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An Extensive and Comprehensive Review on RDS: Techniques, Applications and Problems

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Abstract

Respondent Driven Sampling (RDS) is becoming a widely used method to sample hard-to-reach populations, especially in Public Health and Social Science research. The purpose of this systematic review is to present an overall view of RDS methodology, its applications in different areas as well as the difficulties faced when implementing it. A search of electronic databases was conducted systematically to find relevant studies for synthesis that would provide insight into the strengths, limitations and future directions of RDS.

Keywords: Respondent-Driven Sampling; RDS Methodology; Hidden Populations; Epidemiology; Social Science; Sampling Bias; Network Analysis.

INTRODUCTION

Respondent-Driven Sampling (RDS) refers to a sampling strategy which can be used in cases where hidden or hard-to-reach populations are involved.[1] Its use has grown from sociology to several other fields such as public health, epidemiology and social science research because it provides population characteristics that are not biased. Present review will aim at reviewing the methodology of RDS critically, its applications in research and challenges accompanying its implementation around the world. Respondent-Driven Sampling (RDS) emerged in the late 1990s as a novel sampling methodology designed to overcome the challenges of sampling hidden or hard-to-reach populations. Developed by Douglas D. Heckathorn, RDS was introduced as a systematic approach to studying populations for which traditional sampling methods were ineffective or impractical.[2] The history of RDS is characterized by its evolution from conceptualization to widespread adoption across various disciplines.

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MATERIALS AND METHODS

A systematic search was conducted across academic databases including PubMed, Web of Science, and Google Scholar. Keywords such as "respondent driven sampling", "RDS methodology", and "RDS applications" were used to identify

relevant published articles. Articles were screened based on inclusion criteria focusing on RDS methodology, applications, and challenges.[3] These published articles were then regorusly reviewed to find out the key findings.

Key Findings: Methodology of Respondent Driven Sampling (RDS)

Respondent Driven Sampling (RDS) is a sampling method designed to study hidden or hard-to-reach populations by leveraging social networks within these populations. RDS involves a systematic process of participant recruitment, whereby initial participants (seeds) recruit their peers, who in turn recruit additional participants, creating a chain referral sampling approach.[4] This methodology aims to overcome the limitations of traditional sampling methods and provide unbiased estimates of population characteristics. Below, we outline the key steps and principles of RDS methodology:

Selection of Seeds: RDS begins with the selection of a small number of initial participants, known as seeds. Seeds should be well-connected within the target population and possess diverse characteristics to ensure the representativeness of the sample.[5] The selection of seeds is critical, as they initiate the recruitment process and influence the composition of the sample.

Recruitment of Participants: Seeds recruit their peers from within their social networks to participate in the study. Each recruited participant, known as a "wave 1" participant, is given a limited number of coupons or referral cards to distribute to their peers.[6] Participants are incentivized for successful recruitment, typically through monetary compensation or non-monetary incentives.

Snowball Effect: As recruitment progresses, participants recruit their peers, who in turn recruit additional participants, creating a snowball effect. This chain-referral process continues until the desired sample size is reached, resulting in a network based sample.[7]

Monitoring and Tracking Recruitments: Throughout the recruitment process, researchers monitor and track the flow of participants through the social network. Data on the number of coupons distributed, redeemed, and remaining are collected to assess the progress of recruitment and adjust sampling efforts as needed.[8]

Dual Incentive System: RDS employs a dual incentive system to motivate participation and ensure the integrity of the sampling process. Participants receive incentives for both their own participation and for successfully referring their peers to the study.[9]

Weighting Adjustments: To account for biases inherent in the sampling process, weighting adjustments are applied to the collected data. Weighting adjustments correct for differences in individuals' network sizes and recruitment probabilities, ensuring that estimates are representative of the target population.

Statistical Analysis: Statistical methods specific to RDS are employed to analyze the collected data and derive population estimates. Commonly used estimators include the RDS-II estimator, which incorporates weighting adjustments, and bootstrapping techniques to assess the uncertainty of estimates.[10]

Convergence and Sample Adequacy: Convergence is assessed to determine when recruitment has reached equilibrium and the sample is adequately representative of the target population. Adequacy of the sample is evaluated based on criteria such as network size, recruitment diversity, and convergence of key characteristics.[11]

Ethical Considerations: Ethical considerations are paramount in RDS studies, particularly concerning participant confidentiality, informed consent, and protection of privacy. Researchers must adhere to ethical guidelines and obtain approval from institutional review boards to ensure the welfare and rights of participants.[12]

Applications of Respondent-Driven Sampling (RDS)

Respondent-Driven Sampling (RDS) has diverse applications across public health, social science, and market research domains. By providing a systematic approach to sampling hidden populations, RDS enables researchers to generate valuable insights into health disparities, social determinants of health, consumer behavior, and political attitudes. RDS studies contribute to evidence based interventions, policies, and programs aimed at addressing the needs of marginalized and underserved populations. By leveraging social networks within these populations, RDS offers a unique approach to data collection that overcomes many of the limitations of traditional sampling methods.[13] The key applications of RDS in research are as follows:

Public Health Research:

HIV/AIDS and STI Surveillance: RDS is extensively used in epidemiological studies to estimate the prevalence of HIV/AIDS, sexually transmitted infections (STIs), and other infectious

diseases among high-risk populations such as men who have sex with men (MSM), people who inject drugs (PWID), and sex workers. These estimates are crucial for monitoring disease trends, assessing the effectiveness of prevention programs, and allocating resources for targeted interventions.[14]

Substance Use and Harm Reduction: RDS is employed to study substance use patterns, prevalence of drug use disorders, and access to harm reduction services among marginalized populations, including PWID and individuals experiencing homelessness. These studies inform the development of harm reduction strategies, needle exchange programs, and substance use treatment services.[15]

Maternal and Child Health: RDS is utilized to study maternal and child health outcomes among vulnerable populations, such as pregnant women living with HIV, undocumented immigrants, and refugees. These studies assess access to prenatal care, maternal health services, and pediatric healthcare interventions, contributing to efforts to reduce disparities in maternal and child health outcomes.[12]

Social Science Research:

Migration and Mobility: RDS is employed to study migration patterns, social networks, and health outcomes among migrant and mobile populations, including undocumented immigrants, refugees, and temporary workers. These studies explore the social determinants of health, access to healthcare services, and experiences of discrimination and marginalization among migrant populations.[16]

Sexual and Gender Minority Health: RDS is utilized to study the health needs, experiences, and disparities among sexual and gender minority populations, including LGBTQ+ individuals and gender nonconforming individuals. These studies examine access to healthcare, prevalence of mental health disorders, and experiences of stigma and discrimination, informing policies and programs to promote LGBTQ+ health equity.[17]

Criminal Justice and Incarceration: RDS is employed to study the health and social needs of individuals involved in the criminal justice system, including current and former inmates, justice involved youth, and individuals on probation or parole. These studies assess access to healthcare services, prevalence of substance use disorders, and barriers to reintegration into society post release.[18]

Market Research

Consumer Behavior and Market Segmentation: RDS is utilized in market research to study consumer behavior, preferences, and purchasing patterns within hard-to-reach populations, such as niche markets or subcultures. These studies provide insights into consumer motivations, brand loyalty, and product preferences, guiding marketing strategies and product development efforts.[19]

Opinion Polling and Political Campaigns: RDS can be employed in opinion polling and political campaigns to survey hard-to-reach populations, such as minority voters, young adults, and low-income communities. These studies assess public opinion, political attitudes, and voting behavior, informing campaign strategies and policy advocacy efforts.[20]

Challenges in Respondent-Driven Sampling (RDS) Implementation

Respondent-Driven Sampling (RDS) offers a promising approach to sampling hidden or hard-to-reach populations, but its implementation presents several challenges that researchers must navigate. These challenges can impact the validity, reliability, and generalizability of study findings.[16] The key challenges in RDS implementation are depicted as:

Seed Selection Bias

Challenge: The selection of initial participants (seeds) can introduce bias if seeds do not adequately represent the diversity of the target population. Biased seed selection may lead to underrepresentation or overrepresentation of certain subgroups in the sample.[21]

Mitigation: Researchers should employ strategies to ensure diverse seed selection, such as purposive sampling based on key demographic or network characteristics. Additionally, researchers may consider recruiting seeds through community-based organizations or key informants to enhance representativeness.[22]

Recruitment Heterogeneity

Challenge: Variability in participants' social networks, recruitment patterns, and willingness to participate can impact the efficiency and representativeness of RDS. Certain subgroups within the population may be more connected or active in recruitment, leading to sampling biases.

Mitigation: Researchers should monitor recruitment progress and assess recruitment diversity to identify potential biases. Strategies to enhance recruitment heterogeneity may include increasing the number of recruitment waves, implementing targeted recruitment efforts, and offering incentives for participation and recruitment.[23]

Network Assumptions

Challenge: RDS relies on the assumption of a connected social network where individuals know each other and can effectively recruit one another. However, in some contexts, social networks may be fragmented, decentralized, or difficult to access, challenging the feasibility and validity of RDS.[24]

Mitigation: Researchers should conduct formative research to assess the structure and connectivity of social networks within the target population. Adaptations to the RDS methodology, such as increasing the number of seeds or implementing chain-referral reminders, may be necessary to overcome network limitations.[25]

Sample Size and Convergence:

Challenge: Achieving adequate sample size and convergence in RDS studies can be challenging, particularly in populations with small network sizes or low recruitment efficiency. Failure to reach convergence may compromise the validity and generalizability of study findings.

Mitigation: Researchers should carefully consider sample size calculations and recruitment targets based on population size, network connectivity, and expected design effects. Monitoring recruitment progress and implementing strategies to enhance recruitment efficiency, such as increasing incentives or expanding recruitment chains, can help facilitate convergence.[26]

Statistical Complexity:

Challenge: Correcting for biases and estimating population parameters through RDS can be statistically complex, requiring specialized weighting adjustments and estimation techniques. Inadequate understanding or application of RDS specific statistical methods may lead to biased or unreliable estimates.

Mitigation: Researchers should seek expert guidance or consultation from statisticians with experience in RDS methodology. Utilizing software programs designed for RDS analysis and conducting sensitivity analyses to assess the robustness of estimates can help ensure the validity and reliability of study findings.[27]

Ethical Considerations

Challenge: RDS raises ethical concerns related to participant confidentiality, privacy, and informed consent. Participants may be reluctant to disclose sensitive information or refer their peers due to fear of stigma, discrimination, or legal repercussions.

Mitigation: Researchers should prioritize participant confidentiality and privacy by implementing strict data security measures, anonymizing data collection instruments, and obtaining informed consent from participants. Building trust and rapport with the target population through community engagement and partnership with local organizations can also facilitate ethical recruitment and data collection practices.[25]

DISCUSSION

Respondent-Driven Sampling (RDS) has proven to be a valuable tool for sampling hidden or hard-to-reach populations and generating population estimates. However, like any research methodology, RDS has its limitations. While RDS aims to achieve a form of random sampling through peer recruitment, it does not guarantee a truly random sample. The initial selection of seeds and the recruitment process may introduce biases, particularly if certain subgroups within the population are over or under represented. RDS relies heavily on the social networks of participants for recruitment. If the social networks are fragmented or if there are subgroups within the population that are disconnected from the main network, RDS may fail to reach certain segments of the population. RDS assumes that every individual in the population has a nonzero probability of being connected to every other individual. However, in reality, social networks may be fragmented, leading to limitations in the reach and representativeness of the sample.[28] RDS assumes that the recruitment process reaches equilibrium, meaning that recruitment patterns stabilize, and the composition of the sample reflects the underlying population distribution. Achieving equilibrium can be challenging, particularly in populations with high levels of mobility or turnover.

The findings from RDS studies may not be generalizable to the broader population due to the non-random nature of sampling and the reliance on

social networks for recruitment. While RDS provides valuable insights into hidden populations, caution should be exercised when extrapolating findings to other contexts. The effectiveness of RDS relies on participants' willingness to recruit their peers, which may be influenced by the incentive structure. If incentives are not perceived as sufficient or if there are concerns about confidentiality or safety, recruitment efforts may be compromised. RDS raises ethical considerations related to participant confidentiality, informed consent, and privacy. The use of peer recruitment may inadvertently disclose sensitive information about participants' social networks or behaviors, raising concerns about privacy and confidentiality. Analyzing data from RDS studies requires specialized statistical methods to account for the complex sampling design and biases inherent in the recruitment process. Researchers must carefully consider weighting adjustments and other statistical techniques to ensure the validity and reliability of estimates.[27]

Despite these limitations, RDS remains a valuable methodology for studying hidden populations and generating population estimates when traditional sampling methods are impractical or ineffective. By understanding and addressing these limitations, researchers can maximize the validity and utility of RDS studies in informing public health interventions, policy development, and social science research. Future research should focus on refining RDS methodology, exploring alternative sampling approaches, and addressing emerging issues in sampling hard-to-reach populations.[29]

CONCLUSION

Respondent-Driven Sampling (RDS) offers a valuable approach for studying hidden populations and estimating population parameters in public health, social science, and market research. The future of Respondent-Driven Sampling (RDS) holds exciting possibilities, with ongoing advancements and innovations aimed at addressing current limitations and expanding the methodology's applicability. Future advancements in RDS methodology will focus on addressing existing limitations and improving the validity and reliability of estimates. This includes refining techniques for seed selection, optimizing recruitment strategies, and developing innovative statistical methods to account for biases and uncertainties inherent in the sampling process.[26] The integration of digital technologies, such as mobile apps and social media platforms, presents opportunities to enhance the efficiency and reach of RDS. Digital platforms can streamline participant recruitment, facilitate data collection, and enable real time monitoring of recruitment dynamics, thereby improving the timeliness and accuracy of RDS studies. Adaptive sampling designs, will allow dynamic adjustments to sampling procedures based on ongoing data collection, offer potential improvements to RDS. By incorporating feedback mechanisms and adaptive strategies, researchers can optimize recruitment efforts, achieve equilibrium more efficiently, and enhance the representativeness of the sample.[30]

Multiplex sampling involves simultaneously sampling multiple populations within a network or community, allowing for the study of interconnected subgroups and their interactions. Future advancements in multiplex sampling techniques will enable researchers to capture the complexity of social networks more comprehensively and explore the dynamics of diverse populations within a single study. Moreover, the integration of RDS with network analysis methods will offer opportunities to gain deeper insights into the structure and dynamics of social networks. By combining RDS data with network modeling techniques, researchers can elucidate patterns of social connectivity, identify influential nodes or clusters, and assess the impact of network structure on health outcomes and behaviors.[24] Future efforts might be focused on fostering global collaboration and standardization in RDS research, enabling cross-country comparisons and harmonization of data collection protocols. By establishing best practices, guidelines, and quality assurance mechanisms, researchers can enhance the reliability and validity of RDS estimates and facilitate evidence synthesis across diverse settings and populations.[31]

As RDS continues to evolve, it will be essential to prioritize ethical considerations and meaningful participant engagement. Future advancements will emphasize the importance of respecting participants' autonomy, ensuring confidentiality and privacy, and fostering partnerships with communities to co-create research protocols and interventions that are culturally sensitive and responsive to community needs. While RDS has been instrumental in advancing knowledge in various domains, challenges such as bias, network assumptions, and[32] ethical concerns warrant continued attention. By addressing these challenges and advancing methodological innovations, RDS can continue to serve as a robust tool for sampling hard-to-reach populations and informing

evidence based interventions and policies.[33]

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Survey Studies for the Prevalence of Parasites from Commercially Edible Fishes in West Bengal, India

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Abstract

Indian major carps (IMCs) are one of the most economically important as well as edible food fishes all over the world. IMCs are showing different disease problems that cause harm for healthy fishes. The present study was carried out from 12 months of survey study between June 2021 to May 2022 for investigations of different causative fish parasites. In this section, our area of concerning fishes were cyprinidae family fishes the ectoparasites infect them which belong to groups of myxozoan, and protozoan fish parasites that lead to cause severe damage and finally, mass mortality has been observed. In *Labeo catla* (Hamilton, 1822) showing highest *Myxobolus* infection was observed in the rainy season (65.55%), and *Thelohanellus* infection was abundant in winter (65.83%). Another way, *L. rohita* showed a massive *Myxobolus* infection rate in January (56.66%), *Thelohanellus* and *Trichodina* infections highest in April (63.33% and 53.33%). In this article, the prevalence of parasitic studies has been dealt with. As per seasonal diversity, host age, size of the hosts and sex-wise prevalence of parasitic infection over the fish sectors. Here, parasitic infections were shown at different time scales which are stated in this research article. As per the sex wise study, the highest infection was observed in females as compared to male fishes. The large sized fishes were highly prone to parasitic diseases as subjected to small young fishes.

