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UNLOCKING THE HEALING POWER OF DRAGON FRUIT: A LOOK AT ITS MEDICINAL BENEFITS

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Abstract

Dragon fruit also known as pitaya or pitahaya, is a tropical fruit gaining widespread popularity not only for its unique taste and vibrant appearance but also for its remarkable medicinal properties. This article explores the diverse health benefits associated with dragon fruit consumption. Rich in antioxidants, vitamins, and essential minerals, dragon fruit exhibits anti-inflammatory and immune-boosting effects. Studies suggest its potential in managing chronic diseases, including diabetes, cardiovascular disorders, and obesity. Additionally, the fruit's high fibre content contributes to digestive health and weight management. Phytochemicals present in dragon fruit demonstrate anti-cancer properties, making it a promising candidate for cancer prevention and treatment. Furthermore, the fruit's impact on skin health and its role in promoting overall well-being are discussed. As dragon fruit continues to captivate global attention, understanding its medicinal values provides valuable insights for both nutritional and therapeutic applications.

Introduction

Dragon fruit, *Hylocereus spp*., is a climbing vine in the Cactaceae family (Fig.1). It originates mostly in Latin America's tropical and subtropical regions, including North, Central, and South America. It is a long-day plant with lovely night-blooming flowers known as "Noble Woman" or "Queen of the Night" and because of the bracts on the fruit skin, the fruit is also known as pitaya and is referred to as "the scaly fruit." The bioactive compounds derived from dragon fruit peels for human consumption to prevent chronic diseases such as cardiovascular disease, diabetes and cancer. It is a rich source of nutrition such as Vitamin C, calcium, and phosphorous. It has higher medicinal values like reducing hypertension, diabetes. It has greater anti-oxidant activity. Also it has beneficial effects in carbohydrate metabolism, strengthening of teeth and bones, helps in formation of heart tissues (Kumar et al., 2018).

The dragon fruit was introduced to India during the late 90's but the area under dragon fruit is still scanty and limited to a few areas of Karnataka, Kerala, Tamil Nadu, Maharashtra, Gujarat and Andhra Pradesh. The total area under dragon fruit cultivation in India is more than 3084 ha with 12113 MT of production and 1.5-3.1 MT/ha in 2020. In recent times, Gujarat and Karnataka are fast emerging states as the country's leaders in dragon fruit cultivation, which is spread over 1214 and 485 ha respectively (Wakchaure et al., 2021).

Fig.1. Dragon Fruit

Nutritional properties of dragon fruit

Dragon fruit is a nutrient-rich tropical fruit, offering a plethora of minerals, glucose, fructose, dietary fibre, and vitamins (Table 1). Notably, it is renowned for its abundance of vitamin C, phosphorus, calcium, and antioxidants. The red layer of the fruit boasts vitamins B1, B2, B3, and C, along with essential minerals. Compared to other subtropical fruits, dragon fruit stands out for its relatively high antioxidant activity. Enriched with nutrients such as vitamins and high fibre content and minerals like calcium, iron, and phosphorus, dragon fruit maintains a low carbohydrate and fat profile. Its seeds contribute essential fatty acids, including linoleic acid and linolenic acid, at a significant 50%. Additionally, the premature stem of dragon fruit exhibits a higher content of ascorbic acid than the flesh, potentially aiding in preventing conditions such as scurvy, anaemia, and weakness. Furthermore, dragon fruit emerges as a noteworthy source of pectin, particularly in the peel, which can serve as a thickening agent in low-viscosity food and beverages. Globally recognized for its polyphenolic components and antioxidant properties, dragon fruit holds its ground as a healthful addition to diverse diets (Hossain et al., 2021).

Table 1. Nutrient content of 100 g edible portion of dragon fruits

Components	Amounts	Components	Amounts
Water	87 g	Iron	1.9 mg
Protein	1.1 g	Vitamin B1	0.04 mg

Components	Amounts	Components	Amounts
Fat	0.4 g	Vitamin B2	0.05 mg
Fiber	3.0 g	Vitamin B3	0.16 mg
Carbohydrate	11 mg	Vitamin C	20.5 mg
Calcium	8.5 mg	Phosphorus	22.5 mg

Source: Thokchom et al. (2019).

Medicinal properties of dragon fruit:

Dragon fruit is regarded as a heavenly fruit on earth because of its excellent nutritional and therapeutic qualities. The fruits have the ability to control breast cancer, colon cancer, diabetics, high cholesterol, anaemia as well as blood pressure. Its popularity is growing as a result of health benefit claims such as lowering dyslipidemia. It is thought to reduce blood sugar levels in people with type 2 diabetes. Red dragon fruit juice increased the haemoglobin and erythrocyte levels in pregnant women, implying an alternate treatment for pregnant women with anaemia. It offers many other health benefits including anti-inflammatory, chemoprevention of cancer, reduction in the mortality risk of cardiovascular disease and neutralising toxic substances such as heavy metals (Table 2).

Table 2. List of medicinal properties of dragon fruit

Medicinal properties	Biochemical responsible	Effect	Reference
Anti-Cancer property	High antioxidant activity (polyphenol and flavonoid) and high values of anthocyanins	Dragon fruit showed high inhibitory activity on MCF-7 cell proliferation and inhibited the melanoma cell (B16F10) cell growth.	Rajarajeswaran & Kannthimathi, 2011
	B-amyrin, β-sitosterol and stigmast-4-en-3-one	Demonstrated good cytotoxic effects against human prostate cancer (PC3), human breast cancer (Bcap-37) and human gastric cancer (MGC-803) cell lines in a dose-dependent manner.	
Anti-diabetic activity	Polyphenols	Reduced total cholesterol, triglyceride as well as low-density lipoprotein (LDL) levels	Khalili et al., 2009

Medicinal properties	Biochemical responsible	Effect	Reference
		and increased high-density lipoprotein (HDL) in rats	Alireza et al., 2014
	Antioxidant and soluble dietary fiber contents	Fresh pitaya attenuated the insulin resistance, hypertriglyceridemia and atherosclerotic changes induced by fructose supplement in rats.	
	Red dragon fruit peel powder	Decrease in total cholesterol levels, triglycerides and LDL-c and increase in HDL-c levels.	
Prebiotic Effect	Mixed oligosaccharides	Pitaya oligosaccharides have higher resistance towards the human salivary αamylase and stomach acids compared to inulin.; promoted the growth of good bacteria such as Lactobacillus delbrueckii and Bifidobacterium bifidium; promote gut health and corrects gastrointestinal motility disorders.	Wichienchot et al., 2010
Antimicrobial Property	Betalain, polyphenol and flavanoid	Exhibited inhibition zone of about 7 to 9 mm against Grampositive (Staphylococcus aureus, Bacillus subitilis, Bacillis cereus, Listeria monocytogenes, and Enterococcu faecalis) and Gram-negative (Escherichia coli, Vibrio algynolyticus, Salmonela typhimurium, Yersinia enterocolitica and Klebsiella pnuemoniae) bacteria.	Hendra et al., 2019

Medicinal properties	Biochemical responsible	Effect	Reference
Wound healing property	Aqueous extracts of leaves, rind, fruit pulp and flower	Topical applications of H. Undatus produced increases in hydroxyproline, tensile strength, total proteins, DNA collagen content and better epithelization thereby facilitating healing in streptozotocin-diabetic rats	Perez et al., 2005
Anaemia among pregnant women	Iron	Significant effect of red dragon fruit juice on haemoglobin and erythrocyte levels.	Widyaningsih et al., 2017
Antiparkinson's activity	Alkaloids, Terpenoids and Steroids, Glycoside and Flavanoids, Tannins and phenolic compounds	H. undatus extract was found to decrease the duration of catalepsy significantly	Kanchana et al., 2018
Laxative property	Oligosaccharides and Saponins	Dragon fruit increases the number of faecal matters, faecal weight and decreases the water content of faeces which were indications of laxative activity; acts as a bulk-forming laxative and increases intestinal motility in mice.	Kanchana et al., 2018

Conclusion

The medicinal properties of dragon fruit underscore its potential as a valuable addition to a health-conscious diet. Its antioxidant-rich composition not only addresses concerns related to cancer prevention but also exhibits anti-diabetic activity, offering promising avenues for managing metabolic disorders. The prebiotic effect of dragon fruit contributes to gut health, while its antimicrobial properties showcase potential in combating various infections. Moreover, the fruit's wound healing properties highlight its

role in supporting the body's regenerative processes. With considerations extending to addressing anaemia among pregnant women and potential antiparkinson's activity, dragon fruit emerges as a versatile fruit with diverse health benefits. Additionally, its laxative activities contribute to digestive well-being. As research continues to unravel the intricacies of dragon fruit's medicinal values, it stands as a compelling candidate for promoting holistic health across various domains.

References

Alireza, O., Rokiah, M. Y., Shahin, R., Amin, I., Mohd, Z. A. & Bekhit, A. (2014). Anti diabetic activity of red pitaya (Hylocereus polyrhizus) fruit. *RSC Advances*, 4: 62978.

Hendra, R., Masdeatresa, L., Abdulah, R., & Haryani, Y. (2019). Antibacterial activity of red dragon peel (*Hylocereus polyrhizus*) pigment. *Journal of Physics: Conference Series*, 1351: 1, 012042.

Hossain, F. M., Numan, S. M. N., & Akhtar, S. (2021). Cultivation, nutritional value, and health benefits of Dragon Fruit (*Hylocereus spp*.): A Review. *International Journal of Horticultural Science and Technology*, *8*(3), 259-269.

