Trends in Chemical and Biological Research

Edited by

Hari Shankar Biswas
Sandeep Poddar
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Amiya Bhaumik

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Edited by

Dr. Hari Shankar Biswas
Assistant Professor, Department of Chemistry
Surendranath College
Kolkata, India

Dr. Sandeep Poddar
Deputy Vice-Chancellor (Research & Innovation)
Lincoln University College
Malaysia

Dr. Ravinder Nath Anisetty
Vice-Chancellor
Central University of Kashmir
Jammu & Kashmir, India

Dr. Amiya Bhaumik
President
Lincoln University College
Malaysia

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Editors

Hari Shankar Biswas

Rtn. Dr. Hari Shankar Biswas, M.Sc. Ph.D.
Assistant Professor (II) and Ex-Head of the Department
Department of Chemistry, Surendranath College, Kolkata, India

Dr. Biswas received his B.Sc. degree in Chemistry Honours (2005) from R.K.M.V.C. College and his M.Sc. (2008) degree from Presidency College, Kolkata. He was awarded a Ph.D. (2015) in Material Science from the Saha Institute of Nuclear Physics, University of Calcutta. From 2008 to 2010 he was a School teacher at Keshub Academy, Kolkata, then he joined assistant professor in Chemistry at Surendranath College. His current research topic is Carbon thin Film, Graphene, Graphene Oxide-based nano Composite, Synthesis Characterization, and Application in the Environmental, Biological, and Technological fields. He is the author of 10 book chapters and has published more than 23 papers in International Journals. He is a life member of the Indian Science Congress Association and the Indian Chemical Society and an affiliated member of the Royal Society of Chemistry. He is a series book Editor and reviewer of reputed international publishers and journals. He is an Editorial board member of the American Journal of Chemical Engineering, Lincoln University College, and a member of Rotary International. He is also an editor of some books namely: Emerging Concepts in Chemical and Biological Science; Modern Approaches in Chemical and Biological Sciences; Advances in Science Education and Modern Approaches in Chemical and Biological Sciences Vol.2

Sandeep Poddar

Deputy Vice Chancellor (Research & Innovation)
Lincoln University College, Malaysia

Prof. Dr. Sandeep Poddar, presently the Deputy Vice Chancellor (Research & Innovation) of Lincoln University College, Malaysia. He also served as Senior Research Director and Executive Editor(Publications), Lincoln University College, Adjunct Faculty (Honorary), Bharat Center Canada. He has graduated from University of Calcutta in 1993 with Honours in Zoology, he has obtained Post Graduate Diploma in Dietetics from All India Institute of Hygiene and Public Health 1995, Master of Science in Zoology with specialization in Biochemical Genetics from Dayalbagh Educational Institute 1998 with distinction. In addition to this he also obtained Master of Business Administration (MBA) from Lincoln University College in 2021. He has completed Ph.D. in Zoology from Vivekananda Institute of Medical Sciences on Cytotoxicity in 2004. After completing Ph.D. he pursued Post Doctoral Research in different projects on Hemoglobinopathies and Oral Cancer mutation. He is serving as reviewer of several International Journals. He has published several research papers, organized international conferences, and edited books in Malaysia, Australia and India. Dr. Sandeep is founder Assistant Secretary of Dr. Tarak Nath Podder Memorial Foundation, Kolkata, India.

Editors

Ravinder Nath Anisetty

Vice-Chancellor
Central University of Kashmir, Jammu & Kashmir, India

Prof. Ravinder Nath received his B.Sc., Industrial Chemistry from Kakatiya University, India, M.Sc., Ph.D., Chemistry from Osmania University, M.Tech., Biotechnology from JN Technological University, Hyderabad and completed his Post Doc in Pharmacy from Stony Brook University (Formerly SUNY), Stony Brook, New York, USA. He was trained at Oxford University and University of Cambridge. Prof. Ravinder Nath served as an Expert Member & Resource Person at University, State and National level for Higher and Technical Education Governing Regulatory Bodies and also served as Chairman, Convener and Member of various Committees. He held number of honors and awards as a scholar and faculty; He received Visiting Scientist Travel Award by the UGC and PDF Award under Exchange Visitor Program of US Department of State, USA. Teacher's Excellence Award by Confederation of Educational Excellence, New Delhi and Professional Achievers Award by Association Pharmacy Professionals, Bhopal, India. He served as a Member of Academic Senates of Telangana University, Osmania University and also as a Member of Academic Council of Maulana Azad National Urdu University. He is also an active member of various Professional Bodies in the areas of Science and Technology.

Amiya Bhaumik

President
Lincoln University College, Malaysia

Prof. Dr. Amiya Bhaumik is the Founder and Former Vice-Chancellor of Lincoln University College. He is purely from the field of education. Dr. Bhaumik is Executive Vice President of the International Education Consulting Group, St. Louis, USA since 1999. Dr. Amiya Bhaumik was Research Fellow of UNESCO, Paris. During this tenure, Dr. Bhaumik has traveled extensively to Europe, Africa, Asia and Latin America. He has served as Professor of Business Administration in University of Lucknow, India and in University of Malaya and many other places. Dr. Amiya Bhaumik is a very dynamic personality. He has authored numerous book chapters and has huge number of publication in many national and international journals. He has also edited several books.

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Preface

“All our science measured against reality, is primitive and childlike - and yet is the most precious thing we have.” - Albert Einstein.

The pursuit of science represents humanity's most significant collaborative endeavor. Through scientific knowledge, we gain the ability to advance technology, address real-world challenges, and make well-informed decisions, both at the individual and societal levels. Science encompasses the systematic observation, experimentation, and measurement of the characteristics and actions exhibited by the material and physical universe. It also involves formulating general laws to accurately represent these observed facts.

In her paper titled "Oscillatory Systems: Approach from Nonlinear Dynamics," Shrabani Sen provides a concise overview of the fundamental theories necessary for comprehending various non-equilibrium phenomena, including chemical and biological oscillations, as well as spirals. Oscillations, which have been observed in both chemical and biological systems since the emergence of life, are explored in her study. The paper aims to offer a comprehensive understanding of the underlying principles and mechanisms governing these oscillatory systems, highlighting their significance in the field of nonlinear dynamics.

Moumita Roy discusses about a comprehensive review on the significance of chiral vicinal diol units as key intermediates in natural products and drugs. In her paper titled "Immobilized Osmium Catalyst for Asymmetric Dihydroxylation of Olefins," she explores various synthetic routes for the synthesis of enantiomerically enriched 1,2-diols, with a particular focus on the widely favored osmium-catalyzed Sharpless asymmetric dihydroxylation (AD) of alkenes. The paper explores recent advancements in supported osmium catalysts for asymmetric dihydroxylation (AD) reactions, providing insights into their advantages and limitations. It serves as a valuable guide for advancing catalytic systems to improve the efficiency and selectivity of these transformations.

Hydrogen bonding (HB) has been extensively studied for its crucial role and widespread presence in biological structures and reactions for over a century. In the paper entitled "Hydrogen Bonding Probe: Effect of Polarity," Debarati Dey demonstrates that while hydrogen bonding stands as the primary weak force at play in biological environments, many commonly employed probes for detecting hydrogen bonding are influenced by the polarity of the medium. Consequently, the photochemical characteristics exhibited by these probes are influenced by both the dielectric properties and the hydrogen bonding capacity of the medium.

Ants are vital for mangrove ecosystems, yet knowledge about ants in the Indian Sunderbans remains limited. In the paper titled "Abundance of Ants (Hymenoptera: Formicidae) during Pre-Monsoon and Post-Monsoon Seasons in the Mangrove Patches of Indian Sunderbans", Damayanti Bakra explores the topic of ant abundance in the Indian section of the Sunderbans during the pre-monsoon and post-monsoon periods. The study aims to enhance our comprehension of ant populations in this unique environment, shedding light on their distribution and behavior during different seasons.

With approximately 415 million people worldwide affected by diabetes, the monitoring of blood sugar levels is crucial. Nano-sized inorganic materials, such as Fe_2O_3, CuS, CdS, and FeS nanoparticles, have emerged as nano-enzyme models for measuring human blood glucose levels. In the paper "Colorimetric Measurements of Human Blood Glucose Level in the Presence of Nano-Scaled Inorganic Materials," Amit Kumar Dutta discusses the use of these

nano-enzymes as a convenient method for monitoring blood sugar levels. The research highlights the potential of nano-enzymes to revolutionize diabetes management.

Arsenic is a major health concern for a large population, especially in economically developing countries, with potentially severe and fatal consequences. It can be released into the environment through both natural and human activities, involving various processes. In the paper titled "Arsenic Toxicity: Its Existence, Permeability, and Adverse Effects on Human Health - A Brief Overview," Jayita Dutta aims to provide a concise summary of the health risks associated with arsenic poisoning. The paper offers an overview of the detrimental impacts caused by arsenic exposure on human health.

Butterflies, belonging to the Phylum Arthropoda, Class Insecta, and Order Lepidoptera, are a vibrant and significant group of insects. They are not only visually striking but also highly responsive to environmental changes, often serving as bioindicators of climate and playing a crucial role as pollinators. The article titled "Diversity and Ethological Study of Butterfly Species Found in Sree Chaitanya College Campus, North 24 Parganas, West Bengal" authored by Priyankar Sanphui focuses on exploring the diversity and behavioral patterns of butterflies in the specified location.

Conservation plays a crucial role in managing bioresources to support the needs of the bioeconomy. A study was conducted on the ichthyofaunal diversity of the Mridangabhanga River and its surrounding water bodies, which are part of the Ganga distributary. Unfortunately, the area is under constant anthropogenic pressure, leading to the degradation of natural resources. As a result, water quality and fishery potential have been adversely affected. In the paper titled "Study of Fish Diversity of a Major Distributary of River Ganga that Needs Proper Management and Rehabilitation," Lina Sarkar explores the presence of diverse edible fish species in the river, including both freshwater and marine species. The study highlights a concerning trend of declining fish catch, posing a significant threat to the livelihoods of villagers who heavily rely on fishing as their primary source of income.

Turmeric, scientifically known as Curcuma longa, has been widely utilized by humans throughout history for its beneficial phytochemicals. This versatile medicinal plant serves various purposes, ranging from a common household spice to a valuable therapeutic resource. Sonali Ray's study, "Hepatoprotective Effects of Curcumin from Curcuma longa L.: A Comprehensive Account," compiles evidence from the past two decades to provide an insightful and comprehensive overview of the hepatoprotective effects of curcumin derived from C. longa.

Probiotics, non-pathogenic microorganisms, have the potential to improve health, immunity, and mental function when consumed in significant amounts with food. In this study named “Isolation and Characterization of Some Food Grade Lactic Acid Bacteria for their Application as Probiotics” by Shamba Chatterjee and Sucheta Das, seven microbial strains (L1, L2, C1, C2, C3, X, and Y) were isolated from fresh palm sap obtained locally. These strains were then characterized through morphological and biochemical analyses. Bacterial isolates were assessed for their survival in the presence of bile salt, and it was found that strain L2 exhibited the highest tolerance to 0.3% bile salt. Consequently, this strain holds potential for further in vivo investigation regarding its suitability as a probiotic.

Hari Shankar Biswas
Sandeep Poddar
Ravinder Nath Anisetty
Amiya Bhaumik

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Oscillatory Systems: Approach from Nonlinear Dynamics

Shrabani Sen
Department of Chemistry, Rammohan College, Kolkata, West Bengal, India

Corresponding Author's Email: sen.shrabani@gmail.com

ABSTRACT

The aim of this chapter is to present a brief outline of the background theories essential for understanding numerous non-equilibrium phenomena, such as chemical and biological oscillations, spirals and so on. Oscillations are one of the most common phenomena in chemical and biological systems. Oscillations of chemical origin have been present since life originated. Every living system contains thousands of chemical and biological oscillators. The systematic study of oscillating chemical reactions and subsequently the broader field of Nonlinear Dynamics is of considerably fundamental domain of research in recent decades. The chapter starts with a brief outline of some background history of chemical oscillations followed by the basic thermodynamic explanations and stability analysis. A short glimpse of Phase plane analysis and Limit cycles are given. One dimensional stability analysis is followed by two dimensional one. Relaxation oscillation is explained both theoretically and graphically.

Keywords: *Steady State; Equilibrium; Phase Plane; Non-Equilibrium Process; Oscillatory Systems*

Introduction

Dynamics is an interesting topic which explains how a physical variable of interest progresses with time. Harmonic oscillator is an example of linear motion which is well known from Newtonian Mechanics. In this motion the frequency of the oscillator is not dependent onamplitude. Whenever the system is not linear its motion changes the behavior. It is not so simple because of the dependency of its frequency over its amplitude. The result is the motion may vary from purely rhythmic to chaotic region. Nonlinear dynamics has its origin in Physics to Newtonian mechanics developed earlier in mid-1600s, it is now treated as an interdisciplinary subject today (Epstein 1998, Strogatz 1995, Murray 1993, Gillespie 1977, Goldbeter 2006) which has its application in almost all branches of Physics, Chemistry and Biology (Grossman 1990, Hillborn 1994, Jalan 2005, Julvez 2015). Although it developed early but the subject could not found its applicability because the classical three-body system was not under the ability of the Newtonian method. The Poincare developed a new geometric technique in studying such systems (Strogatz). From this a new area of Nonlinear Dynamics has been developed which has enlarged application in different area of science specially in chemistry and biology.

In order to go into the depth of aforementioned people have to get idea about the differences between temporal and spatio temporal oscillations. Oscillations are of two types. One is temporal that means it oscillates in time (Sen *et al.,* 2008, Sen *et al.,* 2009, Dhatt, Sen &

Chaudhury 2020, Murray 1993, Scot 1994) and the other is spatio temporal that means it can oscillate both in space and time. Temporal oscillation implies it is homogeneous in space (Bray 1921) where as the spatio-temporal oscillation has its coupling of the space part with the temporal part with diffusion (Epstein 1998, Ghosh *et al*., 2009, Sen *et al.,* 2010). A chemical or biological oscillation is a periodic far-from-equilibrium phenomena which is part of non equilibrium thermodynamics. After looking back at the history of the subject it shows that long time has been taken to establish the fact that the oscillatory phenomena has no contradiction with the classical second law of thermodynamics. Belousov (1893-1970) submitted the manuscript of continuous conversion of yellow Ce^{+4} to colorless Ce^{+3} in 1951 but it was rejected. The editor outwardly stated that his work was simply impossible. The paper could be published on submission of some additional evidence which can contain some snapshots of different phases of oscillation. Tragic fact s that after laboring for six more years the work was again rejected, At 1961 Zhabottinsky a graduate student of Biophysics began looking at the same system. He replaced citric acid with malonic acid and obtained a better formulation. Zhabotinsky wrote his manuscript and sent to Belousov for his comments. More than ten papers on Belousov Zhabotinsky reaction had been published since 1951 moreover the the manuscript that Belousov originally wrote in 1951 was posthumously published in 1985 (Belousov 1951).So the above elaborative study described how difficult it was for the science community at that time to accept the oscillatory phenomena.

The classical theoretical model was first proposed by Alfred Lotka (1925) (Lotka, 1920). The model is used to explain predator prey system and the basic pillar to elaborate oscillation in chemistry and biology. It has three irreversible steps. X is the population of rabbits. It can reproduce auto catalytically, G is the grass. Since it is present in large it is assumed to be constant. Y represents the lynxes and D the dead lynxes.

$$G+X \rightarrow 2X$$

$$X+Y \rightarrow 2Y$$

$$Y \rightarrow D$$

The above kinetic elementary steps are not reversible: X will not convert into G, nor D convert into live ones. The behavior of the predator and prey species has been described in ecology. The following is the set of differential equations

$$\frac{dx}{dt} = k_x ax - k_y xy$$
$$\frac{dy}{dt} = k_y xy - k_d y$$

Here $k_x$is the rate constant of how fast rabbits reproduce; similarly k_y is the rate constant which indicates how fast lynxes reproduce and k_d is the mortality rate of lynxes. The predators and preys will oscillate in time for a particular set of parameters i.e. rate constants (Figure 1).

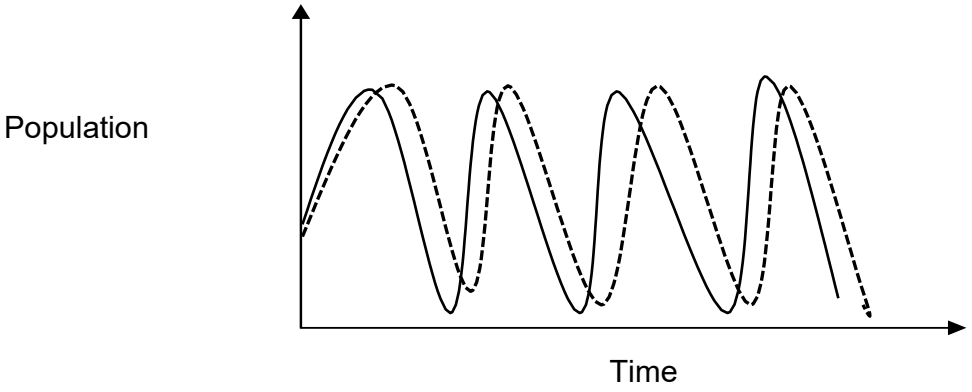

Figure 1: Oscillations of Predators and Prey with time in Lotka Volterra model (Epstein, 1998)

While recalling the violation of second law of thermodynamics in chemical oscillations it is obvious that only oscillate are the intermediates. Reactants and products become steady after some time. There is vast differences between this chemical oscillator and physical one. Physical oscillator as it is known from simple pendulum always pass through equilibrium. Chemical oscillation is not an equilibrium phenomena rather it is away from equilibrium. During the oscillation of intermediates of chemical oscillation the free energy gradually decreases. Conversion of reactants having higher free energy to products having lower free energy leads to the oscillation of the intermediates. To maintain continuous oscillation supply of substrate concentrations must be thoroughly kept. That means the system must be open (Figure 2). Further chemical and thermodynamical study of BZ reaction made significant improvement in the domain of chemical oscillation (AM, 1964).

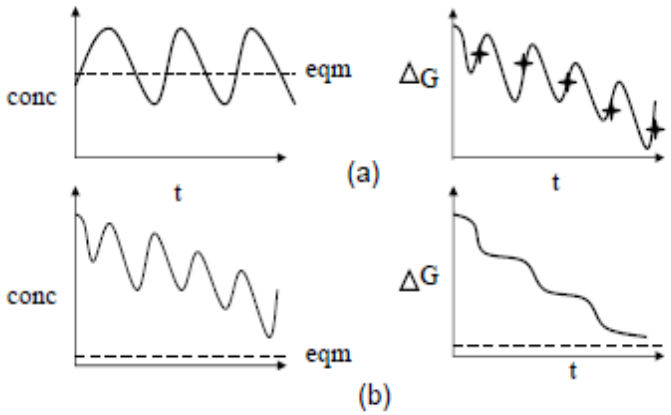

Figure 2: Explaining two different types of Oscillations. (a) Physical Oscillations where it Oscillates around Equilibrium, Inconsistent with Thermodynamics (b)Oscillations far from Equilibrium, Consistent with Thermodynamics. (Epstein, 1998)

Discussion

Linear Stability Analysis

For a system the equilibrium state is unique in nature but the steady states of a nonlinear system may not unique. The steady state of a dynamical system is also known as fixed point.(Epstein 1998, Strogatz 1995).Although at the fixed point the dynamics seizes but near the vicinity of the steady state the trajectories direction changes. Emphasis has been given on the dynamics around the fixed point or the domain of the steady state region. Generally the system is not exactly solvable. Approximation techniques has been taken or linearize the system truncating the nonlinearities. An one-dimensional dynamical system can be represented as,

$$\dot{x} = f(x)$$

Here *f*(*x*) can be a nonlinear function of *x*. The function *f* may not be explicitlytime dependent. Then the system is autonomous or time independent and if it depends on time then it is non-autonomous or time dependent in nature. At the fixed point $f(x) = 0$

Let at a fixed point x_s=0. So $\frac{d}{dt}(\delta x) = 0$ for the above system. It has been disturbed the above system about $x_s$asx_s=$(x + \delta x).\delta x$ is the very small amount of perturbation. Thus it has been obtained

$$f(x + \delta x) = f(x_s) + f'(x_s)\delta x + O(\delta x^2)$$

where $O(\delta x^2)$ is the higherorder Taylor series expansion term and will generally negligible when $f'(x_s)$is nonzero. Since $f(x_s)$is zero at the steady state the following linear equation will arrive as $\frac{d}{dt}(\delta x) = f'(x_s)\delta x$

This linear equation easily tells that the extent of stability of the fixed point. That means If $f'(x_s)$is positive then the fixed point is unstable; the system will go away from it whereas if it is negative then the fixed point is stable means the system will converge in it. Actually, for a one dimensional system the magnitude of $f'(x_s)$gives a measure of stability and$1/f'(x_s)$is a characteristic time scale which determines howsignificantly the solutions will grow or decay in the neighborhood of the fixed pointx_s. A very important point about the one-dimensional flows is that the flow happens monotonically towards and away from the fixed point and existence of oscillatory solutions are thus forbidden.

Two Dimensional System

Two-dimensional systems are analyzed for the stability of fixed points, in almost the same manner as done in one dimensional ones, by linearizing them near those fixed points. Suppose a two-dimensional system of the form

$$\dot{x} = f(x, y)$$

$$\dot{y} = g(x, y)$$

and let $(x_{0,}y_0)$be the only fixed point of the system such that $f(x_0,y_0) = 0 = g(x_0,y_0)$. Now to analyze the stability of the fixed point let people perturb the system around the fixed point by setting $x = x_0+\delta x$and $y = y_0+\delta y$. After linearizing the system around the fixed point it has been obtained the dynamical equations for

the perturbations δ*x*and*δy as* $\begin{pmatrix} \dot{\delta x} \\ \dot{\delta y} \end{pmatrix} = \begin{pmatrix} f_x & f_y \\ g_x & g_y \end{pmatrix} \begin{pmatrix} \delta x \\ \delta y \end{pmatrix}$

as where the matrix containing the terms f_x, f_y, $g_x$and g_y(indicating the values of partial derivatives with respect to *x* and *y* of the functions *f*(*x,y*) and *g*(*x,y*) at the fixed point) is known as stability matrix (*M*). It is assumed the solutions ofδ*x*and δ*y*of the form $\delta x = Ae^{\lambda t}and\delta y = Be^{\lambda t}$then the eigenvalues are given by

$$\lambda = \frac{(TrM) \pm SQRT(TrM^2 - 4\Delta)}{2}$$

Where $TrM = f_x + g_y$ and$\Delta = f_x g_y - g_x f_y$. Now different possibilities of the solutions may arise for different values of *TrM*and∆ . Now different possibilities of the solutions may arise for different values of *TrM*and ∆.

(A) If $(TrM)^2$>4 ∆, then there is always two real valued λ-s where dependinguponthe signs of the corresponding eigenvalues three different kinds of stability of the fixed pointsare possible.

(i) If (*TrM*) >0 and ∆>0 both eigenvalues are positive leading to an unstable node
(ii) If (*TrM*) <0 and ∆>0 both eigenvalues are negative leading to a stable node
(iii) For (*TrM*) >0 and ∆<0 or (*TrM*) <0 and ∆<0 or (*TrM*) = 0 and ∆<0, one eigenvalue is positive and the other one is negative leading to saddle node.

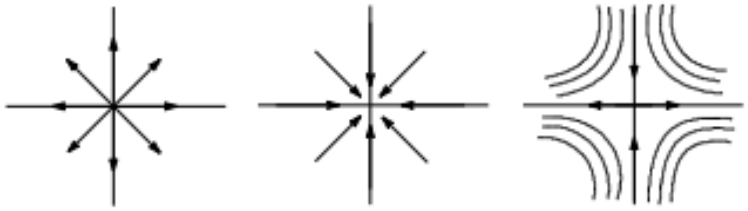

Figure 4: Stabilities of the Fixed Point when $(TrM)^2$>4 ∆ (Strogatz 1995)

(B) If $(TrM)^2$ <4 ∆, then the eigenvalues are always imaginary and appear as complex conjugate pair. Here also stability of the fixed point can be of different types.
(i) If (*TrM*) >0 the both the imaginary eigenvalues have positive real part leading to a stable spiral

(ii) If (*TrM*) <0 the both the imaginary eigenvalues have negative real part leading to a stable spiral
(iii) If (*TrM*) = 0 and ∆>0, then this situation leads to a center.

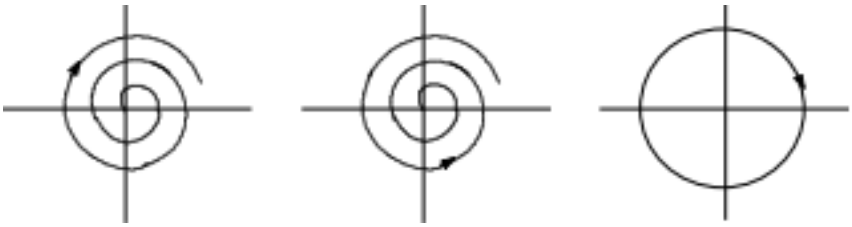

Figure 5: Stabilities of the Fixed Point When $(TrM)^2<4\Delta$ (Strogatz 1995)

Phase Plane Analysis; Limit Cycle: Excitable Systems and Relaxation Oscillations

Limit cycles are isolated closed trajectories in phase space i.e. limit cycle is the only closed trajectory in the neighborhood. This attractor can be stable or unstable depending upon the fact that all the neighboring trajectories can either get attracted to go or away from it (Figure 3).

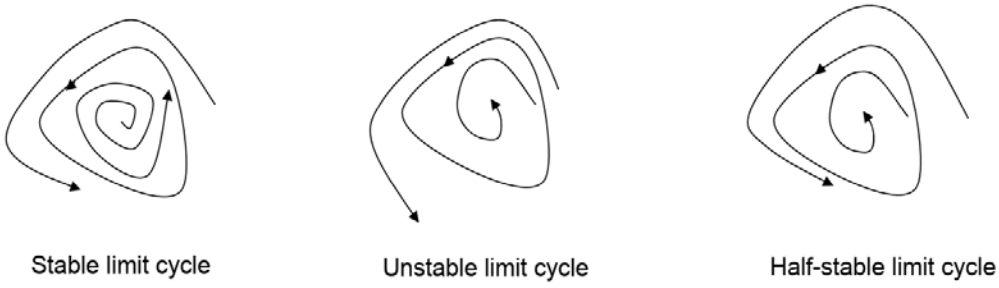

Figure 3: Different Possible Limit Cycles (Strogatz 1995)

Let people consider the equations of model be

$$\frac{du}{dt} = \frac{1}{\epsilon} f(u, v)$$
$$\frac{du}{dt} = g(u, v)$$

Here *f* and *g* are functions of the dynamical variables *u* and *v*. $\epsilon$is a small number introduced as a scaling factor to maintain different time scales of dynamics. *u* reaches fast to a steady state, while *v* changes much more slowly. The term nullcline is defined as the curves on which the rate of changes of each of the dynamical variables are zero. From the intersections of the two nullclines one can obtain steady states. If the steady state falls on any one of the left or right branch then it is stable because all dynamical trajectories in its surroundings point in directing toward the intersection of the two nullclines. These types of systems are known as the excitable systems. On small perturbations of an excitable system it quickly returns to the steady state. As shown in Figure 6, upon applying small perturbation to the steady state the system jumps back to u nullcline because of the faster change of *u* due to $\frac{1}{\epsilon}$ term. Thus the steady state is concluded to be stable.

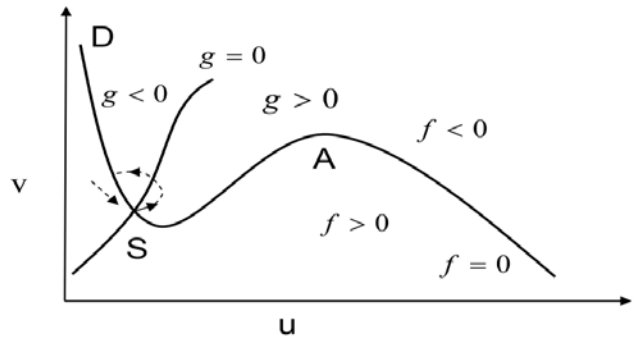

Figure 6: Phase Plane Analysis of Small Perturbation in the Steady States of Excitable Two Variable Model (Epstein 1998)

Now investigate what happens when large perturbation is applied to the steady state. Upon application of large perturbation the system reaches to the point A which is positive for $f(u,v)$. Thus the perturbation continues to grow, pushing the system toward the right branch of the $f = 0$ nullcline. The concentration of v changes slowly compared to u. When the system reaches the $f= 0$ nullcline at B, it starts to go up the nullcline as v increases ($g(u;v) >0$), until it reached to the maximum at C, then it comes rapidly to the left-side branch of u-nullcline. The system then slides along this nullcline again back to the fixed point (Figure 7).

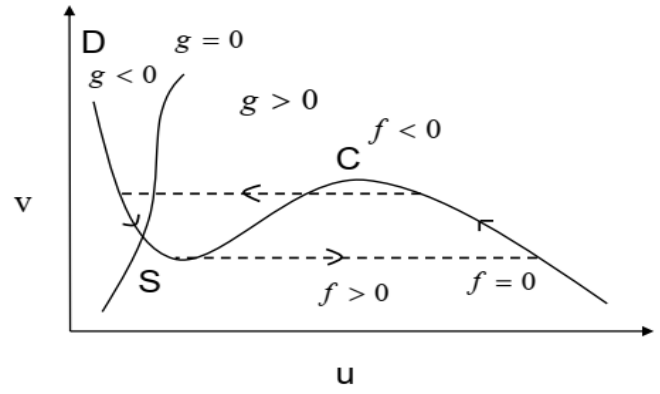

Figure 7: Phase Plane Analysis of Large Perturbation in the Steady States of Excitable Two Variable System (Epstein 1998)

If the intersection of the nullclines lies on the middle branch, the trajectories point awayfrom the steady state making it unstable. Since u changes very rapidly the analysis has tostart from the point A in Figure 6. The system will move slowly along the nullcline as v decreases from A to B. Upon reaching B, u will rapidly increase as the system jumps to the other branch of the $f = 0$ nullcline (C). This branch is in the positive region of $g(u;v)$, so the system will move along the u-nullcline toward D as v slowly increases. Upon reaching D as v slowly increases, the system again ‘falls off’ this branch of the u nullcline and makes a rapid transition to E. It then proceeds back toward A, and the cycle repeats. Periodic temporal oscillations are obtained (Figure 8). These types of oscillations are called relaxation oscillations.

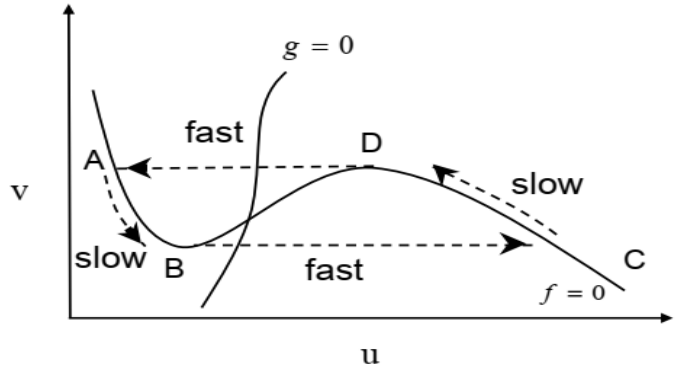

Figure 8: Phase Plane Diagram of Relaxation Oscillations (Epstein 1998)

A note has been taken on the extent of usefulness of the results of linear stability analysis. So long as a fixed point is a node or saddle or a spiral it is stable enough and the nonlinear terms left do not generally affect its stability. But when the fixed point is a center or degenerate nodes or star or non-isolated fixed point its stability is very much dependent on the nonlinear terms left. They should be taken care of to see what happens near such a fixed point.

