ISBN: 978-93-95847-54-4

FRONTIERS IN ANIMAL SCIENCE: RESEARCH AND DEVELOPMENT VOLUME I

Editors:
Dr. Rajendra Salunkhe
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Dr. Shrikant Verma
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BHUMI PUBLISHING, INDIA
FIRST EDITION: AUGUST 2024

Frontiers in Animal Science: Research and Development Volume I

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Bhumi Publishing

August, 2024

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Published by:

Bhumi Publishing

BHUMI PUBLISHING

Nigave Khalasa, Tal – Karveer, Dist – Kolhapur, Maharashtra, INDIA 416 207

E-mail: bhumipublishing@gmail.com

PREFACE

The dynamic field of animal science is at a pivotal moment in history, where the confluence of technological advancements, environmental challenges, and evolving societal needs is shaping the future of livestock production, animal health, and sustainable practices. "Frontiers in Animal Science: Research and Development" is a compendium of cutting-edge research, innovative practices, and forward-looking strategies that are redefining the landscape of animal science.

This book aims to provide a comprehensive overview of the latest developments in various sub-disciplines of animal science, including genetics, nutrition, physiology, behavior, and welfare. It seeks to bridge the gap between fundamental research and its practical applications, offering insights that are not only scientifically rigorous but also relevant to the real-world challenges faced by farmers, veterinarians, policymakers, and researchers.

Each chapter in this volume is authored by experts who are at the forefront of their respective fields, ensuring that the content is both authoritative and current. The topics covered range from the molecular mechanisms underlying animal growth and development to the application of precision agriculture technologies in livestock management. Additionally, the book addresses the ethical and environmental implications of modern animal production systems, emphasizing the importance of sustainable practices in ensuring food security and animal welfare.

As we look to the future, it is clear that animal science will continue to play a critical role in meeting the global demand for animal-derived products while minimizing the environmental footprint of animal agriculture. This book is intended to serve as a valuable resource for researchers, students, and professionals who are dedicated to advancing the field and contributing to the sustainable development of animal production systems.

We hope that "Frontiers in Animal Science: Research and Development" will inspire new ideas, foster collaboration, and drive the next wave of innovation in this vital field.

Editors

TABLE OF CONTENT

Frontiers in Animal Science: Research and Development Volume I
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NOVEL HEPATOPROTECTIVE POTENTIALS OF *CALLIANDRA SURINAMENSIS:* A GROUNDBREAKING STUDY AGAINST CCL4-INDUCED LIVER INJURY IN RATS

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Abstract:

The liver is in charge of many functions, including creating digestive enzymes, primary detoxification of different metabolites, and protein synthesis. The main function of the liver is to detoxify the blood from alcohol and drugs. Almost 2 million people are dying each year from chronic liver illnesses, which pose a significant global health threat. If liver functions are disturbed, it has serious consequences on a variety of homeostatic mechanisms. *Calliandra surinamensis* (CS) is a medicinal plant in the Fabaceae family. The plant's rootbark included secondary metabolites like flavonoids, -sitosterol 3-O-, and -azinobis 3-Ethylbenzothiazoline-3-O-. The hepatoprotective properties of the flavonoids and phenolic compounds present in *C. surinamensis* leaves may offer therapeutic advantages, according to this study. When it came to treating liver injuries, *C. surinamensis* extract performed better than silymarin, the typical medication. Preliminary phytochemical investigations looked into the hepatoprotective efficacy of CS leaf methanolic extract against carbon tetrachloride (CCl4)-induced liver injury in rats. The extract was found to have a protective effect on the liver injury induced by CCL4 in Wistar rats. Moreover, the antioxidant activity of the extract against CCL4-induced hepatic injury was also investigated.

Keywords: Liver Detoxification, Hepatoprotective Property, *Calliandra surinamensis*, Methanolic Extract Hepatoprotection, Protective effects against CCL4-Induced Damage

Introduction:

The liver, which is located in the right upper quadrant of the body and immediately below the diaphragm, is in charge of several functions, including the creation of digestive enzymes, primary detoxification of different metabolites, and protein synthesis (Schaefer *et al.,* 2021). It is the important organ in the body and

essential for the body's maintenance, functionality, regulation (Al-Mehdar *et al.,* 2015) and also necessary for both endogenous and exogenous substances detoxification and excretion (Chen *et al.,* 2020). Its major function is to detoxify the blood from alcohol, drugs, and different toxins. It participates in practically all metabolic processes that are involved in growth, disease prevention, nutrition uptake, energy production, and reproduction (Hugade & Kuthar, 2021). The digestive system is where most xenobiotics get into the body and, after being absorbed, transfer to the liver via the portal vein. Toxin-metabolizing enzymes, which are highly concentrated in the liver, are able to transform xenobiotics into molecules of minimal toxicity and eliminate them. If liver functions are disturbed, it has serious consequences on a variety of homeostatic mechanisms. The metabolic function aberrations are linked to hepatic injury. (Zodape & Bhise, 2018). Consequently, liver disease is one of the most severe illnesses in existence today (Raj *et al.,* 2016). Every year, more than 2 million people worldwide pass away from liver diseases, including 1.3 million cases of liver cirrhosis and more than 0.8 million cases of liver cancer (Im *et al.,* 2021). Drug use, alcohol intake, and exposure to environmental toxins all contribute to the high rate of liver damage, which in turn causes disorders like hepatitis. The majority of hepatotoxic chemicals result in liver cell death by increasing tissue lipid peroxidation, oxidative stress, and serum levels of many biochemical markers including transaminases, alkaline phosphatase, bilirubin, triglycerides, and cholesterol (Ouassou *et al.,* 2021). The liver has a remarkable capacity to scavenge the free radicals produced during the metabolism of numerous medicines. The evolution of oxidative stress, which results in numerous liver dysfunctions, is promoted by the increasing manufacturing of free radicals and the reduced antioxidant defense in hepatocytes. As a result, oxidative stress is linked to a number of liver diseases, including cirrhosis, necrosis, fibrosis, and hepatocellular cancer. The hepatoprotective principles have been well-documented in experimental and clinical studies for their antioxidant capability against a variety of liver diseases. For example, silymarin, silibinin, curcumin, resveratrol, green tea, etc., have all undergone considerable clinical and experimental research against liver disease and have shown promising benefits. These medications are predominantly antioxidants, which indicate and highlights the importance of antioxidant therapy in oxidative stress-induced liver disease (Ezhilarasan, 2018). The liver experiences severe oxidative stress because of its exposure to a variety of prooxidant toxic compounds, as well as because of its metabolic

capacities and ability to transform some xenobiotics into reactive harmful metabolites like ROS (Simeonova *et al.,* 2014). There are many methods to induce liver injury in the model organisms such as drug-induced liver injury the most common drug which is used mostly in UK & USA is paracetamol (Rivera *et al.,* 2020), alcohol-induced liver injury (Fairfielf & Schnabl *et al.,* 2021) and CCl4 is also used to produce liver disease (Ullah *et al.,* 2020). We just focus on CCl4 to produce liver disease models, consumption of CCl4 in rats model results in necrosis, which then progresses to steatosis, fibrosis, and cirrhosis and then transformed into the hepatocellular carcinoma (Nazeer *et al.,* 2015). The xenobiotic-induced free-radical-mediated hepatotoxicity in animal model that is most understood is carbon tetrachloride- (CCl4) stimulated liver disease. By means of cytochrome P450, CCl4 is transformed into two free radicals, trichloromethyl radical (CCl3-) and proxy trichloromethyl radical OOCCl3; (Fouad *et al.,* 2019). These free radicals can trigger lipid peroxidation and cause harm to the liver (Muriel., 2017). Plants have crucial role in our life. Herbal medicines derived from plants are being promoted to treat clinical diseases. More attention has been paid to the protective effects of natural anti-inflammatory and anti-oxidants against liver diseases. Pharmaceutical and cosmetic industries production depend on plants ayurvedic (Mangal *et al.,* 2017) herbal and homeopathic industries production completely dependent on medicinal plant extraction. According to other studies, antioxidants can lower the risk of developing liver illnesses and protect the liver from oxidative damage (Li *et al.,* 2015). Alternative treatments for hepatotoxicity that use natural remedies made from medicinal plants are thought to be effective and secure (Mensah *et al.,* 2016). The importance of natural antioxidants has received so much attention. Previous studies have demonstrated the abundance of antioxidant chemicals in medicinal plants, which have been found to have strong hepatoprotective effects via improving antioxidant status (Al-Snai *et al.,* 2019). The World Health Organization (WHO), claims that more than 75% of the population, especially in regions in Latin America, Africa, and Asia, uses herbal remedies as part of their traditional primary healthcare system (Karunamoorthi *et al.,* 2013). Pakistan has a rich genetic diversity of plants, and due to poor access to medical care, almost all populations have made extensive use of therapeutic herbs. The majority of treatment and prevention regimens for various ailments continue to be based on natural ingredients (Mustafa *et al.,* 2022). The plants have traditionally been used to cure a number of pathological conditions, such as

gonorrhoea, migraines, bowel parasites, and skin illnesses (Qasem *et al.,* 2015). There are numerous medicinal plants, and one of them is *Calliandra surinamensis* (CS), a specie in the Fabaceae family (Procopio *et al.,* 2018). The genus Calliandra is among the biggest in the Fabaceae family with almost 200 species with a small native population also found in Madagascar and India, it grows primarily in the tropical and subtropical regions of America. The treatment of eye disorders, diarrhoea, indigestion (Allen *et al.,* 1981), diabetes and obesity has been reported for a number of species in the genus (Santos *et al.,* 2021). Different kinds of chemicals, including flavonoids, diterpenes, triterpenes, amino acids, and polyphenols, were found in previous investigations on the phytochemistry of many Calliandra species (Omar *et al.,* 2016). Previous research revealed that folklore utilized the stem bark's antioxidant and antibacterial properties as traditional medicine to treat a variety of illnesses and infections (Alzahrani *et al.,* 2016). According to a preliminary investigation, the plant's rootbark included secondary metabolites like flavonoids, -sitosterol 3-O- D-glucopyranoside, and sterol (Omar *et al.,* 2016). The organic soluble components of a methanolic leaf extract and its various organic soluble partitionists were examined in the previous research for their antioxidant activity in terms of total phenolic content, ability to stabilize membranes, antimicrobial, cytotoxic, and thrombolytic activities (Penu *et al.,* 2020). There are following biological properties of *C. surinamensis* extracts such as antioxidant, antibacterial, anti-inflammatory, and cytotoxic effects which are mentioned above. We investigate the hepatoprotective effect of methanolic extract of *C. surinamensis* leaves against CCl4-induced liver damage in rats based on these biological activities because, to our knowledge, no research on the hepatoprotective activity of *C. surinamensis* leaves has been reported to date.

Materials and Method:

1. Chemicals used

Methanol (CH3OH), carbon tetrachloride (CCl4), chloroform (CHCl3) and formalin (CH2O), these all chemicals purchased from local medical store. All reagents produced were analytical grade.

2. Selection of plant

The plant *Calliandra surinamensis* was locally obtained from the University of Lahore, Lahore, Pakistan, between July 15 and July 30, 2022. The plant was identified by

the expert Botanist (Dr. Iram) from the Department of Botany, University of the Punjab, Pakistan, with specimen voucher number Lah#2002.

3. Extract preparation

The *C. surinamensis* leaves were thoroughly cleaned in tap water, then allowed to dry in the shade before being ground into a powder. This powder was steeped in 1250 ml of methanol for 72 hours while being stirred and mixed on a regular basis. The solution was then filtered using Whatman® filter paper as the next step. Three times through to the process, the filtrates were collected, and they were then heated to 40 °C in a rotary evaporator before being placed onto petri dishes with labels. The petri dishes were properly dried at 40 °C in an incubator. The extract, or yield, made up 15.7% of the plant powder's dry weight. After that, the extract was kept at 4 °C until it was required.

4. Animals

Twenty five male albino rats (Wistar strain) of age 7±1 each weighing 150 - 180g were purchased from Faculty of Veterinary Medicine, Ripha International University, Lahore, Pakistan. The animals were divided into five groups of five rats each. The rats were kept in wired cages with a controlled temperature (22.2 C) and light cycle (12 h light and 12 h dark). Rats were permitted to consume regular lab food and tap water. They were given a typical diet to eat.

5. Experimental study

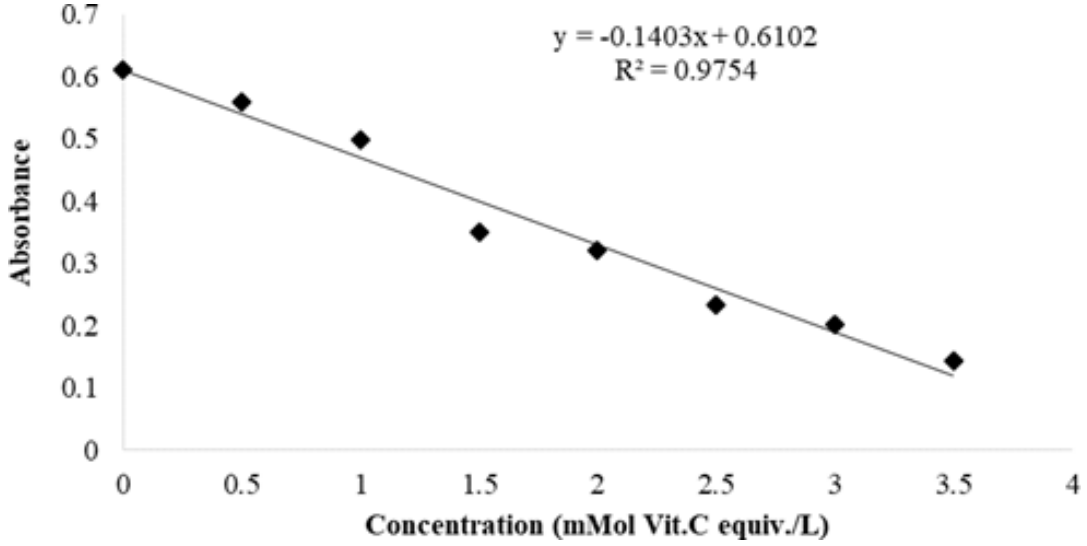
The rats were divided in five groups as follows:

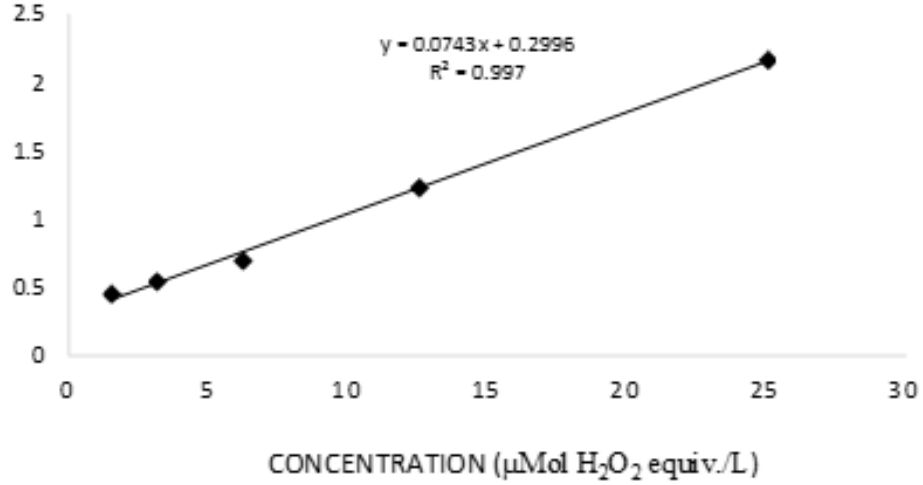
1. First group, known as the negative control group, that received no treatment and was given olive oil; they were given a regular food and access to water for 21 days. Animals from all the remaining groups received an intraperitoneal injection of CCl4 (0.5ml/kg body weight) along with an equivalent volume of olive oil for one time.
2. Second group was standard control group receiving silymarine (100 mg/kg) as a standard hepatoprotective drug.
3. Third group was the positive control group and received only CCl4 (0.5ml/kg body weight): olive oil (1:1) intraperitoneally injected.
4. Fourth group was the treatment group-I, receiving only CCl4 (0.5 ml/kg body weight): olive oil (1:1) intraperitoneally administered just for one time, and *C.*

surinamensis extract dose of 200 mg/kg for 21 days for liver treatment orally by stomach tube.

5. Fifth group was the treatment group-II and received CCl4 (0.5ml/kg body weight): olive oil (1:1) intraperitoneally administered once, and *C. surinamensis* extract dose of 400 mg/kg for 21 days for liver treatment orally by stomach tube.

Previous research on antioxidant properties of methanolic extracts from fabaceae plants involved daily preparations in distilled water, with rats' body weight considered. After 21 days, blood was collected from the jugular vein and tested for ALP, AST, and ALT in the serum, which was then isolated at 2000 g for 10 minutes.





6. Total Antioxidant Capacity (TAC; mmol Trolox equivalent / L)

The assay involves the use of two reagents: Reagent I (R-I, Acetate buffer) and Reagent II (R-II, ABTS). Reagent I is a pH 5.8 sodium acetate solution and a reagent-grade glacial acetic acid solution, which are combined to create a buffer solution.

Reagent II is a commercial H2O2 solution, which dissolves 0.549 g of ABTS in 100 ml, resulting in a final concentration of 10 mM/L. The TAC is calculated using a semi-auto-analyzer. The delta absorbance, or final concentration, is determined by calibrating vitamin C standards.

7. Total Oxidant Status (µmol H2O2 equiv. L-1):

This assay detects the total oxidant status of a sample by analyzing the oxidation of ferrous ion O-dianisidine complex to ferric ion. The oxidants are detected through color intensity, quantified spectrophotometrically, and expressed as micromolar hydrogen peroxide equivalent per liter. The test has a sensitivity of 1.13 mol H_2O_2 equivalent per liter, a precision of 3%, and linearity up to 200 mol H_2O_2 equivalent per liter. Reagents used include NaCl and H_2SO_4, ferrous ammonium sulphate, and O-dianisidinedihydrochloride. A standard curve was created by diluting 100 ml of H_2O_2 and diluted twice. The assay procedure involves combining serum samples with reagent 1, measuring the absorbance at bichromatic wavelengths, and calculating the concentrations using the delta change in absorbance.

8. Statistical analysis

The analysis was carried out using one-way ANOVA, and the findings were presented as "mean standard deviation". The threshold for significance was taken as ($P<0.05$). A post hoc test was used to undertake extra analysis. Using the statistical analysis programmed SPSS 16.0, all of the calculations were done.

Results:

Phytochemicals

The methanolic extract of *C. surinamensis* leaves was investigated in preliminary phytochemical studies, and it contains flavonoids, diterpenes, triterpenes, amino acids, and polyphenols. Effects of *C. surinamensis* methanolic extract on CCl4-induced liver injury in rats with relation to alterations in blood biochemistry are show in Tables 1, 2, 3, 4

Biochemical parameters

The study found that rats exposed to CCl_4 orally induced liver injury, leading to increased bilirubin levels and enzyme activity. However, when rats were given methanolic extract of *C. surinamensis*, these elevated levels were significantly reduced, indicating a high degree of defense against CCl_4's hepatotoxic effect. The higher level of protection was demonstrated at a dose of 400 mg/kg body weight.

Table 1: Bilirubin level in different groups of rats

Groups no.	Treatment groups	Bilirubin (mg/dl)
1	Negative Control	0.77±0.03[a]
2	Stand. control Silymarine (100mg/kg)	0.8±0.058[a]
3	Positive Control (CCl4)	1.8±0.06[b]
4	T-I: *C. surinamensis* (200mg/kg)	0.73±0.07[a]
5	T-II: *C. surinamensis* (400mg/kg)	0.77±0.033[a]

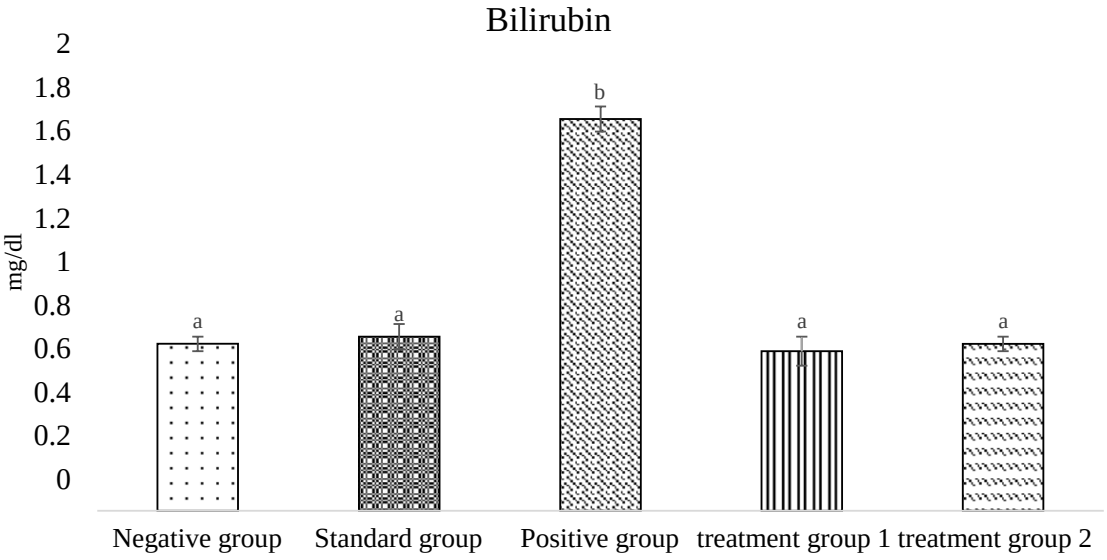


Figure 1: Effect of *Calliandra surinamensis* leave extract on Bilirubin level in CCl4 intoxicated rat. The alphabets a-b on the bars show level of significance (P<0.05) among different groups

Table 2: ALT level in different groups of rats

Groups no.	Treatment groups	ALT (U/L)
1	Negative control group	109.67±22.06[a]
2	Stand. control Silymarine (100mg/kg) + CCl_4	75.67±6.97[a]
3	Positive control group (CCl_4)	263.67±3.28[b]
4	T-I: *C. surinamensis* (200mg/kg) + CCl_4	73.33±12.35[a]
5	T-II: *C. surinamensis* (400mg/kg) + CCl_4	109.33±23.82[a]

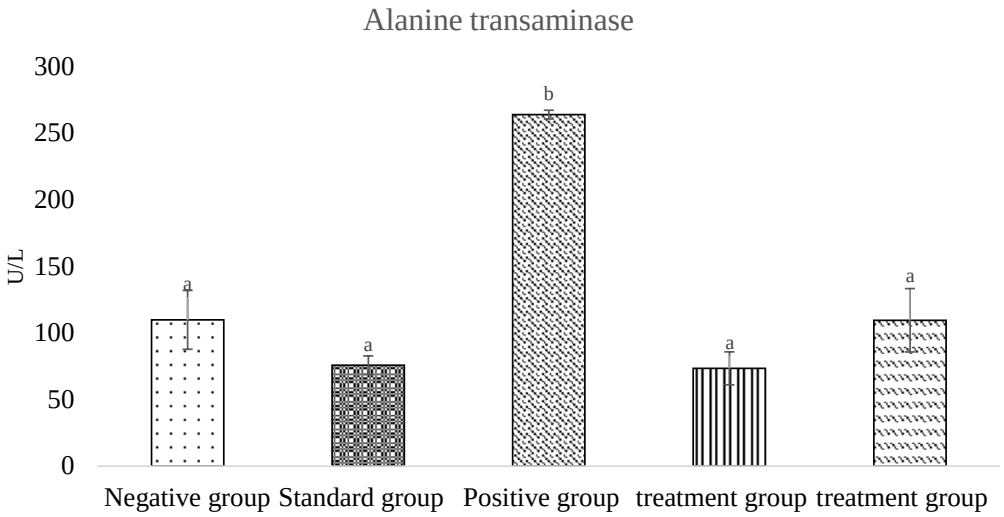


Figure 2: Effect of *Calliandra surinamensis* leave extract on ALT level in CCl4 intoxicated rat. The alphabets a-b on the bars show level of significance (P<0.05) among different groups

Table 3: AST level in different groups of rats

Groups no.	Treatment groups	AST (U/L)
1	Negative Control	112 ± 8.72^{b}
2	Stand. control Silymarine (100mg/kg)	91.33 ± 12.25^{ab}
3	Positive Control (CCl_4)	265.67 ± 7.75^{c}
4	T-I: *C. surinamensis* (200mg/kg)	70.67 ± 2.85^{a}
5	T-II: *C. surinamensis* (400mg/kg)	100.67 ± 4.26^{ab}

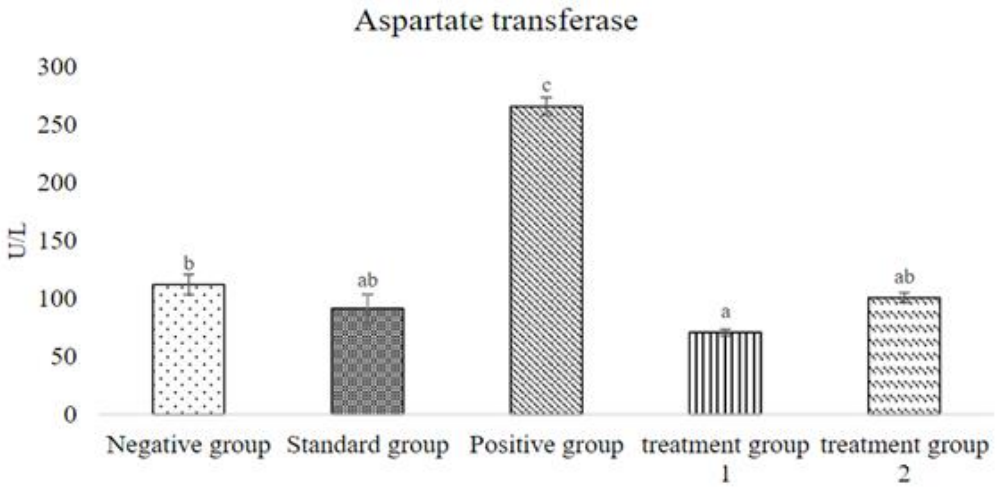


Figure 3: Effect of *Calliandra surinamensis* leave extract on AST level in CCl4 intoxicated rats. The alphabets a-c on the bars show the level of significance (P<0.05) among different groups.

Alkaline phosphatase (ALP)

Groups no.	Treatment groups	ALP (U/L)
1	Negative Control	225.33±32.95[a]
2	Stand. control Silymarine (100mg/kg)	231.66±17.4[a]
3	Positive Control (CCl_4)	467±7.77[b]
4	T-I: *C. surinamensis* (200mg/kg)	195±2.52[a]
5	T-II: *C. surinamensis* (400mg/kg)	234±8.33[a]

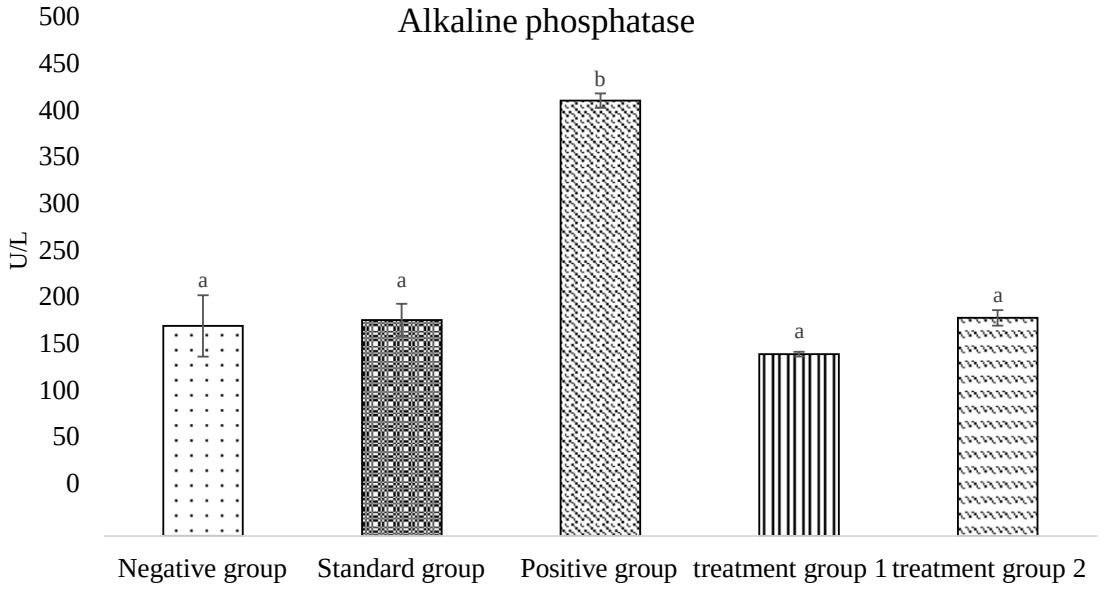


Figure 4: Effect of *Calliandra surinamensis* leave extract on ALP level in CCl4 intoxicated rat. The alphabets a-b on the bars show the level of significance (P<0.05) among different groups

Total antioxidant capacity and oxidative status

The study found that the positive control group showed low liver toxicity, with a mean decrease of 1.1065±0.31536 compared to the negative control group. The *Calliandra surinamensis* leaves extract treatment resulted in a significant decrease in toxicity, with a dosage of 200 mg being very close to the standard silymarin concentration of 1.8257±0.06984. (Table 5; Figure 5 for antioxidant capacity) (Table 6; Figure 6 for oxidative status).