Kanchana, P., Devi, S. K. S. V., Latha, P. P. & Spurthi, N. (2018): Phytochemical evaluation and pharmacological screening of antiparkinson's and laxative activities of *Hylocereus undatus* (White Pitaya) in rodents. *IOSR Journal of Pharmacy*, 8: 78–92.

Khalili, M. A. R., Norhayati, A. H., Rokiah, M. Y., Asmah, R., Muskinah, M. & Abdul, M. A. (2009). Hypocholesterolemic effect of red pitaya (*Hylocereus sp.*) on hypercholesterolemia induced rats. *Int. Food Res. J.*, 16: 431-440.

Kumar, S. B., Issac, R., & Prabha, M. L. (2018). Functional and health-promoting bioactivities of dragon fruit. *Drug Invention Today*, *10*.

Perez, G. R. M., Vargas, S. R. & Ortiz, H. Y. D. (2005). Wound Healing Properties of *Hylocereus undatus* on Diabetic Rats. *Phytotherapy Research: PTR*, 19(8), 665–668.

Rajarajeswaran, J. & Kanthimathi, M. S. (2011). Inhibitory effects of fruit extracts on nitric oxide-induced proliferation in MCF-7 cells. *Food Chem*., 126: 956-960.

Thokchom A, Hazarika B.N, Angami T. 2019. Dragon fruit-An advanced potential crop for Northeast India. *Agriculture & Food: eNewsletter*. 1(4), 253-254.

Wakchaure, G. C., Satish Kumar., Meena, K. K., Rane, J. and Pathak, H. (2021). Dragon fruit cultivation in India: Scope, constrains and policy issues. Bulletin No. 27. ICAR-*National Institute*

Wichienchot, S., Jatupornpipat, M. & Rastall, R. A. (2010). Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. *Food Chem*., **120**: 850–857.

Widyaningsih, A., Onny, S., Umaroh, Muchlis, A. & Faisal, A. (2017). Effect of consumingred dragon fruit (*Hylocereus costaricensis*) juice on the levels of haemoglobin and erythrocyte among pregnant women. *Belitung Nursing J*., 3(3): 255-264.

EFFECTS OF HEAVY METAL EXPOSURE ON HEALTH AND WELL-BEING

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Abstract

Background: Heavy metals, with a density of 5 or higher and an atomic number exceeding 11, are substances that pose serious risks to both the environment and human health. They enter ecosystems through natural processes and human activities like mining, industrial discharge, and agriculture. While some heavy metals like vanadium, manganese, selenium, nickel, and arsenic have essential biological roles in small amounts, surpassing these levels can lead to harmful health effects, including cancer.

Objective: This study aims to explore the consequences of heavy metal exposure on human health and the environment. It focuses on understanding how heavy metals cause toxicity, how they enter our bodies, and potential ways to mitigate their effects.

Results :Exposure to heavy metals such as lead, cadmium, arsenic, mercury, and chromium can disrupt vital organ functions and cause oxidative damage, resulting in various health issues. These metals accumulate in the body over time, increasing health risks. Additionally, heavy metal pollution in the environment can contaminate soil and water, harming biodiversity and ecosystem health.

Conclusion :The contamination of heavy metals poses significant threats to both human health and the environment. Strategies may involve enforcing stricter regulations on industrial waste, promoting sustainable mining practices, and adopting advanced methods for treating wastewater. Furthermore, raising public awareness and educating people about the dangers of heavy metal exposure can help minimize risks to both health and the environment.

Overview

Heavy metals, defined as those with a density of 5 or higher and an atomic number exceeding 11, present significant environmental and human health risks. They enter ecosystems through natural processes like soil erosion and human activities such as mining and industrial discharge. While some heavy metals play essential roles in trace amounts, excessive exposure can lead to serious health effects, including carcinogenicity **(Martin and Griswold 2009).** Lead, cadmium, arsenic, mercury, and chromium are particularly concerning due to their ability to disrupt organ functions and induce oxidative damage. Exposure occurs mainly through ingestion and inhalation, particularly for those living or

working near industrial sites or improperly disposed areas. Bioaccumulation in organisms, especially in hyperaccumulator plants, further exacerbates the risk. Contamination of water, soil, and organisms in urban areas amplifies concerns about human exposure.

ARSENIC: Arsenic, ranked as the twentieth most abundant element globally, presents significant environmental and health hazards in its inorganic forms, such as arsenite and arsenate compounds. Besides occurring naturally, arsenic can be released into the environment through volcanic activity, erosion, fires, and human activities like mining and industrial processes. Arsenic acts as a potent poison, disrupting cellular functions such as respiration and enzyme activity.

MERCURY: Mercury,a naturally occurring metal, is a shiny silver-white, odorless liquid that transforms into a colorless and odorless gas when heated. Highly toxic and bio accumulative, it adversely affects marine environments. Anthropogenic activities such as agriculture, mining, and industrial discharges contribute to mercury pollution. It exists in metallic, inorganic, and organic forms, each with varying toxicity and bioavailability. In water bodies, microorganisms convert mercury into methylmercury, which bio accumulates in aquatic life, posing a threat to humans through consumption.

CADMIUM: Cadmium, ranked as the seventh most toxic heavy metal, by-product of zinc production and poses risks to humans and animals both in occupational settings and the environment. Cadmium finds contemporary applications in rechargeable batteries, special alloys, and is present in tobacco smoke. Inhalation and ingestion are primary exposure routes for humans, leading to acute and chronic intoxications. Once dispersed in the environment, cadmium persists in soils and sediments for decades, gradually accumulating in plants and subsequently along the food chain, ultimately impacting human health.

LEAD: Human activities like mining, manufacturing, and fossil fuel combustion have led to the widespread accumulation of lead and its compounds in the environment, including air, water, and soil. The Environmental Protection Agency (EPA) has classified lead as a probable human carcinogen. Lead poisoning primarily affects the central nervous system and gastrointestinal tract, with potential sources of exposure including drinking water contaminated by lead pipes. Lead primarily accumulates in skeletal bones and chronic exposure can result in severe health issues including mental retardation, birth defects, and kidney damage.

CHROMIUM: In terms of health effects, chromium(VI) compounds are acknowledged as toxins and human carcinogens, whereas chromium(III) is essential for bodily functions. Occupational exposure to chromium commonly occurs in industries dealing with metal

coatings, alloys, paint pigments, rubber, cement, and metal plating. While chromium may be present in cigarettes, the extent of inhalation exposure remains uncertain.Exposure to chromium compounds, especially chromium(VI), can result in ulcer formation upon skin contact, particularly notable among chromate workers with nasal septum ulcers. Elevated exposure levels can impede erythrocyte glutathione reductase activity, affecting the conversion of met hemoglobin to hemoglobin. Research indicates that chromium compounds induce DNA damage through diverse mechanisms, including the formation of DNA adducts and chromosomal aberrations.

The sources, impacts of health effectspermissible limits of, arsenic, mercury, lead, cadmium,chromium and barium in different environmental matrices suggested by various international reputed agencies such as EPA (Environmental Protection Agency), Food and Drug Administration (FDA) and OSHA (Occupational Safety and Health Administration) are presented in Table 1**(Sall *et al.*, 2020 and Jyothi, 2020).**

Table 1: Sources, Impacts of Health Effects and Permissible limits in environmental matrices

Heavy metals	Sources	Health effects	Regulatory limits		
			EPA	FDA	OSHA
Arsenic (As)	Atmospheric deposition Mining pesticides	Cancers of the skin, lungs, liver, and bladder. Brain and Cardiac Problems	0.01 ppm in drinking water	-	10 µg/ m3 of workplace air
Mercury (Hg)	Coal combustion Fish Mining Paint industry Paper industry Volcanic eruption	Damage the brain, kidneys, changes in vision or hearing, and memory problems,Respiratory Problems, damage the liver, kidneys, circulatory system, nerve tissues, and cause skin irritation.	2 (ppb) in drinking water	1 ppm	Organic mercury 0.1 mg/ m3 and Non organic- 0.05 mg/ m3 in air
Cadmium (Cd)	Plastic Fertilizers pesticides Smoking	Kidney disease, lung damage, and fragile bones.	5 ppb or 0.005 ppm drinking water	Bottled drinking water should not exceed 0.005	5 µg/ m3 workplace air

Heavy metals	Sources	Health effects	Regulatory limits		
			EPA	FDA	OSHA
				ppm (5 ppb).	
Lead (Pb)	Batteries Coal combustion Paint industry	Alzheimer's disease, Decreased performance in nervous system functions, weakness in fingers, wrists, or ankles, slight increases in blood pressure, and anemia,miscarriage.lowersperm production.	15 ppb drinking water 0.15 µg/ m3 in air	-	-
Chromium (Cr)	Steel Fabrication Electroplating Textile	Lung disorders (bronchitis,cancer), Renal and reproductive system	0.1 ppm in drinking water.	1 mg/L per L in bottled water	0.0005 and 1.0 µg/ m3 of workplace air

Treatment and Medication Options for Heavy Metal Poisoning

When addressing heavy metal poisoning, the initial focus is on reducing exposure to the toxic substance.In cases of acute poisoning, treatment typically involves the administration of chelating agents. These agents bind to the metal in the body, facilitating its excretion through urine. Chelating drugs are available in pill and injection forms. Commonly used chelating agent drugs include dimercaprol (Bal In Oil), edetate calcium disodium (Calcium Disodium Versenate), and penicillamine (Cuprimine).It's important to note that chelating drugs are reserved for acute poisoning situations due to potential risks **(Jaishankar *et al.*,2014).** These risks include the depletion of essential minerals such as iron and the possibility of metals entering the bloodstream, potentially reaching organs like the brain.Other treatment options for heavy metal poisoning may include stomach pumping to remove ingested metals, the administration of activated charcoal orally, hemodialysis or specialized treatments if kidney failure occurs, and intravenous fluid administration. These interventions aim to mitigate the effects of heavy metal exposure and promote recovery.