Conclusion

So far it has been discussed the stability analysis of fixed point upon perturbed by small and large perturbations. Different types of dynamical behaviours are obtained. Oscillations are amongst them. The importance of oscillatory dynamics is noteworthy in the physical world and its origin is also supposed to be easily understood. A short glimpse has been given. Study of Nonlinear dynamics of oscillatory systems can reveal various phenomena. More insights will help to understand deeply the oscillatory phenomena and it will be done the same in upcoming issues.

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Immobilized Osmium Catalyst for Asymmetric Dihydroxylation of Olefins

Moumita Roy
Department of Chemistry, Ramsaday College, Amta, Howrah, West Bengal, India

Corresponding Author's Email: moumitaroy.iict@gmail.com

ABSTRACT

Chiral vicinal diol units are important intermediates in different natural products and drugs. Among various synthetic routes available in the literature, osmium-catalyzed Sharpless asymmetric dihydroxylation (AD) of alkenes is the most popular method among organic synthetic communities to afford diversified enantiomerically enriched 1, 2-diols. However, the high cost of osmium and chiral ligands, as well as the high toxicity of osmium, can contaminate target products, limiting the application of AD reactions in large-scale processes. Heterogeneous catalysts have an added advantage over homogeneous catalysts due to easy recovery and recyclability, which are beneficial in terms of economic, and environmental concerns. In this direction, various research groups from different parts of the world have worked to overcome the demerits of the homogeneous osmium catalysts by immobilizing the active catalytic center onto heterogeneous support, and have achieved very good success. This review discusses recent work on supported osmium catalysts for AD reactions, highlighting their benefits and drawbacks. It serves as a useful guide for the continued development of improved catalytic systems.

Keywords: Asymmetric Dihydroxylation; Chiral Vicinal Diols; Osmium; Heterogeneous; Catalyst

Introduction

Catalytic asymmetric reactions by transition metals are of immense importance to synthesize enantiomerically pure molecules. Several advantages of such strategies include their economic benefit, potential environmental friendliness, and simplicity of purification (Lapuh, Mazeh & Besset, 2020; Cabré, Verdaguer & Riera, 2022; Fu, Chen & Nishihara, 2021). The fascinating transition metal complex catalysts, visualized to mimic enzymes in many complicated asymmetric reactions have been realized with high success. Unlike with enzymes, the synthesis of either of the desired antipodes of the chiral molecule using transition metal complexes, unlike with enzymes, forms a significant advancement (Leenders *et al*., 2015). In addition to this, the metal complexes have a widened scope of applicability that includes a large variety of reactions with a broader substrate choice. It is due to this distinct presentation of their features that these catalysts are ideal for making organic molecules. Hydrogenation catalysts (Knowles & Sabacky, 1968; Miyashita *et al.,* 1980) and oxidation catalysts (Kolbe, VanNieuwenhze & Sharpless, 1994; Wail *et al*., 1989; Wang & Sharpless, 1994; Lu, Xu & Yang, 2000; Zhang *et al*., 1990; Jacobsen *et al*.,1991) are some

of the synthetic asymmetric catalysts, which compete with enzymes that have long been considered monopolists in terms of enantioselectivity.

Chiral vicinal diol units are important intermediates in different natural products and also in drugs e.g. Bicalutamide, Diltiazem hydrochloride, and Taxol. Asymmetric cis-dihydroxylation of olefins using osmium compounds is highly significant in this direction (Mushtaq *et al.,* 2023). The first catalytic version, one of the cornerstones for fine organic synthesis, i.e. AD of alkenes is a well-established method for the synthesis of various enantiomerically pure vicinal diols (Willingh, 2021). The importance of the process lies in the utilization of cinchona alkaloid-based chiral ligands, which opened an alternative path to allow the catalytic use of toxic and volatile osmium (Schroder, 1980; Bolm & Gerlach, 1997; Bolm & Gerlach, 1998). However, the large-scale application of homogeneous catalytic systems faced challenges due to the prohibitive cost of osmium, and chiral ligands. In addition, there is always a probability of contamination of the target product with toxic osmium.

Therefore, there is an urgent need to replace soluble catalysts with heterogeneous catalysts that are recyclable and easily separated from the reaction mixture with little to no effluent emissions. Hence, the future and well-being of mankind largely depend on the development of science and technology, especially in the field of catalysis, which is related to the fight against environmental pollution, and the conservation of natural resources and energy.

Initial attempts were made to immobilize the ligand on a heterogeneous support, e.g., polymer, silica gel and subsequent heterogenization of Os via complexation with ligands but with very limited success or no success in terms of recovery and re-use of the precious Os. This was explained due to the equilibrium between anchored osmium tetroxide and soluble osmium tetroxide during the reaction (Han & Janda, 1996; Bolm & Gerlach, 1998; Kim & Sharpless, 1990; Bolm, Hildebrand, & Muniz, 2000). The microencapsulation technique (Nagayama, Endo & Kobayashi, 1998; Nagayama, Endo & Kobayashi, 1998; Kobayashi, Ishida & Akiyama, 2001; Ishida, Akiyama & Kobayashi, 2003) first adopted by Kobayashi *et al*. addressed the issue and opened up a new direction towards supported osmium catalysts. Different types of materials, such as polymers, silica-based materials, ion exchangers, ionic liquids, etc. were explored.

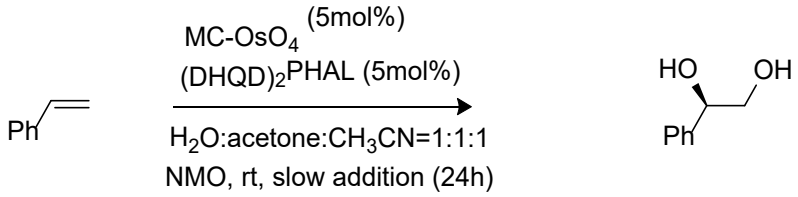
Developing good reoxidation systems for Os (VI) is also highly desirable. Numerous reoxidation systems were evolved, of which the two maximums generally used are based totally on NMO (N-methylmorpholine-N-oxide) (Knowles & Sabacky, 1968) and potassium ferricyanide (Minata, Yamamoto & Tsuji, 1990; Sharpless *et al.,* 1991). Using ferricyanide in AD reactions has certain drawbacks that encompass managing huge amounts of salts (1.4 g/mmol alkene) and effluent disposal, which makes ferricyanide oxidant no longer a real desire for large-scale utility. Of late, the NMO-based hydroxylations are revigorated due to the simplicity of the unit operation. *N-methylmorpholine* (NMM) formed as a byproduct from the reduction of NMO is easy to remove and recycle after oxidation to NMO in an attempt to further add value.

Literature Review

Immobilized Osmium Catalyst on Polymeric Support

Microencapsulation of OsO_4 in polymer capsules *via* the interaction of pi (π) electrons from polystyrene benzene rings with empty d-orbitals of osmium provides a recyclable and reusable osmium catalyst for AD reactions (Nagayama, Endo & Kobayashi, 1998; Kobayashi, Nagayama & Endo,1998; Kobayashi, Ishida & Akiyama, 2001; Ishida, Akiyama & Kobayashi, 2003). This initial attempt with polystyrene microencapsulated catalyst (PS-MC Os) (Nagayama, Endo & Kobayashi, 1998) afforded low yields, selectivity as well as recovery of the catalyst. Later on,they introduced acrylonitrile-butadiene-polystyrene encapsulated osmium catalyst (ABS-MC Os) (Nagayama, Endo & Kobayashi, 1998), and poly[4-(phenoxyethoxymethyl)styrene-co-styrene] microencapsulated osmium (PEM-MC Os) (Kobayashi, Ishida & Akiyama,2001) which showed improved activity (Table-1).

Table 1: AD Reaction of Styrene by Microencapsulated Osmium Catalysts (MC-OsO_4)



Entry	MC-OsO_4	Yield (%), (ee (%), recovery (%))		
		1st run	2nd run	3rd run
1.	PS	4 (-, 97)	-	-
2.	PEM	85 (78, 100)	66 (78, 100)	84 (78, 100)
3.	ABS	81 (94, 88)	83 (94, 74)	84 (94, 74)

*PS: Polystyrene; PEM: Poly[4-(phenoxyethoxymethyl)styrene-co-styrene]; ABS: Acrylonitrile-butadiene-polystyrene

The same group further reported the development of air-stable, nonvolatile and less toxic “polymer-incarcerated osmium (PI Os) catalysts” (Ryo *et al*., 2012) for AD reactions. The catalysts were prepared using microencapsulation followed by cross-linking so that they became insoluble in common organic solvents. Acute toxicity assays showed these catalysts were benign. XAFS analysis of the catalyst revealed the reduction of Os (VIII) to Os (IV) during catalyst preparation.

The catalyst produced excellent yield as well as enantioselectivity for different olefins with very low leaching of Os (Figure 1).

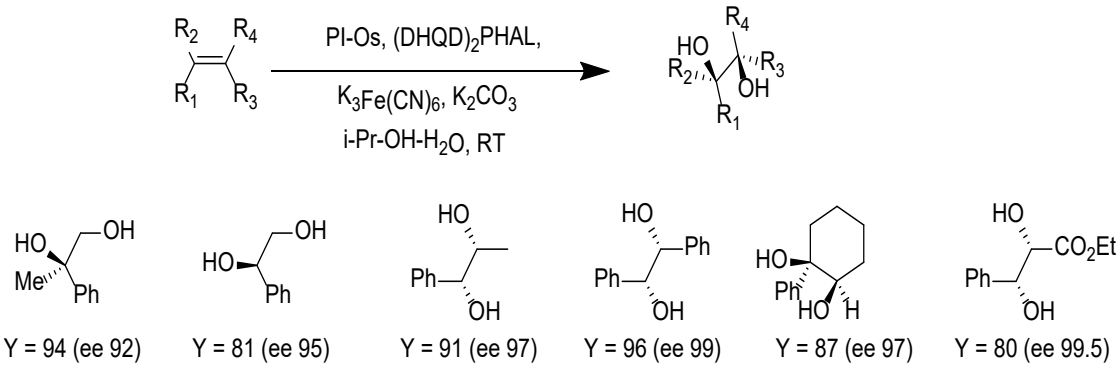


Figure 1:AD of Alkenes Using PI Os Catalyst

PI Os found its relevance in the high-yielding and enantiomerically enriched scale-up synthesis of an important intermediate of Camptothecin which is a known anti-cancer drug (Figure 2).

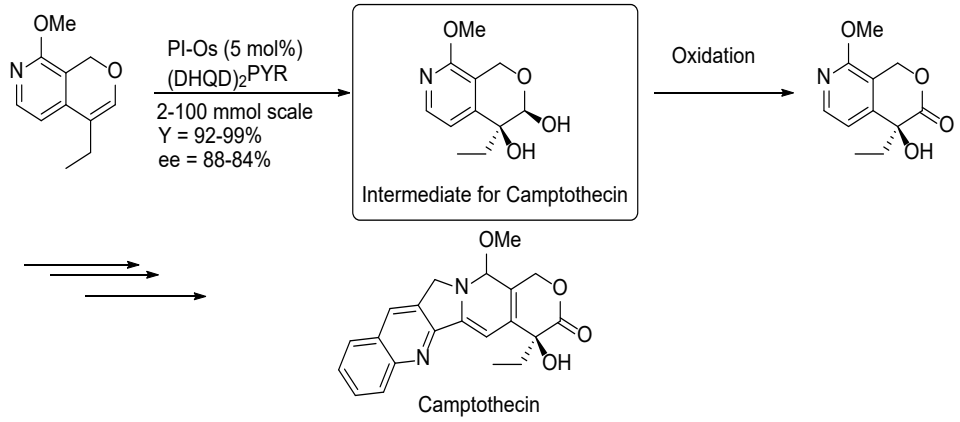


Figure 2: Synthesis of Camptothecin Intermediate Using PI-Os Catalyst

Polyaniline (PANI), which is prepared from readily available commodity chemicals like aniline, is extensively studied as a conducting polymer for applications in the fields of electronics and optics. PANI has excellent environmental stability and attractive redox properties. In addition, PANI exhibited modular doping levels through acid and de-doping through base, inertness and insolubility in aqueous and common non-aqueous (organic) solvents. Such characteristics are paramount for qualification as support in heterogeneous catalysis.

By exploiting modular redox behavior of polyaniline, a simplified and convenient protocol for the immobilization of osmium onto polyaniline was developed by Choudary *et al*. (2006). The catalyst was well characterized by different instrumental techniques namely FTIR, XPS, UV-VIS-DRS, and EDAX. The FT-IR spectrum of PANI-Os showed the presence of the Os-O bond. UV-Visspectrum of the catalyst also supported the same. XPS analysis of PANI-Os revealed the presence of Os (IV) and Os (II) oxidation states (Figure 3).

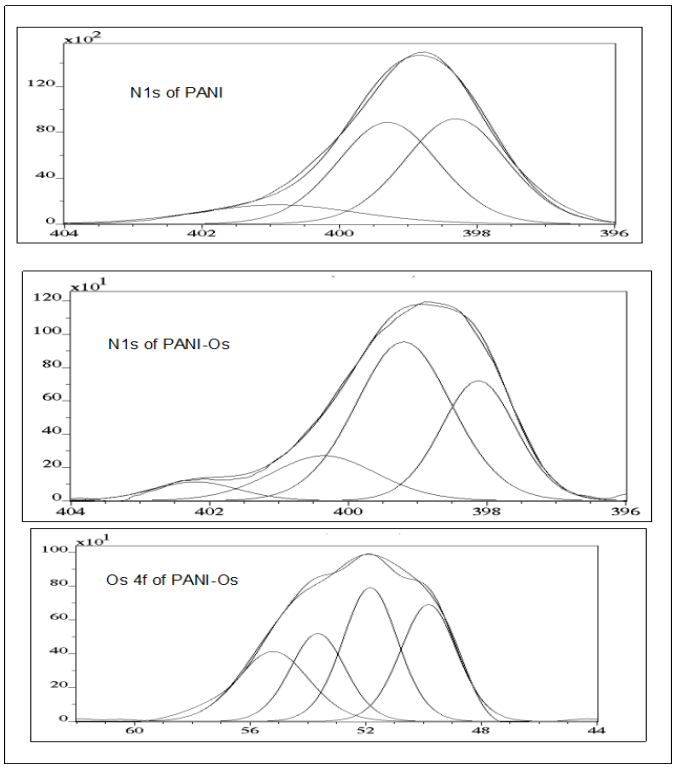


Figure 3: XPS Spectrum of PANI-Os

When this polyaniline-anchored osmium catalyst was used in the AD of alkenes, it exhibited very good reusability up to 5 cycles. They have prepared one bi-functional PANI-Os-Re catalyst which can utilize H_2O_2 in place of NMO as the co-oxidant (Figure 4).

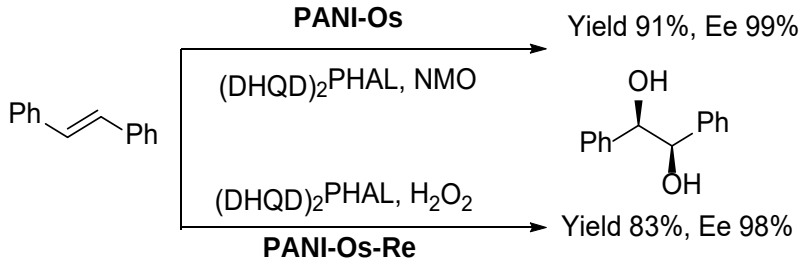


Figure 4: PANI-Os and PANI-Os-Re Catalyzed Dihydroxylation of Trans-stilbene Ion-exchanger-supported Osmium Catalysts

Choudary and co-workers contributed a lot (Choudary *et al*., 2001; Choudary *et al*., 2001; Choudary *et al*., 2002; Choudary *et al*., 2004) to asymmetric dihydroxylation catalyzed by ion-exchanger osmium catalysts. In their first paper (Choudary *et al*.,2001a) they explored layered double hydroxides of Mg and Al (LDH), chemically functionalized silica, and polymeric resin as support material for osmium in catalytic AD of alkenes. OsO_4^{2-} is swapped with LDH-Cl to afford LDH-OsO_4. Similarly, the OsO_4^{2-} is also anchored onto quaternary ammonium groups of silica and organic resin to obtain SiO_2-OsO_4 and resin-OsO_4. All of these catalysts were studied using FTIR, and UV-DRS, which indicated that most of the osmate was

unaffected during the exchange process. SEM-EDX was used to evaluate the osmium content and it was observed that LDH-OsO_4 had the maximum osmium content. All the catalysts were explored for the Sharpless AD of *trans*-stilbene and afforded excellent yield and enantioselectivity (Figure 5).

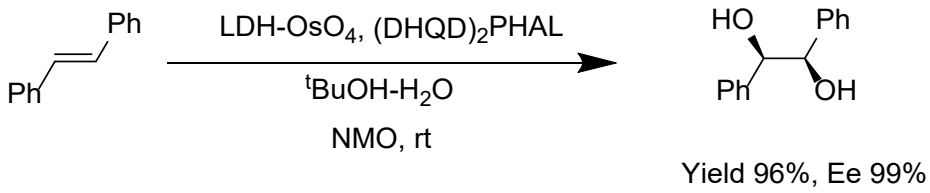
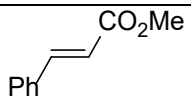
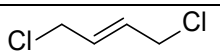
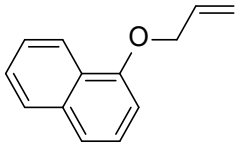
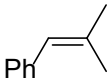


Figure 5: AD of Trans-stilbene Using LDH-OsO_4

To check the catalytic efficacy, LDH-OsO_4 is further used in the dihydroxylation of other alkenes (Table 2).

Table 2: AD of Alkenescatalyzed by LDH-OsO_4

Entry	Substrate	Yield (%)	Ee(%)
1.		89	90
2.		96	97
3.		94	95
4.		90	82
5.		94	77
6.		92	91

Catalysts were almost quantitatively separated by filtration and reused with retention of catalytic activity over a number of cycles. In their follow-up article (Choudary *et al*, 2002), they published a detailed study on the use of various co-oxidants to understand the scope, limitations, and activity of the catalyst. The combination of NMO and LDH-OsO_4exhibited xcellent activity over a number of cycles whereas when $K_3Fe(CN)_6$ and O_2 were utilized as co-oxidants, the catalyst lost its activity after the first use only (Figure 6).

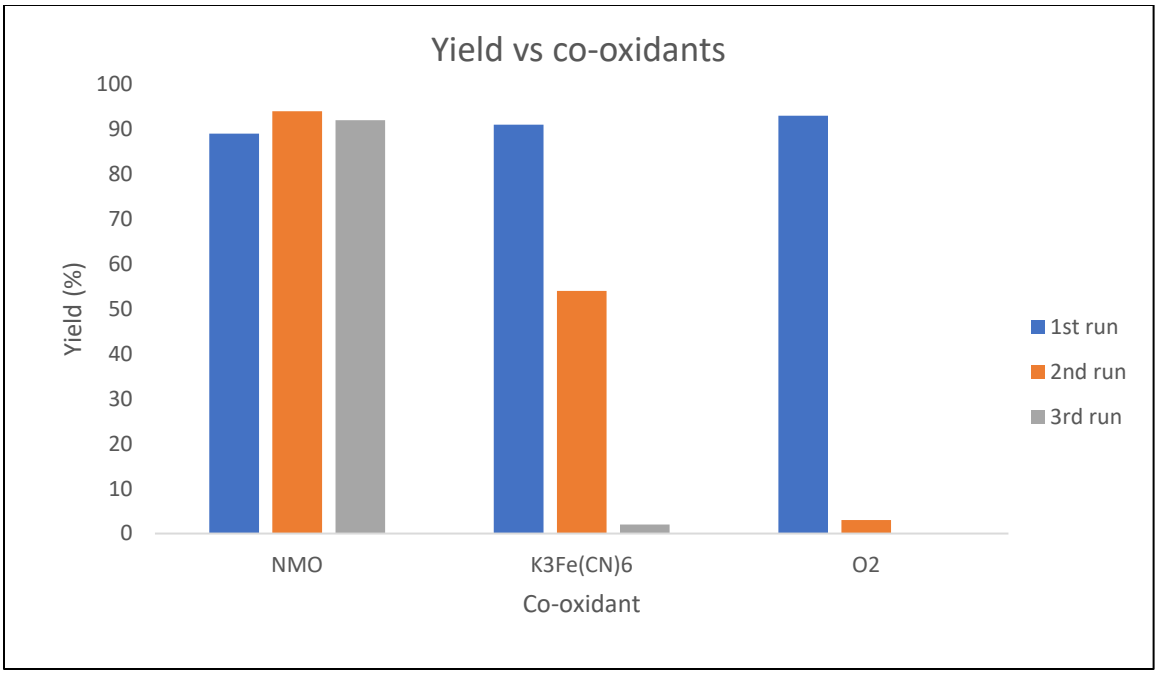


Figure 6: Schematic Presentation of Effect of Different Co-oxidants in the Dihydroxylation of α-methylstyrene Using LDH-OsO_4

As envisaged that leaching of osmate from LDH-OsO_4 in the presence of $K_3Fe(CN)_6$ and $O_2$was the main cause of the deactivation of the catalyst, they designed polymeric resin and chemically modified silica-supported osmium catalysts (Figure 7) which showed far better activity in AD of alkenes in presence of $Fe(CN)_6^{3-}$ and oxygen co-oxidants. Resin-OsO4 showed very high reusability with NMO, $K_3Fe(CN)_6$, and O_2 (Figure 8).

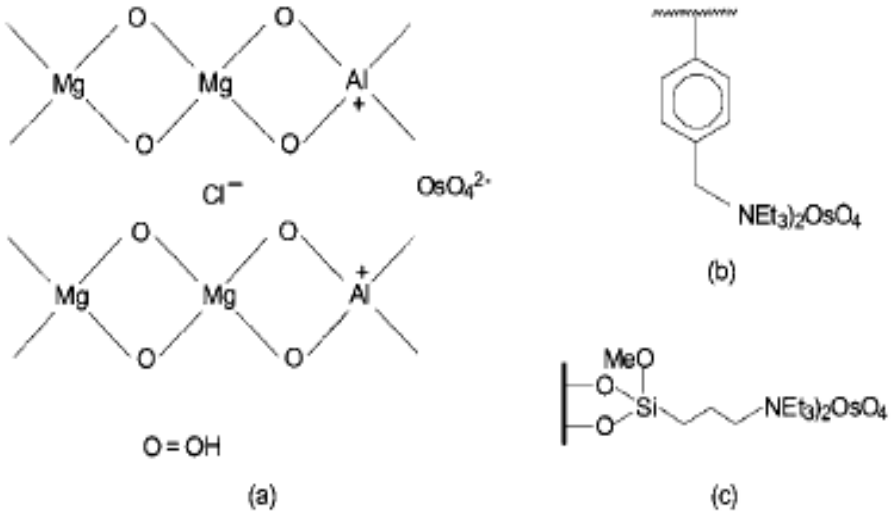


Figure 7: Schematic Presentation of Different Supported Osmium Catalysts

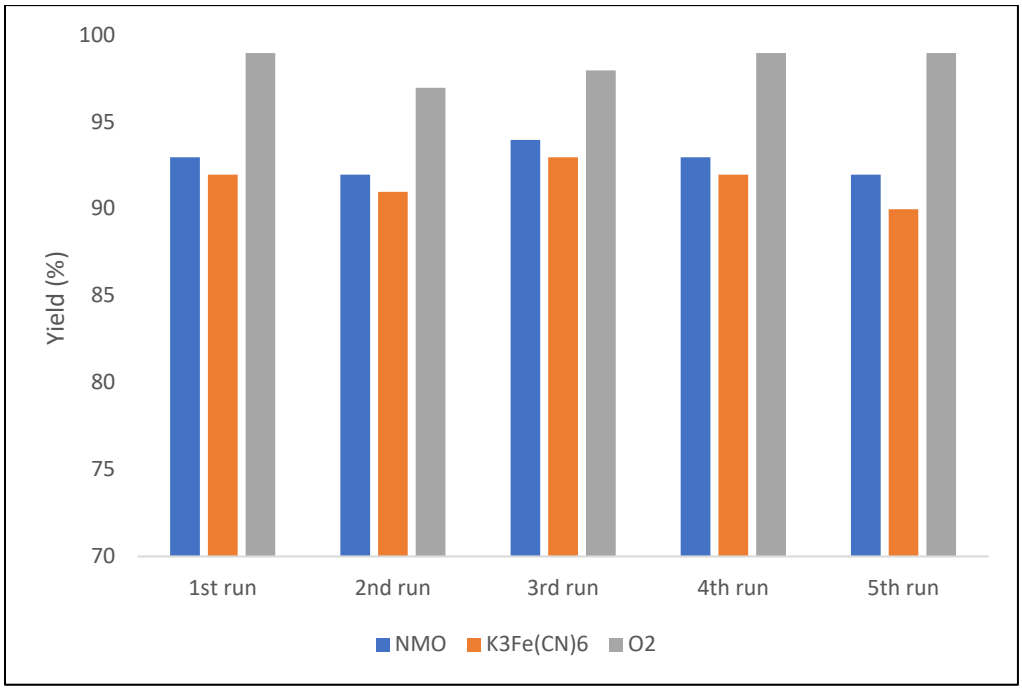


Figure 8: Reusability of Resin-OsO4 Catalyzed Dihydroxylation of α-methylstyrene Using Various Co-oxidants

The same group has further reported a trifunctional catalyst to achieve multistep reactions in one pot. A trifunctional LDH-PdOsW catalyst was prepared by exchanging $PdCl_4^{2-}$, $OsO_4^{2-}$and WO_4^{2-} from Na_2PdCl_4 and K_2OsO_4 and Na_2WO_4 respectively, onto chloride-saturated LDH. The catalyst was characterized using various instrumental techniques, such as X-ray photoelectron spectroscopy which showed the oxidation state of the catalytically active metals such as Pd, Os, and W. XRD data categorically identified the edge alignment of the $PdCl_4^{2-}$, OsO_4^{2-} and WO_4^{2-}.Tandem Heck, AD, and N-oxidationafforded the desired diol with excellent yield and enantioselectivity (Figure 9 & Table 3).

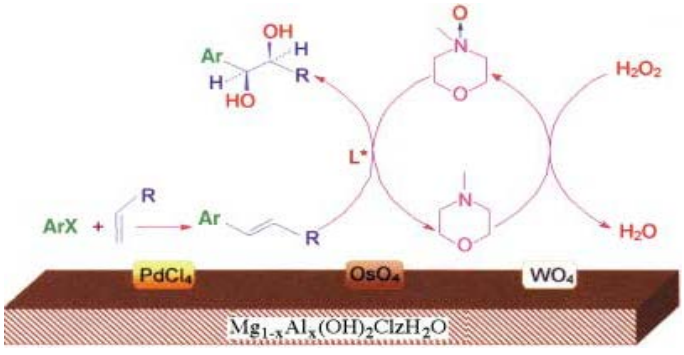
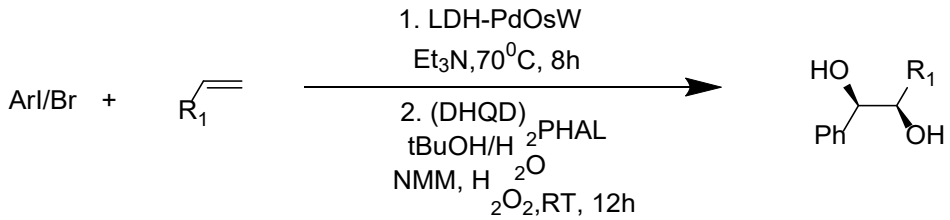


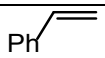
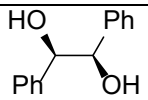
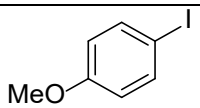
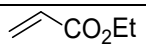
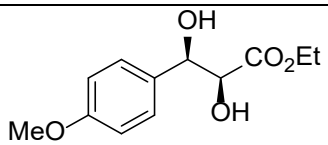
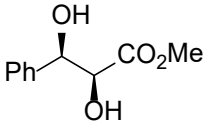
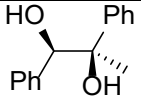
Figure 9: Proposed Reaction Sequences Involving LDH-PdOsW

This novel technique allowed low-priced bulk chemicals for the in-situ preparation of chiral diols. Cheaper H_2O_2 is used to oxidize NMM to NMO which completes the catalytic cycle to

produce the end product i.e., substituted optically pure diols. As per the authors, controlled addition of H_2O_2 promotes hydrolysis of osmium mono-gylcolate ester and eventually higher ee was achieved. Catalyst was recovered almost quantitatively via filtration and repeated use for five times showed only 3% yield loss but ee remained 99%.

Table 3: LDH-PdOsW Catalyzed Heck-AD Reactions



Sl. No.	Haloarene	Alkene	Di-ol product	Yield (%)	ee (%)
1.	PhI			85	99
2.				93	99
3.	PhBr			90	99
4.	PhI			90	47

Choudary *et al.* (2004) reported nanocrystalline magnesium oxide (NAP-MgO) supported bifunctional catalysts to promote one-pot sequential Heck and AD reactions to afford diversified optically active diols. Nanocrystalline metal oxides have interesting properties as support materials because they have many surface sites, such as crystal corners, edges, ion vacancies, etc., which improve surface reactivity. NAP-Mg-PdOs and NAP-Mg-OsW catalysts were obtained by treating NAP-MgO with an aqueous solution of Na_2PdCl_4, K_2OsO_4, and Na_2WO_4 (Figure 10).

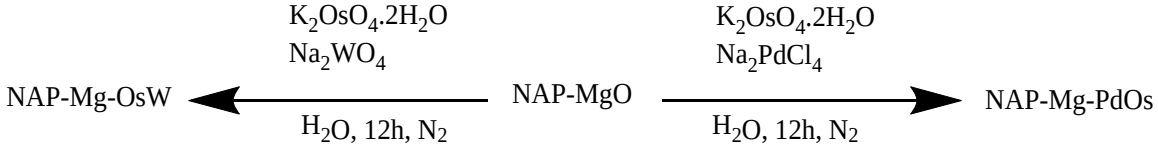
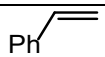
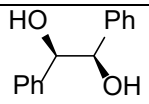
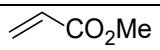
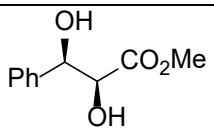
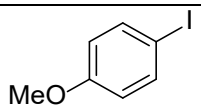
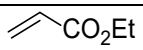
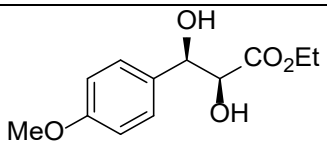


Figure 10: Synthesis of NAP-Immobilized bi-functionalCatalysts

Using NAP-PdOs in the one-pot Heck followed by dihydroxylation starting from aryl iodides and alkenes, diversified diols were obtained in the same reaction vessel (Table 4). Similarly, NAP-OsW was utilized in the concomitant dihydroxylation and N-oxidation (Figure 11).

Table 4: Tandem Heck-AD reaction using NAP-PdOs

Entry	Aryl halide	Olefin	Product	Yield%/ee%
1	PhI			80/85
2	PhI			85/73
3				82/78

Rection condition: NAP-PdOs (3 mol %), aryl halide (1 mmol), olefin (1 mmol) and Et_3N (1.3 mmol) in CH_3CN (2 mL) were stirred at 70 °C for 12 – 16 h. After completion of the Heck coupling, the heating was stopped and NMO (1.3 mmol) and $(DHQD)_2$ PHAL (7.8 mg, 0.01 mmo) in t-BuOH-H_2O (5 : 1, 6 mL) were added under stirring

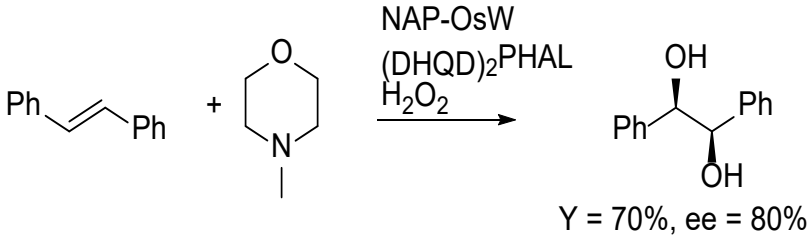


Figure 11: One Pot N-oxidation and AD Using NAP-OsW

Dehury and Hariharakrishnan, (2007) reported a recyclable “osmate-exchanged chloroapatite (CAP-OsO_4) catalyst” for AD reaction of alkenes. Weakly amphoteric apatites can act as supports for diversified ions *i.e.,* cations and anions as they can be easily placed into the apatite framework owing to its high propensity for ion exchange. CAP-OsO_4 was prepared by exchanging OsO_4^{2-} onto chloroapatite. The catalyst was explored in AD reactions on a variety of alkenes containing diversified functional groups e,g. α,β-unsaturated carbonyls, amides, carbonyls, and esters. A good to moderate yield of product was obtained along with high

optical purity. The catalyst was recovered and reused for multiple times without a noticeable drop in activity. Osmium leaching from the reaction mixture was also tested and found to be nominal.