Table 5: Effect of *Calliandra surinamensis* on TAC on CCl4

Groups no.	Treatment groups	TAC
1	Negative control	1.8306±0.04201
2	Standard control	1.8257±0.06984
3	Positive control	1.1065±0.31536
4	T-I: *C. surinamensis* (200mg/kg)	1.804±0.10505
5	T-II: *C. surinamensis* (400mg/kg)	2.1754±0.02926

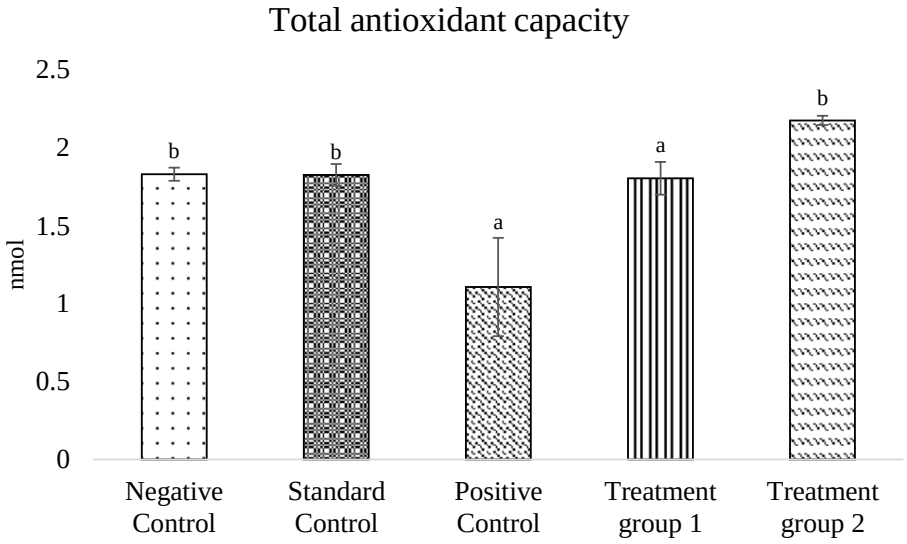


Figure 5: *Calliandra surinamensis* leaves extract affects TAC level in CCl4 intoxicated rats. The alphabets-b on the bars show level of significance (p≤0.05) among different groups

Table 6: Effect of *Calliandra surinamensis* on TOS on CCl_4

Groups no.	Treatment groups	TOS
1	Negative control	23.8143±0.37519
2	Standard control	22.6175±0.53779
3	Positive control	35.4305±0.23758
4	T-I: *C. surinamensis* (200mg/kg)	19.8097±0.11854
5	T-II: *C. surinamensis* (400mg/kg)	15.6695±0.30401

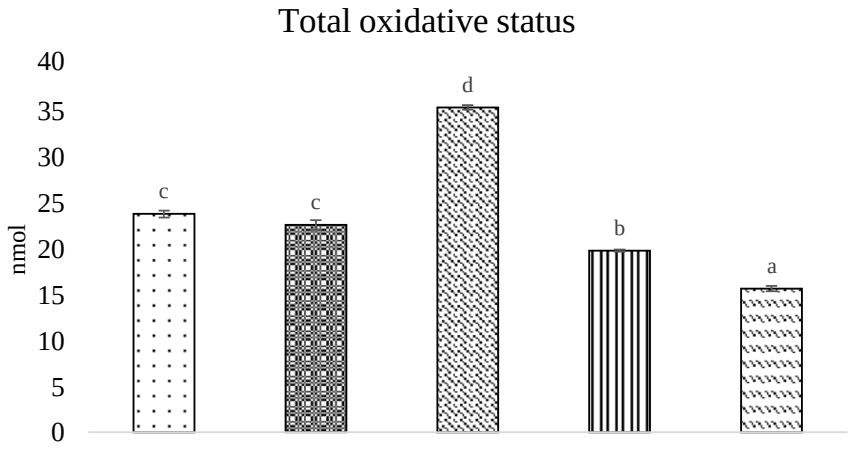


Figure 6: *Calliandra surinamensis* leaves extract affects TOS level in CCl4 intoxicated rats. The alphabets-d on the bars show level of significance (p≤0.05) among different groups

Discussion:

Hepatic cells appear to participate in a range of enzymatic metabolic processes and both alcohol and CCl_4 produced marked liver damage at the given doses as expected (Abdelhafez *et al.,* 2018). Alcoholic liver disease has been linked to the production of reactive oxygen species (ROS), oxidative stress, and hepatocellular damage. Kupffer cells have been shown to be the primary ROS generators with chronic alcohol use, and they are primed and activated for increased production of pro-inflammatory molecules (Moreno *et al.,* 2014). Numerous chemicals can damage the liver, and extensive research has been done on the mechanisms of action that lead to CCl_4-induced liver disease (Meng *et al.,* 2018). The hepatotoxicity induced by CCl_4 is due to its metabolite free radical CCl_{3-} that binds to lipoprotein and leads to peroxidation of lipids of endoplasmic reticulum (Ighodaro *et al.,* 2020). Briefly, CCl_4 is metabolized by cytochrome P450 enzymes in the liver, where it is bio transformed into CCl3 and then oxygenated to form $OOCCl_3$. Both of these radicals are extremely reactive and can deplete reductants, inhibit antioxidant enzymes, cause lipid peroxidation, hypomethylate proteins, and mutate nucleic acid. In the body, there is a complex defense system consisting of antioxidant enzymes that can protect against oxidative damage (Grasselli *et al.,* 2014). leading to oxidative stress, inflammation, apoptosis, and necrosis (Scholten *et al.,* 2015). Enzymes including AST, ALT, ALP, and bilirubin level have been connected to liver structural integrity damage as a result of CCl_4 administration since they are found in the cytoplasm and released into circulation after cellular damages. The emergence of hepatotoxicity is

indicated by this (Iqbal *et al.,* 2022). The current study reveals that pretreatment with a methanolic extract of leaves from *C. surinamensis* has been effective in providing protection, comparable to silymarin (Table 1, 2, 3, 4). The methanolic extract of *C. surinamensis* leaves showed a substantial dose-dependent hepatoprotective effect at 200 and 400 mg/kg to lower the serum level of ALT, AST, ALP, and bilirubin when administered orally to rats. At a dose of 200 mg/kg, this *C. surinamensis* extract is remarkably effective in reducing the elevated levels of bilirubin and liver enzymes (ALT, AST, and ALP). The doses of plant extract used in the current trial were chosen in agreement with those prescribed in the preceding study (Mustafa *et al.,* 2022). The current study's findings revealed a statistically significant ($p<0.05$) elevation in bilirubin levels in the PC group compared to the NC group. In addition, when compared to the PC group, the bilirubin level was significantly ($p<0.05$) decreased in the SC, T1, and T2 groups. Standard control group received silymarin, while treatment groups T1 and T2 received dosages of 200 and 400 mg/kg BW, respectively, of the methanolic extract of the plant *Calliandra surinamensis*. In comparison to the PC group, this extract significantly ($p<0.05$) reduced the levels of bilirubin in T1 (0.73±0.07 mg/dl) and T2 (0.77±0.033 mg/dl) respectively. An earlier study demonstrated that leaf extracts from Citrus hystrix and C. maxima decreased significantly ($p<0.05$) the bilirubin level (1.11 ± 0.01 mg/dl) of plant treated group as compared to the bilirubin level (2.18 ± 0.02 mg/dl) of hepatotoxic positive control group (Abirami *et al.,* 2015). A significant increase in ALT levels in the PC group compared to the NC group, as ALT is a more specific parameter for assessing hepatic damage in the liver. The study found that the methanolic extract of *Calliandra surinamensis* significantly decreased ALT levels in the SC, T1, and T2 groups compared to the PC group. The extract, which contained cold-pressed Syzygium aromaticum oil, also reduced ALT levels in the CCl4-treated group. The extract's dosages of 100 mg/kg and 200 mg/kg significantly decreased ALT levels in the SC and T2 groups. The study found that liver enzyme aspartate transaminase (AST) levels increased in the PC group compared to the NC group, and decreased in the SC, T1, and T2 groups. Treatment groups received *Calliandra surinamensis* methanolic extract, which significantly reduced AST levels in T1 and T2 compared to the PC group. Lobularia maritima also reduced AST levels in the treatment group compared to the CCl_4-treated group. The study found that alkaline phosphatase (ALP) levels were significantly elevated in the PC group compared to the NC group, and decreased in the

SC, T1, and T2 groups. Treatment groups received a methanolic extract of *Calliandra surinamensis*, significantly reducing ALP levels in T1 and T2 compared to the PC group. Morus nigra aqueous methanolic extract was also used to lower ALP levels in the treatment group.

Conclusion:

The increase in liver function specifications and the unfavorable histopathological alters in the liver tissues of the rats in the positive control group in this study demonstrated that CCl_4 induces liver hepatotoxicity. The liver structure of the hepatotoxic rats was protected against CCl_4 toxicity by administering a methanolic extract of the plant *C. surinamensis*. It is concluded that methanolic extract treatment reduces the biochemical parameter increase caused by CCl_4. These results imply that the methanolic extract successfully improved the functional state of hepatocytes. Histological findings supported the extract's ability to treat illness. The study shows that the flavonoids and phenolic chemicals found in the leaves of *C. surinamensis* may have therapeutic benefits due to their hepatoprotective effects. *C. surinamensis* extract had better results than the standard drug silymarin for liver injury treatment.

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UNRAVELLING THE MYSTERIES OF ALKAPTONURIA: FROM GENETICS TO THERAPEUTIC INSIGHTS

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Abstract:

A very rare autosomal recessive condition of tyrosine metabolism in the liver caused by a lack of homogentisate 1,2 dioxygenase (HGD) activity identified as alkaptonuria (AKU). HGA circulatesthroughout the body, entering a variety of tissues, primarily cartilage and connective tissues, where its oxidation products polymerize and deposit as a pigment resembling melanin. A minimal quantity of HGA is also excreted in urine. The three main features of AKU are: darkening of the urine at birth, ochronosis (blue-dark pigmentation of the connective tissue), which becomes clinically noticeable in the ear and eye around the age of thirty and a severe ochronotic arthropathy with involvement of the spine and large joints around the age of fifty.Several case report studies have detailed renal and cardiovascular problems.Recent research on the disease provides the insight about the disease facilitating the better understanding of the disease.

Keywords: Alkaptonuria, Homogentisic Acid, Ochronosis, Ochronotic Arthropathy, Nitisinone.

Introduction:

Alkaptonuria, also known as black urine disease, is a rare inherited metabolic disorder characterized by the deficiency of homogentisate 1,2-dioxygenase (HGD) enzyme activity, leading to the accumulation of homogentisic acid (HGA) in the body. This condition follows an autosomal recessive pattern of inheritance, with an estimated incidence ranging from 1 in 250,000 to 1 in 1,000,000 individuals worldwide [1]. First described by Sir Archibald Garrod in 1902, alkaptonuria represents one of the earliest documented instances of an inborn error of metabolism. The hallmark feature of this disorder is the excretion of dark urine upon exposure to air due to the oxidation of HGA, which imparts a characteristic black colour. However, the consequences of alkaptonuria extend beyond its visible manifestation, encompassing a spectrum of multisystemic complications [2]. Ochronosis or the deposition of a yellow-brown pigment within

connective tissues as a result of HGA oxidation and polymerization, is the clinical feature that distinguishes this condition. The second stage of evolution is known as ochronesis and it manifests itself as a brownish sclera and a bluish pinna around the ages of twenty and thirty.

This review aims to provide a comprehensive overview of alkaptonuria, encompassing its molecular basis, clinical manifestations, diagnostic approaches, management strategies, and ongoing research endeavours. By synthesizing current knowledge and recent advancements, this review seeks to enhance understanding of alkaptonuria and stimulate further research efforts towards improved management and therapeutic interventions.

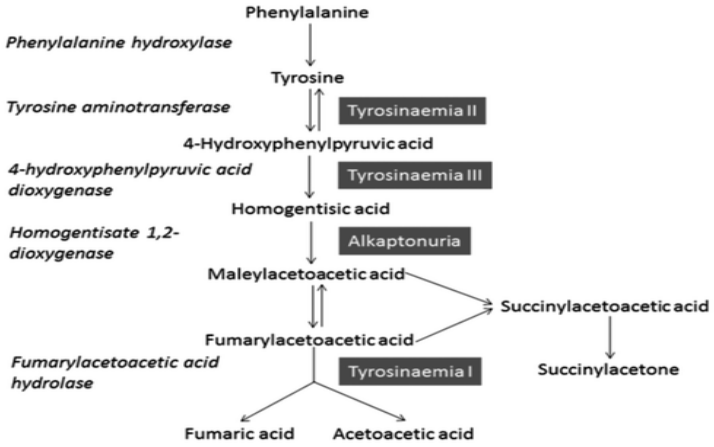

Figure:1 Breakdown of Phenylalanine pathway

Figure 1 sourced from Suwannarat *et al*. (2005). The typical process for the breakdown of phenylalanine and tyrosine and the enzymes involvedin the pathway is explained. The potential abnormalities are highlighted in the diagram. HGA is metabolised and HGD is expressed as an enzyme in liver.

Epidemiology:

AKU affects one instance out of every 2,50,000–10,00,000 newborns globally. As of now, 40 countries are home to 950 AKU patients [4][5]. There are reports that Slovakia, the Dominican Republic, India and Jordan have higher rates of this ailment. Slovakia has the highest rate, with up to 1 in 19,000 people afflicted [4]. After a study of the afflicted families showed that they frequently reside in remote hamlets, it was determined that the founder effect, the loss of genetic variety brought on by genetic isolation was mostly to blame for the generally high occurrence [6]. Additionally, there is no genotype-phenotype association; all mutations result in the development of ochronosis, a task that is challenging and gives the rarity of the disorder [7].Research is still needed to

comprehend the process underlying the disease's progression completely. According to published literature, a higher profile of the disease's characteristic symptoms may be the reason for the rise in the number of new cases reported [4]. But even with the rise in new case reports, the number of people officially diagnosed with the illness is far lower than what would be predicted based on the event.

Clinical manifestations:

Despite having a biochemical deficit since birth, AKU patients are typically asymptomatic until their third decade of life, with the exception of consistent dark urine [8]. The Subclinical Ochronotic Features in Alkaptonuria study [9] found eye pigmentation in a 22-year-old patient and ear pigmentation in a 20-year-old. This suggests that ochronosis begins earlier in life than the major clinical indications. AKU typically does not impact longevity, but the quality-of-life declines with age due to increasing arthropathy.

1. Ocular manifestations:

Two-thirds of the AKU patients were reported with ocular symptoms [10]. The most common ocular characteristics reported were conjunctival and scleral pigmentations. Cataract was not commonly recognized as a prevalent characteristic in AKU until a recent study of the United Kingdom National Alkaptonuria Centre (NAC)cohort indicated that 76% of patients had cataract at baseline. This is assumed to be the result of lifelong exposure to HGA, which mediates the oxidative stress pathway that finally leads to cataract development. Accumulation of ochronotic pigment in the lens has been suspected but not proven. Glaucoma, acute recurrent anterior uveitis, central vein blockage and progressive astigmatism are among the other described visual issues [10].

2. ENT manifestations:

About 90% of AKU patients exhibited ENT indications or symptoms. The most prevalent ENT findings include discoloration of the pinna and cerumen. There have been reports of pigmentation in the tympanic membrane, middle ear and nasal septum. Nearly one-third of the reported cases had minor hearing loss. This was primarily high-frequency sensorineural loss [11]. Pigmentation of the laryngeal cartilage was also documented [12].

3. Respiratory involvement:

The respiratory system, especially the upper airways, might be affected by alkaptonuria. Pigmentation of the bronchi, trachea, and larynx may result from

homogentisic acid (HGA) deposition in the respiratory mucosa. As a result, there may be respiratory symptoms such persistent coughing, hoarseness, and even obstructive airway disease [13].

Figure 2: Ochronotic Pigmentation of Sclerae and Ear Cartilage at Various Ages

4. Spondyloarthropathy:

Low back pain is extremely frequent, affecting 94% of AKU patients before the age of 40 in a US cohort [1]. All patients in the NAC cohort showed uptake on PET-CT. However, only 85.7% reported pain, indicating that the spondylo-arthropathic disease process occurs before symptoms arise. The latter cohort experienced pain in joints such as knees, shoulders, hips, ankles, feet, elbows and wrists. Pain was most commonly reported in the lumbar region (80%), followed by cervical (60%), thoracic (~40%) and sacroiliac (~20%). In the NAC cohort, 36.8% of patients suffered arthroplasty, with nearly one-third experiencing several procedures. Knees were most typically replaced, followed by hips and shoulders [4].

5. Cardiovascular manifestations:

Alkaptonuria has been associated with cardiovascular complications, including valvular heart disease. The deposition of HGA in heart valves can lead to thickening, calcification, and dysfunction, predisposing individuals to conditions such as aortic stenosis and mitral valve regurgitation [14].

6. Urolithiasis:

In a case series, roughly one-third of male participants had prostate stones.AKU-related instances can be more extensive than non-AKU cases. Half of AKU patients have a history of renal stones by the age of sixty four [15].

7. Thyroid disorders:

Primary hypothyroidism is over four times more prevalent than in the general population. HGA accumulation may interfere with thyroid hormone synthesis. The

prevalence of thyroid nodules and malignancies was similar to the overall population [16].

8. Acute haemolysis and methaemoglobinaemia:

The kidneys eliminate HGA predominantly through tubular secretion, which is supplemented by glomerular filtration. There have been 12 recorded cases of haemolysis with methaemoglobinaemia. This fatal consequence is caused by deteriorating renal function, elevated HGA levels and oxidative stress [17].

9.Neurological manifestations:

Parkinson's disease is about 20 times more prevalent in AKU patients than in the general population. This is believed to be linked to lifelong exposure to HGA. Dura mater coloration has been documented [18, 19].

Diagnosis:

1. The urine test for HGA is the gold-standard test to diagnose alkaptonuria in which urine turns black colour when reacted with air. The amount of homogentisic acid excreted is analysed using GC-MS and the amount of HGA excreted each day in patients with AKU is usually between 1 and 8 grams.
2. Molecular genetic testing.
3. CT scan or MRI helps in assessing the severity of joint involvement in AKU.
4. 2D-Echocardiography can be used to detect valvular abnormalities.
5. CT Angiogram can be used to detect calcification of coronary vessels [20].

Treatment:

Patients with AKU have undergone a number of treatment interventions, albeit with mixed results. Palliative care is the norm for current treatments, of which there are four varieties:

1. Decreased production of HGA i) One possible approach to treat AKU symptoms is to follow a low-protein diet with minimal tyrosine consumption, but this is a tough diet to stick to over time and has no proven effect on the symptoms.ii) Alternatively, Oral nitisinone reduces urine HGA excretion in a murine model of AKU by roughly 80%, but as a side effect, plasma tyrosine levels rise. Nitisinone has the same pharmacological action in humans, yet in a three-year randomized treatment trial involving forty patients with alkaptonuria, one patient experienced classical keratopathy due to tyrosine toxicity [21]. Nitisinone is well tolerated when taken orally at a dose of 2 mg per day; it reduces urine HGA excretion by >95% and its average plasma tyrosine level is 800μM.Early nitisinone treatment is

recommended to avoid ochronosis andrelated joint arthropathies. It has recently been demonstrated that nitisinone totally prevents pigment deposition in the chondrocytes within the knee's articular cartilage in a mouse model of AKU[22].

2. Pain control is essential in the day-to-day lives of AKU patients and is addressed by a wide range of analgesic medications: paracetamol, non-steroidal anti-inflammatory drugs and opioids [23]. Other methods of pain reduction include physiotherapy, rest and/or conventional analgesic drugs. It has also been demonstrated that physiotherapy increases activity.
3. Reduction of physical impairments by hip, knee and/or shoulder replacements. 3-5 surgical procedures are relatively common in older AKU patients [23]. Injections of hyaluronic acid into the joints have been shown to be beneficial in the short term in a patient with early ochronotic arthritis [24].
4. Liver transplantation or gene therapy can replace lost genes or enzymes, but these treatments are not yet widely accessible [25, 26].

Conclusion:

Over the last 10-15 years, there have been significant breakthroughs in our understanding of AKU's pathophysiology and clinical therapy. International efforts between academic, clinical and industry partners, as well as multiple patient societies, have led to the identification of nitisinone as an effectiveHGA-lowering agent and facilitated multiple international clinical trials, eventually resulting in the approval of nitisinone for treatment of AKU.

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TREATMENTS OF PHENYLKETONURIA: A REVIEW

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Abstract:

Phenylketonuria is one of the inborn errors of metabolism of amino acids that affects approximately 1 in 100,000 live births. This well-known inherited disorder occurs due to the absence of a deficient enzyme called Phenylalanine Hydroxylase (PAH). If left untreated, it may lead to several risks, like hyperpigmentation of the skin, intellectual disability, behavioral disorders and seizures. This article focuses on the treatments that effectively help in managing the disorder.

Keywords: Phenylalanine, Phenylalanine Hydroxylase, Amino Acid Metabolism Deficiency, Genetic Disorder

Introduction:

The normal functional aspects of the body are affected by any changes or defective metabolic pathways that result in the formation of metabolic intermediates that are toxic to the body and have adverse effects on the physiology of our body [1].these changes in the metabolic pathway are due to mutations in the genes that encode proteins or enzymes that play a key role in metabolism. The defect in the gene, which is caused by mutation or any other factors, is the driving reason behind this autosomal recessive genetic disorder, phenylketonuria, (PKU) [2]. This leads to the accumulation of toxic products or undesirable metabolic products. The enzyme Phenylalanine Hydroxylase (PAH) is the major factor that regulates the metabolism of phenylalanine. The enzyme regulates the conversion of phenylalanine to tyrosine. When the gene responsible for the production of PAH enzyme is mutated, it results in a deficiency or decreased enzymic activity [4].

Phenylalanine and Phenylalanine hydroxylase

Phenylalanine is an essential amino acid that can only be obtained by feeding on its dietary sources. It is metabolized into acetic acid and fumaric acid by having tyrosine and an intermediate metabolite[1]. On the other hand, tyrosine is yet another important metabolite that is involved in a network of biological reactions like the production of DOPA, melanin, thyroid hormones and other metabolites[3].

The enzyme Phenylalanine Hydroxylase (PAH) catalyzes the hydroxylation of phenylalanine to tyrosine. Tetrahydrobiopterin serves as the cofactor of PAH. The deficiency of PAH or its cofactor results in the accumulation of phenylalanine in the blood and tissues, leading to hyperphenylalanemia[6]. The PAH gene is located on the 12q22–12q24 chromosome, which was determined by mapping studies. Due to a defect in the above gene, PAH is not produced or deficient, leading to the formation of phenyl pyruvate, which is excreted in the urine[3].

Phenylketonuria classification

Depending upon the plasma concentration of phenylalanine, PKU is classified as Mild PKU, Moderate PKU & Severe/Classic PKU[4].

Treatment for PKU

Phenylalanine deficit diet

Patients with PKU are recommended to follow a diet that's completely devoid of phenylalanine since it's an essential amino acid that can only be obtained through eating habits[7]. If the quantity of phenylalanine amino acid is little to no, there is no need to metabolize it into its byproducts, so the doctors advise the patients to follow a strict diet. Foods high in protein, such as meats, fish, eggs, and dairy products, must be firmly limited for patients with PKU. Natural low-protein, high-starch meals like potatoes and some vegetables (like peas) are can be taken in moderation [9]. Protein consumption is severely restricted in PKU patients, so medical food substitutes that include the proper ratio of necessary amino acids, vitamins, minerals, and trace nutrients must be given to them. Tyrosine supplementation is an essential component of treatment since, on average, 90% of the dietary phenylalanine intake is transformed into tyrosine[5].Tyrosine is an important precursor for the synthesis of DOPA, Catecholomines and other metabolites[10].

Treatment with tetrahydrobiopterin

Apart from PAH, its cofactor, tetrahydrobiopterin, plays a vital role in phenylalanine metabolism[9]. The treatment of patients with a low diet of phenylalanine is ineffective for patients for whom there is a deficiency in tetrahydrobiopterin (BH4 deficiency). The BH4 test was performed to differentiate this subclass of the disorder[3]. For these patients, treatment with the cofactor BH4 or sapropterin has been proven successful. Though this treatment has been found, its efficacy is low, so doctors prefer other alternative methods[5].

Large Neutral Amino Acid Therapy (LNAA)

LNAA supplements may be used, particularly for PKU adult patients who are unable to adhere to a Phe-restricted diet[7]. LNAAs comprise the aromatic amino acids (tyrosine, tryptophan, threonine, methionine, and histidine) and the branched-chain amino acids (valine, leucine, and isoleucine) Depending on the plasma concentrations of these supplements, they compete to bind the LNAA transporters[3]. Elevated amounts of LNAA supplementation limit the amount of Phenylalanine that passes across the blood-brain barrier and block plasma Phenylalanine transport[8].

Enzyme therapy

Enzyme therapy, a PKU treatment strategy, modifies the metabolic profile of PAHs to lower elevated Phe levels in plasma[2]. PAH-fusion proteins and PAL-replacement treatment are two forms of enzyme therapy. On the other hand, oral PAL is linked to enzyme breakdown, whereas injectable PAL stimulates immunological responses[1]. Immune responses are reduced when polyethylene glycol and PAL are combined. In clinical trials, PKU gene therapy has produced encouraging outcomes[10].

Gene therapy

Over the last 20 years, PKU gene therapy has garnered significant attention in research, with advancements made in hepatic gene therapy through the use of adenoviral vectors in PKU mouse models[6]. However, reliable transduction of the gene in a variety of cell types has been demonstrated by lentiviral vectors, which makes them interesting targets for gene therapy. Given what has been shown in mouse skeletal muscle cells, muscle cells could potentially make appealing targets for gene therapy[8]. The potential for human PKU gene therapy trials stems from encouraging developments in gene therapy research[4].

Conclusion:

PKU is a hereditary condition caused by a lack of the enzyme phenylalanine. One in 100,000 live births has phenylketonuria. Phenylalanine hydroxylase (PAH) is an enzyme that catalyzes the hydroxylation of phenylalanine to tyrosine. Its deficiency causes PKU, a genetic disorder. Reduced or absent enzymatic activity is caused by a mutation in the PAH gene. Three categories of PKU exist: mild, moderate, and severe/classic. 90% of phenylalanine is converted to tyrosine during treatment, which involves a low-phenylalanine diet. LNAA supplements, enzyme therapy, and gene therapy are available forms of treatment. Enzyme therapy alters the metabolic profiles of PAHs, but LNAA supplements restrict the transfer of phenylalanine. Clinical trials on

gene therapy have yielded encouraging results, especially with regard to lentiviral vectors.

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FISHERIES EDUCATION AND IT'S NEW BRANCH

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Abstract:

Education in fisheries refers to all instruction in fishery resource management, including sustainable production, conservation, processing, and trading. By utilizing resources, contemporary cultural systems, cutting-edge harvesting and post-harvesting procedures, marketing, and the social growth of the fishing sector, fisheries education works to improve fish productivity. Professional fisheries education, a burgeoning field, offers immense future potential. Graduates can find employment in academic institutions, industry research facilities, and state and federal fisheries departments, with a likely increase in the number of degree holders. The graduates appear to need a more advanced system of administrative practices and more actual experience with commercial operations. Currently, our country requires technically skilled fisheries professionals to meet future industry challenges. However, the traditional teaching methods lack strength due to outdated tools, causing a disconnect between coursework and practical application. Consequently, students often lack crucial problem-solving skills necessary for rational judgment and planning in higher fisheries education. Revitalized by the Fifth Deans' Committee, India's agricultural education aims for excellence and relevance. By 2022, fisheries schools are expected to produce about 2,820 M.F.Sc. and 220 Ph.D. graduates annually.

Keywords: Fisheries Education, Sustainable, Professional Fisheries

Introduction:

Ensuring sustainable nutritional security is crucial for developing nations. The marine and aquatic environments offer abundant, protein-rich food. With India's population projected to reach 1.6 billion by 2050, boosting food production is vital. Fisheries are significant for income, employment, subsidiary industries, affordable nutritious food, and foreign exchange [1]. The Indian government launched two All

India Fisheries Training Courses in 1945 and established the Central Institute of Fisheries Education (CIFE) in Bombay in 1961 and the Central Institute of Fisheries, Nautical and Engineering Training (CIFFNET) in Cochin in 1963. Today, India has 18 fisheries colleges, two fisheries universities (Kerala and Tamil Nadu), and CIFE Mumbai, conferred deemed university status by the UGC in 1989.

India's fisheries education has rapidly advanced, leading to a project funded by the National Agricultural Innovation Project to develop e-courses for the B.F.Sc. degree program. Involving the College of Fisheries, Mangalore, and the Fisheries College and Research Institute, Thoothukudi, this initiative aims to create e-learning modules to enhance traditional classroom teaching. This approach addresses limited teacher-student interaction and poor comprehension by blending classroom instruction with self-directed learning, keeping teachers updated on industry advancements and improving student-teacher interactions [2].

Objectives

- To comprehend the significance of Fisheries education in our lives.
- Boost fish production through modern culture systems, advanced harvesting techniques, effective marketing, and social progress for the fishing community.
- Prepare fishery science graduates for careers in government, NGOs, private businesses, banking, insurance, and self-employment within the fishing industry.
- Offer students opportunities for higher studies leading to postgraduate and Ph.D. degrees in both state and non-state institutes.