Here are some recommendations to help prevent heavy metal poisoning:

1. Utilize masks and protective attire when working with heavy metals.
2. Many metals gather in dust and soil, minimize their presence in your home (consider removing shoes before entering).
3. Stay informed about local fish advisories concerning mercury contamination.
4. Be mindful of potential sources of lead exposure in your surroundings.

5. Scrutinize product labels for any indication of heavy metal content before introducing them into your household.

Detoxification of heavy metals with nutrition

Certain foods are recommended for their potential to assist in the detoxification of heavy metals. These include coriander, garlic, blueberries, lemonade, spirulina, green tea, tomatoes, and probiotics. Moreover, vitamins B, B6, and C are essential for supporting the body's detoxification mechanisms. Deficiencies in these vitamins can weaken the body's defenses against heavy metal exposure. Vitamin C has been found to have a chelating effect. However, it's important to exercise caution when supplementing with vitamins and to seek advice from a healthcare professional to avoid potential adverse effects. Alongside dietary modifications, increasing the intake of fruits and vegetables can aid in heavy metal detoxification. Conversely, processed foods high in fats should be avoided as they can hinder the detoxification process and lead to increased toxin accumulation in the body.

Conclusion

The contamination of water, soil, and organisms with heavy metals emphasizes the necessity for comprehensive risk assessments and mitigation strategies, particularly in urban regions and near industrial facilities. Implementing preventive measures dietary adjustments counteract heavy metal toxicity.A multi-faceted approach incorporating diverse preventive measures is vital for protecting environmental and human health from the adverse effects of heavy metals. These endeavors are crucial for addressing the far-reaching implications of heavy metal toxicity on ecosystems and human well-being.

References

Martin, S., & Griswold, W. (2009). Human health effects of heavy metals. *Environmental Science and Technology briefs for citizens*, *15*(5).

Sall, M. L., Diaw, A. K. D., Gningue-Sall, D., Efremova Aaron, S., & Aaron, J. J. (2020). Toxic heavy metals: impact on the environment and human health, and treatment with conducting organic polymers, a review. *Environmental Science and Pollution Research*, *27*, 29927-29942.

Jyothi, N. R. (2020). Heavy metal sources and their effects on human health. *Heavy Metals-Their Environmental Impacts and Mitigation*.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary toxicology*, *7*(2): 60.

BIOMINERALIZATION: THE BLUEPRINT OF OCEANIC LIFE

Banothu Divya, Manoj P. Brahmane, Borra Tejaswi, Siyag Dhere, Tuturanjan Gogoi

Abstract

Marine invertebrates employ biomineralization, a biological process that creates shells and skeletons from calcium carbonate dissolved in saltwater. This mechanism is critical to ocean health and is jeopardized by ocean acidification induced by anthropogenic CO2 emissions. In contrast to non-biological processes, biomineralization requires precise control over mineral production. The most prevalent biominerals are calcium carbonate polymorphs such as calcite and aragonite. Molluscs, for example, employ mantle tissue to form complex shell structures. Biomineralization is divided into two types: biologically induced, in which animals have minimal influence over mineral production, and biologically regulated, in which organisms carefully control the process. Ocean acidification destroys the delicate equilibrium required for biomineralization, which harms marine life.

Keywords: Biomineralization, marine invertebrates, calcium carbonate, shells, skeletons, ocean acidification

Introduction

Biomineralization is the process by which organisms produce minerals. It is a crucial process in the world's seas; it is the biological mechanism that converts calcium dissolved in saltwater into solid crystal mineralized structures known as skeletons and exoskeletons. Until the early 1980s, the topic was referred to as "calcification," owing to the prevalence of biologically generated calcium-containing minerals. As more biogenic minerals with different cations were identified, the area became known as "biomineralization." Many species exercise influence over mineral formation, distinguishing these processes from abiotic mineralization.

Biomineralizing organisms are abundant in the world's seas and extremely biodiverse, having a wide range of physiologies, anatomy, and habitats. They also play a crucial role in the global carbon cycle. The mechanism by which our oceans absorb CO2 serves as a key buffer for future increases in atmospheric CO2, making it a vital weapon in global resistance to human climate change. Marine macrofauna, with their strongly calcified exoskeletons and prolonged life cycles and generation durations, are often regarded as the most sensitive species to changing environmental conditions, especially ocean acidification.

Principle groups of biominerals

The shells are generated using a biologically regulated process (biomineralization), which produces a composite material composed of roughly 95% calcium carbonate (CaCO3) and 1% to 5% organic components. The composition of calcified marine macroinvertebrate skeletons varies greatly in terms of calcium carbonate crystal shapes (polymorphs), crystal orientations, organic matrix components (proteins), and so on. Calcium is the preferred cation for most organisms. Calcium carbonate minerals are the most prevalent biogenic minerals, both in terms of production and distribution across many taxa. In nature, CaCO3 appears in several crystal polymorphs such as aragonite, calcite, and vaterite, which, combined with crystal size and organic matrix, give these biomineralized structures their distinct physiochemical characteristics. Material scientists are particularly interested in the fact that bivalves' aragonite and calcite shells are far more robust than geological inorganic forms. CaCO3 crystals in shells are stacked in layers with a specific pattern to create complex microstructures.

Phosphates make for around 25% of the biogenic mineral types. Except for struvite and brushite, most phosphate minerals are formed by controlled mineralization. The iron biominerals are particularly important since they account for around 40% of all minerals generated by organisms, and magnetite production is thought to be the most ancient matrix-mediated biomineralizing mechanism. Biominerals match the requirements for real minerals, but they may also have additional features that set them apart from their inorganically created counterparts. The most noticeable characteristic is that biogenic minerals have distinctive exterior morphologies. Perhaps it is the complexity and diversity of bio-originated structures.

Shell Formation in Bivalve Molluscs

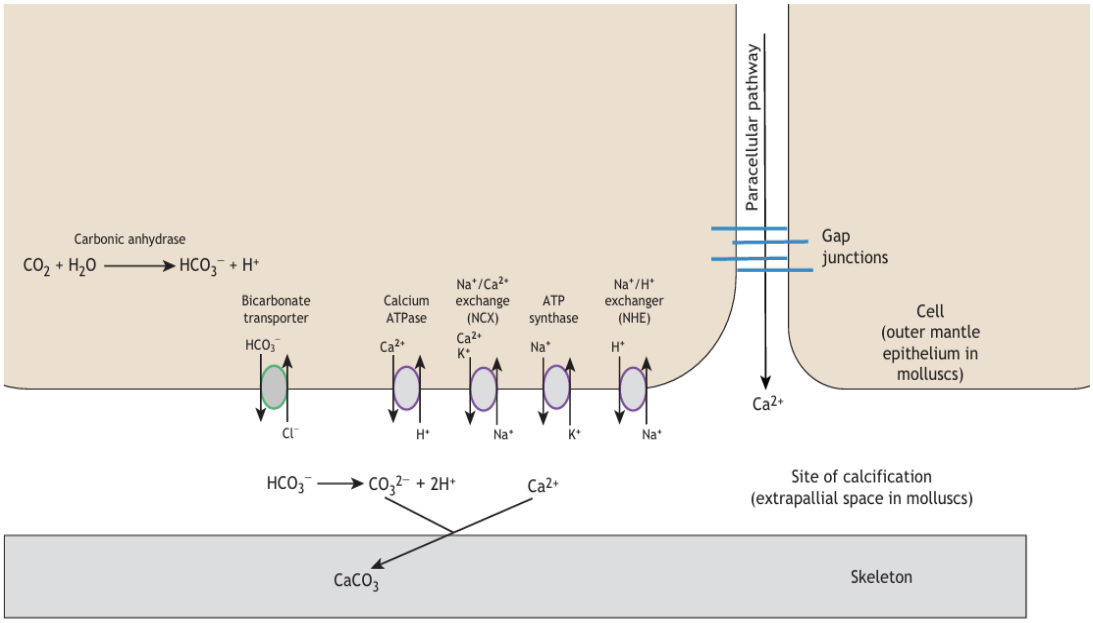
The first known biomineralizing bilaterians are molluscs. The shells not only hold the delicate body but also protect it from predators and other environmental stresses. The phylum Mollusca encompasses a varied variety of animals that create calcareous shells. It is widely acknowledged that shell formation is physiologically regulated by a variety of substances, including proteins, glycoproteins, lipids, and carbohydrates. Molluscs use mantle tissue to form their extraordinary three-dimensional (3D) shell formations. In bivalve molluscs, a thin layer of mantle tissue surrounds all internal organs, with a more complicated and considerable outmost border involved in shell secretion. This mantle edge is separated into folds, with the inner and middle folds primarily engaged in water input and secretory processes, while the outer mantle fold secretes the shell through the outer mantle epithelium.