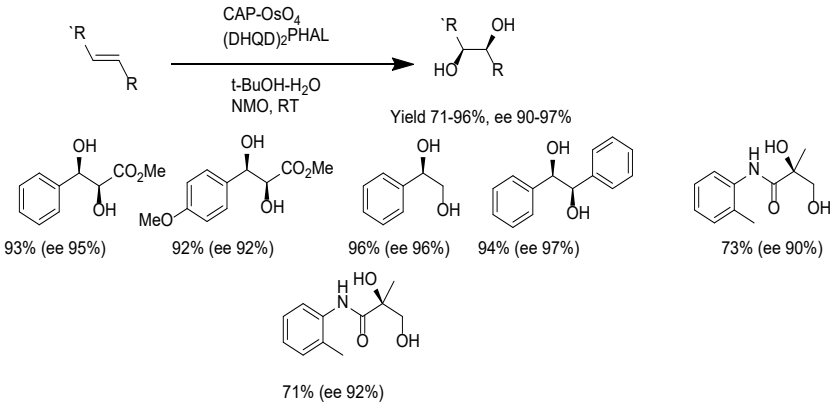
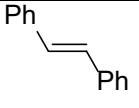
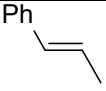
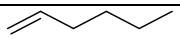


Figure 12: CAP-OsO_4 Catalyzed AD Reaction

Shilpa, Manna and Rana (2015) reported a bioinspired nanoparticle assembly route to obtain a highly effective immobilized osmium catalyst for the AD reactions. Biomaterials have gained immense significance in organic synthesis due to the control achieved over the reaction can be used to develop new routes to functional materials. Thus Poly (allylamine) hydrochloride (PAH) and colloidal silica were used to get a microsphere structure to provide the required textural property and stability to hold osmium securely. Different analytical methods such as DLS, SEM, TEM, EDX, FTIR, UV-Vis, XPS are used to characterize the catalyst thoroughly. The SEM images depicted spherical morphology and TEM analysis showed the formation of silica nanoparticles. XPS analysis revealed the presence of hexavalent osmium. FTIR analysis showed an effective interaction between osmate and PAH. The scope of the catalytic system was studied for AD reaction utilizing NMO as a co-oxidant (Table 5). The efficiency of the catalyst is explained by the advantage of the encapsulation of the catalytic center by polyamines leading to increased activity and the structural stability provided by the inorganic material.

Table 5: Synthesis of Chiral Diols Using Microsphere Encapsulated Os Catalyst

Entry	Alkene	Conversion of alkene (%)	Yield of diols (%)	Ee (%)
1.		99	94	98
2		99	96	98
3		99	99	>99

4		99	98	96
5		99	97	99

Reaction condition: Alkene (0.2 mmol), NMO (1.3 equiv.), Os@MS prepared using PAH (0.118 mol-% of Osw.r.t. olefin), CH_3COCH_3/H_2O (12:1 v/v, 0.6 mL), room temp. $(DHQD)_2PHAL$ (0.01 equiv.) was used as the ligand and olefin was added slowly within a reaction period of 8 h.

Ionic Liquid-modified Osmium Catalysts

Ionic liquid-mediated organic transformations draw attention due to their easy recovery and environmental concern. Qiu *et al*., (2011) reported "1-methyl-3-(trimethoxysilyl) propylimidazolium chloride ($MTMSPIm^+Cl^-$)" ionic liquid modified bimodal mesoporous silica (FBMMs) to anchor osmium and the chiral ligand for the AD reaction. To prepare the catalyst, FBMMs were stirred with the acetonitrile/water solution of $((QN)_2PHAL)$ and $K_2Os(OH)_4.2H_2O$. Immobilization of the ionic liquid on to the mesoporous silica established through analysis by FTIR and pore sizes of the catalyst were calculated using nitrogen adsorption and desorption studies. XRD revealed the reduction in the mesostructured after the introduction of OsO_4-$(QN)_2$ PHAL. Catalytic efficacy was demonstrated in the AD reaction of trans-stilbene. It showed good retention of chiral efficiency over five recycles but product formation dropped significantly e.g. yield loss over six cycles: 99% to 25%. This is attributed to the leaching of the osmium metal as well as the chiral ligand from the catalyst matrix.

Conclusion

This review elucidates the importance and evolution of the supported osmium catalysts for the synthesis of syn diols which are the important building block for numerous biologically relevant organic molecules. Supported catalysts exhibited good to excellent recyclability which further reduces the cost of the goods as well as metal effluent. This is expected to serve as a guiding tool for further development in this field of osmium catalysis.

Acknowledgement

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Hydrogen Bonding Probe: Effect of Polarity

Debarati Dey
Department of Chemistry, Vidyasagar College, Kolkata, West Bengal, India

Corresponding Author's Email: debaratidey07@gmail.com

ABSTRACT

Millions of compounds have been found in literature that show solvatochromic shift in absorption and /or emission spectra due to hydrogen bonding. Almost all of them are sensitive to the polarity of the medium as well. A large number of fluorophores show quenching of emission intensity on hydrogen bonding, while in case of a few increments in fluorescence is observed. However a hydrogen bonding probe, Dibenzo [a,c] phenazine, that cannot sense the polarity of the environment, has been reported which is unique in this respect. This probe can determine only the hydrogen bonding donating capacity of a solvent irrespective of its polarity.

Keywords: *Hydrogen Bonding; Excited State; Polarity*

Introduction

Hydrogen bonding (HB) has been key of interest for more than a century due to its importance and prevalence in numerous biological structures and reaction pathways. The unique properties of water, as solvent and biological medium, are due to the tetrahedral hydrogen bond network. However recent theoretical studies reveal that hydrogen bond is not present in supercritical water (Schienbein & Marx, 2020). Especially in biological sciences, it is perhaps the most abundant weak chemical interaction. It is involved in three-dimensional structure of DNA, RNA, secondary structure of proteins etc and mechanistic pathway of many enzyme catalysed reactions. Isotope-edited IR spectroscopy has recently been used to probe the HB environment of individual bases in DNA duplexes (Fick *et al*., 2021). Even atmospheric chemistry use HB network as a probe to characterise the nature of glycerol-water aerosols (Weeraratna *et al*., 2021). The identification of these interactions are manifested on the photophysical properties of the fluorophores involved there in. However, HB can broadly be classified into two categories: a) ground state HB in and b) excited state HB. The HB interaction of any fluorophore both in ground and excited state can be identified by solvatochromic effect on absorption and fluorescence spectra. This implies that depending on nature of the solvent, whether it is polar / nonpolar and protic / nonprotic, the peak position, shape and intensity changes.

Review of literature

Conditions Required for HB Formation and Solvatochromic Effects

In high school studies, students learn about HB as an electrostatic interaction between a hydrogen atom, which forms a covalent bond with an electronegative element such as N, O, or F and another electronegative element that may be Cl, Br etc. The first hydrogen atom is known as hydrogen bond donor and the later electronegative atom is called hydrogen bond acceptor. HB can be inter- or intra- molecular, that change various physic-chemical properties of a molecule viz. melting point, boiling point, acidity, basicity etc.

Although the charge separation and consequently the charge distribution were first identified in the ground state (S_0), sometimes the effect is enhanced in the excited state (S_1, S_2 ...). The change in the charge distribution pattern is reflected by an enhancement of dipole moment in the excited state (μ_e) compared to ground state (μ_g). This is called intra-molecular charge transfer. Molecules showing such transition contain distinct donor (D) and acceptor (A) moieties. The highest occupied molecular orbital (HOMO) is mainly localised on D and lowest unoccupied molecular orbital (LUMO) has mainly acceptor character (Rohatgi-Mukherjee, 1978).

When solvent interaction comes into effect, the situation becomes a bit complicated. Two cases may arise:

Case I: If $\mu_g < \mu_e$, change from a nonpolar to a polar solvent increases the solvent interaction and get greater stabilisation in the excited state, resulting in a bathochromic or red shift of absorption spectra. Such characteristics are observed for π→π* transitions, as the charge separation occurs in an enhanced region (figure 1).

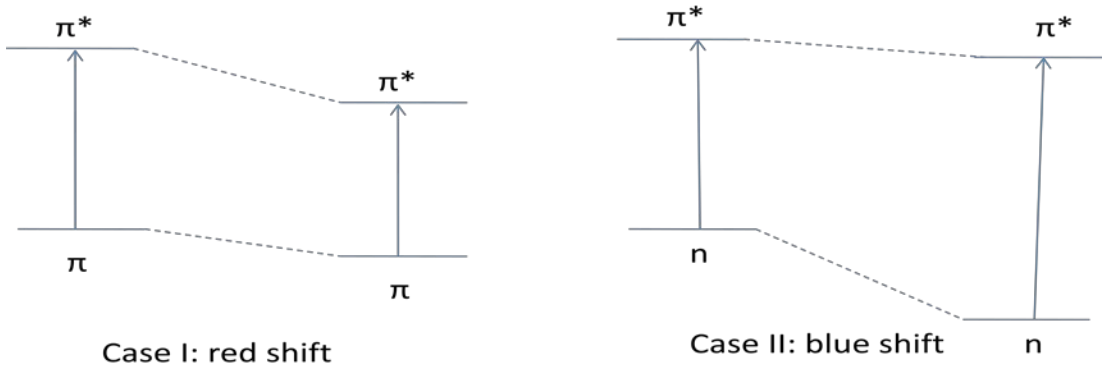


Figure 1: Energy Level Rearrangement on Solvation (Rohatgi-Mukherjee, 1978)

Case II: If $\mu_g > \mu_e$, the ground state is more stabilised in polar solvents. For n→π* transitions, non-bonding lone pair of an heteroatom like N, O remain hydrogen bonded in the ground state. This gives more stability to ground state and excited state is not much stabilised as promotion of nonbonding electrons to π* orbital reduces the hydrogen bonding forces in the excited state. This results in a hypsochromic shift in changing from nonpolar to a polar solvent (figure 1).

Types of HB

Conventionally two kinds of HB are possible: in-plane HB and out of plane HB. In-plane HB is the classical type of HB that occurs with the non-bonding lone pair of any donor and electron deficient hydrogen atom. While out of plane HB occurs with the π* orbital of the donor that is perpendicular to the molecular plane. Theoretical studies revealed that these two kinds of HB with a same fluorophore vary in HB length and also HB strength. Beside the classical HB, recently π-hydrogen bonding used as a probe to understand the reactivity of differently substituted benzenes towards nitration (Galabov *et al*., 2019).

DISCUSSION

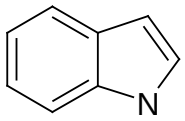
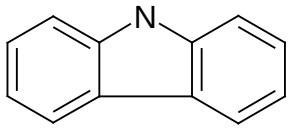
Simultaneous Polarity and Hydrogen Bond Sensor

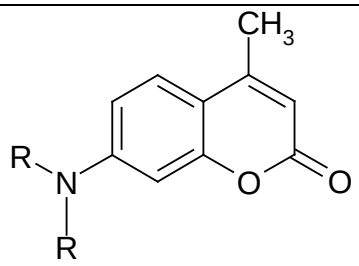
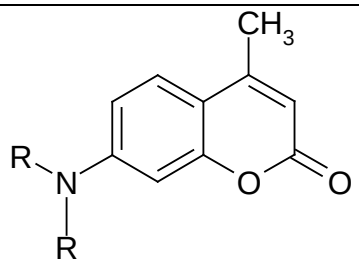
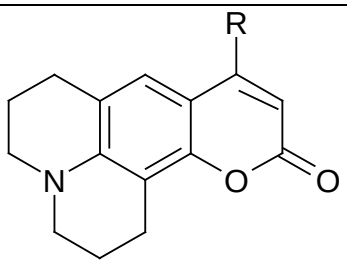
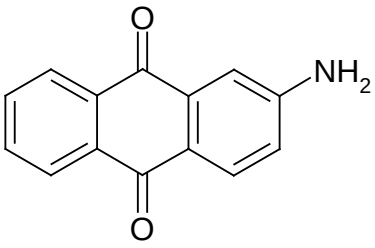
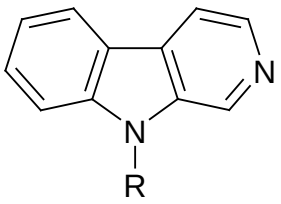
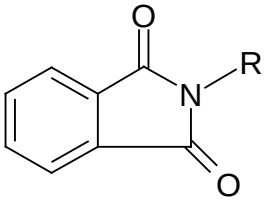
In literature millions of compounds are there that can sense the dielectric of the solvent involved. They are used in various unknown environments to detect the polarity. These polarity probes may behave differently when kept in protic environment. For some compounds the emission intensity decreases in protic medium while some compounds show enhanced fluorescence (Han & Zhao, 2011).

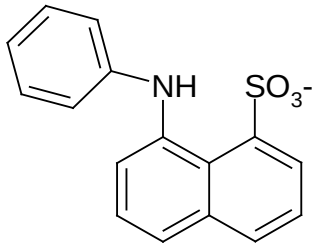
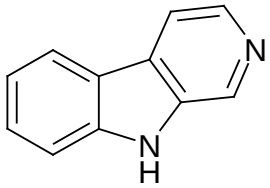
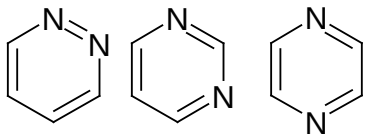
Fluorescence Quenching on Hydrogen Bonding

There are several classes of compounds and their derivatives that are found to be very suitable for analysing solute-solvent interactions in the ground and excited state. Few of them are listed in Table 1. The compounds listed are sensitive to polarity of the solvent and mostly show red shift on switching from nonpolar to polar solvent. In presence of polar protic solvents, HB occurs even in the ground state. On photoexcitation that HB strength may increase or decrease

Table 1: Structure of Different Fluorophores Showing Quenching on Hydrogen Bonding (Han & Zhao, 2011)

Sceletal fluorophore	**Structure**
Indole derivatives (eg. Tryptophan)	
Carbazole derivatives	

Fluorenone derivatives	
Aminocoumarin derivatives (flexible)	
Rigid coumarin derivatives	
Aminoanthraquinone derivatives	
Harmane derivatives	
Phthalimide derivatives	

Aminonaphthalene	
β-carbolines	
Diazines: pyridazine, pyrimidine, pyrazine	

depending on the nature of the compound. In most of the reported cases the emission intensity decreases in protic solvents due to predominant non-radiative transition from S_1 state as it becomes more stable. The HB in protic solvents induces internal conversion and hence quantum yield decreases.

Fluorescence Enhancement on Hydrogen Bonding

There are some compounds where HB with the fluorophore enhances the fluorescence (figure 2). The proposed mechanism suggests that internal conversion and intersystem crossing, both nonradiative channels are stopped. This mechanism named ‘close proximity effect’ happens if the first two excited states (singlet as well as triplet) are very close apart due to their structure. On changing to polar protic solvents the energy states interchange their positions. In nonpolar solvents the nπ* state was the first excited singlet state, having lower quantum yield. After rearrangement of the energy levels the ππ* becomes the S_1 and hence fluorescence quantum yield increases (Han *et al*., 2008; Sikorska *et al*., 2004).

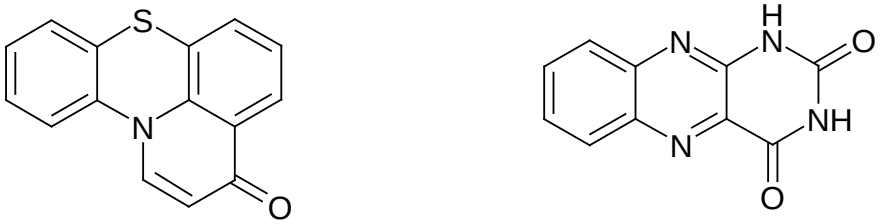


Figure 2: Compounds having increased fluorescence on hydrogen bonding (Han *et al*., 2008; Sikorska *et al*., 2004)

Polarity Insensitive Hydrogen Bonding Probe

The fluorescent probes discussed above are unable to differentiate between dielectric of the medium and its hydrogen bonding capacity. A very few probe, viz, dibenzo[a,c]phenazine (DBPZ) (figure 3) (Dey *et al*., 2007) have been proved to sense only the HB donating capacity of the solvent . The precursor phenazine molecule exhibits almost no change in absorption spectra with different solvents of varying polarity and hydrogen bonding capacity.

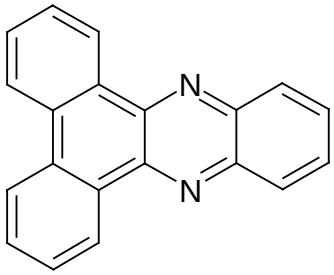


Figure 3: Dibenzo[a,c]Phenazine: Polarity Insensitive Polarity Probe (Dey *et al*., 2007)

Phenazine shows only ~ 30nm blue shift in fluorescence spectra in water compared to acetonitrile. This indicates that the first excited singlet state of phenazine is of $n\pi^*$ character (Choudhury & Basu, 2005). The fluorescence maxima of phenazine remains same in acetonitrile and in ethanol but only quantum yield increases in the later. Thus, the parent phenazine molecule is not very much informative about the dielectric and the hydrogen bonding capacity of its environment. Beside this there are quite a large number of small organic molecules that show significant variation in their dipole moments on photoexcitation and hence may be used as a polarity sensor (Aaron *et al*., 1995; Carvalho *et al*., 2000).

However, DBPZ is such a molecule that has unchanged absorption spectra in solvents of different polarity, e.g. cyclohexane (ε ~2), acetonitrile (ε ~37), methanol (ε ~24) and up to a certain concentration of water (ε = 80). In cyclohexane and acetonitrile, having widely different polarity, the fluorescence maxima and the fluorescence quantum yield of DBPZ show no change. This observation leads to the conclusion that DBPZ cannot sense the polarity of its surrounding environment. However, emission spectra of DBPZ change drastically in hydrogen bond donating solvents. The fluorescence maximum shows bathochromic shift with increase in quantum yield as the hydrogen bond donating capacity of the medium increases. These spectroscopic changes are function of the hydrogen bond donating capacity of the solvents and also the steric effects around the hydrogen bonding site. Though trifluoroethanol has greater hydrogen bond donor capacity than water, (Kamlet *et al*., 1983) water shows higher quantum yield as it can approach towards the probe more easily, due to its smaller size than the bulkier trifluoroethanol.

The dipole moment of DBPZ is much greater in first excited singlet state compared to the

ground state. So hydrogen bond in the excited state is much stronger than in the ground state. However the molecule remains insensitive to the environment polarity due to its peculiar structure. The two bulky phenyl rings in ‘a’ and ‘c’ position make the lone pair of the phenazine nitrogens inaccessible to solvents. Only small hydrogens of the solvent can interact with the lone pair of the nitreogen and build hydrohen bond. This can be proved from ^{1}H NMR study (Dias & Liu, 1990). Excited state hydrogen bonding is reflected from the shortening of hydrogen bond length in the higher state (Parthasarathi, Subramanian & Sathyamurthy, 2006).

Conclusion

Although hydrogen bonding is the most fundamental weak force active in biological medium, most of the probes used to detect hydrogen bonding is also affected by the polarity of the medium. Thus, the photochemical properties shown by those probes are blended with both dielectric and hydrogen bonding capacity of the medium. However Dibenzo[a,c]phenazine is reported to be the polarity insensitive hydrogen bonding probe. The structure of the molecule makes the lone pair of nitrogen unavailable to other atoms except hydrogen.

Acknowledgement

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Abundance of Ants (Hymenoptera: Formicidae) during Pre-monsoon and Post-monsoon Seasons in the Mangrove Patches of Indian Sundarbans

Damayanti Bakra
Assistant Professor, Vidyasagar Metropolitan College, Kolkata, West Bengal, India

Corresponding Author's Email: damayanti.zoology@gmail.com

ABSTRACT

Ants are a social insects that are widely varied and abundant. Although they play a significant role in the mangrove environment, little is known about ants in the Indian Sundarbans. Understanding ant abundance in the Indian portion of the Sundarbans during the pre- and post-monsoon is the goal of this study. 35 species and 21 genera of ants from 5 subfamilies were discovered from five different locations of Sunderbans. Myrmeciinae is the subfamily with the highest number of species (16) followed by Formicinae (8). In the current investigation, five invasive species were discovered: *Monomorium floricola*, *Solenopsis geminata*, *Paratrechina longicornis*, *Tapinoma melanocephalum*, and *Trichomyrmex destructor*.

Keywords*: Sunderbans; Mangrove; Pre-Monsoon; Post-Monsoon*

Introduction

Ants are eusocial hymenopterous insects of the family Formicidae found all over the world, except in the Polar Region. The diversity of ants, as seen by the enormous number of species, subspecies, and variants, and by their tremendous geographic ranges, demonstrates their abundance and ecological dominance. Significant contributions are made to soil aeration, nitrogen deposition, and ecosystem structure. In addition to spreading seeds, they are effective decomposers. Despite its manifold importance, the ant fauna of West Bengal especially the Sunderbans is poorly studied. The author's initial effort was to report 64 species from 30 genera from the Indian portion of the Sunderbans (Bakra, Sheela & Bhattacharyya, 2022). The objective of this study is to find out the abundance of ant species in the mangrove patches of selected localities in the Indian part of the Sunderbans during the pre-monsoon and post-monsoon seasons**.** Further, the study will be the baseline for future studies in this region.

Methodology

Site 2: Bakkhali (88°17'30'E/21°34'45'N),

Site 3: Pathar Pratima (88°18'40'E/21°43'25'N),

Site 4: Gosaba (88°50'32'E/22°07'53'N), and

Site 5: Hingalganj (88°58'18'E/22°20'4'N

Sites 1, 2, and 3 are located in the western sector of the Sunderbans, Sites 4 are in the centre and Site 4 is in the eastern sector of the Indian Sunderbans.

Collection of Ants

The "all- out search' method was used to collect ants. Collection took place during the pre-monsoon (April–June) and post monsoon (October-November) seasons in 2019–2020.

Identification

The ants were identified up to genus level using taxonomic keys by Bolton (1995) and species identification done by using Bingham (1903).

Calculation of Relative Abundance (RA)

The relative abundance of species refers to the number of individuals per species. The relative abundance of ants was calculated using the following formula:
Relative Abundance (%) =
Total number of individuals of the species/Total number of Individuals of all species×100

Results

Ants were found in the mangroves of the Indian Sunderbans in a total of 35 species, 5 subfamilies, and 21 genera (Table-1). Myrmeciinae is the subfamily with the highest number of species (16) followed by Formicinae(8), Ponerinae(4), Pseudomyrmecinae(4) and Dolichoderinae(3). The subfamily Myrmeciinae had the highest abundance (46%) followed by Formicinae (23%),Ponerinae (11%), Pseudomyrmecinae (11%), and Dolichoderinae (9%) (Fig.-1). Pre-monsoon and post-monsoon ant species composition were compared, and it was found that post-monsoon ant species richness was comparatively high (30 species) (Table-2). A total of 30 ant species were discovered during the post-monsoon season but only 23 different ant species detected during the pre-monsoon (Table-2). During pre-monsoon, the highest abundance was found in *Crematogaster rogenhoferi* (24.8%), followed by *Camponotus compressus* (14.37%), *Monomorium indicum* (11%), *Paratrechina longicornis* (9.5%), and *Tetraponera rufonigra* (8.68%). But during the post-monsoon season, the most prevalent species was *Paratrechina longicornis* (27.4%), followed by *Monomorium floricola* (7.8%), *Lepisiota sericea* and *Tapinoma melanocephalum* (both 7.6%) (Fig.-2).There were 5 invasive species: *Monomorium floricola*, *Solenopsis geminata*, *Paratrechina longicornis*, *Tapinoma melanocephalum, and Trichomyrmex destructor*(Acc. to IUCN GISD) in the mangrove patches of Sunderbans (Table-3).

Table 1: List of The Ant Fauna of Mangroves in Sundarbans

Subfamilies	Genera	No. of species
Dolichoderinae	*Iridomyrmex*	1
	Tapinoma	2

Formicinae	*Camponotus*	2
	Paratrechina	1
	Nylanderia	1
	Lepisiota	2
	Oecophylla	1
	Polyrachis	1
Myrmeciinae	*Crematogaster*	3
	Carebara	1
	Dilobocondyla	1
	Monomorium	4
	Meranoplus	1
	Trichomyrmex	2
	Pheidole	3
	Solenopsis	1
Ponerinae	*Anochetus*	1
	Diacamma	1
	Leptogenys	1
	Pseudoneoponera	1
Pseudomyrmecinae	*Tetraponera*	4
Subfamilies- 5	**Genera-21**	**Species-35**

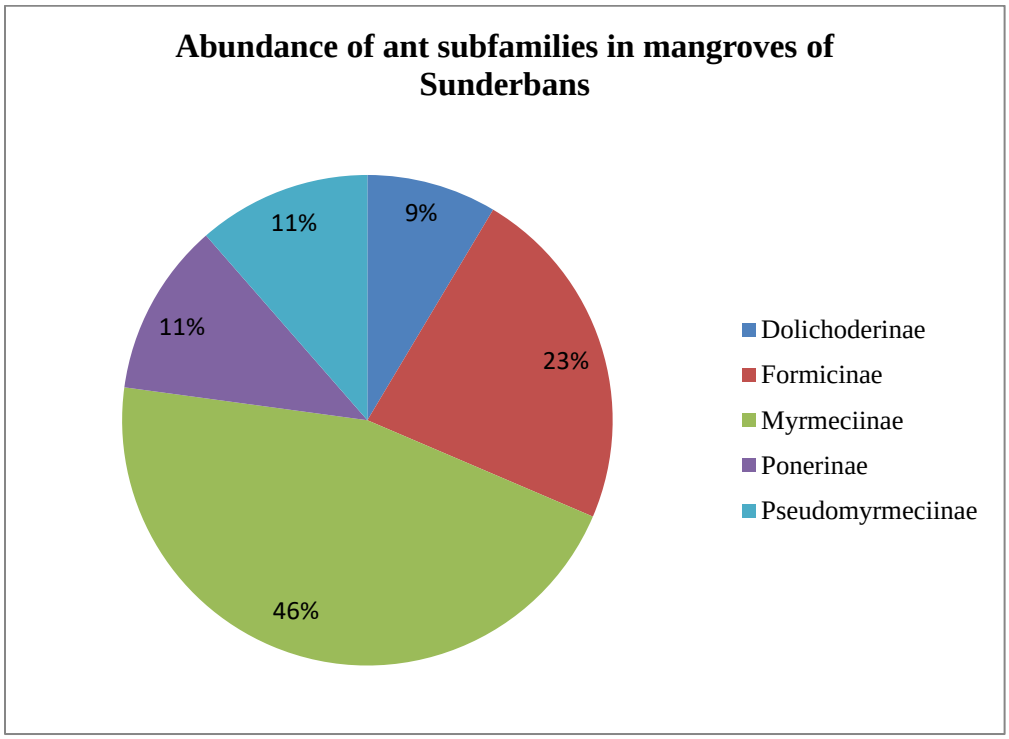

Figure 1: Abundance of Ant Subfamilies in Mangroves of Indian Sundarbans

Table 2: Comparison of Ant Species Abundance during Pre-monsoon and Post - monsoon Seasons of Sundarbans

Ant Species	RA during Pre-monsoon	RA during Post-monsoon
*Camponotuscompressus*Fabricius	**14.37126**	2.349869
*Camponotussericeus*Fabricius	3.892216	3.916449
*Crematogasteranthracina*Smith	2.39521	2.872063
*Crematogasterrogenhoferi*Mayr	**24.8503**	7.049608
*Crematogasteraberrans*Forel	0	0.522193
*Carebaraaffinis*Jerdon	0	0.78329
*Dilobocondylagastroreticulatus*Bharti & Kumar	0	0.522193
*Diacammarugosum*Le Guilou	3.892216	2.088773
*Iridomyrmex anceps*Roger	0.598802	0
*Lepisiotasericea*Forel	1.796407	**7.571802**
*Lepisiotaopaca*Forel	3.293413	0
*Leptogenyshisterica*Forel	0	1.044386
*Meranoplusbicolor*Guerin-Meneville	0.898204	0
*Monomorium floricola*Jerdon	0.598802	**7.832898**
*Monomorium indicum*Forel	**11.07784**	4.699739
*Monomorium atomum*Forel	0	1.56658
*Monomorium latinode*Mayr	**6.886228**	1.044386
*Nylanderia indica*Forel	1.497006	3.655352
*Oecophyllasmaragdina*Fabricius	0.5988024	1.0443864
*Solenopsisgeminata*Fabricius	0	1.827676
*Paratrechinalongicornis*Latreille	9.580838	**27.41514**
*Pheidoleparva*Mayr	0	1.305483
*Pheidolesagei*Forel	0.299401	0.78329
*Pheidolewatsoni*Forel	1.197605	0.78329
*Pseudoneoponerarufipes*Jerdon	0	2.088773
*Polyrachisrastellata*Latreille	0.598802	0
*Tapinoma indicum*Forel	0	0.522193
*Tapinoma melanocephalum*Fabricius	0.898204	**7.571802**
*Tetramoriumlanuginosum*Mayr	0.598802	0
*Tetraponerarufonigra*Jerdon	**8.682635**	5.483029

*Tetraponeraallaborans*Walker	0	1.56658
*Tetraponera nigra*Jerdon	0	2.088773
Tetraponera nitida Smith	0	0.522193
Trichomyrmex destructor Jerdon	1.497006	0
*Trichomyrmexscabriceps*Mayr	0.598802	1.56658
*Anochetusmadaraszi*Mayr	0	0.522193

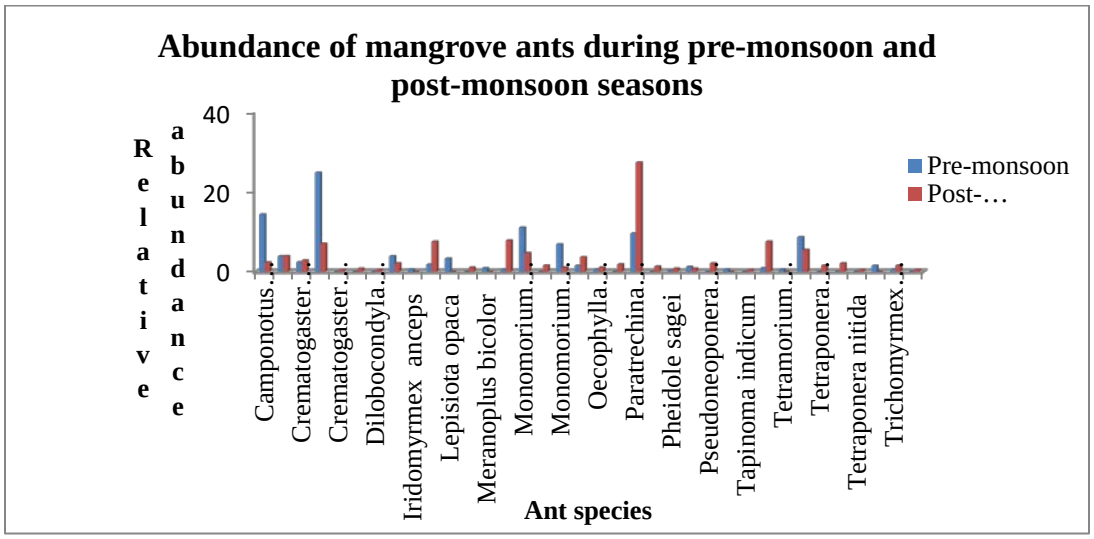

Figure 2: Abundance of Ant Species during Pre-monsoon and Post-monsoon Seasons in the Mangrove Habitat of Sundarbans

Table 3: List of Invasive Species Sampled and Their Subfamilies

Subfamily	Genera	Species
Myrmeciinae	*Monomorium*	*Monomorium floricola*
	Solenopsis	*Solenopsis geminata*
	Trichomyrmex	*Trichomyrmex destructor*
Dolichoderinae	*Tapinoma*	*Tapinoma melanocephalum*
Formicinae	*Paratrechina*	*Paratrechina longicornis*

Discussion

There has been a lot of study done on the diversity and distribution of ants from throughout the world.The ant fauna of a mangrove community in Darwin Harbour was reported by Clay and Andersen in 1996, and they contrasted it with the local savanna and rain forest fauna. In mangroves, they found 16 different ant species, including 3 savannah species, 6 rain forest species, 5 habitat generalists, and 2 species that are only found there. According to Hashim, Jusoh & Nasir (2010), mangrove forests and oil palm plantations are home to a total of 9 species.Fitri *et al.* (2021) reported 11 species of ants belonging to 3 subfamilies and 10 genera from mangrove forest of Pariamon. From West Bengal, Roy *et al.* (2018) first report on mangrove inhabiting ants. They reported total 12 species of ants under the subfamilies Formicinae, Myrmeciinae, Pseudomyrmecinae and Dolichoderinae.The results of the current study showed that the ant community in the Sunderban mangrove is more diverse than other mangroves of the world.