Goals

1. To explore the new frontier of knowledge in fisheries.
2. Transfer technology regionally through high-quality education and training in fisheries, aquaculture, and related industries.
3. Conduct research to address technological gaps in fisheries management, aquaculture, and related sectors to enhance regional socio-economic conditions.

Professional fisheries education in India

The fisheries sector is crucial to India's economy and the livelihoods of fisherfolk, supporting about 20 million people, including 7 million in indirect employment. Recognizing its economic significance, the Union Government has prioritized developing trained manpower for fisheries development. Professional fisheries education began in 1969 with the establishment of the first College of Fisheries in Mangalore, offering

B.F.Sc., M.F.Sc., and Ph.D. programs. The Fisheries College and Research Institute, Thoothukudi, established in 1975, also offers these programs. Currently, India has 18 Fisheries Colleges, including 14 under State Agricultural Universities, all providing comprehensive fisheries education. India currently has 30 professional fisheries colleges, including two Central Universities: Dr. Rajendra Prasad Central Agricultural University (Dr. RPCAU) in Samastipur, Bihar, and Central Agricultural University (CAU) in Imphal, Manipur. Eleven colleges offer only the four-year B.F.Sc. undergraduate course, while sixteen offer both B.F.Sc. and the two-year M.F.Sc. postgraduate courses. Seventeen colleges also offer Ph.D. programs, which include one year of coursework alongside research. All colleges operate on a semester system. The College of Fisheries, Kochi, originally under Kerala Agricultural University, is now part of the Kerala University of Fisheries and Ocean Studies (established in 2011). Tamil Nadu Dr. J. Jayalalithaa Fisheries University, founded in June 2012, includes a constituent college in Nagapattinam focused on Fisheries Engineering-a unique initiative in India aimed at training engineers for the fishing industry. Projections indicate a rising annual output, targeting approximately 2,820 B.F.Sc., 450 M.F.Sc., and 220 Ph.D. graduates by 2022 [3,4].

Dean Committee on fisheries

The Committee on Fisheries (COFI), under the FAO Council since 1965, advises governments, regional fishing agencies, civil society, and the commercial sector on international policies for resource sustainability and biodiversity conservation. In India, the Indian Council of Agricultural Research forms Deans Committees to enhance educational standards by revising course curricula. Stakeholder input from agricultural education at universities and colleges ensures graduates are prepared for self-employment, rural livelihoods, food security, sustainable agriculture, and entrepreneurial roles, emphasizing essential skills and innovation.

Fifth Deans' Committee report

Chaired by Prof. R. B. Singh, the Fifth Deans' Committee has updated curricula, course contents, and degree nomenclature while advocating reforms in admission, assessment, pedagogy, faculty requirements, and governance to enhance excellence and relevance in fisheries education. Since 2016–17, its recommendations have been widely adopted by professional fisheries colleges across India, with a few exceptions among private institutions. The Bachelor of Fisheries Science (B.F.Sc.) course spans four years,

with the first three years focused on coursework and the final year dedicated to the Student READY Programme across different departments [5].

Minimum requirements

Course name: Fisheries

Course level: Bachelor of Fisheries Science (B.F.Sc.)

Master of Fisheries Science (M.F.Sc.)

Eligibility criteria:

1. **Bachelor of Fisheries Science (B.F.Sc.)** - 10+2/Intermediate with PCMB/PCB/Agriculture (P-Physics, C-Chemistry, M-Mathematics, B-Biology) from a recognised Board / University with minimum 50% marks in science stream.
   Duration: 4 years
2. **Master of Fisheries Science (M.F.Sc.)** - The student should have a Bachelor's degree in science with 50% minimum marks.
   Duration: 2 years

The medium of instruction: English

Minimum intake: 40 students per year

Admission process: Merit based/entrance exam

Examination types: Semester System

Departments

1. Department of Aquaculture (AQ)
2. Fisheries Resource Management (FRM)
3. Department of Aquatic Animal Health Environment (AAHM)
4. Department of Aquatic Environment Management (AEM)
5. Department of Fish Processing Technology (FPT)
6. Department of Fish Engineering (FE)
7. Department of Fisheries Extension Economics & Statistics (FEES)

Deemed university

The ICAR-Central Institute of Fisheries Education in Mumbai, established in 1961, is India's leading Deemed University specializing in fisheries education. Originally offering a two-year PG Diploma in Fisheries Science, it now exclusively offers M.F.Sc. degrees in 11 specializations such as aquaculture and fisheries biotechnology. Admission to M.F.Sc. and Ph.D. programs is through national-level tests conducted by

ICAR and CIFE respectively. The institute phased out its two-year B.F.Sc. program in 1998–1999 to focus on expanding master's degree offerings [7].

Table 1: Course offered in M.F.Sc. and Ph.D.

SI. No.	Specialization in M.F.Sc./ Ph.D.
1.	Aquaculture
2.	Fish Nutrition and Feed Technology
3.	Fish Physiology and Biochemistry
4.	Fish Genetics and Breeding
5.	Fish Biotechnology
6.	Fisheries Resource Management
7.	Aquatic Animal Health Management
8.	Aquatic Environment Management
9.	Post-Harvest Technology/ Fish Processing Technology
10.	Fisheries Economics
11.	Fisheries Education

(Source: Fifth Dean' Committee Report, ICAR, 2017) [6]

Carrier opportunities in fisheries

For those who have graduated in fisheries, there are numerous job prospects in India in the public, private, and research sectors. The following is a list of fisheries careers.

Teaching and Research

B.F.Sc. graduates can work as biochemists, technicians, research assistants, and biologists in academic institutions. M.F.Sc. graduates often become Assistant Professors in fisheries departments at institutes and colleges. Passing the ICAR Agricultural Research Service (ARS) exam qualifies individuals to become scientists at top Central Research Institutes such as CMFRI, NFDB, CIFT, CIFA, CIFE, and CIBA.

Government sector

Fisheries graduates have numerous job opportunities in state and central government fisheries departments, including roles such as District Fisheries Development Officer, Fisheries Extension Officer (FEO), and Assistant Fisheries Development Officer (AFDO). UG and PG graduates can also seek employment in agencies like Fisheries Survey of India (FSI), NABARD, FAO, WHO, MPEDA, NIO, and NACA. PG holders in Fisheries Science are eligible for positions like Assistant

Superintendent of Fisheries (ASF), Fisheries Officer (FO) and Assistant Fisheries Officer (AFO).

Private sector

Fishery graduates from B.F.Sc. and M.F.Sc. programs have diverse career opportunities in the private sector. They can work in quality control labs, fish breeding farms, seafood processing companies, pharmaceutical firms, fish farms, fishing industries, hatcheries, and seafood export businesses. National and commercial banks in India also hire them as agriculture officers and fisheries officers [8].

Jobs in foreign countries

Fisheries experts find abundant job opportunities in foreign countries like China, Japan, the United States, Australia, Canada, several nations in Africa, and Europe. Private companies, export-oriented businesses, and colleges abroad often recruit graduates in the fishing industry. Research scholars also have promising career prospects in overseas research centers.

Self – employment

Graduates with a B.F.Sc. or M.F.Sc. degree can launch their own fisheries and aquaculture businesses, fish processing and marketing businesses, feed manufacturing businesses, and other ventures to become entrepreneurs.

Non-fisheries employment

Fisheries graduates can pursue administrative positions in federal and state government departments through competitive exams. Additionally, some graduates find employment in non-technical roles at commercial banks.

Prospective after an education in fisheries

- Many positions in the department of fisheries.
- Faculty in Indian colleges for fishery.
- Small-scale fisheries-related enterprises include aquaculture businesses.
- Fisheries Subject Matter Expert at Krishi Vigyan Kendra.
- Marketing departments of several banks.
- Fisheries scientists are employed in central government fisheries research institutes and Agricultural University research stations.
- Management of seafood quality in the fish processing industry [9].

Fisheries related other courses

Several Indian universities offer specialized subjects like "Fisheries," "Fishery Biology," or "Ichthyology" within their M.Sc. programs in Zoology. Examples include Mangalore University's M.Sc. in Biological Sciences and Madurai Kamaraj University's M.Sc. in Bio-Sciences, which include coursework related to fisheries science. Many of these universities also provide opportunities for research-oriented Ph.D. programs focused on fish or fisheries. Students can pursue doctoral studies at institutions such as the National Institute of Oceanography (NIO), Goa, and central fisheries institutes. However, these degrees are housed within broader fields such as zoology, marine science, marine biology, bio-sciences, or biological sciences, rather than being specifically labeled as fisheries science programs [10].

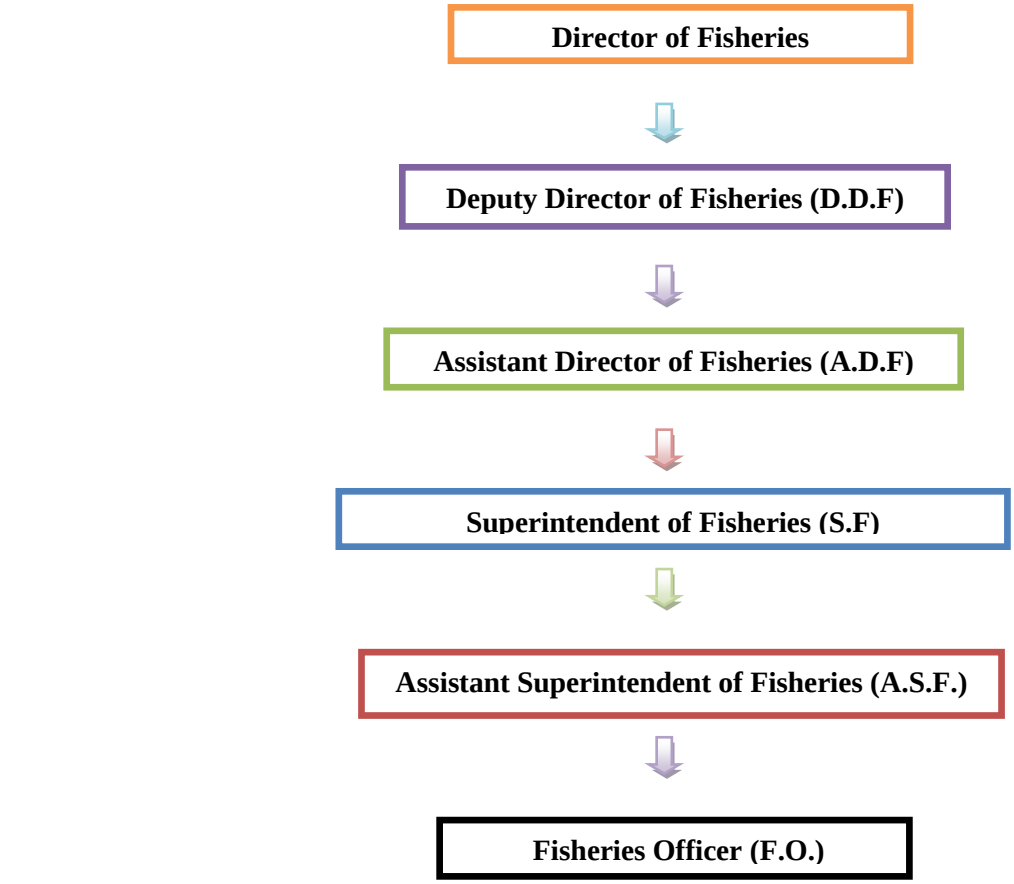
In addition to the Professional Fisheries Colleges, some of the institutions listed below in Table 2 also offer courses in India connected to fisheries.

Table 2: Professional Fisheries Colleges in India

SI. No.	Name of the College/University	Courses Offered
1.	The Indian Institute of Technology, Kharagpur, West Bengal	4 Year B. Tech. Degree programme in Naval Architecture & Marine Engineering M.Tech. & Ph.D. programme in Aquaculture Engineering
2.	The College of Engineering, Waltair	B.E. degree with Fishery Engineering & Naval Architecture as special subject.
3.	Indian Institute of Technology, Chennai	M. Tech in Coastal Engineering
4.	The University of Madras	PG diploma courses in Coastal Aquaculture

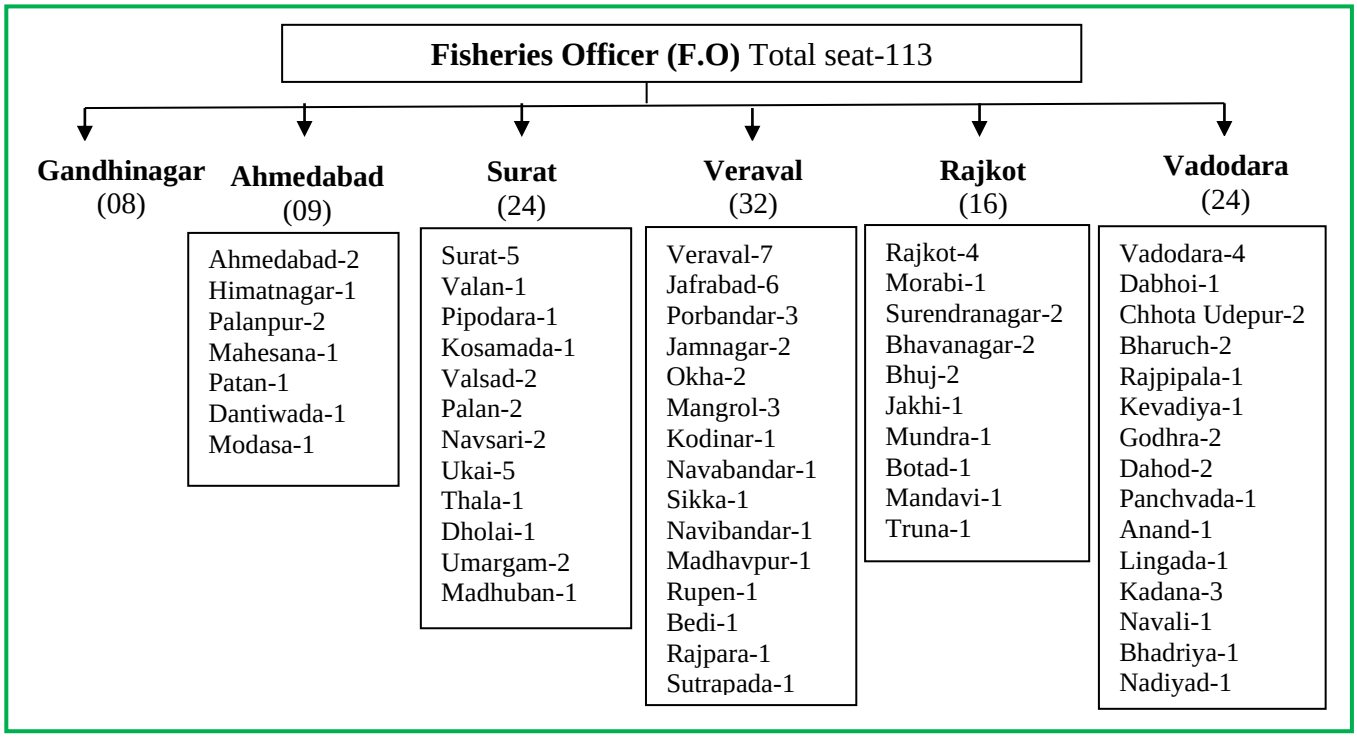
(Source: Handbook of Fisheries and Aquaculture, 2011 and update based on personal communication) [1]

Organizational chart for the department of fisheries



Assistant Superintendent of Fisheries (A.S.F) Total seat-109

Gandhinagar (15)	**Ahmedabad** (12)	**Surat** (25)	**Veraval** (18)	**Rajkot** (14)	**Vadodara** (25)
	Ahmedabad-4 Himatnagar-2 Prantij-1 Palanpur-1 Khedbrahma-1 Patan-1 Dharoi-1 Dantiwada-1	Surat-3 Kakarapar-1 Valan-1 Pipodara-1 Kosamada-1 Valsad-4 Palan-1 Motiwada-1 Navsari-3 Ukai-2 Thala-1 Dholai-1 Fadvel-1 Umargam-2 Madhuban-2	Veraval-5 Junagadh-1 Jafrabad-2 Porbandar-4 Jamnagar-3 Salaya-1 Okha-1 Mangrol-1	Rajkot-4 Bhadar-1 Morabi-1 Surendranagar-1 Bhavanagar-2 Bhuj-2 Jakhi-2 Mundra-1	Vadodara-4 Dabhoi-1 Umarwada-1 Chhota Udepur-1 Bharuch-4 Rajpipala-1 Kevadiya-1 Godhra-2 Panam-1 Dahod-1 Anand-2 Lingada-1 Kadana-1 Navali-1 Bhadriya-1 Nadivad-2



Conclusion:

The expansion of fisheries education to include new branches ensures graduates are well-prepared for the industry's challenges. By integrating technology, sustainability, policy, global perspectives, and practical experience, these programs produce skilled professionals ready to contribute to sustainable fisheries management and foster future innovation.

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METHODS OF MILKING AND FACTORS AFFECTING OF MILKING

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Introduction:

The amount and quality of milk produced at the dairy farm are influenced by the milking employed. Proper milking technique not only help to avoid damage to the teat and udder and improve udder health but also ensure that all of the milk that is present in the udder is extracted, maximizing milk production during lactation. To optimize milking, it is important to reduce disturbances to the cows, such as loud noises, staff movement during milking, and occasionally holding the milk

Milking methods

Teats may be milked either diagonally or by the forequarters first, followed by the hind quarter. To prevent teat injuries, they should be squeezed out rather than dragged. The initial few strips of milk from each teat are collected on strip-cup to check for mastitis and to discharge any bacteria that may have entered and accumulated in the teat canal. Dry milking is preferred because it avoids water contamination. Wet milking, where the udder is wetted beforehand, is not recommended since the water can introduce contaminants. The different milking methods are:

1. Stripping method
2. Full hand method
3. Knuckling method
4. Machine milking

1. Stripping method

Stripping involves firmly grasping the teat at its base between the thumb and forefinger and drawing them down along the entire length of the teat. This simultaneous pressing causes the milk to be ejected and flow down in the streams. The process is repeated continuously. Typically, the stripping method is used for small cows with narrow teats. It is also employed to extract the last strips of milk, which contain more fat. Combining initial full-hand milking with stripping at the end is an effective milking method.

2. Full hand method

The full hand method is considered the best technique as it minimizes injuries to the teats. This method involves encircling the teat with the thumb and index finger at the point where it meets the udder, then closing the rest of the teat with the remaining fingers and pressing it against the palm on both sides. Full hand milking mimics the natural suckling of calf and is used for cows with large teats and buffaloes. It removes milk more quickly than stripping because there is no time lost in changing the hand position. Even with efficient milking methods only about 85% of milk is extracted from udder, leaving 15% residual milk.

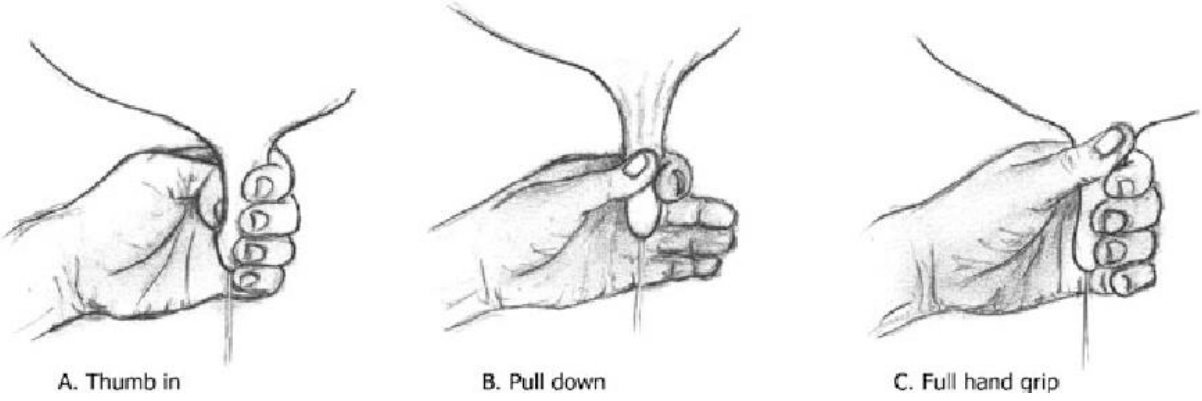

Figure 1: Methods of Milking

3. Fisting/Knuckling

This method involves pressing the thumb against the teats while they are held between thumb and fingers. It can cause injury to the teat. The initial strips of milk from each quarter should not be discarded because they contain the highest number of bacteria. This method is not advisable and is least recommended techniques.

4. Machine milking

In most western and industrialized countries, modern milking machine are widely used. These machines employ alternating negative and atmospheric pressure through a double- chambered teat cup assembly. A continuous partial vacuum is maintained inside the inflatable rubber tube or teat cup liner, where the teat is inserted. A pulsator alternates partial vacuum and normal atmospheric pressure in the space between the rubber liner and the metal shell of the teat cup. When negative pressure is applied between the liner and the shell, milk flows from the teat. When atmospheric pressure enters the chamber, the rubber liner collapses, compressing and massaging

the teat. Continuous vacuum without alternating pressure would cause congestion and irritation of the teats.

Ideal practices used in machine milking

- Prepare the cow by gently wiping and massaging the udder and teats for 30 to 60 seconds using a cloth dipped in warm, mild antiseptic solution.
- Conduct a strip cup test: draw the first milk from each quarter into the strip cup and inspect it for any abnormalities.
- Put on the teat cups promptly
- It is a good practice using a timer to avoid over use of machine.
- Release the vacuum first. Lower the teat cups and massage the udder by hand.
- Apply teat dips to protect the teats.
- Record the milk weight.
- After each milking session, thoroughly clean the milking machine.

Factors affecting milk yield and composition of milk

Variations in milk content and daily production are common in all milking animals. There are two elements that influence milk yield

- Physiological factors include genetics, age, pregnancy, and more.
- Environmental factors, including climate, nutritional status, management, and so on.

Factors affecting milk production:

Normally, milk production increases during the first six weeks of lactation before gradually decreasing. A variety of factors influence the volume of milk

Physiological factors

1. Species: Milk yields vary by species. Buffalo produce more than the typical pure indigenous dairy cattle

2. Individuality of animal: The strain and individuality of a cow within a breed also affect total production. A larger cow typically produces more milk. A cow can secrete up to 8% of its body weight in milk each day.

3. Dry period: Dairy cows normally dry off for two months before their next calving. A rest interval is required to enhance milk production in following lactation. Milk output is typically lowered when the dry period lasts fewer than 40-60 days (25-40% less milk). A dry season lasting more than 60 days does not result in a considerable rise in

milk production. Long dry times reduce the average annual production of the cow by extending the calving interval beyond the normal 13–14-month interval.

4. Age and body weight at calving: The amount of milk produced by the cow rises with each successive lactation (age). This is related to a rise in body weight, which leads in a larger digestive system and a larger mammary gland responsible for milk production. Recurrent pregnancies and lactations can contribute to increased milk production with age.

5. Lactation number: Milk output increases with lactation number, peaking in the fourth or fifth lactation. This is due to the rising development and size of the udder, as well as the increased body size compared to the first lactation animal. The estimated mature yield (mature equivalent) of a primiparous cow (meaning a cow giving birth for the first time), calving at two years of age can be estimated by multiplying yield of first lactation by 1.3. First lactation = 1300 kg. Fourth/ fifth lactation = 1300 X 1.3 = 1690 kg

6. Season of calving: This is most likely due to an interaction between daylight and ambient temperature. Seasonal disparities have diminished as a result of improved dairy cow diet and management.

7. Temperature & Humidity: The effect of ambient temperature on milk output varies by breed. Holsteins and other larger breeds are more tolerant of lower temperatures, whereas smaller breeds, particularly Jerseys, are significantly more tolerant of high temperatures. The optimal temperature for a Holstein cow is around 10 °C. When the temperature rises above 27 °C, milk output declines. The decline in milk output is primarily attributable to a decrease in feed intake. High temperatures have a greater

Environmental factors

1. Feed and water supply: Inadequate feed nutrients are likely to decrease milk production. Galactopoisis is strongly linked to a sufficient feed intake by the lactating animal. The most striking effect is caused by a lack of water, which the cow has no way of storing. Withholding access to water or a lack of water for a few hours will result in a quick decline in milk production.

2. Milking frequency: Cows are typically milked twice daily. Milking twice a day produces at least 40% more milk than once a day. Increasing milking frequency to three times per day improves milk output by up to 20% (range 5-20%). The rise is usually greatest for the first lactation cow and decreases as the cow becomes older

3. Milking intervals: Cows are normally milked at equal intervals (12 hours for two milkings). Cows milked at uneven intervals make less milk than cows milked at equal intervals. The decline in milk yield is greater in high-producing cows than in low-producing ones. Incomplete milking on multiple consecutive days might permanently lower milk supply during the lactation. The average milking duration is 5-6 minutes per cow.

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THE ROLE OF OXIDATIVE STRESS IN HUMAN PATHOLOGY WITH A SPECIAL ATTENTION TO THYROID DISORDERS

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Abstract:

Oxidative stress is caused by the production and accumulation of oxygen-reactive species (ROS) in cells and tissues, as well as an imbalance in the biological system's ability to detoxify these reactive byproducts. Although ROS are often produced as by-products of oxygen metabolism and can perform several physiological activities, including cell signalling, in addition to this, xenobiotics (such as antiblastic medications) and environmental stressors (such as UV, ionising radiations, pesticides, and heavy metals) also significantly boost ROS production, resulting in the imbalance that causes damage to cells and tissues (oxidative stress). A biological system's capacity to detoxify these reactive products is out of balance, which leads to the condition known as oxidative stress. Lipid peroxidation is elevated due to the pro-oxidant impact of thyroid hormones. The various systemic signs of hyperthyroidism, such as myopathy and cardiac insufficiency, may be caused by lipid peroxidation, a very harmful process linked to numerous disorders. Hyperthyroidism and hypothyroidism also substantially impact the functions of antioxidant-scavenging enzymes that prevent lipid peroxidation, such as glutathione peroxidase, catalase, and erythrocyte superoxide dismutase. Therefore, further research is required to determine how oxidative stress-related signalling might affect thyroid problems to develop efficient therapeutic measures for both prevention and treatment.

Keywords: Oxidative Stress, ROS, Thyroid Disorders, Antioxidants, Environmental Toxicants.

