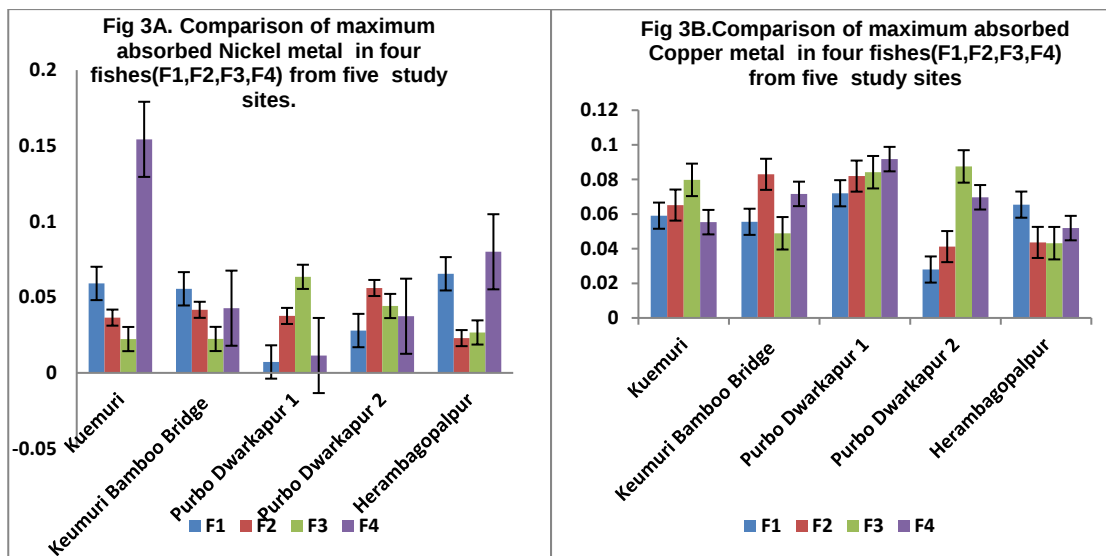
Lead causes harm to the aquatic organisms even when present in a lesser amount in an aquatic body and is also lethal to the consumers (Hossain *et al.*, 2018). The present study determined that lead in river water showed the highest value at Kuemuri Bamboo Bridge (0.001 mg/l), which was within the permissible limit and remained within the permissible limit for five stations as well.

Discussion

The hazardous effect of toxic metals that get easily absorbed in the aquatic fauna and flora is of great concern to the consumers. Rohani *et al.* (2022) and Saha *et al.* (2009) observed that nonessential metals Cd, Cr, Ni, and Co, when consumed in excess by consuming contaminated food fishes, may have carcinogenic effects, cardiovascular dysfunction in adults and in addition, retarded cognitive power in children. The research indicated that the heavy metals do not get removed and are easily transferred to consumers at the next level through the food chain. The effect of metal toxicity studied by different authors revealed that metals are very much hazardous to human life; when exposed to higher levels, it might cause damage to GI system, endocrine system, and appendicular system. Also, long-term exposure may damage grey cells and cause disorientation in normal cognition in man (Akoto *et al.*, 2014). Akter *et al.* (2021) opined that lead accumulation, when exceeding the permissible limit, may affect children's health adversely, like constant pain in the abdomen, constipation, forgetfulness, and

depression, and cadmium and metals lead and chromium might cause heavy damage to the kidney and urethral system and reproductive system, which may lead to carcinoma. The detailed study of metal contamination in fish muscle tissue by Shaltout (2024) and Emon *et al.* (2023) opined that metals ultimately cause damage to the consumers at the level of DNA and cause serious pollution that threatens life.

The studied fishes *Chela labuka*, *Mistus tengara*, *Amblypharyngodon mola*, *Puntius ticto* are the most important high-value fish species and its catch density showed very profitable data in this river. The previous study (Sarkar, 2025) indicated the Kakmara River as the best breeding ground for almost all the fish of Mridangabhangha River, therefore, proper conservation and restoration of the native fish species diversity and their breeding ground should be the utmost priority. Moreover, Kakmara River is affected less by anthropogenic intervention and in addition to it, the river gets a high-tide flow flush from Mridangabhangha River, which definitely increases faunal diversity as well. The four studied fish species have high demand in the local fish market as well as in the fish market of Kolkata as food fishes and also for its esthetic value as ornamental fishes. The fish species *Amblypharyngodon mola* (Mola carplet) is highly preferred fish for its good taste and high protein value and is also a good source of minerals (Fe, Zn, Ca) and vitamins (Alam, 2004). They are herbivorous fish and often planktivorous (Kanwal & Pathani, 2012); therefore, it is very much evident that these fishes are always exposed to absorbed metals by the water as well as planktons and weeds. But the recent study reveals that only zinc metal showed highest bioaccumulation value, which is also within permissible limit. The other fish species *Puntius ticto* is a very common minnow, rich in protein, phosphorus and minerals and also easily available in good quantities from this river. This fish is planktivore (Hoque *et al.*, 2016), and its habitat is surface water. A study of the absorption of toxic metals in the muscle tissue of this fish revealed that all the studied metals are within the permissible limit. The glass barb, *Chela labuka*, is a very common, easily available fish also found in the upper surface and bottom depth of river. This planktivore fish species is rich in protein content, fat and minerals (Epa & Narayana, 2016). Statistical analysis of bioaccumulation of metals in the muscle tissue of this fish indicated safe results, as the value was within the permissible limit. The popular catfish *Mystus tengara*, is a very good source of protein and is available in abundant quantities from this river.



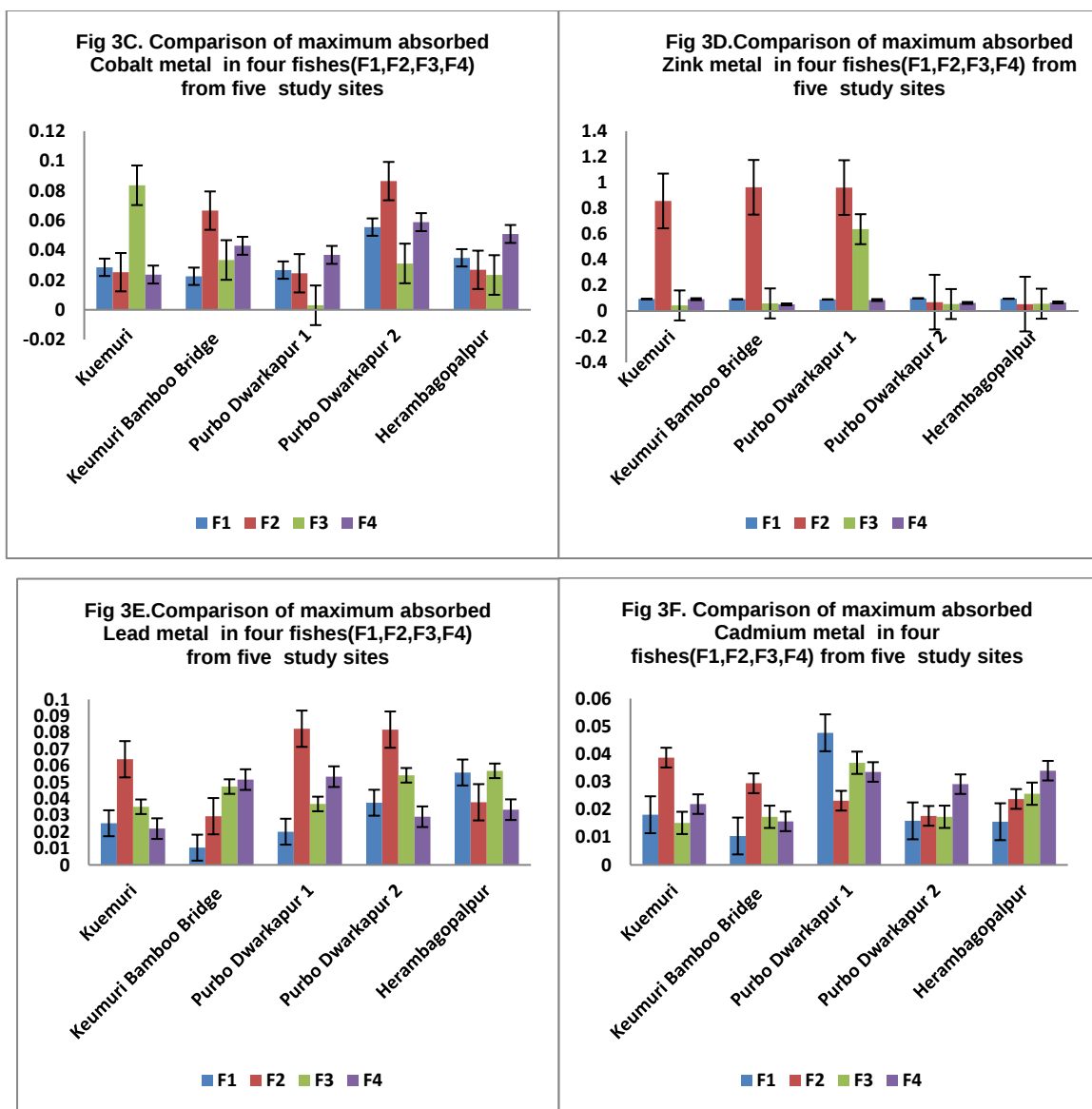


Figure 3: Comparison of Maximum Absorbed Metals (Copper, Lead, Nickel, Zink, Cobalt, Cadmium) in Four Fishes (F1-Mystus tengara, F2-Amblypharyngodon mola, F3- Puntius ticto, F4 - Chela labuka) from Five Study Sites

This species shows a variety of feeding habits (Majumder *et al.*, 2024); they feed on detritus matter, fecal debris and small fishes. Figure 3 A-F showed the graphical representation of the comparison of maximum metal absorption in muscle tissue of four studied fishes. The study indicates that the fish *Mystus tengara* showed the highest metal absorption of metal zinc (0.0983 µg/g), nickel (0.063 µg/g), and cadmium (0.047 µg/g); fish *Chela labuka* showed maximum absorption of metals lead (0.082 µg/g) and cobalt (0.086 µg/g); and fish *Puntius ticto* showed maximum limit of copper metal 0.087 µg/g (Figure 3. A-F) absorption in muscle tissue.

Conclusion

This Kakmara River flows through the area where a concrete road for vehicles is absent. Also, there are no major or small factory outlets that lead to the river; anthropogenic infiltration is also limited, which might be the reason for the lowered metal value in river water. The study of dissolved oxygen, free carbon

dioxide, and pH values of river water in three seasons—pre-monsoon, monsoon, and post-monsoon—indicates a healthy aquatic environment for the Kakmara River. The mangrove forest on both sides of the river, stretching about six km from Herombogopalpur and Purbodwarkapur 2, might be the suitable environmental cover for flowing water as well as aquatic organisms, and that might also be the cause for lowering the contamination of toxic metals. Analysis of heavy metals in the water of the Kakmara River showed a nominal (within permissible limit) value of the absorption limit for all the metals studied. The study determined that bioaccumulation of metal in muscle tissue of all the studied metals is within the permissible limit. Fig. 3.A-F showed the comparison of maximum absorbed metals (Cu,Pb,Ni,Zn,Co,Cd) in four fishes (F1-*Mystus tengara*, F2-*Amblypharyngodon mola*, F3- *Puntius ticto*, F4 - *Chela labuka*) from five study sites; all the values were within permissible limit.

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Conflicts of Interest

The authors declare no conflict of interest.

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Recent Trends in Green and Multicomponent Approaches for the Synthesis of Pyrano[2,3-c] pyrazoles

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Abstract

Among the fused heterocycles, pyrano[2,3-c]pyrazoles are the most significant scaffold because of their biological potential. Recently, growing interest in sustainability has strengthened the green methodology of the multicomponent reactions for the synthesis of these frameworks. This review summarizes an extensive and updated overview of the advancement of green synthetic strategies of these pyrazole-fused heterocycle derivatives in the period of ten years. The use of alternating energy sources, e.g., microwave and ultrasound irradiation, recyclable nano catalysts, metal-free organic and biocatalysts, ionic liquids, water as a green solvent and solvent-free and catalyst-free conditions are highlighted in the review. The descriptive and comparative study of these methodologies unveils significant improvements in the atom economy of the reactions, reduced energy consumption and catalyst recyclability. The critical analysis of the current trends and limitations, this review aims to guide the future design of sustainable, high atom economy synthetic strategies for this biologically significant heterocycle.

Keywords: Green Solvent; Green Synthesis; Microwave; Multicomponent; Nanocatalyst; Solvent Free Ultrasound

Introduction

Heterocyclic molecules are abundantly found in nature, and these are very essential in our biological systems. Due to their structural diversity and functional versatility, they became the indispensable components of pharmaceuticals, agrochemicals, dyes, and advanced materials (Pozharskii *et al.*, 1997; Biswas *et al.*, 2022). Over the last two to three decades, the chemistry of heterocyclic compounds has been extensively studied (Katritzky *et al.*, 2001). The synthesis and application of medium-sized heterocyclic rings, especially the fused heterocyclic rings, have attracted significant attention among the researchers involved in pharmaceutical chemistry as well as drug discovery. The pyrano-fused pyrazoles have four isomers (Figure 1), and among them, pyrano[2,3-c]pyrazole is reported to be the most biologically significant and so the synthesis of this heterocycle becomes of great interest. The incorporation of the pyrazole nucleus imparts diversified applications in different areas such as technology, medicine and agriculture (Abhinav *et al.*, 2020) and agrochemical research (Nassar *et al.*, 2022).

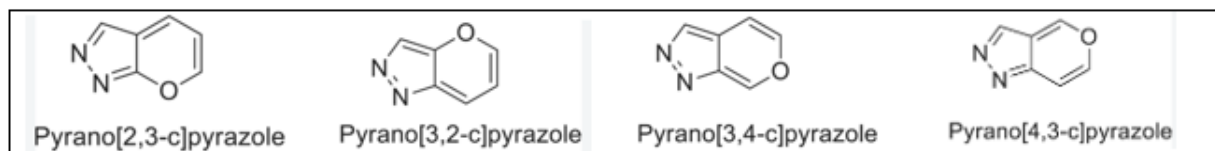


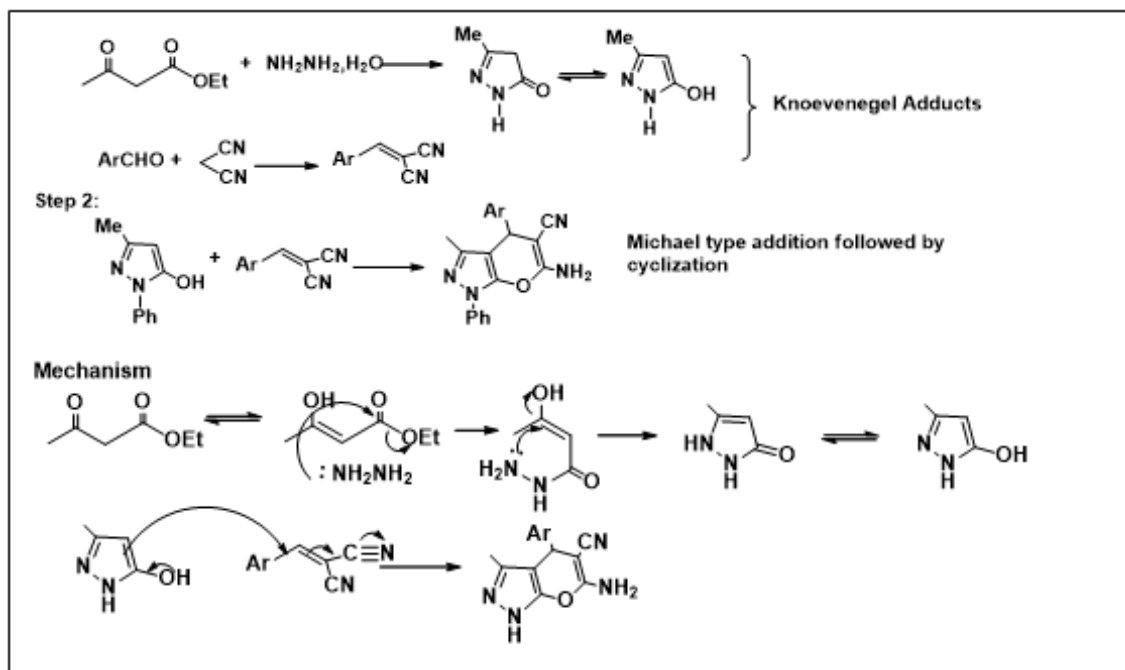
Figure 1: Isomers of Pyrano[2,3-c]pyrazole

The molecules having a pyranopyrazole nucleus are found to exhibit a broad spectrum of biological activities such as cholinesterase inhibitory activity (Derabli *et al.*, 2018), antibacterial (El-Assaly *et al.*, 2021), antimicrobial (Alzahrani *et al.*, 2025; Nguyen *et al.*, 2022), anticancer (Fouda *et al.*, 2022), anti-inflammatory, and analgesic (Kumar *et al.*, 2012) properties. Recent studies have also highlighted their

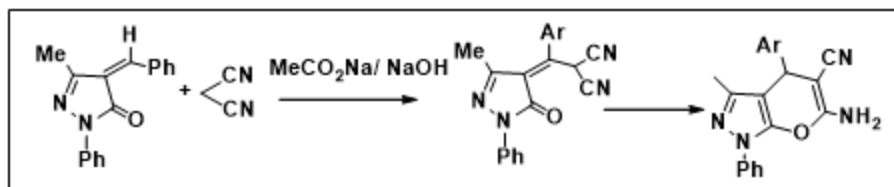
fungicidal, bactericidal, and herbicidal properties (Nicolaou *et al.*, 2002), which make the molecules agrochemically important. Interestingly, recent research of these molecules reveals that these derivatives may be used as the inhibitor of human coronavirus 229E (Allayeh *et al.*, 2024). Such wide-ranging activities have encouraged extensive research on the synthesis of this valuable scaffold in efficient and sustainable methods.

Multicomponent reactions (MCRs) are recognized as the most powerful tool in synthesizing pyranopyrazole due to their inherent benefits—operational simplicity, increased atom economy, reduced waste production, minimization of reaction time and energy consumption. The conventional methods often involve hazardous solvents, strong acids, and extended reaction time (Kharissova *et al.*, 2019). The modern green technologies (Mamaghani *et al.*, 2021; Bhaskaruni *et al.*, 2020) encourage the use of recyclable catalysts, alternating energy sources, green solvents, etc. Consequently, numerous green methodologies have been reported using bio- and asymmetric catalysis (Varma, 2016; Kate *et al.*, 2022), microwave, ultrasound, ultraviolet, green solvents, e.g., water and ionic liquids (ILs) (Romney *et al.*, 2018).

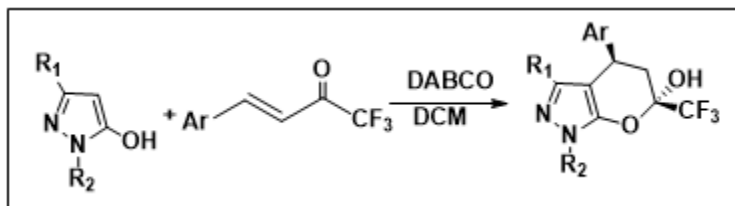
This review will cover a detailed and updated account of the green MCR strategies for synthesizing pyranopyrazole derivatives by using MW, organo or biocatalysts, or nanocatalysts. solvent-free or using water and ultrasound technique. The MCR synthesis of this molecule involves generally four components – (i) aldehyde, (ii) malononitrile, (iii) β -ketoester, e.g., ethyl acetoacetate and (iv) hydrazine hydrate. The reaction takes place in the presence of an appropriate catalyst or promoter (Bhope *et al.*, 2022). The conventional method of synthesis of pyranopyrazole is the Michael addition of a pyrazolone nucleus with malononitrile in the presence of a base (Scheme 1). This is the first approach to synthesizing this molecule by Otto (1974). The cyclization reaction of 4-arylidene-5-pyrazolone with malononitrile was carried out in the presence of base (Scheme 2). Wang *et al.* (2015) further modified the synthesis where the base used was DABCO and the reaction of pyrazolone and α,β -trifluoromethyl ketones took place in DCM as a solvent (Scheme 3).



Scheme 1: General Multicomponent Synthesis



Scheme 2: First synthetic approach of pyrano[2,3-c]pyrazole



Scheme 3: Modified Approach of Synthesising Pyrano[2,3-c]pyrazole Using DABCO as Base

Methodology

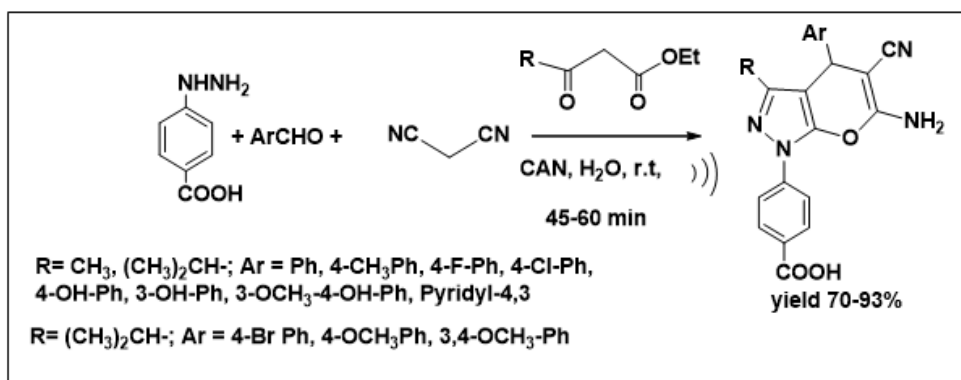
By Using Ultrasonic Sound Technique

Ultrasound irradiation increases the reaction rate and selectivity of the reaction by acoustic cavitation and reduces the reaction time and temperature (Puri *et al.*, 2013). Several catalyst-based and catalyst-free ultrasonically driven MCRs have been developed, providing excellent yields under mild and eco-friendly conditions.

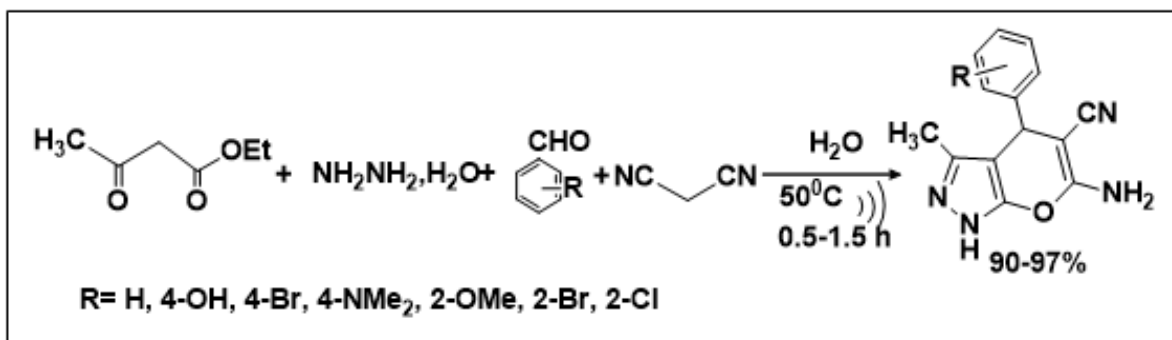
Ablajan and his team (Ablajan *et al.*, 2013) recently outlined a method for synthesizing pyrano [2,3-c] pyrazole derivatives from phenylhydrazine-4-carboxylic acid at 250°C in aqueous medium in the presence of CAN by using ultrasound techniques (Scheme 4).

Shabalala and his group synthesized pyran pyrazoles from different aromatic aldehydes via four-component synthesis in a water medium under ultrasonic irradiation (Shabalala *et al.*, 2015) (Scheme 5). Gujar *et al.* (2014) invented a new approach by using molecular sieves (40 Å) as a catalyst. The advantage of this catalyst is that it can be reused with the same efficiency up to the third run (Scheme 6).

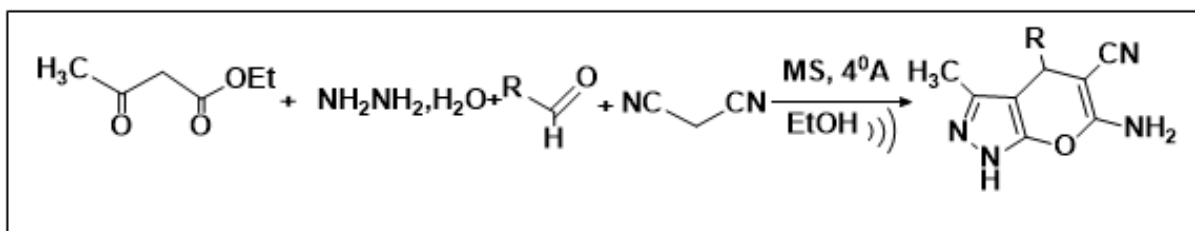
Four-component synthesis of the molecule was also reported using Mn-doped zirconia (Mn/ZrO₂) in aqueous ethanol at room temperature and the recovered catalyst was reported to be recycled for six consecutive times (Maddila *et al.*, 2019). (Scheme 7).



Scheme 4: Pyranopyrazole Derivatives Synthesis in Presence of CAN under Ultrasonic Technique

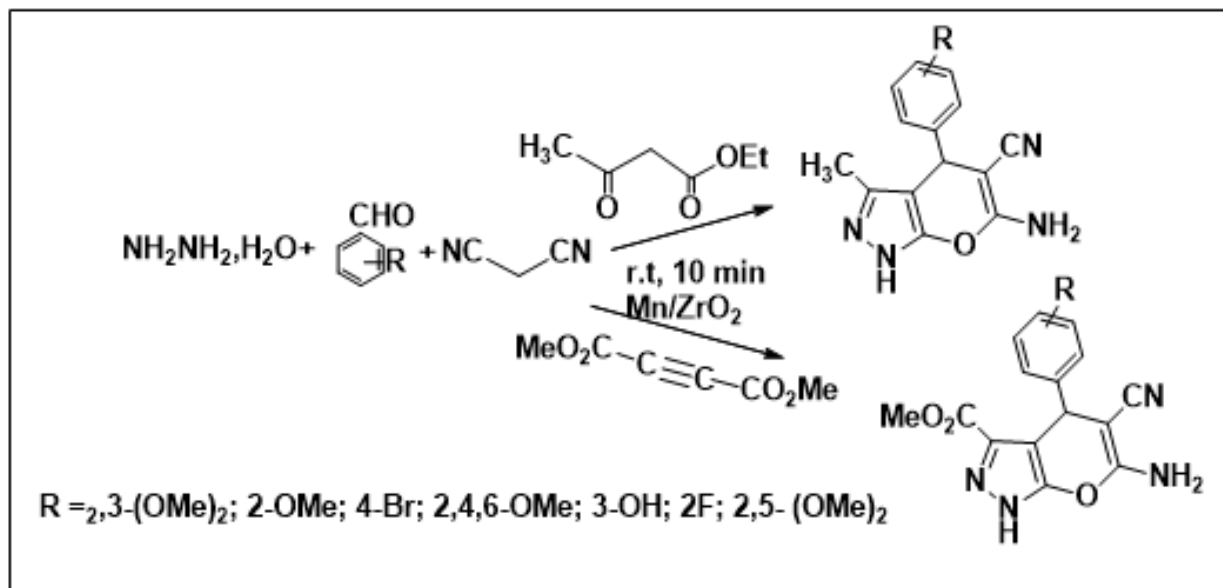


Scheme 5: Pyranopyrazole Synthesis Under Catalyst-Free Condition in Water

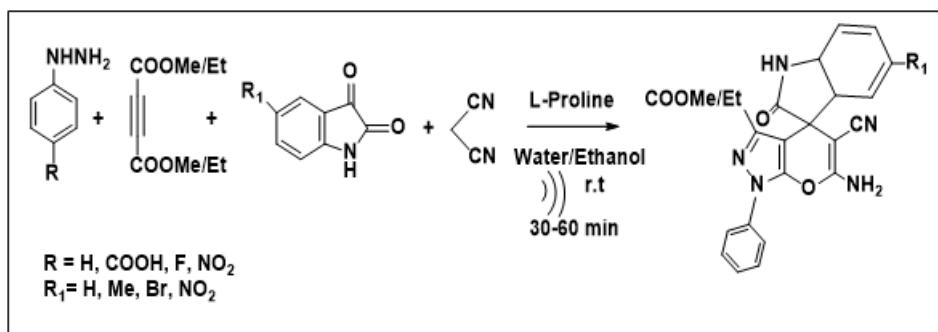


Scheme 6: Pyranopyrazole Synthesis Using Molecular Sieves

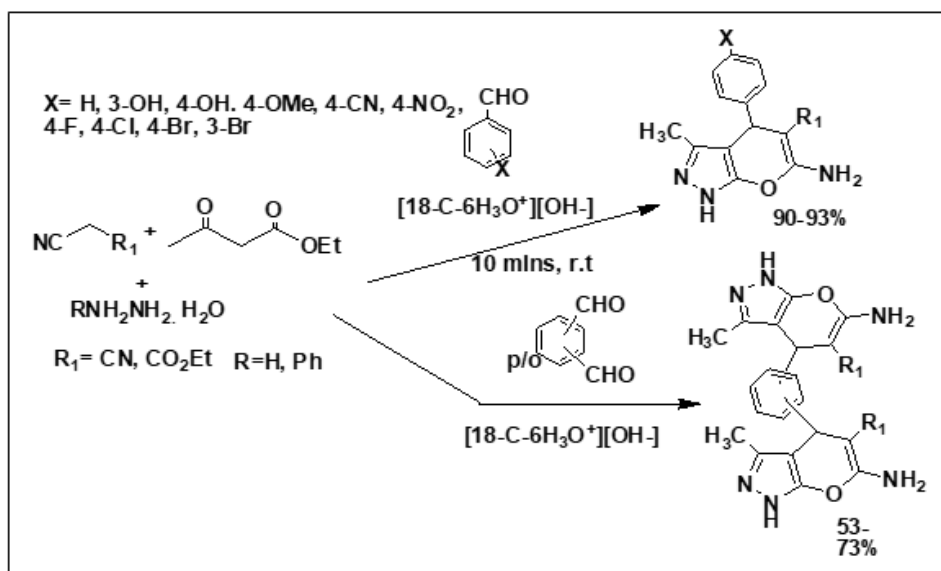
Water is found to be the best medium among other ecofriendly solvents like methanol, acetonitrile, toluene, DMF and DMSO. Spiro pyrano [2, 3-c] pyrazoles were reported to be prepared under ultrasound using L-proline with 1:1 v/v ethanol/water at room temperature (Liju *et al.*, 2015) (Scheme 8). Mishra *et al.* reported the generation of the hydronium ion of crown ether in situ and its catalytic activity towards the preparation of pyranopyrazoles (Mishra *et al.*, 2020) (Scheme 9). Khare *et al.* introduced 1,2,3-triazolyl pyranopyrazoles (Scheme 10) with 92–98% yield at 30°C in water with NaHCO₃ as a catalyst (Khare *et al.*, 2019).