The presence of invasive ants, such as *Paratrechina longicornis*, *Tapinoma melanocephalum*, *Monomorium floricola*, *Trichomyrmex destructor* and *Solenopsis geminata* which are highly adaptable to disturbed habitats, also can cause the loss of other ant species from a habitat due to competition. (Siddiqui *et al.* 2021). Early detection through surveys may target those invasive species (Reaser *et al.* 2020).

Conclusion

Therefore it can be concluded that a wide range of varied species of ants are sustained by the mangrove habitat in Indian Sunderbans. For the purpose of preserving biodiversity, it is therefore strongly advised to expand and create a sustainable mangrove forest to assess ant diversity and abundance in Sunderbans. Non-native species, however, raise problems, especially given how quickly their abundances are growing. As a result, their role in mangrove environments to be discussed, and the variables that promote their occurrence and have a negative impact on native species should be investigated.

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Colorimetric Measurements of Human Blood Glucose Level in Presence of Nano-Scaled Inorganic Materials

Amit Kumar Dutta
Department of Chemistry, Bangabasi Morning College, Kolkata, West Bengal, India

Corresponding Author's Email: akdutta84@gmail.com

ABSTRACT

Nowadays, about 415 million people suffer from diabetes throughout the world. So, the diagnosis and control of diabetes through monitoring of blood sugar levels are of most importance. Several nano-scaled inorganic materials, such as iron oxide (Fe_2O_3), cupric sulphide (CuS), CdS and FeS nanoparticles (NPs) have been established as enzyme models i.e., nano-enzyme for measurements of human blood glucose level. These nano-materials behave like horseradish peroxidase enzyme (HRP) which is commonly used at pathological laboratories during blood sample analysis. To test the enzyme-mimic properties, the experiments were carried out through the reaction between per-oxidase substrate, 3,3′,5,5′-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in the presence of a nano-catalyst, which can be monitored colorimetrically and follow Michaelis–Menten kinetics. Based on this TMB–NPs–H_2O_2 catalysed coloured–reaction, a new analytical method has been explored for identification and quantitative measurement of the concentration of glucose in human blood sample. The probable mechanism of this enzymatic procedure has also been discussed through the detection of hydroxyl radical (OH•). On the basis of the developed methodology, the human blood sugar level can be easily monitored using the attractive nano-enzymes.

Keywords: *Nano-Enzyme; Horseradish Peroxidase; Michaelis–Menten Kinetics; Nonlinear Least Square Fitting; Hydrogen Peroxide; Human Blood Glucose*

Introduction

Diabetes mellitus is a serious disease throughout the world. A higher or lower value of glucose concentration from the normal range (80 to 120 mg/dL or 4.5–6.5 mM) in serum sample is responsible for hyper- and hypo-glycemia disease, correspondingly. So, patients should be cautious to reduce disease-related complications through strict control of blood sugar level, which can be possible through regular diagnosis and monitoring of blood glucose amount (Xu *et al*., 2007 and Badugu; Lakowicz; Geddes, 2004). Again, the persistence of sugar units in human urine samples is similarly more dangerous, because it indicates a serious condition of diabetes. A preliminary screening can easily be done with patients having renal glycosuria which means having high level diabetes (Heller & Feldman, 2008).

Under these circumstances, too many research groups have paid much more attention to developing reliable, cost-effective tools such as titrimetric, spectrophotometric,

electrochemical, etc. (Rivas *et al.*, 2007) for glucose measurement using the horseradish peroxidase (HRP) enzyme (Wang *et al.*, 2000) in the field of medical diagnostics and biotechnology. But one bad luck is that the HRP enzyme holds some drawbacks due to lack of stability, difficult to produce in large quantities and gets easily denatured.

Very recent, Gao *et al.,* (2007) stated that Fe_3O_4 nano-materials show horseradish peroxidase (HRP) enzyme mimetic activity and exposed the entry of different nano-scaled inorganic materials, which have attractive chemical and physical properties, in the bio-medical field.

Keeping this in mind, In the subsequent episode, they have enlarged on details about the enzyme-like activities of our synthesized several nano-scaled inorganic materials and there uses for measurements of human blood glucose level and hydrogen peroxide. How the nano-materials were established as mimic-enzyme by comparing the enzyme kinetic parameters, Michaelis–Menten constant (K_m^{app}) and maximum initial velocity (V_{max}) have been discussed.

Based on these kinetic reactions, this chapter also presented how the calibration curves have been constructed and the blood-sugar level have been measured.

Nano-Scaled Metal Oxides, Metal Sulfides

For colorimetrical measurements of blood-sugar level, the most widely studied nano-scaled inorganic materials include transition-metal oxides, sulfides for instance, iron oxide (Fe_2O_3), cupric sulphide (CuS), CdS and FeS nanoparticles (NPs).

Iron oxide (Fe_2O_3) nanoparticles have been manufactured in laboratories from a trinuclear iron (III) precursor complex through a hydrothermal process using a Teflon PTFE autoclave at 150 °C (Dutta *et al.*, 2012a). The powder X-ray diffraction (XRD) pattern confirmed the formation of the pure maghemite (Fe_2O_3) (JCPDS ID. 39 1346) phase of iron oxide with one major orientation along the (311) plane (Figure 1a). The size and morphology of the nanomaterial has been investigated using transmission electron microscopy (TEM), High-resolution TEM (Figure 1b) which indicate that the sample is composed of well-dispersed hexagonal nanoparticles with an average size about 40 nm.

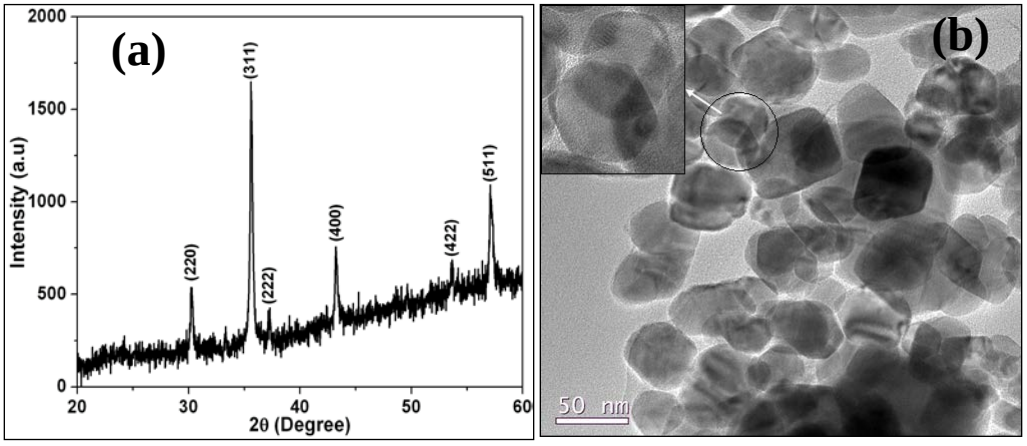


Figure 1: (a) XRD Outline and (b) TEM and HRTEM Pictures (inset) of the Fe_2O_3 NPs (Dutta et al., 2012a)

Another precursor complex $[CuL_2(H_2O)_2]Cl_2$ [HL = pyridine 2-carboxamide] (Dutta *et al.*, 2013) has been used as copper source to prepare cupric sulfide (CuS) nanoparticles via a solvothermal technique using thiourea at pH=8.

CdS NPs (Maji *et al.*, 2012) were synthesized by solvothermal degradation of single-source precursor $[Cd(ACDA)_2]$ using ethylenediamine (EN) or hexadecylamine (HDA) or dimethyl sulfoxide (DMSO) as the solvent at 120 °C.

In another study, FeS NPs (Dutta *et al.*, 2012b) was prepared by dissolution of a trinuclear iron(III) precursor complex in water followed by addition of thiourea, polyvinylpyrrolidone (PVP) and hydrothermal heating in a Teflon PTFE autoclave at 150 °C.

Horseradish Peroxidase–Like Behaviour Measurements

The horseradish peroxidase–like behavior of the proposed nano-scaled inorganic materials was investigated spectro-photometrically through the catalytic reaction of the oxidase substrate 3, 3', 5, 5'–tetramethylbenzidine (TMB) in presence of hydrogen peroxide which generates blue colouration (Figure 2a inset). The reactions were checked by observing the growth of abs (A) value at λ_{max} = 653 nm with time as exposed in Figure 2a. Among the proposed nano-scaled inorganic materials, a comparative performance has been illustrated in Figure 2b and c with iron oxide (Fe_2O_3) and cupric sulfide (CuS) NPs as example and summarized in Table 1.

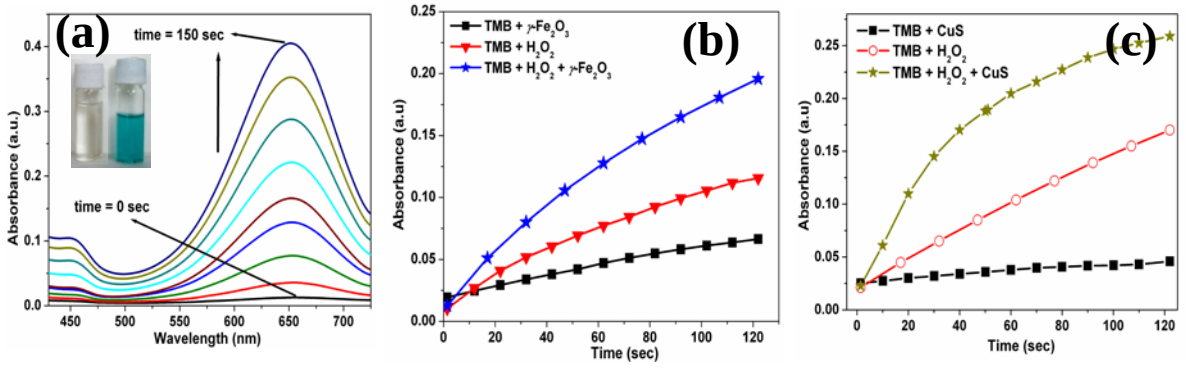


Figure 2: (a) UV–Vis spectral changes of TMB–H_2O_2 system with time, catalyzed by Fe_2O_3 nano-enzyme. Inset: Taking photographs of TMB solutions, before and after the reactions. (b) and (c) absorbance vs. time diagrams of TMB systems using different nano-enzymes under different conditions (Dutta et al., 2013 and Mitra et al., 2014)

To investigate the kinetics of the reaction, the time–stopwatch was started immediately and the absorbance (A) value changing with time was continuously observed at λ_{max} position. The experiments were repeated with fixed amount of H_2O_2 and varying amount of TMB or contrariwise in presence of Fe_2O_3 or CuS nano-enzymes and every-time and the absorbance data were used to calculate kinetic parameters according to Beer–Lambert Law with the help of molar absorption coefficient (ε = 39,100 $M^{-1}cm^{-1}$ at λ_{max} = 653 nm) (Karaseva *et al.*, 2002) of the oxidized product of TMB. It has been found that within the appropriate range of TMB

(Figure 3a, 4a) and H_2O_2 (Figure 3b, 4b) amount, the plot of initial rate vs. TMB or H_2O_2 amount, shows typical Michaelis–Menten like behaviour. i.e.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

E = mimic-enzyme (Fe_2O_3 or CuS NPs), S = substrate (TMB or H_2O_2), ES = enzyme–substrate complex and P = product. The initial rate, $V_0 = k_2[ES]$ can be improved to kinetic equation (2), commonly known as Michaelis–Menten kinetic model.

$$V_0 = \frac{V_{max}[S]}{K_m^{app} + [S]}$$

When the kinetic data were fitted to the above model using nonlinear least squares fitting way, the corresponding parameters (Michaelis–Menten constant) (K_m^{app}) and maximum initial velocity (V_{max}) with TMB as the substrate were acquired. In Table 1, the data have been compared with usually used Horseradish peroxidase (HRP) enzyme and those of previously reported nano-mimetics. All these values can be re-checked from the Lineweaver–Burk (Lineweaver & Burk, 1934) double–reciprocal plot ($1/V_0$ vs. $1/S_0$) (insets of Figure 3 and 4). Actually, Michaelis–Menten constant indicates the enzyme affinity to substrate. Here, a lower value of Michaelis–Menten constant than HRP suggests that the Fe_2O_3 or CuS NPs have greater affinity to TMB. Hence, it has been concluded that the proposed NPs possess inherent peroxidase–like behavior and could be used as artificial peroxidase mimics.

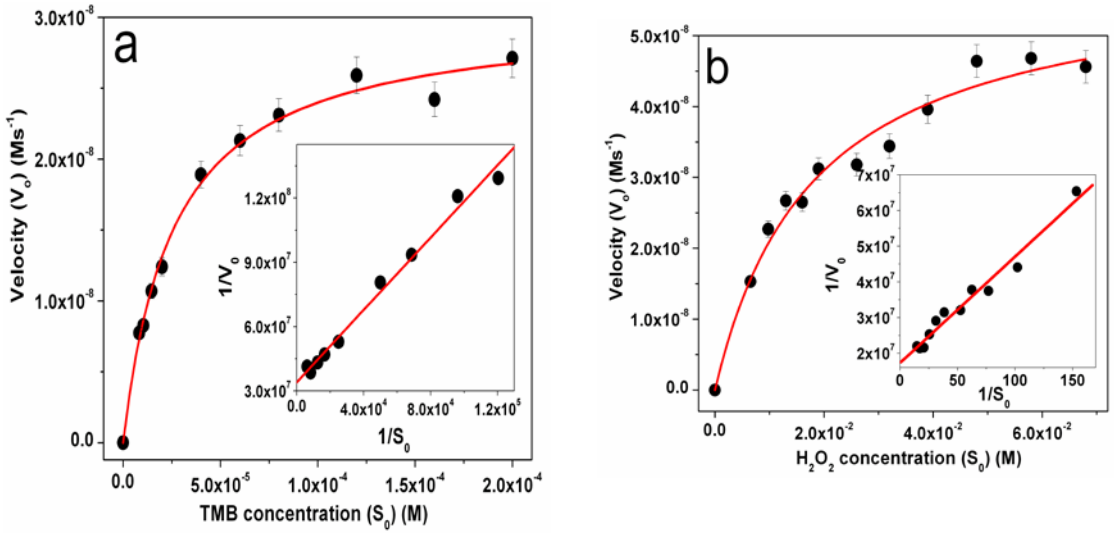


Figure 3: Kinetic analyzed data with the help of the Michaelis-Menten and Lineweaver-Burk model (insets) using γ–Fe_2O_3 NPs (a) changing the amount of TMB with fixed amount of H_2O_2 and (b) changing the amount of H_2O_2 with fixed amount of TMB (Mitra et al., 2014).

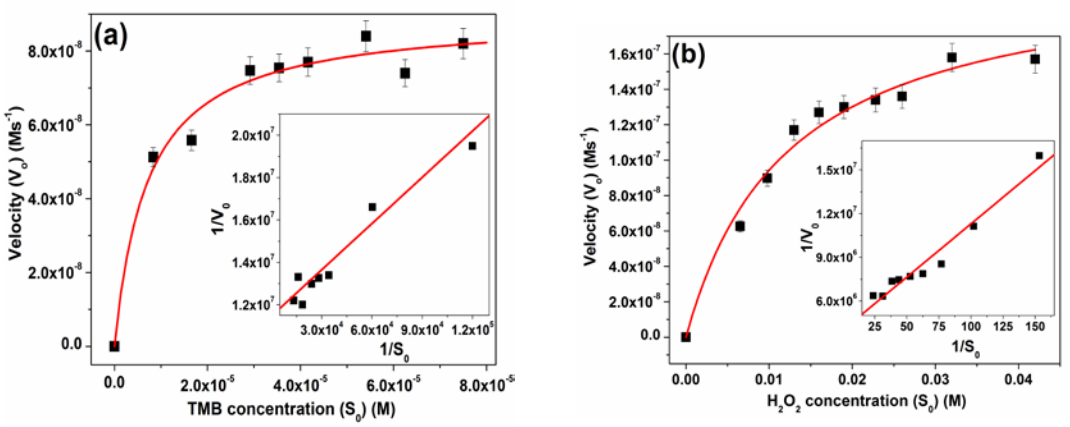


Figure 4: Kinetic analyses plot according to kinetic models via CuS NPs (a) changing the amount of TMB and (b) changing the amount of H_2O_2 (Dutta et al., 2013).

To recognize the mechanism and reason of the enzymatic behavior of the nano-enzymes, Fenton–like operation (He *et al*., 2012) has been proposed and assumed that ferrous ions or cuprous ions, existing at the superficial position of the nano-materials, may react with the substrate with the help of hydrogen peroxide, ensuing in a coloured reaction product.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + OOH^{\bullet} + H^{+}$$

The hydroxyl radical (OH·), produced throughout the reaction sequence, may catalyze the oxidation of the TMB, resulting in a blue color.

Table 1: Assessment of The Kinetic Parameters of The Proposed Nano-Scaled Inorganic Materials Established as Peroxidase-Mimic.

Catalyst	Substrate	K_m^{app} (mM)	V_{max} (M S^{-1})
γ–Fe_2O_3	TMB H_2O_2	0.03 18.0	3.02×10^{-8} 5.9×10^{-8}
CuS	TMB H_2O_2	0.0072 12.0	8.96×10^{-8} 2.09×10^{-7}
HRP	TMB H_2O_2	0.434 3.70	10.00×10^{-8} 8.71×10^{-8}
CdS	TMB H_2O_2	0.0095 3.62	3.57×10^{-8} 5.6×10^{-8}
FeS	TMB H_2O_2	0.0082 9.36	8.70×10^{-8} 1.92×10^{-7}

Colorimetric Glucose Measurements

In this section, the measurement processes of glucose have been demonstrated following the above oxidase–like properties of the CuS or Fe_2O_3 nanoparticles with TMB–H_2O_2 colored-reaction system. Here, a biological enzyme glucose-oxidase (GOx) has been used to oxidize glucose to produce H_2O_2, and that H_2O_2 has been employed in the above TMB–H_2O_2 catalytic reaction in presence of a nano-enzyme instead of external H_2O_2 as follows.

$$\text{Glucose} + O_2 \xrightarrow[\text{pH 7.0}]{\text{GOx}} H_2O_2 + \text{Gluconic acid}$$

$$H_2O_2 + \text{TMB(Colorless)} \xrightarrow[\text{pH 4.0}]{\gamma\text{-}Fe_2O_3\ \text{NPs}} 2H_2O + \text{oxidised TMB(Blue color)}$$

As hydrogen peroxide is the main output of the reaction between glucose oxidase (GOx) and glucose, the amount of H_2O_2 equivalent to amount of oxidized TMB blue color intensity has been employed to measure glucose concentration indirectly. According to Figure 5, a calibration curve has been constructed based on color intensity change i.e., change in abs (A) value at λ_{max} versus glucose concentration in presence of fixed amount of TMB, Gox and nano-enzymes. The response plot is linearly interrelated to glucose concentration from 2-1800 μM using CuS nano-enzyme according to Table 2.

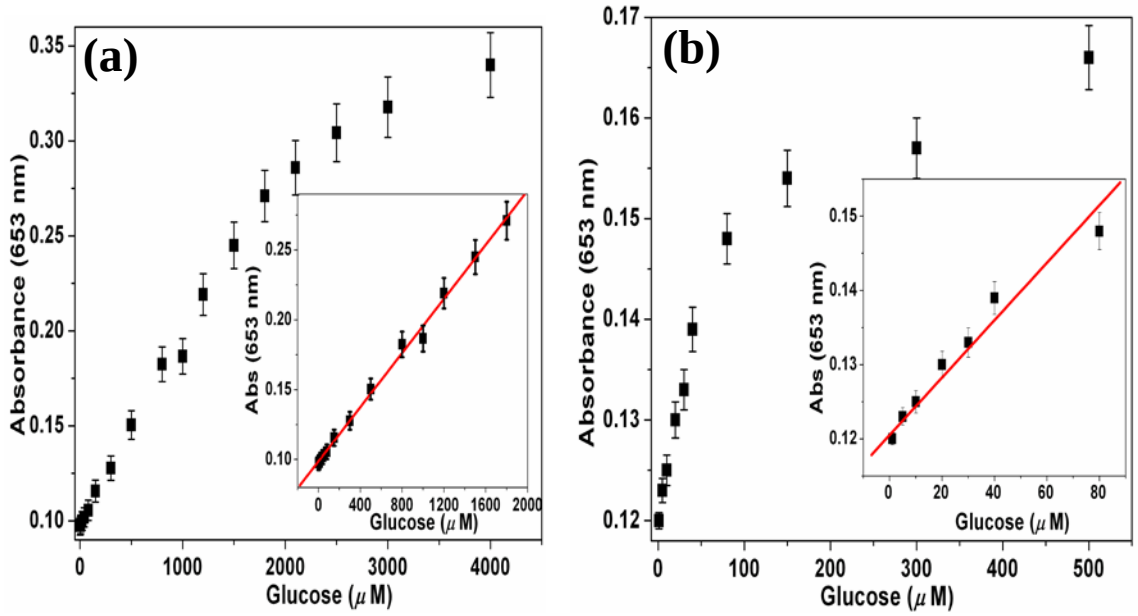


Figure 5: Amount of glucose–response curve using a) GOx/TMB/CuS system and b) GOx/TMB/γ–Fe_2O_3 system. Inset: corresponding linear calibration plot for glucose. (Dutta et al., 2013 and Mitra et al., 2014)

Table 2: Evaluation of Response Parameters towards Glucose Using the Proposed Nano-Scaled Inorganic Materials

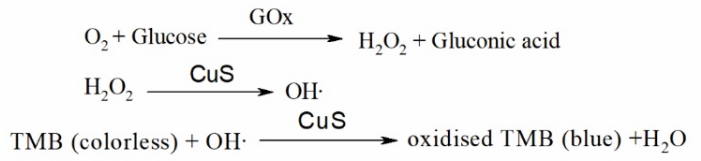
Nano-enzyme	Linear range	Detection limit
γ–Fe_2O_3 NPs	1–80 μM	0.21 μM
CuS nanoparticles	2–1800 μM	0.12 μM
Fe_3O_4 nanoparticles	50-1000 μM	30 μM
CdS NPs	18-1100 μM	4 μM
FeS NPs	2-30 μM	0.5 μM

Using the above glucose measurement technique, an attempt has been made to measure glucose concentration in real samples such as blood and excreted urine of healthy human volunteers. In its place of glucose, when the diluted urine and blood solutions have been used with GOx like glucose-GOx system described above, the abs (A) values at λ_{max} = 653 nm were collected. From the calibration curves, the amount of glucose in the above blood and urine are calculated which matched well with pathological lab report (Table 3).

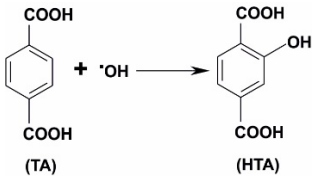
Table 3: Measurements of Sugar Level in Real Samples using γ –Fe2O3 Nano-Enzyme (Dutta et al., 2013)

Sample	Pathological Lab report	Colorimetric measurements
Blood A	5.93 mM (106.0 mg/dl) (GOD-POD process)	6.20 mM (111.5 mg/dl)
Blood B	5.15 mM (92.0 mg/dl) (Hexokinase way)	5.37 mM (96.5 mg/dl)
Urine A	4.90 mM (89.0 mg/dl) (Hexokinase way)	4.73 mM (85.0 mg/dl)
Urine B	5.22 mM (94.0 mg/dl) (Hexokinase way)	5.06 mM (91.0 mg/dl)

The above glucose reaction may also occur through the production of hydroxyl radicals from the decomposition of hydrogen peroxide similar to the enzyme like activity.



The generation of OH· has been recognized by terephthalic acid (TA) photoluminescence analytical systems (Barreto *et al*., 1994) where TA combined with hydroxyl radicals and formed 2–hydroxy terephthalic acid (HTA) as stated by following equation.



Conclusion

In summary, the proposed nano-scaled inorganic materials are widely explored as an peroxidase–mimic like HRP, which exhibits effective peroxidase–like properties towards conversion of peroxidase substrates TMB with the help of H_2O_2 producing a blue-coloured solution. They show various advantages such as low–cost, easy to synthesis, non–toxic, high catalytic efficiency, excellent steady to biodegradation, and not being liable to denaturation. Based on this TMB–NPs–H_2O_2 catalysed colour–reaction, new analytical platform has been established for glucose identification. Human blood glucose and urine glucose levels have also been measured successfully with the help of glucose oxidase (GOx) and a proposed nano-scaled mimic enzyme. All these studies suggest that the proposed nano-scaled inorganic materials are attractive materials which will help their application in the biomedical and bioengineering fields.

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Arsenic Toxicity: Its Existence, Permeability and Ill Effects on Human Health – A Mini Overview

Jayita Dutta

Department of Chemistry, Ranaghat College, Ranaghat, West Bengal, India

Corresponding Author's Email: jayita.chems87@gmail.com

ABSTRACT

Arsenic has an awful influence on millions to billions of people throughout the world, with a special emphasize on the population of economically developing countries like India because of its poisonous nature, which leaves destructive to deadly health effects on human beings. Mobilization of this metalloid contaminant is possible from both natural and anthropogenic sources following various processes. Absorption of arsenic in the human body occurs either through inhalation of air coming out of various wastes of industry, soil, fossil fuels etc., or ingestion of contaminated food, and most importantly, via drinking water having a higher arsenic concentration. Long back, almost a century ago, arsenic was used as a homicidal agent. Exertion of such a toxicant is also possible through its vast agricultural use as a pesticide and its use as a chelating therapeutic agent used to save human lives from arsenic spreading in different body parts. The toxic nature of inorganic trivalent arsenic is much more dominant over pentavalent as well as its organic species. Its adverse health effects on humans are visibly of two types: acute and chronic. Depending upon the nature of exposure and absorption of such a lethal dose in the human body, it causes several disorders, including gastrointestinal, skin, pulmonary, cardiovascular, hepatic, neurobehavioral, diabetic, reproductive etc. along with carcinogenicity of the lung, bladder, skin, kidney, etc. Preventive measures are necessary by enhancing the awareness of poor people about the cruel destiny of arsenic intake, providing ample drinking water free from arsenic in the affected areas, and developing initiative by identifying the affected sources of various government and non-government funding agencies. In this article, an attempt has been made to provide an overview of the health impacts caused by arsenic poisoning.

***Keywords*:** *Arsenic Toxicity*; *Drinking Water*; *India*; *Human Health*; *Prevention*

Introduction

Among the metals causing toxicity, arsenic is one of the most important contaminants, leading to extensive endemicity throughout the sphere. The toxic effects of arsenic on human health have now become a worldwide problem due to their severity. Intake of arsenic at elevated level has lots of fatalistic effects on the human body. In the early eighteenth century, arsenic became renowned for its inherent poisonous nature because its sharp potential as a homicidal agent resulted in the recurrent murders of well reputed persons. Arsenic, for having such efficacy in killing people, attained fame as the 'king of poisons' and the 'poison of kings' (Hughes *et al*., 2011). The path of insertion, duration of

exposure to toxic arsenic and its existence in different chemical forms determine the amount of arsenic absorbed in the body.

The naturally existent virulent arsenic metalloid is omnipresent in the environment. It is found to be present in rocks, rock forming minerals, soils, sediments, organisms, surface and ground water in an appreciable amount (Shankar, Shankar & Shikha, 2014). Rock erosion, volcanic eruptions, dust storms, weathering reactions, and forest fires are some of the naturally mobilizing activities of poisonous arsenic (Herath *et al*., 2016). In addition to these natural methodologies, several anthropogenic activities also need to be mentioned as sources of arsenic spreading among humans. Metal mining, burning of coals, smelting, agronomy, use of fossil fuels, manufacture of paper, cement industry, and wood preservatives are the significant anthropogenic origins of arsenic (Chung, Yu & Hong, 2014). The responsibility of individual agricultural industries in the disbursal of vast amounts of arsenic into the environment is worthy of mention. A large amount of arsenic is released during pesticide production in the forms of arsenic acid, dimethylarsenic acid, mono- and di-sodium methyl arsenate etc. Again, utilization of arsanilic acid as an additive to animal feed transmits the threatening toxicity of arsenic (Hughes *et al*., 2011).

There can be several ways of arsenic insertion in human body *via* consumption of food, fossil fuel, drinking water, inhalation, contact through the skin etc., of which drinking water is the most dangerous medium. The route of exertion of noxious arsenic in the human body is highly complicated and does not follow any conventionally accurate technique. It is still not enlightened, evidently, even after going through a long journey of research work. Alarming levels of arsenic are observed to be released mainly from water, either by direct intake or through its usage in cooked things. People procure drinking water from variable sources in nearby localities like wells, reservoirs, rivers, lakes, etc. To date, especially in developing countries like India and others, groundwater is considered to be the main source for drinking purposes. Incidentally, the extent of contamination with deadly arsenic happens to be the maximum in groundwater. In groundwater, elevated arsenic concentrations impose public health threat, mostly to the severely affected millions of people in India and Bangladesh, as they are directly exposed to varying levels of impermissible toxicity (Chowdhury *et al*., 2000). The aquifers of the alluvial Ganges adapted as the source of drinking water supply for the Bengal basin, are immensely polluted with poisonous arsenic. The Bengal belt is one of the most hampered parts by arsenic toxicity because of its rise in concentration in groundwater (Das *et al*., 2009).

Deadly arsenic develops in groundwater pollution from the very well-known natural sources of minerals like arsenic-containing sulfides (red realgar and yellow-orange orpiment), pyrites and iron oxy-hydroxides having arsenic. The mobilization of toxicity arising from arsenic release in groundwater results mainly from arsenic-sulfide oxidation, microbial metabolism, desorption and reductive dissolution of arsenic from its hydroxides, oxides and iron-oxides (Welch *et al*. 2000).

Prolonged swallowing of arsenic by humans in excess amounts causes a large number of diseases, like multiorgan failure, and even a number of deadly health disorders. A rise in the

intake of arsenic through drinking water shows dermatological disorders, melanosis, gastric problems, pancreatic insufficiency, bowel irritation, symptoms like paralysis, diseases related to cardiology, neuropathy, behavioral abnormalities, vascular anomalies, reproductive system, portal fibrosis, hyperkinesias, renal disease, and diabetes (Mandal & Suzuki, 2002). Several carcinogenic syndromes are also caused by toxic arsenic consumption, which results in skin, lung, kidney, liver, and urinary bladder cancer (Mazumder & Dasgupta, 2011). It shows its outcome even in stillbirths, human infant deaths etc. In this article, the main focus is on the relentless dermatological, non-carcinogenic, and carcinogenic effects caused by mankind's chronic arsenic ingestion.