Keywords: Economical; Survey study; Ectoparasite; and Prevalence.

INTRODUCTION

Aquaculture is a very highly produced sector around the world. It is a vast way for economical as well as eco-friendly medium. This helps every fish farmer for their wealth being purposed. Fishes are food products which contain several minerals, which have good nutrient values and several people intakes around the world. Nowadays, fish are infected with several deadly causative pathogens like parasites, bacteria, fungi and viruses. These are caused by major diseases on fish farms. The limitations in are parasitic illnesses in aquaculture because of increased fish density in the lentic areas of water where the fish diseases can spread quickly from one host to another (Sinha, 2018). They interfere with fish growth and decrease immune systems *i.e.*, they damage fish immune profile and as a result, fishes become died.

Parasites are abundant and various, which helps

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to contribute to the natural communities (Kuris *et al.*, 2008). Parasites intake their feed from hosts and due to some extension, they give a potentially negative impression on the infected organisms, it creates dynamicity in the population chain, biodiversity, community structure and food web connectivity (Marcogliese, 2003; Wood *et al.*, 2007; Johnson *et al.*, 2008). Parasites play a key role in ecosystems by interfering with the abundance and density of fish populations and food web establishment (Acosta *et al.*, 2020). So, the population diversity of parasites is often used as an indicator to study species richness and biodiversity, as well as the dynamics of fish populations (Levy *et al.*, 2019; Marcogliese, 2002). Several fish parasites having complex life cycles may contain the definitive fish host and one or more intermediate invertebrate hosts (Juntaban *et al,* 2021). Myxozoans are diverse and abundant cnidarian parasites, they are widely studied for development of the infections (Abdel-Ghaffar *et al.*, 2005; Székely and Molnár, 1999). Myxosporeans are common parasites of fishes around the world (Lom and Dyková, 1994), which creates major damage to the economically important fresh and marine water fish species (Kaur and Attri, 2015). The genera *Myxobolus* (Bütschli, 1882), and *Thelohanellus* (Kudoa, 1933) are among the most studied myxozoan fish parasites. The myxozoan parasite samples are very important, they contain 67 genera and more than 2600 species (Morris, 2010).

This study observed the primary infection in freshwater fishes, which contain parasites. Parasitic infection is the entry point of other secondary infections (e.g., bacteria and fungi). Parasitic infections are predisposed season wise and are generally affected by the physiology and ecology of the host fishes. Therefore, appropriate health status is useful for controlling actions elicited in aquaculture invention. West Bengal is a "rice fish culture". This state is enormously important historically as well as geographically for a long past.

The state of West Bengal has been played centre of attraction for a high amount of freshwater fish production and also has the distinction of a wide range of water areas under traditional shrimp farming. The disease is the only factor that creates constraints in the aquaculture industry, inhibiting economic success in this sector. The objectives of this study were to isolate, and identify diverse parasites from IMCs, and to find out the "Parasitic Frequency Index" (PFI) in the status of months and seasons from selected several water bodies districts of West Bengal. In this study, we aim to identify their prevalence in different time scales. In future prospects, we will focus on the prevention measures pathway.

MATERIALS AND METHOD

2.1 Study location

The study was performed in the district of North 24 Parganas (Monirampore, Barrackpore, Icchapur and Titagarh) area of West Bengal, India, which has several export farms of IMCs. Among them, several of these exporters partially rely on small-scale farms in nearby rural areas to maintain the supply of IMCs, which helps to reduce costs. Around 4500-5000, fish were produced monthly in freshwater farms.

2.2 Experimental setup

A total of 320 fish were collected from four differently situated IMCs farms in the district of North 24 Parganas (Monirampore, Barrackpore, Icchapur and Titagarh) of West Bengal, India was visited during the period from June 2021 to May 2022. At each farm, the water quality parameters were measured. 320 fishes were collected in diverse fish farms, and average body weight (26 ± 2.4 g) and length (15 ± 0.5 cm) were respectively collected for the study. In polyethene bags filled with oxygenated pond water, all fish were transported to the lab for additional examination. After reaching the working station, specimens were stocked in a 5000 litre cultured tank with a submersible water filter system (SOBO, Aquarium Internal Filter WP 1000F) for pumping, aeration, and filtering water continuously and commercially available fed was given 2% of their body weight.

2.3 Collection and processing of specimens

In this current study, we observed the prevalence of parasitic infestation in freshwater IMCs, and it was carried out over 12 months of study between June 2021 to May 2022. The live fish specimens were collected from different waterbodies of Monirampore (n=240; 80 no. each in the month of June, among them male 36, and female 44; 80 no. each in the month of October, among them male 38, and female 42; 80 no. each in the month of February, among them male 40, and female 40), Barrackpore (n=240; 80 no. each in the month of July, among them male 34, and female 46; 80 no. each in the month of November, among them male 38, and female 42; 80 no. each in the month of March, among

them male 36, and female 44), Icchapur (n=240; 80 no. each in the month of August, among them male 38, and female 42; 80 no. each in the month of December, among them male 40, and female 40; 80 no. each in the month of April, among them male 34, and female 46), and titagarh (n=240, 80 no. each in the month of the September, among them male 40, and female 40; 80 no. each in the month of January, among them male 34, and female 46; and 80 no. each in the month of May, among them male 38, and female 42) districts of North 24 Parganas, West Bengal, India. These different sampling sites were selected for concerning purposes. The live specimens were collected regularly once every month and they were collected in live conditions. The fish were brought to the laboratory in live condition with water filled containers and the length and weight of the fish were measured. A total of 44 fish farms were facing problems of fish being lethargic and showing excessive mortality. Fish are intake 1% body weight commercial fed every day, which is available in the market. Each pond's size is around 1.25 bighas.

2.4 Sampling for parasitic study

First, the live fish specimens were put to anaesthetize with a dose of sodium bicarbonate (NaHCO3) buffered MS-222 (Tricaine methanesulfonate, Argent laboratories; 30 mg/l). The skins and gills were thoroughly examined for the presence of different parasites. The collection and preservation methods were opined by Soota (1980). The site of collection and date were noted for future prospects. The live fishes were screened for the presence of parasites within 12 hours. An external sign and health status were well documented. With special interest skin and gills were gently removed at least damaged and kept on separate petri plates containing physiological saline (0.65%) water and thoroughly examined. Each pair of gills were separated and checked thoroughly for the presence of any kind of infection. Morphological characters were well described by Soulsby (1982). Photographs were taken using Magnüs MLX PLUS (India) with an in-built digital camera (Fig. 1).

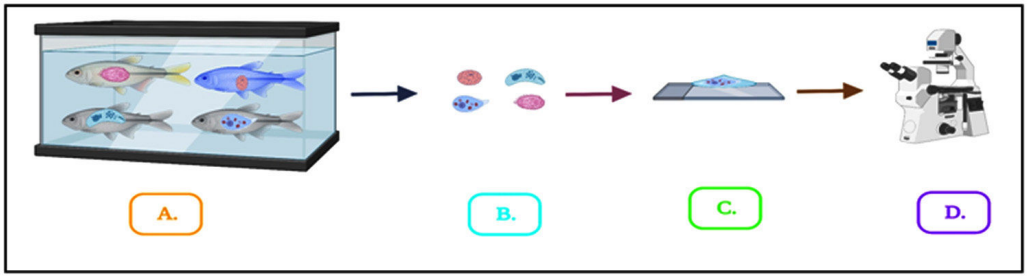


Fig. 1: In this schematic diagram, fishes were infected with different parasites in an aquaculture tank (A), isolation the parasites were gently dissected (B), the fresh smear was made grease free slide with the help of water (C) and finally observed under microscope at different magnifications (D).

2.5 Sex identification of fishes

Fish were tested physically for sex, and the urogenital papillae were used as a test. In addition, the ovaries in females and the gonads in men are visible.

2.6 Determination of Parasite Frequency Index (PFI) and Prevalence Study

The Parasite Frequency Index (PFI) was determined by using the percentage of the number of hosts infected by an individual parasite species against the total number of hosts examined in a particular area under investigation (Vijaysundardeva *et al.*, 2018).

The prevalence rate of parasite infection was measured using the model (Amos *et al.*, 2018):

$$\text{Prevalence (\%)} = \frac{\text{No. of fish host infected}}{\text{Total no. of host examined}} \times 100$$

The prevalence of parasites infection based on the sex of the fish (Amos *et al.*, 2018):

$$\text{Prevalence (\%)} = \frac{\text{No. of a particular sex of fish infected}}{\text{Total no. of a particular sex of fish examined}} \times 100$$

The model was used to estimate the parasite intensity (Amos *et al.*, 2018):

$$\text{Intensity} = \frac{\text{Total no. of parasite species in a sample of fish examined}}{\text{No. of fish host infected}}$$

The frequency index was clarified into rare (0.1-9.9%), occasional (10-29.9%), common (30-69.9%) and abundant (70-100%) (Vijaysundardeva *et al.*, 2018).

2.7 Statistical analysis

It was statistically established that the infection was sex dependent using chi-square analysis.

RESULTS

3.1 Infection of host fishes

Screening of *Labeo rohita* showed infections (e.g., *Myxobolus, Thelohanellus* and *Gyrodactylus*) and *L. catla* (e.g., *Myxobolus* and *Thelohanellus*) were observed (Table. 1).

Table 1: Cyprinidae family fishes showing parasitic infection

Sl. No.	Name of fishes	*Myxobolus* infection	*Thelohanellus* infection	*Gyrodactylus* infection
1.	*Labeo rohita* (Hamilton, 1822)	+	+	+
2.	*L. catla* (Hamilton, 1822)	+	+	-
3.	*Cirrhinus mrigala* (Hamilton, 1822)	-	-	-
4.	*L. bata* (Hamilton, 1822)	-	-	-
5.	*L. calbasu* (Hamilton, 1822)	-	-	-

"+" = infected and "-" = non-infected

3.2 Identification of Parasites

In this current study, different fish skin and gill lesions, such as erosions, ulcerations, haemorrhages, profuse mucus, dullness of the coloring and whitish discoloured areas were documented depending on the strength and method of the parasite attachment, and immunological response of the host. The macroscopic inspection of infected fish also revealed various behavioural abnormalities, including weakness, despair, anorexia, flashing, and an increase in opercular movements.

The microscopic observations of fish that were infected by protozoan *Trichodina* revealed lesions on the skin including increased opercular movement, dull colour, pale spots, and excessive mucus production. *Trichodina mutabilis* which was found in *Poecilia reticulata* (Peter, 1859), was identified using the sticky disk's diameter (60 to 65 m), the denticle ring's diameter (40 to 45 m), and the number of denticles (28 to 30) (Kazubski and Migala 1968). As per microscopical evidence, the presence of polar capsules, and spores indicates that the observed specimen belongs to the class Myxosporean parasites (Lom and Arthur, 1989). Due to this parasitic infection, the gill of fish shows whitish colouration and behavioral abnormalities.

3.3 Month wise parasitic prevalence in Labeo catla (Hamilton, 1822)

The month wise diversity of parasites in *Labeo catla* is given below. The occurrence of parasitic fauna like *Myxobolus* sp. (Fig. 2A) and *Thelohanellus* sp. (Fig. 2B) were obtained from *L. catla* in this current study. The Parasitic Frequency Index (PFI) of *Myxobolus* sp. was found highest in "July" (73.33%) opined as "Abundant" and the lowest prevalence was found in February (3.33%) indicated as "Rare". These findings are fully satisfied with the previous study (Das *et al.*, 1989; Seenappa and Manohar, 1980; Narasimhamurti and Kalavati, 1984; Basu and Halder, 2003) who have documented that myxozoan parasites were highly prevalent in August to January when the ambient temperature was below 25°C and the lowest prevalence in February (3.33%). The PFI range of *Thelohanellus* sp. suggests that the January (76.66%) month was found to be the "Abundant" condition, and the rare condition was found to be in May, August, September, February and March but a previous study (Kaur *et al.*, 2012) showed that 100% prevalence of infection in May against complete 0% prevalence in this study, and the probable reason will be due to the diverse geographical attributes. The earlier report stated that *Thelohanellus* sp. highest prevalence was found in February (31.66%) it opined "Common" distribution and the lowest in August (11.66%), and it stated "Occasional" type of distribution. This will be possible in case of high stocking density, depth of the water, temperature fluctuations and several physicochemical parameters which co-relate with the available literature (Banu and Khan, 2004).