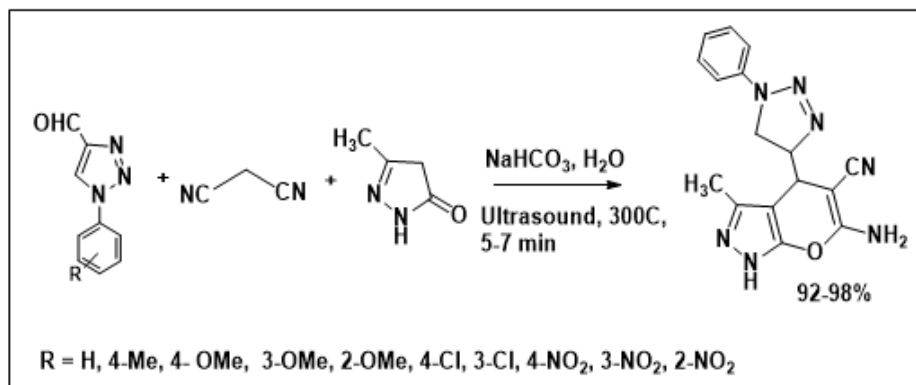
Scheme 7: Preparation of pyrano[2,3-c]pyrazole Derivatives Using Mn/ZrO₂ as Catalyst



Scheme 8: Synthesis of Spiropyranopyrazole Derivatives in Presence of L-proline Catalyst



Scheme 9: Bispyrano[4,3-*c*]pyrazole from Benzene Dicarboxaldehyde (*o/p*)



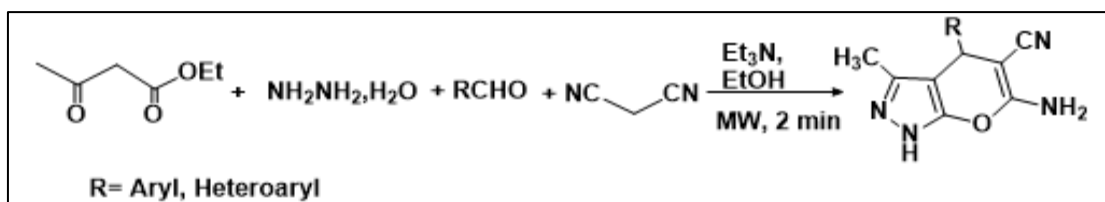
Scheme 10: Preparation of 1,2,3-triazolopyrano[2,3-*c*]pyrazole

Microwave Technique

Microwave irradiation accelerates chemical transformations by rapid internal heating and improved reaction kinetics. Numerous microwave-assisted MCRs for pyranopyrazoles have been reported,

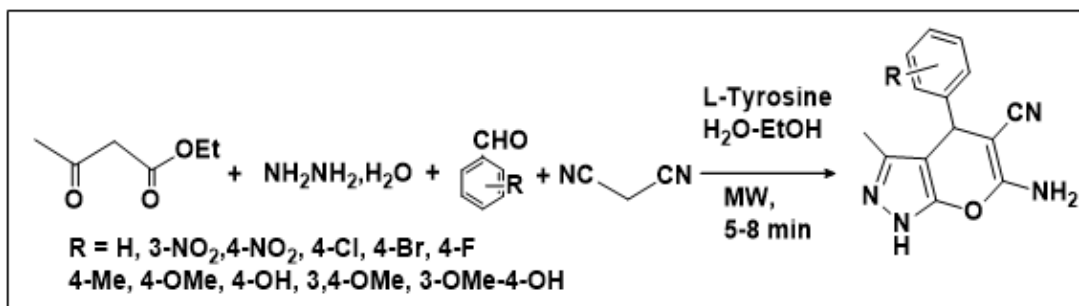
demonstrating shortened reaction times, excellent yields, and operational simplicity. The microwave irradiation increases the rate of reaction and decreases the time of reaction and makes the reaction eco-friendly.

A comparison was made between the microwave method and the conventional method of synthesizing a series of pyrano [2,3-c] pyrazoles by Shukla and his team and they concluded that the conventional method ended with good yields of the product, whereas microwave irradiation reduces the duration of the reaction (Shukla *et al.*, 2015) (Scheme 11). Some of the compounds in this series showed cytotoxic activity against the Hep3B cancer cell lines (Sharma *et al.*, 2016).



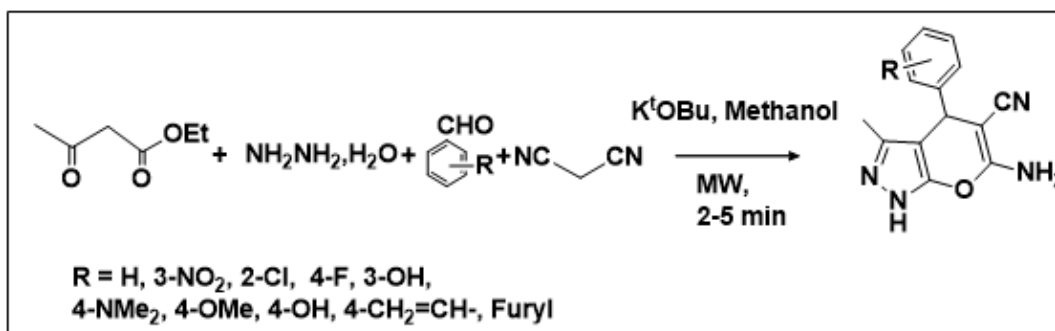
Scheme 11: Base Catalyzed Synthesis of Pyranopyrazoles Under Microwave Condition

The eco-friendly synthetic approach was reported to synthesise pyranopyrazoles under MW technique using solvent mixture water-ethanol system in the presence of L-tyrosine (Rupnar *et al.*, 2017) (Scheme 12).



Scheme 12: MW Preparation of pyrano[2,3-c]pyrazoles in Presence of L-tyrosine

Yallappa *et al.* recently introduced a new method of synthesis of the compounds via four component MCR in presence of K^tOBu under MW condition and found the excellent yield of product in 5-7 mins (Yallappa *et al.*, 2022) (Scheme 13).

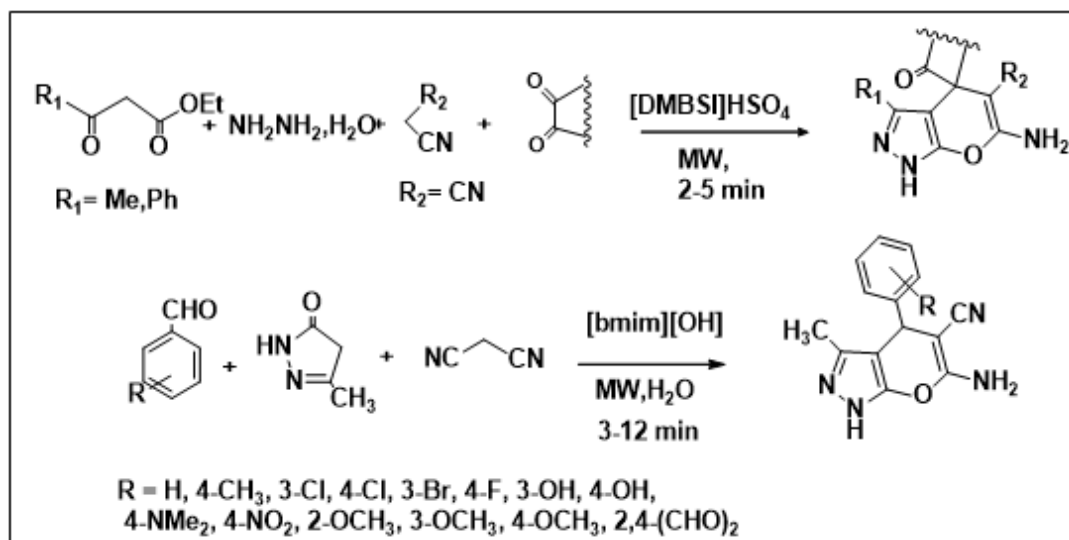


Scheme 13: Base Catalyzed Synthesis Under MW Condition

In the green MCR, ionic liquids have played the excellent role of green solvent in place of common organic solvent due to their favorable properties (Becerra *et al.*, 2022). A rapid, straightforward, one-pot

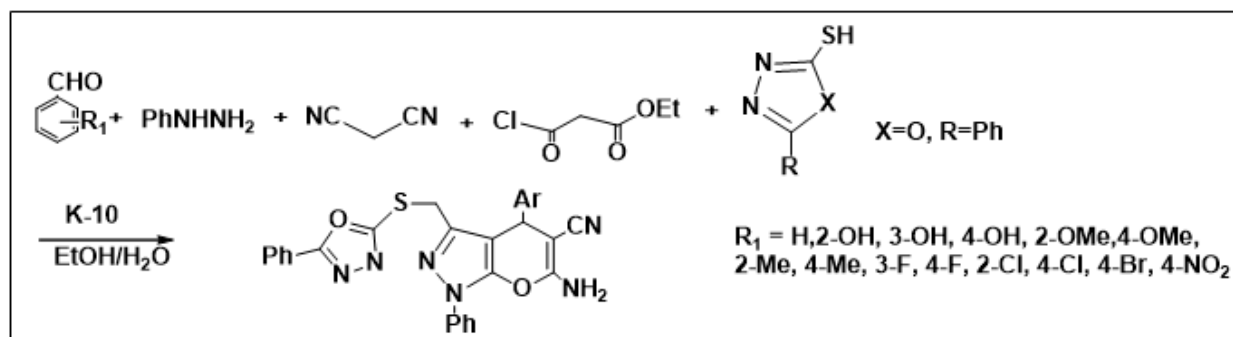
approach using [DMBSI][HSO₄] was reported by Mamaghani and his team (Mamaghani *et al.*, 2015). The reaction was placed at 60°C in MW without solvent (Scheme 14). The spirodiketones were taken by the researchers to produce spiropyranopyrazoles. These compounds showed antibacterial activity against gram-positive and gram-negative bacteria in in-vitro studies using tetracycline and erythromycin as standard drugs.

Imidazole-based ionic liquid [bmim][OH] was also used in the synthesis of pyranopyrazoles under microwave conditions (Aliabadi *et al.*, 2016). The synthesised compounds (Scheme 15) were found to exhibit antioxidant activity.



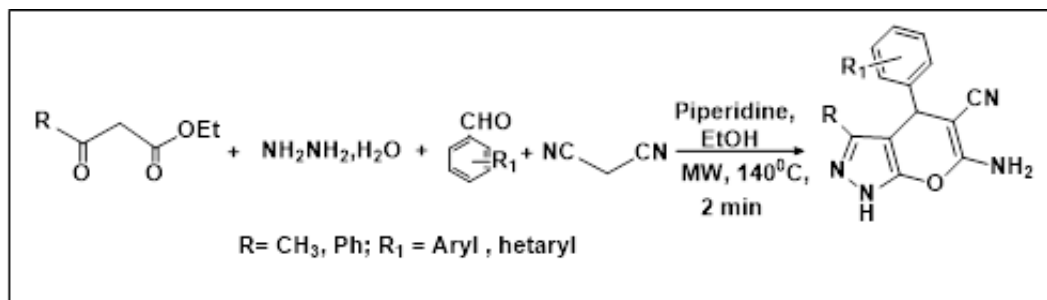
Scheme 14 & Scheme 15: Synthesis of Spiropyranopyrazoles Derivatives in Presence of IL Under MW

There has been a growing emphasis on developing eco-friendly reaction pathways, environmentally sustainable catalysts, and solvents, as well as synthesizing novel biologically active heterocycles. These areas have become crucial focal points in numerous ongoing research programs (Reddy *et al.*, 2017). The new pyranopyrazoles linked with thioether were prepared in the presence of Montmorillonite K-10 clay (Reddy *et al.*, 2019) (Scheme 16). Due to the acid-catalytic nature of the catalyst, the reaction was initiated rapidly. The catalyst K-10 can be reused, is inexpensive, is eco-friendly, and increases the yield of the product. The antibacterial activity was also reported for these compounds.



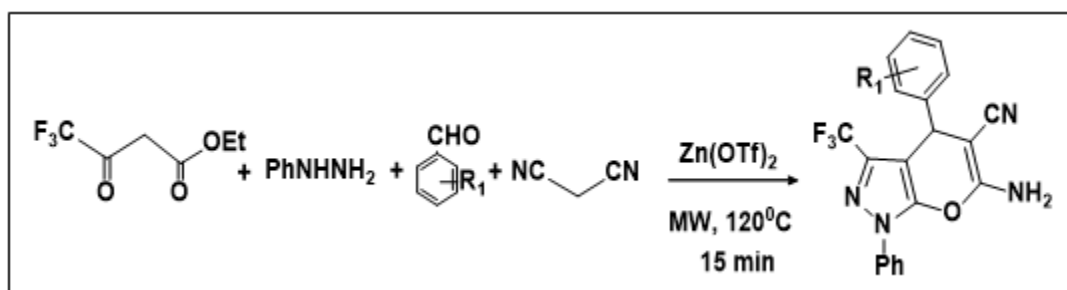
Scheme 16: Thioether-linked Pyranopyrazoles via a Five-Component Reaction

Recently, microwave synthesis of these molecules were reported using piperidine as catalyst at 140°C in 2 min in good to excellent yields (El-Agrody *et al.* 2022) (Scheme 17).



Scheme17: MW Synthesis in Presence of Piperidine

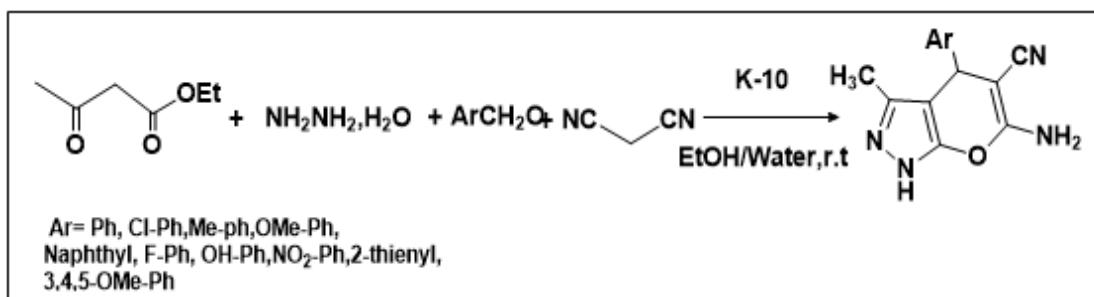
Another multicomponent approach under MW technique was successfully done in two steps: (i) β -ketoesters and aryl hydrazines were irradiated for 10 min at 80°C in the presence of Lewis acid catalyst zinc triflate, (ii) then addition of aromatic aldehydes and malononitrile and after irradiation, the mixture at 120°C for 15 min produced the desired product (Parikh *et al.*, 2022) (Scheme 18).



Scheme 18: Preparation of Pyranopyrazole Derivatives in Presence of Lewis Acid Zinc Triflate Using Nanocatalyst

Among the wide range of catalysts, nanoparticle-derived nanocatalysts are found to be excellent alternatives to conventional catalysts as their surface-to-volume ratio is increased and also for their robustness and stability (Yang *et al.*, 2017; Saxena *et al.*, 2017). Due to the presence of more open active surfaces than bulk catalysts, the contact between the reactant and catalyst becomes very efficient (Narayan *et al.*, 2019). The nanocatalysts show high selectivity, reusability, and control over the reaction routes (Ahmad *et al.*, 2023) and minimize the byproducts. Recently heterocyclic compounds are synthesized by using the nanocatalysts.

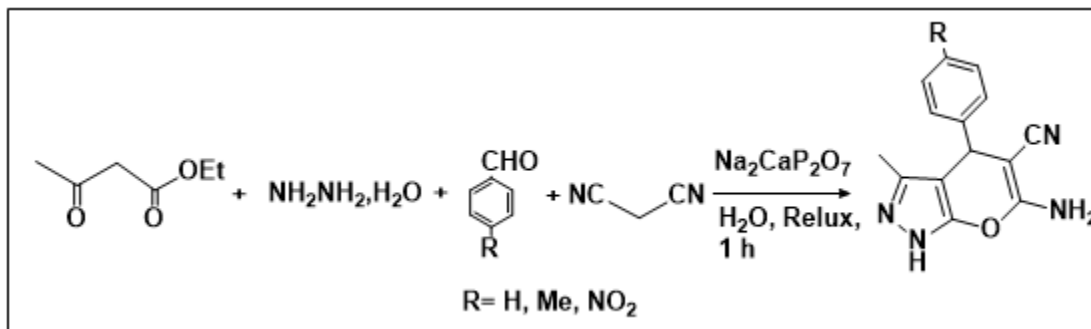
The green heterogeneous acid catalyst Montmorillonite-K10 is used to prepare pyranopyrazole derivatives at 100°C (Moosavi-Zareet *et al.*, 2016) (Scheme 19).



Scheme 19: Preparation of Pyranopyrazole Derivatives in Presence of Montmorillonite-K10 Catalyst

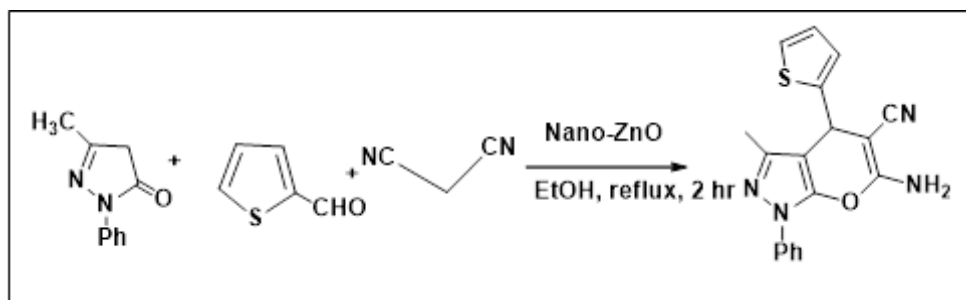
MNPs are now very popular as catalysts in MCRs and another widely used nanocatalyst is heterogeneous magnetic nanocatalysts.

Pyranopyrazoles were successfully prepared in the presence of a $\text{Na}_2\text{CaP}_2\text{O}_7$ catalyst via a four-component reaction in refluxing water for 1 h (Addoum *et al.*, 2021) (Scheme 20).



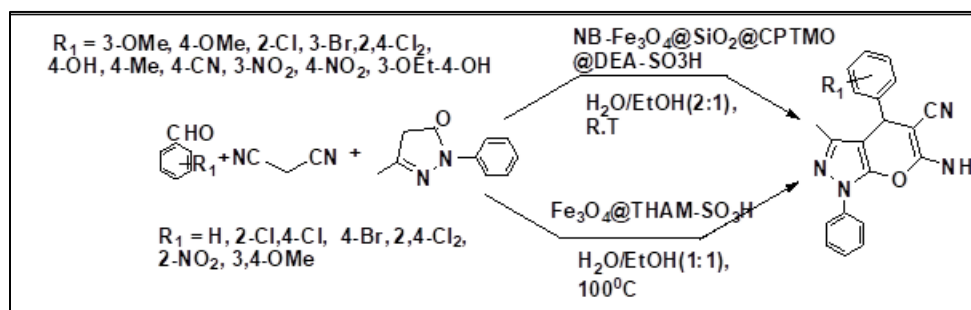
Scheme 20: Pyranopyrazole Derivatives in Presence of $\text{Na}_2\text{CaP}_2\text{O}_7$ catalyst

The thiophene-based pyranopyrazole derivatives were prepared by taking thiophene-2-carbaldehyde and refluxing it with pyrazol-3-one and malononitrile in ethanol in the presence of a nano-ZnO catalyst (Amer *et al.*, 2021) (Scheme 21).



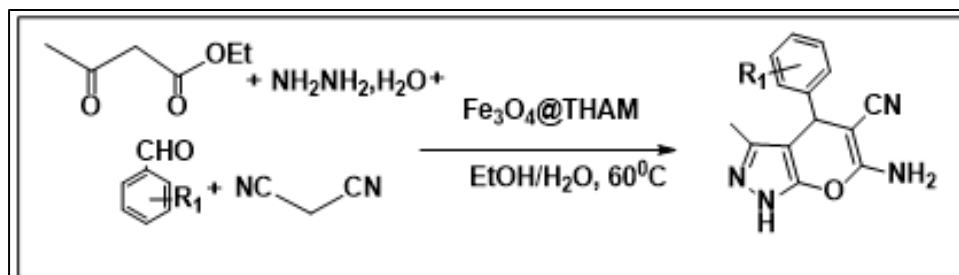
Scheme 21: Pyrano[pyrazole Derivatives Synthesis in Presence of Nano-ZnO catalyst

The nanomagnetic catalysts, e.g., $\text{NB-Fe}_3\text{O}_4@\text{SiO}_2@\text{CPTMO}@\text{DEA-SO}_3\text{H}$ (Eftekhari-Far *et al.*, 2020) and tris(hydroxymethyl)aminomethane (THAM), were prepared and successfully used to synthesize the pyranopyrazole framework (Scheme 22).



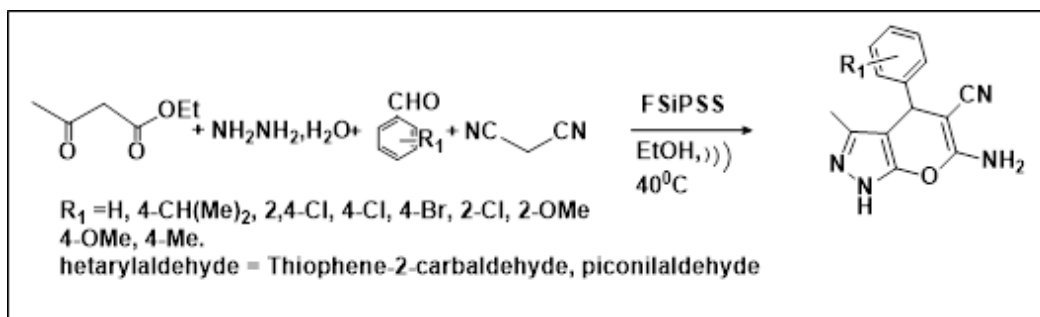
Scheme 22: Pyrano[2,3-c]pyrazole Synthesis in Presence of Nanomagnetic Catalyst

Recently, Fatemeh Mir and her team used another reusable nanocatalyst, $\text{Fe}_3\text{O}_4@\text{THAMPiperazine}$, in an ethanol/water medium to prepare the dihydropyranopyrazole (Mir *et al.*, 2023) (Scheme 23).



Scheme 23: Preparation of Pyrano[2,3-*C*]Pyrazole Derivatives with $\text{Fe}_3\text{O}_4\text{@THAM}$ Nanocatalyst in Aqueous Medium

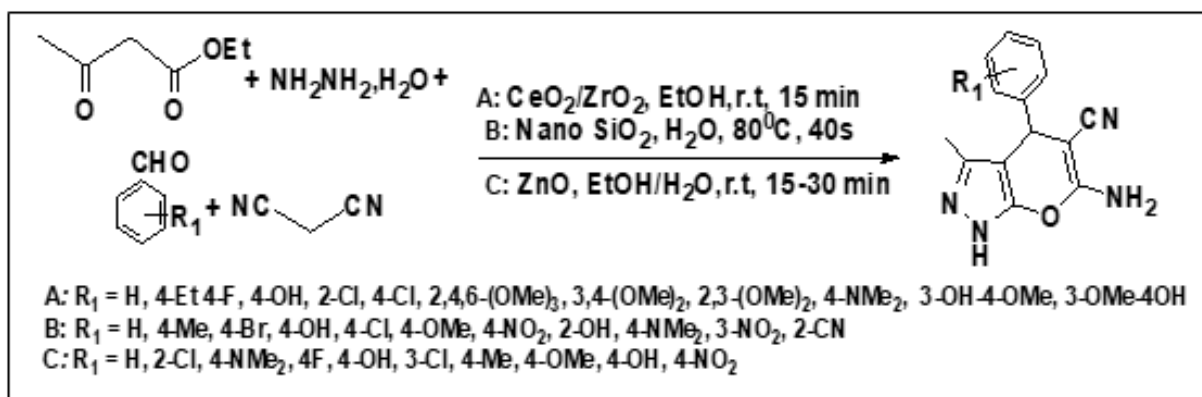
Another efficient and recoverable nanomagnetic catalyst $\text{Fe}_3\text{O}_4\text{@SiO}_2\text{@OSi}(\text{CH}_2)_3\text{-N(3-pyridoyl sulfonic acid)}$ semi carbazide (FSiPSS) was synthesized and characterized by Beiranvand *et al.* (2022) and used in MCR under ultrasonication and produced the desired molecules in very short reaction time. (Scheme 24).



Scheme 24: Pyranopyrazole Derivatives Preparation in Presence of Nanomagnetic Catalyst

The ceria-doped zirconia catalyst introduced by Maddila *et al.* (2017) showed excellent yields of pyranopyrazole derivatives at room temperature within 15 mins. Another nanocatalyst, nano- SiO_2 , was used successfully in water for only 40s by the sol-gel process from wheat-straw agricultural waste. This synthesis took place at room temperature using the ultrasonic technique and leads to good to excellent yields as reported by Patel and his team (Patel *et al.*, 2018).

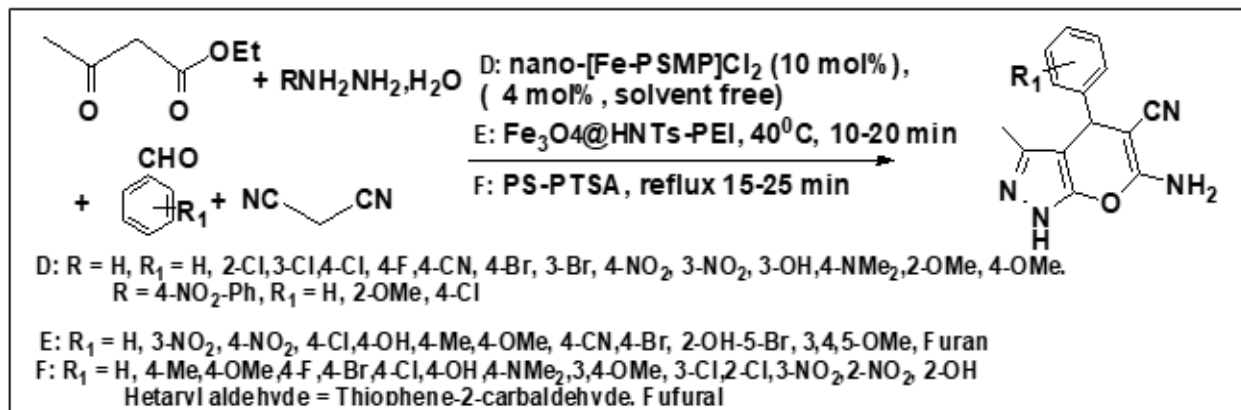
Another synthetic route of pyranopyrazole was designed by Chhattise *et al.* (2020) with four component reagents at room temperature within 15-30 mins, resulting in a high yield of product (Scheme 25).



Scheme 25: Pyrano[2,3-*c*]pyrazole Derivative Synthesis with Different Nanocatalysts

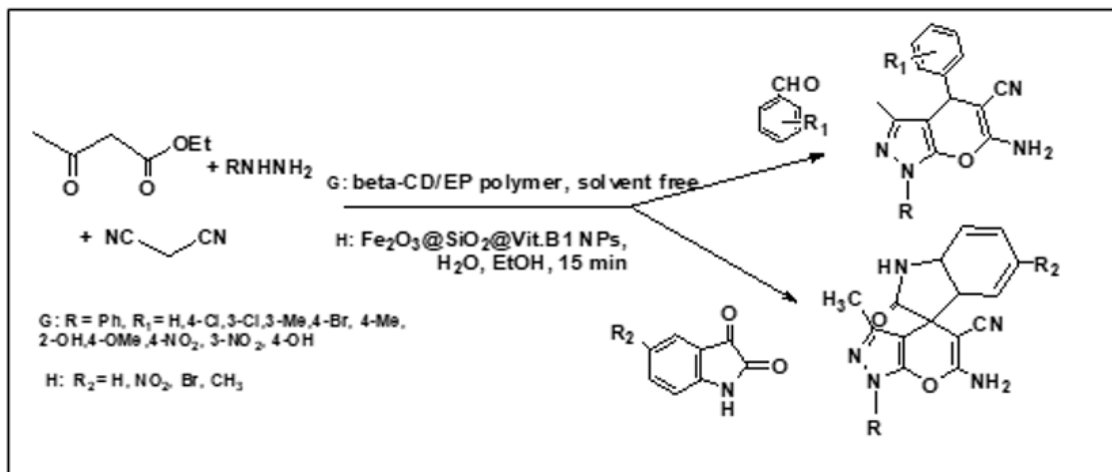
The nano-Schiff base nano-[Fe-PSMP]Cl₂ was applied by Moosavi-Zare *et al.* to prepare a pyranopyrazole derivative under solvent-free conditions at 1000°C (Moosavi-Zare *et al.*, 2018) (Scheme 26).

The heterogeneous nanocomposite Fe₃O₄@HNTs-PEI (Hajizade *et al.*, 2018) was used as a catalyst for the preparation of pyranopyrazole derivatives (Scheme 26). This catalyst can be reused without reducing yield of the product and catalytic activity. A polymer-supported catalyst (Gujaret *et al.*, 2014) was also reported for synthesizing pyranopyrazole derivatives.



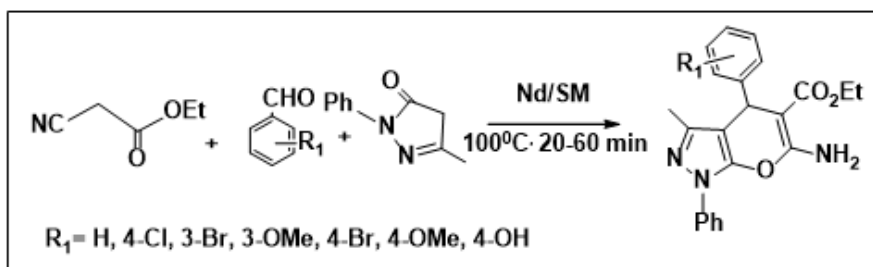
Scheme 26: Pyranopyrazole Derivatives Preparation in Presence of Heterogeneous Nanocomposite

The target molecules were reported to be synthesized in the presence of a heterogeneous catalyst, β -cyclodextrin-epichlorohydrin (β -CD/EP), a nano-sponge polymer, at 1000°C under solvent-free conditions (Jafari Nasab *et al.*, 2018) (Scheme 27). The pyranopyrazoles and spiropyranopyrazoles were also prepared by applying magnetic organo nano catalyst Vit B1 supported Fe₂O₃@SiO₂ NPs (Rahman *et al.*, 2018) in aqueous ethanol at 250°C. This NP increases the electrophilicity of carbonyl carbon and nitrile nitrogen by coordinating with them and facilitates the nucleophilic attack (Scheme 27).



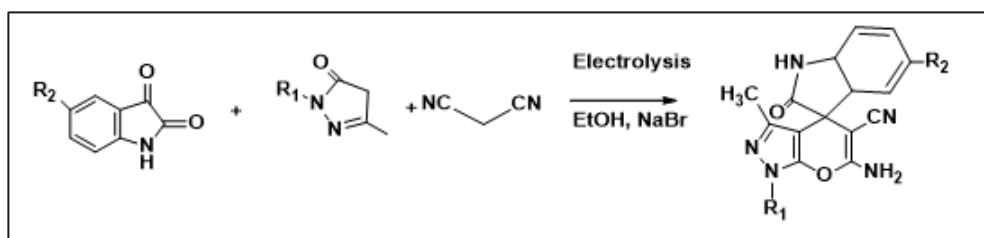
Scheme 27: Pyrano[2,3-c]pyrazole and Spiropyranopyrazoles Derivatives in Presence of Organonanocatalyst

Rather *et al.* (2018) applied a novel Nd-Salen Schiff base complex as a catalyst, prepared on mesoporous SiO₂ by adsorption of NdCl₃, to prepare pyranopyrazoles (Scheme 28).