Literature Review

Chemical Existence of Toxic Arsenic

In conjunction with other elements of the nitrogen group *i.e.*, group 15, arsenic is a fearsome toxic element present in the fourth period of the modern periodic table with its atomic weight of 74.92 and 33 atomic number. This harmful element has an elaborate history of creating vulnerability in human health. This element has neither odor nor taste, so its detection in people becomes very troublesome (Hughes *et al*., 2011). But its poisoning effects leave visible ailments in the human anatomy. This environmentally potent toxicant is basically a metalloid with the dual characteristics of both a metal and a non-metal. It shows the lustrous nature of metal, its ductility and malleability, and its heat and electricity conduction properties, along with the non-ignorant part of having the non-ductility nature of it in its elemental form. Depending on the variability of the existence of different oxidation states, arsenic shows its diversity in the toxicity level. Arsenic can exist naturally in the span of –III, 0, +III and +V oxidation states, of which the most commonly persistent are the +III and +V ones and the more rare are the 0 (elemental) and –III (arsine and arsenide) states. In groundwater, the regularly known chemical forms of arsenic are arsenites and arsenates with their +III and +V valence states, respectively, as inorganic species, which undergo inter-conversion by redox methodology. Organic forms of arsenic derived from the inorganic ones by the method of bio-methylation also exist in nature. In addition to the inorganic forms, organic methylated arsenicals are also found in groundwater in very small amounts. The public is mostly exposed to these two valence states of arsenic (Flora, 2015). Mankind is affected mostly by the consumption of trivalent and pentavalent arsenic compounds, as they are absorbed to a greater extent by human organs. Some well known arsenic compounds with +3 and +5 oxidation states are arsenite(+III) and arsenate(+V), arsenic trioxide(+III) and pentoxide(+V), monomethyl arsonous(+III) and arsenic acid(+V), dimethyl arsinous(+III) and arsenic acid(+V), trimethyl arsine oxide(+V), arsanilic acid(+V), arsenobetaine(+V) *etc*. (Sturgeon *et al*., 1989) In comparison to organic arsenic compounds, the abundance of inorganic arsenic compounds in the environment is much higher. Considering the order of toxicity, it has been noticed that the As(III) is about sixty times more toxic than the As(V), and in the case of methylated arsenic, *i.e.*, the organic one, it is less toxic by almost a hundred times than the inorganic arsenic compounds (Hughes *et al*., 2011).

Permeability Level of Arsenic in Human Body

A huge entity of people globally, exceeding 100 million, and especially a community also in millions of poor developing countries like India are at risk of considerable elevated levels of deadly arsenic exposure, of which maximal absorption occurs through drinking water (Ravenscroft, Brammer & Richards, 2009). Populations higher than two billion are dependent on groundwater as the dominant source of drinking water and this has become a universal challenge to provide drinking water free from toxic metal contamination with such rapid population growth. In accordance with the 1993 World Health Organization (WHO) guidelines and also the US Environment Protection Agency (EPA, 2001), the permissible level of arsenic in the human body has been reduced from 50 ppb to 10 ppb to diminish the carcinogenic hazard to mankind. However, in more than 100 countries, inclusive of the USA, Africa, Argentina, Australia, Bangladesh, Burma, Cambodia, Chile, China, Hungary, India, Mexico, Myanmar, Nepal, Pakistan, Peru, Sri Lanka, Thailand and Vietnam (Shaji *et al*., 2021), in underground water arsenic concentration has been detected in more than the prescribed level by WHO. Taking into account the severity of exposure to the arsenic toxicity of an economically developing nation like India, it has been found that in addition to the four Union Territories, almost twenty states of India, among which Himachal Pradesh, Haryana, Punjab, Arunachal Pradesh, Assam, Bihar, Jharkhand, West Bengal, Chhattisgarh, Odisha, Andhra Pradesh etc. have been affected by the harshness of arsenic contamination in groundwater to a greater extent (Shaji *et al*., 2021). In current times, in countries like India, a huge mass are not at all very mindful of the cruel fate of humankind caused by drinking arsenic contaminated groundwater. In India, it has been well documented that the extent of poisonous arsenic was found is much higher in shallow aquifers than in aquifers more than 100 m deeper (Shaji *et al*., 2021). In 1980, in the eight districts of West Bengal, India, the existence of arsenic was first proclaimed at a very high level (Das, 2019). On both banks of the Bhagirathi River, the districts of Bengal (Howrah, Hooghly, Bardhaman, Malda, Murshidabad, Nadia, 24-parganas) are found to be the most affected by arsenic occurrence in groundwater. The concentration of such a toxicant as arsenic was found to be abnormally high, even greater than 300 ppb in some of the aquifers, which can create disaster and lead to a death toll. This endorsed guideline of the recommended level of arsenic in humans cannot be maintained in various countries, this standard value can only be followed to some extent in well-off countries. In Germany, since 1996, after a series of arguments, they have decided to accept the allowed limit of ingestion of arsenic in people to be 10 ppb as standard, whereas in Australia it has a limit of up to 7 ppb, and in Canada they have considered the permissible limit to be up to 25 ppb (Ravenscroft, Brammer & Richards, 2009). Countries suffering from economic obstacles fail to implement such a level of arsenic absorption because of the unease of immoderate expenditure. It is worthy of mention that 10 ppb is not even the safest guideline for living a healthier life; in the real world, it should have been 0.17 ppb. But failure in the instrumental measurement accuracy for arsenic speciation results in the compromise of such a recommended level (World Health Organization, 1993).

Consequences of Toxic Arsenic on Human Health

In ancient ages and also in modern ages, the characteristics of the insidious arsenic virulence resulting in mortality have remained changeless. The untold suffering of a huge population of millions living in poverty and facing starvation, specifically in the Bengal basin, including India and Bangladesh, is horrendous due to their continuous exposure to arsenic toxicity essentially through drinking water directly or indirectly above its admissible level for survival. Arsenic poisoning in the human body can occur in acute and chronic manners. Acute poisoning by arsenic develops mostly by unintended intake or very rarely by ingestion out of depression with a desire for own destructiveness at an abnormally high concentration (Ravenscroft, Brammer & Richards, 2009). Acute toxicity caused by arsenic absorption in the human body normally shows its harshness in a very short time, sometimes within an hour. Acute arsenic maladies involve impairment of cardiological organs, imbalance of the nervous system, which follow the pathway of fatality in a short duration. Another major dilemma as a symptom of acute arsenic poisoning happens to the gastrological organs of the human body, involving a vicious vomiting tendency, unbearable abdominal pain, diarrhea, nausea, and uncontrollable salivation. It also causes muscular aches, skin disorders, and multi-organ breakdown. In acute syndrome, variability in the amount of consumption of arsenic and its absorption in the human body leads to the loss of life within a time range of one to four days (Ratnaike, 2003).

In accordance with WHO, chronic arsenic syndrome is the outcome of arsenic intake much above the safe level for a long duration, perhaps more than six months. Health complications arising out of chronic arsenic toxicity sometimes remain asymptomatic for even more than ten years. This toxicity has a number of severe human health complications, including disorders in the immune system, bronchitis, hematological imbalance, liver cirrhosis, peripheral neuropathy, non- functional renal system, cardiological failures, dermatological complexity and diabetes mellitus (Ratnaike, 2003). Also, chronic arsenic consumption through drinking water causes detrimental reproductive irregularities, resulting in premature birth, stillbirth, instinctive abortion, retardation in child growth and infections at the infant stage. (Hopenhayn-Rich *et al*., 2000). Chronic arsenic exposure to an extremity increases the probability of malignancy in different body parts of a human being. In 1980, arsenic was perceived as a carcinogenic element and received registration from the International Agency for Research on Cancer (IARC, 2012). Exhaustion of arsenic in chronic fashion leads to the malignancy of different organs of the human body, like the skin, lung, liver, kidney, urinary bladder and colon. The aftereffects of carcinogenic behavior in humans as a result of prolonged arsenic exposure in the initial stages of life last for decades until old age (Ratnaike, 2003).

Effects on Skin

Considering the skin to be the major part of the body, chronic arsenic exposure to it shows a number of dermatological abnormalities, especially in the undernourished people of economically developing countries. In 1983, skin lesions caused by arsenic consumption were first determined in India at the School of Tropical Medicine in the Dermatology

Department, Kolkata (Saha, 1995). Hyperkeratosis, keratosis and pigmentation are the most common forms of skin lesions arising from chronic arsenic poisoning. Keratosis is associated with the development of uniform or sometimes diffused nodular elevations on the upper part of the skin, including the palms and soles, resulting in its thickening. With discrepancies in the level of penetration of the toxic arsenic inside the skin depending upon the extent of exposure, arsenical keratosis can be of various types. When its outcome is seen on both the palms and soles after a decade of chronic exposure, the dryness and thickening of the skin are referred to as diffused keratosis, sometimes leading to severe keratosis. When its visibility is on the soles of the feet or only on the dorsal part of the palms, soles, and legs with sensible nodules, those are referred to as partial and dorsal keratosis, respectively (Ravenscroft, Brammer & Richards, 2009). Another variety is the spotted keratosis, with a greater number of enlarged, rough, dry, nodular appearances. Hyperkeratosis shows symptomatic skin thickening with visible cracks and fissures (Saha *et al*., 1999). Hyperpigmentation emerging out of long-term chronic arsenic exposure appears as dispersed dark brown spots, skin darkening in a diffused manner noticeably on limbs and trunks, along with peculiar appearances of colorless spots following somewhat like a raindrop pattern (Ratnaike, 2003). The rise in exposure to ultraviolet radiation and the presence of a greater quantity of melanin in the skin raise the probability of comparatively rare Bowen's disease, mostly in people of Asian countries. In de-pigmented skin, *i.e*., in the absence of melanin, dermal arsenic absorption accelerates the tendency for squamous and basal cell carcinoma (Ratnaike, 2003). Another symptomatic malady termed "Mee's line due to the accumulation of toxic arsenic in the keratin-enriched part of the human body arising from its chronic ingestion shows marked white lines appearing crosswise in the finger and toe nails (Ratnaike, 2003). A reported observation of the gender-based effect of arsenic contamination presents a slight predominance of skin lesions in males over females (Rahman *et al.*, 2006).

Effects on Neurological System

Arsenic absorption in different parts of the body for a lengthy period can affect the brain markedly because of its wide range of toxicity. Its effect appears to be a distraction of the concentration of the mind and learning abilities of the affected person, as it can efficiently penetrate the blood-brain barrier (Mundey *et al.*, 2013). Intake of arsenic and its agglomeration for a prolonged period of time from early childhood can show several neurobehavioral changes upon reaching the early adulthood stage (Tsai *et al.*, 2003). The maximum deposition of toxic arsenic in the brain occurs in the pituitary gland. Chronic inhalation of more poisonous inorganic arsenic can develop complications related to sensory and symmetrical sensorimotor neuropathy, which affects the muscles and sensations (Guha Mazumder, 2003). Numbness, muscle weakness, loss of reflexes, pain in the soles of the feet and a feeling of paraesthesia are some of the common symptoms caused by neuropathy (Vahidnia *et al.*, 2007). Arsenic consumption above its recommended level by WHO can also cause central and peripheral neuropathies, of which peripheral neuropathy is the prevalent one affecting the peripheral nerves (Mathew, Vale & Adcock,

2010). Extensive study on this reveals that the symptoms arising from peripheral neuropathy have very much in common with Guillain–Barré syndrome, which has its own calamitous consequence of showing little weakness and sometimes resulting in terrible paralysis. In West Bengal, India, a detailed survey reported that severe complications caused by peripheral neuropathy emerge mainly from chronic arsenic ingestion through drinking contaminated groundwater (Mukherjee *et al.*, 2003).

Effects on Respiratory System

Chronic inorganic arsenic ingestion in elevated concentrations through drinking water or through the exhaustion of dust and fumes in the industrial area is a worthy factor in growing respiratory complexities, causing malfunctionality of the lungs and leading to fatalities. A longitudinal study related to respiratory troubles due to arsenic absorption reveals some prevalent clinical symptoms like trachea and asthmatic bronchitis, laryngitis, chronic cough, wheezing chest sounds, breath shortness and rhinitis (Guha Mazumder *et al*., 2000). A long-term research report admits that numerous people from the Bengal basin with harsh skin lesions arising from arsenic intake at elevated levels suffer from adverse pulmonary diseases (Guha Mazumder, 2007). Chronic obstructive pulmonary disease (COPD) is a very well-known respiratory illness that has an acceptable connection with chronic arsenic toxicity. Arsenic intake unconsciously from very early childhood can enhance the chances of bronchiectasis (Guha Mazumder *et al*., 2005).

Effects on Cardiovascular System

Chronic penetration of poisonous inorganic arsenic in its trioxide form rapidly enhances the probability of human expiration, arising from the impairment of the cardiovascular system and disrupting the normal flow of the blood vessels. An enhancement in the accumulation of platelets in the human body due to prolonged arsenic intake also hampers normal cardiological activities (Lee *et al.*, 2002). Thickening and stiffening of arteries, myocardial infarction and ischemic heart diseases disrupting the blood flow to the heart muscle are also caused by the severity of arsenic consumption. Another endemic cardiovascular disease is Blackfoot disease, which results in foot gangrene, and this is mainly confined to the coast of the western part of Taiwan (Rahman *et al.*, 1999).

Effects on Renal System

Kidney irregularities, as a virulent outcome of chronic arsenic absorption in the human body, have now been gradually increasing in the present days. Arsenic intake as a result of long term exposure to it causes its simultaneous liberation through renal functioning along with its aggregation in the kidneys, damaging the renal tissues (Madden & Fowler, 2000). In kidneys as well, the conversion of less toxic pentavalent arsenic to more toxic, sparingly soluble trivalent arsenic takes place. Hypourea, urea nitrogen level in blood, proteinurea and rise in the level of serum creatinine are some of the kinds of renal injuries caused by the poisonous nature of arsenic trioxide intake in various ways (Sasaki, Oshima & Fujimura, 2007). Deposition of arsenic in the renal organs to a greater extent also causes destruction of the kidney capillaries, glomeruli and tubules. At the beginning, tubular damage occurs

followed by brisk tubular cell regeneration, which is accompanied by concurrent thickening of the glomerular basement membrane and interstitial fibrosis as an alarming consequence of chronic arsenic saturation in human body organs (Fowler & Weissburg, 1974).

Effects on the Reproductive System

Arsenic is considered an embryo-toxic element due to its detrimental effects on the overall reproductive procedure involving pregnancy and childbirth, hampering the maturity of the fetus depending upon the nature and span of exposure to it. Abnormalities related to pregnancy resulting in stillbirth, conceptus mortality, preterm

birth, and neonatal death are the crucial effects of arsenic absorption by pregnant women through drinking water (Ahmad *et al.*, 2001). The probability of these critical effects is reported to be almost six times higher for arsenic exposed pregnant women as compared to non-exposed pregnant ones (Von Ehrenstein *et al.*, 2006). Chronic exposure to arsenic, even a little above the recommended level via drinking water, has the effect of reducing infant birth weight and retarding the growth of the fetus in the uterus. Arsenic chronic exposure to pregnant women also hampers the excretion process through urine and creates disturbance in the distribution of metabolites, leaving toxic effects on the growing fetus, which has an impact at the different stages of pregnancy. So far, the discussion has been confined to the toxic effect of arsenic on females, but it is also prominent in the case of males showing the symptoms of improper functioning of the gonad due to a failure in testosterone synthesis along with symptomatic apoptosis and necrosis (Shen *et al.*, 2013).

Carcinogenic Effects

Arsenic is a very familiar toxic element because of its disastrous carcinogenic effect on humans since the late seventeenth century (Kligerman & Tennant, 2007). Since 1980, arsenic has been recognized as an element highly responsible for malignancy in different human organs by the International Agency for Research on Cancer (IARC). Risk of cancer enhances depending upon the medium of arsenic intake and its absorption in different body parts at a raised concentration, whether it is via inhalation involving people grasping arsenic-contaminated air through their exposures to different industrial operations or by ingestion of toxic arsenic polluted groundwater for drinking purposes for a lengthy duration. It has been evidenced from the exhaustive research conducted in different countries worldwide, like Argentina, Taiwan, Chile, USA, India and Bangladesh that there is a close attachment between exposure to arsenic and malignancy (Martinez *et al.*, 2011). In West Bengal, India, a huge mass faced the cruelty of death caused by the carcinogenic outcome of horrible skin lesions due to arsenic intake from drinking water or food; though the carcinogenicity arising from skin abrasion for chronic arsenic exposure could not be identified by those living in poverty because of economic obstacles (Rahman *et al.*, 2011). The noxious mechanisms of arsenic carcinogenicity in the human body involve abnormal chromosomal arrangements, oxidative stress, DNA repair inhibition, slow progressive alteration in DNA methylation pattern, transformed growth factors, rapid rise in cell generation, promotion or progression, gene amplification and its suppression (Kitchin,

2001). Several inorganic arsenicals, inclusive of arsenite, arsenates, and arsenic trioxide, result in malignancy of the skin, lung, liver, kidney, urinary bladder and prostate. In addition to inorganic arsenic compounds, some organic methylated trivalent arsenic species also show a probability of developing carcinogenic effects (Mass *et al.*, 2001). Genetic disorders due to transformations in the DNA structure and irregularities in the arrangement of chromosomes are found to be more prominent in symptomatic people suffering from arsenic induced skin diseases as a result of drinking polluted water than in asymptomatic ones because of their non-exposure to poisonous arsenic (Mahata *et al.*, 2004).

Preventive Measures

The current scenario of the universally perilous aftereffects of arsenic ingestion on human health needs extraordinary attention to protect mankind. Intake of a balanced diet with the requisite amount of vitamins and mineral supplements and pure drinking water are the necessities for saving themselves from the arsenic chronic syndrome. Considering all the different means of arsenic exposure for people, the spreading of a considerable number of deadly ailments through drinking water has become an alarming concern. Several preventive measures are very essential for weakening the severity of arsenic poisoning in humans. In the detected localities with unsafe levels of arsenic concentration in drinking water sources, there should be a rapid enhancement of chemical examination of water quality to reduce the concentration level by taking appropriate measures. The foremost objective is to identify the wells that are in use with a non-permissible level of arsenic present, providing the public with an alternative source of arsenic-free water. Digging deeper wells is a necessary factor for lowering the amount of arsenic, as shallow aquifers have a higher risk of arsenic presence. Harvesting rainwater can also be considered an alternative source for drinking purposes other than contaminated groundwater. Proper application of the methodologies for providing safe drinking water should be implemented on an immediate basis, as symptoms and signs arising from arsenic poisoning have been found to be reduced after 3–4 years after improvement of drinking water quality, with a reduced rate of fatalities. One notable way of keeping people safe from the curse of arsenic toxicity is the enhancement of awareness among the common people by increasing the publicity regarding the unfortunate effects caused by arsenic consumption well above the accepted guidelines by WHO in order to reduce their further exposures.

Since long ago, one promising way of intoxicating arsenic metal by isolating it from blood proteins is by chelation therapy using the common antidote British anti-lewisite (BAL) or dimercaprol (chemical name). In spite of its potency in protecting from chronic arsenic toxicity, BAL causes the simultaneous partial distribution of poisonous arsenic to the brain. Due to the toxic activity of BAL, other chelating agents such as DMPS (dimercaptopropane-1-sulfonate) and DMSA (dimercaptosuccinic acid) with a greater therapeutic index are used to reduce arsenic toxicity. With an increase in the time gap between exposure to toxic metals and chelation, the efficiency of the chelating agent gradually decreases or sometimes disappears (Kosnett, 2013). In addition to chelating therapy, biological monitoring is also needed for the detection of arsenic levels through the collection of hair,

blood, urine and toe nail samples. Whole hearted initiative is very much obligatory from government and non-government ends for inspecting the water quality of neglected rural areas through proper identification via research, considering it a challenge to provide arsenic-free or at least within the safe limit drinking water.

Conclusion

The global devastating essence of arsenic poisoning by acute and chronic ingestion leaves a matter of serious health concern predominantly to the people of rural as well as urban areas of countries like India and others, which are socially and economically unprogressive. This destructiveness caused by toxicity, showing the path of individual physical botheration even up to death, can take the shape of severity because of a huge gap between the awareness of people about the fate of such poisoning and the urge of the government, through its initiatives, to view an arsenic-free country by taking necessary precautionary measures. The poisoning nature of inorganic arsenic has been realized to be higher than that of organic ones. Arsenic intake above its permeable limit through food or drinking water causes sincere health anomalies related to the skin, respiratory system, nervous system, even loss of memory, cardiovascular system, hepatic system, renal system, reproductive system and deadly cancer of some organs, including the skin, lungs, liver, kidney and urinary bladder. The supply of ample quantities of nutritious food and pure drinking water should be enhanced in the affected areas in order to get some relief from the curse of arsenic virulence. A sincere approach to extensive, meticulous chemical testing of usable water and also biological analysis of human samples is very much recommended by the government and other laboratories at ground level to fight against such a slow-killing toxicant. More focus is needed on the research and development activities to counter the arsenic toxicity that is destroying humankind by increasing the accuracy of its detection, the application of chelation therapy, and other versatile, affordable scientific technologies without hampering the environmental ecology.

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Diversity and Ethological Study of Butterfly Species Found in Sree Chaitanya College Campus, North 24 Parganas, West Bengal

Priyankar Sanphui
Department of Zoology, Sree Chaitanya College, North 24 Pargana, Habra, West Bengal, India

Corresponding Author's Email: sanphuip@gmail.com

ABSTRACT

Butterflies are an extremely important group of colorful insects belonging to Phylum – Arthropoda, under Class- Insecta and order- Lepidoptera. They are highly sensitive to environmental change and sometimes considered bio-indicators of climate and are potential pollinators. This work was aimed at studying the diversity and common behavioral aspects of butterflies. The study was conducted in Habra and around Sree Chaitanya College from February to September 2022. A total of 35 different species under 25 genera belonging to five families were reported. The maximum number of species belonged to the family Nymphalidae. The ethological aspects of some butterflies were studied in detail. Six butterfly species belonging to three families were studied. The butterfly species studied were the tawny coster, plain tiger, common jezebel, common palm fly, common silverline. A specific behavioural pattern like hovering, basking, resting, flight and nectarine was documented. Observation revealed that plain tiger, tawny coster, common silverline spent most of the time resting. The preference of the butterfly species for nectar-collecting plants was also studied. It was observed that, despite having a number of flowering and non-flowering plants in the area, each butterfly species has a specific preference for their nectar plants.

Keywords: *Butterfly; Diversity; Sree Chaitanya College; Ethology*

Introduction

Butterflies are one of the best taxonomically studied groups of insects (Robbins & Opler, 1997) There are more than 28000 species of butterflies worldwide and India having different terrain, climate and vegetation, houses about 1500 species of butterflies (Tiple, 2011). The butterflies have been regarded as indicators of the health and quality of their host plant and the ecosystem as a whole. The habitat ecology of butterfly species is governed by nectar as well as host plant preference. A report suggests that the butterfly species increase as per the availability of plant species in semi-urban and urban areas (Kuussaari *et al*., 2007). Butterflies show a preference for nectar flowers and do not feed indiscriminately on any flowers available (Tiple, Deshmukh & Dennis, 2005). However, very little information is available regarding the feeding habits of adult butterflies (Kunte, 2000).

With developmental activity and anthropogenic reasons greenery is lost. In addition, the increase in pollution of the air, water and soil, poses threats to wildlife. This ultimately results in an ecological imbalance. Even in such a situation, the institutional campuses with relatively undisturbed natural vegetation are providing a potential habitat for insects, including the butterfly population (Nair, Mitra & Bandyopadhyay, 2014).

Sree Chaitanya College, Habra is located in a sub-urban area in the North 24 district of West Bengal. The campus is spread over a large area with natural green vegetation as well as a managed garden, providing a good habitat for the butterflies. As the campus has a good source of nectar and plants preferred by butterflies for completing their life cycle, along with no use of pesticides, different types of the butterfly are found in this area.

This study was aimed at preparing a checklist of butterflies found on the campus of Sree Chaitanya College, Habra as there is no published checklist of butterflies of the area. The present study was also aimed to document the behavioural attributes of some selected butterfly found in this area.

Materials and methods

Study Site

Sree Chaitanya College campus in Habra, North 24 Parganas, West Bengal, India was selected as the study site. The campus has buildings along with a large ground in the front, a managed garden area, a number of open areas with wild vegetation and a medicinal plant garden. The campus has good vegetation with different herbs, shrubs and trees. The present study was conducted on the campus of the college,

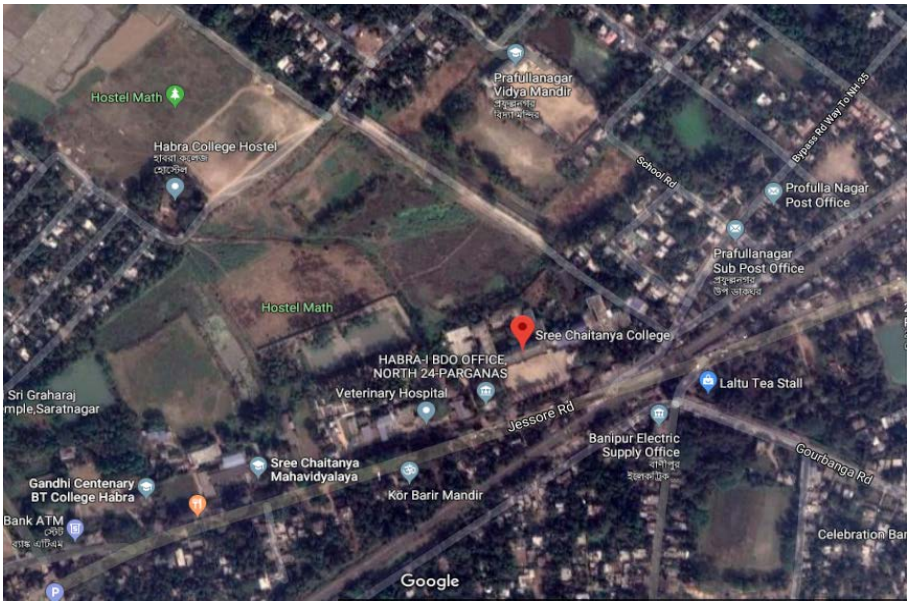

Figure1: Study Site. Red Pin Indicates Sree Chaitanya College

Study Period

The study was conducted from February to September 2022.

Field Survey for Eco-Ethology

The butterfly diversity was determined by an extensive field survey. VES or visual encounter survey technique was used as described earlier (Sanphui, Kabir & Saha, 2021). Briefly, the method involves walking through the study sites systematically searching for butterflies. The butterflies were identified by taking pictures and matching them with the published literature and books. Photographs were taken using the camera.

The different behavioural activities of the butterflies were studied using the focal animal sampling method. A time budget of different behaviours like resting, flying, basking and nectaring was recorded and represented graphically.

RESULTS

Butterfly Diversity in the Study Area

The names of the observed butterfly species is listed in Table 1. 34 species of butterflies in 25 genera and 5 families were reported. The five families were Nymphalidae, Pieridae, Papilionidae, Lycaenidae, Hesperiidae. Out of all the five families, majority of the species belonged to the Nymphalidae family. Out of 34 species, 17 belonged to the family Nymphalidae. 7 species belonged to the family Pieridae, 4 species belonged to the family Papilionidae and 3 species belonged to the families Lycaenidaeand Hesperiidae each. *Hypolimnasbolina, Hypolimnasmisippus, Papiliopolytes, Papiliopolytes, Graphiumagaphium* were the most rarely sited species in the study area.

Table 1: List of Butterflies Observed

Sr. No.	Family	Name of Butterfly	Scientific Name	Status
1	Nymphalidae	**Angle cestor**	*Ariadne ariadne*	Very common
2		**Common palmfly**	*Elymniashypermnestra*	Common
3		**Tawny coster**	*Acraea terpsicore*	Common
4		**Plain tiger**	*Danaus chrysippus*	Common
5		**Common evening brown**	*Melanitisleda*	Common
6		**Striped tiger**	*Danaus genutia*	Common
7		**Peacock pansy**	*Junoniaalmana*	Very common
8		**Grey pansy**	*Junoniaatlites*	Common
9		**Common crow**	*Euploea core*	Common
10		**Common threering**	*Ypthimaasterope*	Common
11		**Common four ring**	*Ypthimabaldus*	Common
12		**Great eggfly**	*Hypolimnasbolina*	Rear
13		**Danaid eggfly**	*Hypolimnasmisippus*	Rear
14		**Common bush brown**	*Mycalesisperseus*	Common

15		**Commander**	*Moduzaprocris*	rare
16		**Castor**	*Ariadne merione*	very common
17		**Common baron**	*Euthaliaaconthea*	common
18	Pieridae	**Common jezabel**	*Delias eucharis*	Common
19		**Bengali albatros**	*Appiasolferna*	Very common
20		**Three spot grass yellow**	*Euremablanda*	Common
21		**Striped albatros**	*Appiaslibythea*	Very common
22		**Common grass yellow**	*Euremahecabe*	Very common
23		**Common wanderer**	*Pareroniavaleria*	very common
24		**Psyche**	*Leptosianina*	very common
25	Papilionidae	**Common mormon**	*Papiliopolytes*	Rear
26		**Lime**	*Papiliodemolues*	Very Rear
27		**Common joy**	*Graphiumdoson*	Rear
28		**Tailed joy**	*Graphiumagaphium*	Rear
29	Lycaenidae	**Common silverline**	*Cigaritisvulcanus*	Common
30		**Common pierrot**	*Castaliusrosimon*	Common
31		**Pale grass blue**	*Pseudozizeeriamaha*	Common
32	Hesperiidae	**Indian plam bob**	*Suastusgremius*	Common
33		**Small banded swift**	*Pelopidas mathias*	common
34		**Rice swift**	*Borbocinnara*	Common

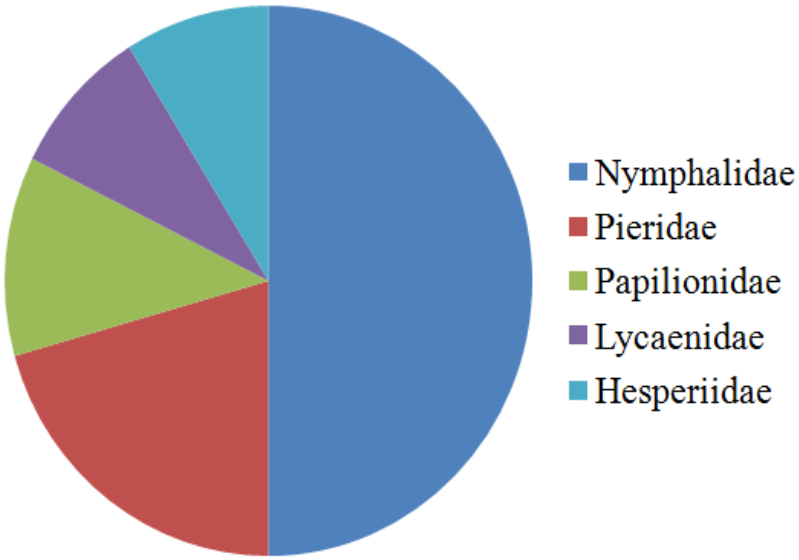

Figure 2: Graphical Representation of Percentage of Different Species of Butterfly Species under Various Families

Figure 3: Some of the Observed Butterflies 1. Pale Grass Blue 2. Common Crow, 3. Striped Albatros, 4. Indian Plam bob, 5. Lime, 6. Common Joy, 7. Common Grass Yellow, 8. Danaid Eggfly 9. Tailed Joy, 10. Great Eggfly, 11. Common Mormon, 12. Bengali Albatros, 13. **Common fourring, 14. Common silverline 15. Angel Castor, 16. Common Jezabel, 17. Grey Pansy, 18. Common Plam fly, 19. Tawny Coster, 20. Peacock Pansy, 21. Plain Tiger**

Behavioural Activities of Butterfly Species in the Study Garden

The different behavioural activities of some selected butterfly species were studied. Various activities such as flight, resting, nectering and basking were studied for selected species found in the study area. Different species of butterflies were observed during the study period. Many were visitors of short duration who came for nectaring, but did not stay, most probably due to a lack of suitable host plants and other conditions. Seven species that were regularly seen were chosen for behavioral study. The seven species selected were Common jezebel, Grey pansy, Angle castor, Tawny coster, Common palmfly, Plain tiger, Peacock pansy.