The periostracum is an important structural component of this production process. This is a thin layer of quinone-tanned proteins, mucopolysaccharides, and lipids released by specialized cells located between the middle and outer mantles. This periostracal sheet reflects dorsally, enveloping the outer mantle fold and forming and confining a very small region (the extra pallial space), which contains a thin layer of fluid that secretes the shell. The periostracum is commonly seen on the exterior of shells in certain species (e.g., blue mussels and *Myochama anomioides*) as a thin black protein coating that protects against rusting. The outer mantle epithelium deposits the calcium carbonate shell mostly on the periostracum.

The majority of the study to far has been on the shell matrix proteins (SMPs) and sugar moieties found in the shell matrix space. Despite being a tiny component of the shell matrix, SMPs play an important role in CaCO3 crystal nucleation, growth, and the formation of various crystal polymorphs. SMPs are secreted in the mantle, which is an epithelial tissue that lines the shell, and then transferred to the shell matrix. These molluscan shell proteins (MSPs) are ultimately responsible for the formation of various shell microstructures and mechanical strength. Mantle tissue expresses and secretes molluscan shell matrix proteins (MSPs), which permeate the densely packed molluscan shell microstructures. As a result, identifying and quantifying MSPs has been identified as an important step toward better understanding biomineralization.

Shell formation procedure

Calcium ions from the inner mantle move passively into Crassostrea gigas' outer mantle epithelial cells via voltage-gated calcium channels. Calcium is subsequently actively excreted via calcium ATPases and Na+/Ca2+ exchangers. The transmembrane sodium gradient generated by basal Na+/K+ ATPases drives the exchangers' activity. This basic cellular process for calcium transport is augmented by regionalization of the mantle tissue and selective protein release, resulting in a diverse range of mollusk shell morphologies.



Clark *et al.*, 2020

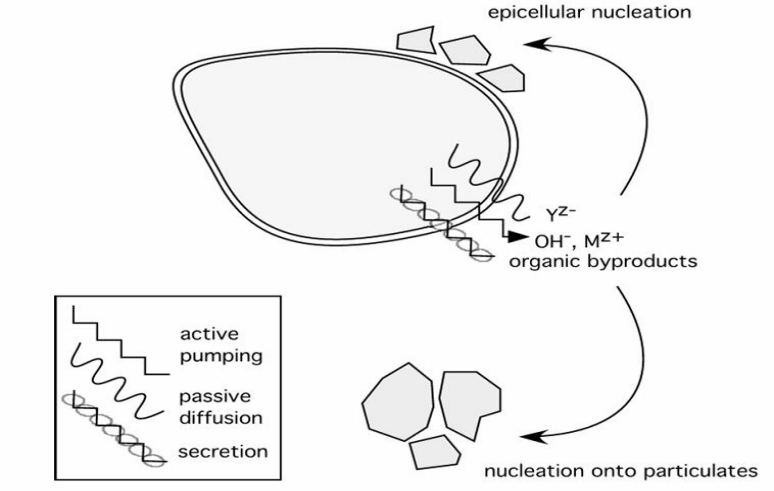
Fig.1. Illustration of the key ion transporters involved in biomineralization

Diverse Pathways of Biomineralization

The two basic categories of biomineralization processes are distinguished by the level of biological control they exhibit. These were first described as "biologically induced" and "organic matrix-mediated" by Lowenstam (1981); Mann (1983) expanded on the latter term to refer to "biologically controlled" mineralization.

Biologically induced mineralization

"Biologically induced" mineralization refers to the secondary precipitation of minerals caused by interactions between biological activity and the environment. In this case, cell surfaces frequently function as nucleation agents, resulting in mineral development. The biological system has limited influence over the type and distribution of minerals deposited. In some circumstances, biological surfaces are critical during the induction stage because nucleation frequently occurs directly on the cell wall, allowing the emerging biominerals to remain securely attached.



Weiner & Dove, 2003

Fig.2. Illustrating physiologically induced mineralization

Biologically controlled mineralization

In "biologically controlled" mineralization, the organism employs cellular processes to influence mineral nucleation, growth, shape, and ultimate deposit position. Biologically regulated mineralization processes can be classified as extracellular, intercellular, or intracellular. These distinctions apply to the positions of the mineralization site in relation to the cells responsible for mineralization. In certain circumstances, mineral formation begins inside the cell and subsequently moves outside.

Conclusion

The saturation levels of calcite and aragonite minerals (Ωcal and Ωarg) are quickly falling in worldwide seas and oscillating at an unprecedented scale in coastal waters due to excessive human CO2 absorption. This human-induced alteration in ocean carbonate chemistry is commonly referred to as ocean acidification (OA). The decreasing saturation condition of CaCO3 minerals under OA poses a danger to biomineralization, not only in molluscs but also in various other calcifying marine animals. Under OA, internal pH at the calcification site decreases, as does the capacity to ingest dissolved inorganic carbon. Thus, OA compels organisms to expend more energy to maintain internal pH homeostasis/acid-base balance, drastically impairing or altering the biomineralization process and the energy budget. Metamorphosis, the energy-intensive process that turns pelagic larvae into benthic juveniles, makes them more vulnerable to OA. Simultaneously, the larvae must change their shell composition from "aragonite" to "calcite". Furthermore,

aragonite larval shells are 50% more soluble than calcite, making larval stages particularly susceptible in future oceans.

References

Arivalagan, J., Yarra, T., Marie, B., Sleight, V. A., Duvernois-Berthet, E., Clark, M. S., Marie, A., & Berland, S. (2017). Insights from the Shell Proteome: Biomineralization to Adaptation. *Molecular Biology and Evolution*, *34*(1), 66–77. https://doi.org/10.1093/molbev/msw219

Chandra Rajan, K., & Vengatesen, T. (2020). Molecular adaptation of molluscan biomineralisation to high-CO2 oceans – The known and the unknown. *Marine Environmental Research*, *155*, 104883. https://doi.org/10.1016/j.marenvres.2020.104883

Clark, M. S. (2020). Molecular mechanisms of biomineralization in marine invertebrates. *Journal of Experimental Biology*, *223*(11), jeb206961. https://doi.org/10.1242/jeb.206961

Gilbert, P. U. P. A., Bergmann, K. D., Boekelheide, N., Tambutté, S., Mass, T., Marin, F., Adkins, J. F., Erez, J., Gilbert, B., Knutson, V., Cantine, M., Hernández, J. O., & Knoll, A. H. (2022). Biomineralization: Integrating mechanism and evolutionary history. *Science Advances*, *8*(10), eabl9653. https://doi.org/10.1126/sciadv.abl9653

Weiner, S. (2003). An Overview of Biomineralization Processes and the Problem of the Vital Effect. *Reviews in Mineralogy and Geochemistry*, *54*(1), 1–29. https://doi.org/10.2113/0540001

INTRODUCTION TO CRISPR-CAS9: GENOME EDITING

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Introduction

Over past decades, scientific endeavours have persistently aimed at establishing economical and dependable methods for exact genomic modifications in live cells. Such genome editing holds significant potential across diverse domains of life. In the context of human gene therapy, this technology shows promise for eradicating genes associated with specific genetic disorders. Conversely, in agriculture, genetic manipulation of plant genomes could significantly boost crop production and mitigate plant diseases. This has been used as a new technique in stock enhancement of aquaculture species (Diwan *et al*., 2017; Gratacap *et al*., 2019; Mokrani and Liu, 2024). A groundbreaking advancement in this field is the emergence of a tool derived from a bacterial system known as clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR/Cas9), originally identified in *Streptococcus pyogenes*. This tool has recently garnered considerable attention due to its precision, versatility, and efficiency in gene editing applications.

Since its inception in 2012, CRISPR/Cas9 technology has revolutionized the field of biology, providing unparalleled precision in DNA sequence manipulation within living cells. While CRISPR has become almost synonymous with gene editing, it represents an evolution rather than the inception of this scientific concept. The journey of genome editing dates to the 1970s, marked by significant milestones such as the successful creation of transgenic mice (Friedmann, 1992). However, these early techniques lacked the ability for targeted genomic insertions, a limitation that catalyzed the global scientific community's pursuit of more refined gene targeting technologies. This collective endeavor ultimately led to the development of advanced tools like CRISPR/Cas9, which now dominate the landscape of genetic engineering. In 2012, an important discovery was made regarding the bacterium *Streptococcus pyogenes*, unveiling its potential for genetic engineering. This system encompasses a component known as "Clustered Regularly Interspaced Short Palindromic Repeats" (CRISPR), which functions as a guiding RNA sequence. Paired with this is the Cas9 protein, acting as an endonuclease. This dual-component system is targeted at inducing double-strand breaks (DSBs) in DNA, a crucial