Scheme 28: Pyrano[2,3-*c*]pyrazole Derivatives in Presence of Nd-Salen Schiff Base Complex

The novel electrolytic method was applied by Elinson *et al.* (2009), where he used sodium bromide as an electrolyte and indolone derivative as one of the components to synthesize spirocyclic indole-linked pyranopyrazole compounds (Scheme 29).



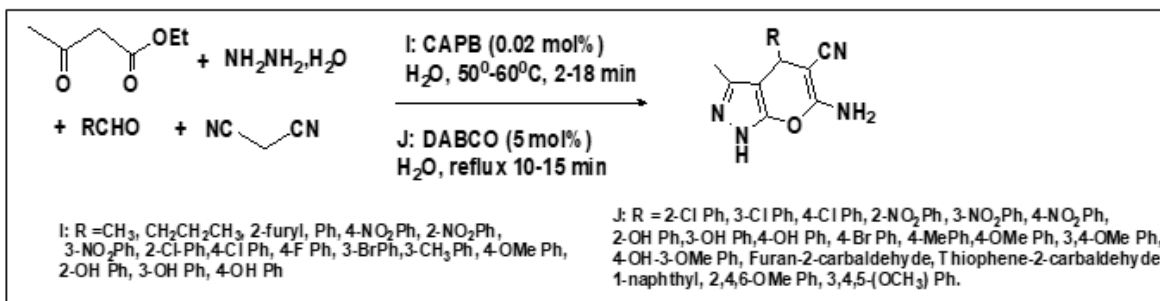
Scheme 29: Electrolytic Method of Pyrano[2,3-*c*]pyrazole Derivatives Synthesis

Using Organ Catalysts

Recently, organ catalysts are very popular for chemical reactions and have become an emerging area in green chemistry. Organocatalysis method is extensively used in organic and medicinal chemistry to design MCRs to avoid heavy transition metals.

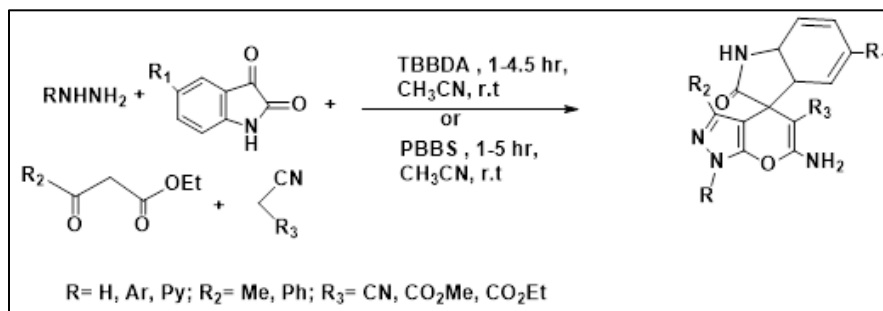
Organocatalysis offers metal free and environmentally friendly alternatives for facilitating MCRs. Catalysts such as DABCO, aspirin, maltose, sodium lactate, and zwitterionic surfactants have been successfully employed to achieve high yields with benign conditions (Tao *et al.*, 2016; Fiorani *et al.*, 2015).

Tamaddon and Alizadeh (Tamaddon *et al.*, 2014) designed a synthetic method of pyranopyrazoles using zwitterionic surfactant CPBA in water at 50^o-60^oC. The advantages of the use of CAPB as reported by them are (i) reaction condition became milder, (ii) catalyst may be reused, (iii) products were isolated easily (Scheme 30). DABCO is the most popular organo catalyst used in the MCRs to synthesize pyranopyrazoles. DABCO was applied by Waghmare *et al.* for one pot synthesis from four component reactions in water (Waghmare *et al.*, 2017) (Scheme 30).



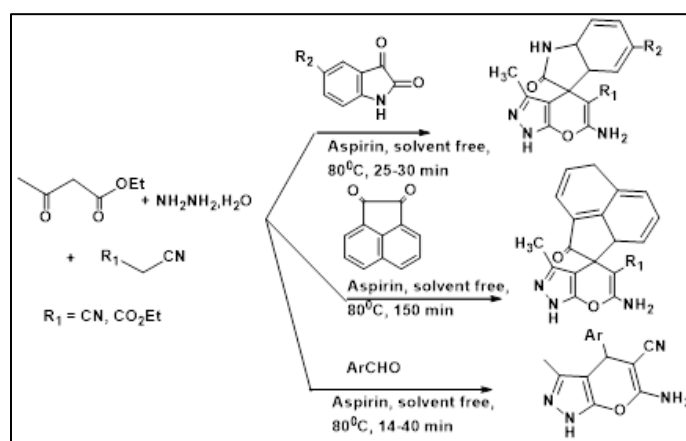
Scheme 30: Pyrano[2,3-*c*]pyrazole Synthesis Mediated by Organocatalysts

A new series of pyranopyrazoles from isatin derivatives was prepared successfully when TBBDA (N,N,N',N'-tetrabromobenzene-1,3-disulfonamide) and PBBS poly(N,N'-dibromo-N-ethyl-benzene-1,3-disulfonamide) were employed as organocatalysts (Ghorbani-Vaghei *et al.*, 2017). These catalysts were recovered and reused easily several times. The reuse of these catalysts did not change the yield of the desired product (Scheme 31).



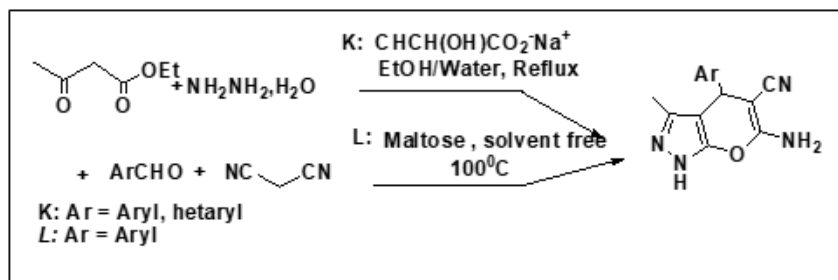
Scheme 31: Pyrano[2,3-*c*]pyrazoles from Isatins Mediated by Organocatalysts

Fatahpour *et al.* (2017) used aspirin as organo catalyst under solvent-free conditions at 80°C (Scheme 32).



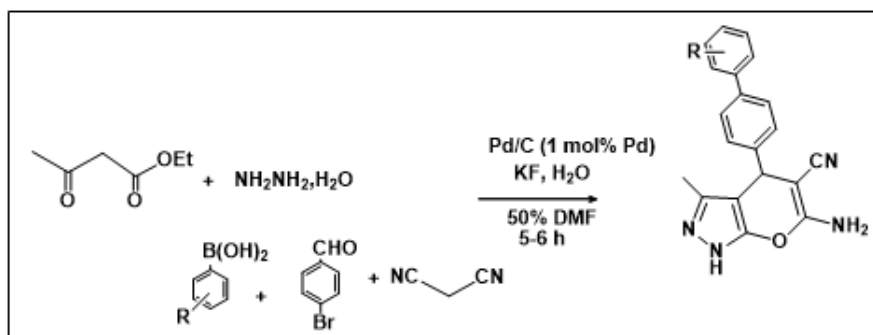
Scheme 32: Preparation of Pyrano[2,3-*c*]pyrazole Derivatives Employing Aspirin

Sonar *et al.* conducted the condensation reaction under reflux conditions in an aqueous ethanolic solution involving sodium lactate as an organocatalyst to synthesize pyranopyrazoles (Sonar *et al.*, 2019). Salahi and his team performed this reaction using maltose as a catalyst under solvent-free conditions (Kangani *et al.*, 2015) (Scheme 33).



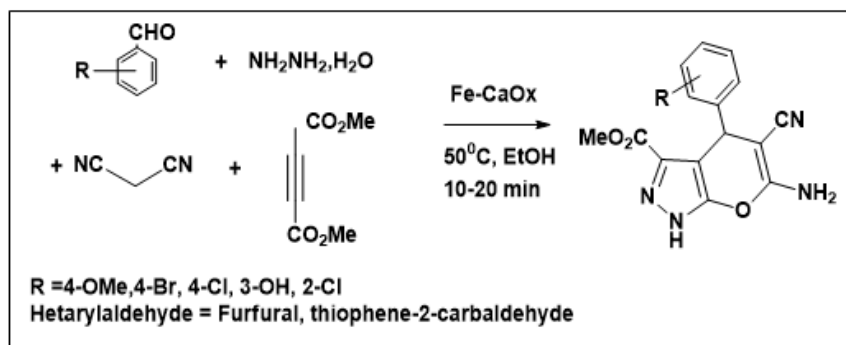
Scheme 33: Synthesis of Pyrano[2,3-*c*]pyrazole Derivatives Using Sodium Lactate, Maltose

A new series of biaryl-substituted pyranopyrazole derivatives was synthesized by using the inorganic catalyst $\text{KF} \cdot 2\text{H}_2\text{O}$ (Lu *et al.*, 2015). The biaryl system was prepared in situ by a Suzuki coupling reaction (Scheme 34).



Scheme 34: Using Inorganic Catalyst KF to Synthesise Pyranopyrazoles

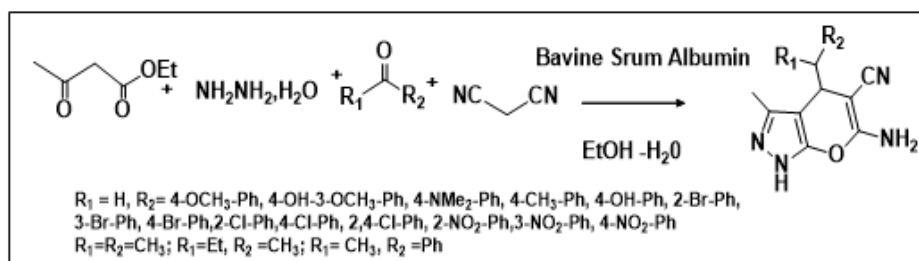
Gangu *et al.* (2017) performed a new synthetic approach by using dimethyl acetylene dicarboxylate as one of the components in presence of heterogeneous catalyst Fe-CaOx (Scheme 35).



Scheme 35: Pyranopyrazole Derivatives Synthesis Under Heterogeneous Catalyst Fe-CaOx

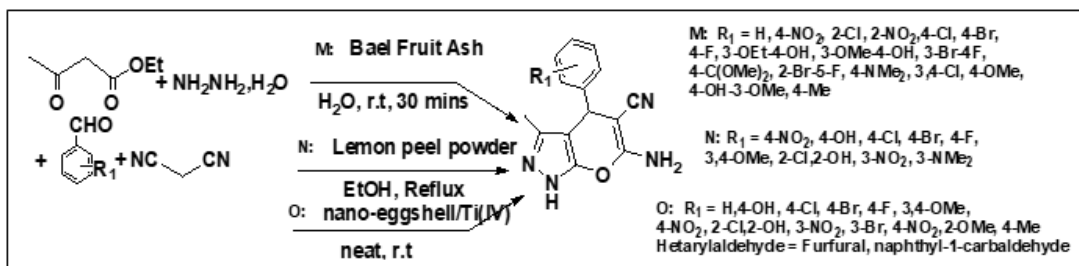
Using Biocatalyst

Biocatalysts are natural catalysts, such as enzymes, and they can catalyze chemical transformations. Biocatalysts have become increasingly popular because these are environmentally friendly and reduce the formation of by-products and waste in a chemical reaction. Biocatalysts also offer region and stereoselectivity, allowing for precise and complex transformations (Jumbam *et al.*, 2020). Xingtian *et al.* synthesized pyranopyrazole derivatives from various carbonyl compounds catalyzed by bovine serum albumin (BSA) at 45°C for 45 min in ethanol and reported good to excellent yield of the products (Huang *et al.*, 2016). BSA recovered easily and used another five times without affecting the yield (Scheme 36).



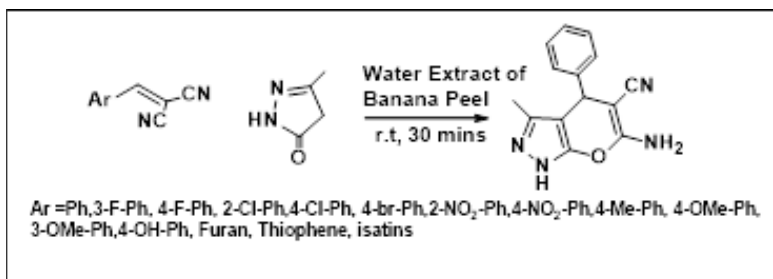
Scheme 36: Preparation of Pyrano[2,3-*c*]pyrazole Derivatives Employing BSA as a Catalyst

The successful application of natural catalysts, e.g., bael fruit ash (Shinde *et al.*, 2017), lemon peel powder (Ghodke *et al.*, 2020), and nano-eggshell/Ti(IV) (Tafti *et al.*, 2021), was reported for the synthesis of pyranopyrazole derivatives in mild conditions (Scheme 37).



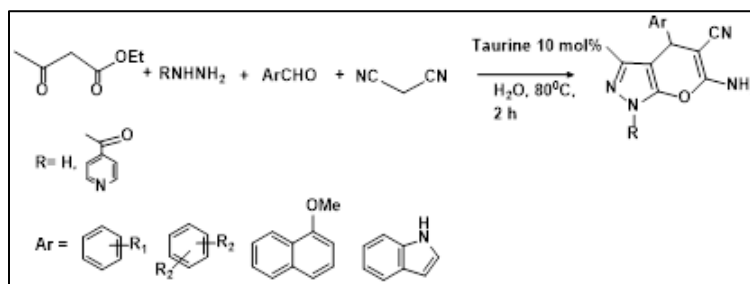
Scheme 37: Some Natural Catalysts to Synthesise Pyranopyrazole Derivatives

Another excellent natural catalyst, Water Extract of Banana Peels (WEB) was reported in the preparation of pyranopyrazoles by Chowhan and his group (Dwivedi *et al.*, 2020) (Scheme 38).



Scheme 38: Water Extract of Banana Peels used as natural catalyst

The use of taurine as a catalyst in the production of densely substituted Pyrano[2,3-c]pyrazoles has been described recently (Mali *et al.*, 2021) (Scheme 39).

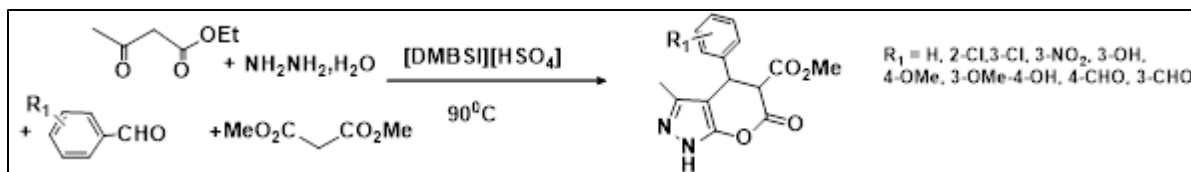


Scheme 39: Synthesis of Pyrano[2,3-C]Pyrazole Derivatives in Presence of Taurine

The biocatalysts, being biodegradable with minimal waste, have excellent selectivity. The challenges include issues related to enzyme stability and the difficulty of reusing enzymes compared to nanocatalysts.

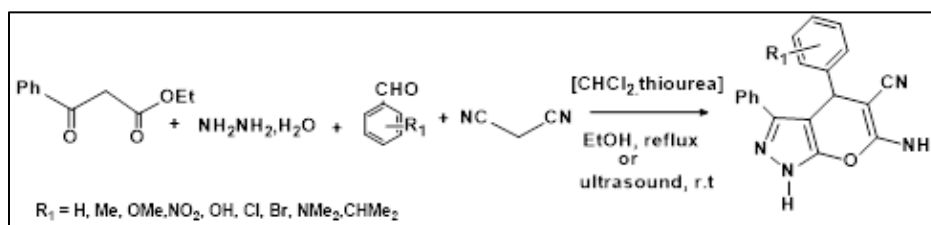
Using Ionic Liquids

In modern research, ionic liquids (ILs) are being successfully utilized as environmentally friendly catalysts and solvents in chemical reactions. The SO₃H-functionalized ionic liquid ([DMBSI]HSO₄) was reported to be employed in the synthesis of pyranopyrazoles under different green technical conditions (Farokhian *et al.*, 2018) (Scheme 40) (Zakeri *et al.*, 2017) (Scheme 42).

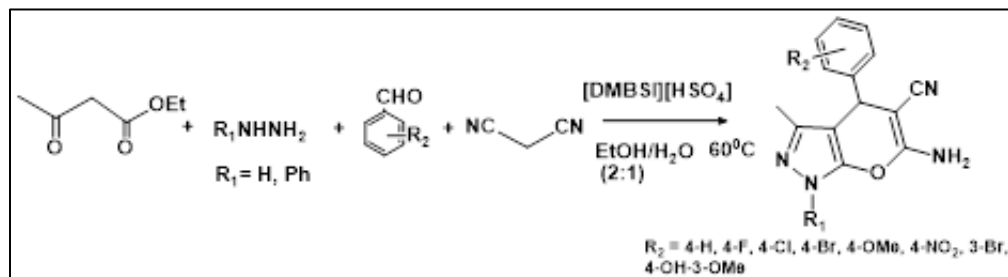


Scheme 40: Pyranopyrazole Derivative Synthesis Applying Ionic Liquid ($[\text{DMBSI}][\text{HSO}_4]$)

Alikarami and his team described a synthetic route of a new series of substituted pyranopyrazoles using choline chloride-based thiourea ($\text{ChCl}_2\cdot\text{Thiourea}$) (Dehbalaei *et al.*, 2017). The synthesis was carried out at room temperature under ultrasound, and the catalyst was recovered easily from the reaction mixture and reused successfully for the next reaction (Scheme 41).

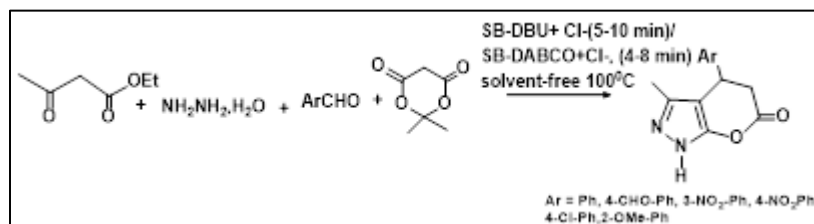


Scheme 41: Preparation of Pyranopyrazole Derivatives Mediated by Choline Chloride-Based Thiourea



Scheme 42: Pyranopyrazole Derivative Synthesis Catalyzed by $[\text{DMDBSI}].2\text{HSO}_4$

The silica-supported ionic liquids have become very popular ILs recently (Mamaghani *et al.*, 2021), and a number of synthetic strategies were developed by applying these catalysts, resulting in a high yield of the products (Rigi *et al.*, 2017). (Scheme 43).



$\text{SB-DBU}^+\text{Cl}^-$ = silica bonded diazabicycloundec-7-enium chloride;

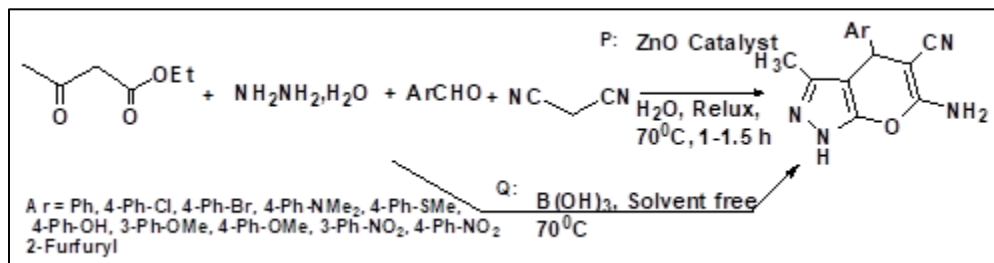
$\text{SB-DABCO}^+\text{Cl}^-$ = silica bonded *n*-propyl-4-aza-1-azoniabicyclo [2.2.2] octane chloride

Scheme 43: Synthesis of Pyrano [2,3-*C*] Pyrazole Derivatives Using Silica-Supported Ionic Liquids

Ionic liquids play dual role as solvent and catalyst. But high cost, toxicity is considered as the limitations of using these.

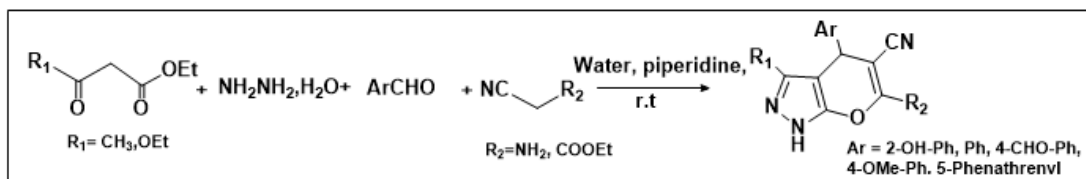
Using water as green solvent and solvent free condition

Recently, water has been widely used as a green solvent since it is inexpensive, environmentally friendly, non-toxic and cost-effective. The S. U. Tekale group reported new pyranopyrazole derivatives in water in the presence of a ZnO catalyst (Tekale *et al.*, 2013). A new approach to using boric acid solution in solvent-free conditions was applied as a green acid catalyst (Scheme 44).



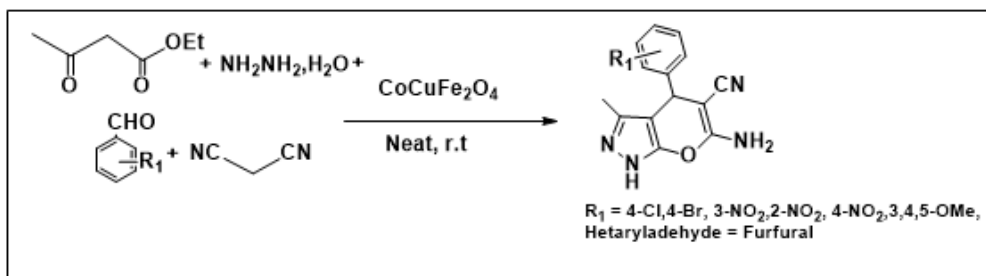
Scheme 44: Synthesis of Pyranopyrazole Derivatives Using ZnO and Boric Acid as Acid Catalysts

A base-catalyzed one-pot preparation of pyrano[2,3-c]pyrazole derivatives in a water-piperidine system has recently become very effective owing to its simplicity (Scheme 45).



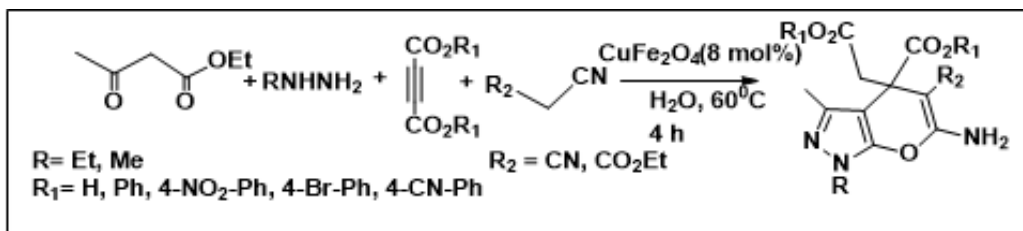
Scheme 45: Pyranopyrazole Derivative Synthesis in Water

Another solvent-free synthetic route was described by Dadaei where amagnetic nanocrystals $\text{CoCuFe}_2\text{O}_4$ were applied (Dadaei *et al.*, 2022) (Scheme 46).



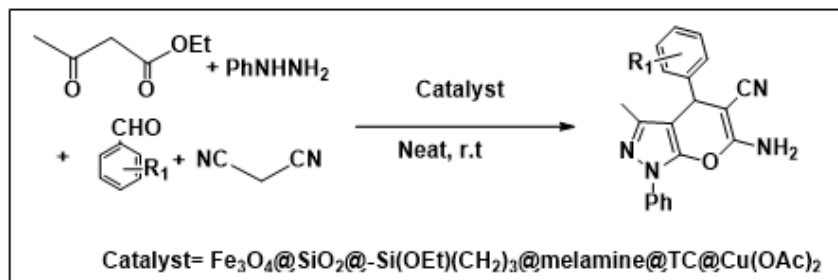
Scheme 46: Pyranopyrazole Derivatives Using $\text{CoCuFe}_2\text{O}_4$

Pradhan *et al.* synthesized pyrano[2,3-c]pyrazoles in the presence of a CuFe_2O_4 nanocatalyst in aqueous medium (Pradhan *et al.*, 2014) (Scheme 47).



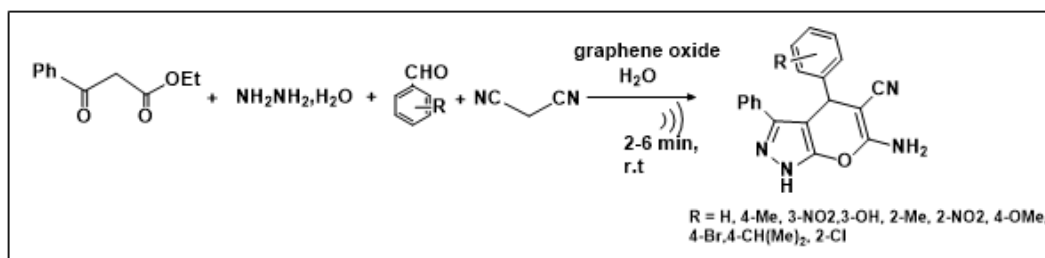
Scheme 47: Pyranopyrazole Synthesis in the Presence of Nanocatalysts in an Aqueous Medium

Nanomagnetic catalyst $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-Si}(\text{OEt})(\text{CH}_2)_3\text{@melamine@TC@Cu}(\text{OAc})_2$ was used to produce pyranopyrazole in high yield under solvent-free conditions. (Soleimani *et al.*, 2024) (Scheme 48).



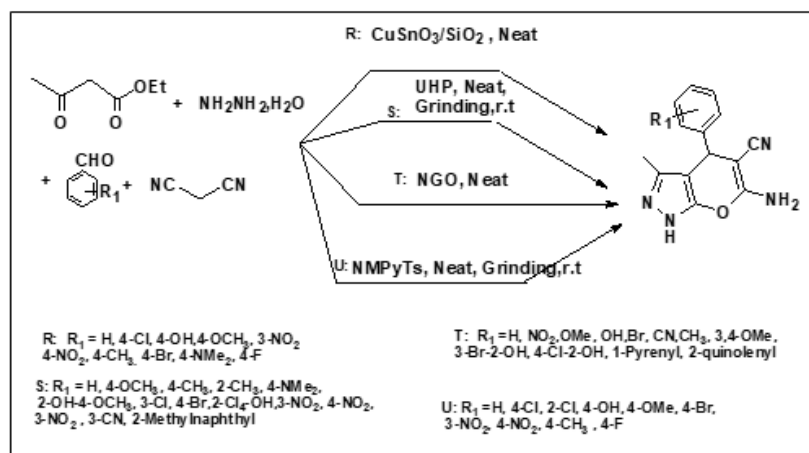
Scheme 48: Pyranopyrazole Derivatives Preparation Using Nanomagnetic Catalyst

Foroughifar's group synthesized, under ultrasound assistance, the target molecules catalyzed by 10 mol% graphene oxide with vigorous stirring. The reaction was performed at room temperature in an aqueous medium for 2–6 min with excellent yield (Dehbalaei *et al.*, 2018) (Scheme 49).



Scheme 49: Pyrano[2,3-*c*]pyrazole Derivatives on Graphene Oxide

The pyranopyrazole derivatives were synthesized in solvent-free conditions by applying the $\text{CuSnO}_3\text{:SiO}_2$ catalyst (Sangle *et al.*, 2022). Verma *et al.* (2022) designed a solvent-free, eco-friendly synthesis of pyranopyrazole derivatives mediated by urea hydrogen peroxide. The solid-base heterogeneous catalyst nitrogen-doped graphene oxide (NGO) was utilized by S. Ganesan and his team (Saravana *et al.*, 2020), whereas Sapkal *et al.* (2020) introduced ionic liquid (NMPyTs) as a catalyst to prepare pyranopyrazole derivatives by grinding technique at room temperature (Scheme 50).



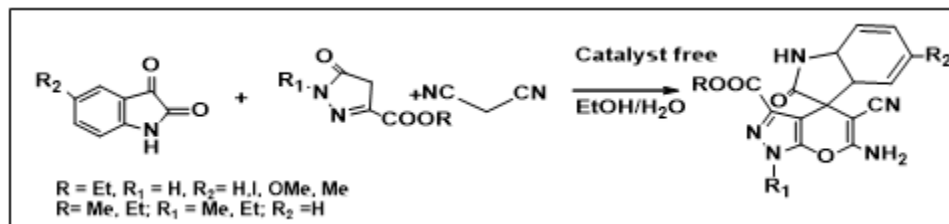
Scheme 50: Pyrano[pyrazole] Derivative Synthesis Without Solvent

Pyrano[pyrazole] derivative synthesis without solvent Most of the reactions, except reactions with hydrophobic substrates, have taken place in water with excellent atom economy. The advantages of using water as a green solvent are that (i) it is non-toxic and (ii) inexpensive.

Using Catalyst Free Condition

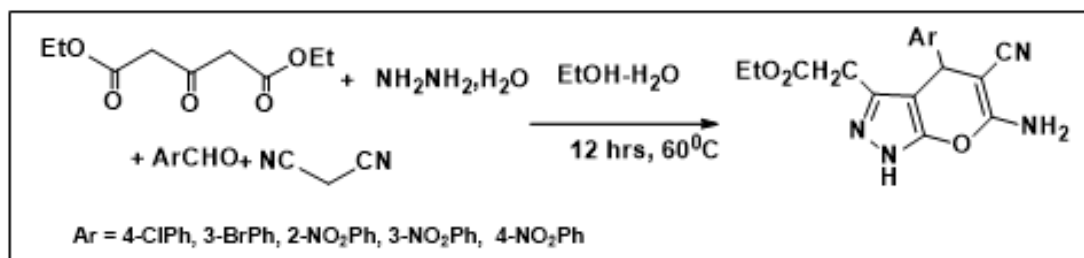
Several catalyst-free protocols—including ball milling, magnetized water, and thermal MCRs—have been introduced as the ultimate expression of green chemistry, eliminating the need for added reagents while achieving high efficiency.

Pore *et al.* designed a green catalyst-free synthesis for pyranopyrazole using isatin and DMAD (Pore *et al.*, 2013) (Scheme 51).



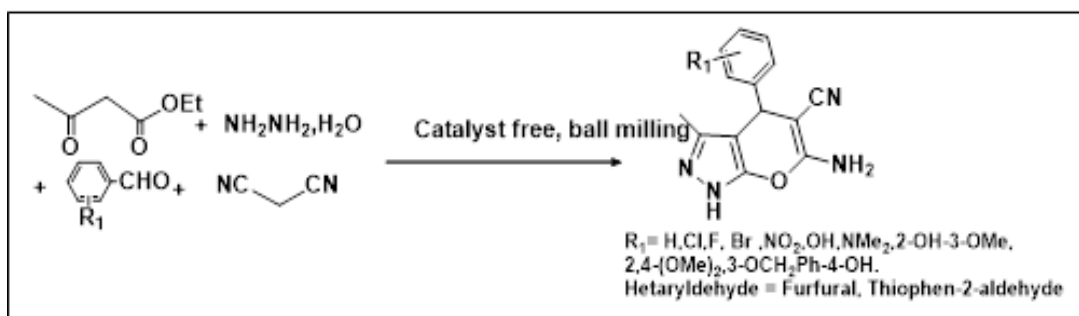
Scheme 51: Pyranopyrazole Derivatives from Isatins without Using Any Catalyst

A region and stereoselective synthesis were done taking dialkyl 3-oxopentanedioate in water/ethanol without catalyst. It took 12 hours to get to the target molecules (Kooreshari *et al.*, 2014) (Scheme 52).