It was observed that the common Jezabel spent the maximum time in nectering where as the common palmfly spent most of the time in resting. Common Jezabal and Peacock Pancy spent the least percentage of time in resting. Among all the 7 observed butterfly species Angle castor spent the highest percentage of time in flying, Common Jezabel and Peacock pancy were the highest percentage of time nectering. Common palmfly was the species that spent the highest percentage of time resting.

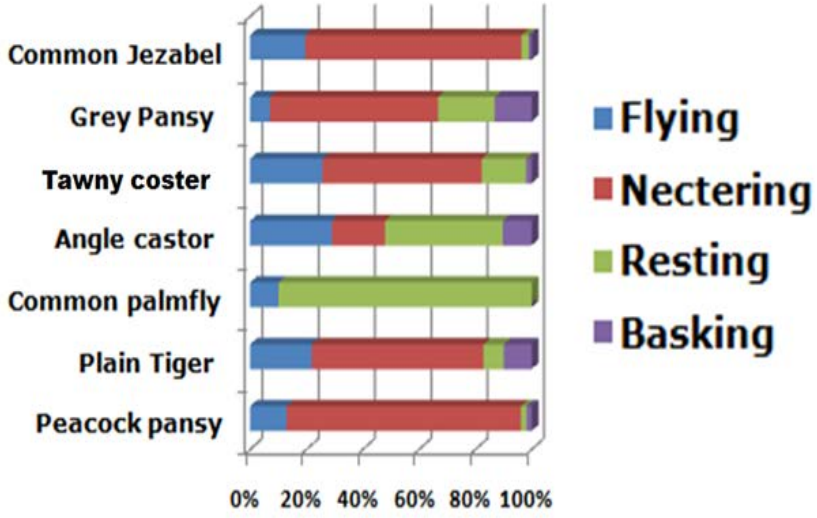

Figure 4: Comprehensive Representation for Several Behaviouralactivities of Different Butterfly Species During the Studyperiod in the Study Area

Discussion

The butterflies are one of the prominent biodiversity markers (Kunte, 2000), major pollinators of both wild and cultivated plants (Tiple, Deshmukh & Dennis, 2005) and also act as naïve gardeners. An abundance of butterflies usually indicates a healthier ecosystem. The preference of butterfly species towards particular habitats is associated with the availability of host plants on which the larva can feed and the adult can collect nectar. There is seasonal variation in the diversity of butterflies. In some months, the number of butterfly species is abundant, however, in other months, they are rare or absent. (Kunte, 2000). It has been shown by Wynter-Blyth (1957) that there are two peak seasons for the abundance of butterfly species in India, one is March–April and another in October.

The present study provides an account of butterfly diversity inside Sree Chaitanya college campus. Observation reaveals presence of 34 species of butterfly under 25 genera and 5 families. The highest number of species belonged to family Nymphalidae. The geographical location of any area, its climatic conditions and vegetative composition are essential requisites for supporting a rich diversity of butterflies.

In the study it was seen that inspite of environmental stresses, the butterfly diversity may be high if suitable nectoring as well as host plant is found for a sustainable life. Apart from feeding on nector, the diet of adult butterfly may vary from rotton fruit to vegetable, mineral from soil etc. Drinking at wet soil patches is an important feeding activity in many butterflies The butterfly diversity along with different behavioural activity was documented in the present study. Foraging behaviour of selected butterfly was observed on different flowers like *Lantana camara*, *Mikania micrantha, Melochiacorchorifolia* etc. The colour and fragnance of flowere employ strong signal for foraging of butterflies (Begum, Habiba & Howlader, 2014 and Goulson & Cory 1993). The butterfly diversity in the study site indicated a varied assemblage of floral species. The flora in the study area was a mixed type with herbs and shrubs dominating the vegetation. The number of trees are comparatively lesser. Although information regarding species specific habitat study and different ethological activities of butterfly is available (Young, 1975) studies on comprehensive eco-ethology of different butterfly species is very less.

Conclusion

The study throws light on nectar as well as host-plant preferences and the behavioural activities of several butterfly species. The study also underline the importance of institutionalcampuses as a preferred habitat for butterflies. If these campuses are maintained properly the butterfly diversity may increase in the study area providing a rich ground for butterfly conservation and research. Future attempts would be taken in uderstanding the complex mutualistic interaction between the different butterfly species and the flowering plants. This is the first effort in exploring the butterfly diversity in Sree Chaitanya college campus. The present list of butterfly species is not conclusive and future studies will be conducted to update this list.

Acknowledgment

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Study of Fish Diversity of a Major Distributary of River Ganga that Needs Proper Management and Rehabilitation

Lina Sarkar
Department of Zoology, Sree Chaitanya College, Habra, West Bengal, India

Corresponding Author's Email: sarkarlina321@gmail.com

ABSTRACT

Conservation is curtailed for bioresource management that enhances the bioeconomy's needs. The ichthyofaunal diversity of one of the major distributaries of the Ganga, the Mridangabhanga River and its adjacent areas of water bodies was studied. The river flows through the area, which is the world's heritage site, the Biosphere Reserve, and the largest forest area in the world, the Sunderban. A constant anthropogenic pressure is destroying the natural resources of this area. This alteration has adversely affected water quality and, definitely, its fishery potential. The present study reveals that the river houses a good number of edible fish, which belong to fresh water as well as marine water. The fish catch survey reveals that the quantity of fish caught was decreasing rapidly, which was a threat to the villagers as fishing was the basic tool for earning bread. The causes of these destructions should be identified and eradicated with immediate effect to conserve the natural breeding grounds. Therefore, implementation of strong conservation strategies is earnestly required to revive river wealth; on the other hand, sustainable use of natural resources is the only solution for the restoration of this bioresource. This study reveals that the river houses 110 fish species belonging to 33 families and 63 Genera. The study also reveals that river pockets in this catchment area are safer places for fish spawns to survive. The heavy metal analysis of the fish tissue shows that the upper range limits of some metals are higher than the permissible limits.

***Keywords**: Distributaries; Tidal Effect; Estuarine Fishes; Fish Seeds; Breeding Ground*

Introduction

The Sunderban is the pride of West Bengal. It is the wealth of India as well. The Sunderban is situated between N' 21º 30' to 22 º 40' and E' 88º 05' to 89 º 55'. This is one of the largest forests in the world and covers an area of about 140,000ha. This area was inscribed as a biosphere reserve and world heritage site in 1987. The Sunderban lies on the delta of the Ganges, Brahmaputra, and Meghna rivers. The total area, which includes the area between the Bay of Bengal in India and spreads throughout the south-west of Bangladesh between the river Baleswar in East and Harinbhanga in the West. The area is a mosaic of large rivers, many rivulets, various tributaries, and many distributaries. Mridangabhanga is one of the most important distributaries of river Ganges which originates from Ganga and flows south wards and meets Bay of Bengal at Sunderban. This area houses an immense treasure of flora and fauna. It is a unique habitat and also a good breeding ground for a

large number of threatened and endangered species. Being a unique ecological sphere, Sunderban is always an undulating ecosystem moving through an ongoing process in a wider range. The meshwork of rivers and rivulets has formed the world's largest delta and this area houses the largest faunal composition of the Sundarban. In this area, the aquatic flora and fauna are enriched due to the constant inflow of fresh tidal water from the sea and the Bay of Bengal. The present study was conducted on the Mridangabhanga River and also on the large Bheris, backwater areas, and ponds where local people use those waterbodies for fish culture. The study also included lowland areas, marshy lands, and canals. The Mridangabhanga River is one of the distributaries of the River Ganga; it joins Mathurapur and Pathorprotima blocks of South 24 Pargana, West Bengal. The studied river and the adjacent area river are fed by sea tides twice daily, which is an important factor that influences the aquatic population of this river. In the valley of the Mridangabhanga River, during high tide, the river water rises and covers lowland, marshy areas, and other water bodies. When low tide comes, the water ceases, and the lowland area becomes exposed as semi-dried muddy patches, except for the ponds and bheries. Moreover, during high tide, many estuarine and marine fish enter the river flow, which might increase the diversity of the fish population. The research findings of Allison *et al.* (1998), Allison and Kepple (2001), and Allison (1998) revealed that the topography as well as the extent of the forest boundary had changed due to anthropogenic intervention. The yearly fish catch data reveals that this resource is a good revenue earning system for the state. A large group of residents of this area are dependent on capture fishery activity, which is the backbone of the Sundarban economy as well as the state of West Bengal. A year's worth of 3355 MT of fish catch is recorded from the Sunderban area (Huq *et al*., 2004). The diversity of fish fauna in several rivers was studied by several authors, and results indicated that the meshwork of rivers in Sunderban houses a rich diversity of ichthyofauna (Chakraborty *et al*., 2021; Paul *et al*., 2021; Sen & Mandal, 2019; Saha *et al*., 2018; Islam *et al*., 2017; Mishra & Gopi, 2017; Chakraborty & Adhikary, 2014; Rahaman *et al*., 2012; Sarkar & Banerjee, 2012, Dhara & Paul, 2016; Mitra, Banerjee & Banerjee, 2006).

A detailed study on fish diversity and its metal absorption limits in fish tissue was conducted during the present research, which aimed to satisfy the local people's needs and also stratify the conservation strategies for the river valley.

Methodology

The recent study was on the River Mridangabhanga, one of the distributaries of the River Ganga. The study sites, 1. Bolerhat Bazaar Bridge, 2. Dwarakapur Purbo Haribasar, and 3. Kedarpur (Figure 1), were chosen at proper intervals and covered the area of the riparian zone. Figure 1 depicts the sampling sites along with the adjoining lowland area and fish culture ponds. Fishermen were engaged in fishing from 6 a.m. to 6 p.m. Each month for 10 days (Sarkar & Banerjee, 2012) at each landing station, sampling was done at local fish landing stations. Fish were caught by various gears (Cast net, Lift net,Gill net) and traps were used for catfish and mud species collection. All collected fish were assorted into three groups: oozing females, adults, and fries. Identification was done according to the standard

taxonomic procedure (Day, 1876; Talwar & Jhingran, 1991; Jayaram, 1999; Sarkar & Banerjee, 2012). The fish diversity of the Mridangabhanga River is listed in Table 1. The toxic metals were estimated by following standard methods (American Public Health Association, 1999). Quantitative estimation of Cu, Zn, and Ni in two fish samples was done using an atomic adsorption spectrophotometer.

Figure 1. Map showing the study sites (Google Map)

Table 1: List of fishes of Mridangabhanga River valley

Sl.No.	Order	Family	Name of the Fish	Status
1	Beloniformes	Belonidae	***Xenentodoncancila***	LC
2	Synbranchiformes	Mastacembelidae	***Macrognathuspancalus***	LC
3	Synbranchiformes	Mastacembelidae	***Mastacembelusarmatus***	LC
4	Synbranchiformes	Synbranchidae	***Monopteruscuchia***	LC
5	Perciformes	Latidae	***Lates calcarifer***	LC
6	Perciformes	Serranidae	***Epinepheluslanceolatus***	LOC
7	Perciformes	Serranidae	***Epinephelusmalabaricus***	LC
8	Perciformes	Ambessidae	***Ambassiskopsii***	NE
9	Perciformes	Terapontidae	***Terapon puta***	LC
10	Perciformes	Ambessidae	***Chanda nama***	LC
11	Perciformes	Ambessidae	***Parambassisbaculis***	LC
12	Perciformes	Ambessidae	***Parambassisranga***	LC
13	Perciformes	Polynemidae	***Polynemusparadiseus***	LC
14	Carcharhiniformes	Carcharhinidae	***Carcharhinus limbatus***	VU
15	Carcharhiniformes	Carcharhinidae	***Carcharhinus leucas***	LC
16	Carcharhiniformes	Carcharhinidae	***Carcharhinus sorrah***	LC

17	Carcharhiniformes	Carcharhinidae	***Carcharhinus hemiodon***	LC
18	Torpediniformes	Narcinidae	***Narcinebrunnea***	LC
19	Rhinopristiformes	Pristidae	***Pristis clavate***	EN
20	Rhinopristiformes	Rhinobatidae	***Rhinobetosannadalei***	DD
21	Myliobatiformes	Gymnuridae	***Gymnura japonica***	VU
22	Osteoglossiformes	Notopteridae	***Notopterusnotopterus***	LC
23	Osteoglossiformes	Notopteridae	***Chitalachitala***	NT
24	Anguilliformes	Anguillidae	***Anguilla bengalensis***	NT
25	Anguilliformes	Anguillidae	***Anguilla bicolor***	NT
26	Clupeiformes	Clupeidae	***Coricasoborna***	LC
27	Clupeiformes	Clupeidae	***Gonialosamanmina***	LC
28	Clupeiformes	Clupeidae	***Gudusiachapra***	LC
29	Clupeiformes	Clupeidae	***Nematalosagalatheae***	LC
30	Clupeiformes	Clupeidae	***Tenualosailisha***	LC
31	Clupeiformes	Pristigasteridae	***Llisha elongate***	LC
32	Clupeiformes	Engraulidae	***Setipinnaphasa***	LC
33	Clupeiforms	Engraulidae	***Setipinnatenuifilis***	DD
34	Clupeiformes	Engraulidae	***Setipinnataty***	
35	Clupeiformes	Danionidae	***Parluciosomadaniconius***	LC
36	Cypriniformes	Cyprinidae	***Amblypharyngodon mola***	LC
37	Cypriniformes	Cyprinidae	***Aspidoporiajaya***	LC
38	Cypriniformes	Cyprinidae	***Chaguniuschagunio***	LC
39	Cypriniformes	Cyprinidae	***Barilusbarila***	LC
40	Cypriniformes	Cyprinidae	***Barilusbarna***	LC
41	Cypriniformes	Cyprinidae	***Barilusvagra***	LC
42	Cypriniformes	Cyprinidae	***Cirrhinusreba***	LC
43	Cypriniformes	Cyprinidae	***Esumusdanricus***	LC
44	Cypriniformes	Cyprinidae	***Laubukalaubuca***	NT
45	Cypriniformes	Cyprinidae	***Labeopangusia***	LC
46	Cypriniformes	Cyprinidae	***Labeoangara***	LC
47	Cypriniformes	Cyprinidae	***Puntius sarana***	LC
48	Cypriniformes	Cyprinidae	***Puntius chelynoides***	LC
49	Cypriniformes	Cyprinidae	***Puntius chola***	LC
50	Cypriniformes	Cyprinidae	***Puntius conchonius***	LC
51	Cypriniformes	Cyprinidae	***Puntius amphibious***	DD
52	Cypriniformes	Cyprinidae	***Puntius binotatus***	LC
53	Cypriniformes	Cyprinidae	***Puntius puntio***	NE
54	Cypriniformes	Cyprinidae	***Puntius phutunio***	LC
55	Cypriniformes	Cyprinidae	***Puntius sophore***	LC
56	Cypriniformes	Cyprinidae	***Puntius terio***	LC
57	Cypriniformes	Cyprinidae	***Puntius ticto***	LC
58	Cypriniformes	Cyprinidae	***Salmostomabacaila***	LC
59	Cypriniformes	Cyprinidae	***Salmostomaphulo***	LC
60	Cypriniformes	Cyprinidae	***Tor putitora***	EN
61	Cypriniformes	Cyprinidae	***Tor tor***	EN
62	Cypriniformes	Cyprinidae	***Pethiaconchonius***	LC
63	Cypriniformes	Cyprinidae	***Pethiagelius***	LC
64	Cypriniformes	Cyprinidae	***Securiculagora***	LC
65	Cypriniformes	Cyprinidae	***Rasbora daniconius***	LC
66	Cypriniformes	Cyprinidae	***Danio rerio***	LC

67	Cypriniformes	Cyprinidae	*Chela laubuca*	LC
68	Cypriniformes	Cyprinidae	***Danio aequipinnatus***	LC
69	Cypriniformes	Cyprinidae	***Lepidocephalichthysguntea***	LC
70	Cypriniformes	Cyprinidae	***Pethiaconchonius***	LC
71	Siluriformes	Siluridae	***Wallago attu***	VU
72	Siluriformes	Siluridae	***Ompokpabda***	NT
73	Siluriformes	Siluridae	***Ompokbimaculatus***	NT
74	Siluriformes	Bagridae	***Batasiobatasio***	LC
75	Siluriformes	Bagridae	***Mystusbleekeri***	LC
76	Siluriformes	Bagridae	***Mystuscavasius***	LC
77	Siluriformes	Bagridae	***Mystusgulio***	LC
78	Siluriformes	Bagridae	***Mystustengra***	LC
79	Siluriformes	Bagridae	***Mystusvittatus***	LC
80	Siluriformes	Bagridae	***Ailiacoila***	NT
81	Siluriformes	Bagridae	***Siloniasilondia***	LC
82	Siluriformes	Pangasilidae	***Pangasius pangasius***	LC
83	Siluriformes	Clariidae	***Clarias batrachus***	LC
84	Siluriformes	Heteropneustidae	***Heteropneustesfossilis***	LC
85	Aulopiformes	Synodontidae	***Harpadonnehereus***	NT
86	Mugiliformes	Mugilidae	***Planilizamacrolepis***	LC
87	Mugiliformes	Mugilidae	***Chelonparsia***	LC
88	Mugiliformes	Mugilidae	***Rhinomugilcorsula***	LC
89	Mugiliformes	Mugilidae	***Valamugilbuchanani***	LC
90	Gobiliformes	Oxudercidae	***Apocryptesbato***	LC
91	Gobiliformes	Oxudercidae	***Apocryptodonmadurensis***	LC
92	Gobiliformes	Oxudercidae	***Glossogobiusgiuris***	LC
93	Gobiliformes	Oxudercidae	***Parapocryptesserperaster***	LC
94	Gobiliformes	Oxudercidae	***Parapocryptesbatoides***	LC
95	Gobiliformes	Oxudercidae	***Periophthalmuskalolo***	LC
96	Kurtiformes	Kurtidae	***Kurtus indicus***	NE
97	Anabantiformes	Osphronemidae	***Trichogasterfasciata***	LC
98	Anabantiformes	Osphronemidae	***Trichogasterlalius***	LC
99	Anabantiformes	Osphronemidae	***Trichogasterchuna***	LC
100	Anabantiformes	Channidae	***Channa orientalis***	VU
101	Anabantiformes	Channidae	***Channa punctate***	LC
102	Anabantiformes	Channidae	***Channa striata***	LC
103	Anabantiformes	Badidae	***Badisbadis***	LC
104	Anabantiformes	Nandidae	***Nandus nandus***	LC
105	Pleuronectiformes	Cynoglossidae	***Cynoglossusmacrostomus***	VU
106	Pleuronectiformes	Cynoglossidae	***Cynoglossus lingua***	LC
107	Pleuronectiformes	Cynoglossidae	***Cynoglossusarel***	LC
108	Pleuronectiformes	Soleidae	***Synapturaalbomaculata***	NE
109	Tetraodontiformes	Triacanthidae	***Triacanthusbiaculeatus***	NE
110	Tetraodontiformes	Tetraodontidae	***Leiodoncutcutia***	LC

Table 1 shows 7 species Nearly Threatened Category (NT) , 4 species Vulnerable category(VU) , 3 Endangered category species (EN), 5 species Not Evaluated Category

(NE), 3 species Data Deficient Category() , (LC)least concerned category species, according to “The IUCN Red List of Threatened Species” 2021.

Result and discussion

The River Mridangabhanga houses several breeding grounds and pockets of fish in its valley. The huge primary productivity from mangrove habitat, with high organic and inorganic nutrients, might have enriched this river ecosystem, which in turn provides good nurturing grounds for fish. The findings of Mitra, Banerjee and Banerjee (2006) and Sen and Mandal (2019) indicated that the pelagic region of the deltaic region is highly productive due to high nutrients derived from mangrove plants, surface run-off, and anthropogenic origin. The monsoon storm and heavy rainfall damage the embankment of the river, and inundation increases the salinity of river water, which acts as a threat to the natural breeding grounds.

The study of fishes in Mridangabhanga reveals a rich and diverse list of freshwater, estuarine, and marine fishes. A total of 110 fish species belong to 19 orders, 33 families, and 63 genera. Cypriniformes were the most dominant order, comprising 31.8% of the total fish population, followed by the Siluriformes (12.7%), Clupeiformes (9.09%), Anabantiformes (7.27%), Gobiliformes (5.45%), Perciformes (8.18%), Mugiliformes (3.63%), Carcharhiniformes (3.63%), and Pleuronectiformes(3.63%).Synbranchiformes (2.72%), Tetraodontiformes, Rhinopristiformes, Osteoglossiformes, and Anguilliformes represent (1.81%), and Beloniformes, Aulopiformes, Myliobatiformes, Kurtiformes, and Torpediniformes represent 0.9% of the total diversity of the fish fauna.

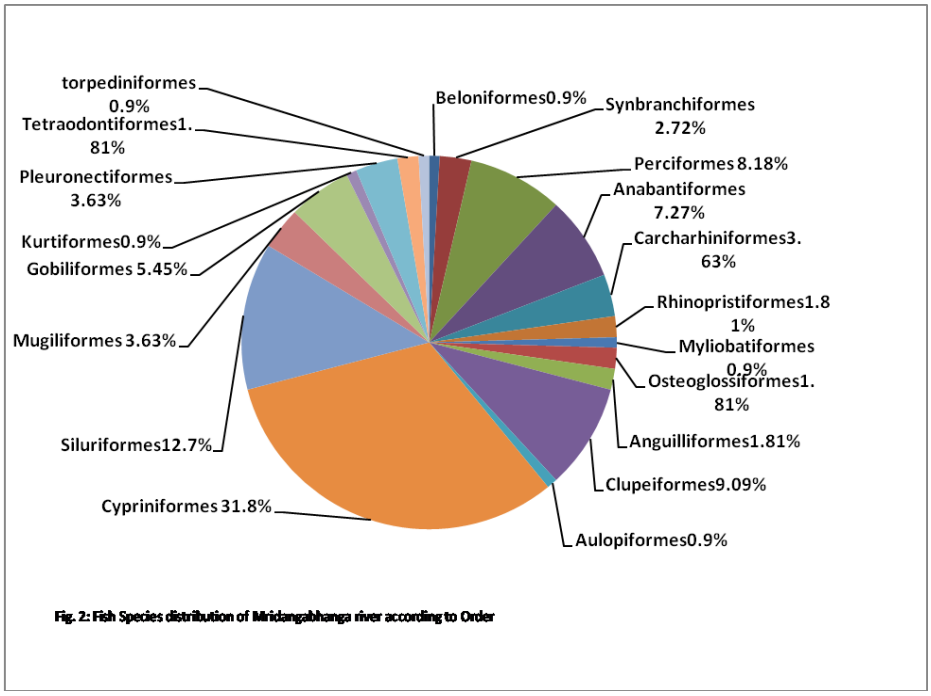

Figure 2: Fish Species Distribution of Mridangabhanga River According to Order

Order Cypriniformes represents 2 families; among them, the Family Cyprinidae was the most dominant family, having a total of 21 Genus and 35 Species, and the Family Danionidae represents 1 Genus and 1 Species. Order Siluriformes represents 5 Families among them family Bagridae (4 Genus 8 species), family Siluridae (2 Genus 3 species), Order Clupeiformes represents 4 Families, among which Family Clupeidae represents 5 Genus 5 species. Family Pristigasteridae represents 1 Genus 1 Species, and Family Engraulidae represents 1 Genus 3 Species. The order Anabantiformes represents 4 families, Badidae with one genus and one Species, Nandidae with 1 Genus 1 Species, Osphronemidae 1 Genus 3 Species and Channidae with 1 genus 3 species.Order Gobiliformes represents the Family Oxudercidae with 6 Genus 6 Species,OrderKurtiformes represents 1 Family Kurtidae with 1 Genus 1 Species,Order Pleuronectiformes represents 2 Families among them Family Cynoglossidae represents 1 Genus 3 species and Family Soleidae 1 Genus 1 Species. The order Tetraodontiformes represents 2 families: the Family Triacanthidae with 1 Genus 1 species and the family Tetraodontidae with 1 Genus 1 Species. With 4 genera and 4 species, the order Mugiliformes represents the only family Mugilidae. The order Aulopiformes also represents the only family Synodontidae with 1 Genus 1 species. The order Carcharhiniformes represents Family Carcharhinidae with 1 Genus 4 Species.Order Rhinopristiformes represents two families and both the Families Rhinobatidaeand Pristidaerepresents 1 Genus and 1 Species. Order Osteoglossiformes represents the Family Notopteridae representing2Genus and 2 Species. Order Myliobatiformes represents Family Gymnuridae with 1 Genus and 1 Species, Order Synbranchiformes represents two Families, Mastacembelidae with 2 Genus and 2 Species and Family Synbranchidae with 1 Genus and 1 species.Order Perciformes represents 6 families family Latidae with 1 Genus 1 Species, and Family Ambessidae with 3 Genus 4 Species, family Terapontidae 1 genus 1Species. Order Beloniformes represents Family Belonidae with1 Genus, 1 Species.

During the present study, 110 fish species were recorded from the Mridangabhanga river (Table 1). Among these fishes 7 species Nearly Threatened Category, 4 species Vulnerable category, 3 Endangered category species, 3 least concerned category species, 5 species in the Not Evaluated category, and 3 species in the Data Deficient category, according to "The IUCN Red List of Threatened Species" (IUCN, 2021).

The fish, which are found only during the monsoon season in this area, are sporadic visitors to this river, and they show a higher population. Similarly, the population of freshwater fish species is also found to increase during the heavy flow of the monsoon. There are several pockets of small temporary pools connecting channels at PurbaDwarakapur which houses a large number of oozing females during monsoon and fingerlings were found in the shallow back water in these pockets during the post monsoon. The brackish water fishes as well as spawns of *Anguilla bengalensis, Chanda nama, Xenentodoncancila, Gudusiachapra, Setipinnaphasa, Labucalabuca, Puntius sarana, Mystustengara, Heteropneustesfossilis, Rhinomugilcorsula, Cynoglossuscynoglossus,* were recorded in the backwater of Purbadwarakapur area gives shelter to the freshwater were found during low tide at post

monsoon. The waterlogged area trailing bandh area of the riverbank shows a rich diversity of fishes, and they showed a heterogeneous composition of freshwater, estuarine, and marine fishes. The fish farmers from the local area get those spawns easily and cultivate them in the culture ponds. These indicated a good source of natural fish seeds, which will not only be a source of livelihood but also increase the fish population also. Gram Panchayat controls the fishery at Purba Dwarakapur, and it aims to conserve those spawns and increase production on a large scale. The graphical representation of toxic metal absorption in fish muscle tissue of the Mridangabhanga River (Figure 3A-R) shows that the values were within limits according to the World Health Organization (2008).