Introduction:

In the present scenario, harmful Environmental toxicants affect human physiology by inducing the cells to generate reactive oxygen species, which enhance oxidative stress. Free radicals are frequently produced internally by intracellular metabolism when the antioxidant mechanisms become exhausted, or externally by

contaminants like cigarette smoke. The two sources of ROS exposure are endogenous and exogenous, respectively. The increase in ROS generation in cells may be caused by a variety of environmental factors, including exposure to cigarette smoke, UV radiation, heavy metal ions, ozone, allergies, medications or poisons, pollutants, pesticides, or insecticides (Antunes dos Santos *et al.,* 2018; Mahajan *et al.,* 2019; Tobore, 2019). ROS are produced in mitochondria during aerobic metabolism. ROS are produced in mitochondria during aerobic metabolism. (Rodriguez & Redman, 2005). Oxidative metabolism and ROS generation inside mitochondria are intimately related, and ATP synthesis is known as oxidative phosphorylation. The coupling of these processes serves as the primary energy source in aerobic organisms (Papa, 2012). Mitochondria not only serve as important ROS producers but also as important ROS receivers. Illness is caused by covalent and enzymatic changes to proteins during or after protein creation, during protein cleavage or breakdown, and by oxidative damage and mitochondrial breakdowns. These post-translational changes collaborate with other messengers, like as free radicals, to regulate mitochondrial activity. (HU *et al.,* 2016). In each cycle of ATP generation, 0.2–5% of the electrons pass via ETC due to the leaky nature of oxidative phosphorylation. This results in a partial O_2 decrease (Hamanaka *et al.,* 2013). The primary endogenous sites for the production of cellular redox-reactive species, including ROS and reactive nitrogen species (RNS), are the mitochondrial electron transport chain (ETC), endoplasmic reticulum (ER), peroxisomes, membrane-bound NADPH oxidase (NOX) isoforms 1–5, dual oxidases (Duox) 1 and 2 complexes, and nitric oxide synthases isoforms 1–5. (NOS1–3). The mitochondrial ETC complexes I and III produce superoxide anions (Rodriguez & Redman, 2005). In the past thirty years, oxidative stress has become popular in medical sciences. It actively participates in the physiology of many common disorders, including Alzheimer's, Parkinson's, high blood pressure, preeclampsia, atherosclerosis, and acute renal failure. Reactive species of oxygen (ROS), which the cells produce by metabolizing oxygen, have the potential to be hazardous. Under typical conditions, the rate of oxidant removal balances out the rate and amplitude of oxidant creation. Oxidative stress is brought on by a lack of pro- and antioxidant equilibrium. High ROS concentrations in biological cells have a significant influence on how well they operate, which can result in faulty cell function, aging, or illness (Sharifi-Rad *et al.,* 2020)

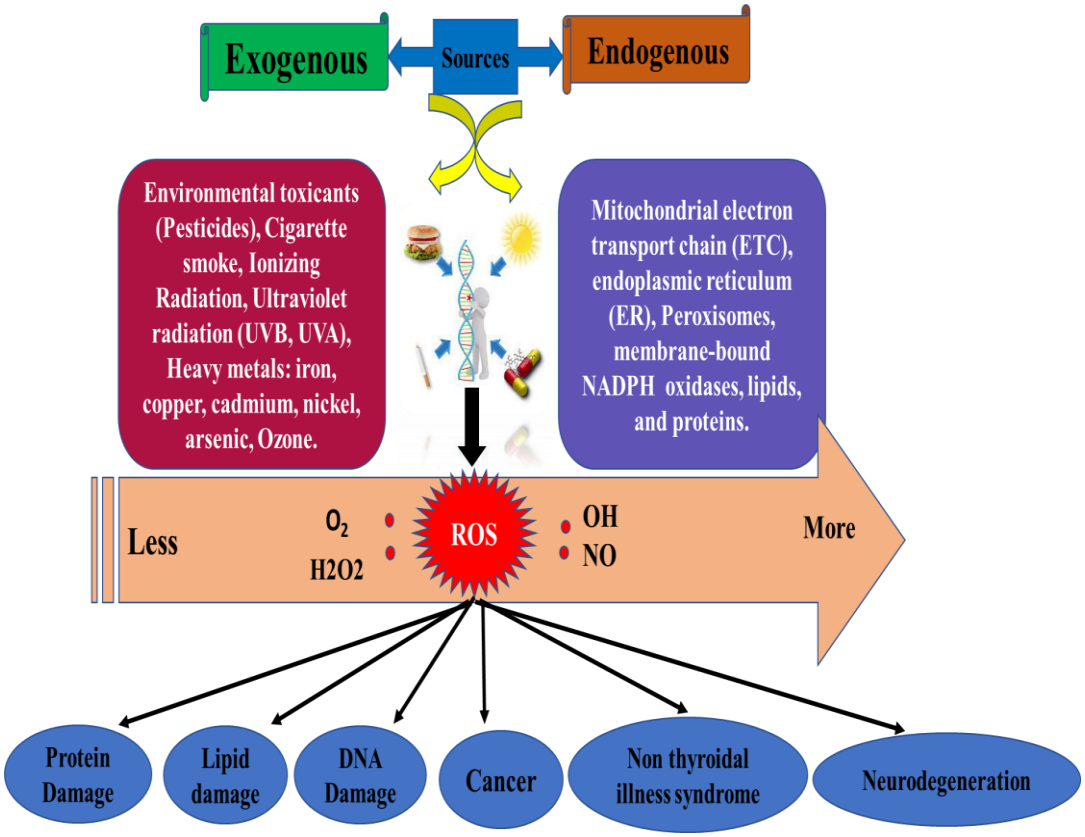

Figure 1: Impacts of ROS Via different sources

Oxidative stress:

Oxidative stress is the result of a discrepancy between the body's ability to neutralize reactive oxygen species (ROS) and their production. Damage to cell structures and components including lipids, proteins, and DNA results from an imbalance between too many reactive chemicals and a poor endogenous defense system, which eventually aids in the development of a variety of illnesses. When present in the proper low concentrations, ROS function as molecules that drive signal transduction within cells and also protect them (van der Vliet *et al.,* 2018). However, if produced in excess as an inflammatory response, ROS can cause the emergence of additional highly reactive species (van der Vliet *et al.,* 2018). Notably, the oxidative modification of necessary enzymes or regulatory sites is crucial because redox modification causes cell signaling to change and causes programmed cell death and oxygenative stress. Reactive oxygen species (ROS) generation and neutralization capacity are out of balance in living things, which causes oxidative stress. Damage to cell structures and molecules is caused by the discrepancy between too many reactive chemicals and inadequate endogenous defense. Inflammation and oxidative stress are intimately related. Oxidative stress can lead to inflammation, which can lead to

oxidative stress (Ishibashi, 2013; Petrie *et al.,* 2018), creating a vicious cycle that damages cells and fosters an inflammatory environment (Ng *et al.,* 2012). Reactive oxygen species' systemic expression and a biological system's ability to promptly detoxify the reactive intermediates or repair the resulting damage are not matched, which leads to oxidative stress. A cell's natural redox state can be upset, producing peroxides and free radicals that damage all of the components of the cell, including DNA, lipids, and proteins, and can have harmful effects. The effects of oxidative stress from oxidative metabolism include nucleotide damage and DNA strand breaking. Reactive oxygen species, such as the superoxide radical O_2 hydroxyl radicals and the H2O2 (hydrogen peroxide), which are produced, are mostly indirect causes of damage (van der Vliet and Janssen-Heininger, 2014). In addition, several reactive oxygen species function as messengers for cells in redox signalling. As a result, oxidative stress may interfere with cellular signalling's regular functioning. The development of attention deficit hyperactivity disorder, cancer, Parkinson's disease, Lafora disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction, fragile X syndrome, sickle-cell disease, and lichen planus are all believed to be influenced by oxidative stress in humans. Reactive oxygen species, however, can be helpful since the immune system uses them to combat and eliminate microorganisms in conditions including vitiligo, (Birnboim, 1986) autism, (Arican and Kurutas, 2008) infection, chronic fatigue syndrome, (James *et al.,* 2004) and depression. (Kennedy *et al.,* 2005). Through the stimulation of a process known as mitohormesis, (Jimenez-Fernandez *et al.,* 2015) short-term oxidative stress may also play a significant role in the prevention of aging. It is also necessary for plants to start their stress response mechanisms. (El-Bahr and El-Deeb, 2016) Normally, mitochondrial ETC transfers electrons for oxygen reduction to water, however 1% to 3% of the time, electrons escape from this system and generate superoxide" (Homa *et al.,* 2019). Other than this, Humans include a variety of internally produced ROS, such as: During the I oxidative burst from phagocytes (white blood cells), Bacterial and viral destruction and foreign protein denaturation; (ii) xanthine oxidoreductase (XOR) metabolism; (ii) arachidonate pathways; (iii) peroxisome metabolism; (iv) detoxification of harmful chemicals (i.e., strenuous exercise, persistent inflammation, and infections) (Birben *et al.,* 2012).

Antioxidants

Antioxidants have the function of neutralizing free radicals in biological cells, which have a detrimental effect on living things. The enzyme known as superoxide dismutase (SOD) has a specific function in reducing the consequences of oxidative stress caused by the presence of free radicals. These metalloenzymes, which control oxidation processes in living cells primarily, have a subunitary structural organization. The oxygen radicals' recombination process is catalyzed by this enzyme. SOD is effective in treating a variety of pathological conditions in the human body as well as in preventing them from occurring the formation of hydrogen peroxide and triplet oxygen. Antioxidants reduce oxidative stress-related damage by breaking down radical chain events (Da Pozzo *et al.,* 2018). Their function necessitates operating in both hydrophilic and their chemical makeup is highly diverse as a result of their hydrophobic cellular habitats. Antioxidants can be enzymatic or non-enzymatic (Madhu *et al.,* 2022). But from a nutritional standpoint, a distinction between endogenous and exogenous classes is more instructive. All antioxidants that cells can produce from smaller building components are included in the first class. As a result, all enzymatic antioxidants as well as certain non-enzymatic ones (such as thiol antioxidants and coenzyme Q10) are endogenous. Exogenous antioxidants, on the other hand, must be consumed through the food because eukaryotic cells are unable to synthesize them. Therefore, this last point requires special consideration as this is the part of cellular redox balance that is most uncertain. Depending on how soluble they are, antioxidants may be categorised into two groups: liposoluble and water soluble (Lazzarino *et al.,* 2019). Because the vegetables and fruits that contain these antioxidants also include water, the water-soluble antioxidants are best absorbed in the body. They are, however, quickly removed from the body through the urine. Polyphenols are a kind of water-soluble antioxidant, as is vitamin C. (Lazzarino *et al.,* 2019)Antioxidants that are absorbed in the presence of lipids are known as liposoluble or fat-soluble antioxidants. As a result, the body is unable to absorb and use these antioxidants in the absence of lipids. It's crucial to remember, though, that they are difficult to eliminate from the body and can build up over time, surpassing the recommended threshold. An illustration of a fat-soluble antioxidant is vitamin E. (Lazzarino *et al.,* 2019; Vona *et al.,* 2021).

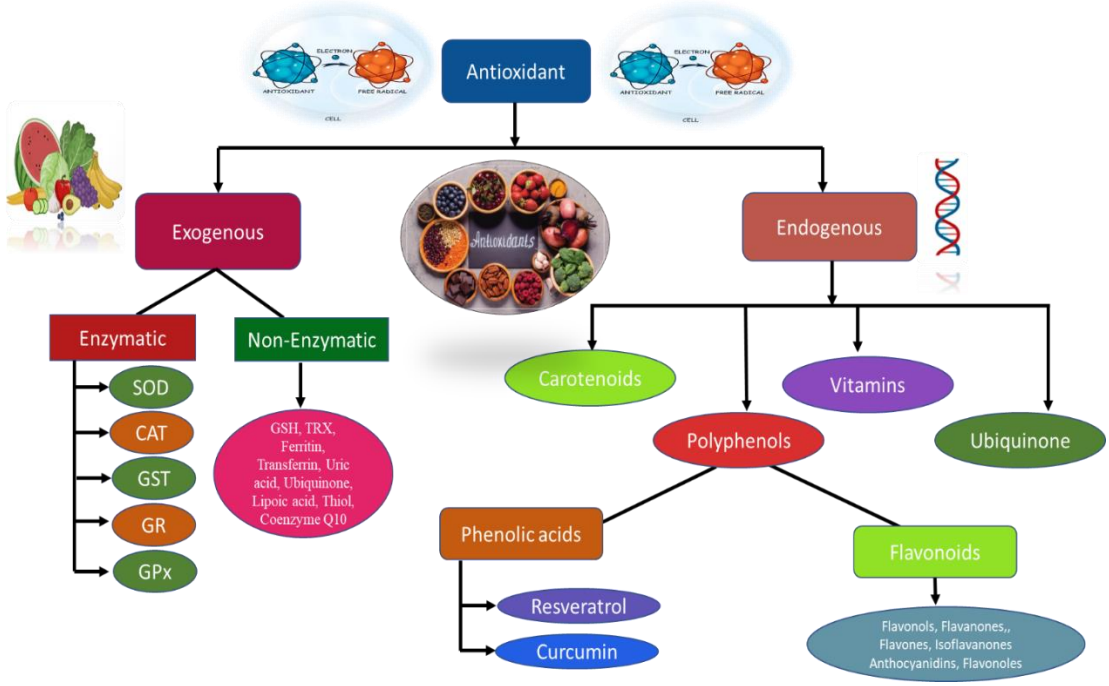

Figure 2: Different types of Antioxidants: Exogenous and Endogenous

Oxidative stress in thyroid gland:

Iodine complexes (IC) are a class of signal transduction based on the inclusion of an organic molecule formed from the element iodine with one to four iodine atoms, generally the tyrosine amino acid. The thyroid gland is the primary source of iodine elements in the systems of vertebrates. It produces considerable amounts of tetraiodothyronin (thyroxin, T4) and a considerably lower triiodothyronin quantity, which are released into the bloodstream (T3). These two substances work at the cellular level by attaching to specific receptors that connect to both genomic and nongenomic signalling pathways. In addition to these processes, thyroid hormones (THs) undergo several changes in peripheral tissues, mostly deiodination but also decarboxylation, which result in a variety of derivatives with signalling potential (Gems and Partridge, 2008; Segal, 2005). These distal conversions may even produce local levels of certain derivatives that are higher than the levels of THs that are in circulation (Wrzaczek, 2021). THs have a broad range of actions that affect almost every tissue in the body. The many ICs formed from the THs have unknown behaviours that appear to vary significantly. For instance, thyronamines counter its effects (Empson *et al.,* 2007), at least at the mitochondrial level, whereas diiodothyronin (T2) generates metabolic effects comparable to those of T3 (Kasagi *et al.,* 2009)Further research into this component of thyroid physiology is required. The known effects of THs may be roughly

divided into two categories: the regulation of metabolism and the regulation of growth and development. ROS generation and oxidative stress in the body are intimately related to the metabolic consequences of THs. First, the overall metabolic impact of THs and IC in general is a relative acceleration of the basal metabolism, which includes a rise in the rate of metabolism responses, both catabolic and anabolic (Rostami *et al.,* 2013). Increased oxygen intake, respiratory rate, fuel mobilization, fuel oxidation for energy extraction, and heat generation and release are the effects (El Hassani *et al.,* 2019)). It seems sense that increasing the respiratory rate would increase the generation of ROS, but as was already said, there is no linear relationship between these two factors. The inconsistent results in the studies might be explained by the fact that the THs simultaneously alter many oxidative stress-related factors, resulting in distinct, even opposing effects reports on oxidative stress and hypo- and hyperthyroidism exist in the literature. For instance, hypothyroidism has been linked to oxidative damage in metabolically active tissues that is either unaffected (Ameziane-El-Hassani *et al.,* 2016; Frijhoff *et al.,* 2015; Massart *et al.,* 2013) reduced (Ates *et al.,* 2018; Sies, 2017) or increased (Radák *et al.,* 2002).

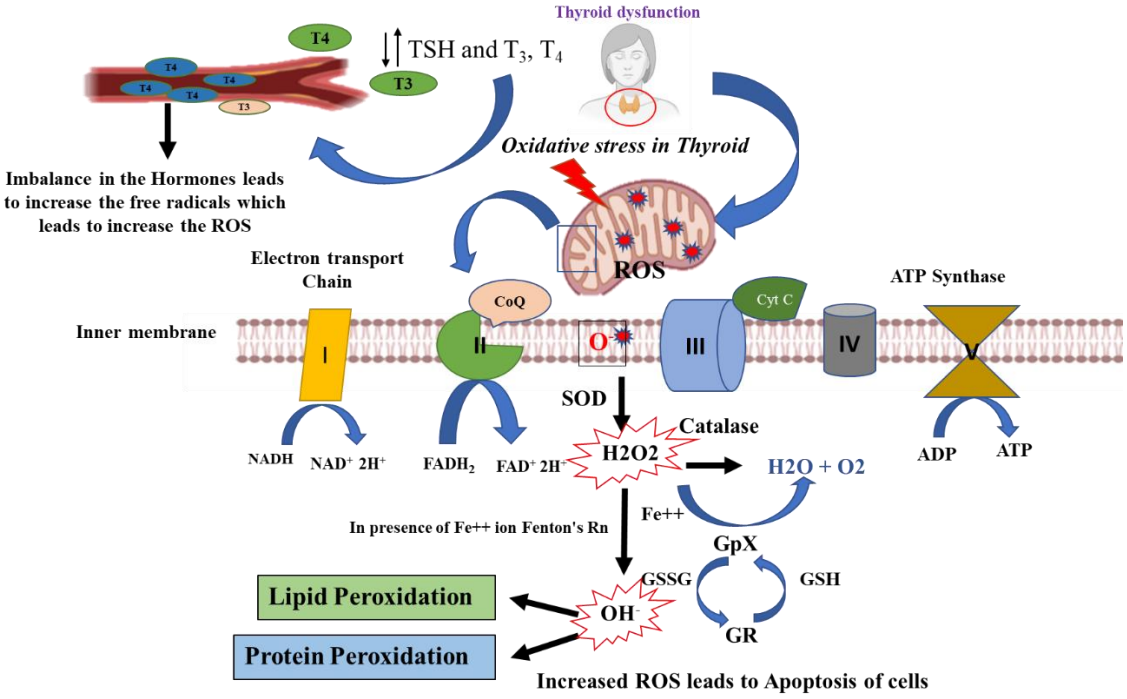

Figure 3: Mechanism Showing Oxidative Stress in Thyroid Gland

Mechanical oxidative signaling and the function of ROS in the thyroid:

In order for the thyroid to function normally, ROS is crucial. Oxidase synthesis is catalyzed by releasing enzymes from thyroid cells (Ameziane-El-Hassani *et al.,* 2016).

Inositols are also involved in the generation of thyroid hormones and proper thyroid function, triggering a series of events that include controlling TSH-dependent signalling (as a TSH transmitter) and producing H2O2 is employed to iodinate and couple iodotyrosine and iodothyronine (Zinellu *et al.,* 2021)– (El Hassani *et al.,* 2019). Hypothyroidism may arise from inadequate thyroid hormone synthesis caused by an inositol deficiency or impairment of the inositol cascades, which may be made worse by an increased requirement for inositols in response to high TSH levels (Tronci *et al.,* 2021), (El Hassani *et al.,* 2019). Treatment for hypothyroidism with inositol substantially reduces TSH levels. Its effectiveness has been established compared to therapy without inositol when used with metformin and selenium (Luti *et al.,* 2021; Pace *et al.,* 2020). In thyroid follicles, thyroid peroxidase (TPO) catalyses the highly complicated process of thyroxine (T4) and triiodothyronine (T3) production, which involves ROS, particularly H2O2 (Figure 3) (Thanas *et al.,* 2020). During the first phases of the iodide oxidation process, which produces thyroid hormone, ROS are already crucial (Massart *et al.,* 2013) Through their impact on mitochondrial activity, thyroid hormones also play a regulatory role in metabolism (Venditti *et al.,* 2006). Since ROS is essential, the thyroid is particularly vulnerable to oxidative injury while performing its function (Paunkov *et al.,* 2019).

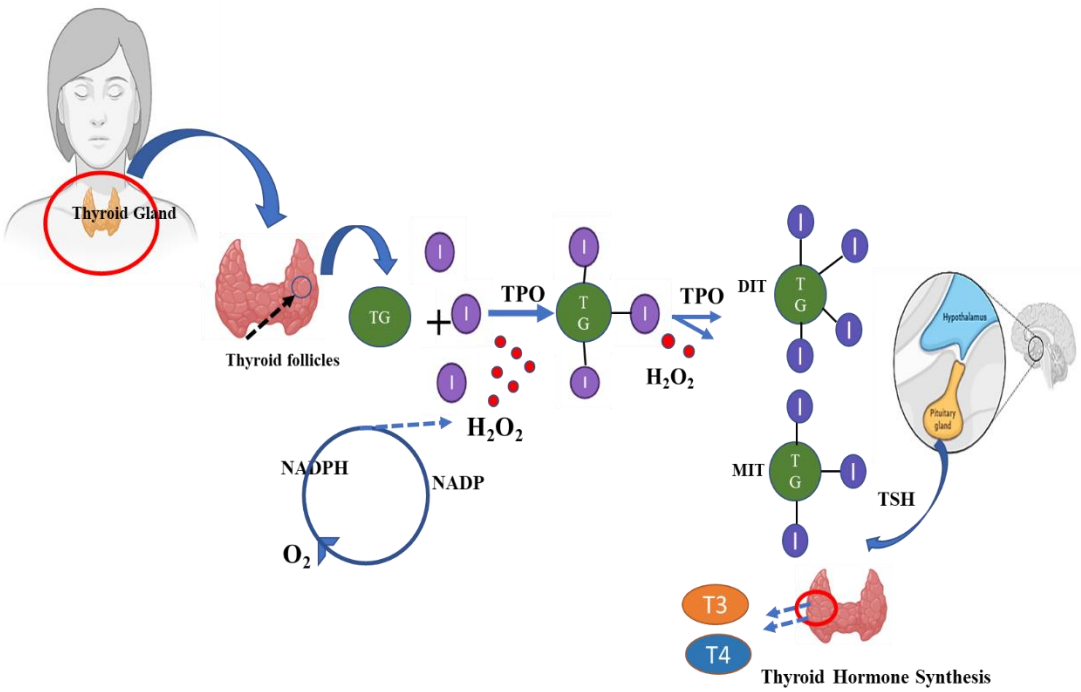

Figure 4: Role of ROS in Thyroid Hormone Synthesis

Correlation between thyroid diseases, ROS, and oxidative stress:

Hypothyroidism:

According to previous research increased levels of MDA have been seen in hypothyroidism, particularly its subclinical form, when compared to healthy people. In addition to insufficient antioxidant defence, thyroid cells' altered lipid metabolism may also be involved (Torun *et al.,* 2009). Despite decreasing lipid peroxidation levels, therapy for hypothyroidism does not reduce blood MDA amounts to those seen in healthy people, although it may considerably increase SOD activity (Baskol *et al.,* 2007). The endogenous antioxidant system's decreased activity, which leaves cells unprotected against the buildup of free radicals that causes oxidative damage, is likely the cause of the association between hypothyroidism and oxidative stress (Mancini *et al.,* 2016). Similarly, a mutation in the gene that codes for NOX activity may be a factor in the overstimulated generation of ROS. Oxygen-free radical buildup may limit TPO function, which would interfere with the generation of thyroid hormones and result in the onset of hypothyroidism (Fortunato *et al.,* 2014; Mancini *et al.,* 2012; Ohye *et al.,* 2010).

Hyperthyroidism:

Additionally, thyroid hormones promote mitochondrial respiration, which increases the release of ROS into the respiratory chain. In contrast to hypothyroidism, where the redox imbalance can be attributed to an ineffective antioxidant defence mechanism, excess thyroid hormone synthesis induces oxidative stress by increasing the formation of free radicals (Mancini *et al.,* 2016). Consequently, oxidative harm to cell structures may be related to thyroid hormone overproduction (hyperthyroidism). Indicative of oxidative damage to membrane lipids, hyperthyroid people have more excellent rates of lipid peroxidation than euthyroid persons (Piazera *et al.,* 2018; Wang *et al.,* 2011).

Conclusion:

Free radicals and oxidative stress are recognised to be harmful to human health. Numerous studies show that free radicals do contribute to the onset and development of several diseases, ranging from cardiovascular disease to cancer. Many of the processes involved in developing thyroid diseases are probably currently unknown. However, there is a clear link between elevated ROS production and evidence of oxidative damage and the onset of thyroid cancer and other illnesses listed here. Thyroid problems may start or intensify ROS generation and oxidative stress, which will cause oxidative damage. The most current research indicates a direct connection

between oxidative stress and thyroid disorders. A viable strategy for avoiding the onset of several chronic thyroid disorders appears to be preventive nutrition treatment against redox imbalance, which involves enhancing the daily diet with items that have a high antioxidant value and supporting the body's natural antioxidant defences. In order to cure and prevent thyroid illnesses and other oxidative diseases, it may be possible to design strategies that are carefully aimed at the free radical background.

Acknowledgement:

Authors gratefully acknowledge the HOD, Department of Zoology, Dr. Harisingh Gour Vishwavidyalaya, Sagar, (M.P.) India for providing infrastructure and experimental facility and ICMR for providing SRF fellowship.

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A REVIEW ON NAVIGATING THE CORTISOL CASCADE: UNDERSTANDING ITS INFLUENCE ON MENTAL HEALTH

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Abstract:

Cortisol- the stress hormone causes brain disorders which can lead to depression. Few physiological changes are noted when affected with high cortisol levels and needs immediate recovery or it can cause damages in human beings. Many teens are now been focused upon as they are the fallen victims to depression. Females are mostly prone to depression due to sex specific cortisol levels in them. Psychological therapy is used as prevention and treatment in patients.

Keywords: Cortisol, Depression, MDD (Major Depressive Disorder), Corticosteroids.

Introduction:

In recent years, the role of cortisol has been a significant attention in the reality field of mental health research. Studies have increasingly linked with elevated cortisol levels to various mental disorders, with depression which is emerging as a prominent concern [1]. In particular, adolescents become a focal point of investigation, given their susceptibility to mental health challenges [2]. Furthermore, sex-specific differences in cortisol levels have underscored the need for tailored approaches to understand and address depression [3]. This review serves as an overview of the relationship between cortisol, brain disorders and depression, emphasizing the critical importance of early intervention and psychological therapy involving the potential adverse effects of high cortisol levels, especially among vulnerable populations.

Effect of cortisol:

In a survey taken in 2020 it is proven that MDD (Major Depressive Disorder) has become the second leading cause of disease in different countries. Depression is said to have a direct control over these cortisol levels [4]. Our brain and other related organs in our body are said to be really sensitive to this cortisol level stress or corticosteroids which are associated with both reversible and irreversible changes in the hippocampus [5].

Cortisol activity:

Dysregulation of hypothalamic pituitary adrenal axis has been the major cause for the development of MDD. Many youths are being focused upon due to a survey leads to youth having higher depression levels than other age groups. An elevated morning cortisol level precedes depression in adolescence. The morning and nocturnal cortisol were the risk factors for depression rather than the existence of MDD itself. The hyper activation of HPA axis is said to increase the circulating inflammatory cytokines, which leads to the pathogenesis of depression. Another underlying development of depression is low level of BDNF (brain derived neurotrophic factor) - an important neurotrophic factor [6]. Cortisol levels is said to be associated with age related factors. PTSD (post - traumatic stress disorder) and MDE (major depressive episodes) - both have higher rate of suicidal behaviors in humans [12].

Adult patients whoever was suffering from depression, the HPA axis activity is said to be at peak, as evidenced by excessive secretion of the cortisol [11]. The depression in childhood is related with BMI (body mass index). Obesity can be a lead to be having a depressed mood [10].

Maternal cortisol:

Maternal cortisol and depression during pregnancy were tested by saliva and blood of morning samples during the second trimester. Elevated cortisol levels in mothers during pregnancy is an indicator of depression[8]. Preterm infants are mostly affected with poor growth and neuro development [9].

Diagnosis:

Patients with high cortisol level are prone to more depression. The assessment of the cortisol in our saliva has proven a reliable and valid reflection of the respective unbound hormone in the blood. Diagnosis using saliva can replace the blood sampling from infants for endocrine test like the dexamethasone suppression test [13].

Treatment:

Many patients with depression show resistance to psychological therapy. It is related with age and illness. But to now only psychological therapies have more effect in treatment than with other modes [7].

Conclusion:

In conclusion, cortisol, the stress hormone, plays a significant role in the development of brain disorders, including depression. High cortisol levels can induce

physiological changes that may lead to long-term damage if not addressed promptly. Teenagers, particularly females, are particularly susceptible to depression due to their vulnerability to stress and sex-specific cortisol levels. Psychological therapy serves as a crucial preventive measure for individuals at risk of depression, emphasizing the importance of early intervention and support in mitigating the negative impact of chronic stress on mental health.

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WATER POLLUTION AND ITS MANAGEMENT

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Introduction

Water is an integral part of our life and has been well recognized as nectar. Therefore, conservation and management of water is necessary for the general wellbeing of all life. Water is a very important resource, which is used for number of uses, like domestic use, agricultural use, industrial use etc. It is very important for sustenance of life.

Water is present only on earth. This source, therefore, needs to be protected. However, in the world scenario, the picture is different. Under the name of development, this vital source gets polluted by man-made activities. According to the scientists of National Environmental Engineering Research Institute, Nagpur, India, about 70 % of the available water in India is polluted (Pani, 1986).

Water pollution is a contamination of water bodies due to manmade activities. When pollutants are directly or indirectly discharged into water bodies without adequate treatment leads to problem of water pollution.

Water quality problem is a critical issue in India. Fluoride and arsenic contamination of groundwater is widely cited as one of the major challenges in drinking water quality regulation. The reported population directly affected and at risk in case of fluoride and arsenic contamination is estimated as 25.1 and 71.3 million respectively (Panda, 2002). Handa *et.al*. (1986) studied the few places of India for groundwater quality and observed that land use practices affected the groundwater quality in that area.

Many times, industrial discharges may contain the pollutants like oils, phenols, plastics, suspended solids, heavy metals, traces of pesticides etc. These pollutants are released from different processes carried out in industrial area, e.g. process like manufacturing, sizing, cooling, dying and bleaching at different stages of production. Such effluent cause surface as well as groundwater pollution, directly and indirectly (Pawar *et.al*., 2014).

In Gujarat, there are more than 90,000 industrial units, in which about 8,000 are polluting units. All the major rivers and streams of this state are in a bad condition due to discharge of industrial effluents to Kolak, Mahi, Daman Ganga or Amalakhadi. In the Ankleshwar industrial estate, Bharuch, water in past seven years have become so polluted that one cannot even wash their hands (Sindhu, 2010).