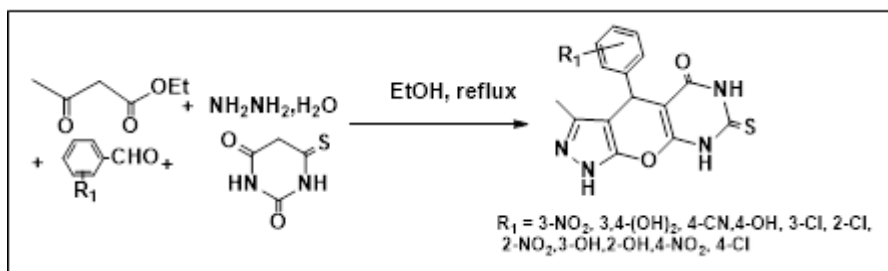
Scheme 52: Catalyst Free Synthesis of Pyranopyrazole Derivatives Using Aqueous Ethanol

Ball milling technique (Dekamin *et al.*, 2016) was used to synthesize pyranopyrazole (Scheme 53).



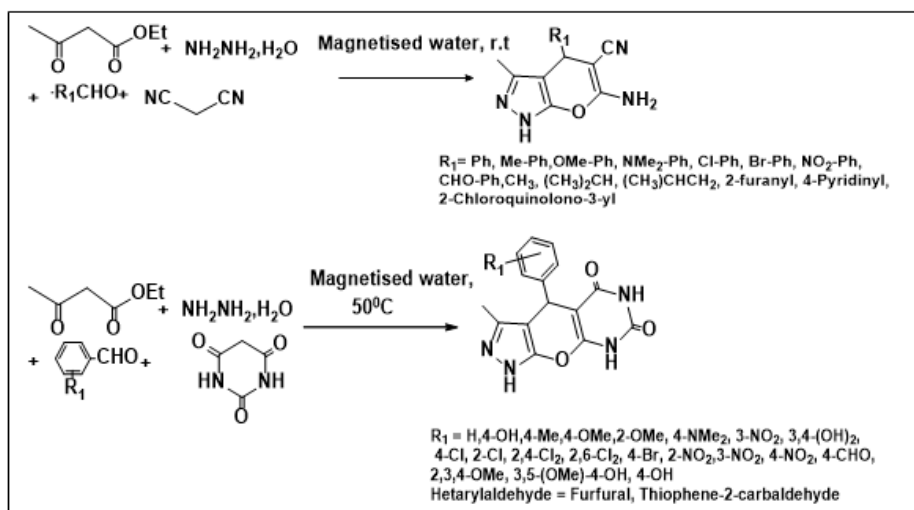
Scheme 53: Catalyst Free Preparation of Pyranopyrazole Derivatives with Ball Milling Technique

A catalyst-free four component synthesis of pyrazolo thiopyrimidines was reported (Patil *et al.*, 2017) and the compounds were found to exhibit antituberculosis activity (Scheme 54).



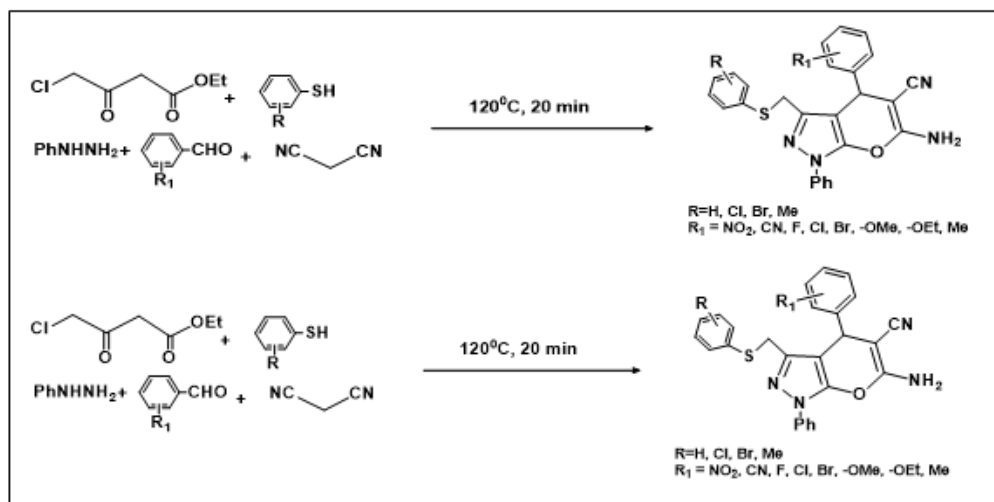
Scheme 54: Catalyst Free Synthesis of Pyrano[Pyrazoles from Thiopyrimidine

The synthesis of the new series of pyranopyrazole and pyranopyrimidine was also reported using magnetized water at room temperature (Bakherad *et al.*, 2017) (scheme 55).



Scheme 55: Synthesis of Pyrano[2,3-C]Pyrazole and Pyrano[2,3-C]Pyrazolo[2,3-D]Pyrimidine Using Magnetized Water without Using a Catalyst

Ramesh *et al.* (2016) described a solvent-free as well as catalyst-free synthetic route for the synthesis of thioether-linked pyranopyrazoles (Scheme 56).



Scheme 56: Catalyst-free Preparation of pyrano[2,3-c]pyrazole Derivatives

Catalyst free reactions are free from the contamination of catalysts and often takes longer time.

Table 1: Critical Comparison of Green Multicomponent Strategies for the Synthesis of Pyrano[2,3-*c*]pyrazoles

Green Technique	Conditions	Advantages	Major limitations	Sustainability Assessment
Ultrasound-assisted MCRs	Aqueous /aqueous–alcoholic media, r.t.–60 °C	- Short reaction times; - enhanced mass transfer; - often catalyst-free or mild catalysts	- Limited scalability; - Equipment dependency; - reduced efficiency for poorly soluble substrates	High
Microwave-assisted MCRs	Solvent-free or green solvents; short irradiation time	- Dramatic reduction in reaction time; - high yields; - energy efficiency	- Scale-up challenges; - uneven heating in some systems	High–Moderate
Nanocatalyst-based MCRs	Water or ethanol; r.t.–reflux	- High catalytic activity; - recyclability; - mild conditions	- Catalyst synthesis may be multistep; - long-term environmental impact uncertain	Moderate
Organocatalyst-based MCRs	Water, ethanol, or solvent-free; mild temperatures	- Metal-free; - inexpensive catalysts; - operational simplicity	- Catalyst loading sometimes high; - limited asymmetric versions	High
Biocatalyst-based MCRs	Aqueous or aqueous–alcoholic media; mild temperatures	- Excellent selectivity; - biodegradable catalysts; - minimal waste	- Narrow substrate scope; - enzyme stability - reuse issues	Very High
Ionic liquid-mediated MCRs	Solvent-free or aqueous systems; moderate heating	- Dual role as solvent and catalyst; - high efficiency; - recyclability	- Toxicity concerns; - high cost; - challenging purification	Moderate–Low
Water as green solvent	r.t.–reflux; simple bases or acids	- Non-toxic; - inexpensive; - excellent atom economy	Poor solubility of hydrophobic substrates	Very High
Solvent-free protocols	Thermal, MW, grinding	- Minimal waste; - high atom economy; - fast reactions	- Mixing issues; - temperature control limitations	High
Catalyst-free approaches	Water, ethanol, ball-milling, magnetized water	- Ultimate green strategy; - no catalyst contamination	- Often longer reaction times; - limited substrate scope	Very High

Scope and Outlook

From a comparative perspective, the recent study reveals that the multicomponent reactions offer distinct advantages in terms of green chemistry principles. Nevertheless, the sustainability of certain methodologies remains limited by some factors such as catalyst synthesis complexity, scalability constraints and also long-term environmental impact of some nanomaterials and ionic liquids.

Looking ahead, the recent studies will help the researchers to prioritize the development of genuinely benign and scalable catalytic systems derived from renewable and biodegradable resources along with mechanistic understanding. Improving catalyst durability and recyclability as well as expanding

asymmetric and enantioselective multicomponent protocols may lead the future research on the synthesis of these heterocycles to promising directions.

Conclusion

This review critically summarizes the recent trends in green multicomponent approaches to biologically active pyrano[2,3-c]pyrazole derivatives. The integration of alternative energy sources like MW and ultrasound together with nanocatalysts, organocatalysts, biocatalysts and ionic liquids has increased the efficacy of the reaction.

Instead of high yield, shortened reaction times, and mild reaction conditions, challenges remain with respect to nanocatalyst preparation as well as the use of ionic liquids. So, researchers should focus strongly on developing universally benign catalysts in the future to improve the industrial applicability.

Overall, the study discussed herein underscores the potential of green multicomponent reactions as powerful tools for the sustainable synthesis of pyrano[2,3-c]pyrazole derivatives.

The author looks forward to this review encouraging the researchers to embrace these eco-friendly methods, paving the way for cleaner and more sustainable practices.

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Conflict of Interest

The author declares no conflict of interest.

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Task Specific Ionic Liquids (TSILs) used as Reaction Media and Catalyst

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Abstract

Task-specific ionic liquids (TSILs) have gained more attentiveness in recent years due to its specific physical as well as chemical properties. They are employed in many specific reactions of synthetic organic chemistry due to their distinctive nature. They also serve as a good catalyst in many reactions due to their excellent catalytic properties. The ionic liquids consist of unique properties, including very low vapor pressure, reusability, more thermal stability and distinct structural characteristics. These properties make them more multifaceted and green solvents. The applications of ionic liquids segregate them from the other conventional solvents used in synthesis. The ionic properties of ionic liquid furnish a special solvent environment for several reactions of synthesis. Ionic liquids also appear as a remarkable material due to its special characteristics such as low flammability, less volatility and ability to dissolve in a large number of organic and inorganic substances. This review is an attempt to propose some developments in the area of TSIL in regard to catalysis and reaction media for the synthesis of organic compounds.

Keywords: *Catalysis; Ionic Liquids; Organic Synthesis; Task Specific Liquids*

Introduction

In the last few decades, ionic liquids (ILs) received more attention in catalysis and reaction media for synthetic reactions. They have wide application in pharmaceuticals, biomedicines, lubrication technology, electrochemistry and industries that carry out the synthesis of a large number of compounds (Zhuo *et al.*, 2014; Greer *et al.*, 2020). The use of ILs is a greener process in the manufacturing of a large number of compounds due to their noticeable features, including low vapor pressure, low in flammability, reusability, low melting point, low boiling point and thermal stability. They are treated as green solvents due to acting with less harmful emissions and low effluent discharge in organic synthesis processes. However, many chemical industry processes have been or still are highly polluting the environment. Moreover, their ability to dissolve in a range of organic and inorganic compounds makes them a significant material. Although the term "ionic liquids" has been popular in recent years, the history of ionic liquids most likely was initiated by Paul Walden in 1914. He reported the synthesis and various properties of the ionic liquid [EtNH₃][NO₃]. Later on, Gorden (1969) critically studied the ionic liquids. Wilkes and coworkers (1982) reported many significant reactions and referred to them as a green solvent. The first effective use of an ionic liquid, dialkylimidazolium chloroaluminate, as a catalyst in Friedel-Crafts acylation reactions was reported by Boon *et al.* (1986). The term "Task Specific Ionic Liquids" (TSILs) was introduced to describe the characteristics of ionic liquids by Davis (2004). He significantly reviewed the various syntheses of TSILs and their applications in catalytic reactions and synthetic organic chemistry. TSILs are the type of ionic liquids structured and designed to perform specific actions or tasks in synthetic reactions. In recent years, TSILs, a class of ionic liquids, have received more attention in synthetic reactions because of their specific characteristics. Winkel *et al.* (2008) reported the asymmetric synthesis involving the utilization of task-specific chiral ionic liquids. In our previous review (Chaturvedi *et al.*, 2014; Kumar, 2025), the catalytic properties and application of

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ionic liquid. This review is an attempt to explain some developments of TSIL and their use in reaction media and catalysis.

TSILs as Reaction Media and Catalyst

Task-specific ionic liquids (TSILs) are also referred to as functionalized ionic liquids. They are specific salts having functional groups for specialized tasks/reactions that act as versatile solvents as well as catalysts to perform many reactions. They have focused applications in the specific reactions. TSILs furnish properties like high stability, great solubility in most reactants, and superior selectivity for reactions. They are treated as green solvents because they involve less environmentally harmful reactions than the conventional organic synthesis. Bellina *et al.* (2009) had prepared ionic liquids shown in Fig. 1 that carried glycerylimidazolium. They extensively studied their physicochemical features such as thermal stability, hydrogen bonding, and conductivity. It has been demonstrated that ionic liquid has application in Heck reaction and the palladium-catalyzed coupling reactions.

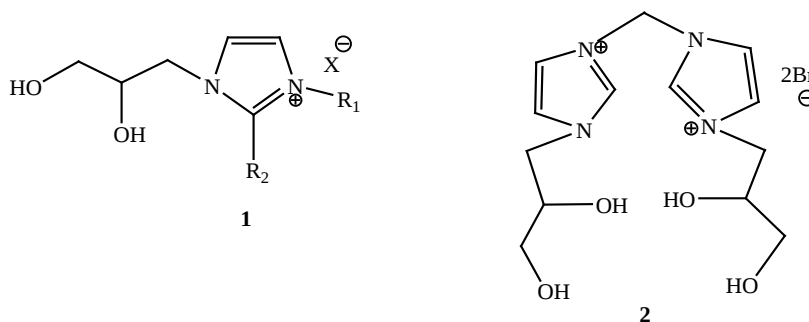
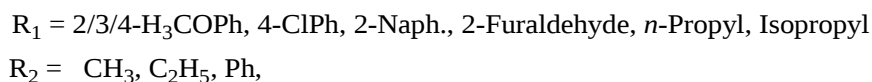
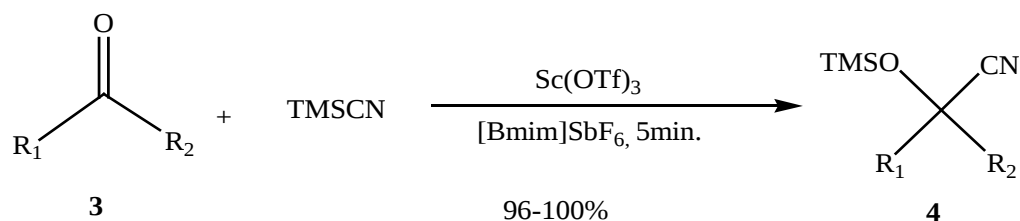


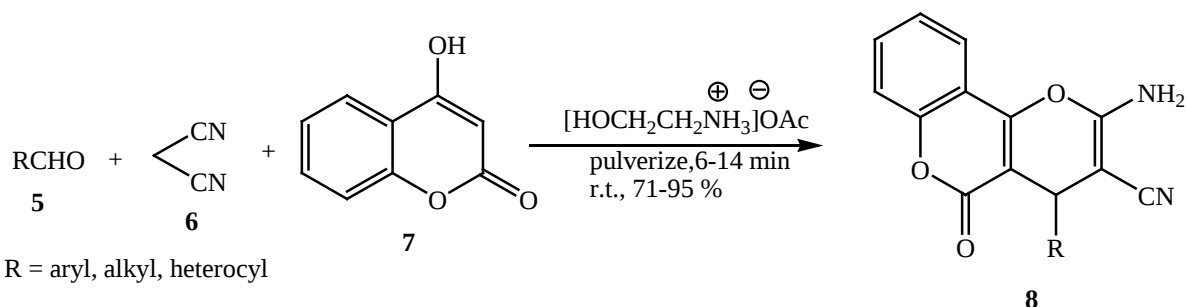
Figure 1: Glycerylimidazolium

Park *et al.* (2009) applied scandium triflate as a catalyst in the cytosilylation reaction of carbonyl compound **3** to yield compound **4**. When the ionic liquid 1-butyl-3-methylimidazolium hexafluoride [Bmim]SbF₆ was used as a catalyst in this reaction, it was found that the product formed easily (**Scheme 1**). It was revealed that ionic liquid increased the catalytic activity of the reaction effectively. It was suggested this was due to the increase of Lewis acidity of IL and anion exchange between scandium triflate and ionic liquid.

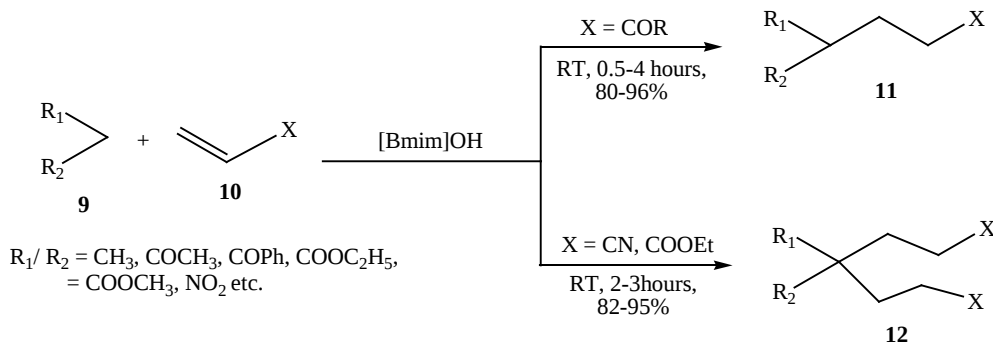


Scheme 1

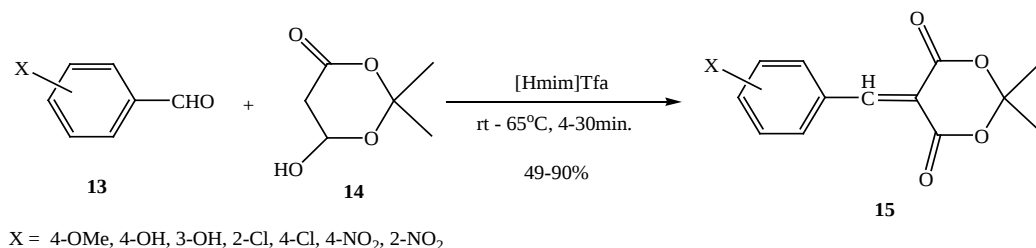
Shaterian and Honarmand (2011) reported that task-specific ionic liquid [HOCH₂CH₂NH₃]⁺[OAc]⁻ is a reusable and effective catalyst for one-pot synthesis of dihydropyrano[3,2-c]chromene derivatives **8**. This synthesized product was produced with the condensation of 4-hydroxycoumarin **7**, various aldehydes **5**, and malononitrile **6**. This reaction occurs in a combinatorial fashion. The reaction at room temperature gave excellent yield (**Scheme 2**).

**Scheme 2**

The distinct selectivity of task-specific ionic liquid, 1-butyl-3-methylimidazolium hydroxide [Bmim]OH, was reported by Ranu and Banerjee (2005) in a well-known Michael addition reaction. They conducted the reaction of active methylene compounds **9** with conjugated ketones/esters/nitriles **10**. They suggested that vinyl ketones and chalcones yield mono-addition product **11**, while the unsaturated esters and nitriles yield *bis*-addition product **12**. These reactions were carried out at room temperature and give excellent quantitative yields (**Scheme 3**).

**Scheme 3**

Darvatkar *et al.* (2006) conducted the Knoevenagel condensation consisting of Meldrum's acid **14** with a range of aldehydes **13**. They used the different kinds of ionic liquids, such as 1-butyl-3-methylimidazolium hexafluoroborate [Bmim]PF₆, 1-methylimidazolium trifluoroacetate [Hmim]Tfa, 1-butyl-3-methylimidazolium tetrafluoroborate [Bmim]BF₄, and 1-hexyl-3-methylimidazolium tetrafluoroborate [Hmim]BF₄ in this reaction. The corresponding derivatives of ylidene **15** were produced (**Scheme 4**). They observed that at room temperature, use of ionic liquid [Hmim]Tfa as a catalyst yields product effectively. The utilization of ionic liquids, the product produced easily, was reused.

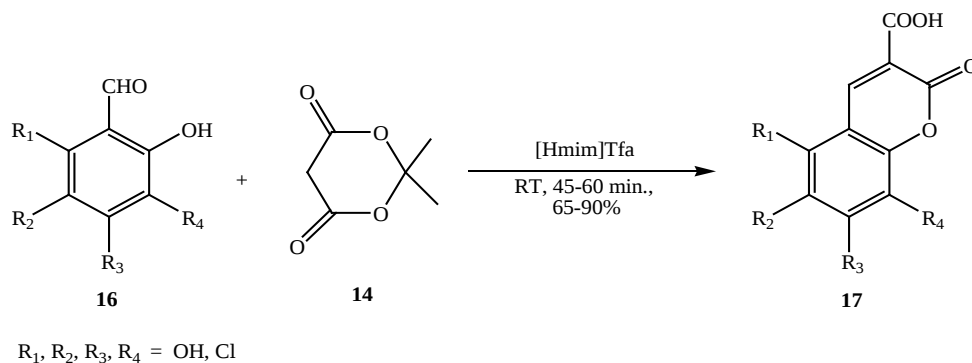
**Scheme 4**

Darvatkar and their associates (2008) had again used TSIL [Hmim]Tfa in the reaction of *o*-hydroxy aryl aldehydes **16** and Meldrum's acid **14**. The derivatives of coumarin-3-carboxylic acid **17** were produced

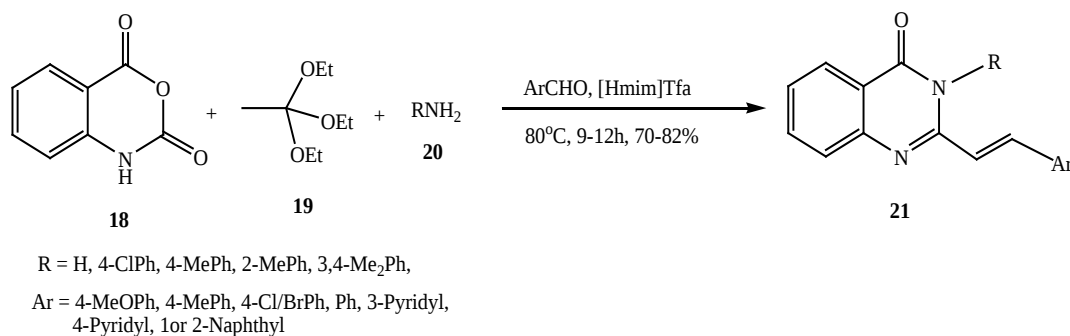
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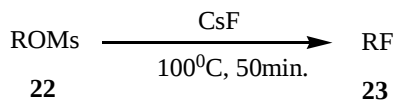
effectively. They conducted this reaction at room temperature and produced the product in good yield (**Scheme 5**). They concluded that the use of this ionic liquid acts as an effective catalyst.

**Scheme 5**

Dabiri *et al.* (2008) used [Hmim]Tfa as a TSIL in a reaction of isatoic anhydride **18**, triethyl orthoformate **19**, and amine derivatives **20**. This reaction produced 2-styryl-4(3H)-quinazolines (**21**) in the presence of different substituted aromatic aldehydes. The reaction was carried out at 80°C, and the product yield was satisfactory (**Scheme 6**).

**Scheme 6**

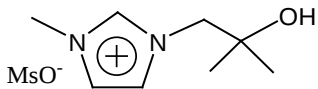
Shinde *et al.* (2008) reported the effect of ionic liquid and alcohol in nucleophilic substitution reactions. This reaction involved the conversion of mesylates **22** to the corresponding fluoro-substituted compound **23** in the presence of cesium fluoride at 100°C (**Scheme 7**). It was found that the combination of tertiary butanol and ionic liquid accelerated the reaction effectively, as shown in Table 1. The advantage of this reaction is very few side products. It was suggested that the weak hydrogen bond of HF reduces nucleophilicity and decreases the basic character of fluoride, which leads to fewer side products.



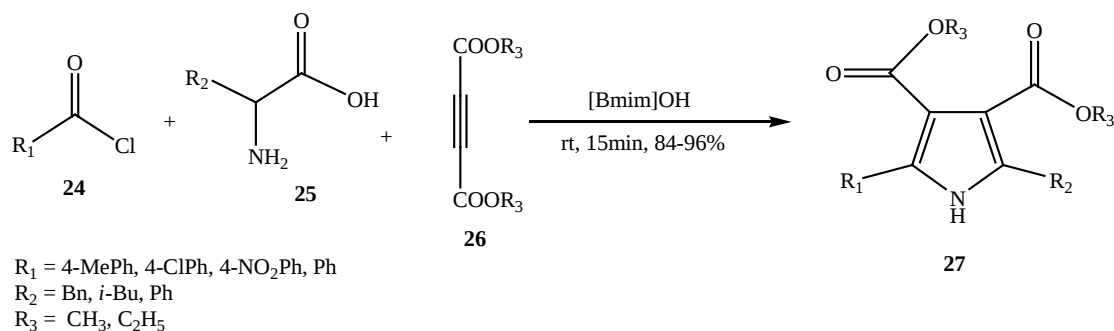
R = 2-Naphthyloxypropane, protected steroids and protected glycosides

Scheme 7

Table 1: Effect of Solvent and IL on Nucleophilic Fluorination

Solvent/Ionic liquid	Equivalent	Yields in %
Tertiary Butanol	0.5	22
[Bmim]OMs	0.5	30
Tertiary Butanol: [Bmim]OMs	0.5:0.5	37
	0.5	97

Yavari and Kowsari (2008) utilized 1-butyl-3-methylimidazolium hydroxide [Bmim]OH, an ionic liquid, as a catalyst for the condensation reaction of acid chlorides **24**, dialkyl acetylene dicarboxylates **26** and amino acids **25** in aqueous solution. The pyrrole derivatives were formed with sufficient yields. They studied this reaction with ten different types of ionic liquids, in which [Bmim]OH was most effective. At 80°C, pyrrole derivatives have good yield. It was reported that benzoyl chloride having an electron-withdrawing substituent reacts faster (**Scheme 8**).

**Scheme 8**

Conclusion

Ionic liquids are generally molten salts, which are composed entirely of ions, consisting mainly of organic or inorganic cations and anions. Ionic liquids are referred to as a green solvent due to their low vapor pressure, being environmentally less harmful, low volatility and recycling properties. They have characteristics to dissolve in most of the organic and organic compounds, making them fabulous for many reactions. They have noticeable catalytic properties in most of the synthesis. Task-specific ionic liquids are a type of ionic liquid that performs specific tasks for several synthetic reactions and has wide applications.

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Tibetan Gazelle (*Procapra picticaudata*) in Indian Trans-Himalayan region: Current Status, Threats, Conservation Strategies and Human Perspective

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Abstract

Many endemic species, including the Tibetan gazelle (*Procapra picticaudata*), inhabit the Himalayan region, one of the world's biodiversity hotspots. It is a high-altitude antelope endemic to the Tibetan Plateau. In India, it is mostly found in the Changthang region around Hanle in Ladakh. Its population has gone down drastically due to anthropogenic reasons and is on the verge of local extinction in India. The present study investigates the distribution, ecology, population, conservation status, threats, and conservation initiatives of Tibetan gazelle in Ladakh, India, along with assessing the perception of the local people regarding the gazelles. Field observations in the Changthang wildlife sanctuary near Hanle recorded multiple sightings, which confirm the restricted distribution and small group size. Hunting in earlier times, extensive grazing of livestock, harsh weather conditions, and a very small population size were identified as the major threats to the Indian population of Tibetan gazelle. Conservation initiatives like the establishment of protected areas, regulation of overgrazing and awareness programs have resulted in limited but encouraging results. A survey among the locals revealed that though the locals are familiar with the Tibetan gazelle and hold a positive attitude toward conservation of these animals, they are not well aware of the critical conservation status of the animal. The study emphasizes the necessity of integrated conservation strategies, monitoring, and long-term scientific research, as well as habitat management and dynamic community engagement to prevent the local extinction of this high-altitude endemic ungulate.

Keywords: Changthang; Conservation; Hanle; Local extinction; Tibetan Gazelle

Introduction

The Himalayas, spreading across 2400 km from Kashmir in the northwest to Arunachal Pradesh in the east, are one of the 36 biodiversity hotspots of the world (Wanjari *et al.*, 2026). Several unique and endemic species inhabit the western and Trans-Himalayan regions of India.

The Tibetan gazelle is a small, high-altitude antelope endemic to this region of India. The Tibetan gazelle, *Procapra picticaudata*, belongs to the family Bovidae, subfamily Antilopinae and tribe Antilopini. The Tibetan gazelle belongs to the genus *Procapra* which is a primitive lineage of antelopes quite different from the African gazelle (*Gazella*). The genus *Procapra* includes three closely related species of Asian gazelle: *Procapra picticaudata* (Tibetan gazelle), *Procapra gutturosa* (Mongolian gazelle) and *Procapra przewalskii* (Przewalski's gazelle), all of which are found in high-altitude cold arid environments. The Tibetan gazelle is endemic to the Tibetan Plateau. They are mainly found in the Qinghai, Tibet, Gansu, Xinjiang and Sichuan regions of China. A small population of Tibetan gazelles lives in Ladakh and North Sikkim, India (Jiang *et al.*, 2019).

Reports suggest that the number of Tibetan Gazelles in the wild and their habitat have decreased significantly since 1970 (Zhang & Jiang, 2006). Several factors contribute to this decline, with human

activities being the most significant. Habitat loss, grassland fragmentation, hunting and overgrazing have been reported to be the main threats to the survival of the gazelles. According to the International Union for Conservation of Nature and Natural Resources (IUCN) Red List, the Tibetan gazelle is listed as near threatened (NT) globally (IUCN SSC Antelope Specialist Group, 2016). The animal is protected in India and China under the Wildlife Protection Act (Anon, 2023).

Presently a small but major population of Tibetan gazelles in India is found in Ladakh, more specifically in the Hanle region in the southeastern part of the Changthang Plateau. Ladakh is a union territory in the northernmost part of the Republic of India. It is a high-altitude cold desert, experiencing extreme temperatures, very low rainfall and severe solar radiation (Singh *et al.*, 2011). Ladakh is marked by rugged barren mountains, vast plateaus and sparse vegetation, making it a unique ecosystem with harsh climatic conditions (Sahana *et al.*, 2023). Hanle is a remote village in the Union territory of Ladakh. Hanle is known for having the Indian Astronomical Observatory, which is one of the highest observatories in the world and also for the Changthang wildlife sanctuary, which is home to many rare and endemic flora and fauna, including the Tibetan gazelle.

Regardless of its ecological significance, the Tibetan gazelle is one of the least studied ungulates of the Indian Trans-Himalayas. The present study is an account of the Tibetan gazelle with respect to its distribution, habitat preference, population, conservation status, threats and conservation initiatives, focusing mainly on the Ladakh population. A semi-structured survey was also conducted to understand the perspective of the local people towards the Tibetan gazelle and its conservation.