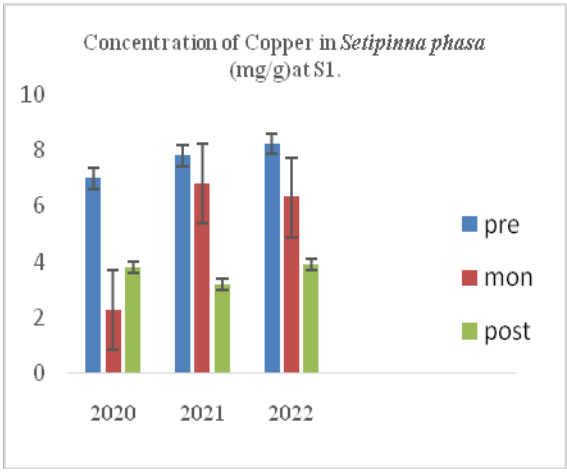

Figure 3A: Copper accumulation limit in Fish Tissue, Station S1

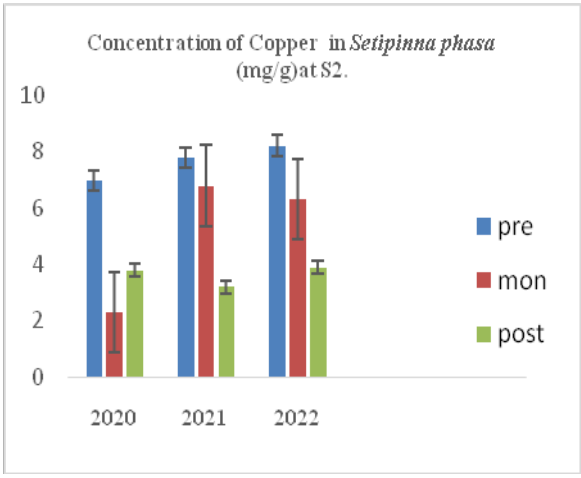

Figure 3B: Copper accumulation limit in Fish Tissue, Station S2

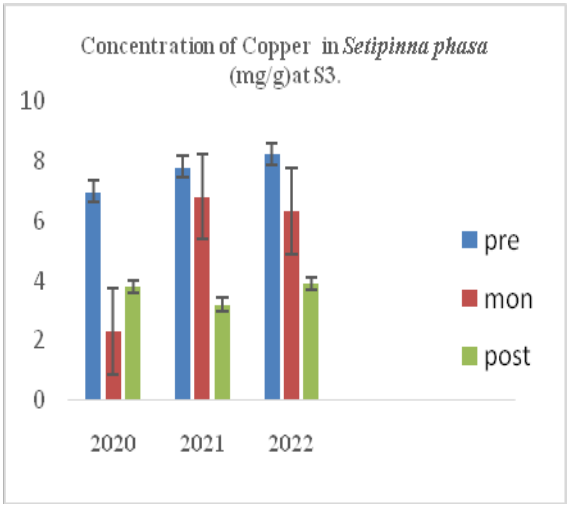

Figure 3C: Copper accumulation limit in Fish Tissue Station, S3

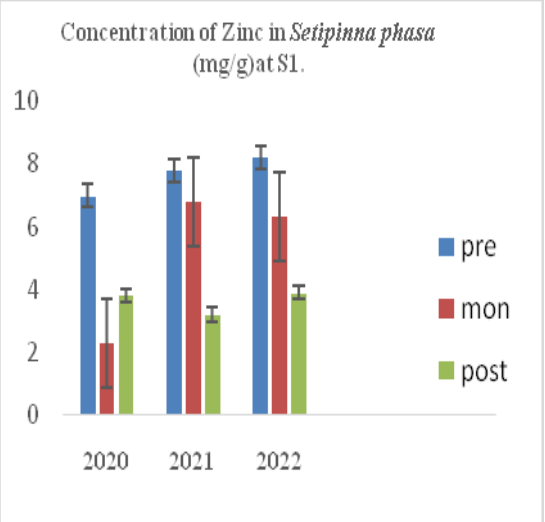

Figure 3D: Zinc accumulation limit in Fish Tissue Station, S1

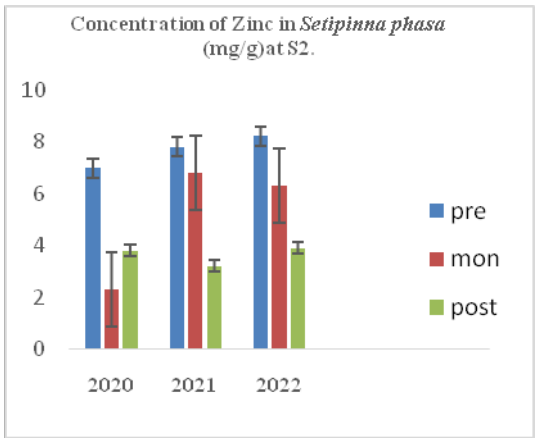

Figure 3E: Zinc accumulation limit in Fish Tissue Station, S2

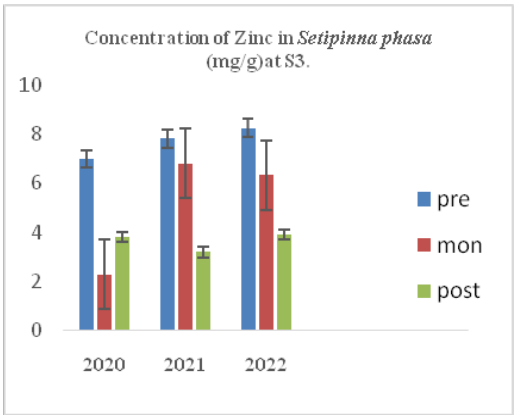

Figure 3F: Zinc accumulation limit in Fish Tissue Station, S3

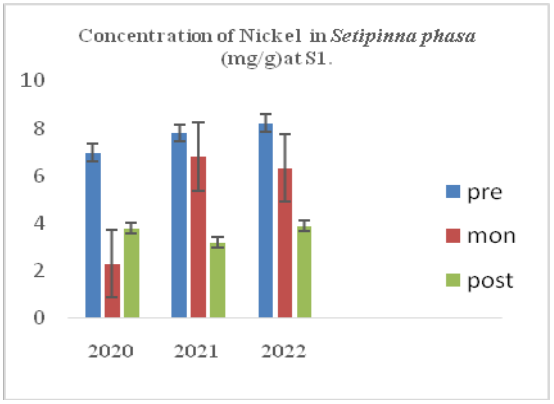

Figure 3G: Nickelaccumulation limit in Fish Tissue Station, S1

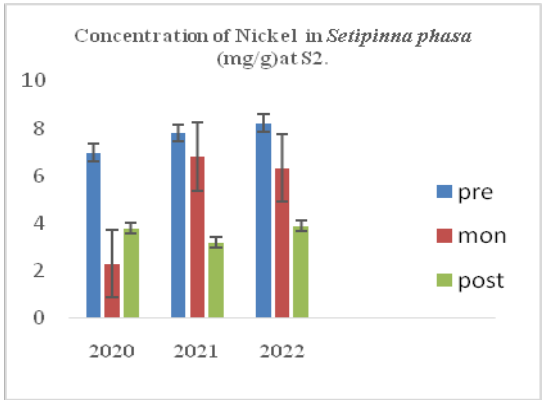

Figure 3H: Nickelaccumulation limit in Fish Tissue Station, S2

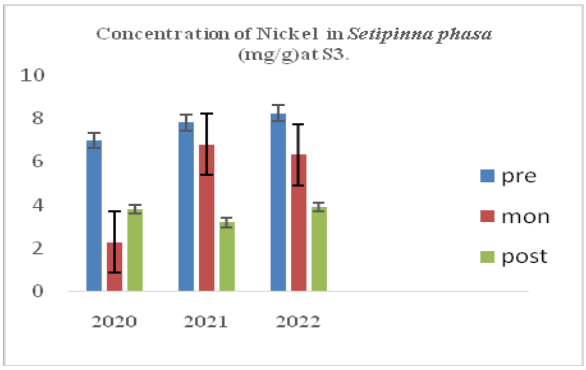

Figure 3I: Nickel accumulation limit in Fish Tissue Station, S3

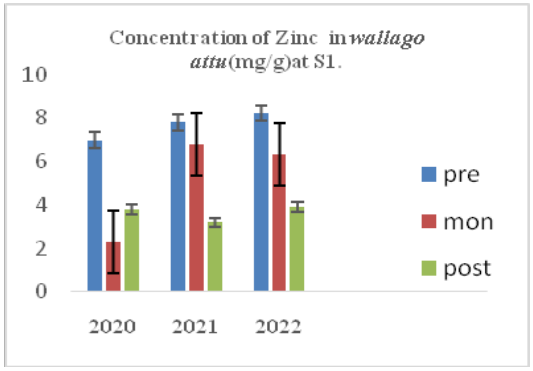

Figure 3J: Zinc. accumulation limit in Fish Tissue Station, S1

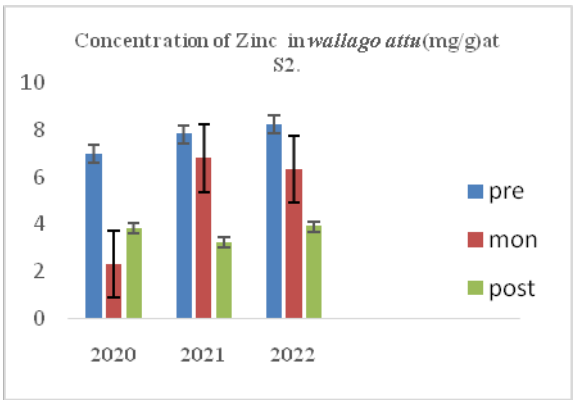

Figure 3K: Zinc accumulation limit in Fish Tissue Station, S2

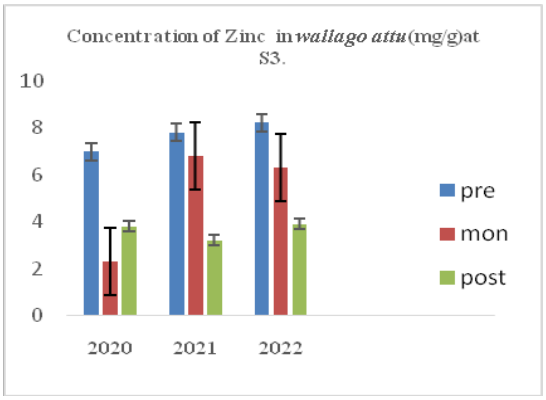

Figure 3L: Zinc accumulation limit in Fish Tissue Station, S3

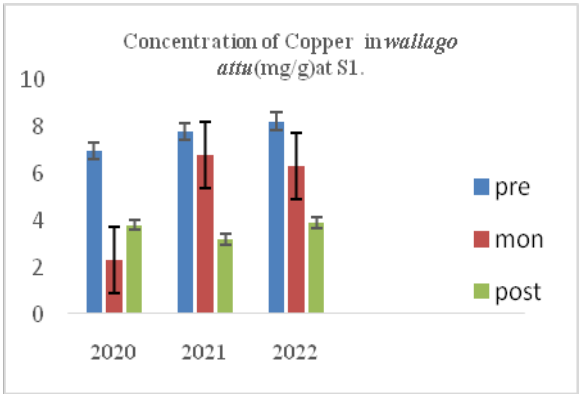

Figure 3M: Copper accumulation limit in Fish Tissue Station, S1

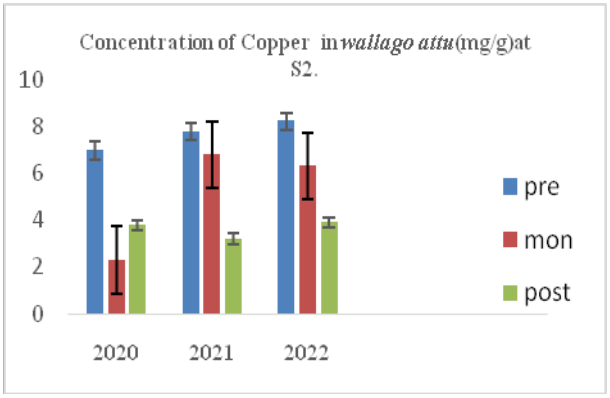

Figure 3N: Copper accumulation limit in Fish Tissue Station, S2

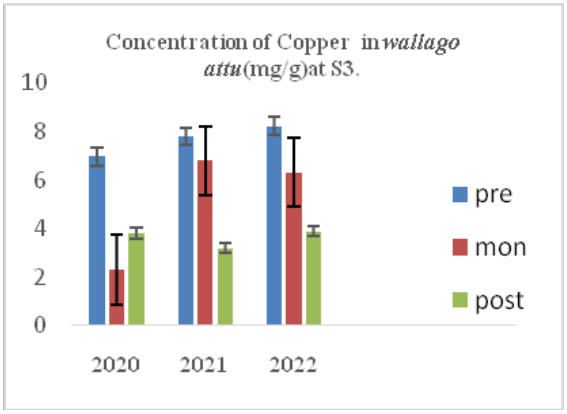

Figure 3O: Copper accumulation limit in Fish Tissue Station, S3

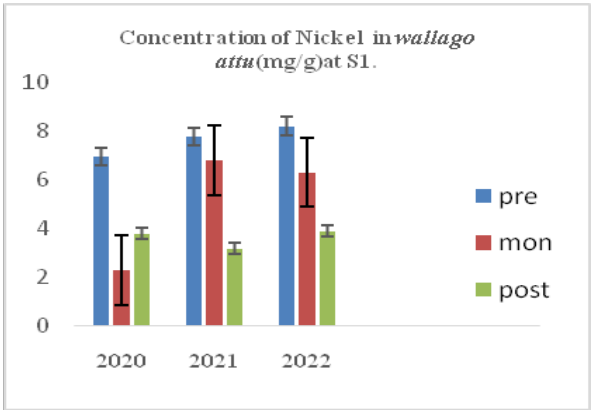

Figure 3P: Nickelaccumulation limit in Fish Tissue Station, S1

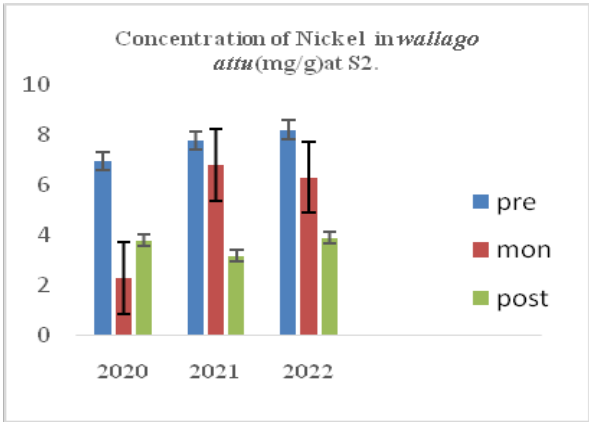

Figure 3Q: Nickelaccumulation limit in Fish Tissue Station, S2

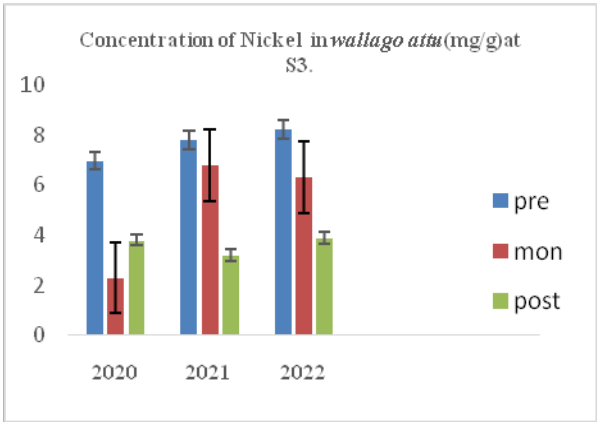

Figure 3R: Nickel accumulation limit in Fish Tissue Station, S3

Conclusion

The anthropogenic interventions have caused undesired changes in the atmosphere and irregular undulations of the sea and have adverse effects on water bodies, which cause irreversible damage to aquatic biota as well as fisheries. The sea has adverse effects on biodiversity, sustainability, and the livelihood of fish resources and fishermen's communities on a large scale. The factors that influenced the fish catch in the Sunderban area were undulating environmental factors. The present study reveals that the ichthyofaunal diversity of the river Mridangabhanga is a good source of freshwater and estuarine food fish. This river also nurtures the breeding grounds of some of the fish, which tolerate a wide range of salinity variations. This river has to face soil erosion every monsoon season. As a result, it affects the aquatic ecosystem, which has an adverse impact on aquatic fauna. The anthropogenic intervention in this river is less, but the environmental hazards are more powerful in damaging the riverine ecosystem. The river shows steady productivity throughout the year, so it may be conserved as a good nurturing ground. The metal absorption limits are within limits. Effective conservation measures are urgently needed to save the natural breeding grounds and protect the diversity of the fish.

The river Mridangabhanga experienced immense damage due to natural disasters as well as anthropogenic adversities. The freshwater fish as well as the brackish-water fish of this river have to withstand wide hydrological changes. Those anthropogenic activities that caused much destruction of the river biota should be stopped immediately, all sorts of threats should be taken care of, and strict conservation actions should be introduced with immediate effect.

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Hepatoprotective Effects of Curcumin from *Curcuma Longa* L.: A Comprehensive Account

Sonali Ray

Assistant Professor, Department of Botany, Surendranath College, Kolkata, India

Corresponding Author's Email: sonyyrr@gmail.com

ABSTRACT

Since time immemorial, man has benefited from the active phytochemicals derived from plants. One such novel medicinal plant is *Curcuma longa,* or turmeric, which has a diverse usage from being a household spice to being a therapeutic gem. The medicinal value of the plant is due to curcumin, a polyphenol. It has remarkable pharmacological properties like antimicrobial, anti-inflammatory, antidiabetic, antimutagenic and hepatoprotective, among many others. In the present account, the hepatoprotective potential of the novel plant has been focused upon due to the increasing cases of various liver diseases in humans that lead to fatalities. The present work attempts to summarize the evidence of hepatoprotective effects of curcumin derived from *C. longa*, mostly in the last two decades, and thereby provide an insightful and comprehensive account. The enumeration of the evidence of curcumin's hepatoprotective properties will open many new avenues for further in-depth investigations.

Keywords: *Curcuma Longa; Curcumin; Hepatoprotective; Liver*

Introduction

Curcuma longa, commonly known as turmeric, is a significant member of the ginger family, Zingiberaceae. The plant, being a native of southeast Asia, is widely cultivated in India, Sri Lanka, China, Jamaica, Indonesia, and Taiwan. This perennial rhizomatous plant reaches a height of up to 1 meter with long, curved alternate leaves. The yellowish-orange rhizome is cylindrical and aromatic.

Taxonomic Position:

Division: Magnoliophyta

Class: Liliopsida

Subclass: Zingiberidae

Order: Zingiberales

Family: Zingiberaceae

Genus: *Curcuma*

Species: *C. longa*

Figure 1: Rhizome of *Curcuma longa* L.

C. longa is known to be a very significant member of the family for its wide spectrum pharmacological properties. Turmeric has been very well documented in Ayurveda for its therapeutic potential and described in Dashemani Lekhaniya (emaciating), Kusthagna (anti-dermatosis) and Visaghna (anti-poisonous), as presented by YT (1994) in the Charak Samhita of Agnivesh. In the traditional practice of medicine, it is documented to be useful for gastric and hepatic related issues, along with blood-related problems, the wound-healing process, and many dermatological infections (Aggarwal & Sung, 2009; Tung *et al*., 2019).

In Indian homes, this plant is being used in everyday practice for treating various ailments (Krup *et al*., 2013). It is also a household spice that has been used in almost all cuisines in many parts of India as well as other countries like Thailand, China, Iran, etc. for hundreds of years. It is also used cosmetically and in dermatologic diseases (Kocaadam & Şanlier, 2017).

For many centuries, this plant has been used as a potent cure for inflammation and infectious diseases. Among the diverse pharmacological activities like antimicrobial, antioxidant, anticarcinogenic, anti-inflammatory, and antidiabetic, this review is concentrated on the hepatoprotective potential of the novel plant. Since liver damage is lethal for living organisms, it is very important to provide as many possible ways of treatment as possible to prevent liver injury resulting from various causes. So, this review is an attempt to summarize the significant evidence where the role of curcumin derived from *C. longa* has proven to exhibit its protective action in the liver.

The therapeutic value of turmeric is due to the presence of curcuminoid, which is the major bioactive phenolic compound derived it. It is composed of curcumin (1,7-bis(4-hydroxy-3-methoxypheny1)-l,6-heptadiene-3,5-dione) and its derivatives bis-demethoxy-curcumin (BDMC) and dimethoxy-curcumin (DMC).

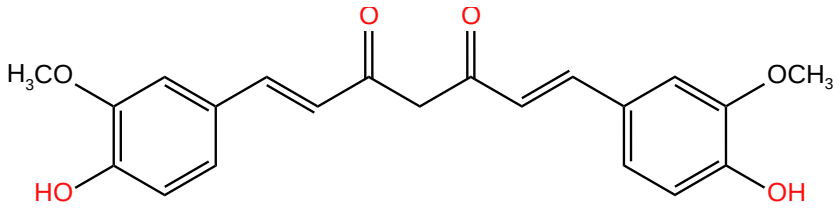

Figure 2: Structure of Curcumin

Extensive research is being done on the cellular, molecular, and biochemical mechanism of curcumin, to infer on the virtue of the active principle (Joe *et al*., 2004; Rivera & Muriel, 2009; Patel *et al*., 2020).

Girish and Pradhan (2008) While studying the efficacy of herbal drugs for treating liver diseases, I have thoroughly focused on the effects of curcumin. They reported that curcumin proved to be very efficient in treating hepatotoxicity that was induced by various toxic drugs, plant toxins and viral agents. Though it has to be also noted that the efficacy of curcumin could be lower due to temperature and light sensitivity and poor bioavailability.

Literature Review

Different Hepatoprotective Activities of *C. Longa*:

Several studies and experimental trials have found that curcumin is successful in attenuating liver damage that is induced by ethanol, carbon tetrachloride (CCl_4) intoxication, thioacetamide, iron overdose, etc. The following discussion accounts for some of the significant reports and findings on the hepatoprotective properties of curcumin from *C. longa*.

Hepatoprotective Activities against Carbon Tetrachloride (CCl_4)-Induced Liver Damage:

Acute liver damage can be induced in wide varieties of laboratory animals by a well-known hepatotoxic drug, carbon tetrachloride (CCl_4). The liver damage is caused by the generation of reactive oxygen species that cause oxidative stress and eventually cellular damage. It hampers the integrity of the hepatocytes and thereby leads to the release of liver enzymes into the blood serum. Several studies have reported the hepatoprotective effects of curcumin on CCl_4-induced liver damage in many experiments on animal models (Park *et al.*, 2000; Chattopadhyay *et al.*, 2004; Fu *et al*., 2008).

Kang *et al*., (2002) assessed the effect of curcumin on the process of collagen synthesis in rat livers injured by CCl_4 induction. Curcumin was observed to have an inhibitory effect on the collagen synthesis and activation of the hepatic stellate cells in both in vivo and in vitro conditions of induced rat liver injury. and thereby, can be used as an anti-fibrogenic agent.

Similarly, in 2004, Gaedeke *et al.*, It was found that curcumin could block the expression of many mediators that were induced by TGF-β in renals cells and thereby exhibiting antifibrotic property.

In a study carried out by Kamalakkannan *et al*., (2005), CCl_4 was given to rats for three months at a dosage of 3 ml/kg/week. There are certain enzymes like aspartate transaminase (AST), alkaline phosphatase (ALP), and γ-glutamyl transferase (GGT), that act as markers, and their concentrations increased. The levels of thiobarbituric acid reactive substances (TBARS) and hydroperoxides in the liver and kidney also increased with a sharp decrease in the activities of enzymic antioxidants like superoxide dismutase (SOD), catalase, etc. Curcumin and its synthetic analogue of bisdemethoxy curcumin (BDMC-A), when orally administered to CCl_4 exposed- rats for three months, were capable of increasing the activities of tissue enzymic antioxidants and glutathione concentration. The levels of marker enzymes and the plasma TBARS also were seen to have increased. This study revealed that BDMC-A had more potential effect than curcumin.

The concentrations of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) drastically increase in CCl_4-administered rats, that result in inflammation and damage of liver. This effect was observed to be negated by the administration of curcumin, which prevents liver damage by its antioxidant property and by inhibiting the activation of NF-κB. These observations of the prevention of the production of proinflammatory cytokines were made by Reyes-Gordillo *et al*., in 2007.

In a similar study by Elaziz *et al*., (2010), The effect of *C. longa* extract was observed against the acute hepatotoxicity of CCl_4 which decreased protein content, immunoglobulins, the activity of superoxide dismutase (SOD) and glutathione level. Production of nitric oxide (NO) production, levels of γ glutamyl transferase (γGT), glutamate oxaloacetate transaminase and glutamate pyruvate transaminase were also increased by the effect of carbon tetrachloride. The oral dose of 80 mg per Kg of the powder of *C. longa* for four weeks daily before the carbon tetrachloride injection allowed the activity of superoxide dismutase and glutathione level to elevate, along with prevention of nitric oxide production, and the levels of γ glutamyl transferase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase also coming to normal ranges. The curcumin of *C. longa* could reduce the effects of CCl_4 of lowering the red blood cells, white blood cells and haemoglobin content. All these findings and parameters well established the protective action of *C. longa* against hepatotoxicity caused by CCl_4.

Sengupta *et al*. (2011) also studied the effect of curcumin in carbon tetrachloride-intoxicated mice, in terms of liver damage. The aqueous extract of turmeric has been found to reduce the levels of SGOT, SGPT and bilirubin, which increase in the serum due to the intoxication of CCl_4, thereby giving a protection from liver damage.

Curcumin was effective in elevating the levels of total antioxidant status (TAS) while reducing the levels of total oxidant status (TOS) and malondialdehyde (MDA) in serum and liver extracts in CCl_4 -induced liver damage. CCl_4 also causes necrosis, fibrosis, along with steatosis and degeneration of hepatocytes. The mitotic activity and cirrhosis in liver also increases due to its effect. Treating the rat models with curcumin showed alleviation of inflammation and steatosis (Hismiogullari *et al*., 2014). Similar reports were put forward by

Fu *et al.,* (2008) where it was reported that curcumin successfully reduced the pathological indexes for hepatocytic death caused by CCl_4 in rat models.

Lee *et al.* (2016) also documented the hepatoprotective activities of curcumin and turmeric extract, from the results of their experiment in CCl_4-induced liver damage in animal model. The peroxidated lipids and the oxygen species produced cause hepatocyte necrosis, inflammation and hepatic fibrosis. Lipid accumulation also occurred in the hepatic damage process. The elevated levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were restored after curcumin treatment. The lipid accumulation was also found to be attenuated.

Recently, Ibrahim *et al*. (2020) has reported that the treatment of crude extracts of *C. longa* and curcuminoid could increase the levels of superoxide dismutase (SOD), catalase and GPx activities, which decrease significantly in CCl_4-induced hepatotoxic rats. As mention ed earlier, the increase in the levels and activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum shows the histochemical alteration of the liver. It was also specifically reported that the curcuminoids and silymarin could restore the activities of AST to its normal levels. Histopathological observations also confirmed the protective effect of curcuminoids against histological distortion of the liver in such rats.

Hepatoprotective Activities in Alcoholic Liver Disease (ALD):

Ethanol oxidation generates free radicals and induces the production of toxic metabolites. Oxidative stress leads to the pathogenesis of alcoholic liver disease (ALD). Curcumin, through its antioxidant and anti-inflammatory effects, along with its ability to scavenge free radicals and antifibrotic activity, can exhibit its hepatoprotective nature. (Baliga *et al*., 2018).

An experimental study was carried out by Nanji *et al.,* (2003) in alcohol-induced liver male Wistar rats, where curcumin is reported to inhibit the expression of NF-κB-dependent genes like Tumour Necrosis Factor-α (TNF- α), interleukin 12 (IL-12), monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-2 (MIP-2) and nitric oxide synthase (iNOS). The lipid peroxidation leading to liver damage could be prevented by the treatment of curcumin. Curcumin was also observed to have a significant effect on the accumulation of fat on liver. This was found to be due to the inhibition of TNF-α leading to the reduction of fat storage in liver. The antioxidant mechanism also aided in the reduction of the fatty liver condition in the rats that were treated with curcumin. It was also reported for the first time that curcumin would successfully prevent alcoholic liver disease. The study emphasized on the further clinical trials with curcumin treatment on humans with similar liver disease conditions, to establish its therapeutic usage.

Samuhasaneeto *et al*., (2009) studied liver pathology in the early stages of ethanolic liver damage and the effect of curcumin on it. It was concluded that curcumin improved the histopathological status of the liver by reducing oxidative stress and inhibiting NF-kB activation in female Sprague-Dawley rats.

Curcumin treatment for six weeks in mice that were exposed to ethanol for six weeks in specific dosages, could reverse the effects of liver damage. In this experiment conducted by Rong *et al*., (2012), curcumin was shown to lower reactive oxygen species (ROS) production and enhance antioxidative defense mechanism.

Uchio *et al.* (2017) reported about the inhibition of hepatic oxidative stress and inflammatory cytokine production in mice, suppressing its acute ethanol-induced liver injury by the application of hot water extract of turmeric.

Similar hepatoprotective effect of curcumin in liver damage caused by alcohol in mice was observed by Wang *et al*. (2019). They concluded that curcumin inhibited the endoplasmic reticulum stress and regulated the mitochondrial dysfunction.

Salehi *et al*. (2021) reported the crucial role played by curcumin in inducing the biogenesis of mitochondria, by which the intrinsic and extrinsic apoptotic pathways in alcohol-affected hepatic cells are activated. In this way, curcumin prevents liver cells' degeneration from alcoholic induction.

Very recently, a study done by Chen *et al*. (2023) revealed that curcumin/cyclodextrin polymer complex (CUR/CDP) can inhibited or down regulated the expression of proteins related to DNA damage, and thereby attenuate liver injury caused by ethanol.

Hepatoprotective Activities in Non-Alcoholic Fatty Liver Disease (NAFLD):

Non-alcoholic Fatty Liver Disease (NAFLD) is one of the most prevalently occurring liver disorder which encounters excessive fat accumulation in the liver, without any significant alcohol consumption. An amorphous formulation of curcumin was subjected to patients with NAFLD for 8 weeks to assess its efficacy, in a trial done by Rahmani *et al*. (2016). The fat content of liver of the patients was observed to reduce, along with the total body mass content, cholesterol, glucose and triglycerides.

Mansour-Ghanaei *et al*. (2019) examined that curcumin treatment in a dose-dependent manner could reduce the levels of the liver enzymes like alanine aminotransferase and aspartate aminotransferase.

It has been reported through a detailed investigation by Ahsan *et al.* (2020) that curcumin could prevent the progression of fibrosis, preventing the oxidative stress in liver induced by 8-OH-deoxyguanosine.

Lee *et al*. (2022) recently had validated a quantification method for curcumin and all its derivatives. They also reaffirmed the protective ability of curcumin against NAFLD.

Hepatoprotective Activities in Thioacetamide-Induced Liver Damage:

Thioacetamide (TAA) is a hepatocarcinogenic agent that has a necrotic effect on the liver cells. This necrotic effect was modified into apoptosis, which is caused through the release of cytochrome from mitochondria, followed by the activation of caspases.

Shapiro *et al.* (2006) reported that curcumin directly played a role in improving the survival status of rats by lowering oxidative stress, liver necroinflammation which occurred due to thioacetamide induction.

Thioacetamide-induced liver cirrhosis in rats was examined and found to be prevented by curcumin. Curcumin could prevent liver damage through its anti-inflammatory activity. Hydroxyproline levels and the weight of the spleen were found to be lower, along with reduced oxidative stress, when treated with curcumin (Bruck *et al*., 2007).

Infection due to virus or bacteria, or any toxic chemical damage the hepatocytes. These hepatocytes start an inflammatory response which activate the production of collagen by hepatic stellate cells. It was reported by Wang *et al.* (2012) that curcumin inhibited the activation of hepatic stellate cells, suppressed the inflammatory activity, and also induced apoptosis of hepatocytes, already damaged by thioacetamide. Thereby, curcumin was established its role in inhibition of the thioacetamide-induced hepatic fibrosis as studied in the BALB/c mice.

The ethanolic extract of the rhizome of *C. longa* exhibited hepatoprotective properties on thioacetamide-induced liver cirrhosis of 8 weeks in rats, in an experiment conducted by Salama *et al*. (2013). They showed hepatoprotective effects at oral doses of 250 mg per kg and 500 mg per kg. It worked by inhibiting the proliferation of hepatocytes. The extract could also rise the levels of glutathione and help in hepatic detoxification.

Farjam *et al.* (2014) examined the effect of curcumin treatment in thioacetamide-induced hepatic encephalopathy. This study was done in male Sprague Dawley rats, which showed inflammation and necrotic hepatic tissue after thioacetamide induction. Both the inflammation and necrosis were significantly reduced along with lowering of the levels of ammonia, ALP, ALT and AST, by the treatment of curcumin, in a dose dependent manner.

Hepatoprotective Activities Against Iron and Heavy Metals Toxicity:

A very common effect of toxicity in liver is due to excessive iron deposition in hepatocytes, that produces fibrosis and cirrhosis. In a study done by Reddy and Lokesh (1996), it was inferred that curcumin could prevent the serum levels of AST and ALT, which are important parameters to assess liver damage. The lipid peroxidation levels induced by iron administration in wistar rats, were remarkably reduced by a dosage of curcumin of 30 mg per Kg of body weight for 10 days.

García-Niño *et al*. (2014) investigated the protective action of curcumin on the liver against heavy metals like arsenic, cadmium, chromium, copper, lead, and mercury. It was observed that curcumin could reduce the hepatotoxicity caused by the heavy metals, could prevent histological damage induced by them, and prevent histological injury along with lipid peroxidation and glutathione depletion. The liver's antioxidant enzymes status was also seen to be maintained.

Effect of Curcumin Against Paracetamol, Concanavalin A, and Nicotine:

Oxidative stress often leads to hepatotoxic conditions in liver disorders. Such hepatotoxity was induced by the administration of 500mg/kg of paracetamol in mice, due to which there was a noticeable upgradation in the activities of certain marker enzymes like alanine transaminase, etc. and decreased activity of reduced glutathione and catalase levels. When the mice were pre-treated with curcumin, along with picroliv and ellagic, at doses of 50 mg per Kg and 100 mg per kg administered orally, the levels of these enzymes changed to normal ranges, supporting their hepatoprotective property. This study put forward the suggestion of using these phytochemicals in treating liver ailments (Girish *et al*., 2009).

Concanavalin A is a potent polyclonal mitogen that damages the liver parenchyma through a gradual activation of T lymphocytes, followed by cytokine secretion. Li *et al.* (2014) presented reports of curcuma oil exhibiting hepatoprotective properties in such Con A-induced injury and also chemotherapeutic effect against inoculated hepatoma in mice. They observed that curcuma oil had anti-inflammatory, anti-oxidative and antitumor properties along with several advantages of addressing multiple targets and with minimum side effects.

Curcumin was reported by Salahshoor *et al*., (2016), to increase the liver weight, decrease the levels of nitric oxide in nicotine-treated male mice, thereby posing to be hepatoprotective in nature against nicotine-toxicity.

A widely used pesticide, Carbofuran, exerts harmful effects on the liver of humans along with other animals. The toxic effect of the chemical on blood and liver can be ameliorated by the usage of turmeric extract (Hossen *et al*., 2017).

Some other Significant Hepatoprotective Activities:

Colpitts *et al.* (2014) documented that curcumin of turmeric was able to inhibit the entry of hepatitis C virus of all genotypes into the liver cells of humans, by disrupting virus binding through regulation of membrane fluidity.

Taebi *et al.* (2020) reported that the extract of *C. longa* and curcumin could positively lower the proliferation of human hepatocellular carcinoma cell line (HepG2), in a concentration-dependent manner. They were also observed to increase fatty acid oxidation and reduce the lipid synthesis gene expression.

Discussion

Among many different diseases, those that lead to liver damage are the most deleterious. Since liver diseases due to diverse causes are responsible for many of the human deaths worldwide (Farzaei *et al*., 2018), it is very essential to provide as many ways of treating or preventing liver damage as possible in both modern and traditional medicine. Curcumin derived from *C. longa* has been documented to exhibit hepatoprotective properties against alcoholic as well as non-alcoholic liver disease. It is also reported by many workers to be potent against the damaging effects of the hepatotoxic drug - carbon tetrachloride and also against thioacetamide, paracetamol, concanavalin A, nicotine, iron, heavy metals like arsenic, cadmium, chromium, copper, lead, mercury, etc.