3.4 Season-wise occurrence of parasites in Labeo catla (Hamilton, 1822)

The seasons' change is highly influenced by the occurrence of parasites which has been available

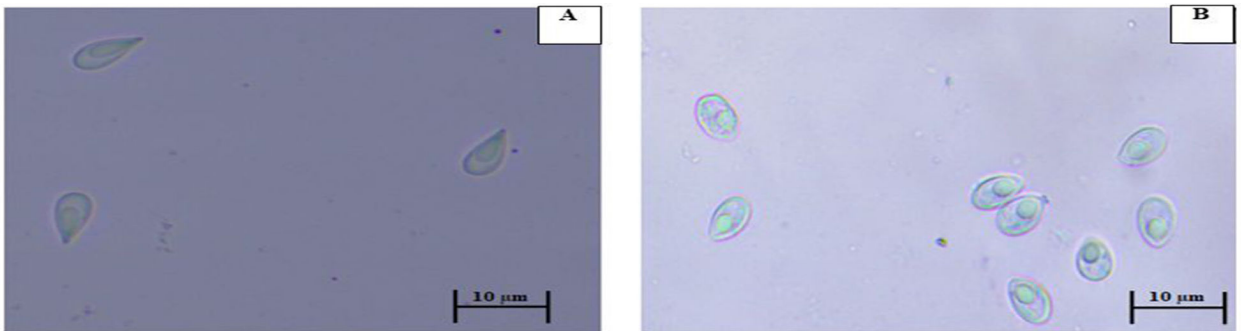


Fig. 2: In these figs., *Myxobolus* sp. infection was observed in *Labeo catla*, scale bar 10 micrometre (µm) 2A; and *Thelohanellus* sp. infestation was observed in *Labeo catla*, scale bar 10 micrometre (µm) 2B.

in several research literatures (Bhuiyan *et al*., 2007; Banu *et al*., 1993; Chandra *et al*., 1997). The total study period was separated into four seasons, they were-summer (April-June), rainy (July-September), winter (October-January) and spring (February-March).

The infection cycle of *Myxobolus* sp. was remarkably shown as a seasonally constant increase from summer to winter with the highest during rainy (65.55%), and it shows "common" the lowest observation in the season of spring (6.66), and it shows "rare" distribution. The fluctuations of the parasitic prevalence due to seasonal, ecological and physiological conditions play a vital role in the fish (Ahmed *et al*., 1991; Wisheiwski, 1958). The variety of parasitic fauna determination is involved in the diet and lifespan of the host, large hosts need more habitats appropriate for parasites than do small ones (Polanski, 1961).

Thelohanellus sp. highly increased its observation in the winter (65.83%) season and it opined "common" type of distribution and in the spring (4.99%) season it shows a "rare" kind of distribution. The presence of the parasite hosts showed parasitic specific nature.

The result suggested that infection site specificity of the hosts for parasite attachment. The direction of the water force moves towards the respiratory current over the gills.

3.5 Parasitic prevalence in Labeo rohita (Hamilton, 1822)

The majority of parasitic taxa well reported in *L. rohita* during this current study period are *Myxobolus* sp. (Fig. 3A), *Thelohanellus* sp. (Fig. 3B), and *Trichodina* sp. (Fig. 3C) The PFI values of the isolated parasites showed the abundance values in the host specification and gradually common, occasional and finally rare status is also given as per PFI values. Infection with *Myxobolu 6s* sp. was documented as a "common" situation in the *L. rohita* as the PFI values reach their maximum in the month of January (56.66%). The occasion and occurrence of the *Thelohanellus* sp. were documented lowest in the month of April (63.33%), which was present throughout the year except for September. The establishment of *Thelohanellus* sp. infection "Common" was observed in the month of April (63.33%). Our findings, corroborated with the available researcher articles (Akther *et al*., 1997; Hossain *et al*., 1994; Bhuiyan *et al*., 2007; Banu *et al*., 1993; and Chandra *et al*., 1997) in *L. rohita*. PFI of *Trichodina* sp. highest in the month of April (53.33%), which was established as a "Common" condition.

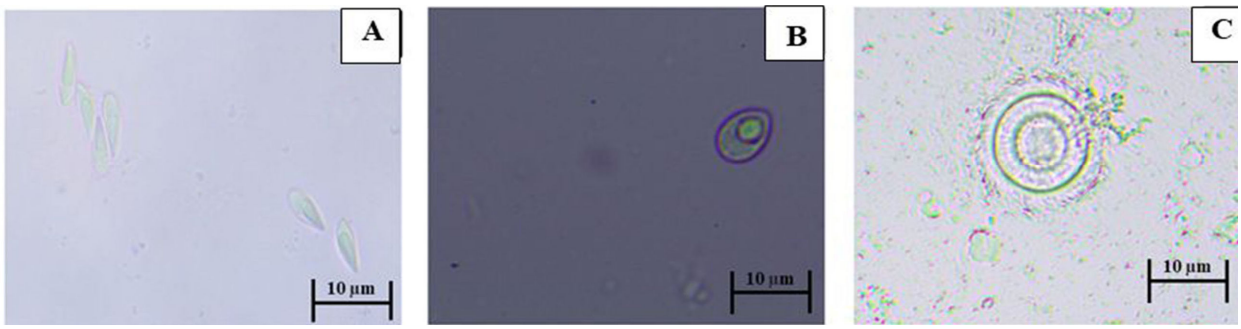


Fig. 3: Here, *Myxobolus* sp. infection was observed in *Labeo rohita*, scale bar 10 micrometre (µm) (3A); and *Thelohanellus* sp. infestation was observed in *Labeo rohita*, scale bar 10 micrometre (µm) (3B); and *Trichodina* sp. infected was observed in *Labeo rohita* scale bar 10 micrometres (µm) (3C).

3.6 Season-wise occurrence of parasites in Labeo rohita (Hamilton, 1822)

The seasonal prevalence of parasites in *L. rohita* was not diverse. In the case of *Myxobolus* sp. it shows baseline levels distribution in the all over seasons. The winter and spring seasons show peaks for *Myxobolus* sp. infection in fish. These results are strongly supported by another research article (Bhuiyan *et al.*, 2007) in *L. rohita*. The prevalence of *Thelohanellus* sp. infection in fishes shows peaks. The decreases in water volume led to nutritional disruption which declines food fish production, in another hand fall in water temperature and declines in the immune response caused by more disease-prone fishes, then mortality will be rise. The prevalence peak of *Trichodina* sp. showed moderate peaks in the spring and summer seasons. This protozoan parasite is observed in the skin of *L. rohita* because the ectoparasitic in nature and the only protozoan parasite which was found throughout the study times.

In the consideration of sex-wise study, the compared to males **(Figs. 8, 9, 10, and 11),** which is fully satisfied with the previous study (Wahab *et al.*, 2021).

3.7 Age-wise study of parasitic variations

In this current study, it is shown that parasite infections are highly correlated with the age of fish. The prevalence of parasites was lower in young fish than in adult fish greater than six months (Fig. 12). These findings were fully satisfied with the previous study (Saha *et al.*, 2015).

3.8 Length-wise infections of parasites

The length of the fish is highly related to parasitic infections. It is revealed that lengthy fishes are highly susceptible to infections as compared to small fishes. Here, we concluded that the smallest fishes are significantly less susceptible to the infection than the other length groups and the length of the fishes are highly important for the study of parasitic study (Saha *et al.*, 2015) (Fig. 13,14).

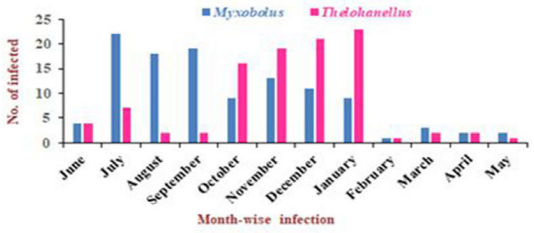
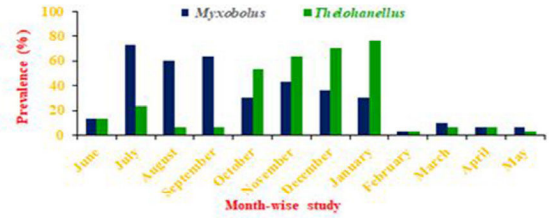


Fig. 4



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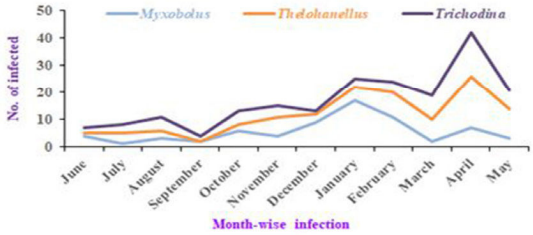

Fig. 6

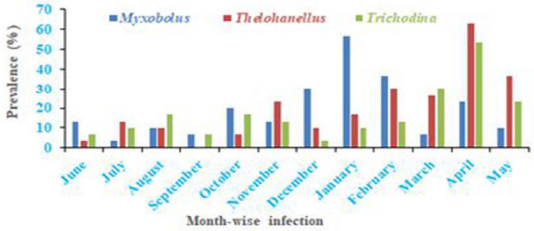

Fig. 7

Fig. 4: Shows month-wise infected individuals at different time scales in *Labeo catla*. Here, it showing a number of infections in fish of different months. In comparison with fig. 3, it shows that month-wise parasite infection is highest in the months of April and May. In fig. 2 months of July to January showing the highest number of parasitic observations but in another way **Fig. 5** Showing number of parasitic incidence highest in April and May as compared to other months. **Fig. 6,** Month-wise prevalence (%) rates is shown in *Labeo catla*. Here, the highest peak is observed in the month of July for *Myxobolus* infection. In the month of January showed the highest abundance of *Thelohanellus* infection as compared to other months in *Labeo rohita*. In **Fig. 7** *Myxobolus* infection is highest in January. The month of April shows highest rate of *Thelohanellus* infection and also *Trichodina* showing highest peak in April as compared to other months in *Labeo rohita*.

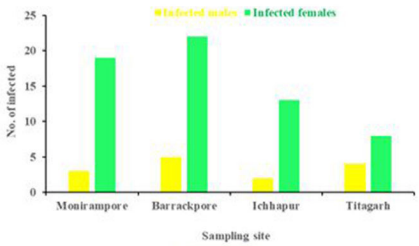

Fig. 8

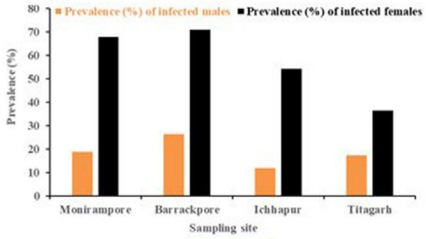

Fig. 9

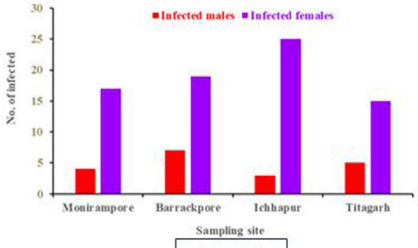

Fig. 10

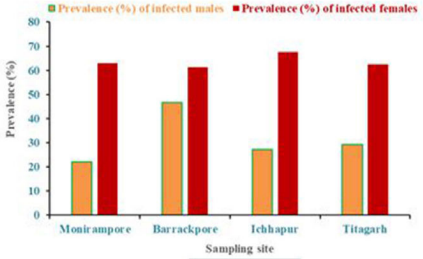

Fig. 11

In **Fig. 8**, diseased fish farms of the Barrackpore were the highest female (22) infections observed and lowest female infections were found in Titagarh (8). Highest male fishes' infection is observed in Barrackpore (5) and lowest infection is observed in Ichhapur (2) of *Labeo catla*. sampling site-wise female fishes of *Labeo catla* is showing highest number of parasitic incidences as compared to the male sex.

In **Fig. 9**, female fishes of Ichhapur (25) were observed highest number of infections and lowest number of infections is observed in Titagarh (15). Highest number of infected male fishes is found in Barrackpore (7) and lowest number of male infections is found in Ichhapur (3) of *Labeo rohita*.

In **Fig. 10,** highest prevalence rate of females is observed in Barrackpore (>70%) as compared to other sampling sites and lowest in Titagarh (>30%). In another way, highest prevalence rate of males is observed in Barrackpore (>20) as compared to other sampling sites and lowest is observed in Ichhapur (>11%) of *Labeo catla*.

In **Fig. 11**, highest prevalence rate of female fishes is found in Ichhapur (>65%) and lowest prevalence rate of female fishes 61.29%. Highest prevalence of male fishes is found in Barrackpore (>40%) and lowest observation is found in Monirampore (22.22%) of *L. rohita*.

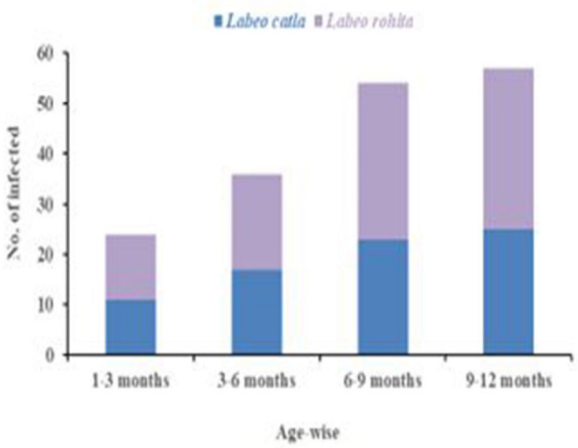

Fig. 12

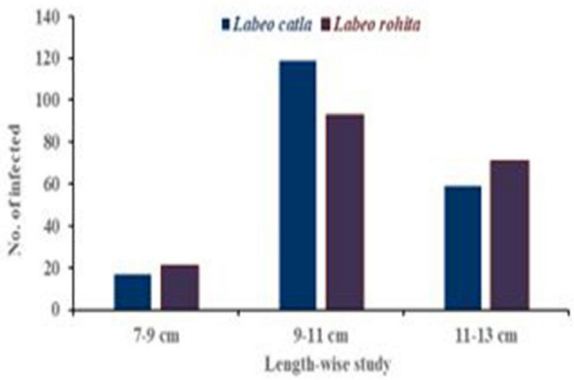

Fig. 13

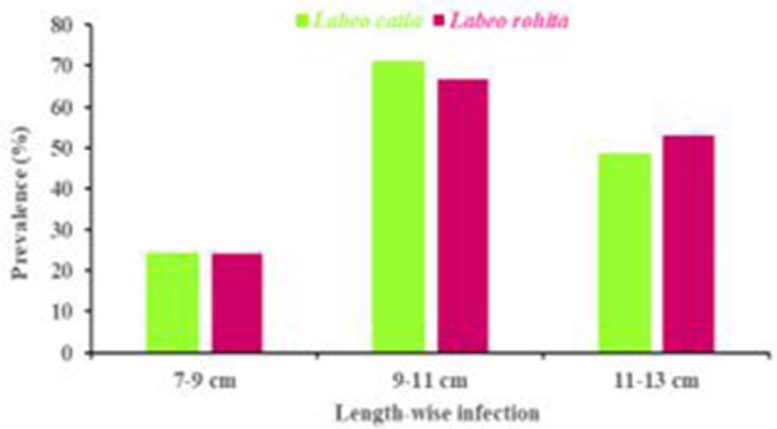

Fig. 12 In this graph comparison study between age-wise in *L. catla* and *L. rohita*. As per this study, the age group between 9-12 months showed the highest number of parasitic infections in both fishes.

Fig. 13 In this pictorial presentation, a contrasting study between ***L. catla*** and ***L. rohita*** regarding length-wise revealed abundance level of parasitic infections showed 9-11 cm fishes.