Methodology

Study Site and Time

The study was conducted in Hanle Village and the southeastern part of the Changthang wildlife sanctuary located near Hanle Village. Hanle is a high-altitude remote village within the union territory of Ladakh with the coordinates 32°47'N 79°00'E. It is approximately 260 km from Leh, the summer capital of Ladakh. Situated at an altitude of 4500 m (~14760 ft) above sea level (Bhatnagar *et al.*, 2006a). Hanle is known for its clear sky, stargazing and wildlife. The study was conducted during September 2019.

Data Collection and Survey

Primary data was collected as described earlier by Jiang *et al.* (2019) from Hanle Village and by visiting different parts of the Changthang Wildlife Sanctuary located near Hanle. Photographs were taken by a mobile phone camera. For gathering information and understanding the perception of local peoples, a small survey was conducted using a semi-structured questionnaire with closed and open-ended questions among the locals of Hanley and nomads living nearby following Dash *et al.* (2025). A random sampling method (Browne-Nuñez & Jonker, 2008; Hasan & Csányi, 2023) was used to select the respondents. Secondary data were gathered from the very few published papers and documents that are available in the public domain.

Results and Discussion

Local Name and General Morphology of Tibetan Gazelle

The Tibetan gazelle (*Procapra picticaudata*) is locally known by its Tibetan name “Gua” or “Goa” (sometimes also written as “Gowa”) in the Hanle region. Local communities use the Tibetan and related Himalayan dialects to derive the word “Goa.” These animals form a natural part of the traditional ecological knowledge of the people of the Changthang region. Most of the locals recognize the animal as Gua and are unaware of the English name.

The Tibetan gazelles are small-sized antelopes who have compact bodies with relatively short limbs and weigh about 13–16 kg. (Schaller, 2000; IUCN SSC Antelope Specialist Group, 2016). They have a light

fawn to greyish brown coat color on the dorsal side and white on the ventral side. The males possess cylindrical, ringed, pointed, backward-curved horns (about 20-30 cm in length). The females are usually hornless. Both sexes have a very distinct heart-shaped white rump patch, which acts as a visual signal for group cohesion.

Habit and Habitat Preference

The Tibetan gazelles live in high-altitude environments between 3500 m and 5500 m above sea level in cold desert conditions with winter temperatures as low as -20°C and very low precipitation (Michel, 2024; Bhasin *et al.*, 2024). The Tibetan gazelle prefers to stay in open plains and gentle slopes with sparse vegetation of grasses and sedges (Schaller, 2000; IUCN SSC Antelope Specialist Group, 2016). They avoid dense vegetation, steep slopes and rugged terrain. They avoid areas with high human and livestock presence. Though they can coexist with other wild ungulates, they compete with domestic livestock (Qiao *et al.*, 2024; Yan *et al.*, 2024).

They feed on grasses, forbs and sedges and can survive with minimum water, deriving moisture from the plants and grasses they feed on. They generally live in small groups, though solitary males can also be seen. They follow a polygynous mating system, with females usually giving birth to one fawn at a time (Schaller, 2000).

They are among the very few ungulates adapted to live in extreme hypoxic conditions. They have increased lung capacity, resulting in efficient oxygen utilization and higher hemoglobin affinity for oxygen (IUCN SSC Antelope Specialist Group, 2016).

Distribution

The Tibetan gazelles are endemic to the Tibetan Plateau and majorly found in China (housing the primary population) and India (with a marginal population). However, some sources also mention the presence of a very small population in Nepal.

In India, they are primarily found in eastern Ladakh, especially the Hanle Valley of the Changthang region, and very rarely in North Sikkim, near Gurudongmar and Tso Lhamo (Qiao *et al.*, 2024).

Previously, they had a much wider distribution, ranging over 20000 sq. km in the early 20th century (Bhatnagar *et al.*, 2007), which reduced to about 1000 sq. km by the late 1980s (Bhatnagar *et al.*, 2006a; Fox *et al.*, 1991). Presently they are restricted to 100 to 150 sq km in the Kalak-Tartar plateau of the Hanle region.

Population Status

The Tibetan gazelle is one of the rarest large mammals in India. Though globally listed as near threatened (IUCN), it is critically endangered in India.

The last and probably the only documented survey of the gazelle, in 2000 by Bhatnagar *et al.* (2006a), revealed the occurrence of this species only in Hanle, Eastern Ladakh. It is on the verge of local extinction with only about 50 individuals in Ladakh (Namgail *et al.*, 2008). The two populations reported previously (Fox *et al.*, 1991) near Dungti and Tso Kar in the 1980s were found to be extinct during the survey done in 2000.

The other populations seem to be declining. The population at Kalak Tartar region of Hanle Valley was estimated to be 30 animals in 2000 (Bhatnagar *et al.*, 2006b), which was at least 68 individuals in 1997 and at least 36 seen in one group in 1998 (Bhatnagar *et al.*, 2006a).

According to the previous red list assessments of the IUCN, the Tibetan gazelle has been listed as least concerned since 2003. From the subsequent red list assessments in 2007, 2008, and 2016 (latest), the

animal has been assessed as near threatened globally. The current population trend is declining according to IUCN (IUCN SSC Antelope Specialist Group, 2016).

Tibetan gazelles are protected under Indian wildlife laws and are listed under Schedule I of Indian wildlife (Protection) Act 1972 (Bhatnagar *et al.*, 2006a).

Sighting During Field Visit

A field visit was conducted in the Changthang wildlife sanctuary (Figure 1) by vehicle for about 4 hours along with an experienced guide. It was found that the driver and the guide were very well aware of the habitat of the gazelle. During the study, 5 sightings at 5 different places (Figure 2) were recorded. The details are given below:

Table 1: Field Visit Sightings of Tibetan gazelle in Changchang Wildlife Sanctuary

Sighting	Number	Details
1	4	1 male, 2 female and 1 fawn
2	2	1male and 1 female
3	1	1 male
4	3	1 male and 2 female
5	3	1 female 2 fawn

As the gazelles live in a range of a very small area, spotting them becomes very easy if someone is aware of their habitat, making hunting relatively simple, which definitely raises concern. But the guides and drivers were aware of the animals' dwindling population and wildlife laws. They strictly abide by the rules and regulations of the forest department and never take tourists very close to the gazelles. As the land has no large vegetation, observing these animals even from quite a distance is possible for the tourists.



Figure1: Changthang Wildlife Sanctuary, Hanle, Ladakh



Figure 2: Two of the Sightings of Tibetan Gazelle in Changthang Wildlife Sanctuary. A: A Group of Two Gazelles, B: A Solitary Male Gazelle

Major Threats to Indian Population

Hunting in Historical Times:

One of the major causes for the massive decline in the population is due to the extensive hunting in the 20th century during the 1962 Sino-Indian War and other socio-political disturbances. Reports indicate that such disturbances affected ration supplies, prompting the hunting of gazelles as an alternate food source (Bhatnagar *et al.*, 2006b). The local interviews revealed that hunters found the gazelles to be easy targets, as they could approach the animals easily in vehicles. As the animals live in an almost flat area without any large vegetation, hideouts are not available, making hunting even easier. Although legal protection for these animals has reduced hunting in recent times after the 1980s, the historical impact is severe.

Overgrazing of Domestic Livestock:

As mentioned previously, the Tibetan gazelle can coexist with other wild ungulates, but the domesticated livestock compete with the gazelles. Overgrazing of the domestic livestock in the area, particularly the *pashmina* goats, reduces the forage availability.

The sudden increase in the livestock grazing resulted due to the infiltration of Tibetan refugees in this area who were also pastoral. Additionally, the government implemented measures to boost *pashmina* production in the region. Reports suggest that the domesticated goats of the Changthang region produce the finest cashmere or *pashmina* in the world (Namgail *et al.*, 2007). This resulted in a huge increase in farming of *pashmina* goats and, in turn, intensified the grazing cycle.



Figure 3: Grazing of Livestock within Changthang Wildlife Sanctuary

As the vegetation on which the gazelles feed is limited in such cold deserts, livestock removes a significant amount of plant biomass, directly affecting the survival of Tibetan gazelles (Hagalia 2004). In the current study, two large herds of goats were observed grazing very close to one of the locations where Tibetan gazelles were spotted (Figure 3). Even in present times, the livestock grazing is occurring in almost the same area where the gazelles are present. This certainly raises concern.

Small Population Size:

Extremely low population (about 50 individuals) in the entire Ladakh region raises the risk of a genetic bottleneck and inbreeding, which poses a severe threat to the Indian population of Tibetan gazelles.

Forage Scarcity During Severe Winter:

Tibetan gazelles inhabit very high altitudes with harsh weather conditions and prolonged winters. Reports suggest that during 1998-99, heavy snowfall in the area covered the sparse and dry forage, resulting in the death of a large number of Tibetan gazelles.

Conservation and Management

Establishment of Protected Area (only for Insitu Conservation):

The Changthang Wildlife Sanctuary, established in 1985, is a high-altitude protected area at about 14600 ft. This sanctuary is the only major habitat of the Tibetan gazelles in India.

It is to be noted that Tibetan gazelles are exclusively conserved in the wild. None of the zoos in India house these animals and therefore there is no possibility of captive breeding programs like other Antilopes. None of the Indian zoos can house Tibetan gazelles, as replicating the extreme habitat conditions of the gazelles are not possible. The three high-altitude zoos of India (Padmaja Naidu Himalayan Zoological Park in Darjeeling, Himalayan Zoological Park in Gantok and Pt. G.B. Pant High Altitude Zoo in Nainital) are below the altitude of 7000 ft., whereas the Tibetan gazelles are adapted to live at an altitude of ~ 14000 ft.

Conservation Initiatives:

A conservation initiative was undertaken in 2000 jointly by the Jammu and Kashmir Department of Wildlife Protection (JKDWP), the Nature Conservation Foundation, Mysore (NCF) and the International Snow Leopard Trust-India (ISLT) for the Tibetan gazelles. As an outcome of the initiative, a workshop was held on August 22, 2005, in Hanley, Ladakh, to formulate strategies for the conservation of Tibetan gazelles based on their research. A number of suggestions, like the establishment of a grazing-free area, posting of guards (gazelle guards), putting up of informative sign boards, initiation of a separate project on gazelle recovery, were put forward. Following this, a meeting of conservationists, the wildlife department and village leaders in Hanle Valley was held on 25th August 2005 at Tashi Choeling monastery, Hanle, to inform the locals regarding the findings and discuss the conservation strategies and seek their active participation (Bhatnagar *et al.*, 2006b).

Supplemental Winter Feeding:

Probability of winter starvation of the gazelles due to heavy snow covering the forage needs to be taken care of. Despite efforts to provide winter supplementary feed to gazelles, none were successful. Large amounts of alfalfa brought from different parts of Ladakh were provided by the wildlife department for the 2004-2005 winters but were not accepted by the gazelles. Similar efforts were successful with Tibetan wild asses (kiangs) inhabiting the same place (Bhatnagar *et al.*, 2006b). An alternative can also be providing supplementary fodder to local livestock to reduce the grazing pressure.

Establishment of 'Grazing Free' Areas:

Creating 'grazing-free' areas as suggested in the workshop of NCF-ISLT in 2005, in the areas inhabited by the Tibetan gazelles through community participation, can be an effective step towards the conservation of the gazelles. NCF, along with pastoral communities (Changpas and Tibetan refugees), has set up a small grazing-free reserve in the area and has maintained it for the past few years (Trivedi, 2012).

Community Awareness and Involvement:

Awareness of the local community is needed regarding the conservation of Tibetan gazelles. Local peoples can also be involved in the conservation of the gazelle or sustainable tourism centered on the Tibetan gazelle.

The study revealed a gradual increase in awareness about these animals among the locals of Hanley. The locals are now finding an alternative livelihood by serving as driver-cum-guides for the tourists who intend to see the Tibetan gazelle. The locals now comprehend those numerous tourists flock to this remote village to catch a glimpse of the Tibetan gazelle. This has resulted in an increase of homestays and a source of income for the locals. In fact, some of the homestays are named after this rare endemic gazelle (Figure 4). Locals are slowly acknowledging the importance of the conservation of these animals and sometimes taking part in such initiatives directly or indirectly.

Apart from the locals, the Changpa, a semi-nomadic community of Buddhist pastoralists inhabiting the high-altitude areas of Changthang and the personnel from the armed forces need to be made aware of the present situation and status of the Tibetan gazelle and the importance of their conservation in Hanle. Some informative hoardings and posters focusing on the Tibetan gazelle and their last refuge in Ladakh could be a beneficial step to increase awareness.

During this study, researchers found a board from the J&K wildlife protection department that displayed the name of the Changthung wildlife sanctuary and the animals present (Figure 5), probably following the suggestion of the NCF-ISLT workshop in 2005. The board was not in a very satisfactory condition due to lack of maintenance and harsh weather conditions. Very few banners or posters were observed in the public areas. However, many of the homestays in Hanle display images and posters of Tibetan gazelles.



Figure 4: Home Stay in Hanle, Named after Tibetan Gazelle



Figure 5: A Board Near the Entry of the Changthang Wildlife Sanctuary

Continuous Research and Monitoring:

Despite some initiatives, the research and information regarding Tibetan gazelles is limited and more research is needed to understand the ecology and behavior of these animals. A monitoring program for the gazelles and land use should be undertaken. To understand the population dynamics, need to closely monitor the population and conduct regular censuses of the Tibetan gazelle.

Human Perception on Tibetan Gazelle and its Conservation:

A small survey was conducted among the local people of Hanle to understand the attitude of the people towards the Tibetan gazelle and its conservation. All the respondents were above 18 years of age. The respondents comprised 57.5% male and 42.5% female (n=73). The respondents included homestay owners, their family members and staff, local drivers and guides, Buddhist monks, monastery staff, students, housewives and nomads. The survey's findings are presented in table 2.

The survey data indicates that the locals are quite well aware of the Tibetan gazelle (though they were more familiar with the local name, Goa). The majority of the respondents (84.9%) have heard about the animal and its presence in the Changthang area. Quite a good percentage (63.01%) of respondents have also seen the animal, indicating its visible presence in the landscape.

However, a very small percentage of the respondents were aware that this is the last major population in India and about the conservation status, indicating a significant knowledge gap in conservation-specific aspects. This study shows that though the locals recognize the animal, they are not fully aware of its ecological vulnerability.

73.9% of respondents were aware of the species being legally protected, reflecting some success of the awareness and outreach programs. The majority of the respondents show a positive attitude towards the conservation of the Tibetan gazelle, which is definitely a good sign.

The survey also revealed that tourists mainly visit Hanle for the astronomical observatory, stargazing and Umling La. Tourism here is not wildlife-oriented and awareness about the Tibetan gazelles is still low among tourists. Only a small percentage of tourists visiting Hanle engage in gazelle sightings, which is

probably good from the conservation point of view, as too much tourist attention may affect the animals in a negative way.

Table 2: Survey Findings on Local People's Awareness and Attitudes toward Tibetan Gazelle Conservation in Hanle

Questions		Response		
Close Ended Questions		Percentage of Respondent Answering		
		Yes	No	Indifferent
1	Whether they have heard of Tibetan gazelle or Goa/Gowa?	84.93	15.07	0
2	Whether they have seen Tibetan gazelle (physically)?	63.01	36.99	0
3	Whether they seen Tibetan gazelle in images, paintings etc?	82.19	17.81	0
4	Whether they are aware that Tibetan gazelle lives in Changthang wildlife sanctuary near Hanle?	84.93	15.07	0
5	Whether they are aware that this population is the last surviving population in India?	30.14	69.86	0
6	Whether they are aware of the conservational status of TibetanGazelle?	15.07	84.93	0
7	Whether they are aware that these animals are protected under law and cannot be captured or killed?	73.97	26.03	0
8	Whether these animals need to be conserved?	69.86	0	30.14
9	Will conservation of Tibetan gazelle have any good effect on their livelihood (directly or indirectly)?	69.86	0	30.14
10	Has awareness about Tibetan gazelle and its conservation increased among the locals?	78.08	2.74	19.18
Open Ended Questions				
11	Main tourist attraction of Hanle?	According to most of the respondent the major attraction of Hanle is the Astronomical Observatory, clear sky for stargazing and a stopover for Umlingla, the highest motorable pass in the world		
12	Are tourists aware of Tibetan gazelle?	According to the respondent majority of the tourists are not aware of the Tibetan gazelle and its conservation status in Hanle		
13	Does tourists go for Tibetan gazelle sighting?	According to the respondent, very few tourists go for gazelle sighting, hough this number is increasing.		

Conclusion

The Tibetan gazelle represents one of the most fragile but ecologically important ungulate species that lives in the high-altitude, harsh, cold desert of the Trans-Himalayan region. Presently, their population in India is extremely precarious with only very few living individuals in small pockets of Changthang Wildlife Sanctuary in Ladakh and in Sikkim.

During the last century, the population of the species has drastically reduced due to anthropogenic pressures, including extensive hunting, overgrazing and habitat degradation. Moreover, the critically small population size and the resulting genetic bottleneck bring the species on the verge of local extinction in India.

Despite these challenges, the present study documents several conservation measures, including the establishment of protected areas, community-based grazing managements and awareness programs, which definitely provides a degree of optimism. The survey indicates a gradual but noticeable improvement in the attitudes of the locals toward the species. People are recognizing the gazelle's ecological and economic value, especially in the context of sustainable tourism. The present study also identifies certain gaps in terms of detailed scientific research and long-term monitoring for better understanding and effective implementation of the conservation policies.

In summary, the future of the Tibetan gazelle in India depends on holistic and integrated conservation strategies combining scientific management with active participation from local communities. Sustainable efforts on an urgent basis are crucial to prevent the local extinction of this unique, endemic, high-altitude species and preserve the ecological balance of the Trans-Himalayan environment.

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A Review of Fluorescence Spectroscopic Studies Inside Confined Systems

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Abstract

Fluorescence spectroscopic techniques, when combined with advanced analytical tools, serve as fundamental methods across physics, chemistry, and biology. Fluorescence imaging capable of detecting concentrations as low as the nanomolar range demonstrates exceptional sensitivity in examining complex molecular environments and interactions at the single-molecule level. Photophysical events induced by light, including Fluorescence Resonance Energy Transfer (FRET), Photoinduced Electron Transfer (PET), and solvation dynamics, naturally occur in biological systems. However, studies of these photoreactions in solution often differ from observations in their natural contexts. Interfacial or confined reaction centers, located in close cellular proximity, may govern the outcomes of these processes. A comprehensive understanding of mechanistic pathways within confined regions requires extensive investigation using high-quality fluorescence probes within organized assemblies such as micelles, reverse micelles, and vesicles. Distinct local characteristics of the restricted cellular interface—such as reduced degrees of freedom, increased rate constants, enhanced mobility, and altered proton concentrations—compared to bulk solution, likely contribute to these differing results. This paper provides a broad overview of the prevailing effects of organized assemblies on various photophysical and dynamic processes.

Keywords: *Fluorescence Spectroscopy; Micelle; Organised Assemblies; Reverse Micelle*

Introduction

The confinement effect can do either or both: shape selectivity for reactants and products and modify the compartmentalization of the encapsulated moieties, thus changing the course of the reaction pathway in a unique way (Das *et al.*, 2018). In this chapter, I am interested in representing the effect of organized assemblies on various light-driven processes by using the fluorometric method, a highly sensitive analytical technique (Bhattacharyya, 2001). Researchers will gather mechanistic views using such ultra-sensitive detection tools about molecular anisotropy, solvation, and rotational relaxation of the probe attached to a lipid or surfactant or protein, etc.; structural or spatial disorderliness; and point-to-point measurement inside nanopools (El-Nahass & El-Gamil, 2026). In a high interfacial region with strict molecular geometry, the light-absorbing moieties in subcellular confinement behave differently compared to bulk solvents. To mimic such unique compartmentalization in a laboratory, researchers must study a wide range of systems composed of various types of molecules with distinct polarity and structural flexibility. Only then can one achieve the heterogeneous nature of the working, organized assembly, like a micellar environment comprising hydrophilic heads and hydrophobic tails. Self-assembling molecular aggregates inside polar solvents (micelles) or non-polar solvents (reverse micelles) and vesicular structures with ionic or non-ionic lipids (liposomes) or surfactants (niosomes) are the wide variety of such systems (Schomäcker & Holmberg, 2009; Kalyanasundaram, 2012). Bengal. The present observation also indicated that pollinators play a role in enhancing the pollination ability, which in turn aims to increase the crop's seed yield.

Objectives

Aqueous moieties present in a nanometer-sized system are capable of controlling the reactivity with the representation of unique smaller pockets of water pools. Very often water molecules dictate the biological

functions of many biomacromolecules when confined inside a biomembrane. I am here reviewing a few photoreactions like FRET, PET, and solvent relaxation studies using self-assembling molecular aggregates inside polar solvents (micelles) or nonpolar solvents (reverse micelles), vesicular structures with ionic or nonionic lipids (liposomes) or surfactants (niosomes), etc. Such restricted geometry with unique structural properties of the building blocks creates non-homogeneous behavior. For visual representation, the schematic figures of micelles, reverse micelles (Figure 1), and multilamellar and unilamellar vesicles were given, and such figures help immensely to understand the heterogeneity of such a system with varying polarity, viscosity, etc.

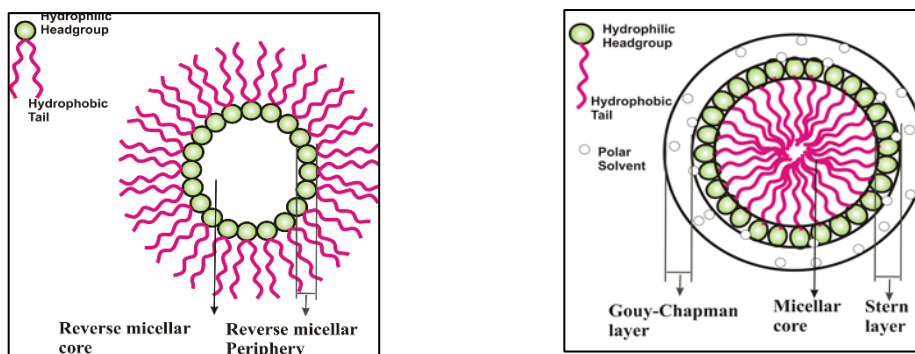


Figure1: Structure of Reverse Micelle (left) and Micelle (right)

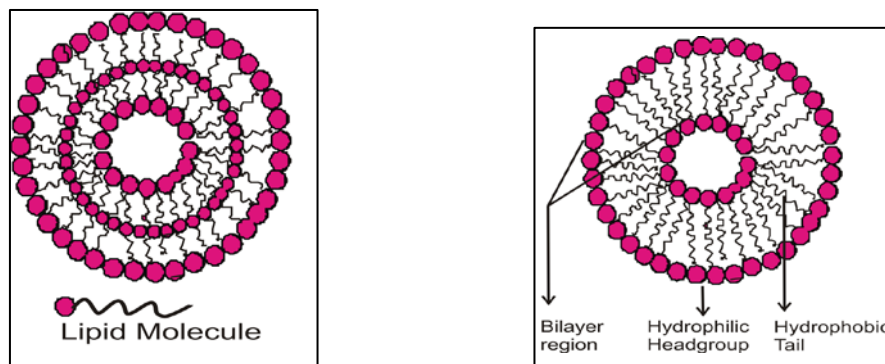


Figure 2: Structure of Multilamellar (left) and Unilamellar Vesicle(right)

FRET Inside Organized Media

In this context, the chapter discusses Förster Resonance Energy Transfer (FRET) within self-assembling molecular aggregates in polar solvents (micelles) and non-polar solvents (reverse micelles), as well as in vesicular structures composed of ionic or nonionic lipids (liposomes, Figure 2) or surfactants (niosomes, Figure 2). It also examines the influence of lipid membrane fluidity on FRET when the donor and acceptor are embedded in small unilamellar vesicles (SUVs) with an approximate diameter of 50 nm. Lipid membranes have a transition temperature (T_m) below which they exist in a solid phase with reduced mobility; above T_m , lipid molecules adopt a more fluid phase with extended hydrocarbon chains. Efficient energy transfer requires donor molecules with high fluorescence quantum yield, while the acceptor—though not necessarily fluorescent—must have a high molar extinction coefficient. FRET occurring over distances of 1–10 nm acts as a “spectroscopic ruler,” being highly sensitive to the inverse sixth power of the donor–acceptor separation (Stryer & Haugland, 1967). The rate of energy transfer is defined by the following equation:

$$k_T(r) = \frac{Q_D \kappa^2 \left(\frac{9000(\ln 10)}{128\pi^5 N n^4} \right) \int_0^\infty F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda}{\tau_D r^6 \int_0^\infty F_D(\lambda) d\lambda}$$

Where (N) is Avogadro's number, (n) is the refractive index, (τ_D) is the fluorescence lifetime of the donor in the absence of the acceptor, and (Q_D) is the quantum yield of the donor in the absence of the acceptor. In this equation, ($F_D(\lambda)$) is the emission intensity of the donor, ($\varepsilon_A(\lambda)$) is the molar absorption coefficient of the acceptor, and (κ^2) is the orientation factor, typically taken as 0.67. This approximation for the orientation factor is valid only when dynamic random averaging of the donor and acceptor is considered.

The expression of the overlap integral $J(\lambda)$ is given by the following equation, where one has to have a considerable amount of overlap between the emission spectra of the donor molecule and absorption spectra of the acceptor molecule:

$$J(\lambda) = \frac{\int_0^\infty F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda}{\int_0^\infty F_D(\lambda) d\lambda}$$

Fluorescence intensity of the donor has no dimension. Where the molar absorption coefficient is in $M^{-1}cm^{-1}$ and the wavelength is in nanometers, then the unit of the overlap integral is in units of $M^{-1}cm^{-1}nm^4$. Energy transfer was observed using self-assembling molecular aggregates inside polar solvents (micelles) or non-polar solvents (reverse micelles) (De *et al.*, 2003), and also a few research studies clearly demonstrated an increased FRET rate by many folds compared to that of the bulk medium (Dar *et al.*, 2018), and such an increment of the rate constant inside molecular aggregates was concluded to be for the restrictive nature of the local environment where the donor-acceptor is embedded and is unable to move too far away from each other. Fluorescence resonance energy transfer study in the presence of TX-100 micelle and reverse micelle was investigated by De *et al.* (2003) with a special type of probe: fluorescein, acridine orange, and a neutral probe, Nile red, and similar to Dar *et al.* (2018), a marked increase in the rate constant has been reported inside such a micellar pool/interface (Fang, 2013). Aerosol octyl sulfate-containing reverse micelles and micelles using sodium dodecyl sulfate (SDS), Pluronic copolymer, etc., were used to check the effect of confinement on FRET and were found to have more efficient FRET parameters inside such an organized environment due to the fact that the donor-acceptor couple is closely packed, having optimal proximity to each other (Mondal *et al.* 2006). Other than micelles and reverse micelles, a FRET study was also conducted inside a small unilamellar vesicle with a completely different objective (Ghatak *et al.*, 2011). The search for the effect of temperature on the rate constant of energy transfer was designed for the Coumarin-153–Rhodamine 6G and Coumarin-151–Rhodamine 6G pairs. Using DMPC SUV, results were shown that there was a prominent effect for the Coumarin-153–Rhodamine 6G pair with the change in temperature compared to those of the other FRET pairs. The deeper location of coumarin-153 inside vesicular architecture is the main cause for the improved FRET parameters. Suggestive data in this work also explained that rigidity of the long alkyl chain changes with temperature, which in turn affects FRET parameters in a larger way for the probe, which is deeply localized, i.e., coumarin 153.

Solvation Dynamics inside Organized Media

The vital role of water molecules in dictating structural and dynamical reactivity motivates the study of solvation, or solvent (water) dynamics. In the solvation process, solvent molecules surround solute particles

(ions or molecules). Specifically, solvation dynamics is the study of the time-dependent, non-equilibrium reorganization of polar solvent molecules around a charged probe molecule (Jimenez *et al.*, 1994). Solvation dynamics typically occurs on picosecond to femtosecond time scales and is often triggered by instantaneously formed dipoles through pulse excitation. This process can affect chemical reactions in multiple ways.

Fluorescent probes that show large changes in charge separation between the ground and excited states are commonly used to study solvent relaxation. Since water molecules dictate the biological properties of many biomacromolecules, probing different regions of such assemblies is crucial. This discussion provides a brief overview of solvation dynamics in confined media or interfacial regions.

Compartmentalization within restricted nanopools or interfacial regions drastically alters physical properties such as polarity, anisotropy, mobility, and acidity/basicity (Hansen, 2025; Smortsova *et al.*, 2017). Confined media commonly used in laboratory studies include micelles, microemulsions, lipid bilayers, surfactant bilayers, and multilayers. Micelles formed from negatively charged, positively charged, or neutral surfactants have been used to study solvent relaxation, often showing significantly slower dynamics than in bulk media (Mandal *et al.*, 2002).

Using femtosecond upconversion setups and streak cameras with highly solvatochromic fluorescent probes such as 4-(dicyanomethylene)-2-methyl-6-(p-dimethylaminostyryl)-4H-pyran and coumarin-153, researchers have reported both fast and slower components of solvation dynamics (Mandal *et al.*, 2002; Mondal *et al.*, 2023). Computational studies employing MD simulations, dynamic density functional theory, and QM/MM (Quantum Mechanics/Molecular Mechanics) models allow atomistic analysis of solute environments and solvent reorganization on ultrafast time scales.

The strength and dynamics of hydrogen bonding between water molecules and the polar parts of micelles are significantly slower than hydrogen bonds between water molecules in bulk (Balasubramanian & Bagchi, 2001). Studies comparing interaction energies, hydrogen-bond breaking/forming dynamics, and solvent reorientation lifetimes in bulk solutions versus confined pools have shown much slower solvation dynamics in smaller water pools, such as in n-heptane/AOT/water microemulsions (Sarkar *et al.*, 1996).

Solvation dynamics in microemulsions containing negatively charged, positively charged, or neutral surfactants also exhibit distinctive preferences and complex behaviors, consistent with the heterogeneous nature of such assemblies. Similar slow dynamics have been reported in non-aqueous reverse micelles and vesicles (Corbeil & Levinger, 2003). Differential polarity gradients and microviscosity within lipid molecules containing chromophores in unilamellar and multilamellar structures offer unique confinement environments, which can be explored through solvation studies.

Sýkora *et al.* (2002) reported bimodal lifetimes for water molecules inside vesicles. Key findings indicate that solvation dynamics inside vesicles are significantly slower and more complex due to the presence of water in different localized regions. Excitation wavelength dependence of solvation and rotational relaxation was also observed. For example, three solvent relaxation components (two slow, one fast) were reported in multilamellar vesicles formed with non-ionic surfactants and long-chain polymers (Ghatak *et al.*, 2011). Detailed analysis revealed that the two slow components arise from differing viscosities in the headgroup regions: one in the internal water pool and one in the external bulk-facing region. Local backbone vibrations and solvent-polymer interactions within the bilayer contribute to rapid conformational changes and ultrafast water orientation, producing the fast solvation component.