Conclusion

The therapeutic power of turmeric is receiving a considerable amount of attention for its manifold benefits in treating various diseases. Various investigations have concluded that curcumin, the phytochemical from *C. longa*, has the ability to modulate the functions of several signal transductions, which ultimately can attenuate acute or chronic diseases. In regard to medicinal usage, *C. longa* has been the focus of investigation, where, several preclinical and clinical studies proved the virtue of curcumin as a hepatoprotectant, with long-term health benefits. This account enumerates the findings to assert that curcumin has significant protective effect on liver-related diseases through many cellular and molecular mechanisms. This review would provide an impetus for further investigative trials and new drug discoveries utilizing the novel phytochemical, curcumin of *Curcuma longa* L.

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Isolation and Characterization of Some Food Grade Lactic Acid Bacteria for their Application as Probiotics

Shamba Chatterjee, Sucheta Das*
Department of Biotechnology, Haldia Institute of Technology, Haldia, West Bengal, India

*Corresponding Author's Email: sucheta.bt@gmail.com

ABSTRACT

Probiotics, which are living, non-pathogenic microorganisms, can enhance a person's health, immunity, and mental function when taken in large quantities together with food. In the present study, seven microbial strains (L1, L2, C1, C2, C3, X, and Y) were isolated from locally collected fresh palm sap and characterized morphologically and biochemically. Among them, two strains were yeast (L1 and C3), two were Bacillus (C2, Y) and three were Cocci (L2, C1, X). All the bacterial isolates were gram-positive and catalase-negative. They showed a broad antimicrobial spectrum against both gram-positive (*Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis MB1, Leuconostoc messenteroides Ly)* and gram-negative bacteria (*Salmonella abony, Escherichia coli).* There was little or no change in the growth after three hours of incubation at pH 2, 2.5 and 3. So all the strains were tolerant of gastric acidity. Bacterial isolates were checked for their survivability in the presence of bile salt. Strain L2 showed maximum tolerance to 0.3% bile salt. So, this strain can be further checked *in vivo* for its usefulness as a probiotic.

Keywords*: Lactic Acid Bacteria; Probiotics; Palm Sap; Antimicrobial Activity*

Introduction

In recent years, consumers have paid more attention to human health and used more natural products. As a result, there is a greater need for natural products in a variety of industries, including those that deal with the food and dairy business, the health sector, the agricultural industry, poultry and fisheries, pesticides and fertilisers. This prompted extensive study to find novel, naturally derived antimicrobial chemicals that might be utilised successfully without endangering human health, the environment, or their foods, for example. Medicinal plants, as well as marine and terrestrial creatures, including fungi and bacteria, are known sources of natural substances with valuable antimicrobial activity. Since ancient times, Lactic acid bacteria (LAB) have been present in different types of fermented foods such as curd, yogurt, cheeses, sauerkraut, sausage *etc.* They also have GRAS (Generally Recognized As Safe) status by the United States Food and Drug Administration (FDA) for human consumption (Rodríguez, 1996). During carbohydrate fermentation, LAB, which is a group of related bacteria, produces mainly lactic acid. They are gram-positive bacteria with DNA that has a GC content of under 50%. Since they lack cytochromes and porphyrins and are therefore catalase and oxidase negative, the majority of LAB are non-

spore forming rods or cocci. They are also aerotolerant anaerobes. Due to their ability to create a variety of antimicrobial substances, including lactic acid, hydrogen peroxide, di-acetyl, and bacteriocins, this group of bacteria can be employed to control microbial growth. Bacteriocins are cationic, hydrophobic, secreted anti-microbial peptides (AMP) of length 20–60 amino acids that are produced by the ribosome, which differs from antibiotics. Both gram-negative and gram-positive pathogenic bacteria that cause food to rot, infections, allergies, and cancer can be inhibited by them. They can be applied alone or in combination with other natural products or chemical drugs.

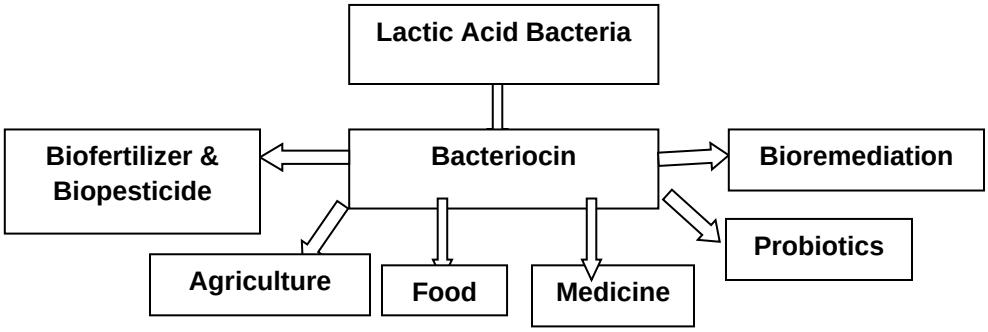

Figure 1: Application of Lactic Acid Bacteria

Probiotic microorganisms assert health benefits for the host when ingested in a suitable amount (Reuben *et al*., 2020). They are live microbial dietary supplements that benefit the host by balancing the microbial population in the intestines (Fuller, 1999). The first observations were made by Elie Metchnikoff, a Nobel Prize laureate, in 1907, who proposed that due to the intestinal microorganisms' reliance on food, it is simpler to change the flora in human systems and replace toxic microbes with beneficial ones. The most accepted probiotic LAB varieties contain various Lactobacillus species. (*Lb. acidophilus*, *Lb. johnsonii*, *Lb. casei*, *Lb. rhamnosus*, *Lb.gasseri*, and *Lb. reuteri*),genus Bifidobacteria (*Bf. bifidum*, *Bf. animalis* subsp. *lactis*, *Bf. longum* subsp. *longum*, and *Bf. longum* subsp. *infantis*) and *Lactococcus* spp. (*L. raffinolactis*, *L. lactis* subsp. *lactis*, and *L. lactis* subsp. *cremoris)* (Ouwehand & Salminen, 2003, Chassard, Grattepanche & Lacroix, 2011, Jung *et al*., 2017). LAB can be used to treat a variety of disorders brought on by pathogenic bacteria that are drug-resistant (Marco *et al*., 2017). The approval process for probiotics requires certain essential characteristics, such as resistance to bile and low pH, antibiotic susceptibility, and antimicrobial activity (Unban *et al.*, 2021). There are numerous sources from which LAB can be isolated, including sugar cane plants, milk products, animal intestines, freshwater fish, fermented foods, and chicken farms (Mulaw *et al.*, 2019).

Palm wine is a very popular drink in different parts of South and Eastern India, Africa and Philippines. It is produced from the sap of different types of palm trees. Though the types and quantity of microorganisms influence the product quality, mainly yeast and some lactic acid bacteria like *Leuconostoc mesenteriodes, Lactobacillus plantarum* etc. have been

found to ferment the sap of palm trees (Sornsenee *et al*., 2021). According to Naknean, Meenune and Roudaut (2010), the product quality is also influenced by the techniques used to tap palm trees and collect their sap, as well as by the air and the surrounding environment. In the recent studies by Fossi *et al*. (2022), it was observed that the probiotic LAB isolated from locally harvested palm wine in Cameroon exhibited cholesterol (LDL)-lowering ability both *in vivo* and *in vitro*. In another study by Ramadhanti *et al*. (2021), lactic acid bacteria with probiotic properties were isolated from palm sugar from West Sumatra, Indonesia. Immunomodulatory activity of the probiotic strains isolated from palm sap in the presence of some prebiotics was also established in a mouse model (Harahap, Munir & Hutahaean, 2023).

This study aimed to determine the types and number of lactic acid bacteria present in a sample of palm wine taken from a local area of Haldia, Purba Medinipur, as well as evaluate certain characteristics that would make them effective probiotic components.

Methodology

Isolation of Lactic Acid Bacteria

Freshly tapped palm sap was collected aseptically in sterile containers from the nearby village of Haldia and immediately brought to the laboratory in an ice box (4°C) to stop fermentation during transportation. Isolation of microbial strains was performed on the same day of collection. With slight changes, the technique of Bromberg *et al*. (2004), was adopted to carry out LAB isolation. To obtain a 1:10 dilution, 1 ml of each sample was diluted in 9 ml of 1% buffered peptone water. Using sterile distilled water, this mixture was further diluted, and 100µl of the diluted samples were applied to Lactobacillus MRS agar plates, a selective medium for lactic acid bacteria. The plates were kept at 30°C for 48–72 hours until growth became visible.

Characterization of Isolates

a) Morphological characterization:

Single colonies of the LAB isolates were picked up and performed Gram staining. The slides were observed under light microscope at 40X and 100X magnifications.

b) Biochemical characterization:

Some biochemical experiments were carried out using each isolate's 18-hour overnight culture in order to properly identify the bacterial isolates, such as Catalase reaction, carbohydrate utilization test, MR-VP test (Methyl red-Voges-Praskauer), indole production, NaCl tolerance study etc. by following standard protocols.

Detection of Antibacterial Activity of the LAB Isolates

Antibacterial activity of the LAB isolates was tested following the agar-cup assay against seven indicator strains. In this method, a bore was made using a borer with a diameter of 5mm on the MRS plates. Cultures of indicator strains were spread over the plates and 50 µl

of cell free supernatant of each isolate was tested by taking both the filtered and boiled cell-free culture supernatant (pH 7) of overnight grown isolates. Then the plates were incubated for 24hrs at 30°C for the formation of a zone of inhibition. The result was further confirmed by Spot on the Lawn method. A direct comparison was made between the diameters of zones of inhibition (mm) produced by different strains after 24hrs incubation.

Micro-organisms used:

The inhibitory spectra of the isolates were evaluated against total seven (7) number of Gram positive and Gram negative bacteria including *Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis, Salmonella abony, Leuconostoc messenteroides Ly, Enterococcus faecalis MB1*and*Escherichia coli*. Bacterial strains were procured from MTCC, Chandigarh and maintained on Trypticase Soy Broth (TSB, Himedia) media.

Acid Tolerance Study

For application as a good probiotic agent, the bacterial isolates should survive in stomach acid (pH 1.5) and bile acids (pH 2.5). So the survivability of the isolates was checked at pH 2, pH 2.5 and pH 3 following the method of Liong and Shah (2005). Isolates were grown in MRS broth for 24 hours at 37°C and centrifuged at 5000 rpm for 10 minutes at 4°C. As inoculums, 1% of this solution was added to MRS broth that had been acidified with concentrated HCl to pH 2, 2.5, and 3, and then incubated for three hours at 37°C. MRS broth that had not been acidified was used as the control. OD values (600nm) were taken at 0 and 3 hours after incubation at 37°C. Strains that showed little or no reduction in OD values were considered acid-tolerant.

Bile Salt Tolerance Study

Following the methodology outlined by Walker and Gilliland (1993), this experiment was carried out. MRS agar supplemented with 0.3% bile salt (Himedia Laboratories Pvt. Ltd., India), was prepared and each isolate was spread over and incubated at 37°C. After this incubation period, growth was compared with that of control plates of MRS agar without bile salt. The percentage of LAB colonies formed on MRS agar relative to the starting bacterial concentration was used to calculate the survival rate:

survival rate (%) = (log $CFUN_1$/ log $CFUN_0$) × 100,

where N_1 is the viable count of isolates after incubation and N_0 is the initial viable count.

Results and Discussion

Isolation of Lactic Acid Bacteria

A total of 89 bacterial colonies were obtained from the isolated plates, of which some were cream in color and some were whitish. Among them, 7 colonies (L1, L2, C1, C2, C3, X, Y) were selected and purified by the streak plate method on MRS medium, and the isolates were maintained in MRS slants at 4°C. Small, white colonies were selected as lactic acid bacteria (Figure 1).

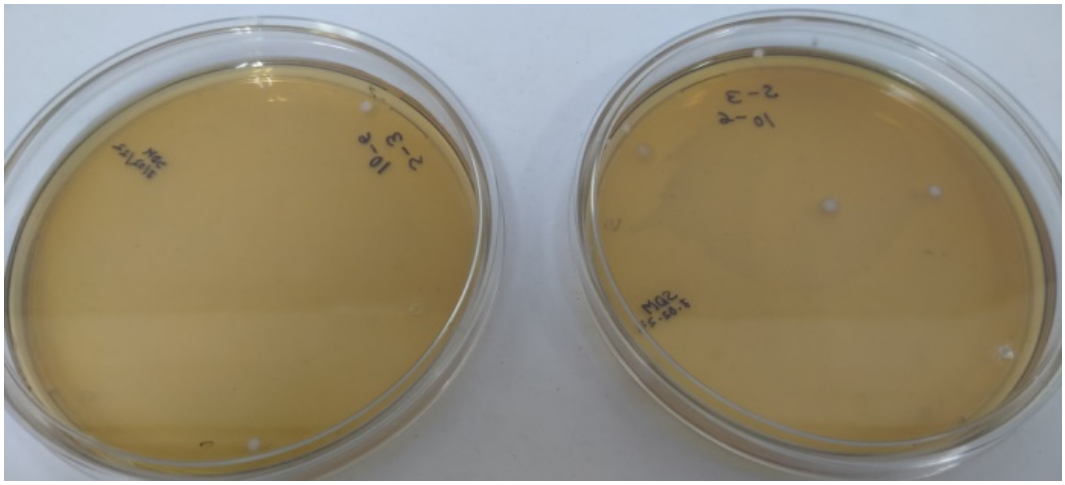

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Figure 2: Lactobacillus MRS Plates with LAB Isolates

Characterization of Isolates

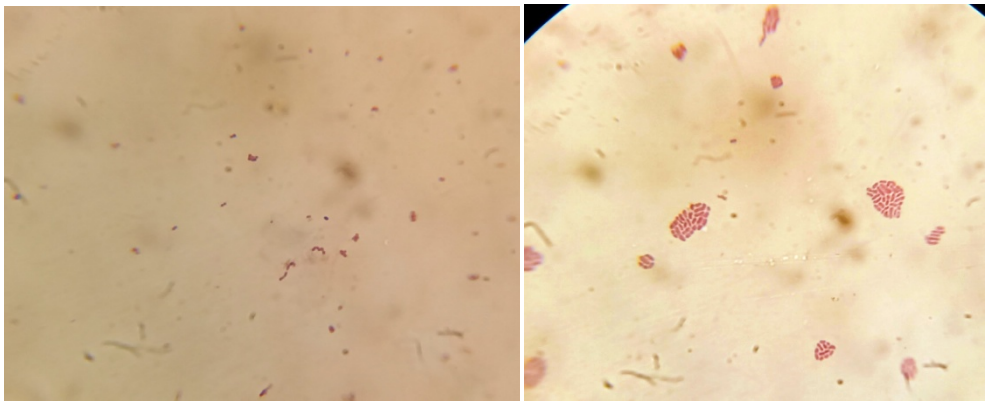
Except for L1 and C3, all the isolates were gram-positive and Catalase negative. Among the isolates L2, X, C1 appeared as cocci and C2, Y as Bacillus and two strains, L1 and C3, were yeast (Figure 2). All the isolates were negative in the Indole test. Except for C1 and Y, all the isolates were Methyl Red positive (Figure 3). All the isolates were VP and Citrate negative (Table 1). All the isolates were able to grow in the presence of 10% NaCl. The carbohydrate fermentation profiles of the isolated strains were shown in Table 2 as evidenced by the color change after 48 h of incubation (Figure 4). The fermentation pattern was compared to the standard lactic acid bacterial strains' fermentation chart. According to this test, it can be predicted that, L2, X, C1 and C3 are *Lactococci* species and C2 and Y are *Lactobacillus* species.

Table 1: Biochemical Properties of Seven Isolates

Tests	L1	L2	C1	C2	C3	X	Y
Morphology	c (y)	c	c	r	c (y)	c	r
Gram staining	+/-	+	+	+	+/-	+	+
Methyl Red	+	+	-	+	+	+	-
Voges Proskauer	-	-	-	-	-	-	-
Catalase test	+	-	-	-	+	-	-
Indole test	-	-	-	-	-	-	-
Gas from glucose	-	-	-	-	-	-	-
NaCl tolerance	+	+	+	+	+	+	+

c: round shaped, cocci; r: rod shaped, Bacillus, c(y): yeast; + : positive reaction, -: negative reaction

Source: Collected by authors



a b

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Figure 3: Observation under Light Microscope (100X) After Gram Staining of L2 (a: chain of cocci) and Y (b: rod shaped)

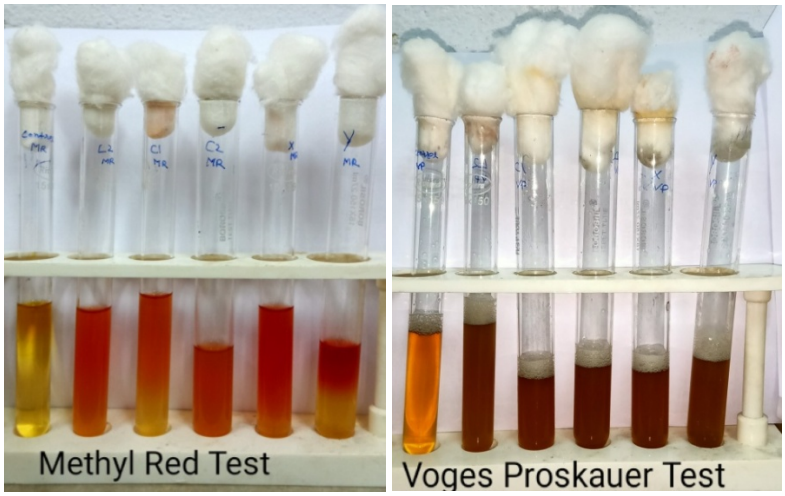

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Figure 4: IMViC Test of Isolates

Table 2: Carbohydrate Utilization Profile of Five LAB Isolates

Carbohydrates	L2	C1	C2	X	Y
Lactose	++	++	++	+/-	+/-
Sucrose	++	++	++	++	++
Maltose	++	++	++	++	++
Fructose	+/-	+/-	+/-	-	+/-
Dextrose	++	++	++	++	++

++: Good utilization, +/- : weak utilization, - : No utilization

Source: Collected by authors

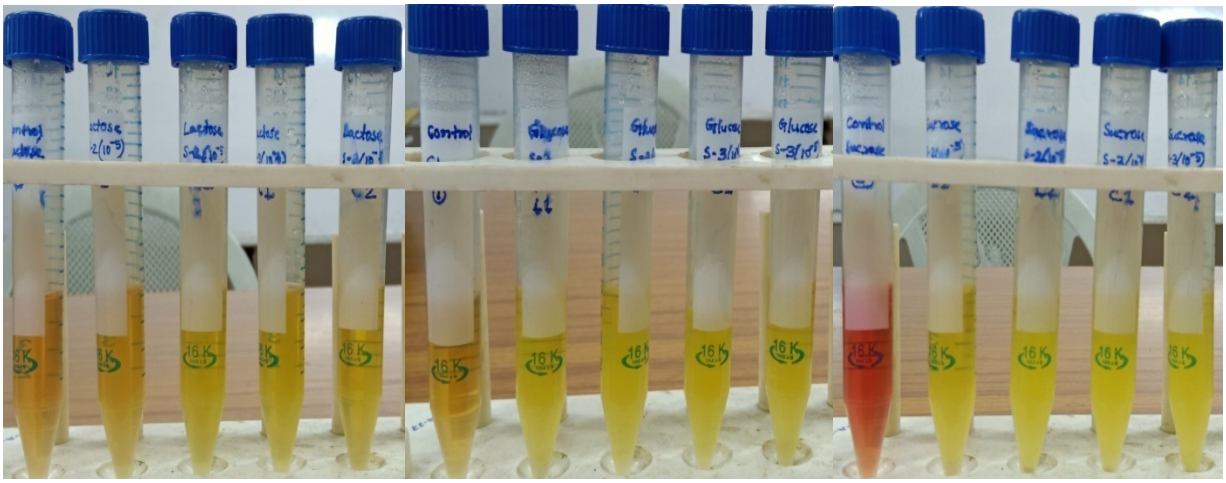

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Figure 5: Carbohydrate Utilization Test of the LAB Isolates

Antibacterial Activity of the LAB Isolates: All the five LAB strains showed broad inhibitory spectra. No isolate showed inhibitory activity against all the tested bacteria (Table 3). LAB C1and C2 has shown maximum antibacterial activity against most of the test organisms. Figure 5 showed some of the antibacterial activity plates with zone of inhibition.

Table 3: Antibacterial Spectra of Different Isolates

Indicator strains	**L2**	**C1**	**C2**	**X**	**Y**
Staphylococcus aureus	++	++	++	++	++
Bacillus subtilis	++	++	++	++	++
Streptococcus faecalis	++	++	++	++	++
Enterococcus faecalis MB1	-	+	+	**-**	**-**
Leuconostocmessenteroides Ly	-	-	+	+	+
Salmonella abony	++	++	++	++	++
Escherichia coli	+	+	-	-	-

++: Zone diameter >2cm; +: <2cm; -: no zone of inhibition

Source: Collected by authors

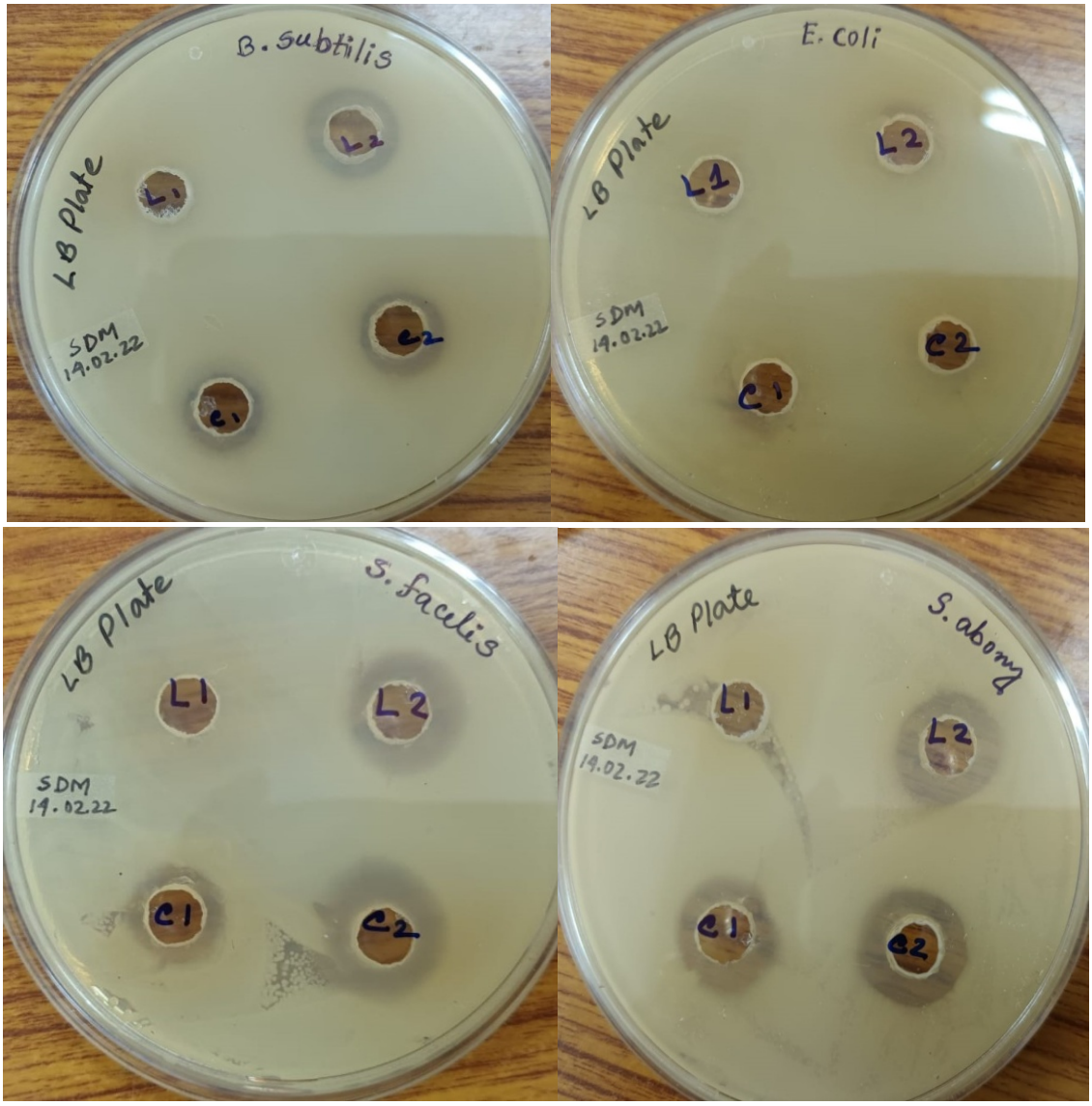

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Figure 6: Antimicrobial Activity of the Isolates Against the Pathogens Tested

Acid Tolerance Study

After 3 hrs incubation period, all the isolates showed a slight increase in OD_{600} values at pH 2 and PH 3, but at pH 2.5, there was little lessen in the OD_{600} values. After 24hr growth ceased in most of the cases, but at pH 2, all the isolates showed a little increase in values (Figure 6). So it can be concluded that all the bacterial isolates were very acid tolerant, though their growth is better at pH 2. In the stomach, where pH is around 1.5, the foods stay for 90 minutes. For this reason, the acid tolerance of the isolates was mainly observed for 3 hours.

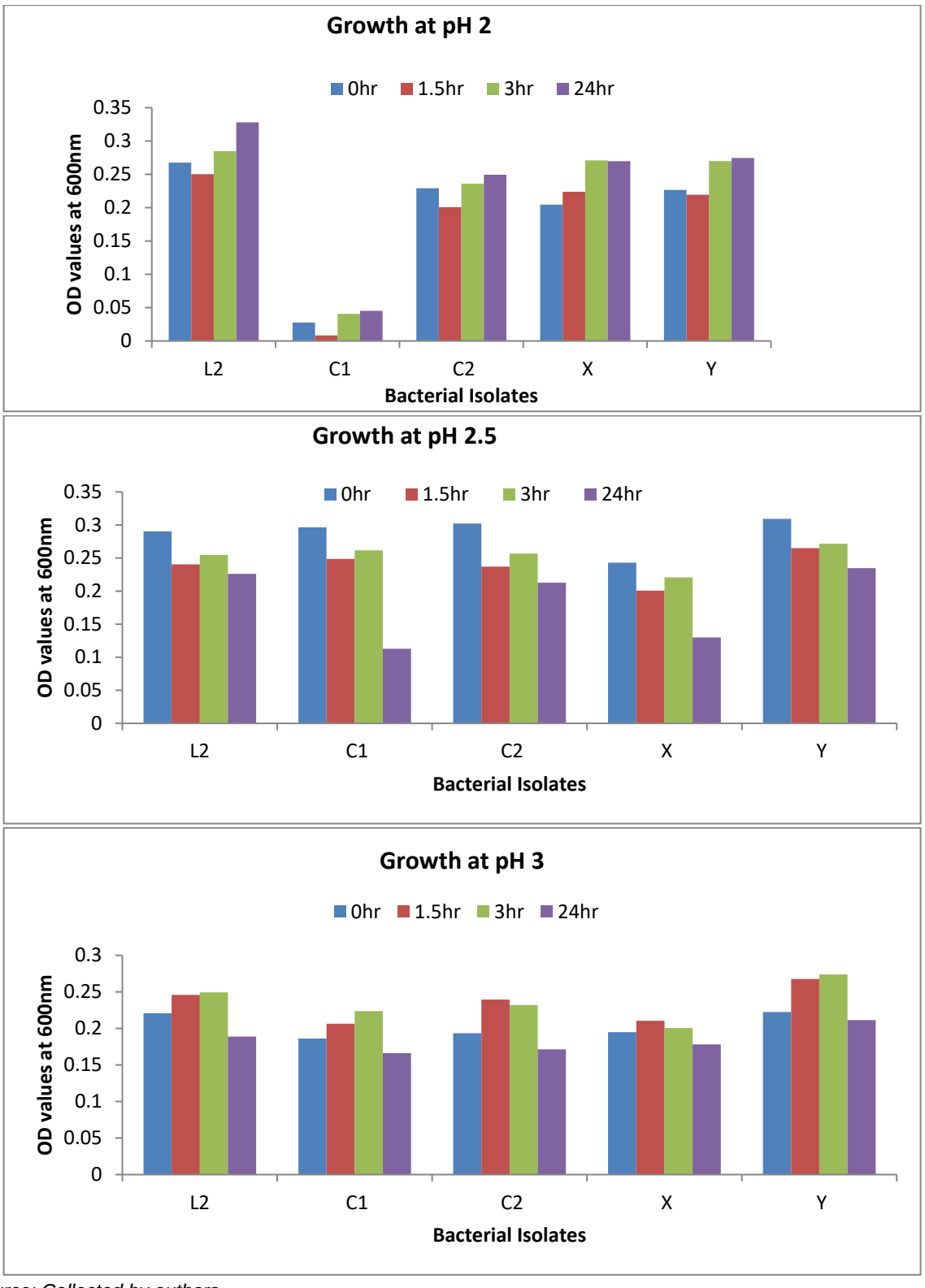

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Figure 7: Growth Pattern of Isolates at pH2, pH2.5 and pH3

Bile Salt Tolerance Study

According to Gilliland, Staley and Bush (1984), 0.3% bile tolerance is necessary for the evaluation of bile-tolerant probiotic LAB. All the isolates survived the tested bile salt concentration (0.3%). Small colonies developed after 48 hours of incubation at 37°C on bile salt agar medium. Growth was also observed in MRS broth containing bile salt. Survival rate was highest for L2, then for Y and least for X (Figure 7).

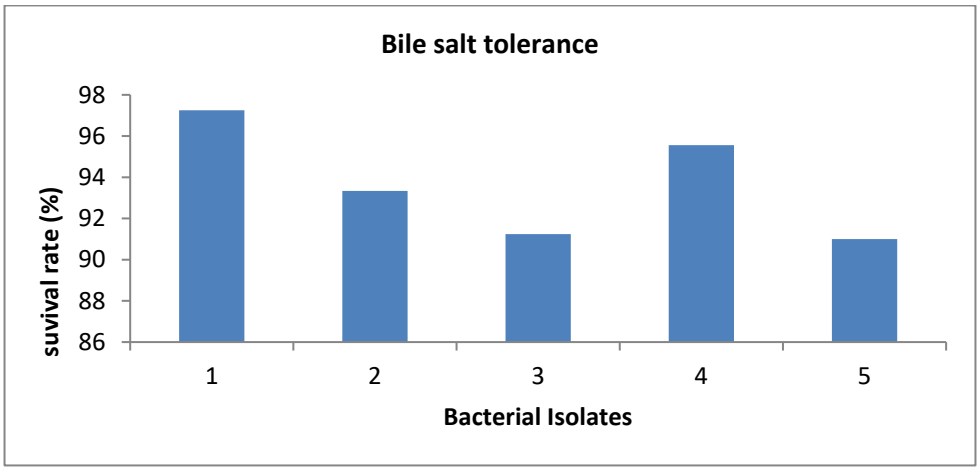

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Figure 8: Survival Rates of Isolates in Presence of Bile Salt

Conclusion

This study demonstrates that palm sap is a potential source of LAB with probiotic properties, especially strong antimicrobial activity against food borne pathogenic bacteria. Among all the isolates, two strains were yeast (L1 and C3), two were Bacillus (C2, Y), and three were cocci (L2, C1, X). All were gram-positive and catalase-negative. All the isolates showed a broad antimicrobial spectrum against both gram-positive (*Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis MB1, Leuconostoc messenteroides Ly)* and gram-negative bacteria (*Salmonella abony, Escherichia coli).* All the isolates showed little or no change in growth after three hours of incubation at pH 2, 2.5 and 3. So all the strains were tolerant of gastric acidity. All the LAB strains were very bile salt-tolerant. Strain L2 showed maximum survivability in the presence of 0.3% bile.

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