Causes of water pollution

1. Industrial waste-Industries are major contributor of water pollution. Pollutants & toxic chemicals are coming out of such industries, if they are not treated properly before discharging into fresh water bodies leads to a water contamination. Many industries are sending effluent without any treatment into waterbodies. Such industries make water unsafe for drinking and further this polluted water get mix with the sea water, as rivers meet to sea, it directly affects the sea water. We have many examples in India where industries lead to severe water pollution problem. Mithi river pollution, Ganga River pollution these are major examples of water pollution by industrial activities.
2. Sewage & waste water- Sewage is also called as a domestic waste water or Municipal waste water. It is waste water that is produced by a community of people and transported through a sewer system it contains bacteria, pathogens, detergents, chemicals etc. If such waste water is treated properly up to tertiary treatment by sewage treatment plant, then it causes less harm to water bodies, but if such waste water sends to water bodies without treatment, then it causes contamination of waterbodies. Bacteria present in waste water causes health related issues. In city like Mumbai sewage water generation is more than the capacity of the sewage treatment plant, in such cases more waterbodies contamination is found. Mithi river not only polluted by industrial waste but also polluted by sewage which completely change the status of river to Nallah. Once upon a time Mithi river had capacity to provide freshwater, but manmade activities changed river into nallah.
3. Dumping of waste material-dumping of solid waste material and litter in water bodies causes water pollution. Nirmalya generated in huge amount during festival time, people discharged such solid material in a waterbody as well as plastic bottles, glass, paper wraps also thrown in water bodies by people lead to severe problem of water pollution. An officer related

AN OFFICER RELATED TO SOLID WASTE MANAGEMENT (SWM) DEPARTMENT SAID, "AROUND 250 METRIC TON GARBAGE HAS BEEN COLLECTED FROM THE BEACHES EVERY DAY. BUT SOMETIMES DURING THE MONSOON SEASON AROUND 400 METRIC TON GARBAGE FLOATS ON BEACHES. A HUGE AMOUNT OF GARBAGE ACCUMULATES ON VERSOVA, JUHU AND AKSA BEACHES." (FREE PRESS JOURNAL, JULY 2023)

4. Oil pollution-oil does not mix with water but sea water get polluted due to oil spillage by ships or accidently also oil layer formed in sea area due to ship accident, It forms thick oil layer on the surface of the oil due to this thick water layer sea animals unable to take dissolve oxygen from water bodies, many birds slipped into this oily area.
5. Acid rain -Acid rain is caused by air pollution, when acid particles from air pollution mix with the water vapour, after condensation it comes as an acid rain, it causes effects like corrosion of statues, affects biodiversity in sea water gradually.
6. Agricultural Waste & Eutrophication-farmers are using Nitrogen phosphorus fertilisers as well as pesticides to avoid diseases on crop, these chemicals seeps down in groundwater or flow towards water bodies in rainy season leads to access growth of algae in water bodies. Accumulation of nitrogen and phosphorus in water bodies which increased level of nutrients in water bodies is called as a Eutrophication. It results in growth of algae bloom in water bodies. It depletes oxygen level in water which affects fish and other aquatic animals.
7. Radioactive waste-Radioactive waste from facilities that create nuclear energy can be extremely hazardous to the environment and must be disposed of properly. Uranium is used in the creation of nuclear energy, it's a radioactive material, lots of precautions are required while handling this material as well its nuclear waste generated in these plants needs to disposed off properly in deep ocean. Unfortunately, if any accidents occur at such plant, radioactive material released into the environment.

The nuclear crisis that occurred in Japan after 2011 Tsunami prompted Japanese government dumped 11 million litres (2 million gallons) of radioactive water into the Pacific Ocean. (Mandal,2016)

Management of water pollution

Water pollution management is essential need of today's time, if today we put our efforts for reducing water pollution, it will give good water quality to our next

generations. Water management is the control and movement of water resources to minimize damage to life and property and to maximize efficient beneficial use. Good water management of dams and levees reduces the risk of harm due to flooding. (https://www.nrcs.usda.gov/water-management)

Awareness in today's generation about water pollution and its prevention is very important. Strict rules and regulations are required to prevent water pollution problem. Use of Precautionary Principle as well as Polluter Pays Principle are very important in reducing water pollution problem. In Precautionary principal industries should take care about pollutants which is coming from their industry then Polluter Pays focuses on whoever cause pollution, he has to pay cost for it. Effective implementation of these two principles is very important in water pollution management, which has check on the industrial activities regularly and minimise problem associated with water pollution.

Water Pollution management includes wise and rational use of water resources as well some measures to avoid pollution problems are required to be adopted. Water use basically have three major areas where water require in higher amount on three level i.e. Drinking water for common public then use of water for agriculture purposes and then for industrial purposes. There are many ways for minimising pollution problem, some of them are discussed below.

1. **Sewage (Waste water) treatment:** Sewage treatment plants collect, treat, and discharge wastewater, providing a service essential to environmental and public health. In urban areas domestic sewage treated by using Municipal sewage treatment plant then such treated water sends to a waterbody. Sewage treatment plants include Preliminary, primary, secondary & tertiary type of treatment it includes steps like Screening and pumping, Grit removal, Primary Settling, Aeration, Secondary settling, Filtration, Disinfection, Oxygen Uptake. Sewage treatment plant play effective role in removing pollutants from waste water. After all these steps if such water sends to waterbodies, it cannot contaminate waterbodies. STPs are present in Urban areas not in all areas, this is main problem as well as they are in limited number in Urban areas, day by day population of urban areas are growing but STPs are in limited number with limited capacity.
2. **Industrial wastewater:** Main objective of treating waste water is to avoid harmful effects of effluent on environment. Every industry has need to have effluent treatment plant, as it avoids impurities to mix with water bodies. Effluent treatment

plant includes preliminary, primary, secondary and tertiary treatment. Preliminary treatment removes solid particles, grease, oil etc. Grit chamber removes inorganic solids such as sand, gravel, big size solid particles, broken pieces of metal which avoid damage problem to pump system.

Primary treatment removes suspended solids and organic matter. Secondary treatment includes use of aerobic microorganisms to remove remaining pollutants such as organic matter and suspended solids. Tertiary treatment removes suspended and dissolved solids, for removal of pathogenic contaminants disinfection process also present in the ETP.

In today's time in ETPs some advance techniques like Zero liquid discharge, Reverse osmosis also involved. Purpose behind Zero liquid discharge is to remove all waste liquid discharge and to get a clean water that can be reused. Filtration process removes pollutants and bacteria from sewage, Coagulation –flocculation eliminates inorganic metallic ions, organic matter, removes particles and infectious agents. Reverse Osmosis removes many contaminants with salinity, such water further possible to do reuse for different purposes. Industries generating huge amount of effluent have their own effluent treatment facilities on industry site.

3. **Agriculture wastewater:** Point sources include pesticides, fertilizers runoff into water bodies. Animal husbandry with agriculture also added waste material like animal waste products into soil which contaminates water bodies.

   Non point pollution sources comes from different sources and locations, they are not from a specific single point. It includes pollutants like insecticides, pesticides, excessive fertilizers. Sediment wash off fields, soil erosion causes pollution. Techniques like crop rotation, ploughing, crop mulching, plantation of perennial crops helps to retain soil and avoid erosion problem. Farmers if use limited quantity of fertilizers, it avoids seepage of excess of fertilizers into the soil as well as use of biological pesticides instead of chemical pesticides reduces chances of seepage of pesticides through soil.

4. **Construction site storm water:** Construction sites cause water pollution in different ways like runoff through construction site, waste disposal. Use of cut off ditches helps to divert water runoff. Proper disposal of waste material without causing any harm to nature is very important, it reduce pollution problem. Covering of drainage channel also avoid water pollution problem.

Conclusion:

Each drop of water is important, in today's time huge quantity of water get polluted by manmade activities, if we want to save our water resources in good condition for next generations, then it's very important to control water pollution problem by using various ways, today's small action can contribute in water saving for next generation. Rational use of water sources with adoption of new techniques which avoid pollution problem is necessary in today's time.

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THE CATALYSTS OF LIFE

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Introduction:

Enzymes are fundamental to all biological processes, acting as nature's catalysts to accelerate chemical reactions. Without enzymes, the biochemical reactions necessary for life would occur too slowly to sustain life processes. This chapter delves into the intricate world of enzymes, exploring their structures, mechanisms, and functions, as well as their vital roles in various biological systems and applications in biotechnology.

1. Understanding enzymes

Enzymes are proteins that catalyse biochemical reactions, lowering the activation energy required for the reactions to proceed. Their ability to increase the rate of reactions without being consumed in the process is what makes them indispensable to life.

1.1. Definition and characteristics

Enzymes are characterized by their ability to:

- **Catalyse reactions**: Enzymes speed up reactions by lowering the activation energy.
- **Be specific**: Each enzyme is specific to a particular substrate or set of substrates.
- **Be reusable**: Enzymes are not consumed in the reaction and can be used multiple times.
- **Operate under mild conditions**: Enzymes function optimally at physiological conditions, such as specific temperatures and pH levels.

1.2. Enzyme classification

Enzymes are classified based on the type of reaction they catalyse:

Oxidoreductases: Catalyse oxidation-reduction reactions (e.g., alcohol dehydrogenase).

Transferases: Transfer functional groups between molecules (e.g., amino transferase).

Hydrolases: Catalyse hydrolysis reactions (e.g., lipase).

Lyases: Break bonds by means other than hydrolysis or oxidation (e.g., decarboxylase).

Isomerases: Catalyse isomerization changes within a single molecule (e.g., phosphoglucose isomerase).

Ligases: Join two molecules together with the help of ATP (e.g., DNA ligase).

2. The structure of enzymes

Enzyme structure is closely related to its function. The structure of an enzyme determines its activity and specificity.

- **2.1. Primary structure**

  The primary structure of an enzyme is its amino acid sequence. This sequence dictates the enzyme's higher-level structures and, ultimately, its function.

- **2.2. Secondary structure**

  The secondary structure refers to local folding patterns within the protein, such as alpha-helices and beta-sheets, stabilized by hydrogen bonds. These structures form the enzyme's basic shape.

- **2.3. Tertiary structure:** The tertiary structure is the three-dimensional shape of the enzyme, resulting from interactions between secondary structure elements. This structure is crucial for the enzyme's active site and overall function.

- **2.4. Quaternary structure**: Some enzymes consist of multiple polypeptide chains or subunits, each with its own tertiary structure. The quaternary structure refers to the arrangement of these subunits in a multi-subunit enzyme, such as haemoglobin.

3. The mechanism of enzyme action

Enzymes catalyse reactions through a specific mechanism that involves several steps.

3.1. Enzyme-substrate binding

Enzymes interact with substrates through their active sites. The active site is a specific region of the enzyme where the substrate binds. This interaction is often described by:

- **Lock and Key Model**: The enzyme's active site is complementary in shape to the substrate.
- **Induced Fit Model**: The enzyme's active site undergoes a conformational change to better fit the substrate upon binding.

3.2. Formation of the enzyme-substrate complex

When a substrate binds to the enzyme, it forms an enzyme-substrate complex. This complex stabilizes the transition state of the reaction, reducing the activation energy needed for the reaction to proceed.

3.3. Catalysis

The enzyme catalyzes the conversion of substrates into products through various mechanisms, including:

- **Proximity and orientation**: Enzymes bring substrates close together in the correct orientation.
- **Strain**: Enzymes can induce strain in substrates, making bonds easier to break.
- **Chemical assistance**: Enzymes may provide catalytic groups or cofactors that facilitate the reaction.

3.4. Product release

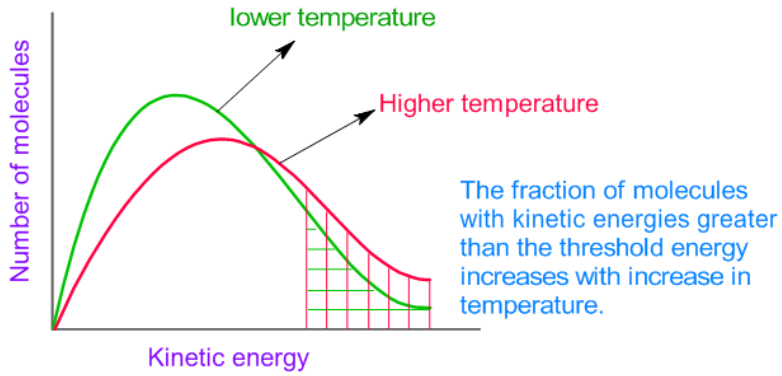
After the reaction, the enzyme releases the products, which are typically less tightly bound to the enzyme than the substrates. The enzyme then returns to its original state, ready to catalyse another reaction.

4. Factors affecting enzyme activity

Enzyme activity is influenced by several factors, including:

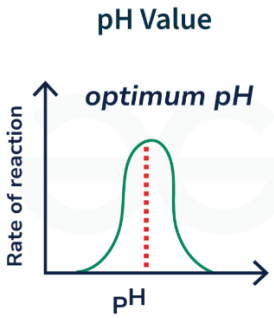
4.1. Temperature

Enzymes have an optimal temperature range. High temperatures can cause denaturation, where the enzyme loses its functional shape, while low temperatures can slow down the reaction rate.



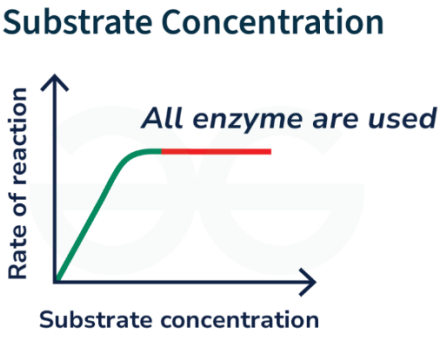
4.2. pH

Enzymes have an optimal pH range. Deviations from this range can lead to changes in enzyme structure and function, often affecting the ionization of amino acid residues in the active site.



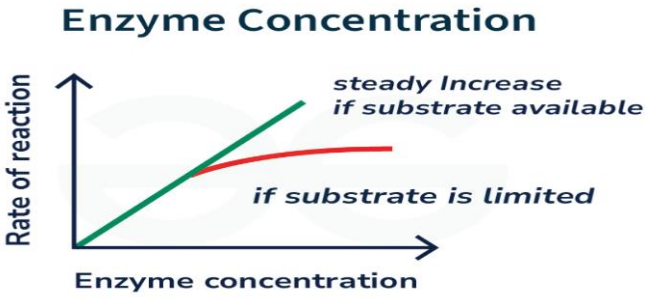
4.3. Substrate concentration

Increasing substrate concentration generally increases the rate of reaction, up to a point where the enzyme becomes saturated. Beyond this saturation point, the reaction rate levels off.



4.4. Enzyme concentration

Increasing enzyme concentration will increase the reaction rate proportionally, provided there is sufficient substrate available.



4.5. Inhibitors

Inhibitors can decrease enzyme activity:

- **Competitive inhibitors**: Bind to the active site, competing with the substrate.
- **Non-competitive inhibitors**: Bind to a different part of the enzyme, altering its activity.

- **Allosteric inhibitors**: Bind to an allosteric site, changing the enzyme's shape and activity.

5. Enzyme regulation

Cells regulate enzyme activity to maintain homeostasis and respond to environmental changes.

5.1. Allosteric regulation

Allosteric regulation involves molecules binding to sites other than the active site (allosteric sites). This binding can either activate or inhibit the enzyme.

5.2. Covalent modification

Enzymes can be regulated by covalent modifications, such as phosphorylation or acetylation. These modifications can activate or deactivate the enzyme.

5.3. Feedback inhibition

In feedback inhibition, the end product of a metabolic pathway inhibits an enzyme involved earlier in the pathway, preventing the overproduction of the product.

6. Enzymes in metabolic pathways

Enzymes play crucial roles in metabolic pathways, which consist of interconnected biochemical reactions.

6.1. Catabolic pathways

These pathways involve the breakdown of molecules to release energy. Examples include glycolysis and the citric acid cycle. Enzymes such as hexokinase and pyruvate dehydrogenase are vital in these processes.

6.2. Anabolic pathways

Anabolic pathways build complex molecules from simpler ones. Examples include the synthesis of proteins and nucleic acids. Enzymes such as DNA polymerase and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) are essential.

7. Enzymes in biotechnology

Enzymes have significant applications in biotechnology and industry.

7.1. Medical applications

Enzymes are used in diagnostics, such as blood glucose tests for diabetes, and in therapeutics, such as enzyme replacement therapies for conditions like cystic fibrosis.

7.2. Industrial applications

Enzymes are employed in processes like brewing, baking, and detergent manufacturing. They enhance efficiency and reduce the need for harsh chemicals.

7.3. Environmental applications

Enzymes are utilized in bioremediation to degrade pollutants and in wastewater treatment to break down organic materials.

Future of enzyme research:

The study of enzymes continues to evolve, with advances in enzyme engineering, synthetic biology, and computational modelling. Researchers are exploring ways to design custom enzymes for specific industrial and medical applications, potentially revolutionizing fields ranging from medicine to environmental science.

Conclusion:

Enzymes are pivotal to the complexity and efficiency of life's biochemical processes. Their ability to catalyse reactions with high specificity and efficiency underscores their importance in both fundamental biology and applied sciences. Understanding enzymes not only reveals the intricate mechanisms of life but also opens doors to innovative applications that can address challenges in health, industry, and the environment.

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A BRIEF REVIEW ON FLUORIDE AND FLUOROSIS

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Introduction:

Fluorine (Symbol F) is an element of halogen group with atomic number 9 and molecular weight 18.998. Fluorine is the lightest and rare element, it is the most electronegative of all elements requiring great care in handling. It exist as a diatomic molecule with remarkably low dissociation energy (38Kcal/mol), as a result it is highly reactive and has strong affinity to combine with other elements to produce compounds known as fluoride.

Fluoride is a ubiquitous element present in soil and atmosphere water. Fluoride is mainly found in ground water in which the solvent action of water on the rocks and soil of earth's crust derive it from the adjacent soil, the porosity of the rocks or the soil through which water passes and the speed with which water flows, the temperature of interaction of rock and water, the hydrogen and calcium ions concentration determine the fluoride content of drinking water. Fluoride is present in the teeth, bones, thyroid gland and skin of animals. On the formation of dental enamel and normal mineralization in bones, it plays important role. [1] It is generally accepted that fluoride stimulates bone formation. [2] For hardening the enamel and reducing the incidences of caries small concentration of fluoride is required. [3] When consumption of fluoride is deficient, children usually suffer from dental caries whereas excessive exposure leads to fluorosis. That is why fluoride is often called a "two-edged sword".

Fluoride may be detrimental to environment and human health when its presence increases due to natural or anthropogenic source. [4] The world health organization [5] and Bureau of Indian standards 10500 [6] permit fluoride content in water 1.5 mg/L and 1.0 mg/L respectively as a safe limit for human consumption, but in excess i.e. >1.5 mg/L creates major problems in animals and human beings. The recommended level of fluoride in drinking water in India is accepted as 0.5 to 0.8 mg/L.

Nomenclature

According to the additive nomenclature, the systematic name fluoride and the valid IUPAC name is determined. However, the name fluoride is also used in

compositional IUPAC nomenclature which does not take the nature of bonding involved into account. Fluoride is also used non-systematically, to describe compounds which release fluoride upon dissolving. Hydrogen fluoride is itself an example of a non-systematic name of this nature. However, it is also a trivial name and the preferred IUPAC name for fluorane.

Origin and occurrence

The soil minerals, drinking water, air, food, beverages, drugs and cosmetics are the sources of fluoride for human exposure. It is the 13th most common element in Earth's crust, comprising between 600 and 700 ppm of the crust by mass. Because its reactivity, it is essentially only found in compounds. The main natural sources of fluoride in soil are fragments of minerals, such as fluorite or fluorspar, fluorapatite, cryolite and topaz. [1] The geological survey of India reveals that topaz, apatite, rock phosphate, phosphate nodules and phosphorite are wide spread in the earth's crust in India and contain high percentages of fluoride. As a result of the rich mineral content and contaminates the soil and water, therefore the source of fluoride in ground water is primarily geogenic. Three minerals exist that are industrially relevant sources: fluorite, fluorapatite and cryolite. [7,8]

- **Fluorite** (CaF_2), also called fluorspar or Blue John, is the main source of commercial fluorine. Fluorite is a colorful mineral associated with hydrothermal deposits. It is common and found worldwide. China supplies more than half of the world's demand; Mexico is the second-largest producer.
- **Fluorapatite** ($Ca_5(PO_4)_3F$) is mined along with other apatites for its phosphate content and is used mostly for production of fertilizers. Most of the Earth's fluorine is bound in this mineral, but because the percentage within the mineral is low (3.5%), the fluorine is discarded as waste. Only in the United States is there significant recovery.
- **Cryolite** (Na_3AlF_6) is the least abundant of the three, but is a concentrated source of fluorine. It was formerly used directly in aluminium production.

Several other minerals, such as the gemstone topaz, contain fluoride. Fluoride is not significant in seawater or brines, unlike the other halides, because the alkaline earth fluorides precipitate out of water.

Organofluorines have been observed in volcanic eruptions and in geothermal springs. Their ultimate origin (physical formation under geological conditions or initial biological production and deposition in sediments) is unclear. They are not a

commercially important source of fluorine, but are trace environmental contaminants whose amount is being studied. [9]

The possibility of small amounts of gaseous fluorine within crystals has been debated for many years. One form of fluorite, antozonite, has a smell suggestive of fluorine when crushed. The mineral also has a dark black color, perhaps from free calcium (not bonded to fluoride).

Fluorite

Fluorapatite

Cryolite

Characteristics

Physical properties

Fluorine forms diatomic molecules that are gaseous at room temperature. The density is about 1.3 times that of air. [10] The element has a "pungent" characteristic odor and fluorine gas is actually a very pale yellow. Fluorine solidifies at −220 °C into a cubic structure, called beta-fluorine. [11]

Chemical reactivity

Fluorine's chemistry is dominated by its tendency to gain an electron. It is the most electronegative element and a strong oxidant. The removal of an electron from a fluorine atom requires so much energy that no known oxidant can oxidize fluorine to any positive oxidation state. [12] Fluorine gas is highly reactive with other substances both because of the strong bonds it forms with other atoms, but also because of the relative weakness of the fluorine–fluorine bond. [13]

Isotopes

Fluorine occurs naturally on Earth exclusively in the form of its only stable isotope, fluorine-19,[14] which makes the element monoisotopic and mononuclidic. Seventeen radioisotopes have been synthesized: mass numbers 14–18 and 20–31. [15]

Applications of fluoride

- Fluoride salts are used to enhance the strength of the teeth by the formation of fluorapatite.

- Sodium fluoride is used to fluoridate water.
- Sodium hexafluorosilicate (Na2SiF6) are commonly used additives in the U.S.
- Sodium fluoride is used as a cleaning agent, e.g. as a "laundry sour."
- Sodium fluoride used in toothpaste to prevent cavities.
- The fluoride is the reagent for the synthesis of fluorocarbons.
- Fluoride is used in medicine and in many foods and beverages.
- Fluoride is used in agrichemicals
- Compounds containing fluorine-18, a radioactive isotope that emits positrons, are often used in PET scanning,
- Synthetic sodium fluoroacetate has been used as an insecticide but is especially effective against mammalian pests.
- A method of archeological dating of bones uses fluoride's tendency to accumulate in calcium-containing organic matter.
- Poisons containing fluorine are well known for killing insects and rodents and the very few organisms that incorporate fluorine in their biochemistry do so to make natural poisons.

Biological aspects

To a certain extent (as per WHO, 0.6 ppm) fluoride ingestion is useful for bone and teeth development but excessive ingestion (>1.5 ppm) causes a disease known as fluorosis. Fluorosis is a chronic metabolic disease of teeth, soft tissue, bone and joints caused by ingesting large amount of fluoride either through water or rarely from foods of endemic areas which affects every organ, tissue, cells and bones in the body and results in health complaints with several other diseases. In short it causes non-skeletal manifestations, dental fluorosis and skeletal fluorosis.

The problem of fluorosis has been known in India for a long time. The disease earlier called "mottled enamel" was first reported by Vishanathan (1935) to be prevalent in human beings in Madras presidency in 1933. Mahajan (1934) reported a similar disease in cattle in certain parts of Hyderabad state. However, Shortt (1937) was the first to identify the disease as "Fluorosis" in human beings in Nellore district of Andhra Pradesh. [16]

International status of fluorosis

Fluorosis is an endemic public health problem in 24 nations around the globe, including India. The following countries have been identified for the problem of fluorosis:

Argentina	Iran	Pakistan
Algeria	India	Palestine
Australia	Jordan	Sri Lanka
African nations	Japan	Syria
Bangladesh	Libiya	Turkey
China	Morocco	Thailand
Egypt	Mexico	UAE
Iraq	New Zealand	USA (Southern States)

National status of fluorosis

The endemic states with the percentage of people at fluorosis risk in India as follows:

S. N.	States	Total population (Millions)	Population at risk (Millions)	% at risk (Millions)
1	West Bengal	56.21	1.65	2.9
2	Uttar Pradesh	130.83	1.77	1.4
3	Tamil Nadu	39.19	7.64	19.5
4	Rajasthan	39.82	10.9	27.4
5	Punjab	16.05	2.07	12.9
6	Orissa	29.8	3.26	10.9
7	Maharashtra	52.84	0.14	0.3
8	Madhya Pradesh	38.36	1.68	4.4
9	Karnataka	34.42	6.9	20.0
10	Haryana	14.57	2.17	14.9
11	Gujarat	29.45	4.78	16.2
12	Delhi	1.23	0.16	13.0
13	Andhra Pradesh	52.31	13.5	25.8
14	All 14 states	535.08	56.62	169.6

Of the 85 million tons of fluoride deposits on the earth's crust, 12 million are found in India. [17] Hence it is natural that fluoride contamination is widespread, intensive and alarming in India. India is one among the 24 nations around the globe, where health problems have been reported due to excessive fluoride in drinking water. Today, millions of men, women and children are crippled and leading a vegetative life

due to fluorosis. Presently fluorosis is prevalent in 19 states of India. [18] The endemic states with the percentage area affected are as follows

1. 50-100% districts are affected - Andhra Pradesh, Tamil Nadu Uttar Pradesh, Gujarat, Rajasthan.
2. 30-50% districts are affected - Bihar, Haryana, Karnataka, Maharashtra, Madhya Pradesh, Punjab, Orissa, West Bengal.
3. < 30% districts are affected - Jammu and Kashmir, Delhi, Kerala.

In India, people of nearly 22,400 villages of 196 districts of 19 states are drinking fluoride contaminated water which is above the maximum allowed concentration (MAC) of 1.5 ppm recommended by WHO. [19] Concentration of fluoride in drinking water in different parts of India varies between 0.5-50 ppm. Out of 6 lakh villages, atleast 50% have fluoride content in drinking water exceeding 1.0 ppm. [20] In India, an estimated 66.62 million people, including 6 million children suffer from fluorosis as a consequence of consuming fluoride-contaminated water and 6 million people in the country affected with skeletal fluorosis and exposed to risk of developing skeletal fluorosis. [21]

Treatment and prevention

There is a crying need to overcome the problem of fluorosis. Human beings can be prevented by using the three approaches i.e. health education, preventive measures and treatment of the disease.

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AMBIENT EFFECTS OF AIR POLLUTION ON HEALTH IN DEVELOPING COUNTRIES

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Abstract:

The deleterious effects of ambient air pollution on human health have been consistently documented by many epidemiologic studies worldwide. It has been calculated that globally, at least seven million deaths are annually attributable to the effects of air pollution. The major air pollutants emitted into the atmosphere by a number of natural processes and human activities include nitrogen oxides, volatile organic compounds, and particulate matter. In addition to poor ambient air quality, there is increasing evidence that indoor air pollution also poses a serious threat to human health, especially in low-income countries that still use biomass fuels as an energy resource. This review summarizes the current knowledge on ambient air pollution in financially deprived populations.

Keywords: Air Pollution; Health; Developing Countries

Introduction:

It is well known that air pollution has a number of detrimental effects on human health and is considered a major issue for the global community. The World Health Organization (WHO) estimated that, in the year 2017, ambient air pollution was responsible for nearly seven million deaths, representing more than 10% of all-cause deaths and more than doubling previous estimates. Air pollution accounts world wide for an estimated 9% of deaths due to lung cancer, 17% due to chronic obstructive pulmonary disease, more than 30% due to is chemic heart disease and stroke, and 9% due to respiratory infections. The Global Burden of Disease report identified in 2017 that air pollution was among the leading risk factors for disease burden, being globally responsible alone for 3.1% of all Disability-Adjusted Life Years (DALYs). All these findings confirm that air pollution is now the world's largest environmental health risk. Outdoor air pollution is a mixture of thousands of components. Among them, airborne particulate matter (PM) and the gaseous pollutants ozone, nitrogen dioxide (NO2),

volatile organic compounds (including benzene), carbon monoxide (CO), and sulphur dioxide (SO_2) are the most important from a health perspective. Primary pollutants such as soot particles and oxides of nitrogen and sulphur are emitted directly into the air by the combustion of fossil fuels. Major sources of primary particles include motorized road traffic, power generation, industrial sources, and residential heating. Secondary pollutants, formed when primary pollutants react or interact in the atmosphere, include mainly ozone (O_3) and PM. Fine particles are derived primarily from direct emissions from combustion processes such as gasoline and diesel fuel, wood burning, coal burning for power generation, and industrial processes.

Fine particles can travel large distances (more than 100 km), with the potential for high background concentrations over a wide area. As a consequence, their composition may be extremely heterogeneous, depending on the meteorological conditions and human activities in a particular geographical area. Ultrafine particles are fresh emissions from combustion-related sources such as vehicle exhaust and atmospheric photochemical reactions and are recognized as important markers of exposure to traffic exhaust along main roads. Fine and ultrafine particles are those associated with the worst effects on health as they can reach the deepest portions of the airways or even reach the blood stream directly. If acute and long-term exposure to ambient air pollution represents a serious threat for health in western industrialized countries, the burden of this problem is even higher in developing countries, where population explosion along with widespread industrialization coupled with urbanization have resulted in dense urban centers with poor air quality. In such developing countries, however, huge economic and social disparities coexist; thus, in addition to the poor ambient air quality, people can be also exposed, especially in rural areas, to high concentrations of indoor air pollution due to the use of biomass fuels (coal, wood, and other solid fuels) as an energy resource. Worldwide, more than three billion people, largely in developing countries, rely on biomass fuels for their domestic energy needs. As a consequence, household air pollution from solid fuel use has become a serious threat to health. In this regard, the best example is provided by Asia, which has experienced rapid and disharmonic industrialization, urbanization, and transportation development in the recent decades, with resulting outdoor and indoor air pollution levels that are constantly well above the upper limits indicated by the WHO guidelines. China, in particular, the Asian country with the fastest industrial development and

population increase, is now facing the worst air pollution problem in the world. This review focuses on this particularly vulnerable population living in low- and Middle-income countries and highly exposed to both household and outdoor pollutants. A number of studies and meta-analyses have shown that increased mortality is associated with short- and long-term exposure to PM, both in developed and developing countries. there was an associated 7.3% increase in all-cause mortality. This robust association was even more evident when the analysis was restricted to the Medicaid-eligible sub-group, documenting that persons with low socioeconomic status are more likely to be exposed to higher pollutant levels than the rest of population. As previously mentioned, this association may particularly important in East-Asian countries, which, due to their rapidly developing economies and dense populations, are exposed to very high levels of air pollution. For instance, with urbanization increasing from 26% in 1990 to 50% in 2010, China has undergone dramatic epidemiological transitions and among the risk factors responsible for DALYs, ambient and household air pollution ranked fourth and fifth, respectively. A recent meta-analysis of 33 time-series and case-crossover studies conducted in China to assess the mortality effects of short-term exposure to air pollution. The short-term effects of air pollution on health have also been the object of intense research in developing countries outside the Asiatic region. Likewise, indoor air pollution has been shown to have a significant impact on the health of populations living in rural areas in less industrialized countries such as Pakistan and India.