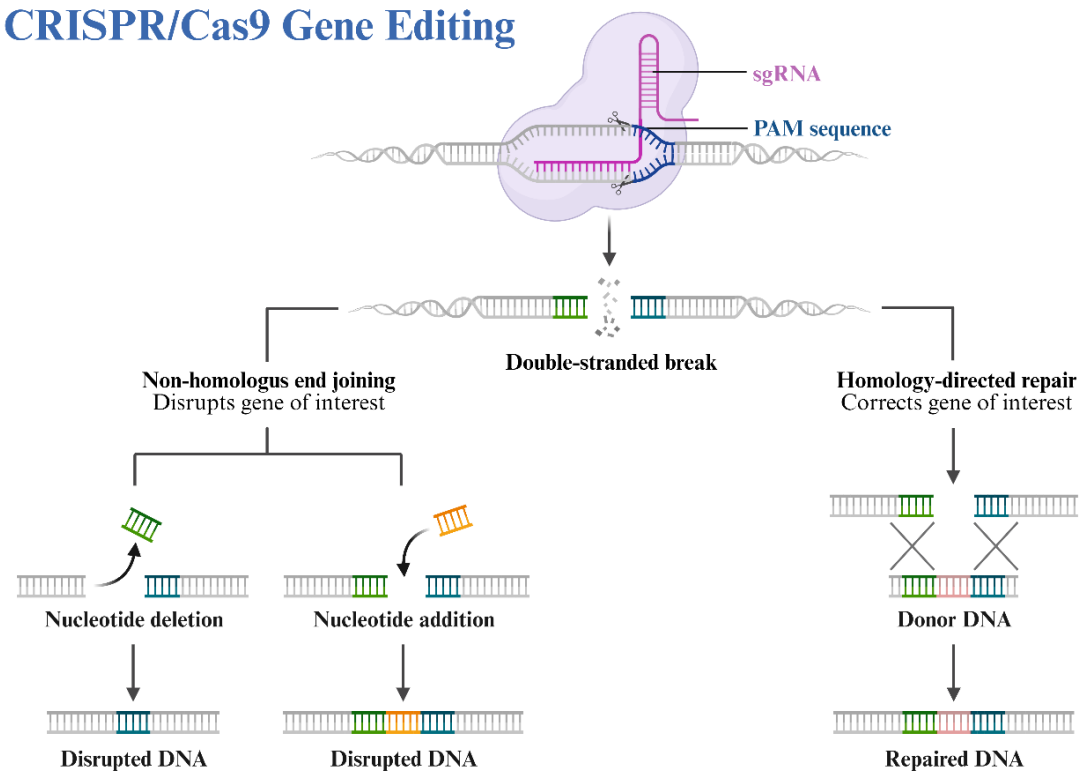
step in genome editing. This groundbreaking discovery of harnessing CRISPR and Cas9 from *S. pyogenes* marked a significant advancement in the field of genetic engineering, providing a powerful and versatile tool for targeted genome modification (Kozovska *et al*., 2021).

CRISPR systems are diverse, classified into six distinct types, further organized into two classes based on the sequence and structure of Cas (CRISPR-associated) proteins (Kozovska *et al*., 2021). While Types IV-VI are recent discoveries, Types I-III have been extensively studied, with the Type II CRISPR system emerging as the most prevalently utilized in practical applications. Type I CRISPR systems, characterized by the presence of the Cas3 protein are known for their intricate mechanism involving a DNase domain and helicase activity, leading to the degradation of the target DNA. However, due to their complexity, they are not commonly used for gene modification purposes. In contrast, the Type II system, known for its relative simplicity and multifunctionality, is widely applied in gene therapy. This system is marked by the presence of the Cas9 gene and other associated genes like Cas1, Cas2, and in the case of the II-B subtype, Cas4. The Cas9 protein's versatility has been enhanced through various point mutations, aiming to increase its specificity in targeting. Type III CRISPR systems, containing the Cas10 protein, exhibits a unique mechanism that enables the simultaneous destruction of both RNA and DNA. This dual action mechanism of Cas10 endonuclease sets it apart from other types. Recent research has focused on identifying novel, more streamlined CRISPR-based technologies. A key goal is to minimize the size of the employed nucleases for easier gene delivery packaging. One such development is the discovery of Cas14 proteins, which are remarkably compact, ranging from 400–700 amino acids. Unique in their function, Cas14 proteins are RNA-guided nucleases that target single-stranded DNA (ssDNA) using a single RuvC nuclease domain and do not require a PAM sequence. Despite their small size, Cas14 proteins operate with larger RNA scaffolds. Another groundbreaking addition is the Class 2 Type VI system, CRISPR/Cas13a (also known as C2c2), derived from *Leptotrichia shahii*. This system is tailored to target single-stranded RNA molecules, functioning as an RNA-guided ribonuclease. The exploration of Cas14 and Cas13a holds significant potential, especially in developing strategies to interfere with plant ssDNA or RNA viruses, marking a crucial step in the economic application of CRISPR technology.

In the CRISPR-Cas9 system, the precise targeting of specific genomic locations is achieved through the coordinated action of two types of RNA molecules: the CRISPR RNA (crRNA) and the trans-activating CRISPR RNA (tracrRNA) (Faure *et al*., 2019). The crRNA plays a crucial role in target recognition, as it contains a 20-nucleotide sequence complementary to the desired target site in the genome. This sequence specificity is what allows the crRNA to accurately guide Cas9 to the intended genomic location. The tracrRNA, on the other hand, serves a dual purpose. Firstly, it acts as a scaffold, linking the

crRNA to the Cas9 enzyme, thus forming a functional complex capable of genome editing. Secondly, tracrRNA is involved in the

maturation process of crRNAs from their precursor forms (precrRNAs), an essential step for the crRNA to become fully functional. In most applications of CRISPR-mediated genome editing, these two RNA components – crRNA and tracrRNA – have been ingeniously fused into a single guide RNA molecule, commonly referred to as sgRNA (single-guide RNA) or simply gRNA. This synthetic sgRNA retains the essential features of both its components: the 20-nucleotide sequence from the crRNA that directs Cas9 to the specific genomic site, and the binding sequence derived from tracrRNA that is necessary for the interaction with Cas9. The development of sgRNA represents a significant advancement in CRISPR technology, simplifying the system and enhancing its efficiency for genome editing applications (Ceasar *et al*., 2016; Nidhi *et al*., 2021).



The effectiveness of the Cas9 endonuclease in targeting specific genomic loci relies not only on the 20-nucleotide complementary sequence provided by the single-guide RNA (sgRNA), but also on a crucial 3-base pair sequence known as the Protospacer Adjacent Motif (PAM). PAM is a short DNA sequence located adjacent to the target site on the DNA strand and is essential for the endonuclease activity of Cas9. In the absence of PAM, Cas9 cannot recognize or bind to its target, even if the sgRNA sequence is completely complementary to the target DNA. This requirement of the PAM sequence thus

represents a significant limitation in the application of the CRISPR-Cas9 system. The native PAM sequence recognized by the Cas9 enzyme from *Streptococcus pyogenes* is 5′-NGG-3′, where 'N' can be any of the four DNA bases, and 'G' represents guanine. However, research has identified over 20 different Cas9 homologs from various bacterial species, each recognizing different PAM sequences. These homologs do not cross-react with one another, making it feasible to use multiple Cas9 enzymes simultaneously in complex genome editing projects. For Cas9 to induce a double-strand break (DSB) in the target DNA, the PAM sequence must be located immediately downstream of the 3′ end of the target site defined by the sgRNA. Upon binding to the target DNA in the presence of the appropriate PAM sequence, Cas9 cleaves the DNA at a site three nucleotides upstream of the PAM motif. This cleavage is facilitated by two distinct endonuclease domains within Cas9: the HNH and RuvC-like nuclease domains, each cutting one strand of the double-stranded DNA (dsDNA). This precise and targeted mechanism of action is what makes CRISPR-Cas9 an invaluable tool for genome editing. The detailed figure showcasing CRISPR-Cas9 gene editing has been shown in the figure above.

As of now, three primary variants of the Cas9 endonuclease have been developed (Anders *et al*., 2016; Kim *et al*., 2020). The Cas9 is the standard form of Cas9 that creates double-strand breaks (DSBs) in DNA at specific locations. The induction of DSBs triggers cellular repair mechanisms. There are two main pathways for DNA repair. One is nonhomologous end joining (NHEJ), which is repair mechanism that often results in insertions or deletions (indels) at the break site, leading to disruptions or mutations in the targeted genomic locus. Similarly, the other one, homology-directed repair (HDR), is when a donor DNA template is provided, HDR can lead to precise substitution mutations, as it uses the provided template to repair the DSB, allowing for specific gene editing. Cas9D10A (Nickase Cas9): This variant was developed by Cong and colleagues and is a mutant form of the original Cas9, known as Cas9D10A. This enzyme has only nickase activity, meaning it cleaves only one strand of DNA due to the inactivation of the RuvC domain of Cas9. As a result, Cas9D10A does not typically activate the NHEJ repair pathway, reducing the likelihood of indels. When paired with a repair template, HDR can be utilized to mend the single-strand break, allowing for precise genome editing. RNA-targeting Cas9 Modifications: Recent advancements have led to the development of modified nickases capable of targeting RNA. These adaptations expand the scope of CRISPR technology beyond DNA to also include RNA editing, offering new avenues for research and therapeutic applications.

The delivery method of the CRISPR-Cas9 editing system is a crucial aspect that significantly impacts the safety and efficacy of its therapeutic applications. There are three primary methods for delivering the CRISPR components into target cells (Chandrasekaran *et al*., 2018). *Plasmid DNA Delivery:* This approach involves using plasmid DNA vectors that

carry the genes encoding both the Cas9 protein and the guide RNA (gRNA). Once the plasmid is introduced into the target cells, the cellular machinery transcribes and translates these genes, producing the functional CRISPR-Cas9 complex inside the cell. This method is widely used due to its relative simplicity and high efficiency of delivery. However, it also poses a risk of random integration of the plasmid into the host genome, which can lead to off-target effects. *Cas9-sgRNA Ribonucleoproteins (RNP):* In this method, the CRISPR-Cas9 system is delivered as a pre-assembled complex of Cas9 protein bound to single-guide RNA (sgRNA). This format ensures immediate activity upon entering the cell, as it bypasses the need for transcription and translation. RNPs have a lower risk of off-target effects and unintended genomic integrations compared to plasmid DNA, making them a safer option for therapeutic applications.