Fig. 14 In this figure showed the highest parasitic prevalence is observed between *L. catla* and *L. rohita* in the case of length-wise evaluation, 9-11 cm length fishes showed the highest peaks of prevalence in both fishes.

3.9 Water quality investigation

An overview of the water quality investigations were obtained in the sampled disease fish farms (Table 2) situated in different districts of West Bengal, India. These findings suggested that the presence of parasites can disturbs water quality.

Table 2. Water quality parameters of different diseased fish farms located in West Bengal, India

Parameter	Monirampore	Barrackpore	Icchapur	Titagarh
pH	8.10	8.08	8.23	8.01
Conductivitya	5.33	5.17	7.91	6.25
TDS	3.44	3.18	5.32	4.67
Alkalinityb	211	177	226	182
Hardnessb	232	169	252	195
Dissolved oxygenb	3.9	4.1	5.2	4.9
Ammoniac	0.446	0.592	0.645	0.539
Total phosphorusc	0.101	0.148	0.145	0.123
Temperature (°C)	27.7	28.1	29.7	29.2

[a]unit µs/cm; [b]ppm (Parts per million); [c]mg/l.

DISCUSSION

Myxozoan infection is a common parasitic disease of freshwater fishes that belongs to different taxa (Kaur and Attri, 2015). These infected fishes are unmarketable because of the presence of visible large cysts in the gill lamellae. As per guidelines of food hygiene regulations, those fishes unable for consumption (Betke *et al.*, 2001). Myxosporean

parasites show strong host-specific interactions (Molnár, 1994). Accumulation of toxic chemicals and water eutrophication with algal blooms contribute to poor water quality that creates stress factors and increases fish susceptibility to parasite stimulation (Coutant, 1998). The myxozoan and protozoan fish parasites are found most abundant from January to April when the temperature diverges between 19 to 27 °C. But, changes in months vary in high temperature reducing fish infections. In high temperatures, the life cycle of these parasites becomes hampered and remains in the dormant stage. In this current study, the parasitic prevalence is carried out in a temperature dependent manner and it occurs mainly during the winter season. While in the rainy season parasitic infection was observed in moderate amounts (Hossain *et al.*, 2008), and also environmental queues play a major role in their living purposes. The high prevalence of protozoan parasites can indicate such factors as environmental parameters like seasonality, fish behaviour and handling management in farmed fish (Florindo *et al.*, 2017). Stress conditions are caused by variations in the aquatic ecosystems that create parasitic infections along with host susceptibility and social status correlated with the fish specimens (Alves *et al.*, 20010; Gómez and Morgan, 2003). In the case of protozoan infection where concentrations of ammonia in the water were at higher levels (Florindo *et al.*, 2017). Due to the use of higher crude protein which increased nitrogenous substances in the water bodies (Florindo *et al.*, 2017). The highly diverse nutrients in the water can elicit the proliferation of protozoan parasites (Smallbone *et al.*, 2016).

Another approach the length and age of the fish play an important factor in varying the prevalence of parasitic infection in IMCs. Here, elevated levels of parasitic prevalence graphs are indicated in adults and large fishes as compared to the younger and smaller ones (Saha *et al.*, 2015) (Fig. 12). In this study, adult fishes are highly infested with parasitic infections as compared to young fishes. The age and length of the fishes are provided significant attributes for parasitic infections. Adult fish are having increased metabolic activity due to a high amount of food intake. These findings correlated with other finds (Bashirullah, 1973; Dogiel, 1961). Their findings also supported that, parasitism is associated with the age and food habits of the fish specimens.

The sex-wise study reveals that female fishes are highly infected by parasitic infestations as compared to males. Due to the strong immune systems in males which protect them from parasitic infections as compared to female fishes (Smith, 1969; Satpute and Agarwal, 1974; Sinha and Chakrabarti, 1984; Rajaiah, 1997). The host's sex has an important bearing on the regulation and recurrence of the parasites. The biochemical differences in the quantity and quality of the steroid hormone presumably present in male and female hosts may account for the differences in myxozoan and protozoan parasite infections in male and female hosts. Thus, it can be concluded that the influence of the seasons, host age, sizes, and sex play a significant role in the prevalence of *Myxobolus, Thelohanellus* and *Trichodina* sp. parasitic infections in *L. catla* and *L. rohita*.

CONCLUSION

In this current study, the winter season with the low water temperature, high level of dissolved oxygen, moderate pH and low hardness provides favourable environmental queues for ectoparasitic infections like *Myxobolus, Thelohanellus* and *Trichodina* sp. Therefore, water quality plays a major role in the increasing rate of pathogens and their capability to survive on the host. So, the concentration of fish seeds and water quality paradigm will be kept appropriately controlled to avoid parasitic infections in the fish hatcheries or ponds (Sinha, 2018). In this study, the addition of diverse key points which are majorly involved in the prevalence study of parasites. This study will help for future enthusiastic researchers who work in that field.

Declaration of Competing Interest

The authors have declared no conflict of interest.

Acknowledgement

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Effect of Dye-effluent on the Amino Acids of the Tadpoles of Rana Hexadactyla

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Abstract

Metamorphosing tadpoles of Rana hexadactyla were exposed to sublethal concentration of dye effluent for different periods. The prometamorphic tadpoles registered elevation in the levels of FAA and depletion of BAA. But the tadpoles in metamorphic climax stage showed abnormal increase in BAA, while the post metamorphic individuals exhibited rise in BAA and fall in the FAA content.

Keywords: Dye-effilent; Metamorphosing; Essential Amiono acid; Tadpoles.

INTRODUCTION

The widespread use of chemicals in agriculture forestry, hydroponics and soil melioration to augment production has inevitably resulted in the pollution of water bodies (Metelev et al, 1983).[6] Morever the industrial effluents are indiscriminately discharged into lentie and lotic systems.

Amphibians are the components of food webs in both aquatic and terrestrial communities (Porter and Mohanson 1976).[7] Mtamorphosis of frog takes place in fresh water bodies which in Madurai city, Tamil Nadu are fed up by the polluted Vaigai River. Exposure to polluted water results in accumulation by absorption through gills and skin in the case of amphibian tadpoles (Light, 1985)[4] thereby causing decline in frog and toad population across their range as suggested by Gibbs et al (1971).[2]

Regarding amphibians, studies on nitrogen metabolism in tadpoles during development alone were focused by Brown (1964)[1] and Weber (1967).[8] The effect of pollutant on amino acid metabolism in metamorphosing tadpole in totally neglected.

The present investigation is to study the impact of toxicity of sublethal concentration of dye effluent (0.009 ppm) on the levels of free amino acids (FAA) and bound amino acids (BAA) in metamorphosing tadpoles of Rana hexadactyla.

MATERIALS AND METHODS

Tadpoles (10 days old) of premetamorphic stage were collected from Narayanapuram Tank, Madurai transported quickly to the laboratory and kept in cement water tanks filled with tap water

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and provided with aeration for 10 days prior to experimentation. During the period acclimatization these tadpoles were fed with fresh leaves of Hydrilla and Nelumbo. Subsequently the water was renewed and excreta as well as unfed were carefully removed daily.

Dye effluent

Dye effluent was chosenfor the present investigation. The concentrated dye effluent was obtained from a reputed dye factory situated by the side of River Vaigai running through Madurai City. The names of chemicals and dyes which are found in the dye effluent are specified.

By appropriate studies it was found that 0.009 ppm was the sublethal concentration at which no mortality occurred for 45 days

Preparation of Experimental tissue

Tadpoles of different stages after being harvested were killed and kept in oven maintained at 50° C+ 2°C for 7 days. The dried materials were than thoroughly homogenized into powder and used later for amino acid analysis.

RESULTS AND DISCUSSION

Levels of FAA in healthy tadpole during development

In premetamorphic tadpole (40 days old) reared in untreated tap water at laboratory temperature for 20 days. The contents of FAA and BAA were found to be 16 mg and 13 mg respectively. In both pools all amino acids were found. In FAA pool, arginine, lysine, glutamate and tyrosine were higher in concentration where as in BAA pool, arginine, histidine, glycine, hydroxyproline and proline were more. The total AA content was 29 mg (Fig. 1)

In premetamorphic tadpole (55 days old) of control group the content of FAA and BAA were found to be 13 mg and 14 mg respectively with an accumulation of 32 mg of total AA when compared to the level in premetamorphic tadpole, reduction in the concentration of FAA from 16 mg to 13 mg accumulation in the content of BAA from 13 mg to 19 mg ; depletion in the concentration of almost all amino acids except isoleucine, leucine, glycine and hydroxyproline in FAA pool and conversely higher concentration of almost all amino acids except histidine, glutamate, glycine and proline in BAA pool were observed in the healthy prometamorphic tadpoles.

According to Brown (1964)[1] new proteins are formed in the course of development and differentiation to yield specialized structural entities and special proteins including enzymes. Since the total AA level is enhanced from 29 mg in premetamorphic stage to 32 mg in premetamorphic stage and depletion in FAA level and elevation in BAA level than in prometamorphic stage it is assumed that protein synthesis seemed to be slowly promoted causing diversion of FAA to BAA.

In the normal tadpoles of metamorphic climax stage, the FAA and BAA and total AA contents were found to be 13 mg, 16 mg and 29 mg respectively. Eventhough the FAA level was retained the BAA level was slightly depressed from 19 mg to 16 mg when compared to previous stage. That depression was mainly due to the lower concentration of

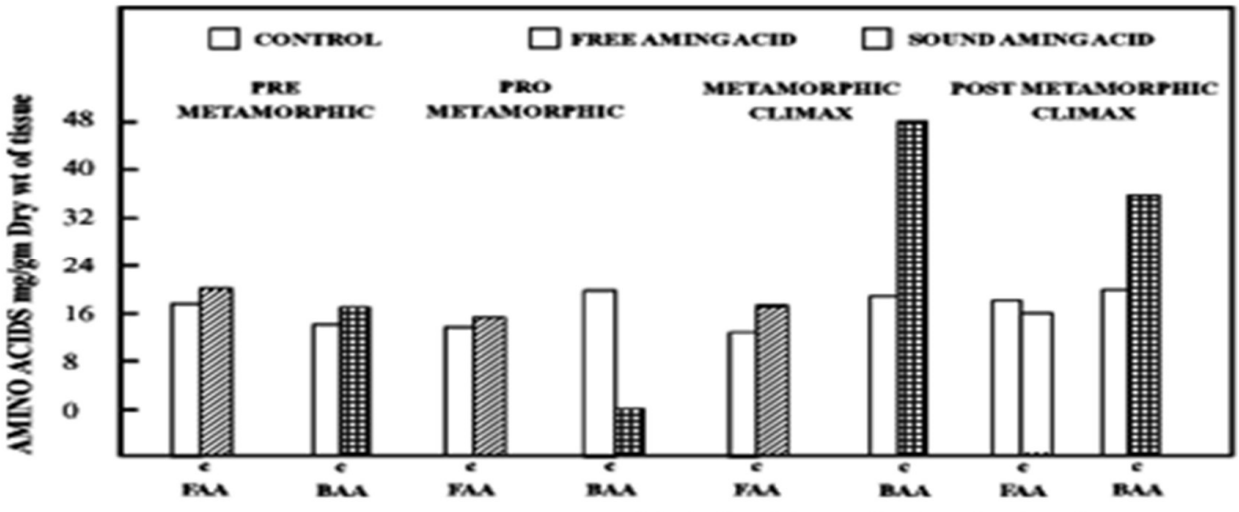

Fig. 1: Variations of the amino acid in healthy tadpoles during development

isolencine, leucine, lysine, methionine, theonine, trypophane, cysteine and tyrosine.

This is the period in which active regression and transformation are predominatly taking places. Weber (1967)[8] suggested that protein is degraded during metamorphosis. The lower level of BAA without increase in the level of FAA is suggestive of active proteolysis as suggested in BAA pool it is assumed that these amino acids may be routed to glucogenic path way via pyrurate or to kreb's cycle via intermediaries of kreb's cycle to generate energy.

In the post metamorphic stage, the newly emerged frog had registered higher concentr ation of FAA (16 mg) and lower content of BAA (11 mg) with a total AA of 27 mg when compared to previous stage. The FAA level was slightly enhanced because of higher concentration of almost all AA except valine, proline and serine with a reduction in BAA level which was due to depression in the concentration of almost all AA except arginine, leucine. Methionine, tryptophane, cysteine, hydroxyproline and serine.

According to Brown (1964)[1] coupled with proteolysis, the amino acids of the newly created pool may now be converted to postmetamorphic type of protein including new enzymes. Since BAA level is further depleted from 16 mg to 11 mg with a slight elevation in the level of FAA from 13 mg to 16 mg. It is suggested that protecolysis seemed to be continued even at this stage perhaps for the generation of amino acids which may be required for the synthesis of post metamorphic enzymes, as suggested by Brown (1964).[1]

Effect of Dye effluent of metamorphosing tadpole

When the prematamorphic tadpole (40 days old) was exposed to sublethal concentration of dye effluent for a period of 20 days. The toxic stress resulted in significant elevation in both FAA and BAA levels by 11% and 14% respectively (Fig. 2). Increase in the levels of FAA and BAA was effected by higher contents of EFAA (+13%) and NEFAA (+10%) in FAA pool and EBFAA (+16%) and NEBAA (+ 14%) in BAA pool. When compared to control the experimental tadpole had registred higher concentration of almost all amino acids in both pools except arginine, histidine, methionine, cysteine, glutamate and tyrosine in FAA pool and arginine, valine, glycine and proline in BAA pool.