Pertaining to the adverse effects on the lungs, air pollution, as previously mentioned, is the cause and aggravating factor of many respiratory diseases like chronic obstructive pulmonary disease, asthma, and lung cancer. Increased ambient O_3, NO_2, PM2.5, and SO2 levels were consistently associated with increased hospital admission for asthma and pneumonia in various studies conducted in Hong Kong and Taipei. A systematic review confirmed that indoor air pollution due to solid fuel combustion was also an important risk factor for chronic obstructive pulmonary disease in adult populations living in low-income countries, particularly in non-smoking women. In addition, a number of studies have consistently documented the association between air pollution and the risk of developing lung cancer; women carry the highest risk, probably due to their increased exposure to indoor air pollution. Time-based multiple risk factor models have shown that smoking and solid-fuel use collectively contributed to 75% of lung cancer deaths in China. In addition to respiratory diseases, there is also increasing

evidence that sustained exposure to ambient and household air pollution has a particularly deleterious effect on the cardiovascular system and an association has been found between hypertension, coronary heart disease, and stroke. Although populations in low- and middle-income countries are highly exposed to environmental pollution, the bulk of evidence that links these exposures to cardiovascular disease is derived mostly from populations in high-income countries. Pertaining to developing countries, for instance, the results of a recent study conducted by Chen and colleagues clearly indicate that life-expectancy in Northern China, where air quality is particularly poor, is 5.5 years lower owing to an increased incidence of cardio respiratory mortality. Notably, a study on long-term exposure to ambient air pollution conducted in Shenyang,

The largest and most heavily industrialized city in China, in addition to the close link between air pollution and arterial thrombosis, there is also some evidence of the association with venous thromboembolism, as suggested by a recent systematic review. A study conducted in Santiago, Chile between 2001 and 2005 reported that the short-term increase in hospital admissions for venous thrombosis and pulmonary events was proportional to the elevations in the concentration of fine PM, documenting that the burden of this phenomenon is significant also for developing countries. If air pollution, both outdoor and indoor, is considered a major health problem in developing countries, the burden of this issue is even greater in those population groups that are particularly vulnerable such as pregnant women, newborns, and children.

In particular, exposure to indoor air pollution from the combustion of solid biofuels is a significant public health hazard predominantly affecting women and small children living in poor households in both rural and urban communities in developing countries. Recent studies have shown that air pollution can affect the developing fetus via maternal exposure, resulting in preterm birth, low birth weight, growth restriction, and potentially adverse cardiovascular and respiratory outcomes. air pollution during pregnancy and low birth weight and still birth and a meta-analysis by pope and colleagues calculated that the RR of low birth weight and stillbirth attributable to indoor air pollution in developing countries was 21% and 26%, respectively. Another more recent systematic review and meta-analysis found a strong association between household air pollution from solid fuel use and the risk of adverse pregnancy outcomes; such exposure resulted in an 86.43 g reduction in birth weight and a 35% and 29% increased risk of low birth weight and stillbirth, respectively. Interventions aimed at

reducing exposure to household air pollution will result in an improvement of survival outcomes for all children.

Notably, a study evaluating the mortality effects of indoor air pollution and ambient urban PM pollution in Mexico estimated that the annual child mortality rate would decrease by 0.1 per 1000 children in the absence of these environmental exposures. There is also consistent evidence that smoke from biofuels can cause acute lower respiratory infections in childhood . Notably, a randomized controlled trial performed in Guatemala found that a reduction in exposure to household air pollution led to a significant one-third reduction of severe childhood pneumonia, with possible important implications for the reduction of child mortality. In addition, air pollution has been found to be an important contributor to the increased prevalence of allergic diseases in children in developing countries, including asthma. In addition, increased ambient O_3, NO_2, PM2.5, and SO_2 levels were associated with increased hospital admission for asthma in children.

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APPLICABLE USES OF ANIMAL CLONING IN CONSERVATION OF ENDANGERED SPECIES AND ITS IMPACT

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Abstract:

This chapter focuses on the applicable uses of animal cloning in conservation of endangered species and its impact, outlining the start of cloning in research. Explaining clone's history, types, vectors, wide applications in several fields and limitations and disadvantages, as it raises many ethical issues in society for producing clones. Cloning itself is not a new technique, it has existed in nature for centuries and decades. For example, in a bacteria which is a single celled microorganisms produce exact copies of themselves when they reproduce. In humans, splitting of a fertilized egg into two halves is similar to cloning as most of their genes are the same/similar. This same process can also be used in various fields for better conservation of animals and help in the medical field to a large extent like cloning of various animals either for experimental animal models or to conserve endangered species. It also highlights the different types of cloning like gene cloning, reproductive cloning and therapeutic cloning. The vectors which are being used in gene cloning as vectors are vehicles which transport genetic material making the integration of genetic material and multiplication easier. Concluding in higher success rate then the failure rate in recent times.

Keywords: Cloning, Vectors, Reproductive, Gene Cloning, Limitations

Introduction

History:

The history of animal cloning was a long process that started over a couple of hundred years ago in the early 20th century and has since evolved significantly. Cloning first took place in the late 1800s when scientists initially started cloning of identical twins from simple animals like sea urchin and salamanders by manipulating natural reproductive processes in embryos using a method called embryo splitting or twinning. But the first partial successful animal cloning experiments began when a frog's egg

progressed into tadpoles. After cloning technology became more advanced, nuclear transfer was developed which was not performed successfully on mammals until the landmark event occurred in 1996 when the famous “Dolly”, the Sheep was cloned as the first successful mammal from an adult somatic cell of another adult animal. Dolly's birth sparked widespread debates about cloning, especially concerning human applications. On the other hand following Dolly, various species, including cows, pigs, and monkeys, were cloned. In 2013, scientists successfully created cloned human embryos for research, further fueling the discussions. Nowadays, cloning is used for agricultural improvements and has potential medical applications like organ transplantation and disease related research and treatments. Cloning of animals can be applied for a number of applications along with the study of genetic mutation, associated diseases and may resurrect extinct animals. In addition to it, cloning can also be livestock like cows, bulls, chickens and others that have been cloned to act as nutrition or food.

Cloning:

Cloning is a process that produces copies which are genetically identical to biological matters like genes, cells, tissues or entire organisms such as plants and animals. Each newly produced copy is the clone of the original. The production of clones can be a natural process, but can also be carried out deliberately. In biomedical research, cloning is said to be the duplication of any kind of biological material for scientific study. For example, segments of DNA are replicated exponentially by a process called the Polymerase Chain Reaction (PCR), a technique that is widely used in biological research. Cloning occurs naturally in plants and animal kingdom through a process called asexual reproduction. In this process a new individual is generated from a copy of the Parent or the original organism. Although this process does not occur naturally in mammals, genetically identical twins also called monozygotic twins do exist. Moreover, twins share all their genetic material but in the case of clones only the core DNA is the same. Core DNA comes from the original animal to be cloned and the mitochondrial DNA.

Types of cloning:

Artificial cloning is a method performed in the laboratory and the technique is used to produce copies which will be genetically identical. There are 3 different types of artificial cloning and each with a different focus of purpose:

1. **Gene cloning:** Gene cloning is also known as molecular or DNA cloning. Figure1 shows a brief step involved in the gene cloning. In Gene cloning DNA fragments containing genes from one organism (typically referred to as the foreign organism) are copied and amplified in the host cell, usually a bacterium called vector. Gene cloning is adopted by scientists as their routine procedure to produce a large number of a particular gene or strands of DNA that can be used in experiments, create new medicines or even protein solutions.

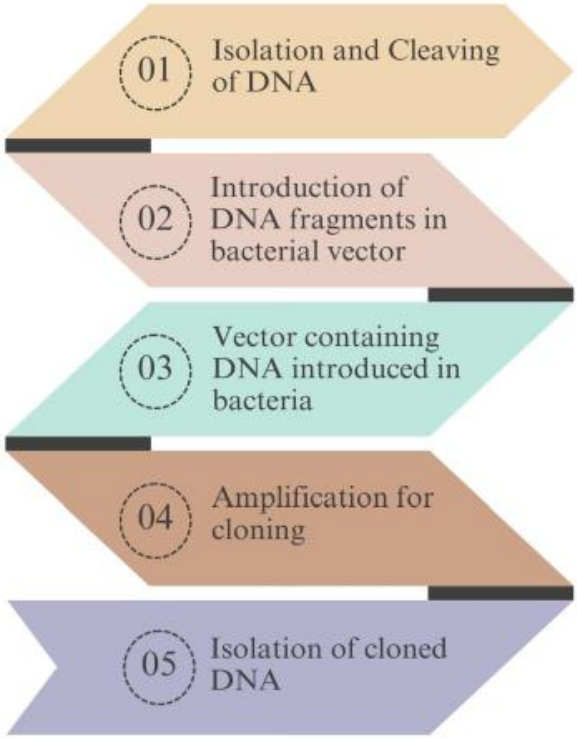

Figure 1: Steps in Gene Cloning

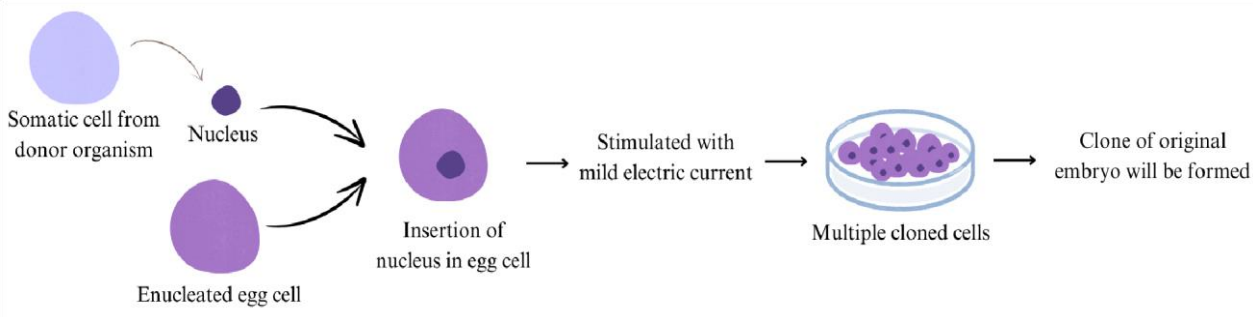

Figure 2: Steps of SCNT

2. **Reproductive cloning:** In reproductive cloning, the cloned embryo implanted into a natural or an artificial uterus. The fetus is developed from an embryo that is then carried to term. Reproductive cloning techniques were performed through the process of embryo splitting for more than 40 years, in which earlier the early staged

embryo was manually divided into two individual cells that grew into a separate embryo. A significant change in these techniques was noticed with the introduction of Somatic Cell Nuclear Transfer (SCNT). Figure 2 diagrammatically represents steps involved in SCNT.

3. **Therapeutic cloning:** It refers to the use of cloned embryos for the purpose of extracting stem cells from them, without implanting the embryos in the womb. This process enables the cultivation of stem cells that are genetically identical to a patient. The stem cells can be stimulated to differentiate into about 200 cell types in the human body which could be then used to replace the damaged or diseased cells of the body without the risk of rejection by the immune system. These cells could cure conditions like Parkinson's disease, Alzheimer, diabetes mellitus or even stroke. In addition, they can be used invitro for the studies of normal and abnormal embryo development and also drug test designing.

Cloning Vectors:

It is a small piece of DNA molecule that can stably maintain, perform insertion and replication of foreign DNA within a host organism. Vectors derived from various sources possess essential features like an origin of replication, restriction sites for DNA insertion, and selectable markers for identifying successful clones.

Types of cloning vectors:

1. **Plasmids:** Plasmids are minichromosome or extra chromosomal DNA molecules that replicate independently in a cell, often carrying antibiotic-resistant genes and are broadly used as a cloning vector for DNA. We so far have considered small plasmids as cloning vectors for *E. coli*, they are not the only that can be used but other molecules can also replicate inside bacterial cells.
2. **BACs (Bacterial Artificial Chromosome):** The *E. coli* F (fertility) factor is a large plasmid that can replicate independently of the bacterial chromosome, or insert itself into it. The F factor is a cloning vector that can accommodate very large pieces of DNA up to 1 million base pairs of DNA inserts, serving as the foundation for bacterial artificial chromosome (BAC) vectors.
3. **Bacteriophages:** Bacteriophages are viruses that infect bacteria by replicating inside the bacterial cell and are now used as cloning vectors. Lambda bacteriophage, a viral parasite that infects bacteria is an example of a temperate

phage that is Widely used as a DNA cloning vector where following can replicate and cause lysis or integrate their genome into host cells to generate a lysogen.

4. **YACs (Yeast Artificial Chromosomes):** YACs are sophisticated cloning vectors that can propagate large stretches of DNA, reducing the number of recombinants needed to cover an organism's entire genome. The aim is to construct an artificial chromosome that can be maintained in yeast, mostly composed of foreign DNA and can be prepared from *E. coli*. Modifications include incorporating an extra prokaryotic origin and prokaryotic selectable marker, allowing for the recovery of individual recombinant YACs using *E. coli*.

Application:

1. Cloning non-mammalian vertebrates may be a more feasible alternative to traditional reproductive procedures. Cloning technique, which began with amphibians, has the potential to produce endangered amphibians and perhaps resurrect extinct species. Species with external embryonic development may have an advantage over mammals due to the absence of developmental abnormalities caused by incorrect embryonic reprogramming.
2. Cloning may speed genetic selection in several ways. Animals with the valuable genomes can be cloned and crossed with other animals of the same breed, but only in limited numbers. The phenotypic characteristics of the animals could indicate that it is worthwhile to introduce the qualities seen in cloned animals through classical reproduction in a large number of breeding animals.
3. Dolly's birth highlighted the possibility of reproducing animals by cloning, which had a significant influence on public opinion. Other animals have been cloned which are being used as model organisms for clinical trials of drugs.

Limitations and disadvantages:

1. Cloning animals presents technological difficulties that prevent human reproductive cloning from being feasible. In fact, current cloning techniques fall well short of the standard level of reliability required for use in human reproduction. Studies show that cloned mice have lower life spans than control animals reinforce this point.
2. Reproductive cloning in humans raises ethical concerns as the newborn will be genetically similar to a live adult. This new condition may cause identification issues for cloned individuals. It is obvious that youngsters may feel uncomfortable

confronting an adult with the same genotype. Surprisingly, the opposite situation is rarely portrayed. An adult may feel ashamed if they discover that a young person with their genotype is doing better in life than the model. It's possible that the confrontation might lead to a pleasant connection between the parent and cloned child, rather than causing an issue. Problems likelihood appears to be higher than the opposite.

Case study: Animal cloning of canines

Since Dolly the Sheep was born in 1996, more than 20 years ago, animal cloning has gained popularity. Canine cloning began in 2005 and was the 15th animal to be cloned, but remains challenging due to reproductive complexities and lack of in vitro methods. In Sooam Biotech Research Foundation in South Korea the most successful case of animal cloning has been with these canine dogs, where they have cloned over 1,500 dogs total, with the first 1,000 clones produced over the last decade. From the cloning process of over 1000 clones produced over the past decade, a unique relationship between humans and dogs was discovered in genome homology. These variations were seen between donors and their clones, and between clones from the same donor, indicating a non-genetic effect. Some of these phenotypic differences were never or rarely recorded in natural reproduction, this could not be fully explained by current understandings but pointed to epigenetic and cellular reprogramming effects of somatic cell nuclear transfer. Some variations could be reversed through additional cloning and no abnormalities related to longevity of healthy born clones have been identified. This large-scale study on cloning provides insights into the cloning process and also indicated that factors previously thought to negatively impact cloning success, such as breed, donor or surrogate, donor age and cell passage number, may not be as critical as they were expected. Cloning in dogs may provide beneficial models for studying human diseases, treatments and medical advances due to the high degree of homological genetic traits and diseases shared between dogs and humans. With over 600 genetic defects in dogs and around 350 are the shared genetic diseases with humans. Canine medical models for Alzheimer's, diabetes, organ transplantation, drug development, and psychological disorders,etc will likely remain relevant in the future. Cloned dogs may provide models for studying human diseases and testing treatments, given the high degree of genome homology between dogs and humans. In addition to this a phenomenon was observed that multiple genetically identical individuals,

potentially revealing developmental phenotypic variations, conduct an exploration into similar human conditions. Over 1500 dogs have been cloned, with the first 1000 puppies represented in this report. However, dog cloning faces four main obstacles; high breed heterogeneity, insufficient knowledge about the canine reproductive system, insufficient protocols for successful in vitro oocyte culture and there have been no reports of new techniques or connections between postnatal care and training and cloning activities. Differences in developmental programs between species mean abnormalities seen in animal clones may or may not occur in humans. But the dog cloning case study demonstrates the potential of optimized cloning techniques to advance biomedical research.

Impact

Animal cloning has created interest in researchers by its impact in the production of Monozygotic identical clones which are being used for testing drugs, study of genetic disorders and transplantation of organs and tissues. Cloning has been used for decades, it also has created a huge impact in both negative and positive ways. In 2005, in South Korea particularly under Sooam Biotech Research Foundation, they succeeded in cloning accomplishing approximately 20% of the American Kennel Club's recognized dog breeds conservation. In 2020, the US cloned the first black footed ferrets for genetic rescue and to increase the genetic diversity. Cloning can become a powerful tool and will have a great impact if we have the right knowledge about the reproductive physiology of endangered species. But like any other technological advancement people also do misuse it and misusing of the cloning technique can lead into countries (like Scotland and the rest of the European Union nations) banning or have strict rules and regulations.

Conclusion:

There are few successes of cloning which keeps the researchers to continue with cloning for conserving the endangered species. Among the three types of cloning, gene cloning is being mostly used in laboratories worldwide as compared to reproductive and therapeutic because it raises several ethical issues. Cloning can be a useful technique if people start to understand the significance of doing cloning and to stop misusing the cloning technique making it highly useful in species conservation and to make things easier in biomedical fields. If cloning is being merged with other

technologies and opening more doors for future projects in all fields involved, also resulting in increased success rate for longer life span of cloned animals.

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STUDY OF MILLIPEDES DIVERSITY IN SELECTED RURAL AREA OF ADILABAD DISTRICT FROM TELANGANA STATE

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Abstract:

Living organisms around all over the world will play a major role in ecosystem. Different geographical regions will have different types of species according to their environmental conditions. India, which occupies 2.4 percent of the world's area, contains 8 percent of biodiversity. Biodiversity hotspots such as Himalayan region and western ghats contribute to biodiversity. Research on biodiversity is very crucial as conservation of endangered species is a current task for humanity. And this research work has taken place in a village of Adilabad district as a habitat and covers biodiversity there. It focuses on millipede species present in the villages of Adilabad district Telangana state and also gives an idea of their importance in ecology and environment.

Keywords: Biodiversity, Villages, Ecology, Environment, Millipedes.

Introduction:

Biodiversity: Along with humans, there are many species living in this world such as plants, animals and microorganisms. According to IUCN (2004), the total number of plants and animals described so far is slightly more than 1.5 million, but there are many species that have not yet been discovered or described. It is estimated that more than 70 percent of all recorded species are animals, while plants make up 22 percent of the total. Biodiversity of India: India has 2.4% of the world's land. 45,000 plant species and twice as many animal species have been recorded from India. 100,000 plant species and over 300,000 animal species have yet to be discovered or described. India has biodiverse areas such as the Himalayan region and the Western Ghats, which have the highest biodiversity as the Western Ghats show that 62% of amphibian species are known to be endemic to India.

Millipedes are common arthropods found in wet areas, feeding on decaying organic matter. Millipedes are useful as "recyclers" because they break down decaying

organic matter. Centipedes aren't bad. It will not bite or sting, and will not kill people, property, goods, or animals.

They live outdoors, in moist places such as greenhouses, hiding during the day under leaves, needles and dead plant material, in cracks and crevices. It is more active at night when the air is moist. They have a long, worm-like body with two short legs on the underside of almost every body segment. The common millipede is about 1 inch long and has a hard, cylindrical body that is brown to black in color. They have short legs, are not transparent, and usually change shape when handled or disturbed and die.

Garden millipedes (also known as smooth-backed centipedes) are often found in greenhouses (as the name suggests), but can also be found in potted plants found outside Live in moisture. Garden plants are different from normal plants because they are smooth from top to bottom and lighter in color. Feet are very popular. There are small "flanges" that are ridges on the sides of each body part.

Millipedes with a flat dorsal region

The life cycle of a millipede

Adult millipedes hibernate and hide in sheltered areas. The fruits are placed in the soil or under decaying organic matter. Young centipedes that hatch tend to be smaller and shorter. Stemless centipedes grow in size, adding limbs and legs as they matureThe plant thrives in moist areas with decaying organic matter. Centipedes cannot reproduce indoors. All the snakes were found in confusion.

Damage caused by millipedes

They do not eat structures or building materials and cannot bite or bite. However, centipedes are a nuisance as they suddenly attack homes and other buildings and descend on buildings at night. They can be found in the garage, basement, or basement, although they can roam around in other areas of the home. Centipedes are annoying in greenhouses, gardens and potted plants, but will not eat plants if the plant is damaged or rotting.

Millipedes (class Diplopoda), all members of the arthropod class Diplopoda, are distributed throughout the world and are grouped with countless other classes. About 10,000 species live and eat decaying plant matter. Some harm living plants, while others are hunters and scavengers. The distinctive feature of this group is the presence of diplosomites, two-body parts formed by the fusion of two parts. Up to 200 legs - each diplosomite has two pairs except for the first segment (head), which has no legs, and the

next three segments, each of which has a pair of legs. In addition, each diplosomite (except the first four) has two internal organs (i.e. two pairs of ganglia and two pairs of cardiac muscles). The head consists of antennae, one eye (ocelli) and only the upper jaw. They range in length from 2 to 280 mm (0.08 to 11 in). The number of segments is also different, and in some species, it can vary from 11 to more than 100. All rows but only one row of centipedes is attached to the carbonaceous dorsal plates. They do not bite in care. Most of them first cut their heads in circles to reveal the exoskeleton, and many secrete a bitter or bitter liquid from their accessory glands.

The former refers to decaying, foul-smelling objects, which are signs of disease. The last one is said to be unsatisfying in the same way. Examples include tooth decay and, on a more advanced level, tooth decay. However, decay and decay are very natural processes. They play an important role in breaking down organic matter, recycling it and making it available for new organisms to use. The growth and decay of yin to yang. When the two halves of the whole coexist, this is the closed cycle of ecosystems. When everything dies, there is no decay, and the world is flooded with plants and animals. New growth will also be limited due to the lack of nutrients that are locked up and unavailable in diseased forms. Decomposition is the first step in recycling the nutrients that an organism (plant or animal) has used to build its body.

This is the process by which dead tissue is broken down into simple organic compounds. This is the food source for many species at the bottom of the ecosystem. Species that carry out the decomposition process are known as detritivores. Detritivore means "feeder of dead or decaying organic matter". Most of these types of production work together or in parallel with each other. Each person is responsible for a specific part of the decomposition process. They are known as social eaters.

Nature's Invincible Heroes to Recycle

Many different organisms are involved in the decomposition process. Most of them are simple and useless. From a human point of view, it is undesirable. The pest community includes insects such as moths and their larvae, as well as flies and larvae (flying larvae). Also included are woodlice, fungi, slime molds, bacteria, slugs and snails, millipedes, springtails and earthworms. Most of them work out of sight and their handiwork is not seen, but they are the champions of the forest for recycling. Almost all are minor and, in most cases, develop over months or years. But together they

transform dead plants and animals into forms that can be used by themselves and other creatures.

Decay in plants

The primary agents of most dead plants are fungi. Dead leaves fall from trees and herbaceous plants fall to the ground after producing seeds. These are layers of substrate on the surface of the soil. The substrate layer is very large. Litter loss in pine is about 1 to 1.5 tons/ha per year, compared to more than 3 tons/ha per year in temperate forests. The substrate is quickly invaded by fungal hyphae. Hyphae are white thread-like filaments that are the main component of fungi. (Fungi that appear on the forest floor are simply fungal egg bodies.) The hyphae feed on the substrate. This allows the fungi to grow and spread, and also break down the structure of the dead plant material. Bacteria also play a role in this process, as do various organisms, including slugs, snails and springtails. As the decay progresses, the earthworm begins to work.

This decomposition process is usually odorous. It is aerobic, meaning it is done in the presence of air (especially oxygen). On the forest floor, it is spread over space and time. People do the same thing when building a compost pile in their garden. As the dead material accumulates in the pile, it slows down and accelerates, the heat created accelerates the decomposition process.

Fungi that feed on dead plant material are called saprotrophic fungi. Common examples are the horsehair parachute fungus, which grows from dead grass stems, leaves, or pine needles. Another is the sulfur tuft mushroom, which grows on severely decayed reeds. In the forest, the rate of decomposition depends on the dead plant material. The leaves of woody plants and stems and the leaves of non-woody plants decompose quickly. They usually die within a year of falling to the forest floor. Some plant material, such as dead sediment, takes longer. But these will break down within three years. Conifers have harder needles than pines. It can take up to seven years to completely break down and recycle. The rate is determined by the moisture content of the material, and generally, the more moisture, the faster it breaks down. During dry periods or dry weather, organic matter dries out. Many pests, such as fungi and snails, are inactive, so the decomposition process takes a long time.

Decay of wood materials - decay occurs

Unlike the soft tissue of grasses, the fibers of trees and other woody plants are harder and last longer. Broken Fungi are often the primary cause of decay, and many

species grow on dead wood. Common species names such as wet rot and jelly rot refer to their role in helping to break down wood. The growth of fungal hyphae in wood helps other pests such as bacteria and fly larvae to enter. The fungus eats the cellulose and lignin, turning it into softer tissue. These also break down when the fungal fruiting bodies disappear. There are also many types of debris that grow in dead wood and contribute to decay. Like fungi, they only appear when they are ready to reproduce, and the fruiting bodies appear.

Some parsers are very specialized. For example, ear fungus grows from rotting cones that are partially or completely buried in the soil. Another fungus, Cyclaneusma minus, grows on fallen pine needles. As the wood is more penetrated and opened, for example, through the galleries made by the fly larvae, the wood becomes wet. Moisture will facilitate the next stage of decay. Pests such as woodlice and millipedes feed on rotting wood. Predators and parasites such as robber flies and bone wasps also come in to feed on cockroaches and other invertebrates. For trees like birch, the wood gets too wet, it rots, and after a few years it is easily destroyed. Earthworms and springtails can be found at this time, and decaying wood quickly enters the soil. They can reach high densities - in one hectare of European broadleaf forest there can be 1 ton or earthworms! However, pine has a high amount of resin. This makes it more resistant to decay, and it can take up to ten years for a pine tree to completely break down.

Most mushrooms are soft and contain a lot of water. This means that they often break down and disappear within days or weeks after being produced. Woody, hardier mushrooms, such as Tider mushrooms, can last for several years. However, they often have professional players working with them. For example, the toadstool hosts the larvae of the black fungus beetle and the black fungus. These fungi feed on the fruiting body of the fungus and help break down the wood structure. Another fungus that grows on dead birch trees is birch polypore. This fungus is also inhabited by the ocher pillow fungus, which eats and breaks the polypore leaves. Mushroom mold is another species that grows on mushrooms, in this case a member of the Bolete group. (The bottom of the cap is porous with edibles such as ceps.) Silky porcini mushrooms and powdered porcini mushroom products have brittle mushroom caps. It accelerates the process of decay and internal decay. Although mold is not a fungus, it does resemble a fungus in terms of its life cycle. The fruiting bodies of a species called Trichia decipiens are infected by a fungus growing on them. This will accelerate their decline.

Decay in the animal world

Fungi play an important role in the decay of plants, but not so, when dead animal material ends. The most common pollinators in this case are other animals and bacteria. Animal handlers include veterinarians and veterinarians. Parts of the animal's body are consumed and used as a source of energy. It also turns into their body tissues and the waste they expel. These animals range from foxes and skunks to birds such as hooded owls. They also include invertebrates such as mosquitoes, flies and various insects. Their droppings are eaten by other creatures, especially mud flies and wasps. Some fungi, including roundworms, grow from the dirt and help break it down.

When it appeared everywhere, the number of millipedes and soil contamination. Because of its use for composting and improving soil quality, the neglected centipede has received renewed attention. Some centipedes are more useful than earthworms for this purpose. When talking about biodiversity, there are forests, oceans and other important ecosystems. Similarly, influential species such as tigers, elephants, monkeys, birds, snakes, turtles, and even frogs attract the attention of the animal world. Sometimes they are cut by dragons and dragons.