Additionally, the transient nature of RNPs reduces the duration of Cas9 activity in the cell, further minimizing potential off-targ*et al*terations. *mRNA Delivery:* This method involves delivering mRNA molecules encoding the Cas9 protein and sgRNA separately into the target cells. The cells then use these mRNA templates to produce the Cas9 protein and sgRNA. The advantage of mRNA delivery is that it avoids the use of DNA, thereby reducing the risk of integration into the host genome. Like RNP delivery, this method also provides a transient expression of the CRISPR components, which can be advantageous in reducing off-target effects.

Protocol for CRISPR-Cas9 Gene Editing

Materials and Reagents

- CRISPR-Cas9 system components:

i. -sgRNA targeting your gene of interest
ii. -Cas9 mRNA or Cas9 protein

- Microinjection setup (microinjector, micropipettes, microscope)
- Fertilized zebrafish eggs
- Embryo medium (E3 medium for zebrafish)
- Pronase (for dechorionation, if necessary)
- Anesthesia solution for fish (e.g., Tricaine)
- Incubator set to appropriate temperature

Protocol Steps

1. sgRNA Design and Synthesis
    - Identify the target sequence in the fish gene of interest.
    - Design sgRNA complementary to the target sequence. Ensure the presence of a PAM sequence adjacent to the target site.
    - Synthesize or purchase the sgRNA.
2. Cas9 Preparation

- Use either in vitro transcribed Cas9 mRNA or commercially available Cas9 protein.
- If using mRNA, synthesize or purchase Cas9 mRNA.

3. Microinjection Mixture Preparation
   - Prepare a microinjection mixture containing sgRNA and Cas9 (either mRNA or protein).
   - Adjust the concentration as per experimental requirements (typically in the range of 10-100 ng/µl for each component).
4. Collection and Preparation of Fertilized Eggs
   - Collect fertilized zebrafish eggs.
   - If necessary, dechorionate the eggs using Pronase to facilitate microinjection.
5. Microinjection into Zebrafish Embryos
   - Under a microscope, inject the CRISPR-Cas9 mixture into the cell or yolk of one-cell stage zebrafish embryos using a microinjector.
   - Inject a calibrated volume per embryo (e.g., 1-2 nL).
6. Incubation and Development
   - Transfer the injected embryos to embryo medium in a petri dish.
   - Incubate the embryos at a suitable temperature (e.g., 28.5 °C for zebrafish).
   - Monitor the development of the embryos.
7. Screening and Analysis
   - Screen for successful gene editing in the developed fish. Techniques may include PCR, sequencing, or phenotypic analysis.
8. Rearing of Gene-Edited Fish
   - Transfer positively edited fish to a rearing tank.
   - Provide appropriate care and monitor for desired gene-editing outcomes and overall health.

Notes and Considerations

1. Ensure all reagents are RNase-free to prevent degradation of RNA components.
2. Practice microinjection technique as it requires precision and skill.
3. Ethical considerations: Follow all institutional and governmental guidelines for genetic modification and animal care.

Salient Achievements of CRISPR-cas9 in Fisheries and Aquaculture

A lot of achievements through groundbreaking research works by various researchers have been done in CRISPR-cas9 for the improvement of aquaculture species. Some of the notable works are mentioned below.

1. *Sterility in Salmonids via CRISPR:* In Atlantic salmon, CRISPR has been utilized to induce sterility by targeting the "dead end" gene (Güralp *et al*., 2020). While, this approach effectively produces sterile salmon, a key challenge is that this genetic modification is not

inheritable, limiting its commercial applicability. However, insights from zebrafish and medaka research suggest potential methods to restore fertility in breeding stocks, which could enhance the utility of this approach in aquaculture.

2. *Use of Sterile Fish as Surrogate Broodstock:* An innovative application of the "dead end" gene editing in fish is creating sterile specimens that can then be implanted with germ cells from donor species (Baloch). This technique has been demonstrated in Japan, where genetically edited grass puffer fish (*Takifugu alboplumbeus*) are used as surrogate broodstock for the more commercially valuable tiger puffer (*T. rubripes*) (Yoshikawa *et al*., 2018; Yoshikawa *et al*., 2020). This approach holds promise for conserving endangered species and enhancing the production of valuable fish species.

3. *CRISPR in Disease Resistance:* The application of CRISPR for enhancing disease resistance in aquaculture is complex due to the multifaceted nature of disease resistance, which involves interactions between the host species, pathogens, and the production environment. Research is ongoing to identify genes in Pacific salmon that confer resistance to sea lice, with the aim of editing these genes into Atlantic salmon to improve their resistance (Straume *et al*., 2021; Turnbull, 2021).

4. *Enhancement of Muscle Mass in Fish:* Japanese researchers successfully used CRISPR to develop a line of red sea bream with increased muscle mass and a shorter body length. By creating deletions in the *Pm-mstn* myostatin gene without using exogenous genetic constructs, they achieved a 16 percent increase in edible muscle tissue. Similarly, Chinese researchers edited the myostatin gene in yellow catfish (*Pelteobagrus fulvidraco*), resulting in a significant increase in body weight and muscle mass (Zhang *et al*., 2023).

5. *Myostatin Gene Editing in Various Fish Species:* Over the past five years, editing myostatin genes has led to a significant increase in muscle mass in several fish species, including common carp, olive flounder, blunt snout bream, Sea bream, mud loach, and channel catfish (Roy *et al*., 2022). This highlights the potential of gene editing to enhance growth traits in aquaculture.

6. *CRISPR Editing of the PI3K Gene in Gibel Carp:* Recent research from China reported using CRISPR to knock out the PI3K gene in Gibel carp (Huang *et al*., 2021). While this gene alteration improves insulin sensitivity in mammals, in carp, it didn't affect glucose levels or uptake. However, it resulted in enhanced somatic growth and better feed conversion efficiency, showcasing a novel application of CRISPR in aquaculture.

7. *Deletion of the t1r1 Gene in Zebrafish:* Another study demonstrated that deleting the *t1r1* gene in zebrafish significantly improved their acceptance of plant proteins (Cai *et al*., 2021). This could have implications for sustainable fish farming practices.

8. *CRISPR in Coloration of Aquaculture Species:* In ornamental fish breeding, coloration is a key trait. CRISPR has been used in China to disrupt carotenoid transport genes in common carp, altering their red and white color patterns (Du *et al*., 2021). Similarly, editing *ASIP* genes in Oujiang common carp eliminated black patches (Chen *et al*., 2019), and knocking out the *tyrosinase* gene in red tilapia resulted in uniformly red fish with no black pigment (Segev-Hadar *et al*., 2021). Additionally, Israeli scientists achieved true albino Nile tilapia by disrupting the *slc45a2* gene (Segev-Hadar *et al*., 2021).

9. *CRISPR in Crustaceans:* The first genome editing in a decapod was reported in 2016 in China on the ridgetail white prawn (*Exopalaemon carinicauda*), focusing on the chitinase compound *EcChi4* (Gui *et al*., 2016). CRISPR was used to induce mutations in the gene responsible for *EcChi4*, with successful heritable changes that did not affect survival or growth. This approach has also been applied in other crustaceans like *Daphnia magna* (Nakanishi *et al*., 2014) and *Parhyale hawaiensis* (Martin *et al*., 2016).

Conclusion

The advancements in CRISPR-Cas9 technology have significantly propelled the field of aquaculture genetics, offering innovative solutions for enhancing various traits in fish and crustaceans. These developments range from inducing sterility in salmonids to improving muscle mass in several fish species and altering coloration in ornamental fish. The successful application of CRISPR in aquaculture demonstrates its potential as a powerful tool for sustainable and efficient food production. However, it also necessitates careful consideration of the ethical and ecological implications of genetic modifications in aquatic species. As research continues to evolve, CRISPR technology promises to play a pivotal role in the future of aquaculture.

References

Anders, C., Bargsten, K. and Jinek, M. (2016). Structural plasticity of PAM recognition by engineered variants of the RNA-guided endonuclease Cas9. *Molecular Cell*, 61: 895-902.

Baloch, A.R.K. (2019). Utilization of CRISPR/Cas9 and novel germ cells labelling technique for surrogate production in sturgeons. Ph.D. Thesis, ISBN-978-80-7514-086-9.

Cai, W.J., Li, J., Li, L., Chen, X., Wei, J.R., Yin, Z., He, S. and Liang, X.F. (2021). Knockout of t1r1 gene in zebrafish (*Danio rerio*) by CRISPR/Cas9 reveals its roles in regulating feeding behavior. *Aquaculture*, 545: 737189.

Ceasar, S.A., Rajan, V., Prykhozhij, S.V., Berman, J.N. and Ignacimuthu, S. (2016). Insert, remove or replace: A highly advanced genome editing system using CRISPR/Cas9. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1863: 2333-2344.

Chandrasekaran, A.P., Song, M., Kim, K.S. and Ramakrishna, S. (2018). Different methods of delivering CRISPR/Cas9 into cells. *Progress in Molecular Biology and Translational Science*, 159: 157-176.

Chen, H., Wang, J., Du, J., Si, Z., Yang, H., Xu, X. and Wang, C. (2019). ASIP disruption via CRISPR/Cas9 system induces black patches dispersion in Oujiang color common carp. *Aquaculture*, 498: 230-235.

Diwan, A., Ninawe, A. and Harke, S. (2017). Gene editing (CRISPR-Cas) technology and fisheries sector. *Canadian Journal of Biotechnology*, 1: 65-72.