PET Inside Organized Media

Photoinduced electron transfer is a photophysical incident where, upon excitation with light energy or a photon, the donor moiety causes electron transfer towards the acceptor part, creating a charge separation, and is frequently observed in various natural and synthetic systems. PET can be intramolecular

(same molecule) or intermolecular (different molecule) (Bolton *et al.*, 1991; Romanetz *et al.*, 2026). The theory regarding the electron transfer was first proposed by R. A. Marcus in a seminal paper. PET can happen either through bonds or space, and such a mechanism involves overlap between orbitals of donor and acceptor molecules, which implies that the D-A distance plays a crucial role influencing the rate constant value. The expression for the free energy change (ΔG) for such a PET process can be calculated by using the Rehm-Weller equation:

$$\Delta G = E(D^+/D) - E(A/A^-) - E_{00} - e^2/\epsilon d$$

The first term and the second term on the right side of the equation represent reduction potentials of the donor and the acceptor molecules, respectively. E_{00} is the threshold photoexcitation energy from the singlet ground state to the first excited state, and the last term is the estimated electrostatic interaction between the excited donor and acceptor after charge transfer, where e is 1.6×10^{-19} C, ϵ is the dielectric constant of the medium used, and d is the separating distance.

The rate (k_{ET}) for the kinetics of PET process can be given by,

$$k_{ET} = k_{el} \nu_n \left(\Delta G^* / k_B T \right)$$

K_{el} is the probability or electronic factor that ensures the continuation of the event towards product state and represents the frequency factor. It reflects the Boltzmann factor. ΔG^* is free energy or the driving force.

It is defined as

$$\Delta G^* = \frac{1}{4\lambda} (\lambda + \Delta G^0)^2$$

Now comparing the equations, the classical Marcus equation is obtained

$$K_{ET} = K_{el} \nu_n \exp \left(\frac{-(\lambda + \Delta G^0)^2}{4\lambda K_B T} \right)$$

The reorganization energy (λ) is the sum of solvent reorganization energy and inner-sphere reorganization energy. The dielectric constant, or polarity, of the medium plays a significant role in controlling the activation barrier for electron transfer (ET) by stabilizing the charged transition state. According to Marcus's theory, the relationship between activation free energy, driving force (ΔG^0), and reorganization energy can be summarized as follows:

1. $\lambda > \Delta G^0$: In the normal Marcus region, $\log k$ increases as ΔG^0 decreases.
2. $\lambda = \Delta G^0$: The reaction becomes barrierless.
3. $\lambda < \Delta G^0$: In the inverted region, $\log k$ decreases with increasing driving force.

To study ET, electron-rich molecules such as aniline, N,N-dimethylaniline, N,N-diethylaniline, N,N-dipropylaniline, and N-deuterio-N-ethylaniline are commonly used as donors, while high-electron-affinity molecules like various coumarin dyes (C-151, C-152, C-481, etc.) serve as acceptors (Chakraborty *et al.*, 2008). The choice of donor-acceptor pair and the polarity of the solvent are critical for efficient redox reactions, solar energy conversion, and biological processes.

Given the structural and electronic heterogeneity inside micellar microenvironments, researchers have explored how ET rates and related parameters change in micelles compared to bulk solvent. For instance, studies using negatively charged and nonionic micelles with N, N-dimethylaniline as the donor and oxazine as the acceptor reported significantly slower ET rates than in bulk solution (Pal *et al.*, 1999). This retardation arises primarily from increased donor-acceptor separation within the micellar or interfacial region.

Nanda *et al.* (2005) conducted experiments and theoretical calculations of ET rate constants in micelles composed of SDS, CTAB, and TX-100. They modeled micelles as heterogeneous systems with three distinct regions: a hydrophobic core formed by densely packed alkyl chains, a headgroup shell or interface with intermediate polarity and viscosity, and the surrounding water representing bulk solvent characteristics. Using Marcus theory, they proposed a two-dimensional electron transfer (2DET) model, where ET dynamics depend not only on the driving force but also on solvent reorganization energy. Such ET involves nonequilibrium solvent configurations during rapid electron transfer, and bimolecular diffusion rates were found to be slower than ET rates, indicating that reactant diffusion plays an insignificant role.

These studies revealed a Marcus inversion region, with a maximum rate at lower exergonicity, reflecting the presence of nonequilibrium solvent states in heterogeneous dielectric environments. Although surfactants like CTAB, DTAB, and TTAB form structurally similar micelles, variations in micelle size and shape can significantly influence ET rates. In some cases, researchers calculated reorganization energies for intermolecular photoinduced ET by presuming the location of donors and acceptors within the micellar environment.

Organized assemblies such as micelles, nanoscopic water droplets in organic solvents, and double-layered vesicles often alter donor–acceptor distances and orientations, resulting in faster or slower ET rates. Recently, photoinduced ET (PET) has been applied in cellular imaging and disease therapy (Niu *et al.*, 2023). Studies of PET in vesicular environments indicate that lipid bilayers or multilayers control ET rates through modifications of reorganization free energy, reaction free energy, nuclear dynamics, and donor–acceptor coupling strength. In these systems, donor and acceptor diffusion remains slower than ET, producing a Marcus inversion curve and highlighting the role of nonequilibrium solvation and conformational dynamics (Ghatak *et al.*, 2012).

Conclusion

In this discussion, results on several photophysical phenomena were carefully presented and analyzed, citing numerous relevant studies to highlight the main findings and objectives of the article. It is evident that studying solvation dynamics and electron transfer in a heterogeneous medium at different excitation wavelengths provides insights into solvent dynamics coupled with electron transfer and energy transfer processes in various microenvironments. Such investigations can ultimately enable non-invasive probing of intracellular microviscosity, cell membrane structure, and molecular interactions. Achieving this requires a detailed understanding of the mechanisms underlying these photoinduced events within restricted or interfacial environments.

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Transformation of Upcycle Plastic Waste into Carbonaceous Materials for Advanced Energy-Storage Applications

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Abstract

Recycling and depositing plastic waste materials into valuable carbonaceous materials is an important method for generating more sustainable machinery. This dynamic is further reinforced by the mounting industrial and societal demand for recycled materials and the crucial need to transition to a more sustainable and elegant route towards demand for recycled materials and the crucial need to transition to a more sustainable and elegant route towards greener synthesis approaches. More specifically, renovating waste plastic materials into upgraded forms for energy-storage devices like batteries and supercapacitors helps reduce pollution while also increasing sustainable resources for current energy systems. Moreover, the plastic waste containing a large quantity of carbon has emerged as a promising strategy to transform through combustion to produce for manufacturing carbonaceous materials with large specific surface area and high electric conductivity, showing great promise for sustainable applications in electrochemical energy-storage devices such as sodium-ion batteries, lithium-ion batteries, and also supercapacitors. The review is focused on the preparation of novel syntheses of active carbonaceous frameworks for energy storage applications from plastic waste and addressed how the use of plastic waste could enhance more sustainable, ecofriendly and highly efficient plastic upcycling technologies.

Keywords: *Applications; Carbon Materials; Carbonaceous Frameworks; Energy Storage; Plastic; Polymer Waste*

Introduction

The ongoing growth of society has led to the rapid accumulation of deposited plastic waste, which has emerged as a significant global environmental challenge, posing threats to ecosystems, social health, and economic stability. The continuous increase in plastic manufacture has become widely used by population and accelerated industrial development coupled with expanding worldwide productivity. One promising method includes renovating plastic waste into highly active carbonaceous frameworks and nanomaterials with high porosity, large surface area, abundant functional groups, and good conductivity, such as graphene, activated carbon, carbon nanotubes (CNTs), carbon quantum dots (CQDs), and porous carbon material. This review provides a wide-ranging overview of generally used plastics, describing their classifications, physicochemical characteristics, buildup pathways, and related environmental and health impacts. Furthermore, it focuses on various recycled waste synthetic approaches and highlights recent advances in the pathway of converting discarded plastics into carbonaceous materials through diverse synthesis methods and their application in energy-storage systems and supercapacitors, followed by insights into future research directions.

Moreover, solid waste, which involves various types of waste from various sources, such as plastic, agricultural, municipal, paper, polymeric wastage, and industrial waste, is predominantly contained in carbonaceous skeletal frameworks, which facilitates the advancement of nanoscience-based technologies. Moreover, especially carbon materials synthesized from waste polymer precursors have recently gathered considerable attention as eco-friendly and cost-effective alternatives for energy-related technologies (Dzikunu *et al.*, 2025; Tatrari *et al.*, 2021). The conversion of polymer waste into carbon frameworks through

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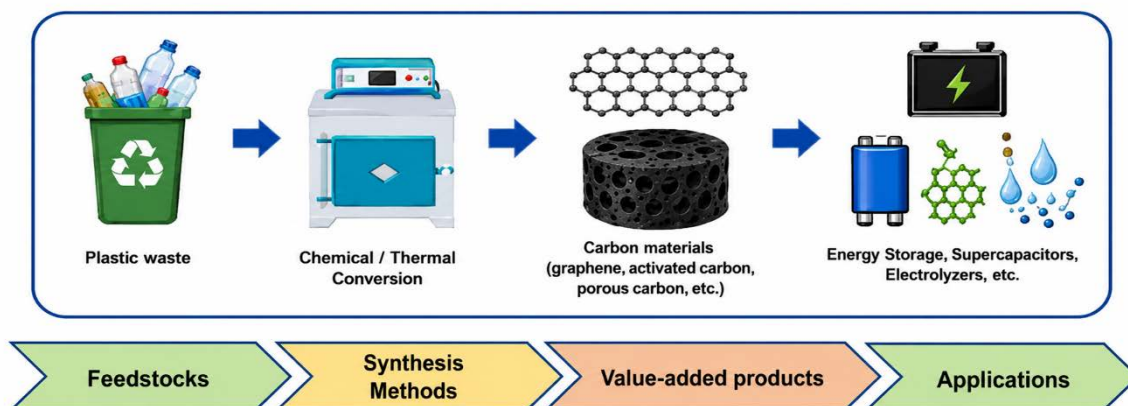


Figure1: Reusing And Advancement of Plastic Wastes to Carbonaceous Materials and Potential Applications

pyrolysis, activation, or templating systems mitigates environmental pollution and synthesizes materials with high surface area, sharp porosity, and excellent electrical conductivity (Ferdous *et al.*, 2024; Zhai *et al.*, 2022). These structural and electronic features show such carbon resources highly suitable for battery electrodes, facilitating higher ion diffusion, charge transport, and longstanding cycling stability (Ruan *et al.*, 2025). The supercapacitors, which have a hierarchical pore structure and large surface area, demonstrate superior capacitance and rapid charge-discharge capabilities (Okonye & Ren, 2024). Additionally, these carbonaceous materials have exposed promising potential in highly energy conversion and storage systems, with fuel cells, metal-air batteries, and electrocatalytic processes. Thus, carbonaceous materials synthesized from unwanted polymers represent a sustainable pathway toward sustainable energy machineries. In contrast, the activation process plays a vital role in tailoring the structural morphology and physicochemical features of the carbonaceous framework, with the final characteristics being basically dictated by the activation approach and processing conditions employed. The current global solid waste situation and its distribution highlight the urgent need for effective management. The current dispositions in solid waste generation and its widespread distribution highlight the urgent stipulation for sustainable waste government strategies. The specific emphasis is located on recent advances in waste-to-resource conversion skills, the synthesis of functionalized porous carbon materials, and their emerging applications like green energy, environmental protection, and also nanotechnologies. These developments expose the immense potential of solid waste as a renewable carbon resource, contributing promptly to waste valorization and eco-friendly sustainability.

Plastic Waste-to-Carbon Nanostructures: A Combustion-Based Approach

The different types of plastic raw materials oscillate in their conformation, molecular structure, and carbonization yield, which pointedly affect their carbonization process. For example, larger macromolecules like polyimides are closely governed by their structural features, such as strong thermal stability and resistance to carbonization, but low-molecular-weight polymers like polypropylene (PP) showed carbonization at elevated temperatures due to their molecular chain configuration. Since plastics are usually structured with high carbon content, converting waste plastics into high-value carbon nanomaterials shows a highly effective approach for both recycling and manufacturing functional carbon-based materials. Furthermore, the structural properties of the resulting carbonaceous nanomaterials depend principally on the type of plastic precursor used under carbonization.

The carbonization yield also fluctuates among raw materials; for example, the yield from waste PP can vary notably under specific processing conditions. Therefore, selecting suitable carbonization techniques and parameters according to the characteristics of the plastic feedstock is critical to maximizing the production of carbon materials. Accordingly, solid waste resources can be repurposed for the large-scale development

of diverse carbon-containing nanomaterials (CNMs). Among these, carbon quantum dots (CQDs), carbon nanotubes (CNTs), and graphene exhibit remarkable physicochemical characteristics, including high aspect ratios, outstanding electrical conductivity, excellent mechanical robustness, and superior thermal transport properties. Over the last decades, enormous efforts have been made to investigate transforming solid waste into carbon nanotube materials and applying their potential in various technological fields, like supercapacitors, fuel cells, solar energy conversion devices and targeted drug delivery (Lobato-Peralta *et al.*, 2025). In 2015, Gong and his teams. Gong and his teams developed a method to transform real-world waste polymers, specifically polyethylene (PE), polystyrene (PS), and polypropylene (PP), into porous carbon nanosheets (PCNSs) that can eliminate organic dyes from wastewater. The resulting PCNSs demonstrated outstanding adsorption performance, attributed to their substantial specific surface area, structural tunability, and multiple adsorption pathways, including pore filling, π - π stacking interactions, hydrogen bonding, and electrostatic interactions, which ease the effective appropriation of diverse contaminants (Gong *et al.*, 2015).

Some important plastic precursors for carbon material synthesis, polyethylene (PE), polystyrene (PS), and polypropylene (PP) are often favored due to their high carbon production. Polystyrene (PS) has been reported to generate aromatic hydrocarbons during pyrolysis, which can reduce its effectiveness as a carbon precursor (Riedewald *et al.*, 2022). Moreover, Veksha *et al.* (2017) explored the impact of various plastic feedstocks on carbon nanomaterial yield under catalytic chemical vapor deposition (CCVD), exploiting non-condensable gases resulting from plastic pyrolysis at 500°C and 800°C. At 500°C, a hybrid plastic mixture (MP) designed carbon nanocages and multi-walled carbon nanotubes along with a large graphitization level. Furthermore, they also examined how various plastic feedstocks influence the formation of carbon nanomaterials (CNMs), as examined by Yao *et al.* (2022) and Sharma & Batra (2020). Their verdicts revealed that polystyrene (PS) formed the highest CNM yield, which was accredited to its high carbon content, low hydrogen concentration, and increased formation of liquid-phase products. polypropylene (PP) and Polyethylene terephthalate (PET) containing approximately 33 wt% oxygen revealed low carbon yield because of its oxygen-rich structure helps carbon oxidation during pyrolysis, leading to excessive CO₂ generation. Therefore, PET was recognized as an unsuitable precursor for CNM synthesis. In addition to this, polystyrene (PS) is non-biodegradable and may persist in the environment for decades. During degradation time, polystyrene materials can release styrene and other harmful substances into the surroundings, potentially contaminating soil and water and posing risks to both humans and wildlife.

The conversion of waste plastics into high-value carbon nanomaterials presents a cost-effective and economically viable strategy for producing advanced functional materials. These carbon nanomaterials derived from plastic waste generally possess superior electrical conductivity and thermal stability, making them highly suitable for high-performance applications, including supercapacitors and conductive composites. Therefore, Hu *et al.* (2024) utilized a salt-assisted carbonization approach to transform waste poly(ϵ -caprolactone) (PCL) into graphene materials. It is seen that the resulting material demonstrated remarkable photothermal properties, including a 98% solar absorption rate, efficient light-to-heat conversion, strong water transport ability, low water evaporation enthalpy, and a thermal conductivity of 0.06 W·m⁻¹·K⁻¹. Its water evaporation rate reached 2.92 kg·m⁻²·h⁻¹, outperforming numerous state-of-the-art photothermal materials. This advancement enables the upcycling of waste plastics and offers a promising route for developing integrated systems for freshwater generation and power cogeneration. Moreover, under the vehicle scrapping, valuable metals and electronic components are typically recovered; however, up to 350 kg of plastic waste often ends up in landfills. In 2020, Algozeeb and colleagues introduced a technique for synthesizing graphene from mixed plastic waste. The process employs rapid thermal discharge to heat waste plastic resistors to exceptionally high temperatures, resulting in highly carbonized materials, which are suitable for graphene formation (Luong *et al.*, 2020).

Graphene material manufacturing from waste plastics signifies a promising strategy for environmental remediation and sustainable applications. Additionally, due to their outstanding electrical conductivity, high porosity, large surface area, and abundant surface groups, they have been widely used as anode materials in lithium-ion batteries, as substrates for photocatalysts, and also used as main components in various fields like supercapacitors, flexible electrodes, and biosensors. This strategy decreases environmental pollution brought about from waste plastics, endorsing the sustainability of our society. However, due to its high practical applicability and excellent performance, graphene derived from plastic waste has emerged as a major topic of research in recent years.

This is particularly evident in the area of catalyst recycling and reuse, where catalysts play a vital role across the chemical, energy, and environmental industries. In response to these demands, recent studies have introduced innovative strategies for recovering precious and critical metals from metal-rich waste sources. Within the sustainable chemistry framework, particular attention has been focused toward changing waste materials into high-performance catalysts capable of operating efficiently under mild reaction conditions. The chemical recycling methods like hydrogenolysis, catalytic cracking, pyrolysis and advanced oxidation help the transformation of plastic waste into good yields, including liquid fuels, carbon-containing constituents, syngas, and hydrogen-enriched gases. However, Cheng *et al.* (2024) investigated that pyrolysis and fluid catalytic cracking restored some part of the energy fixed in packing plastic waste and biomass materials and produced highly active carbon fibers.

The microwave-assisted pyrolysis catalytic process has shown promise in achieving both substantial carbon yields and enhanced hydrogen energy recovery (Yu *et al.*, 2024). Similarly, plasma gasification has gained considerable attention for solid waste processing, as it efficiently converts plastic residues into hydrogen-rich syngas without generating harmful emissions (Qin *et al.*, 2025). Among these technologies, catalytic conversion is particularly crucial, since the choice of catalyst profoundly affects the overall efficiency and selectivity of the plastic-to-carbon conversions. Various transition metal oxides, such as Fe_2O_3 , Co_3O_4 , NiO , $\text{Ni}_3\text{Co}_2\text{O}_x$ and FeAlO_x , have been widely explored owing to their widespread accessibility and cost-effectiveness (Qin *et al.*, 2025). For instance, in 2022, Shen *et al.* synthesized an FeAlO_x catalyst material for the catalytic conversion of plastic waste into carbon nanotubes (CNTs) and hydrogen.

As of now, the majority of reviews concentrate on recent advances in the catalytic transformation of waste plastics into carbon-based nanostructures for the practical applications of the resulting materials. The study specifically focuses on three major products—graphene, porous carbon, and carbon nanotubes—and their applications across various technological sectors.

Although several methodologies have been established for the conversion of plastic waste into functionalized carbon nanomaterials, restrictions in product efficiency and yield remain. For these reasons, continued research is needed to design and synthesize advanced catalytic pathways and improve reaction conditions to achieve superior structural characteristics and enhance production. It enhances conversion efficiency and develops the applicability of these materials in high-performance devices.

The conversion of waste-derived feedstocks into diverse carbon-based nanomaterials (CNMs) and subsequently their integration into polymeric nanocomposites, energy-storage and energy-conversion devices, bioactive systems, and aerospace technologies has significantly improved the value and relevance of waste-based CNMs. Due to the mounting importance of these waste resources as viable precursors for carbon-based nanomaterials synthesis, this review focused on recent strategies in the field. It outlines and hypothesizes various categories of waste materials that can be used to generate carbon-based nanomaterials with specific morphology, structure, and physicochemical properties, while also considering their sustainability and potential applications for environmental remediation. Despite several review articles

on waste utilization, this review also introduces the challenges of implementing carbon-based nanomaterial synthesis and contributes a clearer perspective on their increasing significance to human society.

Recently, extensive research has explored the biomass-resultant materials derived from nanofibers, cellulose, and melanin for application as binders, polymeric electrolytes, or active electrode apparatuses, applying high potential in supercapacitors and rechargeable battery systems (Olazabal *et al.*, 2022). A promising approach for addressing the abounded complications in the plastic residue material is upcycling, which involves converting secondary plastic resources from consumer and industrial feedstocks into high-value products for broader technological applications (Jehanno *et al.*, 2022; Jan *et al.*, 2026). These biomass-derived materials demonstrate noteworthy potential applications for rechargeable battery and supercapacitor systems for increasing the overall sustainability of energy-storage technologies.

Conclusion

The worldwide accumulation of waste plastics has risen to a serious global waste management challenge. Large volumes of mismanaged waste plastics are disposed of in landfills and natural ecosystems, causing environmental pollution and loss of valuable resources. Addressing the global waste crisis needs innovative and sustainable garbage disposal solutions with an environmentally friendly approach. By transforming waste plastics into functional carbon materials, including porous carbon, graphene-like materials, activated carbon, carbon nanotubes and carbon nanofibers obtained through co-pyrolysis of various types of biomass materials and plastics, it helps to generate valuable and sustainable yields for advanced energy-storage systems and also instantaneously reduce plastic pollution. Overall, the valorization of plastic waste into carbonaceous materials signifies a sustainable and green pathway for addressing both plastic waste management and the rising demand for advanced energy-storage technologies. This review approaches the green pathway over the transformation of plastic waste into functional carbonaceous materials, offering a co-friendly sustainable and environmental remediation to effectively reduce environmental pollution.

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Tri-metallic Nanoparticles: A Green Approach to Multifaceted Biological Applications

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Abstract

Trimetallic nanoparticles (TNPs) with three metallic elements show improved properties in catalysis, optics, magnetism and biocompatibility as compared with the mono and bimetallic nanoparticles. The unique properties allow for application in agriculture (e.g., enhancing plant growth, controlling pathogens) and biomedicine (e.g., drug delivery, cancer treatment). Conventional methods of TNPs synthesis involve toxic reagents and consume a high amount of energy. However, green synthesis of TNPs using natural agents like plant extract is a sustainable approach. This green approach leads to the production of versatile nanoparticles with significant applications in improving agricultural methods and health outcomes.

Keywords: *Agricultural Application; Biological Application; Biomedical Application; Green Synthesis; Microorganism-Mediated Biosynthesis; Plant Mediated Biosynthesis; Trimetallic Nanoparticles*

Introduction

Nanotechnology has changed medicine, pharmaceuticals, materials and agriculture. TNPs have collected attention due to its unique physicochemical properties and synergistic effect, which often outperform mono- and bimetallic. TNPs are promising biomedical and agricultural candidates due to their improved catalytic activity, tunable optical and magnetic behavior, thermal stability and biocompatibility (Roy *et al.*, 2023).

Traditional TNPs synthesis uses hazardous reagents, high energy and harmful byproducts. A sustainable, cost-effective alternative is green synthesis, which uses plant extracts, bacteria, fungi and algae. They naturally reduce and cap TNPs, giving them biologically useful size, shape and surface properties (Radulescu *et al.*, 2023).

TNPs biologically synthesized by biologists are promising for targeted drug delivery, antimicrobials, cancer therapy, bioimaging, biosensing and tissue engineering. Green synthesis of three metals improves biological interactions, therapeutic efficiency and diagnostic performance while reducing toxicity (Kulkarni *et al.*, 2023; Vijayaram *et al.*, 2024). As scientists study TNPs' effects on plant health, soil quality and crop productivity, their use in agriculture is growing. Besides fighting phytopathogens, TNPs' catalytic and redox properties help crops deliver nutrients, degrade pesticides, germinate seeds and tolerate stress. For sustainable farming, biocompatibility and environmental responsiveness reduce agrochemical use and promote environmentally friendly agricultural systems (Osman *et al.*, 2024).

In biomedical and agricultural nanotechnology, biologically synthesized trimetallic nanoparticles offer novel solutions to healthcare, environmental sustainability and global food security.

Biological application of Trimetallic nanoparticles (TNPs)

The presence of three different metals in the same nanostructure enhances their physicochemical stability, catalytic activity and multifunctionality that are highly attractive for biomedical agricultural applications.

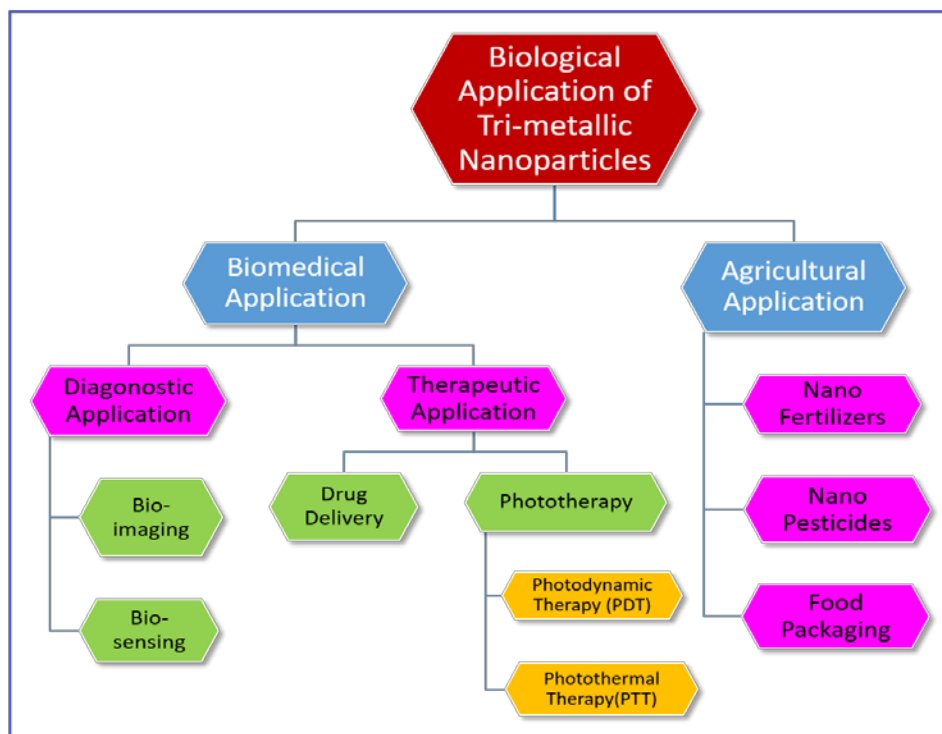


Figure 1: Biological Application of Trimetallic nanoparticles (TNPs)

In medicine, TNPs are studied for targeted drug delivery, antimicrobial therapy, bioimaging, biosensing and cancer therapy, where tunable surface chemistry and optical properties result in improved therapeutic efficacy and reduced toxicity.

In agriculture, TNPs are promising nanofertilizers, nanopesticides and antimicrobial agents for crop protection, thereby establishing the path to sustainable farming practices with a lesser chemical load. These advances together showcase the potential of TNPs as next-generation nanoplatforms for addressing global challenges in health and food security.

Biomedical Application

Anti-Cancer Activity:

Cancer remains a major killer worldwide; early diagnosis and treatment are very important. Biological and green synthesis methods for the synthesis of trimetallic nanoparticles demonstrate promising cytotoxic activity against a variety of cancer cell lines and are considered as promising novel anticancer agents.

Au/CuO/ZnO nanoparticles derived from *Verbena officinalis* (100% cytotoxicity in Jurkat cells at 6 μmol) (Dobrucka *et al.*, 2021) and Au/Pt/Ag nanoparticles derived from *Pleurotus florida* (10% viability decreases in MDA-MB-231 breast cancer cells at 100 $\mu\text{g/ml}$) demonstrated remarkable antileukemia (Chaturvedi *et al.*, 2020). While Ru/Ag/Pd nanoparticles synthesized from garlic tunicate leaves demonstrated antiproliferative effects against HepG2, Caco-2 and K562 cancer cell lines (Hussein *et al.*, 2022). Similarly, Ag/Co/Fe nanoparticles produced using *Citrus lemon* showed notable cytotoxicity against HepG2 and MCF7 cells (Abdelsattar *et al.*, 2023). Trimetallic nanostructures have also been employed for cancer diagnostics. Highly sensitive detection of H_2O_2 from cancer cells using Au-Pt-Pd/rGO nanocomposites and trimetallic hybrid nanoflower-decorated MoS_2 nanosheets (Dou *et al.*, 2018).

These results show the importance of trimetallic nanoparticles for cancer therapy and diagnostic applications.

Sensing Applications:

Biosensors are utilized for qualitative and quantitative detection of biomolecules and trimetallic nanoparticles (TNPs) have emerged as very useful sensing platforms because of their controllable plasmonic and magnetic properties. Plasmonic TNPs use localized surface plasmon resonance (LSPR), where shifts in wavelength occur due to change in refractive index or nanoparticle agglomeration, allowing sensitive analytic detection.

Trimetallic nanoparticles (TNPs) have demonstrated significant potential in biosensing and theranostic applications owing to their enhanced catalytic and optical properties. Cu-Au-Pt nanoparticles exhibited strong NIR-I plasmonic absorption and were successfully applied in glucose sensing, biosensing and photothermal cancer therapy. Au-Ag-Pd nanocomposites enabled the simultaneous detection of multiple biomolecules, including ascorbic acid, dopamine, acetaminophen and tryptophan (Abdelwahab *et al.*, 2020). Pd-Cu-Au nanoparticles showed excellent peroxidase-like activity, allowing ultrasensitive detection of H_2O_2 and glucose with detection limits of 5 nM and 25 nM, respectively (Nie *et al.*, 2020).

Furthermore, Au-Pt-Pd/reduced graphene oxide nanocomposites were used for monitoring H_2O_2 release from live cancer cells. While Pd-Fe-Ni alloy nanoparticles served as effective electrochemical sensors for biotin detection (Gholivand *et al.*, 2015). These findings highlight the versatility of trimetallic nanoparticles as promising platforms for advanced biosensing, diagnostic and theranostic applications.

Antimicrobial Activity:

TNPs showed good antibacterial activity through different mechanisms. They quickly bind to the cell walls of bacteria, disrupting the membrane integrity and causing intracellular components to leak out. In addition, the production of reactive oxygen species (ROS) leads to the oxidation damage of important biomolecules, while direct contact with proteins and DNA also inhibits the growth of bacteria (Slavin *et al.*, 2017). For example, Au-Ag-Cu TNPs showed a wide range of efficacy against both Gram-positive (*Staphylococcus aureus*) and Gram-negative *E. coli* bacteria (Markowska-Szczupak *et al.*, 2025). Similarly, Pt-Ag-Cu TNPs were found to be highly efficient against multidrug-resistant bacteria mainly due to their increased ROS-mediated killing of bacteria (Rai *et al.*, 2012). The results demonstrate the potential of trimetallic nanoparticles as new candidates of antimicrobial agents against drug-resistant bacterial infections.

Antifungal Activity:

Just like their antibacterial potential, TNPs exhibited strong inhibitory effects against pathogenic fungi. They interact with fungal cell walls and change membrane permeability, and their ability to bind intracellular proteins and nucleic acids interferes with metabolic processes and replication. Also, ROS overproduction promotes the apoptosis of the fungal cells (Rai *et al.*, 2017). For instance, Cu-Ag-ZnO TNPs have demonstrated notable inhibition of *Candida albicans* (Ghosh *et al.*, 2020). The results suggest that TNPs have the potential to be used as antifungal agents in both clinical and agriculture settings.

Antioxidant Activity:

In addition to their antimicrobial properties, TNPs display strong antioxidant potential. They effectively scavenge free radicals such as DPPH• and ABTS•+, either through electron transfer or hydrogen atom donation. The multimetallic composition provides enhanced redox potential, mimicking the catalytic activity of antioxidant enzymes such as superoxide dismutase and catalase (Phan-Xuan *et al.*, 2024). For example, Au-Ag-Pd TNPs exhibit superior radical-scavenging ability in DPPH and ABTS assays, while Cu-Fe-Ag TNPs significantly reduce oxidative stress by neutralizing ROS in vitro. This dual ability to combat both pathogens and oxidative stress renders TNPs highly promising for therapeutic applications.