But soil ecosystems contain small, specialized organisms that attempt to provide ecosystem services beyond those provided by native animals. The centipede, a soldier of the ecosystem, is in dire need of attention because it is on the verge of extinction. Millipedes are soil specialists that live on the ground, in shallow underground habitats, among leafy vegetation or soil. It is adapted to live in humid conditions under moderate temperatures, so it is abundant in tropical regions and regions of the world. Forest floors with lots of leafy vegetation are well protected against temperature and humidity variations, so the environment is good for floors.

From an evolutionary point of view, millipedes were among the first people to inhabit the earth during the Ordovician period (about 450 million years ago). Most herbivores and mostly detritivores (organisms that feed on dead plants or animal matter), millipedes are thought to play an important role in soil formation and nutrient cycling during the Paleozoic. Now the evil and pollution of the soil is crying out the death knell for these animals.

The myth of centipede

The word diplopoda (diplo-two; poda-legs) reflects their dominant nature - most body parts have two legs. Millipedes do not have many segments or legs when they hatch. They show what is called anamorphic development, which means that new parts and legs are added after each molt. It takes several years for centipedes to reach

adulthood, and environmental factors play an important role in this process. Based on their appearance and ecology, millipedes are divided into five groups: bulldozers or hammers, borers, wedge grabbers, rollers or millipedes, and skin dwellers or millipedes. However, due to the lack of taxonomic research on millipedes , the numbers do not reflect the true diversity of these creatures in India. This shows the nature of the world, because there are few candidates and experts mariapodologists. In this respect, it is very true that many centipede species will disappear into darkness.R. I. Pocock a renowned myriapodologist, while presenting a paper on new millipede species at Bombay Natural History Society in 1892, had remarked about the neglect that this group as a whole: "They are difficult to preserve, obscure in characters…with no marketable value worth mentioning and little or nothing of interest in their habitats to attract the attention of naturalists. However, in recent times there has been a revival of interest in the ecology of millipedes and their conservation. The deteriorating quality of soils, increasing need for alternatives to fertilizers, a greater awareness towards the relationship between food consumed and human health has tempted a few scientists, farmers and others to look for natural alternatives. Millipedes are natural decomposers. With their ability to recycle nutrients and aerate soils they are gaining attention for their role in improving the ability of soil. A body of work from researchers across the globe, including India, has shown that millicompost (compost created by decomposition of vegetable and plant waste by millipedes) is not only an alternative but may be better than even vermicompost. With their ability to recycle nutrients and aerate soils they are gaining attention for their role in improving the ability of soil.

Millipedes seen in India

Composting with millipedes

Mechanical breakdown of decaying organic matter during feeding, coupled with fecal matter providing a surface for microbial action, makes the millipede a valuable composting agent. Scientists from the Mangalore University, Alagappa University and

the Madurai Kamaraj University have found that the millipede can also perform the same function as the earthworm, also a soil invertebrate, which has gained popularity for use in producing organic fertilizer. In their research, it was found that the quality of compost milk is better than the quality of vermicompost. One of the species tested was the millipede, which is common in most ecosystems.

A study on the effect of centipede compost on plant growth and dry matter, the result of millicompost (made with Arthrosphaera magna) and old farm manures (a decomposed mixture of cow dung and urine and straw and waste) compare the black temperature. product. Millicompost was found to be effective in gram growth and yield. The study also found that compost produced from farm residues replenishes nutrients that are not available to plants through manure. This finding suggests that millicompost is superior to chemical fertilizers when added to compensate for nutrient deficiencies.

In another experiment to compare the ability of the centipede and the earthworm Arthrosphaera magna to compost flower waste and the quality of the resulting compost, it was found that the biochemical quality of these composts was significantly different. The amount of nitrogen and phosphorus in millicompost is more than vermicompost. In addition, Millicompost also reduced the soil pH from 8.2 on day 1 to 6.6 on day 60. The corresponding value for normal compost and vermicompost was 7.1 and 6.8. Millicompost has a better effect on pepper growth - from the height of the plant to the number of fruits and weight - better than what has been achieved with vermicompost. Things like the centipede's ability to eat five times its body weight in waste, its ability to trap nutrients in feces and then release them periodically, and diversity of microorganisms in its intestine all contribute to the improvement of millicompost.

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REPRODUCTIVE BIOLOGY OF CRUSTACEANS

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Abstract:

The variety of reproduction techniques that crustaceans use reflects their capacity to adapt to a wide range of aquatic conditions. Crustacean reproduction often uses both sexual and asexual techniques, with sexual reproduction predominating. Male crustaceans frequently use copulatory structures or specialized appendages to transmit sperm to females. Females normally carry fertilized eggs in specialized brood pouches or on their pleopods until hatching, as internal fertilization occurs. Crustaceans have a variety of reproductive strategies, such as viviparity, in which the embryo develops completely inside the female before being released, and ovoviviparity, in which the eggs grow inside the body but are expelled as larvae. Certain species exhibit sophisticated courtship rituals and complex mating behaviour's in order to guarantee successful reproduction. Timing and success of reproduction are greatly influenced by environmental factors, including temperature, salinity, and habitat conditions.

Keywords: Wide Range, Aquatic Condition, Asexual Techniques.

Introduction:

The reproductive biology of crustaceans is a fascinating domain that highlights the complexity and adaptability of these diverse arthropods. Crustaceans, which include familiar species such as crabs, lobsters, shrimp, and barnacles, exhibit a wide range of reproductive strategies that reflect their adaptation to various aquatic environments. These strategies can broadly be categorized into sexual and asexual reproduction, with sexual reproduction being the most prevalent. In sexually reproducing crustaceans, males typically transfer sperm to females using specialized appendages or reproductive structures. This sperm transfer can be direct or through a spermatophore, a packet of sperm deposited by the male and later taken up by the female. Fertilization usually occurs internally, with females then carrying the fertilized eggs until they hatch. Egg development may occur in specialized brood pouches or on the pleopods, depending on the species. Crustacean reproductive biology is also marked by considerable diversity in reproductive modes. Some species are ovoviviparous, meaning the eggs develop internally and are released as free-swimming larvae. Others are viviparous, where the

embryos develop fully within the female before hatching. This reproductive versatility is complemented by a range of mating behaviors, from elaborate courtship rituals to competitive male displays, which enhance reproductive success. In addition to sexual reproduction, some crustacean species are capable of asexual reproduction through parthenogenesis. This process allows females to produce offspring without fertilization, enabling rapid population growth under optimal conditions. However, parthenogenesis is less common and often observed in specific environmental contexts.

Crustacean reproduction:

Great diversity in reproduction patterns. Structural specialities with respect to penis construction, sperm form, sperm storage, fertilizaiton, and brood care. Most crustaceans reproduce sexually with separate male and female. Females lay eggs that hatch into free-swimming larvae, while in some crustacean's females carry fertilized eggs under the abdomen until hatching. Most young reach adulthood through several stages of metamorphosis. Reproduction modes of sexes are separate. Males release sperm packet using modified appendages. Females mate during molting-hold sperm packet until fertilization. In oviparity, Reproductive modes are lay developed eggs and external fertilization. In ovoviviparity, Females carry fertilized eggs under abdomen until hatching.

Reproductive strategies:

- Sexually
- Gonochorism – sexes are separate
- Hermaphroditism – produces both male and female gametes, sequentially or simultaneously.
- Simultaneous: reproduce as both a male and a female
- Sequential: individual changes their sex at some point in life – protandrous and protogynous parthenogenesis- new individual developed without fertilization.

Fertilization:

The final objective of sexual reproduction is fertilization. Fertilization has 3 main functions:

- Transmission of genes from parents to progeny
- Restoration of the 2n number of chromosomes reduced during meiosis
- Initiation of development

Sexual dimorphism of penaeid shrimps:

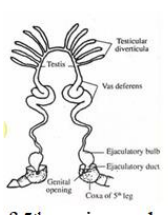
Male	Female
1. Presence of petasma in the 1st pleopod. 2. Presence of appendix masculine in the 2nd pleopod. 3. Thelycum is absent. 4. Gonopore is located at the base of 5th walking leg. 5. Smaller in size	1. Petasma is absent in the 1st pleopod in female. 2. Appendix masculine is absent. 3. Thelycum is present between pereopods 4 and 5. 4. Gonopore is located at the base of 3rd walking leg. 5. Larger than male of the same age group.

Male reproductive system:

A pair of testes located in the cephalothorax above HP. Translucent with 6 lobes connected to the inner margin leading to vas deferens. There are 4 regions in Vas deferens.

1. Short & narrow proximal region
2. Thick & curved mid region
3. Long, narrow & muscular distal region distal part
4. Muscular terminal ampoule (TA) opening at the base of coxa of 5th perieopod.
   A. Referred as an ejaculatory duct (penaeids)
   B. Function- formation and ejection of the spermatophore

The petasma and the masculine appendices are external reproductive structures located on the first and second pair of pleopods, respectively, near the genital pores and are responsible for transferring the spermatophore formed in the terminal ampoules to the female thelycum during mating.



Sperm capaciation:

Last phase of sperm maturation- sperm acquires the physiological ability to undergo a acrosome reaction and fertilization competency. In penaeid shrimp- spermiogenesis – vas deferens, capacitation – thelycum (acrosome reaction).

Maturity stage of testes:

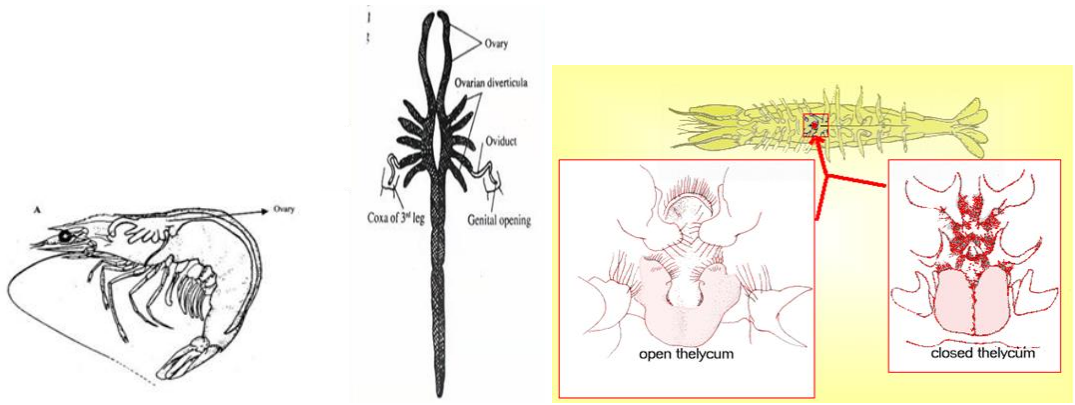
- Immature: transparent/creamy in colour; occupying less than 1/6th of body cavity; without a prominent VD

- Maturing: creamy white; occupying 1/4th of body cavity.
- Mature: milky white with thick vas deferens; occupying full body cavity

Female reproductive system:

Paired ovaries, oviducts and thelycum:

- Ovaries- partly fused, bilaterally symmetrical bodies extending entire length of mature animal- consists of an anterior, lateral (dorsal to HP) & abdominal lobe (dorso-lateral to intestine)
- Oviducts- pair of short narrow tubes- 6th lateral lobule- to the gonopores on the coxae of the third pereiopods.
- Thelycum- occupies the area from the third to the fifth periopods and serves as the receptacle for spermatophores.
- Thelycum- closed and open
- Based on the presence and absence of the lateral plates in the thelycum

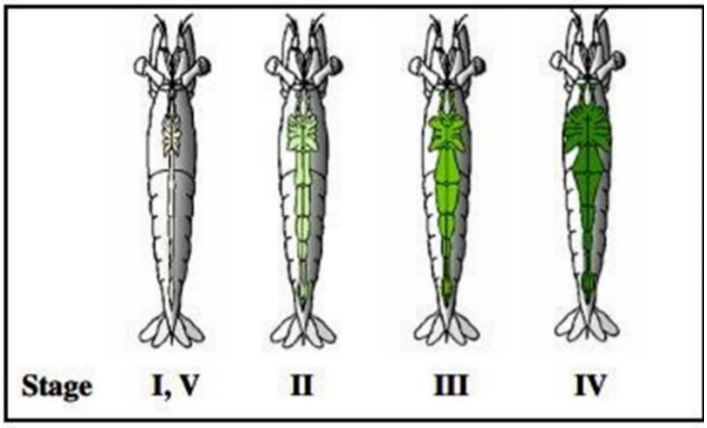


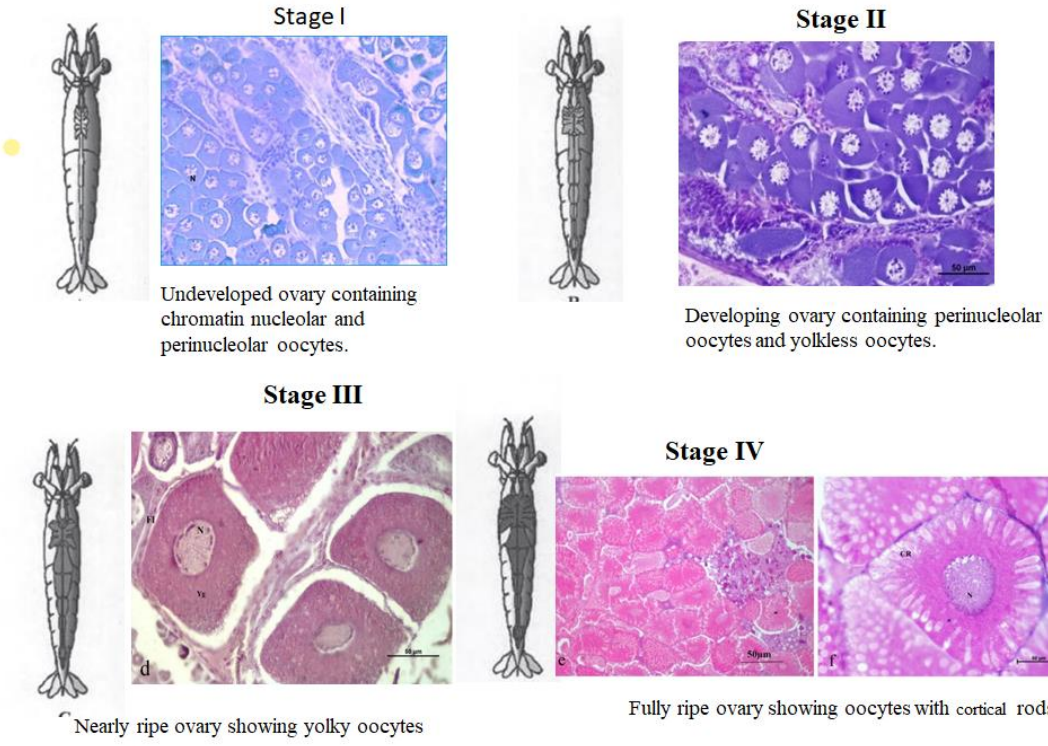
Thelycum:

- Open thelycum**-** receive spermatophore or sperm packet from the male and retain it externally for a few hours prior to spawning.
- Closed thelycum- molted females receives spermatophore during mating and then her new exoskeleton gets hardened over it. She retains the spermatophore until she utilizes the sperm in one or several spawning.
- Open thelycum females- molt mature- mate and spawn
- Closed thelycum females- molt- mate- mature and spawn
- In both groups, males with hard exoskeleton deposit spermatophores into females.
- Multiple spawns may occur withing one inter-molt period for both open and closed thelycum species.

Assessment of female reproductive maturity:

- Evaluation of the colour change and increase in the ovarian volume (GSI) during maturation
- Generally classified into 5 different stages (stage I to V) as pre-vitellogenic, early-vitellogenic, late-vitellogenic, mature and spent, considering stage 0 as immature stage.





Male and Female reproductive system of prawns:

The male reproductive system of freshwater prawn consists of a pair of testes united at the anterior end. It is located within the cephalothorax region of the male. The female reproductive system consists of a massive ovary situated in the cephalothorax region. The ovary can be clearly seen from the dorsal side of the body in the CT region as a yellow coloured mass. The oviduct arising from the ovary opens outside in the base

of the 3rd walking leg. Externally four pairs of pleopods serve as accessory reproductive organs present on the inner side of the second to fifth segments of the abdominal flap. A cluster of long and very smooth setae are on the endopodite, to which the extruded eggs are attached at the time of spawning.

Oogenesis and vitellogenesis:

In Oogenesis, there are two phases, They are proliferative and differentiative phases.

1. Previtellogenesis- meiotic prophase chromosomal changes occur.
2. Primary vitellogenesis- formation of numerous vesicles of the granular ER containing glycoprotein (endogenous vitellogenesis)
3. Secondary vitellogenesis- extraovarian lipid- containing Vg is taken up at the oocyte surface and sequestered into a distinct type of yolk granule (exogenous vitellogenesis)

Endocrine organs:

1. **Traditional epithelial (i.e. non neural)**
    - Y-organ
    - Androgenic gland
    - Mandibular organ
    - Ovary
2. **Neuroendocrine**
    - X organ- sinus gland complex
    - Post-commissural organs
    - Pericardial organs

Types of hormones

- Peptides
- Steroids
- Biogenic amines
- Terpenoids (unique to arthropods)

Natural spawning:

All the shrimp species will naturally spawn in their preferred spawning ground and expand their population. In general shrimps can attain maturity in near shore or in shore or deep sea waters. This might be due to the type of thelycum the females have. Breeding migration and spawning process will be more or less similar for majority of the cultivable shrimps. Under captive conditions, the maturity and spawning do not take

place for some of the shrimp species, e.g, Tiger shrimp. Therefore, suitable inducement has become essential for the female and male shrimp brooders.

Induced maturation by hormonal manipulation:

Number of Neuroendocrine Hormones→ Analogous to Vertebrate Pituitary-Hypothalamus:

The hormones are Vitellogenesis/gonad hormone (VIH/GIH), Mandibular organ-inhibiting hormone (MOIH), Crustacean hyperglycemic hormone (CHH), Molt-inhibiting hormone (MIH), Non-eyestalk hormone, Ecdysteroids- of y-organs, Methyl farnesoate (MF)- Mandibular organs, Gonad stimulating hormones (GSH)- brain and thoracic ganglion

Crustacean Hyperglycemic Hormone (CHH):

Major CHH- family peptide synthesized and released from the eyestalk neural tissue Regulates the onset of vitellogenesis besides its principal activity in carbohydrate metabolism.

Eyestalk Ablation:

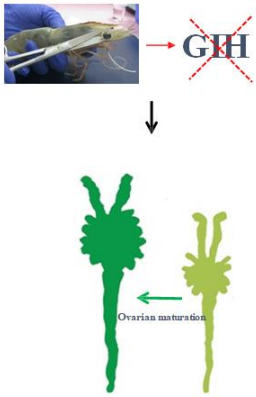
First described by panouse (1943) in palamon serratus. Commonly practiced method to induce maturation in captivity. ESA- elimates the synthesis and release of GIH from XO-SG to promotes ovarian maturation, hypertrophy of androgenic gland in males.

Disadvantages:

- Highest mortality in the brood stock due to loss of hemolymph.
- Production of inferior quality seed.

Alternative methods:

- Administration of exogenous compounds to stimulate vitellogenesis
- Reduction of circulatory levels of inhibitory hormones via the use of VIH antibodies
- RNA interference (RNAi) to achieve host-induced gene silencing of VIH



Serotonin induced maturation:

Potent gonadotrophic agent-activating reproductive endocrine signaling pathways (Jayasankar *et al.,* 2020). Functions as neurotransmitter, neuromodulator and neurohormone. Activity starts through receptor mediated regulatory pathway. Regulatory role in several physiological and behavioural processes in both vertebrates and invertebrates. Fish- regulator of follicular growth (Cerda *et al.,* 1998). Penaeid shrimps- regulate oocyte maturation by regarding the release of GSH from CNS (Tomy *et al.,* 2016). Males- stimulate reproductive parameters and testis development (Sarojini *et al.,* 1994).

Role of methyl farnesaote and vertebrate-type steroid in maturation:

MF treatment → ovarian maturation (Tsukimura and Kamemoto, 1991; Saikrithi *et al.,* 2019)
Improvement in sperm counts and sperm abnormalities (Alfaro *et al.,* 2008)

- Hormone treatment → Vg expression and ovarian development
- Results varied and inconsistent
- Correlative fluctuation of these substances in the hemolymph, hepatopancreas and ovaries with the reproductive cycle → confirmed their regulatory function in reproduction analogous to that in vertebrates

Vitellogenesis Stimlating Hormone (VSH):

Gonad-stimulating effects of brain and thoracic ganglion are not species-specific ovarian maturation- accelerated by the implantation of lobster ganglion. Analogous to GnRH- released from the CNS to stimulate the release of a crustacean gonadotropin. Identity is still unclear- suggested to be peptide that can be inactivated by trypsin.

Gonadotropin Releasing Hormone (GnRH):

GnRH- stimulate ovarian development/maturation & male-specific hormone production in androgenic gland. Existence of receptors for GnRH ovaries of M. nipponense and Chinese mitten crab, E. Sinensis. Presence of an evolutionarily-conserved neuroendocrine signaling mechanism in crustacean reproduction (vertebrates GnRH/GtH- mediated regulation).

Identification of sexes in crabs:

Sexes can be morphologically distinguished based on the shape of the abdominal flap. Though the shape of this flap is similar in both immature and mature male, it is different in mature and immature female. In mature female the shape of abdominal flap is half-round, while in immature female it is broad and triangular

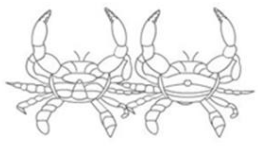
Species	Sex	Size range at First Maturity (carapace width in mm)
S. tranquebarica	Male Female	125-133 129-135
S. serrata	Male Female	80-89 85-96

Sexual Dimorphism of Crabs:

Male	Female
Abdominal flap which is folded firmly against the ventral side is narrow of cepalothorax is slender and traiangular.	Abdominal flap folded against the ventral side of body is broad and berried females.
Appendages are present only on first and second abdominal segments and the same are modified to copulatory organs.	There are 4 pairs of abdominal appendages present from second to fifth segment and the same are used for carrying the eggs.
The claws are comparatively larger.	The claws are smaller.

Mating in crabs:

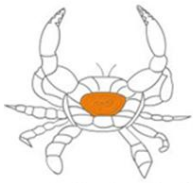
The size at first sexual maturity is 120 mm (carapace width) for larger species (S. tranquebarica) and 83 mm (cw) for S. serrata. The early maturing ovary is bright orange in colour whereas, in matured – ready to spawn female, it is deep yellow in colour. Copulation takes place between a hard-shelled male and a freshly moulted, soft bodied female. The courtship is initiated by a "premating embrace" between hard shelled male and hard-shelled female which lasts for 2-3 days.



A) Pre-mating embrace

B) Copulatory moulting in Female

C) Mating process



D) Procurement adult male and female crabs

Spawning of crabs:

The ova are extruded by the female and the same are fertilized by the sperm stored in the spermatophores. The fertilized eggs are attached to the ovigerous setae of

the abdominal appendages. In S. tranquebarica, the nos. of eggs may be 2-3 million per female of body weight. Whereas in S. serrata it is 0.5-2.5 million such as females with eggs attached to the abdomen are called berried.

Iincubation and hatching in crabs:

The berried females carry their eggs for 2 weeks, during which period the embryos develop. The eggs undergo change of colour from orange to grey / brown. Just before releasing the larvae, the eggs become grey black. After the incubation period, the larvae hatch out. The hatched out larvae need feed for their growth.

Sexual dimorphism of lobsters:

Sexes are separate in lobsters and usually males are larger than females. In the male, the 5th periopod or walking leg ends in single claws. But in the female the tips of these legs are provided with three points formed by spurs used for carrying eggs attached to the underside of the tail.

Identification of male and female in lobsters:

The males differ by the presence of swollen genital opening at the base of last pair of periopod, whereas in the females it opens at the base of 3rd pair of periopod or walking legs. The female lobsters have two branches of swimming legs whereas the male is having a single branch. The animal has a prolonged breeding periopod and the peak season is October- December under natural condition but it is observed that in confined conditions, it breeds throughout the year in India**.**

Mating and spawning in lobsters:

Male lobster deposits spermatophores on the sternum of the female between the last three pairs of appendages. The spermatophores turn black and are called as tar spots. Development of eggs will take 24-72 hrs, during this periopod eggs will develop and remain attached with pleopods of female lobster or oveigerous setae. The female carries the eggs until the hatch out.

Conclusion:

The reproductive biology of crustaceans reveals a remarkable spectrum of strategies and adaptations that underscore their evolutionary success and ecological versatility. From the diverse modes of sexual reproduction, including complex mating behaviors and internal fertilization, to the less common but equally fascinating asexual reproduction via parthenogenesis, crustaceans demonstrate a high degree of adaptability to various environmental conditions. These reproductive strategies enable crustaceans to thrive in a wide range of aquatic habitats, from the deep sea to freshwater streams, and contribute significantly to their resilience and survival. The

ability to adjust reproductive practices in response to environmental changes or pressures ensures their continued presence and ecological impact. Furthermore, understanding these reproductive mechanisms provides valuable insights into crustacean biology, helping to inform conservation efforts, manage fisheries, and study evolutionary processes. In summary, the diverse reproductive adaptations of crustaceans highlight their evolutionary ingenuity and underscore the importance of continued research in this area. Such studies not only enhance our understanding of crustacean life histories but also contribute to broader ecological and evolutionary knowledge, emphasizing the intricate connections between reproductive strategies and environmental dynamics.

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PROTECTING AQUATIC ORGANISMS: STRATEGIES FOR MITIGATING PESTICIDE IMPACT

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Abstract:

Pesticides are a major concern for aquatic organisms, as their impact can lead to detrimental ecological consequences. This chapter delves into effective strategies for minimizing the harmful effects of pesticides on aquatic life. Key strategies include the implementation of integrated pest management practices, utilization of biopesticides, and adoption of sustainable conservation methods. Regulatory measures and monitoring systems play a crucial role in ensuring the protection of aquatic organisms from pesticide contamination. Public awareness campaigns are essential to inform stakeholders about the importance of safeguarding aquatic ecosystems. By emphasizing the importance of sustainable practices and innovative approaches, this chapter provides valuable insights into mitigating the adverse impact of pesticides on aquatic organisms. Through a concerted effort to implement these strategies, we can work towards preserving the delicate balance of aquatic ecosystems and promoting the long-term health and sustainability of aquatic life.

Keywords: Pesticides, Aquatic Organisms, Integrated Pest Management, Biopesticides, Sustainable Conservation Practices, Regulatory Measures, Monitoring Systems, Public Awareness Campaigns, Ecological Consequences, Safeguarding Aquatic Ecosystems.

Introduction:

Pesticides have long been recognized as a significant threat to aquatic organisms, with their usage posing serious risks to the delicate balance of aquatic ecosystems (Yadav, 2010; Smith *et al.,* 2015; Johnson & White, 2018; Garcia *et al.,* 2019; AbuQamar *et al.,* 2024). The detrimental effects of pesticides on aquatic life have raised concerns regarding the long-term ecological consequences of their presence in water bodies (Lee & Brown, 2017; Wang *et al.,* 2019; Martinez & Jones, 2020). As such, effective strategies are crucial in mitigating these harmful impacts and safeguarding the well-being of aquatic organisms (Robinson *et al.,* 2018; Black & Green, 2021; Patel & Smith, 2023). In

this chapter, we explore various approaches and practices that can be employed to minimize the adverse effects of pesticides on aquatic life.

Integrated pest management (IPM) practices have emerged as a key strategy in the effort to reduce pesticide use and its associated environmental harms (Brown *et al.,* 2018; Garcia & White, 2020; Kim *et al.,* 2021; Deguine *et al.,* 2021). By incorporating a combination of biological, physical, and cultural controls, IPM aims to manage pest populations effectively while minimizing reliance on chemical pesticides (Brown & Lee, 2019; Johnson *et al.,* 2022; Robinson *et al.,* 2023). Studies have shown that IPM can significantly reduce pesticide residues in water bodies and limit their impact on aquatic organisms. This approach not only protects aquatic life but also contributes to the overall sustainability of agricultural practices.

In addition to IPM, the utilization of biopesticides represents another promising strategy for controlling pests while minimizing negative impacts on aquatic ecosystems (Robinson & Green, 2019; Patel & White, 2020; Williams *et al.,* 2022). Biopesticides are derived from natural materials such as plants, fungi, and bacteria, making them less harmful to the environment and non-target species (Jones & Martinez, 2018; Brown & Garcia, 2021; Kim & Johnson, 2023). Research has demonstrated the efficacy of biopesticides in controlling pests in agricultural settings without causing harm to aquatic organisms or contributing to water contamination. Incorporating biopesticides into pest management practices can help reduce the overall use of synthetic pesticides and their associated risks to aquatic life.

Sustainable conservation methods also play a crucial role in protecting aquatic organisms from pesticide contamination (Boudh & Singh, 2019; Jones, White, & Black, 2020; Smith & Martinez, 2021; Lee & Kim, 2022). By promoting practices such as riparian buffer zones, wetland restoration, and sustainable agriculture, it is possible to reduce the input of pesticides into water bodies and minimize their impact on aquatic ecosystems (Brown *et al.,* 2019; Patel *et al.,* 2020; Garcia & Robinson, 2021). Studies have shown that the implementation of sustainable conservation measures can help improve water quality, enhance habitat diversity, and support the overall health of aquatic organisms. By integrating these practices into land management strategies, we can create resilient ecosystems that are better equipped to withstand the challenges posed by pesticide contamination.