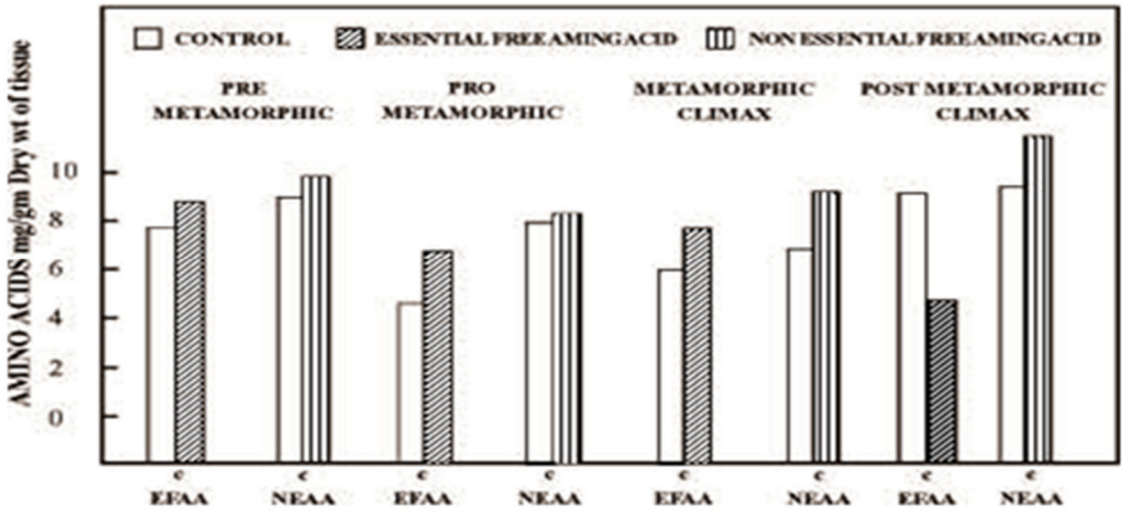

Fig. 2: Flucations of the amino acid in tadpoles exposed to sublethal concentration of the dye effluent

CONCLUSION

Subba Rao and Seyed Quadrin (1984)7 observed that toxicity of toxicant can deplete the levels of protein and amino acids and enhance the activities of proteolytic enzymes Similarly Madhusudhana Rao and Chari (1984)[5] reported that the toxicity of sevin in Lamellidens causes decrease in total protein and FAA Harris (1969)[3] also observed reduction in total FAA concentration in Isopoda due to exposure to 2% salinity. Exposure to pollutant elevates the basic metabolic rate to meet the energy requirements due to physiological stress detoxification and tissue repair. The depression in the level of BAA due to toxicity is suggestive of activated proteolysis which can bring forth accumulation of FAA. Since this

degradation of BAA is not seemed to be inversely related to increase in FAA only it is assumed that most of the AA in the FAA pool may be diverted to metabolic pathways whether to generate non protein metabolites via pyrurate to Carbohydrate or Acetyl – COA to lipid or to generate energy via TCA cycle.

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Role of Different N/P Ratios of some Manures and Phosphate Fertilizer on Bacterial Enzyme and Algal Growth as Eutrophication Control Strategy

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Abstract

The growth responses of green algae and blue green algae were estimated in small simulated eutrophic systems receiving either high N/P ratios maintained with some commonly used manures like Badam Oil Cake (BOC), Mustard Oil Cake (MOC), mixture of BOC and MOC (MIX) or high P/N ratios created by Single Super Phosphate (SSP). Prior to the main experiment a pilot study was carried out with different manures and fertilizers with algal rich pond water to select the suitable N/P ratios of the manures for the main experiment. Based on the results of the primary productivity of the pilot studies, treatments with varying N/P ratios of 60:1(BOC), 50:1(MOC) and 40:1(MIX) were set up in the main experiment along with N/P ratios of 1:20 and 1:40 with SSP. In both the experiments, pond water rich in mixed algal population was added to each jar. The bacterial enzyme activity in the MOC treatment played an important role as a driving factor ensuring the role of microbial heterotrophic pathway in controlling harmful algal bloom. The role of N/P ratio in shifting the food chain from blue green algae to benign algal food chain, *i.e.* green algae is evident from a better positive correlation between the N/P ratio with green algal growth rather than the *Microcystis* sp. bloom. So, the effects of N/P ratios of Badam Oil Cake (60:1), Mustard Oil Cake (50:1) and Mix (Badam Oil Cake + Mustard Oil cake) showed favourable conditions for the growth of green algae thus controlling the major eutrophication syndrome.

Keywords: Eutrophication; N/P ratio; Green Algae; Blue-green Algae; Organic Manures; Fertilizer.

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INTRODUCTION

Eutrophication as a result of pollution from point and non-point sources, possesses serious threat to environment and human being (Harper, 1992; Carpenter et al., 1998). This is specially due to nutrient enrichment of phosphorus (P) and nitrogen (N) in water bodies, more specifically P and N in freshwater and N in oceanic water (Le *et al.,* 2010; Zhou *et al.,* 2022). Tropical eutrophic waters often

experience cyanophycae or myxophyceae blooms, known as Harmful Algal Bloom (HAB) which is the accumulation or aggregations of planktonic blue-green algae at the surface of lakes and reservoirs (Chislock *et al.*, 2013). Because of warmer temperature, they multiply fast and form scum in the surface of numerous stagnant lakes, wetlands and ponds. Cyanophyta algal bloom causes depletion of water quality and serious health hazards of aquatic animals, humans and livestock (Hwang et al., 2020; Wang *et al.*, 2021; Wang *et al.*, 2022).

The growth of blue-green algae has been recorded year-round in some tropical lakes in Uganda (Lake George) and Ethiopia (Lake Aranguadi and Lake Kilotes), although in temperate locations, the production of bloom is seasonal, occurring in the summer when temperatures are high (Ganf 1974). It is common knowledge that cyanophytes spend the winter in the sediments at the bottom of bodies of water, either as dormant spores or as the source of new growth in the spring (Ho *et al.*, 2024). Similarly, for *Microcystis* sp., a successful overwintering is essential because the sediment offers an inoculum for population expansion in the spring (Verspagen *et al.*, 2005). Microcystis colonies were found to double in number after being resuspended from the top 80-100 mm of mud in Lake George (Uganda) by open-water algal blooms with a high concentration of thriving species. (Piehler *et al.*, 2009). Under this backdrop, it is most important to have a lake management plan for controlling eutrophication (Schindler, 1974; Moal *et al.*, 2021).

The release of organic matter in large amount from the decomposed bloom of cyanobacteria can be transformed into inorganic nutrients by bacteria (Wand *et al.*, 2021) which can be utilised by *Microcystis* sp. This decomposition of organic bloom can influence the composition of bacterial community (Shi *et al.*, 2017). The present study envisages to examine the primacy of nutrient manipulation on the growth criteria of Cyanophyceae using particularly organic nutrient manure. The extensive review of literature reveals that N/P regulation have a tremendous regulation on the growth of blue green algae (Plinski and Jozwiak, 1999). One of the big problems of the eutrophication is the wrong direction of food energy as a huge algal biomass or primary producers are not being used by the next trophic level consumers (Liu *et al.*, 2019) and therefore they are not directly linked with grazing food chain. One of the challenge is to direct this wrong pathway of food chain into right direction. In other words, it is possible to shift the food chain from blue green algae to benign algal food chain, *i.e.* green algae.

Therefore, the present study was undertaken to explore the possibility of shifting the food chain by nutrient manipulation with the view of understanding the shifting of the cyanobacterial algal bloom to a benign green algal growth. Due to lack of research in this discipline the experiment was undertaken. The novelty of the present study was to examine the role of organic manure as a nutrient manipulating agent to favour the growth of green algae.

MATERIALS AND METHODS

The present study was conducted in two phases:-

(a) Pilot study

(b) Main experimental trial

(a) Pilot Study

Experimental set-up: Prior to start of the main experiment a pilot study was undertaken to examine the responses of different organic manures and inorganic fertilizers to create an eutrophic condition. 1 litre of pond water was taken in each jar of 3.5 litres capacity in replicate which was treated with following 13 treatments (Table 1).

Table 1: Types and doses of treatments applied in the pilot study

Treatment	Type of manure/fertilizer	Quantity (gm/l)
T-1	Solid Urea	0.20
T-2	Mustard Oil Cake (MOC)	2
T-3	Neem Oil Cake	2
T-4	Badam or Peanut Oil Cake (PNOC/BOC)	2
T-5	Liquid Urea	2 (in ml/l)
T-6	Single Super Phosphate (SSP)	1
T-7	Mix-1:	BOC: 1
	BOC,MOC,SSP and Charcoal	MOC: 1; SSP: 0.5; Charcoal: 1

Table Cont...

T-8	Mix-2: BOC,MOC,SSP and Glucose	BOC: 1; MOC: 1; SSP: 0.5; Glucose: 1
T-9	Mix-3: BOC, MOC, Urea and Charcoal	BOC: 1; MOC: 1; Urea: 1; Charcoal: 1
T-10	Charcoal	1
T-11	Glucose	1
T-12	Tea leaves and Methi leaves	Not measured and given arbitrarily
T-13	Reference or Control	Nothing given

The whole set-up of the thirteen jars were kept under the sun till evening for the next nine days so that the sunlight can penetrate inside the water in order to create algal bloom which is an important indicator of eutrophication. The treatment water was kept stirring in every two days interval so that the nutrients do not settle down at the bottom. Different water quality parameters along with gross primary productivity (GPP), net primary productivity (NPP) and community respiration (CR) during the pilot study were measured following the standard protocol (APHA, 2021) on day 1 before treatment, and then on day 4, day 10. After day-10 the pilot study was terminated.

(b) Main Study

Experimental design: The main study was conducted in eighteen 3.5l plastic jars allotted to 5 treatments and one control in triplicate. The jars were filled with pond water containing dominant blue green algae. Green algae and diatoms were present to a lesser extent. The N/P ratios of the manures were selected based upon the result of primary productivity of phytoplankton in different treatments in the pilot study. The doses of different manure for N/P ratio selection was primarily based on approximate composition of manure. Since the manure is chemically composite in nature, the residual phosphorus content of the selected manure was not considered in the selection of N/P ratio of treatment. Finally following N/P ratios with higher and lower values of both nitrogen and phosphorus were selected and allotted to the five treatments

i) BOC- 60:1,

ii) MOC- 50:1,

iii) MIX of BOC and MOC - 40:1,

In case of SSP fertilizer the ratio was

i) SSP-1:20

ii) SSP-1:40

iii) a set of control.

Each treatment as well as control had 3 replicates. The manures were added during the beginning of the experiment, while second installment was applied on 17th day of the trial. All the jars were exposed to sunlight during the day and transferred to laboratory keeping under artificial light after sunset. The experiment was continued for 30 days.

Water Quality Analysis

The standard protocols of APHA (2021) were followed to examine the different physico-chemical parameters of water such as dissolved oxygen (DO), pH, conductivity, redox potential, total alkalinity, chemical oxygen demand (COD), dissolved organic-C, orthophosphate (PO_4-P), ammonium-nitrogen (NH_4-N), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N). Sampling was done at 5-10 days interval.

Plankton Analysis

Subsamples of plankton were collected from each jar on day 4, day 8, day 13, day 24, day 29 and analyzed for both qualitatively and quantitatively (APHA, 2021). The planktons were categorized on the dominance of Chlorophyceae and Cyanophyceae and remaining few species were expressed as others.

Isolation of bacteria

All the routine procedures were followed (sterilization of glassware, media preparation, inoculation of sample and incubation) for culture of heterotrophic bacteria (HB) and phosphate solubilizing bacteria (PSB). Screening for microbes was done in specific medium in order to isolate the tested groups of microbes.

Quantitative assay of bacterial enzyme

The bacterial strains were individually introduced into nutrient broth within Erlenmeyer flasks and incubated for 24 hours. This is followed by centrifugation of the cultures at 10000 rpm for 15 minutes. The resulting pellets were washed twice with 0.85% saline solution and resuspended to achieve a final optical density of 0.100 at 600 nm.

The resuspended supernatants were utilized for the assessment of extracellular phosphatase activity. Acid and alkaline phosphatase activities were assessed by combining two milliliters of reaction buffer (citric acid/sodium-citrate buffer, pH 5.0 for acid phosphatase evaluation and glycine-NaOH buffer, pH 10.0 for alkaline phosphatase evaluation) with 500 µl of p-nitrophenyl phosphate solution (0.115M pNPP in diethanolamine), followed by the addition of 2 ml of supernatant. The reaction mixtures were then incubated at 37°C for 90 minutes and halted by the addition of 500µl 0.5 M calcium chloride and 2 ml of 3M NaOH. One unit of activity was defined as the quantity of enzyme capable of hydrolyzing 1 µM PNPP per minute. Distilled water was employed in place of supernatants for the preparation of blanks. The hydrolysis of p-nitrophenyl phosphate was quantified by measuring the concentration of p-nitrophenol using a spectrophotometer at a wavelength of 410 nm. The concentration of p-nitrophenol was determined by comparison with a standard curve. The quantity of enzyme (mg) necessary to release 1µmole of p-nitrophenol per minute signifies one unit of enzyme activity.

The acid and alkaline phosphatase activity was calculated using the formula:

Enzyme activity (U/mL) = {OD_{410} nm x 1/0.0208 x V (mL) }/ ε x incubation time (min) x E (mL)

V = Total reaction volume (in milliliters) of assay

1/.0208 = Dilution factor of pNP from standard graph

ε = 18.5 millimolar extinction coefficient of p-Nitrophenol at 410 nm

E = Enzyme reaction volume (in milliliters) used

Statistical analysis

One-way analysis of variance (ANOVA) was done with the help of computer software SPSS (Version 7.5) at 1% and 5% level of significance to find the treatment differences on all days of observation. Standard Error values have been provided for each mean value. Correlation study was done in MSExcel to determine the relationship between the variable observed parameters.

RESULTS

(a) Water Quality

(i) Pilot Study

High primary productivity was recorded from the treatments of MIX-3, MIX-1, BOC, MOC. This formed the basis of the final selection (Table 2) of the manure in the main experiment of the study.