Du, J., Chen, H., Mandal, B.K., Wang, J., Shi, Z., Lu, G. and Wang, C. (2021). HDL receptor/Scavenger receptor B1-Scarb1 and Scarb1-like mediate the carotenoid-based red coloration in fish. *Aquaculture*, 545: 737208.

Faure, G., Shmakov, S.A., Makarova, K.S., Wolf, Y.I., Crawley, A.B., Barrangou, R. and Koonin, E.V. (2019). Comparative genomics and evolution of trans-activating RNAs in Class 2 CRISPR-Cas systems. *RNA Biology*, 16: 435-448.

Friedmann, T. (1992). A brief history of gene therapy. *Nature Genetics*, 2: 93-98.

Gratacap, R.L., Wargelius, A., Edvardsen, R.B. and Houston, R.D. (2019). Potential of genome editing to improve aquaculture breeding and production. *Trends in Genetics*, 35: 672- 684.

Gui, T., Zhang, J., Song, F., Sun, Y., Xie, S., Yu, K. and Xiang, J. (2016). CRISPR/Cas9-mediated genome editing and mutagenesis of EcChi4 in *Exopalaemon carinicauda*. *G3: Genes*, *Genomes*, *Genetics*, *6*: 3757-3764.

Güralp, H., Skaftnesmo, K.O., Kjærner-Semb, E., Straume, A.H., Kleppe, L., Schulz, R.W., Edvardsen, R.B. and Wargelius, A. (2020). Rescue of germ cells in dnd crispant embryos opens the possibility to produce inherited sterility in Atlantic salmon. *Scientific Reports*, 10: 18042.

Huang, J., Shi, C., Gao, Y., Su, J., Shu, Y., Zeng, N., Zhai, G., Jin, X., Han, D. and He, J. (2021). Heterozygous depletion of pik3r1 improves growth and feed conversion efficiency in Gibel carp (*Carassius gibelio*). *Aquaculture*, 545: 737207.

Kim, N., Kim, H.K., Lee, S., Seo, J.H., Choi, J.W., Park, J., Min, S., Yoon, S., Cho, S.-R. and Kim, H.H. (2020). Prediction of the sequence-specific cleavage activity of Cas9 variants. *Nature Biotechnology*, 38: 1328-1336.

Kozovska, Z., Rajcaniova, S., Munteanu, P., Dzacovska, S. and Demkova, L. (2021). CRISPR: History and perspectives to the future. *Biomedicine & Pharmacotherapy*, 141: 111917.

Martin, A., Serano, J.M., Jarvis, E., Bruce, H.S., Wang, J., Ray, S., Barker, C.A., O'Connell, L.C. and Patel, N.H. (2016). CRISPR/Cas9 mutagenesis reveals versatile roles of Hox

genes in crustacean limb specification and evolution. *Current Biology*, 26: 14-26. Mokrani, A. and Liu, S. (2024). Harnessing CRISPR/Cas9 system to improve economic traits in aquaculture species. *Aquaculture*, 579: 740279.

Nakanishi, T., Kato, Y., Matsuura, T. and Watanabe, H. (2014). CRISPR/Cas-mediated targeted mutagenesis in *Daphnia magna*. *PLoS One*, 9: e98363.

Nidhi, S., Anand, U., Oleksak, P., Tripathi, P., Lal, J.A., Thomas, G., Kuca, K. and Tripathi, V. (2021). Novel CRISPR–Cas systems: an updated review of the current achievements, applications, and future research perspectives. *International Journal of Molecular Sciences*, 22: 3327.

Roy, S., Kumar, V., Behera, B.K., Parhi, J., Mohapatra, S., Chakraborty, T. and Das, B.K. (2022). CRISPR/Cas Genome Editing-Can It Become a Game Changer in Future Fisheries Sector? *Frontiers in Marine Science*, 9: 924475.

Segev-Hadar, A., Slosman, T., Rozen, A., Sherman, A., Cnaani, A. and Biran, J. (2021). Genome editing using the CRISPR-Cas9 system to generate a solid-red germline of Nile tilapia (*Oreochromis niloticus*). *The CRISPR Journal*, 4: 583-594.

Straume, A.H., Kjærner-Semb, E., Skaftnesmo, K.O., Güralp, H., Lillico, S., Wargelius, A. and Edvardsen, R.B. (2021). Single nucleotide replacement in the Atlantic salmon genome using CRISPR/Cas9 and asymmetrical oligonucleotide donors. *BMC Genomics*, 22: 1-8.

Turnbull, C. (2021). Genome editing for sustainability: Improving host resistance to combat late blight in potato and sea lice in Atlantic salmon. *Norwegian University of Life Sciences*, Ås. Master thesis.

Yoshikawa, H., Ino, Y., Kishimoto, K., Koyakumaru, H., Saito, T., Kinoshita, M. and Yoshiura, Y., (2020). Induction of germ cell-deficiency in grass puffer by dead end 1 gene knockdown for use as a recipient in surrogate production of tiger puffer. *Aquaculture*, 526: 735385.

Yoshikawa, H., Ino, Y., Shigenaga, K., Katayama, T., Kuroyanagi, M. and Yoshiura, Y. (2018). Production of tiger puffer Takifugu rubripes from cryopreserved testicular germ cells using surrogate broodstock technology. *Aquaculture*, 493: 302-313.

Zhang, X., Wang, F., Ou, M., Liu, H., Luo, Q., Fei, S., Zhao, J., Chen, K., Zhao, Q. and Li, K. (2023). Effects of Myostatin b Knockout on Offspring Body Length and Skeleton in Yellow Catfish (*Pelteobagrus fulvidraco*). *Biology*, 12: 1331.

CLIMATE CHANGE IMPACT AND MITIGATION MEASURES ON INLAND FISHERIES AND AQUACULTURE

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Abstract:

Climate change presents a substantial risk to India's inland fisheries, affecting ecosystems and communities. The utilization of freshwater resources, including rivers, lakes, groundwater and wetlands, for various urban, agricultural, and industrial activities has placed these ecosystems under multiple stressors over time, leading to changes in their habitat conditions. The evolving climate significantly pressures wetland fisheries, ecosystems, and livelihoods at the eco-regional level. The global surface temperature has experienced a more rapid increase since 1970 than in any other 50-year span over the past 2000 years, posing a threat to the future of our food systems. Climate change has caused shifts in hydro-ecological regimes, fish life histories, species composition, adaptation capacities, and the socioeconomics of fishers on a global scale. Key challenges include temperature and oxygen level variations, altered hydrological patterns, and changes in species composition, with consequences extending to livelihoods and food security. Mitigating these challenges involves implementing adaptive management practices, advocating for sustainable fishing techniques, restoring habitats, adopting climate-smart aquaculture, supporting community-based initiatives, integrating policies, building capacity, and sustaining research and monitoring efforts. A comprehensive, multifaceted approach is crucial for ensuring the long-term sustainability of inland fisheries, incorporating scientific research, community engagement, and effective policy implementation. Urgent and coordinated actions are imperative to alleviate the impacts of climate change, enhance resilience, and secure the enduring sustainability of inland fisheries in India.

Keywords: Climate change, Mitigation, Inland, Resources.

Introduction:

Climate change presents significant challenges to India's inland fisheries, impacting ecosystems, fish populations, and the livelihoods of dependent communities. Inland water, a vital resource extensively utilized or impacted across various aspects of human existence, including consumption, agriculture, recreation and more, faces numerous anthropogenic pressures such as extraction, river regulation, damming, pollution, habitat degradation, and fishing. The existing high demand for water is expected to surge in the future due to population growth and development. Without prompt remedial measures, this growth could adversely affect inland fisheries and their associated benefits. Unfortunately, the valuable contributions of inland fisheries are often overlooked or undervalued in the competition for this limited resource. Instead, preference is given to more visible water demands, leading to severe consequences for the sustainability of inland fisheries. Additionally, climate acts as an added stressor, exerting a significant influence on the physical, chemical, and biological processes within freshwater ecosystems. This influence results in alterations to the distribution, abundance, and production of inland fishery resources (Settele *et al*., 2014). Climate change further impacts the global hydrological cycle by altering precipitation and evaporation patterns. These effects manifest through changes in water quality, habitat availability, and fish behavior, driven by rising temperatures, shifting precipitation patterns, and extreme weather events. In instant, climate change induces shifts in species composition, assemblages, abundance, biomass, species distribution, fish yields, and the efficiency of fishing methods and gears.

Addressing these challenges requires a comprehensive approach encompassing sustainable fisheries management, habitat conservation, resilient infrastructure, community-based adaptation, integrated water resource management, research, monitoring, capacity building, policy integration, climate-smart aquaculture, and international cooperation. This multifaceted strategy is essential for bolstering the resilience of India's inland fisheries in the face of climate change. Conversely, prevailing perspectives often predict dire outcomes for inland fisheries due to human-induced threats, with global studies illustrating declining catches, species loss and indications of overfishing. Factors contributing to this decline include inadequate fishery management practices, such as unregulated fishing and the introduction of non-native species. It is imperative to reassess current approaches and implement effective conservation measures to ensure the long-term sustainability of inland fisheries.