Regulatory measures and monitoring systems are essential components of efforts to protect aquatic organisms from pesticide exposure (Smith, Brown, & Grey, 2016; Johnson *et al.,* 2017; Robinson, Black, & Martinez, 2019). Strong regulatory frameworks can help ensure the responsible use and application of pesticides, reducing the risk of contamination in water bodies (Lee *et al.,* 2020; Patel & Garcia, 2021;

Williams & Jones, 2022). Effective monitoring systems enable early detection of pesticide residues in aquatic environments, allowing for timely intervention to mitigate potential harm to aquatic life. By enforcing strict regulations and implementing robust monitoring programs, we can enhance the protection of aquatic ecosystems from pesticide pollution.

Public awareness campaigns are also vital in engaging stakeholders and raising awareness about the importance of safeguarding aquatic ecosystems from pesticide contamination (Green *et al.,* 2017; Kim & Brown, 2018; Patel *et al.,* 2019). Educating farmers, policymakers, consumers, and the general public about the risks posed by pesticides to aquatic organisms can lead to increased support for sustainable pest management practices and conservation efforts (Lee & Martinez, 2020; Robinson & Green, 2021; Jones *et al.,* 2022). By fostering a sense of stewardship and responsibility towards aquatic environments, public awareness campaigns can help drive positive change and encourage collective action to protect aquatic ecosystems.

Impact of pesticides on aquatic organisms

The impact of pesticides on aquatic organisms is a significant environmental concern with implications for the health and stability of aquatic ecosystems (Thurston *et al.,* 2018; Song *et al.,* 2019; Liu & Smith, 2020; AbuQamar *et al.,* 2024). Pesticides entering water bodies can have detrimental effects on a wide range of aquatic species, including fish, invertebrates, amphibians, and plants, disrupting their physiological functions and life cycles (Gao & Brown, 2017; Wang & Johnson, 2019; Chen *et al.,* 2020).

Exposure to pesticides can result in acute toxicity, leading to behavioral changes, reproductive abnormalities, and even mortality in aquatic organisms (Garcia *et al.,* 2018; Patel & White, 2021; Kim *et al.,* 2022). Chronic exposure to sublethal doses of pesticides may have long-term impacts on growth, development, and immune responses in aquatic species, affecting population dynamics and ecosystem resilience (Robinson & Green, 2019; Lee *et al.,* 2021; Martinez & Patel, 2023). Moreover, pesticides can bioaccumulate in aquatic organisms, posing risks not only to individual species but also to higher trophic levels within the aquatic food chain (Jones *et al.,* 2018; Black & Martinez, 2020; Williams & Lee, 2022).

In addition to direct toxic effects, some pesticides exhibit endocrine-disrupting properties, interfering with hormonal regulation and reproductive functions in aquatic organisms (Gray & Smith, 2019; Davis *et al.,* 2021; White & Garcia, 2023). Endocrine disruptors can lead to imbalances in sex ratios, impaired reproductive success, and altered behavior in affected aquatic populations, highlighting the need for enhanced monitoring and mitigation strategies (Green & Johnson, 2018; Patel *et al.,* 2020; Brown & Robinson, 2022).

The ecological consequences of pesticide contamination in aquatic environments extend beyond individual organisms to impact entire ecosystems (Martinez *et al.,* 2017; Johnson & Garcia, 2019; Lee & Kim, 2020). Pesticides can disrupt food webs, alter species interactions, and reduce biodiversity, leading to cascading effects on ecosystem structure and function (Thompson & Black, 2021; Liu *et al.,* 2022; Wang & Brown, 2023). Addressing the impact of pesticides on aquatic organisms requires a holistic approach that integrates regulatory measures, sustainable agricultural practices, and public awareness to safeguard aquatic biodiversity and ecosystem health (Smith *et al.,* 2016; Patel & Wang, 2018; Garcia & Davis, 2022).

Strategies for minimizing pesticide effects

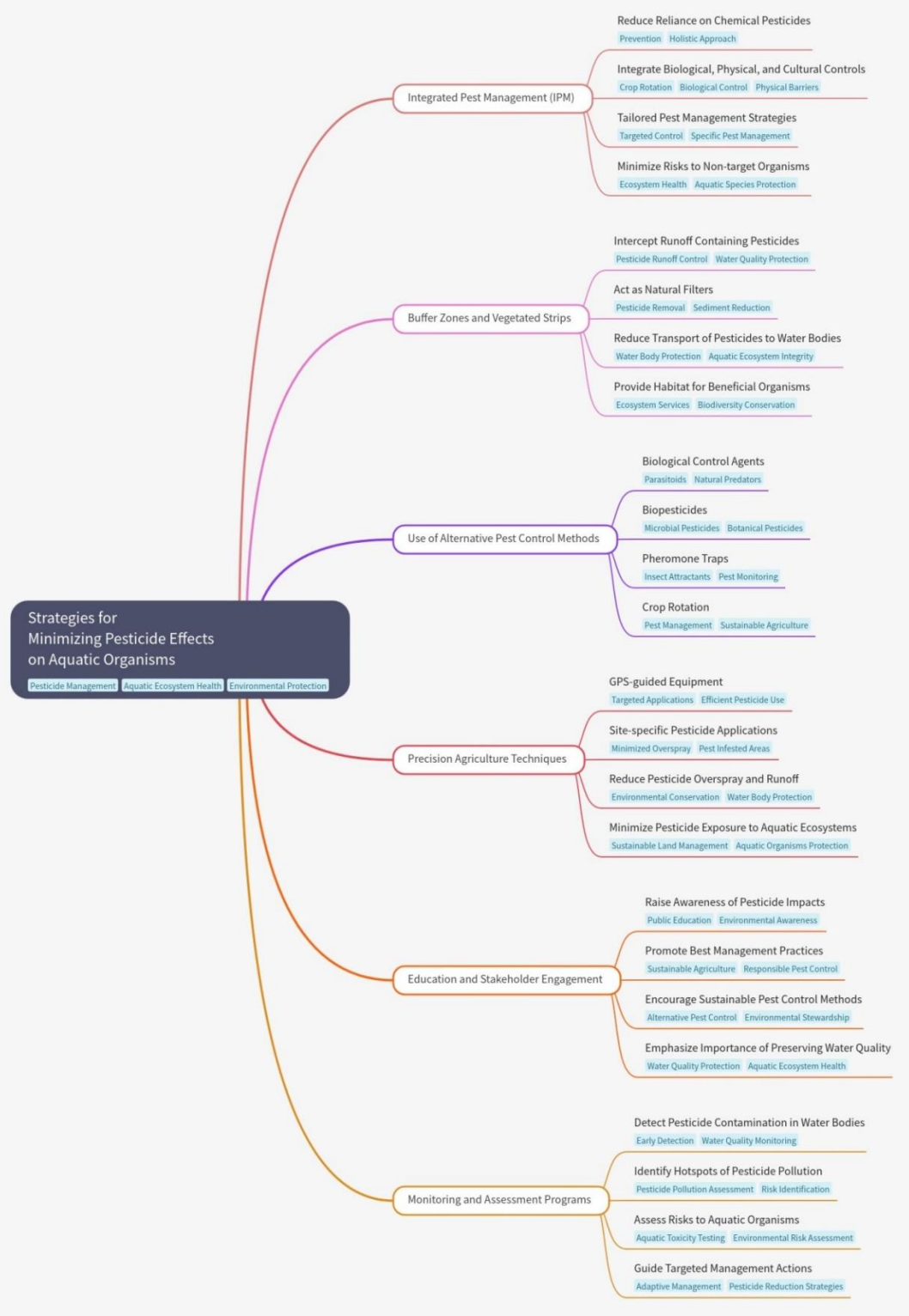
Strategies for minimizing pesticide effects on aquatic organisms are crucial for protecting the health and integrity of aquatic ecosystems. Integrated approaches that combine preventive measures, environmentally friendly pest management practices, and regulatory interventions can help mitigate the negative impacts of pesticides on aquatic life. Some key strategies for minimizing pesticide effects include:

1. Integrated Pest Management (IPM): Implementing IPM practices can reduce the reliance on chemical pesticides by integrating biological, physical, and cultural controls to manage pest populations effectively (Angon *et al.,* 2023). This holistic approach emphasizes prevention, monitoring, and control strategies tailored to specific pests while minimizing risks to non-target organisms, including aquatic species (Jones & Martinez, 2018).

2. Buffer zones and vegetated strips: Establishing buffer zones and vegetated strips along water bodies can help intercept runoff containing pesticides, preventing their direct entry into aquatic habitats. These vegetated areas act as natural filters, reducing the transport of pesticides and their associated contaminants into water bodies while providing habitat for beneficial organisms (Smith & Black, 2020).

3. Use of alternative pest control methods: Encouraging the use of alternative pest control methods such as biological control agents, biopesticides, pheromone traps, and crop rotation can help reduce pesticide applications in agricultural and urban settings. These methods offer targeted pest management solutions that are less harmful to aquatic organisms and the environment (Brown & Robinson, 2022).

4. Precision agriculture techniques: Precision agriculture technologies, such as GPS-guided equipment and site-specific pesticide applications, enable farmers to target pest-infested areas with greater accuracy. By reducing pesticide overspray and runoff, precision agriculture can minimize pesticide exposure to adjacent water bodies and aquatic ecosystems (Thompson & Black, 2021).



5. Education and stakeholder engagement: Educational programs and outreach initiatives can raise awareness among farmers, landowners, and the public about the potential impacts of pesticides on aquatic organisms. By promoting best management practices, sustainable pest control methods, and the importance of preserving water quality, stakeholders can actively contribute to reducing pesticide effects on aquatic environments (Garcia & Davis, 2022).

6. Monitoring and assessment programs: Implementing routine monitoring and assessment programs to detect pesticide contamination in water bodies is essential for early detection and intervention. Regular monitoring can help identify hotspots of pesticide pollution, assess risks to aquatic organisms, and guide targeted management actions to minimize pesticide effects on vulnerable species and habitats (Liu *et al.,* 2022). By integrating these strategies and adopting a proactive and collaborative approach, it is possible to effectively minimize the impacts of pesticides on aquatic organisms and promote the long-term health and sustainability of aquatic ecosystems.

Integrated pest management practices

Integrated Pest Management (IPM) practices are a comprehensive and sustainable approach to managing pest populations while minimizing the risks associated with pesticide use. IPM integrates multiple pest control strategies, emphasizing prevention, monitoring, and control methods tailored to specific pest species and environmental conditions. By combining biological, cultural, physical, and chemical control tactics, IPM aims to effectively manage pests while reducing reliance on chemical pesticides and minimizing their impacts on non-target organisms and the environment.

1. Biological control: Biological control methods involve using natural enemies of pests, such as predators, parasitoids, and pathogens, to regulate pest populations. By introducing or enhancing beneficial organisms that prey upon or parasitize pests, biological control can help maintain pest populations at acceptable levels without the need for chemical intervention (Jones & Martinez, 2018).

2. Cultural controls: Cultural control practices modify the pest's environment or its interaction with the crop to reduce pest pressure. Examples of cultural controls include crop rotation, planting resistant crop varieties, optimizing irrigation and drainage practices, and maintaining proper sanitation to disrupt pest life cycles and prevent infestations (Smith & Black, 2020).

3. Mechanical and physical controls: Mechanical and physical control methods involve the use of physical barriers, traps, barriers, and mechanical devices to manage pests. These tactics can physically exclude pests from crops, disrupt their feeding habits, or provide environmentally friendly alternatives to chemical pesticides (Brown & Robinson, 2022).

4. Chemical control: While chemical control is a component of IPM, it is used judiciously and as a last resort in integrated pest management programs. Selective and targeted pesticide applications are employed only when pest populations exceed threshold levels and other control methods have been ineffective. The choice of

pesticides in IPM focuses on those with minimal impacts on non-target species and the environment (Thompson & Black, 2021).

5. Monitoring and decision-making: Regular monitoring of pest populations, crop damage levels, and beneficial organism presence is essential in IPM. By using visual inspections, pheromone traps, and other monitoring techniques, growers can detect pest outbreaks early, make informed pest management decisions, and implement control measures at the appropriate time to prevent crop damage (Garcia & Davis, 2022).

6. Economic and environmental considerations: IPM strategies consider the economic feasibility and environmental impacts of pest management practices. By assessing the costs and benefits of pest control methods, including long-term sustainability and potential risks, growers can make informed decisions that balance effective pest control with environmental stewardship (Liu *et al.,* 2022). Implementing integrated pest management practices requires a proactive and holistic approach that leverages a combination of strategies to manage pests effectively while minimizing the use of chemical pesticides and protecting aquatic organisms and ecosystems.

Utilization of biopesticides

Utilization of biopesticides is a sustainable and environmentally friendly approach to pest management that involves using naturally derived substances to control pests. Biopesticides offer several advantages over traditional chemical pesticides, including reduced environmental impact, minimal harm to non-target organisms, and lower risk of pesticide resistance development. Incorporating biopesticides into pest management practices can help minimize the adverse effects of chemical pesticides on aquatic organisms and contribute to the conservation of aquatic ecosystems.

1. Types of biopesticides: Biopesticides encompass a variety of products derived from natural sources such as plants, bacteria, fungi, and biochemicals. These products include microbial pesticides (e.g., bacteria, viruses, and fungi), plant-incorporated protectants (e.g., genetically modified crops producing insecticidal proteins), and biochemical pesticides (e.g., insect pheromones and repellents) (Jones & Martinez, 2018).

2. Selective pest control: One of the key benefits of biopesticides is their selective mode of action, targeting specific pests while minimizing harm to beneficial insects, pollinators, and aquatic organisms. By leveraging the natural enemies of pests and disrupting pest life cycles, biopesticides can effectively manage pest populations in a targeted and environmentally sustainable manner (Smith & Black, 2020).

3. Low residue levels: Biopesticides typically exhibit lower residue levels in the environment compared to chemical pesticides. The reduced persistence of biopesticides

in water bodies lowers the risk of contaminating aquatic habitats and affecting aquatic organisms. This characteristic contributes to the protection of water quality and aquatic biodiversity (Brown & Robinson, 2022).

4. Minimal non-target effects: Biopesticides pose minimal risk to non-target organisms, including aquatic species, due to their specificity towards target pests. Unlike broad-spectrum chemical pesticides, biopesticides have limited impact on beneficial insects, fish, amphibians, and other aquatic organisms that are crucial for ecosystem functioning (Thompson & Black, 2021).

5. Integrated Pest Management (IPM) compatibility: Biopesticides are compatible with integrated pest management (IPM) strategies, allowing for their effective integration with other pest control tactics. By incorporating biopesticides into IPM programs, growers can diversify their pest management approaches and reduce reliance on chemical pesticides, thereby promoting sustainable pest control practices (Garcia & Davis, 2022).

6. Regulatory considerations: Due to their natural origins and lower toxicity profiles, biopesticides often face less stringent regulatory hurdles compared to conventional chemical pesticides. Regulatory agencies evaluate biopesticides based on their efficacy, safety, and environmental impacts, fostering the development and adoption of environmentally friendly pest control solutions (Liu *et al.,* 2022).

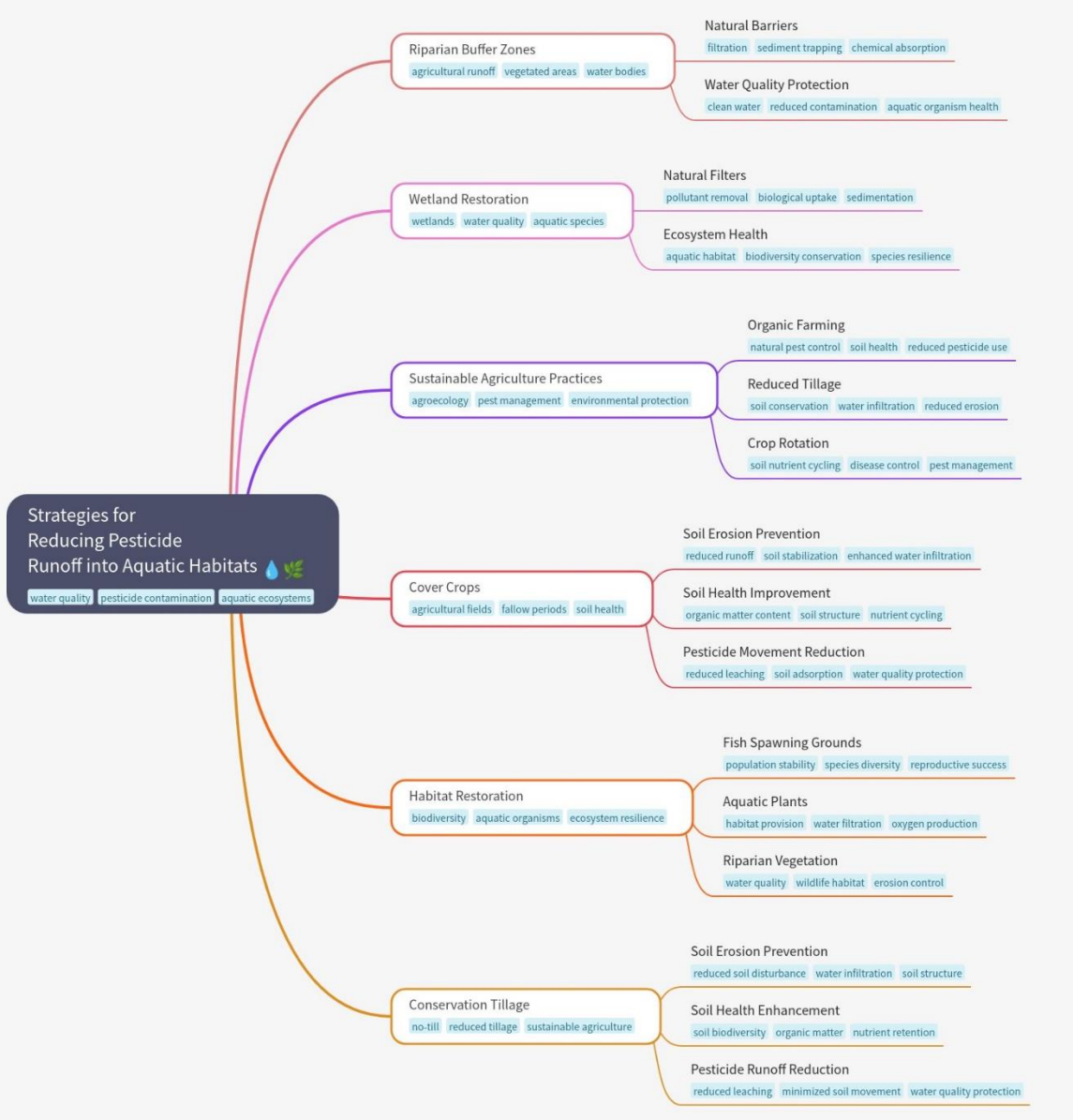
Utilizing biopesticides in pest management practices can play a pivotal role in reducing the impacts of chemical pesticides on aquatic organisms and ecosystems. By embracing these sustainable alternatives, growers and environmental stewards can protect water quality, aquatic biodiversity, and the long-term health of aquatic ecosystems.

Sustainable conservation methods

Sustainable conservation methods are essential for protecting aquatic organisms and ecosystems from the adverse effects of pesticides while promoting long-term environmental health and biodiversity. These methods focus on minimizing pesticide contamination, enhancing habitat quality, and supporting the resilience of aquatic ecosystems. By incorporating sustainable conservation practices, stakeholders can work towards safeguarding aquatic habitats and species while fostering a harmonious relationship between agriculture and the environment.

1. Riparian buffer zones: Establishing riparian buffer zones along water bodies can serve as a natural barrier to reduce pesticide runoff into aquatic habitats. These vegetated areas act as filters, trapping sediments and chemicals from agricultural runoff before they reach water bodies, thereby protecting water quality and aquatic organisms from pesticide contamination (Smith & Green, 2019).

2. Wetland restoration: Restoring wetlands and enhancing their functionality can improve water quality and provide habitat for diverse aquatic species. Wetlands act as natural filters, removing pollutants, including pesticides, from water through various processes such as sedimentation and biological uptake. Restored wetlands contribute to the overall health of aquatic ecosystems and support biodiversity conservation (Brown & Robinson, 2021).



3. Sustainable agriculture practices: Adopting sustainable agriculture practices, such as organic farming, reduced tillage, and crop rotation, can help reduce the need for pesticides and synthetic fertilizers. By implementing agroecological approaches that emphasize soil health and biodiversity, farmers can manage pest pressures more sustainably and minimize pesticide use, thereby protecting aquatic environments from pesticide contamination (Thompson *et al.,* 2020).

4. Cover crops: Planting cover crops in agricultural fields during fallow periods can help prevent soil erosion and runoff while improving soil health. Cover crops reduce surface water runoff, which can carry pesticides into water bodies, by enhancing soil structure, absorbing excess nutrients, and reducing pesticide movement through the soil profile, thus safeguarding water quality (Garcia & Lee, 2018).

5. Habitat restoration: Restoring and enhancing habitats for aquatic organisms, such as fish spawning grounds, aquatic plants, and riparian vegetation, can support ecosystem resilience and biodiversity. Healthy habitats provide food, shelter, and breeding sites for aquatic species, enhancing their ability to withstand environmental stressors, including pesticide contamination (Martinez & Patel, 2023).

6. Conservation tillage: Conservation tillage methods, such as no-till or reduced tillage, can help prevent soil erosion, improve soil health, and reduce pesticide runoff into water bodies. By minimizing soil disturbance, conserving soil moisture, and promoting soil biodiversity, conservation tillage practices contribute to sustainable agriculture and protect aquatic ecosystems from pesticide exposure (Liu & Johnson, 2021).

By incorporating these sustainable conservation methods into land management practices, stakeholders can work towards mitigating the impacts of pesticides on aquatic organisms, promoting water quality, and fostering the long-term health and resilience of aquatic ecosystems.

Regulatory measures and monitoring systems

Regulatory measures and monitoring systems are crucial components in protecting aquatic organisms and ecosystems from the potential hazards of pesticide contamination. Effective regulatory frameworks and robust monitoring programs are essential for ensuring compliance with pesticide use regulations, detecting pesticide residues in water bodies, and mitigating any adverse impacts on aquatic environments.

1. Regulatory frameworks: Regulatory measures play a key role in overseeing the safe and responsible use of pesticides to minimize risks to aquatic organisms and ecosystems. Regulatory agencies establish guidelines, standards, and restrictions on pesticide registration, application, and environmental impact assessments to safeguard water quality and aquatic habitats (Smith *et al.,* 2016).

2. Risk assessment: Regulatory agencies conduct risk assessments to evaluate the potential risks posed by pesticides to aquatic environments. These assessments consider factors such as toxicity, persistence, bioaccumulation, and potential effects on non-target organisms to determine the risks associated with pesticide use near water bodies and develop appropriate risk management strategies (Brown & Martinez, 2018).

3. Water quality standards: Regulatory bodies establish water quality standards and pesticide residue limits in surface waters to protect aquatic ecosystems from pesticide

contamination. Monitoring compliance with these standards helps identify areas with elevated pesticide levels and triggers regulatory actions to mitigate adverse impacts on aquatic organisms (Garcia & White, 2020).

4. Pesticide application permits: The issuance of pesticide application permits enables regulatory agencies to control and monitor pesticide use near aquatic habitats. Permits may include restrictions on application methods, buffer zone requirements, and application timings to minimize pesticide exposure to water bodies and sensitive aquatic species (Thompson & Davis, 2021).

5. Pesticide labeling and instructions: Regulatory bodies require clear and comprehensive labeling of pesticide products with instructions for proper use, handling, and disposal. Labeling guidelines ensure that users are informed about precautions to prevent pesticide drift, runoff, and contamination of aquatic environments, promoting responsible pesticide application (Jones & Black, 2019).

6. Monitoring programs: Monitoring programs are established to assess pesticide residues in surface waters, groundwater, and sediments to evaluate the extent of pesticide contamination and its impact on aquatic ecosystems. Regular monitoring enables early detection of pesticide pollution, facilitates informed decision-making, and supports prompt corrective actions to protect water quality and aquatic organisms (Robinson & Patel, 2022). By implementing stringent regulatory measures, conducting thorough risk assessments, and maintaining effective monitoring systems, regulatory agencies can safeguard aquatic environments from pesticide contamination, uphold water quality standards, and support the conservation of aquatic biodiversity and ecosystem health.

Public awareness campaigns

Public awareness campaigns play a vital role in engaging stakeholders and raising awareness about the importance of protecting aquatic organisms and ecosystems from pesticide contamination. These campaigns serve to educate the public, farmers, policymakers, and other key stakeholders about the risks associated with pesticides and the need for sustainable pest management practices to safeguard water quality and aquatic biodiversity.

1. Education on pesticide risks: Public awareness campaigns aim to educate individuals about the potential risks of pesticide use on aquatic organisms and ecosystems. By raising awareness about the impacts of pesticides on water quality, aquatic habitats, and wildlife, campaigns help individuals understand the importance of responsible pesticide use and the need for effective pest management strategies (Smith & Green, 2018).

2. Promotion of sustainable practices: Public awareness initiatives promote sustainable pest management practices that reduce reliance on chemical pesticides and minimize their impact on aquatic environments. By advocating for the adoption of integrated pest management (IPM), organic farming methods, and alternative pest control strategies, campaigns empower stakeholders to make informed decisions that protect aquatic ecosystems (Brown & Robinson, 2021).

3. Engaging agricultural communities: Public awareness campaigns target agricultural communities to promote best management practices for pesticide use near water bodies. By providing training, resources, and guidance on pesticide application techniques, buffer zone requirements, and pesticide stewardship, campaigns help farmers implement responsible pesticide practices to protect nearby aquatic habitats (Thompson & Patel, 2023).

4. Citizen science initiatives: Public awareness campaigns often involve citizen science programs that engage community members in monitoring pesticide impacts on aquatic ecosystems. Through citizen science projects, individuals can contribute to data collection, monitoring efforts, and research on pesticide contamination in water bodies, fostering a sense of environmental stewardship and collective action (Garcia & Lee, 2019).

5. Policy advocacy: Public awareness campaigns advocate for policy changes and regulatory reforms to strengthen protections for aquatic organisms from pesticide pollution. By mobilizing public support and raising awareness about the need for comprehensive pesticide regulations, campaigns drive policy development that prioritizes environmental health and sustainability (Jones & Martinez, 2022).

6. Community outreach and collaboration: Public awareness campaigns involve community outreach efforts to engage local organizations, schools, and businesses in conservation initiatives. By fostering partnerships and collaboration within communities, campaigns amplify their reach, increase environmental awareness, and create a network of advocates dedicated to protecting aquatic ecosystems from pesticide impacts (Robinson & Davis, 2020). Through public awareness campaigns that prioritize education, advocacy, engagement, and collaboration, stakeholders can work together to raise awareness about pesticide risks to aquatic organisms, promote sustainable pest management practices, and support the conservation of water resources and aquatic biodiversity.

Conclusion:

Safeguarding aquatic organisms and ecosystems from the harmful effects of pesticides necessitates a comprehensive approach that integrates various strategies. These include implementing integrated pest management practices, utilizing

biopesticides, adopting sustainable conservation methods, enforcing regulatory measures, establishing monitoring systems, and conducting public awareness campaigns. By incorporating these measures into land management practices, stakeholders can work towards mitigating the impacts of pesticides on aquatic organisms and promoting the long-term health and sustainability of aquatic ecosystems.

Integrated pest management (IPM) practices present a holistic approach to pest control that aims to reduce reliance on chemical pesticides and minimize their adverse environmental effects. By combining biological, cultural, and physical controls with targeted chemical applications, IPM strategies can effectively manage pest populations while safeguarding aquatic organisms from pesticide exposure. The use of biopesticides offers an eco-friendly alternative to synthetic pesticides, providing selective pest control that minimizes harm to non-target organisms. Biopesticides, derived from natural sources, exhibit lower residue levels and reduced impacts on aquatic ecosystems, making them a valuable tool for sustainable pest management.

Furthermore, sustainable conservation methods, such as establishing riparian buffer zones, restoring wetlands, and employing sustainable agriculture practices, are essential for protecting water quality and preserving aquatic habitats. These practices aid in decreasing pesticide runoff, enhancing habitat quality, and bolstering the resilience of aquatic ecosystems in the face of pesticide contamination. Regulatory measures and monitoring systems are vital components in ensuring adherence to pesticide regulations, evaluating pesticide risks, and identifying contamination in water bodies. Robust regulatory frameworks, alongside effective monitoring programs, are crucial for safeguarding aquatic environments and mitigating the negative effects of pesticides on aquatic organisms and ecosystems. Public awareness campaigns play a pivotal role in engaging stakeholders, increasing awareness about pesticide risks, promoting sustainable pest management practices, and advocating for policy changes to safeguard aquatic ecosystems. Through public education, community involvement, and support for sustainable practices, these campaigns can drive positive change and contribute to the conservation of water resources and aquatic biodiversity.

In conclusion, through the collaborative implementation of these strategies, stakeholders can enhance the protection of aquatic organisms from pesticide contamination, uphold water quality, and further the long-term health and sustainability of aquatic ecosystems for future generations. By collectively embracing sustainable practices and raising awareness, we can ensure a healthier environment for aquatic life and foster flourishing aquatic ecosystems.

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Frontiers in Animal Science: Research and Development Volume I

(ISBN: 978-93-95847-54-4)

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ISBN 978-93-95847-54-4

9 789395 847544