Agricultural Application

TNPs can deliver multiple micronutrients, disease resistance and shelf life while reducing chemical load. Below are three main application areas with recent studies.

Nano Fertilizers:

The use of TNPs in agriculture is increasing as multifunctional nano fertilizers provide micronutrients and enrich plant resistance to biotic and abiotic stress. Completely trimetallic nano formulations are rare, but recent studies show the potential of combining multiple metal oxides. The CMC-Cu-Zn-Fe MNPs showed the potential as a nutrient carrier and nano pesticide, enhancing the tolerance to drought stress and surprising the fungal pathogens in tomato plants (Bouqellah *et al.*, 2023). Zinc-doped calcium phosphate nanoparticles ('nano Zn') also demonstrated improved nutrient supply (Ca, P and Zn) and an antibacterial effect against *Pseudomonas syringae*, with increases in nutrient uptake of up to 65% versus traditional ZnSO_4 . Another study reported that biogenic Cu/Ni/Co oxide TNPs combined with vermicompost with rice straw and press mud significantly increased germination and growth parameters of *Abelmoschus esculentus*, with metal accumulation within plant tissues remaining below WHO safety limits. Moreover, even if not at the nanoscale, a fortified N-P-K fertilizer with a tri-micronutrient matrix (Zn, Fe, Mg) showed evident benefits on tomato growth, underlining the agronomic relevance of the concurrent micronutrient supply and presenting a benchmark for forthcoming TNPs-based nano formulations (Venugopal & Rao, 2021). Collectively these findings highlight the potential of TNPs to enhance nutrient use efficiency, improve plant health and reduce dependence on traditional agrochemicals.

Nano Pesticides / Disease Management:

TNPs, a new class of multifunctional plant disease management agents, can be used to reduce the use of pesticides. For instance, the CMC-Cu-Zn-Fe magnetic nanoparticles have been reported to kill the *Fusarium oxysporum* in tomatoes and relieve drought stress (Bouqellah *et al.*, 2023). The green synthesized CuO-Ag/ZnO nanocomposites with *Ziziphus spina-christi* leaf extract showed broad-spectrum antimicrobial activity against clinical pathogens and also inhibition of virulence factors and ergosterol biosynthesis (5.9 nm) (El-Sawaf *et al.*, 2024). Their trimetallic structure provides to take advantage of their surface functionalization for phytogens. In addition, bimetallic Cu/Zn hybrid nanoparticles have shown to be highly effective against bacterial spot disease of tomato caused by *Xanthomonas spp.* This includes copper-tolerant strains, which demonstrate the benefit of combining materials with different mechanisms of action as antimicrobials. CMC-Cu-Zn-Fe NPs can also provide micronutrients (Cu, Zn, Fe) and thus exhibit dual functionality as nano pesticides and nano fertilizers, leading to reduced requirements of multiple agricultural inputs and enhanced plant health and productivity.

Food Packaging:

Antimicrobial food packaging with trimetallic or Mult metallic nanocomposite film can be used to reduce microbial contamination and extend the shelf life. Embedded films with trimetallic nanoparticles are rare, but some studies showed the advantages of blending metal or metal oxide nanoparticles in polymer matrices. The in-situ synthesized trimetallic Bi-Fe-Sn nanoparticles from *Terminalia arjuna* leaf extract showed significant antimicrobial activity against *Escherichia coli* and *Pseudomonas aeruginosa* in microcrystalline cellulose films, demonstrating that TNPs can be added to biodegradable packaging materials for surface protection (Dar *et al.*, 2023). The composite films with spinel nanoparticles ZnAl_2O_4 and CuAl_2O_4 and chitosan or shellac polymers showed enhanced mechanical strength, lower air permeability, and antibacterial properties against *Bacillus mycoides* and *Canida albicans* (Mohamed *et al.*, 2024).

While they are mixed metal oxides, rather than trimetallic nanoparticles, the structural and functional properties of these spinels make them useful analogs for multifunctional packaging films. A new method to

package grapes was based on chitosan films loaded with ZnO and Ag nanoparticles and citronella essential oil with broad-spectrum antimicrobial activity (Motelica *et al.*, 2020). The multi-component system demonstrates the synergistic formation of trimetallic or hybrid antimicrobial films by metallic nanoparticles and natural biocides. Active packaging films with silver in ZnO nanoparticles and catechin in chitosan/polyvinyl alcohol matrices protected strawberries for 12 days at 4°C, almost completely inhibiting *Staphylococcus aureus* and *E. coli* and improving barrier properties (Meshkini & Khoshshokhan, 2025). Non-trimetallic biohybrids demonstrate the potential of doping and multiple antimicrobial agents in food packaging.

Conclusion

Biosynthesis of trimetallic nanoparticles is an attractive green and sustainable approach for chemical and physical synthesis. Biologically synthesized trimetallic nanoparticles are more stable, synergistic and multifunctional than their monometallic and bimetallic counterparts. The biological agents decreased the environmental impact and enhanced the biocompatibility of these nanoparticles, which are therefore useful in catalysis, environmental remediation and biomedical applications. Green synthesis is a simple, efficient, and scalable approach for producing nanostructures. Further research is required to optimize the synthesis process and to determine their full practical applications.

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Effect of Climate Change on Coral Mortality

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Abstract

Coral reefs are among the most biologically diverse and economically valuable marine ecosystems, supporting nearly one-third of marine fish species and providing essential ecosystem services such as fisheries, coastal protection, and biodiversity maintenance. However, coral reefs are increasingly threatened by climate change-induced ocean warming, ocean acidification, and anthropogenic disturbances, including pollution and overfishing. Coral bleaching, a stress response resulting from the breakdown of the symbiotic relationship between corals and zooxanthellae (*Symbiodinium spp.*), has emerged as one of the most significant causes of coral mortality worldwide. Elevated sea surface temperature (SST), often combined with high irradiance, disrupts photosynthesis in symbiotic algae, leading to oxidative stress, reactive oxygen species (ROS) production, and eventual expulsion of symbionts from coral tissues. This review examines the ecological role of coral reefs, environmental factors affecting coral survival, and the mechanisms underlying coral bleaching. It highlights the importance of coral–algal symbiosis, the role of zooxanthellae in nutrient exchange and calcification, and the influence of thermal stress, ultraviolet radiation, and photoinhibition on coral physiology. Mass bleaching events since the 1980s show the increasing frequency and severity of bleaching under global warming. Some reefs exhibit resilience and adaptive potential, projections indicate that increasing SST and ocean acidification may lead to annual bleaching events in many regions within the coming decades, threatening long-term reef persistence. Therefore, strengthened conservation strategies, climate mitigation efforts, and targeted management of resilient reef systems are urgently required to safeguard coral reef ecosystems and their ecological and socioeconomic functions.

Keywords: *DHW; Oxidative Stress; Resilience; ROS; Symbiotic Algae*

Introduction

Coral reefs are one of the most threatened marine ecosystems because of different factors, including high and destructive fishing, sedimentation pollution and climate change. It has been predicted that seawater temperature will rise further and cause coral death in the next few decades. Elevated temperatures interacting with other physicochemical, biological and anthropogenic factors is the root cause of stress in the coral community and its bleaching. It has been predicted that seawater temperature will increase further and cause coral death in the next few decades. Elevated temperature in association with other physicochemical, biological and anthropogenic factors is the root cause of mortality and bleaching in coral communities (McClanahan *et al.*, 2007).

Climate change also includes rising sea surface temperatures, and global warming poses a major threat to coral communities due to the increased frequency and intensity of coral bleaching events. The continuous warming of oceans is intensifying marine heatwaves, increasing their frequency and duration, and creating stressful conditions that make coral bleaching more frequent and severe (Yu-Long & Chun-Zai, 2026). Thermal stress is one of the major causes of coral bleaching. An analysis of coral bleaching in 81 countries during the period 1998–2017 showed that bleaching is most common in areas where thermal stress events are more frequent and intense. Reefs exposed to high variability in in-sea surface temperature (SST) anomalies showed less bleaching, indicating increased thermal tolerance. Coral bleaching is one of the greatest current threats to reef ecosystems worldwide and occurs when water temperatures exceed the

thermal tolerance of corals (Rivera-Sosa *et al.*, 2025). The highest levels of coral bleaching have been observed in tropical mid-latitudes, approximately 15°–20° north and south, rather than near the equator, despite similar levels of thermal stress. Bleaching started at about 0.50°C higher SSTs than earlier, causing loss of heat-sensitive corals, resulting in higher thermal thresholds in remaining coral populations (Sully *et al.*, 2019).

Coral reefs emit marine biogenic aerosols (sulfur compounds), which play a major role in local climate and influence ocean albedo and cloud formation. Coral reefs are a major source of local biogenic aerosols. A study reveals that beyond the critical thermal stress threshold, corals can no longer influence atmospheric properties. Above this tipping point, corals become vulnerable and undergo mass bleaching. Under this condition, corals can't release aerosols. So, sea surface temperature increase plays an important role in coral bleaching and local climate.

Corals contain a symbiotic alga, and at the time of bleaching, they lose these algae (zooxanthellae), which is responsible for most coral pigmentation. Thermal bleaching occurs due to prolonged exposure to abnormal temperatures, leading to reduced coral biomass. Significant coral mortality has happened in the last 17 years, which are recurrent local, regional, and global bleaching events. The most widespread and severe bleaching occurred during 1997-98, when coral reefs of more than 42 countries were affected by massive coral mortality. The countries that were affected were southern Japan, Sri Lanka, the Maldives, India, Kenya, Tanzania, the Seychelles and other sites in the Indo-Pacific (Fitt *et al.*, 2001).

In this review article, I will search for the root causes of coral mortality and bleaching.

Role of Coral Reefs in the Ecosystem:

Coral reefs are mainly found between the tropics of Capricorn and Cancer and support nearly one-third of all marine fish species as well as thousands of marine organisms. They are ecologically and economically valuable, providing about 6 million tons of fish each year. These resources support both international fishing industries and local coastal communities that depend on reef fish for food and livelihood. In addition to that, coral reefs act as a natural barrier against waves and storms by reducing wave energy through reflection and dissipation, thereby protecting coastal land and nearly half a million people living within 100 km of the reef system (Crabbe, 2008).

Several Environmental Factors Which Influence Coral Survival:

Temperature, light intensity, calcium carbonate saturation, turbidity, sedimentation, salinity, pH and nutrient availability influence the growth and survival of corals. These factors regulate key physiological processes such as photosynthesis and calcification, which limit coral reefs to particular regions of the world oceans. Currently, coral reefs face serious threats from climate change as well as human activities like pollution and overfishing (Crabbe, 2008).

What Is Coral Bleaching?

Most of the color in corals comes from symbiotic algae called zooxanthellae that live within their tissues. Coral bleaching occurs when corals lose these algae. Thermal bleaching happens when corals lose these algae. Coral bleaching occurs when corals lose these algae. Thermal bleaching happens when corals are exposed to temperatures that remain above or below normal for long periods, such temperature stress increases the corals' energy requirements, depletes stored reserves and reduces overall biomass (Crabbe, 2008).

Bleaching can lead to coral death, although some corals may recover, either partially or completely. The process also shows coral growth, recovery from bleaching of coral reefs declines significantly. This trend has been observed during several natural bleaching events, including those in 1983-1984, 1998 and 2005.

Scientists have created mathematical models that examine coevolution to understand how corals might develop resistance to bleaching under rising temperatures (Crabbe, 2008).

Coral-Algal Symbiosis

Corals may adapt to rising temperatures by forming symbioses with more heat-tolerant *Symbiodinium sp.*, although the extent of this adaptation remains debated. Understanding future thermal stress on coral reefs and the level of adaptation required to prevent frequent bleaching is important for both coral reef conservation and global climate policy.

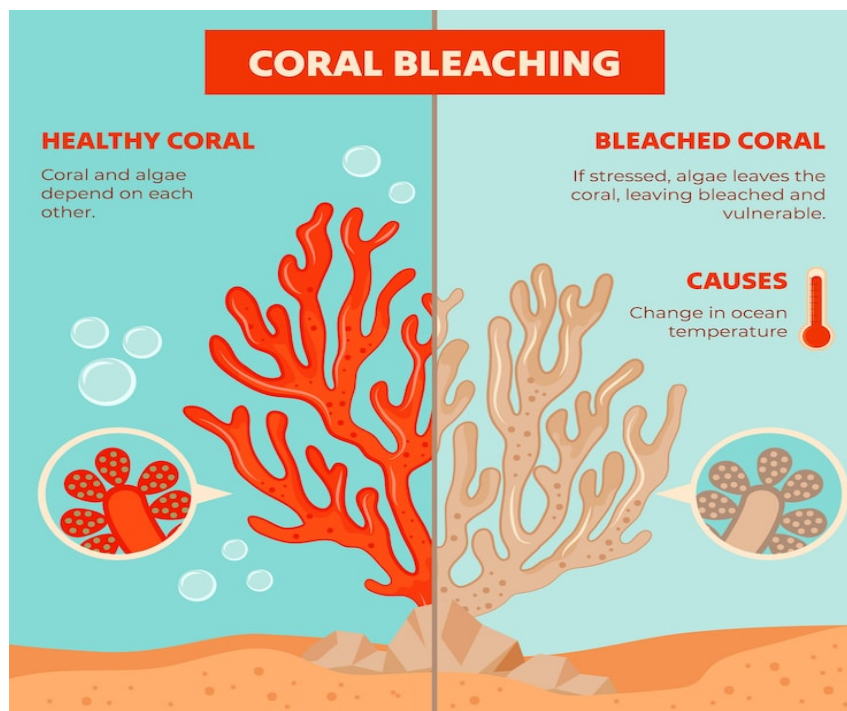


Figure 1: Healthy Vs Bleached Coral

Bleaching Prediction System

The bleaching prediction system developed by NOAA Coral Reef Watch uses satellite data to monitor unusually warm ocean temperatures and measure thermal stress through Degree Heating Weeks (DHW), which effectively predicts coral bleaching events. These real-time warnings are widely used by scientists and marine managers. Historical satellite data from NOAA Coral Reef Watch and global reef maps help to develop methods to project coral bleaching from climate model outputs and forecast the future of mass bleaching under different emission scenarios, as well as the level of increased thermal tolerance needed for coral survival (Donner *et al.*, 2005).

Flexibility of Coral Algal Symbiosis

The flexibility of symbiosis enables the corals to gradually improve their resistance to thermal stress and assist in the recovery of coral cover following bleaching events. Some species of *Symbiodinium* possess higher temperature tolerance, which helps explain why certain corals are able to survive mass bleaching or persist in warmer environments. Corals can acquire a variety of *Symbiodinium* types during their early life stages and may reorganize these symbiotic associations over time in response to thermal stress. They may also obtain different *Symbiodinium* from the surrounding environment when stressed. In some coral communities, shifts towards more heat-tolerant *Symbiodinium* after bleaching have been observed,

suggesting a potential mechanism for adaptation to climate change. However, clear evidence of corals switching completely to entirely new symbiotic partnerships is still lacking (Donner *et al.*, 2005).

Mass Coral Bleaching

Unprecedented mass coral bleaching events associated with increased sea surface temperatures and global warming have been occurring since the 1980s, including three pantropical episodes in 1998, 2010 and 2015-2016. As a result of marine heatwaves or thermal stress, the coral-algal symbiotic relationship with *Symbiodinium spp.* is disrupted, leading to the loss of coral pigmentation. Bleaching causes significant physiological stress to corals and when it persists for extended periods, it often results in high mortality rates. A study indicates that bleaching events are individual disturbances to reefs and focuses on three recurrent bleaching events over the past 18 years along the 2,300 km length of the Great Barrier Reef (Hughes *et al.*, 2017).

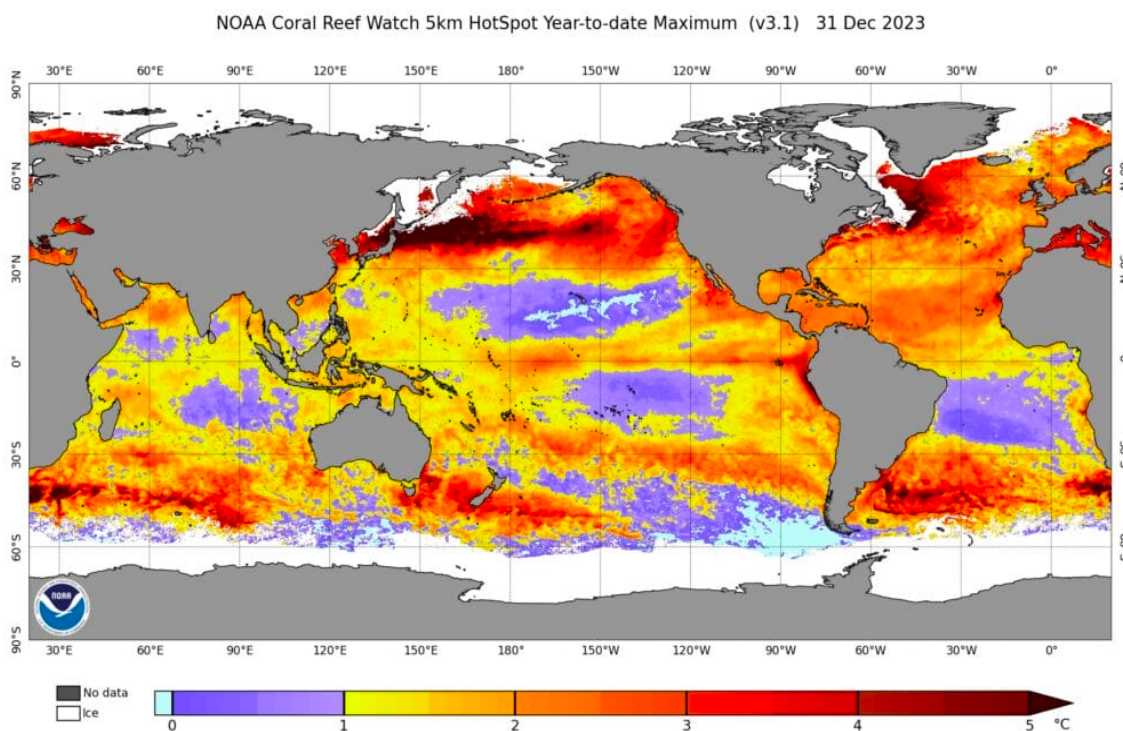


Figure 2: Bleaching Hotspots (Map)

Resistance and Adaptation to Bleaching

Severe bleaching occurred widely across the Great Barrier Reef, regardless of the water quality or protection level. In addition, reefs that experienced bleaching in 1998 or 2002 did not show reduced bleaching in 2016, suggesting that past exposure did not provide significant acclimation or adaptation, likely because the 2016 heat stress was extremely severe. Severe bleaching can affect even very old corals, while milder bleaching is selective, with some resistant species (winners) surviving better than susceptible ones (losers). Fast-growing corals can recover in 10-15 years, but long-lived corals take decades to replace once they die. As the global temperature continues to rise, the long recovery periods are unlikely, meaning coral community structure in the north Great Barrier Reef may shift permanently (Hughes *et al.*, 2017).

Role of Zooxanthellae

Zooxanthellae photosynthesize and live inside the coral host, providing 95% of their photosynthetic production to it. The algae provide amino acids, sugar, complex carbohydrates and small peptides across

the host-symbiont barrier. In return, corals supply the zooxanthellae with their crucial plant nutrients (ammonia and phosphate) from their waste metabolic products, which is very crucial for the survival of these primary producers in marine water.

Photosynthetic oxygen evolution and a loss of variable fluorescence have been reported in cultured zooxanthellae when exposed to temperatures of 34–36°C. The same algae within Caribbean corals are exposed to 32° and 34°C. These studies indicated that corals and these algae, when exposed to heat, caused a malfunction of light reactions. High light intensities will lead to over-reduction of the light reactions and the production of harmful products, like oxygen-free radicals. Temperatures of the tropical seas have increased in the last 100 years; coral cores of the central Pacific confirm this. A 1–2°C sea temperature increase happened due to enhanced concentrations of atmospheric greenhouse gases like CO₂. The emission scenario was specified by the IPCC (Intergovernmental Panel on Climate Change 1992). Greenhouse gases like CFCs and HCFCs will be doubled in the year 2100 (Hoegh-Guildberg, 1999).

Short-wavelength radiation, especially ultraviolet radiation (UVR, 290–400 nm), significantly affects the distribution and physiology of marine organisms, including corals and dinoflagellates. UVR can cause harmful effects, reducing growth rates, chlorophyll content, carbon:nitrogen ratios, photosynthetic oxygen production and the activity of the enzyme Rubisco in these symbionts. Similar negative impacts occur when the dinoflagellates live within the coral tissues. To cope with UVR stress, both corals (hosts) and their symbionts have evolved mycosporine-like amino acids (natural sunscreen compounds that absorb UV radiation) and antioxidant systems that neutralize harmful reactive oxygen species (Hoegh-Guildberg, 1999).

Adaptive Bleaching Hypothesis

It proposes that corals survive stress by replacing sensitive zooxanthellae with more heat-tolerant types, but it lacks strong evidence. There is no direct observation of corals actively switching symbionts during stress. Coral bleaching has a strong impact on coral survival after increasing death rates significantly. The extent of mortality varies on the severity of the bleaching event. In mild cases, coral death may be very slow. In case of severe bleaching events, mortality can reach nearly 100%, as observed in Indonesia and during the 1982–83 eastern Pacific event. In other regions, such as the central and western Pacific (1991 and 1994), coral mortality has ranged between 30 and 50%. Mortality among family-specific staghorn corals (Acroporidae) is the worst affected. Bleaching affected all the colonies of *Acropora hyacinthus* and *A. gemmifera*, and 70% - 80% were dead 5 weeks after the onset of bleaching. The indication of severity of the 1998 bleaching event on the Great Barrier Reef, where corals of 700 years died (Hoegh-Guildberg, 1999).

Different Areas Affected by Coral Bleaching

Projections of sea surface temperature (SST) indicate that coral bleaching events are likely to increase rapidly in frequency. The greatest rise is expected in the Caribbean, Southeast Asia, and the Great Barrier Reef, whereas the Central Pacific is predicted to experience comparatively fewer events. It is anticipated that bleaching may occur every year in most ocean regions by about 2040, with the Caribbean and Southeast Asia potentially reaching annual bleaching conditions by 2020. These bleaching episodes are caused by seasonal increases in seawater temperatures rather than by El Niño events. The results suggest that there is about a 50% likelihood that SST will become high enough to cause coral bleaching by around 2030 in areas such as Comoros and Chagos in the Indian Ocean. The National Oceanic and Atmospheric Administration operates the NOAA Coral Reef Watch program. This system provides satellite-based monitoring and alert services for coral bleaching through online platforms (Crabbe, 2008).

Coral Reefs Produces Aerosol Helps in Cloud Formation

Mass coral bleaching events are becoming increased conditions, and photosystems of symbiotic algae are frequently singular across the Great Barrier Reef (GBR), indicating that continuous ocean warming is surpassing the natural protective capacity of corals. When SST and light intensity rise beyond the physiological tolerance of corals, their stress defense mechanisms begin to fail. Under such stressful conditions, photosystems of symbiotic algae (Zooxanthellae) produce a high amount of Reactive Oxygen species (ROS), which are harmful to coral cells. To counteract this oxidative stress, corals activate their antioxidant defense system. This response increases the intracellular consumption of dimethylsulfopropionate (DMSP), a compound that normally breaks down to produce dimethylsulfide (DMS), an important aerosol precursor gas. Due to high SST and irradiance, a reduction in DMS emission could leave significant climate consequences. Lower DMS emissions may reduce the formation of cloud condensation nuclei (CCN) and low-level clouds (LLC). With fewer clouds reflecting sunlight. So more solar radiation reaches the ocean surface, increasing irradiance and further raising SST. This process can intensify coral stress and create a positive feedback loop, where warming leads to reduced cloud formation (Jackson *et al.*, 2018).

Coral Reef Resilience

Corals that have acclimatized or adapted to thermal stress are more tolerant to high temperatures and radiation, resulting in lower bleaching levels. However, this increased tolerance may lead to the loss of coral species that are less capable of coping with environmental fluctuations. To understand coral reef resilience, researchers have increasingly studied stressed or marginal reef environments. Although such reefs are often less productive and less diverse, they can provide valuable insights into coral resistance. Researchers have increasingly studied stressed or marginal reef environments. More important for conservation and management are reefs that maintain high coral cover, species diversity, and ecological functioning despite disturbances. Reefs in Tanzania, the Comoros, and possibly northern Madagascar show these characteristics, maintaining high coral cover and diversity even after extreme disturbances, making them important priorities for future research and management (McClanahan *et al.*, 2007).

Photoinhibition causes bleaching of symbiotic algae are of two types:

1. Chronic Photoinhibition

- Chronic photoinhibition refers to irreversible photodamage in the photosynthetic system of symbiotic algae, which occurs when the biochemical pathway responsible for synthesis or repair of photosystem components malfunctions.
- The damage can lead to the death of individual algal cells.
- The damaged symbionts are eventually expelled from the coral host through processes such as exocytosis or host cell detachment

2. Dynamic Photoinhibition

- It is a reversible photostress response of algae. Where light energy is diverted away from photosynthesis through nonphotochemical quenching.
- One protective mechanism is the xanthophyll cycle, which redirects excess photons away from photosystem II, especially during midday high-light conditions in shallow-water corals.
- This helps prevent damage to the photosynthetic system, which can also be measured by PAM fluorometry and may act as a protective precursor to chronic photoinhibition.

Putnam and colleagues (2017) suggested that during coral bleaching, the symbiotic algae play a relatively passive role. The algae remain inside host digestive cells that are shed from the coral's gastrodermal layer. Laboratory study indicates that coral hosts are more heat tolerant than symbiotic dinoflagellates (less heat tolerant) (Fitt *et al.*, 2001).

Little work has been done on sensitive indicators of temperature stress in corals and their symbiotic algae, such as induction of heat shock proteins. Such stress proteins are known to confer an ability upon cells to survive previously lethal temperatures, and their induction has been found on exposure of corals to elevated temperatures in the Caribbean genus *Montastrea* sp. (Fitt *et al.*, 2001).

Oxidative Stress and Oxygen Radical Formation

Oxidative stress caused by oxygen radicals in coral-algal symbiotic systems both under normal conditions and during light and temperature stress. Reactive oxygen species such as superoxide and hydrogen peroxide are usually detected indirectly by measuring antioxidant enzymes like superoxide dismutase, catalase, and ascorbate peroxidase, which help remove these harmful molecules (Fitt *et al.*, 2001). Under high temperature and light, chloroplast membranes in symbiotic algae having high levels of unsaturated fatty acids become vulnerable to oxidative damage. The damage can be measured through lipid peroxidation (Fitt *et al.*, 2001).

Coral Disease

Increased temperature can weaken corals and reduce their resistance to pathogens, making them more susceptible to opportunistic diseases. Studies indicate that coral diseases have increased in number and severity, especially in the Caribbean, suggesting that corals are currently experiencing higher physiological stress than in the past several thousand years.

Many coral diseases peak at the end of the warmest season, when coral tissues are thinner and symbiotic algae (Zooxanthellae) densities are lowest, which likely reduces coral resilience (Fitt *et al.*, 2001).

Temperature Stress Caused Decreased Coral Reproduction

Elevated seawater temperature not only causes coral mortality but also significantly reduces their reproductive capacity. Studies following the 1998 bleaching event at Heron Island showed that many bleached coral species (e.g., *Symphyllia*, *Montipora*, *Acropora humilis*, *Favia gonisiastrea*, *Platygyra daedalea*) produced no eggs at all, while others (e.g., *Acropora aspera*, *A. palifera*, *A. pulchra*, *Montipora digitata*) had significantly fewer eggs compared to unbleached corals.

Even after recovering their symbiotic algae (Zooxanthellae), bleached corals often failed to spawn during normal reproductive periods. This indicates that the effects of bleaching persist beyond visible recovery (Hoegh-Guildberg, 1999).

Climate Change Affects Coral Calcification Process

- Photosynthetic activity of zooxanthellae provides them energy for calcification.
- Reduced photosynthesis: during stress or bleaching, corals show reduced growth and calcification. This weakens their ability to compete for space with organisms like microalgae.
- Shift in reef communities: Reduced coral performance can lead to long-term ecological changes, where coral-dominated reefs shift to microalgae-dominated communities, found in the Caribbean and eastern Pacific (Hoegh-Guildberg, 1999).

Discussion

Faster increases in SST are associated with higher levels of bleaching. Global climate models project that SST will increase approximately 0.0270°C/year between 1990 and 2090, which is nearly twice the rate (0.0150°C/year) observed during the previous three decades. If ocean temperatures continue to rise more rapidly, a greater number of reef locations are expected to experience bleaching events (Sully *et al.*, 2019). Studies indicated that equatorial coral reefs show greater resilience to episodes of extreme heat. Because corals in these regions are constantly exposed to variable temperatures, they may develop greater

tolerance to thermal stress. As a result, these regions are considered important priorities for conservation, since those reefs located there are more likely to persist under future climatic conditions (Sully *et al.*, 2019).

Bleaching event in 2016, which was one of the most severe bleaching events ever recorded and was associated with extremely high SST and a strong El Niño. Approximately 93% of reefs within the GBR were highly affected, which resulted in a loss of about two-thirds of corals in the northern Great Barrier Reef and 29% of shallow water corals across the entire reef system. Bleaching was most severe in northern GBR, while central GBR showed moderate impacts (Jackson *et al.*, 2018).

The bleaching event from January to March in 2002 caused moderate to severe bleaching of 41% of offshore reefs and 72% of inshore reefs across the Great Barrier Reef (Jackson *et al.*, 2018).

Elevated temperature combined with high levels of light or irradiance can intensify bleaching. However, ultraviolet radiation is not considered the main cause of large-scale bleaching events. Seasurface temperature analyses show that the warmest parts of the WPWP have warmed less than other tropical ocean areas, suggesting the presence of natural “thermostat” mechanisms that help limit temperature increase beyond certain thresholds (Crabbe, 2008).

Rising sea surface temperatures have been widely predicted to cause significant coral mortality in the coming decades. Elevated temperatures, combined with other physicochemical, biological and human-induced factors, are regarded as the main cause of coral bleaching (McClanahan *et al.*, 2007).

Their study contributes to existing knowledge by analyzing the spatial patterns of temperature variation and the recent increase in temperature within East African coastal current systems between 1951 and 2002. It also examines how that temperature patterns may be linked to spatial differences in coral mortality observed after the 1998 El Niño Southern Oscillation (ENSO) event.

Bleaching event in 2005: In which, after a thermal hotspot developed in the Southern Indian Ocean in January 2005, coral bleaching surveys were carried out in April 2005 on eight reefs along the Kenyan coast, shortly after sea surface temperature reached its peak. Bleaching was characterized as loss of normal coral coloration and estimated by examining color intensity among randomly selected coral colonies. Each colony was categorized into one of six bleaching conditions: 1) unbleached with normal coloration 2) pale or lighter-than-normal color 3) 0-20% of the surface is bleached and 4) 20-50% is bleached 5) 50-80% bleached 6) 80-100% bleached. A difference is observed in bleaching because different genetic types of symbiotic algae may exist within the corals. These symbionts can vary in their environmental tolerance and light absorption capacity (McClanahan *et al.*, 2007).