Table 2: The Primary Productivity Table of Pilot Study

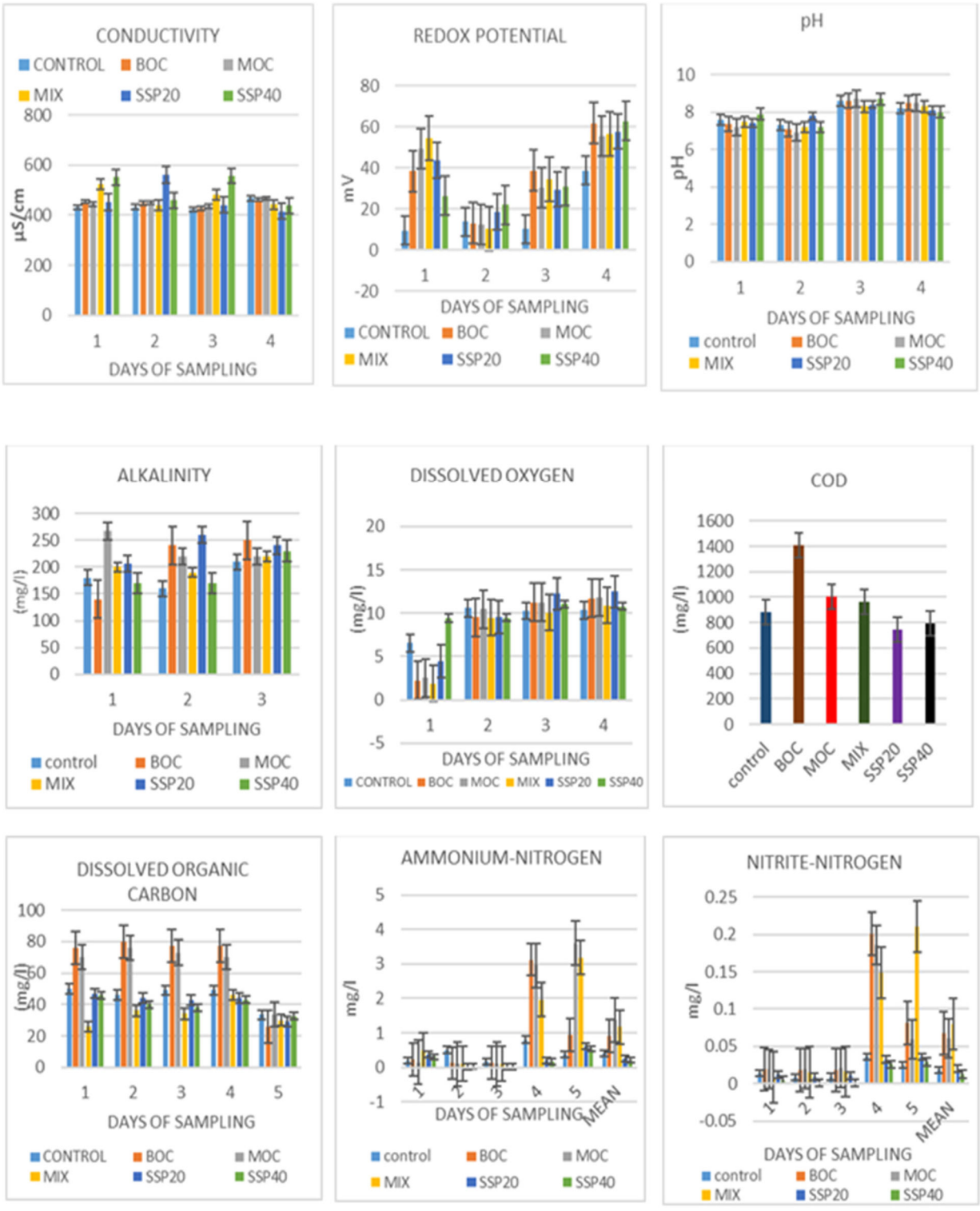
Treatment	NPP (mgC/l/hr)	GPP (mgC/l/hr)	CR (mgC/l/hr)
Solid Urea	0.2	0.389	0.189
Charcoal	0.589	1.369	0.78
Reference	1.2	2.2	1
Mustard Oil Cake	2.42	5.22	2.8
Badam Oil Cake	4.2	12	7.8
Mix-3	7.8	18.2	10.4
Mix-1	12.2	25	12.8

(ii) Main experiment

Conductivity of water ranged from 422.3µS/cm- 523.6 µS/cm in different treatments. One-way analysis of variance carried out on each day which showed a clear-cut differences among the treatments (ANOVA $F_{5,12} \geq 4.873$, $P<0.05$). The highest values differed on different dates of observation. pH of the water ranged from 7.1-8.6. There was no significant difference ($P>0.05$) between the treatments. Redox Potential of water ranges from 9.5 mV- 62.9mV in different treatments (ANOVA $F_{5,12} \geq 109.4$, $P<0.05$). The ranking in values in each treatment was not same on all days of observation and increased considerable towards the end of the experiment (Fig. 1).

Significant treatment differences were observed for PO_4-P ($F_{5,12} \geq 79.54$; $P<0.05$) with the concentrations in BOC, MIX and MOC treatments remained lower than that in the SSP treatments. The concentration of phosphate decreased by 34.22% at the end of the experiment from the 1st treatment. The values peaked in SSP20, SSP40 and MIX after 2nd treatment. The concentration of ammonium-nitrogen ranged from 0.014mg/l to 3.598 mg/l in different treatments employed. The values were consistently higher in MIX and MOC and lower in SSP20 and SSP40 ($F_{5,12} \geq 59.154$; $P<0.05$). After 2nd treatment the values of ammonium-nitrogen peaked. Nitrate nitrogen was entirely absent during the entire period of experiment. Although initially in the pond water it was present at concentration 0.351 mg/l. DO increased sharply on the 4th day of observation showing prominent treatment differences (*ANOVA $F_{5,12} \geq 14.15$, $P<0.05$)* with high values ranging from 14-18 mg/l in MIX, BOC and MOC treatments (Fig. 1).

All the treatments were contributed mainly by chemical oxidation of organic substances rather than biological oxidation since BOD was either negligible or undetected from the samples. The average value of COD was high in BOC followed by MOC and MIX (ANOVA: $F_{5,12} \geq 38.76$: $P<0.05$). Organic carbon of water ranged from 34.4mg/l – 67.2mg/l in different treatments with clear cut differences of treatments on all the days examined ($P<0.05$). The value decreased considerable at the end of the experiment. Alkalinity of water ranged from 140mg/l – 267mg/l in different treatments ($F_{5,12} \geq 5.17$: $P<0.05$) (Fig. 1).



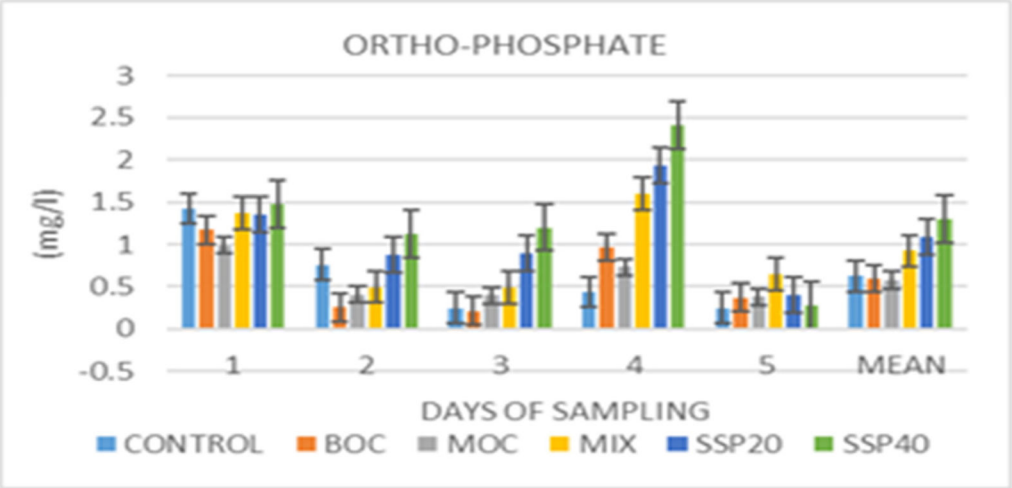


Fig. 1: Results of the different water quality parameters (mean ±SE values) observed on different days of sampling.

(b) Plankton analysis

(i) Qualitative Analysis

The genera present in water of Blue green algae and Green algae (Table 3).

Table 3: Genera of Blue green algae and Green algae found in the water samples.

Genera of Blue Green Algae	Genera of Green Algae
Microcystis sp	Chlorella sp
Oscillatoria sp	Coelastrum sp
Merismopedia sp	Scenedesmus
Anabena sp	Dictyoshaerium sp
	Pediastrum sp
	Ankistrodesmus sp

(ii) Quantitative Analysis

Microcystis Colony Growth

Microcystis Colony ranged from 114 count/l – 3543 count/l in different treatments. One-way analysis of variance carried out on each day showed that the clear cut differences of treatments in all the days examined (ANOVA: $F_{5,12} \geq 1.93$: $P<0.05$). Among the treatments the counts remained quite high on most of the days of observation in BOC, MOC and MIX. Although, differences among treatments were significant on different days of observation, overall mean values for each treatment were not different from some of the treatments to other for example, the overall mean of MIX (913 Count/l) and SSP20 (914 Count/l) respectively (Fig. 2).

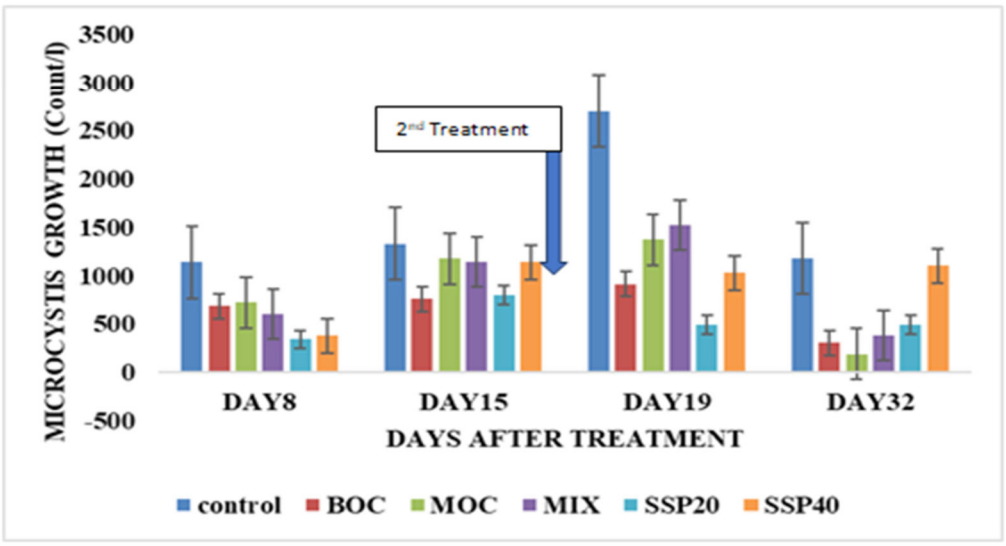


Fig. 2: Treatment wise growth of *Microcystis* sp. (Mean count ±SE) on different days after treatments

Green algae Colony Growth

Green algae ranged from 9240 count/l –69020 count/l in different treatments with significant differences among the treatments on all days of observation (ANOVA: $F_{5,12} \geq 11.12$: $P<0.05$). During the later half of the experiment the population of green algae in BOC and MOC increased sharply in comparison with that in other treatments (Fig. 3).

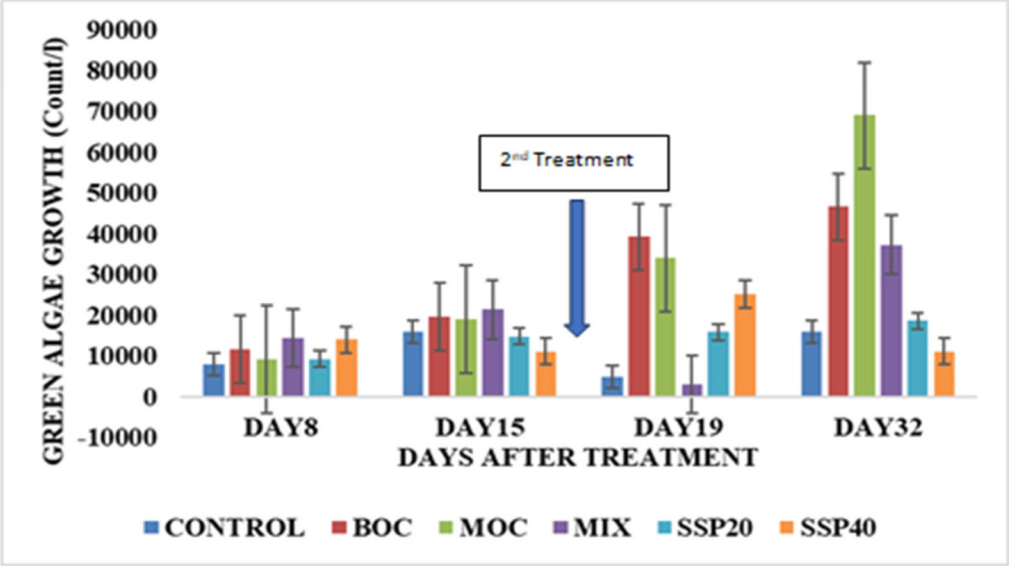


Fig. 3: Treatment wise growth of green algae (Mean count ±SE) in different days after treatments.

(c) Bacterial Enzyme

(ii) Alkaline Phosphatase Enzyme Activity

Initially, from the initial pond water the alkaline phosphatase activity was shown by 3 PSB isolates showing the maximum value of 27.5077 U/ml and lowest value 20.1732 U/ml.

After 1st treatment, each PSB isolates from the treatments SSP20, SSP40, MOC and MIX, the highest value was shown by PSB isolate of treatment MOC (47.747 U/ml) and lowest value by PSB isolate of treatment SSP40 (8.103 U/ml). After 2nd treatment, each PSB isolates from the treatments SSP20, SSP40, MOC and MIX; the highest value was shown by PSB isolate of SSP20 (30.571 U/ml) and lowest value of 17.295U/l by MOC treatment isolate (Table 4)

Table 4: Maximum alkaline phosphatase enzyme activity of different bacterial isolates from the treatments.

Sl. No.	Treatment	Bacteria Group	No of Isolates	Maximum Enzyme Activity (U/Ml)	Day of Maximum Activity
1	Initial	PSB	1	20.544	2nd
2	Initial	PSB	2	20.173	3rd
3	Initial	PSB	3	27.508	4th
Treatment 1					
1	SSP40	PSB	1	8.103	3rd
2	MOC	PSB	1	47.747	3rd
3	MIX	PSB	1	13.024	2rd
4	SSP20	PSB	1	25.372	3rd
Treatment 2					
1	SSP40	PSB	1	20.173	2nd
2	MOC	PSB	1	17.295	3rd
3	MIx	PSB	1	24.908	2nd
4	SSP20	PSB	1	30.571	3rd

(iii) Acid Phosphatase enzyme activity

Initially, from the initial pond water the acid phosphatase activity was shown by 3 PSB isolates showing the maximum value of 66.594 U/ml and minimum value 49.047

After 1st treatment, each PSB isolates from the treatments SSP20, SSP40, MOC and MIX, the highest value was shown by PSB isolate of MIX (57.309 U/ml) and lowest value of 42.746 by SSP20 treatment isolate.

After 2nd treatment, each PSB isolates from the treatments SSP20, SSP40, MOC and MIX, the highest value was shown by PSB isolate of SSP40 (50.068 U/ml) and lowest value of 37.813 U/ml by SSP20 treatment isolate (Table 5).

Table 5: Maximum Acid phosphatase enzyme activity of different bacterial isolates from the treatments.

Sl. No.	Treatment	Bacteria Group	No of Isolates	Maximum Enzyme Activity (U/Ml)	Day of Maximum Activity
1	Initial	PSB	1	63.159	3rd
2	Initial	PSB	2	66.594	4th
3	Initial	PSB	3	49.047	3td
Treatment 1					
1	Ssp40	PSB	1	45.423	4th
2	Moc	PSB	1	48.675	3rd
3	Mix	PSB	1	57.309	3rd
4	Ssp20	PSB	1	42.176	3rd
Treatment 2					
1	Mix	PSB	1	37.813	
2	Moc	PSB	1	44.869	
3	Ssp20	PSB	1	20.173	
4	Ssp40	PSB	1	50.068	

DISCUSSION

Green algae vs Phosphatase enzyme activity

Both the acid and alkaline phosphatase enzyme do not clearly show any inducing correlation (r^2=0.0877; 0.0436) in the growth of the green algae but has selected a favourable growth of the green algae within the range of 1000-1500 which occurred in the treatments of BOC, MOC and MIX (Fig. 4)

Since the plankton growth is limited by the nutrient availability, the interaction between the grazing and heterotrophic pathways being influenced by microbial enzymatic degradation has finally limited the enzyme activity within an optimum organic matter availability resulting in favorable phosphorus concentration for green algae growth. This interaction is mostly based on close nutrient complementary relationship between algae and the bacterial population which might provide scientific support for the research of *Microcystis sp.* bloom control (Cao *et al.*, 2016)

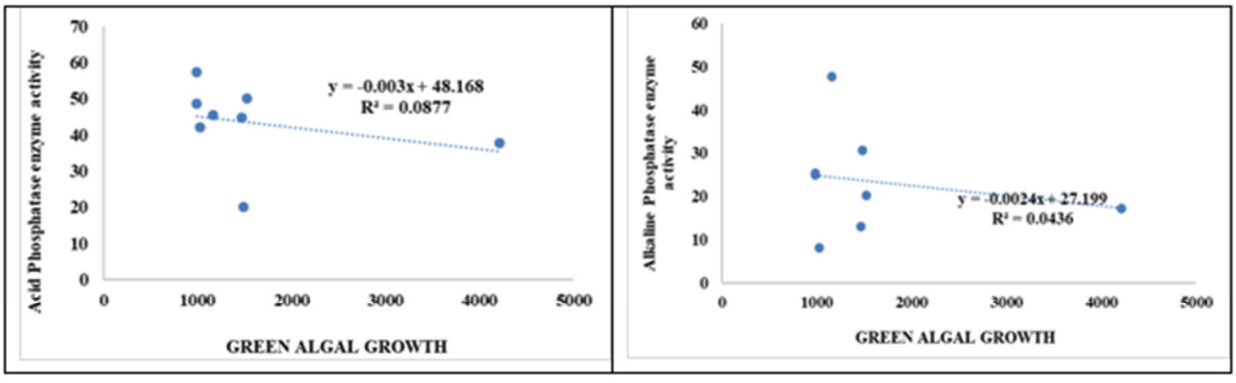


Fig. 4: Correlation between Green algae and Acid and alkaline phosphatase enzyme activity

Studies have found that during the decomposition of single bacterial bloom, large amount of deserved enzymatically hydrolysable phosphorus is released which can be hydrolysed by extracellular alkaline phosphatase producing bacteria can inhibit or promote bacterial growth as well as the alkaline phosphatase activity. PhoX gene in alkaline phosphatase can be used as biomarkers during Microcystis bloom decomposition (Shi *et al.*, 2017).

Therefore, the organic manipulated treatment has favored the maximum growth of green algae within a favourable phosphate range which is being influenced by the bacterial phosphate mineralization metabolic pathways mediated by phosphatase enzymes.

Green Algae vs Phosphate

In low phosphate concentration, green algae population is high. This indicated moderate nutrient concentration is suitable for maintaining the green algae population, this indicates good ecosystem health of the aquatic body showing poor eutrophication syndrome.

Therefore, Badam Oil Cake, Mustard Oil Cake treatments are suitable for favoring the growth of green algae and can be considered as a valuable organic nutrient manipulated eutrophication control strategies (Fig. 5).

Green algae vs N/P and P/N Ratio

The abundance of green algae in all the treatments was directly related to N/P or P/N ratio of water samples regardless of treatments. The relationship was more strongly related when nitrogen was more predominant compared to phosphorus The relation between N/P ratio and green algae has shown a better positive correlation (r^2= 0.7227) (Fig 6A) than the P/N ratio (r^2= 0.5127) (Fig. 6B). Thus N/P ratio has favored the green algae in treatments of BOC and MOC as well as the mixed combination. This implied that green algae had a preference for nitrogen rather than phosphorus in eutrophic conditions.

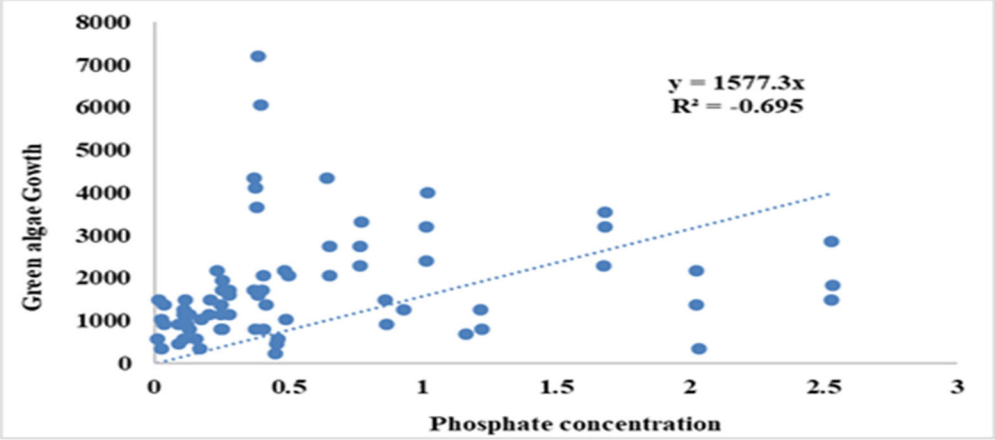


Fig. 5: Correlation between Green algae and phosphate

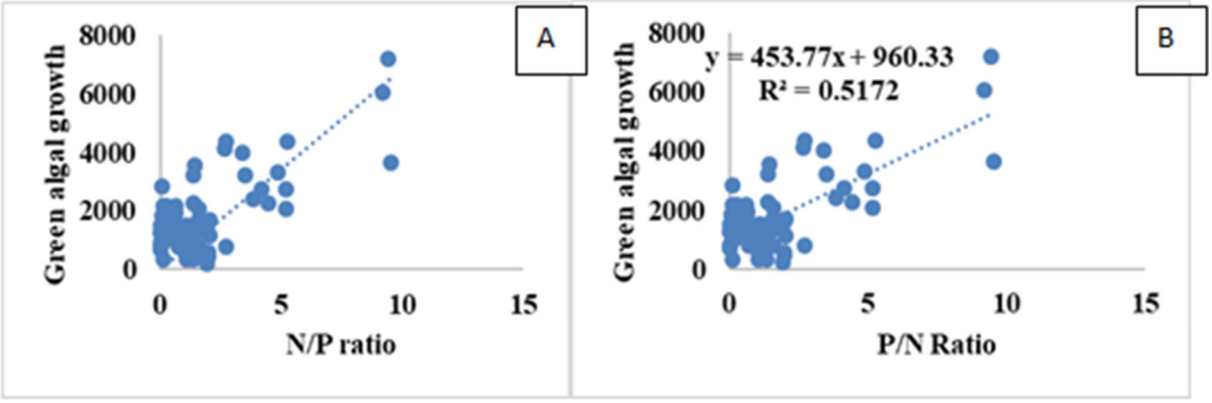


Fig. 6: Correlation between Green algae and (A) N/P ratio (B) P/N ratio

Microcystis vs N/P Ratio and P/N Ratio

The relationship between the Microcystis count and N/P (R=0.0533) (Fig. 7A) or P/N (r^2= 1.76) (Fig. 7B) of water revealed poor correlation. Since, addition of phosphorus or increased in concentration of phosphorus to nitrogen in water is likely to inhibit the growth of blue green algae. More nitrogen than phosphorus was inhibitory to Myxophyceae (Smith, 1983) as well as *Anabaena plantonic* (Wood *et al.*, 2010) at ratio more than N/P 29:1.

In present study, the blue green algae favored the low nitrogen and high phosphorus conditions according to its correlation of growth with ortho-phosphate. The growth of green algae, on the other hand was favored by more nitrogen than phosphorus concentration in water created as a result of nutrient manipulation by organic manure introduction.

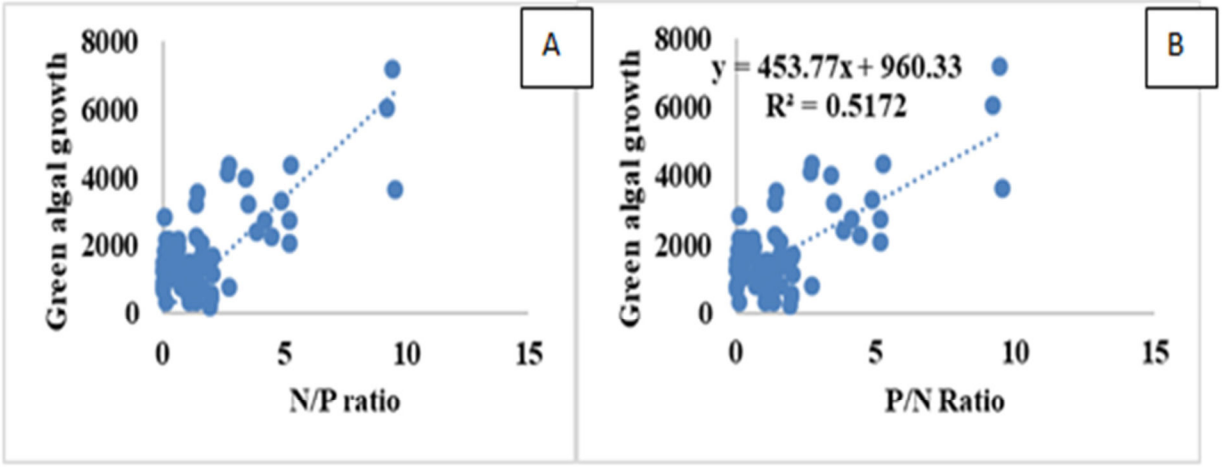


Fig. 7: Correlation between Microcystis growth and (A) N/P ratio (B) P/N ratio.

CONCLUSION

Bacterial enzymes did not play any specific influencing role in shifting the microcystis bloom to green algae population as a result of organic nutrient manipulation but due to interplay of different driving factors based on dynamic interactions between the grazing and heterotrophic pathways, the microbial enzymes have selected a favorable zone for optimum green algae as well as blue green algae growth. The bacterial phosphatase activity in the MOC treatment indicated a role in regulating N/P ratio by their metabolic adjustment for modifying the phosphorus interactive pathways that has driven the growth of Chlorophyceae, although the activity was maximum in SSP treatment. Thus the microbial heterotrophic pathway played a major driving factor in controlling harmful algal bloom.

The relation between N/P ratio and green algae has shown a better positive correlation than the Microcystis bloom. Thus N/P ratio has favored the green algae in treatments Badam Oil Cake, Mustard Oil Cake and the mixed combination of Badam Oil Cake and Mustard Oil Cake. So, the effects of N/P ratios of Badam Oil Cake (60:1), Mustard Oil Cake (50:1) and Mix (Badam Oil Cake + Mustard Oil cake showed favourable conditions for the growth of green algae. Therefore, self-designing of an eutrophic system can be enhanced by nutrient manipulation towards shifting the food chain in a right direction from harmful algal bloom to benign algal bloom.

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Studies on Impact of Insecticides to Indian Honey Bee, Apis Cerana Indica, Fab (Apidae: Hymenoptera) A Survey

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Abstract

Insecticides use, considered as one of the common practice in local farming system, cause detrimental effect on agriculture environment and bio diversity subject. (celli *et al* 2003)[3] Besides that residue sprays on pesticides could also make unintended possible effect to honey bee andother Pollinators. (Stokstad, 2007)[5] So, pollinators have great impact on plant productivity. Because of this main issue to keep the pollinators in healthy and high diversity for sustainable of environment, food and environment. (Celli *et al*, (2003)[3], (Neumann, *et al*, 1994).[55], The field observation a recorded in agriculture crops and laboratory test recorded the effect of common insecticides to mortality and behaviour of honey bees. 96% pesticides traded were applying by our farmers, besides there were 13 active ingredients of fungicides 15, insecticides and 1 herbicides, 1 Molluscuide, they are using twice a week during crop cultivation; 60% of farmers mentioned the presence of honey bees on there land Farmers knowledge in use of insecticides (sethuraj, 2004)[10] is Apporoaiate and safe for bees and farmers when applying insecticides in there field, forager bee move to flowers as the preference (Allen *et al* 1998)[4] to insecticides was 12.5% and 0%, 4.2% mortality test showed LD 5o value O. O1√g/√l insecticides rate is 0.031 and 0.09, which much lower than suggested dosage recommended br insecticides producer. (Said *et al* 2013).[78] This research conculde, that the use of insecticides could lower the pollination service.

Keywords: Apis cerana indica; Behavior; Insecticides pollen; Pollinator; Preference; Lethal dose.

INTRODUCTION

Pesticides including herbicides, insecticides and fungicides are commonly used to prevent or damage pests (Velthuis, 2016)[20], such as weeds, insects and plants pathogens while reducing the amount of labour (Beekman *et al*, 2010)[7], winston, 1991) 60 fuel and machinery used for pest control. Bees had been considered as the premionent t and economic cally most important groups with 35% of the world Food crops productions depends on pollinators by honey bees and more group of insects sigh, 1943) 73 (Van Engeldrop, 2010).[19] In Europe animal pollination could influence the production of 84% of crop species, like honey bees also important pollinators service to wild plants, while they also provide human society by producing various products, honey bees (pests, pathogens, chemical pesticides potts *et al*, 2010) 76 (Haynes 1988)[8] GM crops resource availability habitat Fragementation).

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Therefore it is necessary to maintain the pollinators especially in areas that have large farming field of pollinators depends crops such as mango melon, pumpkin, chilli coconut, carrot, Prini Jal, cabbage, Cauliflower, soya beans, chilly, (Beekman *et al*, 2010)[9]

There are atleast 5 common honey bee species Apis Anderiformis, Apis dorsata, Apis florea, Apis nigracincta, Apis cerana indica, Apis Mellifera. Among them Apis cerana indica is common bees kept by local bee farmers and usually located near agriculture area (Alkinson, 2005)[56], Kubik, 1999)[51], Challa *et al* 2019).[54]

Many agriculture countries the usage of pesticides is common practice is save around 1/5 of total production from pest attack. However in order to create an insurance feeling, the local farmers usually applied higher dosage than recommended dosage. This condition create greater ti honey bee colonies as they were under costant insecticides exposure that may alter (deleterious) behavior and health of colonies, this research consist of couple of parts 1) field observation about Vistiuation rate of honey bees to crops and wild plants 2) Laboratory Test to find the impact of insecticides to direct bee mortality and there behavior.

MATERIALS AND METHODS

This study was divided in to different steps as follows, Preliminary survey; to determine representative location of study, area maruthamalai Lord murga temple hill aera, and also in an around village area coimbatore, Tamilnadu, South India.

Interview of pesticides use the activity was performed to access pesticides use by farmers. The materials used were as follows GPS, Global positioning system, questionnaire, cameras, stationary and other tools Data were collected and then descriptive analysis was performed (Celli *et al*, 2003)

Sampling area and field observation; Field observation was conducted during active period of honey bee 08; 00 to 15; 00AM, the observation and recorded number of bees visitation and plant species were recorded, the activity was carried out for 30 days (Feb 01 to March 03-04- 2023). In order to find type of pollen collected by forager of honey bee, returning forager and kept them inside vial with alcohol 70%, pollen collected and measured by Haemacytometer with formula (AAAS, 2013).[81]

Pollen = Σ A B C D ×2500×df

ABCD = Number of pollen inside four counting chambers

df = dilution factor (in the research dilution factor was calculated was 2)

Survey of Pesticides use

Data regarding on pesticides use was obtained by interview ing the farmers directly in the field, (Frazier, *et al*,2008)[86] Data collected were education background, crop they cultivated pesticides application types of pesticides dose, application, intervals knowledge of farmers related pesticides and farmers perception regarding on the presence of honey bee in the field.