The 1998 coral bleaching event had a strong impact on coral cover, with reefs that originally had high coral cover experiencing the greatest damage. The reefs were dominated by branching and encrusting corals, which possess thin tissues, rapid growth rates and high sensitivity to thermal stress, making them more vulnerable to bleaching.

The 1998 coral bleaching event had a strong impact on coral cover, with reefs that originally had high coral cover experiencing the greatest damage. The reefs were dominated by branching and encrusting corals, which possess thin tissues, rapid growth rates and high sensitivity to thermal stress, making them more vulnerable to bleaching. During typical warm years, seasonal increases in seawater temperatures often cause bleaching in susceptible coral species, but they recover within a few months. During 1997-98 even more heat-resistant coral forms were affected. Mortality was particularly high among fast-growing corals, while slower-growing and more resistant types survived better (McClanahan *et al.*, 2007).

It has been suggested that corals can gradually acclimate or adapt to temperature changes, while the other proposes that corals have rigid thermal thresholds (around 310°C) beyond which bleaching and mortality occur regardless of past exposure (García *et al.*, 2024). Different models agree that reefs in stable, cooler water with few temperature anomalies have the greatest chance of surviving climate change. Scientists are

focusing on identifying reefs that can maintain high coral cover, biodiversity, and ecological functioning. This interest is linked to the concept of resilience, which refers to the ability of ecosystems to recover and regenerate after major disturbances such as coral bleaching. Consequently, resilience has become an important focus in ecological and resource management research aiming to understand and protect reef systems that are better able to withstand environmental stress.

A bleaching survey conducted in 2005 suggests that many reefs in Tanzania demonstrate strong resistance to bleaching, rapid recovery following bleaching events and the ability to sustain high coral cover and species diversity. The 2005 bleaching survey also enabled an analysis of reef community structure, revealing that Tanzanian reefs are among the most diverse in the Indian Ocean. Their generic diversity is comparable to that reported from the Maldives. These findings are also consistent with observations of high species diversity in the region (McClanahan *et al.*, 2007).

Mass coral bleaching events associated with increased sea surface temperatures and global warming have been occurring since the 1980s, including three pantropical episodes in 1998, 2010 and 2015-2016. As a result of marine heatwaves, or thermal stress, which disrupts the symbiotic relationship between corals and their algal partners, *Symbiodinium spp.* Resulting in the loss of coral pigmentation. Bleaching causes significant physiological stress to corals when it persists for extended periods, resulting in high mortality rates (Thirukanthan *et al.*, 2023).

A study indicates that bleaching events are individual disturbances to reefs and focuses on three recurrent bleaching events over the past 18 years along the 2,300 km length of the Great Barrier Reef. Repeated bleaching events have increased in frequency, with the proportion of affected reefs rising from 43% in 1998 to 56% in 2002 and 85% in 2016, indicating a growing loss of potential refuges for coral reefs (Hughes *et al.*, 2017).

Research on the coral *Montastraea annularis* shows how multiple environmental stressors, such as increased temperature and salinity, affect coral physiology. Scientists created a physiological response surface to illustrate the combined effects of different stressors on coral net photosynthesis. Seasonal changes in sea temperature and solar radiation also influence coral tissues and their symbiotic dinoflagellates (Zooxanthellae). The highest symbiont density and pigmentation usually occur during cooler seasons with lower solar radiation, while the lowest densities occur at the end of the warmest season (Hughes *et al.*, 2017).

Coral tissues can only accommodate a limited number of symbiotic dinoflagellates (Zooxanthellae). This means that the population size of these symbionts is physiologically constrained by the amount of host tissue. Therefore, the density of symbionts is directly related to the amount of coral tissue, although different corals may contain different symbiont densities. Environmental factors such as temperature, solar radiation and nutrient availability can cause changes in these stable densities (Fitt *et al.*, 2001).

The ongoing global decline of coral reefs has prompted the development of several strategies aimed at reducing the warming impacts of greenhouse gases (GHGs). One proposed approach involves the artificial release of sea spray or sulfate particles into the atmosphere to imitate natural biogenic aerosols, thereby increasing the local ocean albedo above coral reef regions. Model simulations suggest that injecting 5 Tg of SO₂ annually over Caribbean coral reefs could lower sea surface temperature (SST), reduce solar irradiance, and limit sea-level rise, resulting in almost no predicted coral bleaching events over the next 50 years.

However, applying this method to extensive reef systems such as the Great Barrier Reef (GBR) would likely be costly and inefficient over the long term. Despite these limitations, such interventions may become necessary to safeguard particularly vulnerable or high-value coral reef ecosystems as ocean warming continues (Jackson *et al.*, 2018).

Elevated temperature causes reduced reproduction in corals and as a result reduced production of larvae (reproductive propagules) leads to slower population recovery, making reefs more vulnerable over time. If bleaching events continue frequently as predicted for the coming decades many surviving corals may fail to reproduce altogether, severely threatening the long-term stability and regeneration of coral reef ecosystems (Hoegh- Guildberg, 1999).

Higher temperature enhances bacterial attachment to coral tissue; the pathogen colonizes the coral. It releases extracellular substances that inhibit photosynthesis in symbiotic algae. This causes loss of algal pigments and algal cell lysis, leading to coral bleaching and eventual coral death (Fitt *et al.*, 2001).

In the coral *Oculina Patagonica*, bleaching incidence increases at the end of the warm season. At higher temperatures, the bacterium *Vibrio* AH-1 becomes more virulent. Laboratory studies showed that bacteria grown at 25°C strongly adhere to coral tissues; at 16°C little or no adhesion occurs. It indicates that sea temperatures activate bacterial adhesion genes, enabling infection (Fitt *et al.*, 2001).

Short wavelength radiation, especially ultraviolet radiation, especially UVR (290-400nm), significantly affects the distribution and physiology of marine organisms including corals and their dinoflagellates (Torregiani & Lesser, 2007). UVR can cause harmful effects, such as reducing growth rates, chlorophyll content, Carbon: Nitrogen ratios, photosynthetic oxygen production and the activity of the enzyme Rubisco in these symbionts.

To cope with UVR stress, both corals (hosts) and their symbionts have evolved protective mechanisms, which include the production of mycosporine-like amino acids (natural sunscreen compounds that absorb UV radiation) and antioxidant systems that neutralize harmful reactive oxygen species (Hoegh-Guildberg, 1999).

Climatic factors like ocean acidification cause seawater acidity and reduce carbonate concentration. Lower aragonite saturation rate, which is essential for coral skeleton and decline of calcification rate (calcification reduced by 14-30% by 2050), affecting reef-building capacity.

Conclusion

Coral bleaching has already caused severe and widespread damage to coral populations worldwide and will continue to affect coral reefs in the next few decades. To prevent further degradation of coral reefs, urgent global action to reduce carbon emissions is essential. Without significant effort to limit climate change, a continued rise in ocean temperature will lead to further declines in coral reefs. Current evidence indicates that corals and their symbiotic zooxanthellae cannot adapt or acclimate quickly enough to cope with rapid ocean warming. Continued coral mortality will likely lead to shifts in coral distribution, disrupting reef structure and function.

As corals and zooxanthellae are fundamental to reef ecosystems, these changes will have serious ecological and economic consequences globally by the mid to late 21st century. However, these impacts are still not fully assessed and require urgent research. Climate change can shift coral community structure by favoring more heat-tolerant species while reducing or eliminating sensitive ones. Even under moderate greenhouse gas scenarios (like IS92a), rising sea temperatures are expected to severely impact coral reefs within the next 20–30 years. By around 2040, most reefs will likely experience near-annual bleaching events, exceeding the severity of the 1998 event. Protecting coral reefs, including heavily managed systems like the Great Barrier Reef, requires urgent and rapid global action to reduce climate change and global warming.

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Sustainable Bimetallic Nanoparticles: Synthesis Approaches and Their Medicinal and Catalytic Activities

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Abstract

A novel category of materials has been exclusively studied *i.e* bimetallic nanoparticles (BNPs), which have manifested more embellished performance than their monometallic counterparts. In recent years, the universe witnessed an explosion of inventions and applications of BNPs, as they have much enhanced molecular stability, reactivity, reaction selectivity, and potency in biomedical, industrial and environmental remediation due to their distinctive physicochemical properties. Researchers have proposed various nanoparticle synthesis procedures, but most of them involve expensive and harmful chemicals. Being a significant obstacle to scientific progress, eco-friendly, sustainable approaches are more appreciable for nanomaterials production with minimum environmental impact. This review examines sustainable physical and green synthesis methods for bimetallic nanoparticles, as well as their medicinal and catalytic applications.

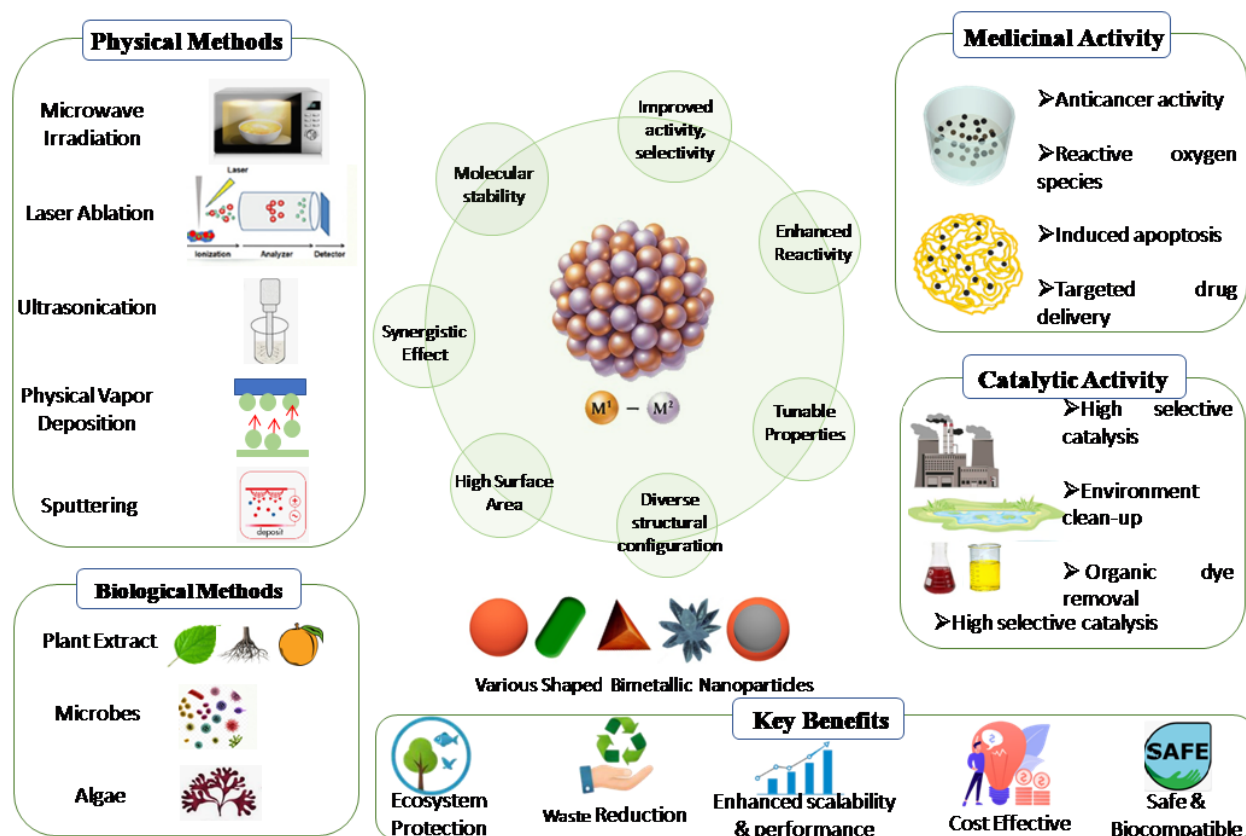
Keywords: *Bimetallic Nanoparticles; Biomedical and Catalytic Activities; Sustainable Synthesis Procedure*

Introduction

Nanotechnology has turned up as one of the most dynamic and fast-paced multidisciplinary fields, creating remarkable prospects for the generation of functional materials with tailored properties (Lim *et al.*, 2018). Nanoparticles, defined by the size range from 1 to 100 nm, are novel materials that have distinct physical and chemical properties and are distinct either from their bulk materials or from the smallest atoms due to their high surface-to-volume ratio. Scientists find nanomaterials more alluring due to the presence of tunable surface characteristics and also they have found widespread application in various fields such as drug delivery (Ponchel & Cauchois, 2017), environmental remediation, energy conversion (Kumar *et al.*, 2022) and catalysis (Lim *et al.*, 2018). Nowadays, researchers have paid attention to metallic nanoparticles among the diverse range of nanomaterials, due to their remarkable magnetic & optical (Srinoi *et al.*, 2018), electrical, catalytic, and biological properties (Thakur & Thakur, 2022). There are three methods—physical, chemical, and biological—to produce metal nanoparticles, which are different from the others in terms of efficiency, cost, environmental impact (Kabir *et al.*, 2018), and scalability. Engaging with toxic (Awasthi & Sharma, 2022) and harmful chemicals, consuming high-energy, environmentally unsafe by-products, and decreasing the acceptability of conventional chemical methods are significant concerns. Sustainable methods, such as physical routes (Krishnia *et al.*, 2022) like microwave irradiation, ultrasonication, and laser ablation, rely on the application of physical energy for nanoparticle synthesis, while biological routes (Salem & Fouda, 2020) that physical routes (Krishnia *et al.*, 2022) such as microwave irradiation, ultrasonication, and laser ablation rely on the application of physical energy for nanoparticle synthesis, and biological routes (Salem & Fouda, 2020) which involve microorganisms are gaining popularity as renewable (Larrañaga-Tapia *et al.*, 2024) and eco-friendly alternatives. They both reduce or eliminate the need for chemical reactions. Here, biological methods exploit plant extracts and other biological agents such as microorganisms (e.g., bacteria, fungi, and yeast) as reducing and stabilizing agents (Salem & Fouda, 2020). Both preparative methods have become highly demanding for the production of sustainable nanomaterials due to attributes such as low cost, biocompatibility, scalability, and minimal environmental impact.

Due to the synergistic and integrated effects attributable to the different metals forming the bimetallic nanoparticle (BNP), BNPs outperform monometallic counterparts in terms of catalytic activities and features

such as stability, biocompatibility, and even therapeutic efficacy. This is very important for applications in catalytic processes and biomedicine. In the biomedical field, BNPs show remarkable anti-cancer activities by producing reactive oxygen species, triggering apoptosis, and specifically exhibiting cytotoxic properties to cancer cells. In addition, their high catalytic efficiency has been applied to pollutant degradation in environmental remediation and various chemical transformation processes. The combination of the synergistic effect and tunable structure leading to enhanced therapeutic and catalytic activities makes BNPs essential materials for future sustainable nanotechnology. This review paper outlines the latest developments in green BNP fabrication processes and discusses their medicinal and catalytic applications.



Scheme 1: Bimetallic Nanoparticles Sustainable Synthesis Procedure and their Applications

Bimetallic Nanoparticles:

The combination of two metals at the nanoscale imparts distinct properties to BNPs; furthermore, synergistic effects (Hao *et al.*, 2024) render them more effective than single-metal systems, enhancing their catalytic, stability-related, optical, and electronic characteristics. BNPs adopt spherical (Długosz *et al.*, 2021), rod-shaped (Elsayed *et al.*, 2022), wire (Tsuiji, 2017), alloy (Abbaspour & Norouz-Sarvestani, 2013) or core-shell (Jiang *et al.*, 2019), flower (Odoom-Wubah *et al.*, 2017), star shape (Censabella *et al.*, 2019) of formation.

The shape and structural arrangement of BNPs play a critical role in regulating nanoparticle activity rates and determining their optical, catalytic, electrical, and biomedical properties (Srinoi *et al.*, 2018). The surface-to-volume ratio, which is fundamentally influenced by nanoparticle shape, governs these activity rates. Distinct nanoparticle morphologies possess unique surface energies, active sites, and crystal planes, all of which significantly affect their effectiveness in applications such as cancer prevention and catalysis.

Physical Synthesis of Bimetallic Nanoparticles:

When conventional chemical synthesis or biological agents are not used to synthesize nanoparticles, physical methods (Nyabadza *et al.*, 2023a), which rely on the "top-down" approach, come to the fore. These methods generally produce high-purity nanoparticles with controlled structure and shape.

Microwave Assisted Synthesis: The microwave-assisted method of BNP synthesis, a physical process, offers a fast, productive, and controllable procedure. This method 1) ensures rapid and uniform heating, which accelerates the reaction rate and provides superior control over nanoparticle nucleation and growth 2) maintains consistency in the particle size and morphology of the nanocomposites; 3) enhances process efficiency, including high selectivity and yield and 4) promotes energy efficiency by enabling faster heating compared to conventional heating methods.

CuPd, CuRh, AuPd, AuRh, PtRh, PdRh, AuPt bimetallic nanoalloys (Abdelsayed *et al.*, 2009) have been prepared by microwave irradiation method. Core-shell AuPd nanoparticles (Harpeness & Gedanken, 2004) and Ag-Rh BNPs (Pande *et al.*, 2018) have been synthesized by the microwave-assisted polyol reduction method.

Laser ablation Process:

Laser ablation is a facile and eco-friendly physical method in which a high-energized laser beam is applied to a solid metal target immersed in a liquid, gas, or vacuum; this causes the emission of atoms and the direct formation of BNPs (Nyabadza *et al.*, 2023b).

Ultrasonication Process:

A simple and fast physical method used for BNPs (Kan *et al.*, 2003), preparation employs high-frequency ultrasound waves, generating localized high temperatures to promote BNPs generation. Because this method uses very few harmful chemicals or stabilizing agents, it produces high-purity products with strong potential and lowers negative effects on the environment.

Physical Vapor Deposition (PVD):

In the Physical Vapor Deposition (PVD) method, BNPs are formed on a substrate by vaporizing a solid source and subsequently condensing and depositing the vapor; resulting in high-purity, defect-free nanostructures (Beyene *et al.*, 2012).

Sputtering:

Sputtering is a physical process used to achieve high purity and control over composition. To achieve excellent purity and composition control, sputtering in this method energetic ions strike a bulk metal target, ejecting atoms that condense into BNPs (Nguyen *et al.*, 2017).

Electrical Explosion of Wire (EEW):

The 'Electrical Explosion of Wire' (EEW) method, also known as 'Pulsed Wire Discharge' (PWD) is a highly effective 'top-down' physical technique for producing high-purity nanomaterial. During this process, a high-density current pulse is applied to a thin metal wire, resulting in rapid Joule heating. The solid wire is converted into a high-pressure plasma and vapor state, after some time, the vapor rapidly condenses to form nanoparticles (Suliz *et al.*, 2022; Pervikov *et al.*, 2020).

Arc Discharge Method:

In this physical synthesis method, a high-temperature (3000–6000°C) plasma arc is generated between two metal electrodes by inducing the electrical breakdown of gas via the 'arc discharge' process. The intense heat turns the anode material into vapor. After some time, the vapor condenses in an inert gas atmosphere, such as helium or argon, to form BNPs (Qu *et al.*, 2020).

Table 1: Biimetallic Nanoparticles Synthesis Using Physical Method

Nanoparticles Type	Method	Size (nm)	Morphology	Applications	References
Ag-Cu and Cu-Ag BNP	Microwave assisted route	Average size 27-97 & 32-184	Spherical	Antiviral activity	Długosz <i>et al.</i> , 2021
Ag-Ir alloy nanoparticle	Microwave irradiation	Average size 20	Spherical, wire	Catalytic activity	Guo <i>et al.</i> , 2018
Ag-Dy BNP	Microwave method	Average size ~ 10	Spherical	Medicinal activity	Mishra & Kannan, 2016
LA@Au-Ag Nanocluster	Microwave assisted method	Average size 1.96-3.30	-	Medicinal activity	Sharma <i>et al.</i> , 2020
Pd-Pt BNP	Laser ablation process	Average size 10-15	starry sky	Catalytic activity	Censabella <i>et al.</i> , 2019
ZnO-Ag BNP	Laser ablation process	Average size 30-130	Nanorods, Nanosheets	Anticancer activity	Elsayed <i>et al.</i> , 2022
Cu-Fe and Cu-Ni	Laser ablation process	Average size 20 & 34	Spherical	Medicinal activity	Ali <i>et al.</i> , 2023
Ag-Pd, Ag-Au, Ag-Pt BNP	Ultrasonic-assisted route	Average size 20-30, 30, 15	Spherical	Catalytic activity	Marimuthu <i>et al.</i> , 2021
Ag-Cu BNP	Physical vapour deposition	Average size ~7	Dot	Medicinal activity	Hao <i>et al.</i> , 2020
Pd-Au BNP	Plasma Sputtering deposition	-	-	Catalytic activity	Guo <i>et al.</i> , 2011
Ti-Ag and Fe-Ag BNP	Electrical explosion of wire	Average size 70, 70-90	-	Antimicrobial activity	Svarovskaya <i>et al.</i> , 2020
Pt-Ni @MWCNT	Arc Discharge Method	Average size 26.55-27.55	Spherical	Medicinal activity	Bekmezci <i>et al.</i> , 2024

Green Synthesis of Bimetallic Nanoparticles:

In response to the escalating demand for the sustainable synthesis of BNPs, 'green synthesis' (Larrañaga-Tapia *et al.*, 2024), an eco-friendly alternative has appeared to replace traditional physical and chemical methods, which are often energy-intensive, hazardous, costly, and environmentally harmful. Green synthesis, also known as eco-friendly synthesis, utilizes biological materials such as plant extracts, bacteria, fungi, yeast, and algae as reducing and stabilizing agents, thereby eliminating the need for toxic or hazardous chemicals. Bioactive molecules, including polyphenols, flavonoids, proteins, enzymes, and polysaccharides. Plants contain bioactive molecules, including polyphenols, flavonoids, proteins, enzymes, and polysaccharides, which help maintain the structure and stability of nanoparticles., contribute to maintaining nanoparticle structure and stability. This approach enables the sustainable production of biocompatible bimetallic nanoparticles for diverse applications (Kumari *et al.*, 2015).

Eco-friendly Plant Based Green Synthesis:

Bioactive organic molecules extractable from plants, such as flavonoids, phenolic acids, terpenoids, alkaloids, proteins, enzymes, carbohydrates, vitamins, and antioxidants are widely used in the synthesis of BNPs as reducing, stabilizing, and coating agents.

Compared to microbe-based methods, using plants to synthesize nanoparticles is simpler, faster, and more cost-effective. This approach does not need culture media and offers better control over the size and shape of the nanoparticles. Various parts of the plant such as leaves (Elemike *et al.*, 2019), roots (Amina *et al.*, 2020), buds, gum, seeds (Emam, 2019), tubers, and fruit peels, have been used successfully to produce

BNP. Plants are promising option for sustainable synthesis of bimetallic nanoparticles because they contain many phytochemicals. These bioactive molecules can be used on a large scale and are environmentally friendly. Still, there are challenges in fully understanding how nanoparticles form and in making sure production is consistent on a larger scale.

Microbes Based Green Synthesis:

Synthesizing BNPs with the assistance of microorganisms like bacteria (Mollania *et al.*, 2024), fungi (Patel *et al.*, 2025), and yeast (Romero-Núñez *et al.*, 2019) is a promising and eco-friendly method. Using whole microbial cells or their extracellular parts in the right conditions is a sustainable and affordable alternative to traditional synthesis. Microbial-assisted production has several clear benefits, such as:

- Rapid microbial growth and ease of cultivation.
- High adaptability to various environmental conditions.
- Inherent metabolic processes that naturally reduce metal ions into nanoparticles.

In this process, the nanoparticles can build up inside the microbial cells or be released outside. This makes microbe-based synthesis a simple, affordable, and eco-friendly way to make high-quality BNPs.

Algae Based Green Synthesis:

Algae are well known for their role in making nanoparticles and are seen as a promising biological source for green synthesis of biogenic nanoparticles (BNPs) (González-Ballesteros *et al.*, 2018; Chaudhary *et al.*, 2020). This method is considered eco-friendly because algae contain many natural reducing and stabilizing agents, i.e., bioactive compounds, such as secondary metabolites, proteins, peptides, polysaccharides, and pigments. Algae exhibit rapid growth, are straightforward to cultivate and harvest, and can be produced on a large scale at relatively low cost, which makes them suitable for sustainable nanoparticle production. As some of the oldest and most widespread photosynthetic organisms, algae are capable of absorbing metal ions and converting them into nanoparticles, providing an efficient and environmentally friendly method for BNP production (Chugh *et al.*, 2021).

Table 2: Green Synthesis of Bimetallic Nanoparticles

Nanoparticles Type	Source	Size (nm)	Morphology	Applications	References
Ag-Au	Stigmaphyllonovatum (amazonvine) leaf	Average size ~15	Spherical	Anticancer activity	Elemike <i>et al.</i> , 2019
Pd-Pt	<i>Cinnamomum camphora</i> (C. camphora) leaf	Average size 20-60	Flower	Electro-Catalytic Performance	Odoom-Wubah <i>et al.</i> , 2017
Ag-Cu	<i>Rheum emodi</i> Root	Average size 40-50	Spherical	Anticancer activity	Sharma <i>et al.</i> , 2021
Au-Pt	<i>Syzygium aromaticum</i> bud	Average size <3	Spherical	Catalytic activity	Sharma <i>et al.</i> , 2020
Ag-Pd, Au-Pd	Kondagogu gum	Average size 2-40	Spherical	Catalytic activity	Velpula <i>et al.</i> , 2021
Cu-Mn	Pumpkin Seed	Average size ~50	Spherical	Anticancer Efficacy	Alafaleq <i>et al.</i> , 2023
Pt-Pd	<i>Dioscorea bulbifera</i> tuber	Average size 10-25	Spherical	Anticancer activity	Ghosh <i>et al.</i> , 2015
Ag-Cu	Banana peel	-	-	Catalytic activity	Su <i>et al.</i> , 2025
Au-Ag	<i>Escherichia coli</i> bacteria	Average size ~23.01	Core-shell	Medicinal activity	Jiang <i>et al.</i> , 2019
Fe & ZnO Nanoparticles	Daedalea Mushroom	Average size ~16.8	Irregular	Antifungal activity	Kamal <i>et al.</i> , 2023

Applications:

Nanoparticles show a lot of promise in biomedicine. Because they are small, have a large surface area, and can be adjusted in different ways, they interact well with biological systems at both the cellular and molecular levels. Among them, bimetallic nanoparticles have demonstrated enhanced efficacy through synergistic effects between constituent metals. Here two potentities, i.e., medicinal and catalytic activities, have been highlighted.

Medicinal Applications:

Bimetallic nanoparticles exhibit significant therapeutic potential, particularly in anticancer, antimicrobial, antioxidant and anti-inflammatory applications. For instance, *Staphylococcus aureus* ("staph") and *Candida albicans* are types of bacteria and yeast that are commonly found in the bodies of healthy individuals. Although usually harmless, it can cause infections ranging from minor skin lesions to severe, life-threatening conditions when it enters the body through cuts, wounds or immune system weakens. Ag-Cu and Cu-Ag BNPs (Długosz *et al.*, 2021) and Ag-Cu BNPs (Sharma *et al.*, 2021) exhibited antimicrobial activity against those microbes. Ag-Cu and Cu-Ag BNPs (Długosz *et al.*, 2021) have shown antiviral efficacy against Human alphaherpes virus 1 & 2 (HHV-1/HSV-1 & HHV-2/HSV-2) which are contagious DNA viruses of the Herpesviridae family. Those viruses can establish lifelong infections with painful sores, blisters, are characterized by recurrent lesions and asymptomatic viral shedding. Many bimetallic nanoparticles (BNPs) have demonstrated enhanced biocompatibility and therapeutic efficacy against cervical cancer (HeLa) (Elemike *et al.*, 2019) and colorectal cancer (HCT116) cells, HT-29 cells, breast cancer cell lines (MDA-MB-231) (Alafaleq *et al.*, 2023). LA@Au-Ag nanoclusters (Sharma *et al.*, 2020) showed antimicrobial activity against Gram-negative bacteria, while Ti-Ag and Fe-Ag bimetallic nanoparticles (Svarovskaya *et al.*, 2020) demonstrated potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA), both are highly pathogenic antibiotic-resistant bacterium. Accurate determination of uric acid (UA) in urine is crucial for diagnosing urinary system disorders. Pt-Ni@MWCNT nanocomposites (Bekmezci *et al.*, 2024) have been employed as sensitive sensors for the detection of UA at low concentration levels.

Catalytic Applications:

The growing environmental threat posed by organic dye pollution from industries such as textiles, paper, and food processing necessitates the development of innovative remediation strategies. Green-synthesized bimetallic nanoparticles serve as highly efficient catalysts for stability and selectivity. They have been extensively employed in oxidation, reduction, hydrogenation of different pollutants via catalytic reactions. These nanoparticles play a vital role in the degradation of organic pollutants, wastewater treatment, and the synthesis of valuable chemical products. Their superior catalytic performance contributes to the development of sustainable and environmentally friendly chemical processes. Several BNPs show catalytic efficiency for hydrogenation reaction (Guo *et al.*, 2018) to produce industrial application, to build electronic device (Censabella *et al.*, 2019), for methane (asphyxiant) combustion (Guo *et al.*, 2011) and for corrosion inhibition at oil field (Su *et al.*, 2025). Some BNPs (Marimuthu *et al.*, 2021) demonstrated degradation of 4-nitrophenol, which is highly toxic and carcinogenic industrial pollutant for environmental remediation.

Future Perspectives and Challenges

Sustainable BNPs have emerged as promising nanomaterials due to their enhanced medicinal and catalytic properties, coupled with environmentally friendly green synthesis approaches. Despite proficient progress, challenges remain in achieving reproducible and scalable green synthesis, precise control over nanoparticle composition and morphology, and a clear understanding of the mechanisms underlying metal-metal synergistic effects.

Despite its advantages, physical synthesis has several limitations, including high initial equipment costs, potential safety risks associated with improper handling of reaction vessels, long-term toxicity,

biocompatibility, high energy consumption and difficulties in real-time monitoring of reaction progress. Additionally, the process often requires chemical precursors and stabilizing agents, which may reduce its overall sustainability. Green synthesis faces challenges like limited control over size and shape, problems with scaling up, inconsistent results, complicated purification, and differences in biological sources. To use nanoparticles in catalytic applications, we need to make them more stable, reusable, and effective in real-world conditions. There is also very few information about how BNPs affect the environment, so more detailed in vivo and environmental studies are necessary.

Future research should focus on creating clear guidelines for "green synthesis" (eco-friendly synthesis) methods that use different biological resources. It should also work to better explain how structure affects effectiveness and improve safety assessment standards.

Conclusion

Bimetallic nanoparticles (BNPs) have played a key role in advancing nanotechnology because of their unique properties and wide range of uses in biomedicine, catalysis, and environmental cleanup. This review covers details of the green and sustainable method of BNP synthesis using both physical and biological methods, their classification, and their applications in medicine and catalysis. It highlights what makes BNPs special and the different ways they can be used. Recently, using green or eco-friendly synthesis methods along with physical techniques has become a promising and sustainable way to produce BNPs. Physical synthesis methods also tend to have less environmental impact and create less waste than traditional chemical approaches.

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