Toxicology and Behavior Test; Relationship with Data Analysis

900 nine hundred Forager Bees were kept in side the bee cub created from plastic cub (lid diameter 8.2 CM bottom diameter 5.8 CM and heights 12 CM during laboratory test, each cub hold 20bees, they feed with 80% of honey and all container kept inside room with average daily temperature 25 C degree temperatures, humidity 60- 80% and photoperiod 12; 12. Like three different types of insecticides were used in this test Active Ingredient 50g/l, active ingredient Profenofos 500g/l : active ingredient Chorotanilprol 100g/l and cyhalotrin 50 g/l. The purpose of the study to find LD50 0f each insecticides to honey bees : also applied bee by microsyringe while adequate applied to throax of control group of honey bees, followed that number of mortality was observed each for 24 hours (feal *et al*, 2011)[94]

Behavioural Test

Impacts of insecticides to behaviour of foragers bees standard Y tube used in this test. Each test used visted cornflower applied with and without insecticides rate was measured (winfree, *et al*, 2007)[98]

Data Analysis Was measured by probit analysis using computer programming POLO PC. Difference among data was tested by t-test with confidence value P value is $P < 0.05$

RESULT AND DISCUSSION

During this study we found that honey bees visted various types of flowers around hive including agriculture crops, plants of resident garden, wild plants, and forest plants (Babacan, 2007)[14] However observation on crops plants showed honey bees

prefer some species than others such as zea mays, capsicum frustescens and cucumis sativus of crop play (Currie, 1999)[15] Based on results on preference of honey bees to crops found that even though bees shows less restrictions, (Al zan *et al*, 2009)[101] this study found that all of most preference plants are located near agricultural field. Also, this research found during dry season that caused great stress as the food and water resources dwindling (Al walli 2004)[13], colony which lead to absconding behaviour response when all colony members for better nest, due to Availablity of nector while crops plants only provide pollen as reward for honey bees (Gouldin *et al.* 2008).[18] Nector is important resources to maintain energy storage in the nest as they change in to honey stock. (Damalaas, 2009).[100]

Farmers Perception for the Presence of Honey Bees in the Field

The results of the interview, showed that 60% of farmers said that the presence of honey bees on their field was abundance (Al Naggar, *et al*)[12], zolalancloumn, 2016)[50] as general information about honey bees from interview showed that Apis cerana indica was more plenty than Apis Mellifera, and Apis dorsata. It is likely that land covered by large forestry aera had much honey bee population. (Willam, 1994)[2], Robinson, 2000)[31], Holy, 2013).[34] During rainy season honey bees were much easier found in the field (Goulson, 2008)[29], while during the during the dry season they could be found also in many agriculture crops (Dinter, 1971)[45], when it rain honey bees will stops foraging and will only stay in the hive, (Evan *et al*,2018)[16] but in the dry season they will gather water to keep he colony cool (pokhrel, *et al*,)[11]

CONCLUSION

We conculde that, the safest compound, honey bee APIs cerana indica, insecticides acetamiprid, mostly suggested for use in crop blooms during insect infestation (cox-Foster 2009)[25] without impacting honey bees. Pesticides are ingested not only by the forager (Alphey, 2002)[35] (honey bees) but also hive bees / larvae who feed on nector and pollen held in honey comb, as a result pesticides impact /effect cause affect the various levels of honey bee (mklein,2007).[1]

Wide variety of pesticides such as insecticides, herbivores, fungicides as well mixed formulation of fungicides and insecticides (Sethuraj, 2004)[10], yang *et al*, 2008).[39] The majority of respondent reported that they usually apply herbicides insecticides and fungicides at least twice during crop season. (Lawrence *et al*, 2007)[21], xaiver, 2020)[42] Al though some farmers may be aware of pesticides hazards but adequate protection is hardly taken minimize risks., (le conte; 2008)[23] further confirmation on abundance and diversity of pollination using most insecticides applied that found from this survey should be performed.

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Carbon Capture and Storage Usage in India to Combat CO_2 Reduction: A Review

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Abstract

An enormous threat to world health and security is posed by the extraordinary rate at which the Earth's atmosphere is changing. Anthropogenic carbon production and natural carbon absorption systems were in equilibrium prior to the industrial revolution. Nevertheless, with an increase in anthropogenic carbon emissions of between 15% and 40% during the industrial revolution, the situation altered. In 2030, coal will still provide for over 60% of India's energy needs, despite the increasing emergence of alternatives like solar and wind. The Earth's atmosphere is changing at an unprecedented rate, posing a huge danger to world health and security. There is broad scientific agreement that human actions, namely how we transform and utilise fossil fuel energy, are to blame for growing CO_2 concentrations in the atmosphere and climate change. Finally, in terms of carbon dioxide emissions, it appears that CCS is currently one of the best available technologies for drastically reducing greenhouse gas emissions from certain industrial processes, and it is a key technology option for decarbonizing the power sector, particularly in countries where fossil fuels are heavily used in electricity production.

Keywords: CO_2 concentrations; CCS; GHG emission; Sequestration; Climate change.

INTRODUCTION

During the next 20 years, it is anticipated that the world's energy demand will climb by 50%, and by 2050, there will be an additional 1 billion people on the planet. 26% of global warming is caused by the atmosphere's current CO_2 content. On the other hand, it is anticipated that the world's energy needs will quadruple by 2030, with the bulk of those needs being satisfied by fossil fuel sources due to their affordability and the availability of existing infrastructure. Just 13% of our overall energy supply comes from renewable sources, with fossil fuels (coal, gas, and oil) making up 80% of the world's energy mix. 52% of the current global CO_2 emissions (15 billion tonnes CO_2 emissions/year) are produced by fossil fuel power plants, heavy industries, and refineries, which leads to climate change. By 2050, emissions must be reduced by 50 to 80 percent from 1990 levels in order to meet the target set forth in the Paris Agreement. As of 2021, there were 27 large-scale carbon capture and storage plants in operation throughout the world. There are 17 CCS operations that are now in operation

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throughout the globe, collecting 31.5Mt of CO_2 annually, of which 3.7 Mt is stored geologically. In order to reduce the cost of collecting and determine the potential for carbon dioxide storage in geological reservoirs, additional research and development in the capturing technology is required. Global CO_2 emissions from energy increased by 0.9% or 321 million tonnes, in 2022, hitting a record high of more than 36.8 billion tonnes. The increase in emissions was far slower than the world economic growth rate of 3.2%. According to IEA estimates, India's total emissions from energy usage in 2018 were 2,251 Mt CO_2 (metric tons of carbon-dioxide). Power generation and industry contributed 53% and 25% of total emissions, respectively. This was followed by transportation and residential sources, which contributed 14% and 4% of total emissions, respectively. To solve the issues that CCS faces in India, an ecosystem supporting CCS facilities in the Indian market must be developed and evolved. The success of CCS is hampered not just by the advancement of technology in the coming years, but also by the lack of a policy environment. The ecosystem should be established and strengthened around the critical pillars of research and development, policy, funding, and governance.

Carbon Capture and Storage (CCS)

CCS is a process of sequestration where carbon dioxide emitted from large power plants or heavy industries is captured and stored before reaching the atmosphere. The goal is to avoid substantial amounts of CO_2 from being released into the atmosphere as a result of the usage of fossil fuels in power generation and other sectors. It is seen as a critical climate protection technology for coal-rich nations such as India, with the ability to significantly reduce CO_2 emissions when compared to any other existing technology.

India's concerns

- India is the world's third largest coal producer, with the fifth largest coal reserves and around 0.5% of the world's oil and gas reserves.
- As of 2018, 66% of India's energy generating capacity comes from thermal power plants, with coal accounting for around 85% of the country's thermal power generation. India is the world's fourth greatest emitter of CO_2.

Components of CCS

Carbon Capture and Storage has 3 components:

Capture: The process of separating CO_2 from other gases produced at major industrial process facilities including steel mills, cement plants, oil and gas plants, coal and natural gas power plants, and coal and natural gas power plants.

Transport: Once separated, the CO_2 is compressed and transported via pipelines, trucks, ships or other methods to a site suitable for geological storage.

Storage/Sequestration: CO_2 is injected into deep underground rock formations, up to at depths of one km or more.

Process Flow of CCS

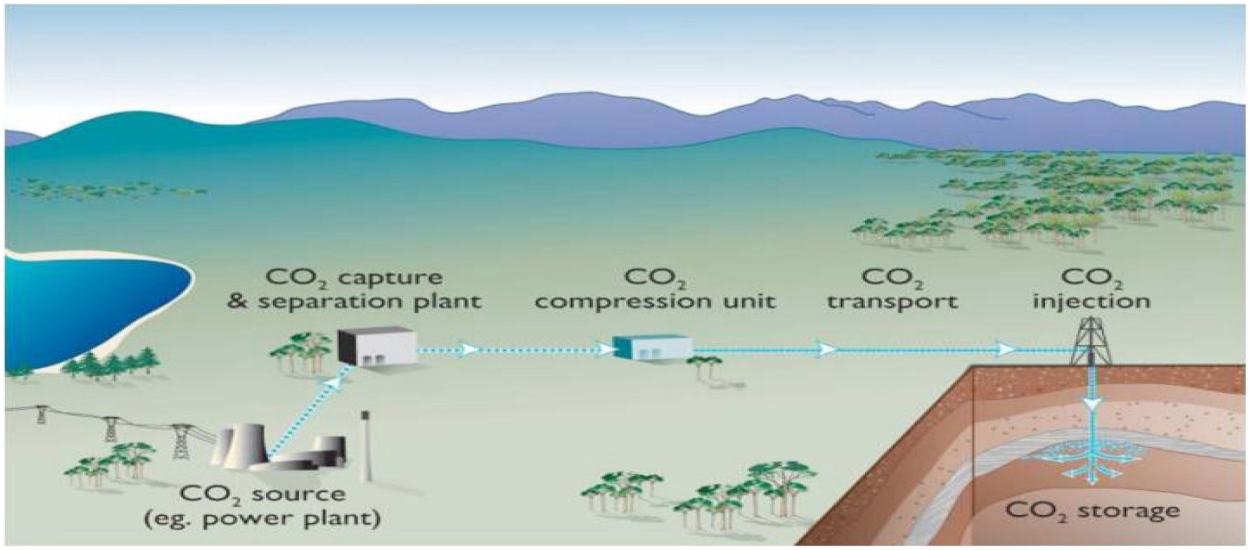

Fig. 1: Different processes/steps of Carbon Capture and Storage

Carbon Capture Technologies:

There are 3 technologies to capture CO_2 such as:

- **Pre-combustion:**

  Where CO_2 is captured before fuel is burned
- **Oxy-fuel:**

  Where CO_2 is captured during fuel combustion
- **Post-combustion:**

  Where CO_2 is captured after fuel has been burned

Why should we go for CCS?

- CCS is a key technology for tackling climate change
- Delivering economic growth and regional prosperity
- The International Energy Agency claimed that CCS might help to reduce world CO_2 emissions by 19% by 2050, making it one of the tools against global warming.
- CCS can be used in Fossil fuel based electricity producing plants, such as coal or gas-fired power plants, which are a key source of energy for our country.
- In the transition to a low-carbon economy, CCS will be crucial for low-carbon power.

Process after capturing CO_2

1. Underground geological storage

- Storage is possible in many different geological settings.
- As they physically trap the carbon dioxide, salt beds, low-permeability shale, and cap rocks are the best places to store CO_2.
- Also used for enhanced oil recovery (EOR).

Stored in: Saline formations, Oil and natural gas reservoirs, Unmineable coal seams and Basalt formations.

2. Ocean storage

- CO_2 stored in the ocean by:
- Direct injection
- Dissolution of carbonate materials
- Production of a CO_2 lake

CCS in India:

- Because India's electricity sector accounts for half of all CO_2 emissions in the country, substantial attention must be paid to this sector in order to minimise (GHG) emissions in the environment.
- Carbon Capture and Storage (CCS) is now used as a bridge technology and a realistic alternative for coal-fired power stations to capture CO_2. However, CCS implementation in coal-fired power plants in India remains low.
- India pledged to reducing its carbon intensity by 30%-33% by 2030 at the UNFCCC Paris Summit.
- The next 10-15 years will be critical for India to achieve technological improvement in order to deploy large-scale CCS plants.
- There are just three commercial CCS plants in India: the Phulpur Urea Plant, the Jagdishpur Urea Plant, and the Aonla Urea Plant.

Challenges of CCS in India:

- In India, public comprehension of CCS technology is quite low, which may lead to significant civilian opposition; consequently, public education on the concerns is required.
- Because of public concerns regarding subterranean CO_2 storage, the government has expressed little interest in domestic demonstrations of the technique.
- Lack of R&D effort
- Need for comprehensive national study on Geological storage
- Lack of financing and inflow of foreign direct investment (FDI)
- Environmental and legal concerns
- Cost scenario
- Political and policy making
- Public opinion
- Foreign policies

Roadmap to Successful CCS in India:

- Policy & Regulatory Framework
- Identification of Suitable CO_2 Storage
- Improvement and Cost Reduction of Capture Technologies
- Development of CO_2 Transport Infrastructure.

Advantages of CCS:

- When CCS is used in current conventional power plants, CO_2 emissions to the

atmosphere can be reduced by 80–90% in comparison to facilities without CCS.

- One of the most effective ways to permanently remove carbon emissions from the environment is carbon capture and storage.
- It produces jobs for qualified engineers, technicians, and labourers.
- It may be possible to generate sustainable geothermal energy by recovering geothermal heat from the location where it was injected using geologically stored carbon dioxide.
- Carbon dioxide from CCS may be used to produce polymers and other compounds, like polyurethanes.
- CCS could reduce the social cost of carbon
- The captured carbon dioxide is incorporated into concrete to reinforce it and increase the infrastructure's endurance.

CONCLUSION

Carbon Capture and Storage will enable us drastically lower our CO2 impact in the future. CCS's importance extends much beyond that of a clean coal technology. The availability of this technology in the future is dependent on current R&D and implementation investments. For more new projects to become operational in recent years, an extended project pipeline is required. Carbon dioxide Capture and Storage is a viable and feasible strategy for mitigating climate change. The post combustion approach is superior than the other two strategies for carbon capture. It is reasonable and doable to use the carbon dioxide capture and storage approach to lessen the effects of climate change. The expense of the CCS is a problem that the government must seriously address. This will give this technology fresh life when Indian policies and attitudes change.

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- Abbreviations spelt out in full for the first time. Numerals from 1 to l0 spelt out
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