Climate change impact on Inland Fisheries:

Temperature Changes and Precipitation Patterns:

Temperature increases change the water's temperature, which has an impact on fish distribution, metabolism, and reproduction. There might be changes in the species composition and a decline in biodiversity if certain species find it difficult to adapt to the warmer waters. Fish habitats and migration patterns are disrupted by variations in precipitation patterns, which can affect river flow, water levels, and seasonal floods. Fish populations might decline, spawning success can be hindered, and habitat degradation can result from floods and droughts. The upper ocean warming (above 700 m) that has been seen since the 1960s (Cheng *et al*., 2017) has been mostly attributed to anthropogenic forcing, with surface waters rising by an average of 0.7 °C per century worldwide between 1900 and 2016. For freshwater systems, an increase of water temperature is expected to occur in most areas, as a result of an increase of air temperature. This is linked to the relatively shallow nature of surface freshwaters and their susceptibility to atmospheric temperature change.

Water Quality:

Climate change can exacerbate water pollution and eutrophication, impacting fish health and habitat suitability. Altered pH levels, oxygen depletion, and increased nutrient runoff can stress fish populations and lead to declines in productivity.

Habitat Loss and Degradation:

The heightened frequency and severity of extreme weather occurrences, such as storms and hurricanes, can inflict damage on aquatic habitats like wetlands, rivers, and lakes. This loss of habitat intricacy and connectivity diminishes areas suitable for fish spawning and nurturing, consequently impacting population dynamics. In India, the inland aquaculture primarily revolves around the Indian major carps, namely *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*, who's spawning traditionally aligns with the monsoon season (June-July) extending through September. However, in recent times, there have been observations indicating that these major carps reach maturity and commence spawning as early as March, potentially enabling them to breed twice annually. This observed increase in breeding activity, compared to previous years (Dey *et al*., 2007), seems to be influenced positively by the changing climate conditions. The Ganga River, India's largest river system, not only serves as a vital water source for millions of people but also sustains a significant population of fishermen. Spanning a total length of 2525 kilometres from its source to mouth, the Ganga River is categorized into three main segments: upper (Tehri to Kanauji), middle (Kanpur to Patna), and lower (Sultanpur to Katwa).

Altered Food Web Dynamics:

Climate change affects aquatic ecosystems' food web structure and energy flow by influencing primary production and prey species availability. Fish growth rates and reproductive success can be impacted by shifts in the distribution and amount of prey. The mechanism at the base of the marine food chain, known as phytoplankton production, regulates the amount of food and energy that is accessible to fish and higher tropic levels. The worldwide marine primary output projected by Earth system models in response to climate change is ambiguous; models predict increases of up to 20 percent by 2100 as well as reductions (Taucher & Oschiles, 2011). This is partially due to the fact that primary production integrates variations in nutrients, light, and temperature, but also because it's unclear how sensitive tropical ocean primary production is to changes in temperature.

Fisheries Management Challenges:

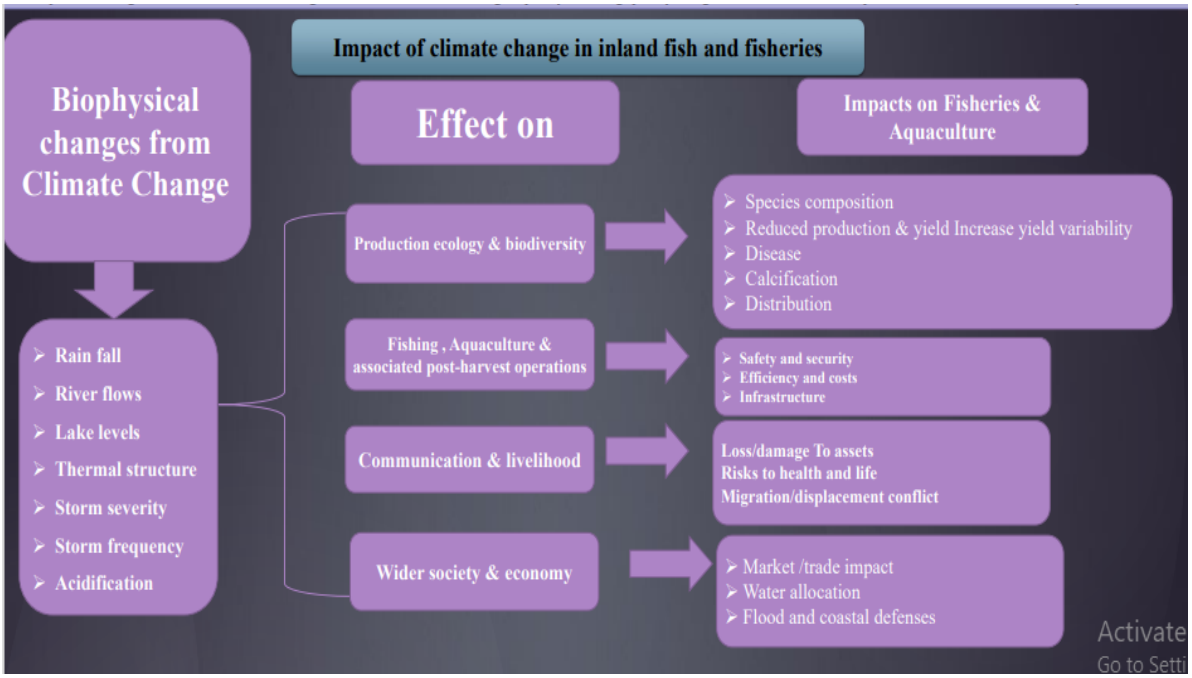
Climate change adds complexity to fisheries management by exacerbating existing pressures, such as overfishing and habitat degradation. Adaptive management strategies are needed to mitigate the impacts of climate change on fish stocks and fisheries-dependent communities. These are some other major challenges are following

- Fish stocks depleted→ Food insecurity → Unemployment → Poverty → Intra and inter conflicts between fishermen and industries.
- Accurate data on assessment of fishery resources and their potential of fish production
- Development of sustainable technologies, yield optimization
- Harvest and post-harvest operations, landing and berthing facilities
- Welfare of fishermen:
    - marginalization, lack of awareness and communication
    - lack of equity and power sharing, empowerment of resource users
- Non-conducive national policy and legislative frameworks
- Insufficient institutional linkages
- Lack of personnel with requisite capacity
- Funding

Mitigation measures

Encourage the use of climate-smart aquaculture techniques to diversify revenue streams and ease the strain on wild fish populations. Troell *et.al.*, (2014). Adopt integrated techniques for managing water resources to ensure that there is enough clean water for fishing. Arthington *et.al.*, (2016). Encourage community participation in fisheries management and resilience-boosting adaptive techniques. Allison & Bassett (2015). Build and modernise the infrastructure supporting the fishing industry to resist harsh weather

and climate change. Bogard *et.al*., 2019. Wetlands and riparian zones are important fish breeding and feeding habitats that need to be preserved and restored. Cowx (2004) to guarantee the long-term sustainability of fish populations, put into effect and uphold sustainable fisheries management techniques (Halls, 2007).



Climate Change impact on Inland Fisheries & Aquaculture

Conclusion:

Mitigating climate change impacts on India's inland fisheries requires coordinated efforts, including sustainable management practices, community involvement, and adaptive strategies. Key measures include regulating fishing, restoring habitats, and creating protected areas. Investment in research and technology is essential for monitoring climate dynamics, and collaboration between stakeholders is crucial for effective policy implementation. Overall, a holistic approach involving sustainable practices, community engagement, innovation, and policy initiatives is vital for preserving the health and viability of inland fisheries and benefiting both biodiversity and local communities.

Reference:

Allison, E.H., & Bassett, H.R. (2015). "Climate Change in the Oceans: Human Impacts and Responses." Science, 350(6262), 778-782.

Arthington, A.H., Bhaduri, A., Bunn, S.E., et al. (2016). "The challenge of providing environmental flow rules to sustain river ecosystems." Ecological Applications, 26(2), 440-455.

Bogard, J. R., Farmery, A. K., Little, D. C., Fulton, E. A., & Cook, M. (2019). Will fish be part of future healthy and sustainable diets?. *The lancet planetary health*, *3*(4), e159-e160.

Cheng, G., Han, J., & Lu, X. (2017). Remote sensing image scene classification: Benchmark and state of the art. *Proceedings of the IEEE*, *105*(10), 1865-1883.

Cowx, I.G. (2004). "The role of fisheries in the rehabilitation and conservation of inland and estuarine waters." Fisheries Research, 66(3), 251-265.

Dey, S., Srivastava, P. K., Maji, S., Das, M. K., Mukhopadhyaya, M. K., & Saha, P. K. (2007). Impact of climate change on the breeding of Indian major carps in West Bengal. J. Inland Fish. Soc. India, 39(1), 26-34.

Halls, A.S. (2007). "Fisheries Management in the 21st Century: Will New Paradigms Apply?" Reviews in Fisheries Science, 15(1-2), 1-9.

Settele, J., Scholes, R., Betts, R., Bunn, S., Leadley, P., Nepstad, D., Overpeck, J.T. & Taboada, M.A. 2014. Terrestrial and inland water systems. In C.B. Field, V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee et al., eds. Climate Change 2014: Impacts, adaptation, and vulnerability. Part A: Global and sectoral aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, UK and New York, USA, Cambridge University Press. pp. 271–359.

Taucher, J., & Oschlies, A. (2011). Can we predict the direction of marine primary production change under global warming?. *Geophysical Research Letters*, *38*(2).

Troell, M., Naylor, R.L., Metian, M., et al. (2014). "Does aquaculture add resilience to the global food system?" Proceedings of the National Academy of Sciences, 111(37), 13257-13263.

GUARDIANS OF THE DEEP: 'PRESERVING MARINE MAMMALS FOR FUTURE SEAS'

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Abstract

Marine mammals represent a captivating and diverse group of animals that have evolved to thrive in the ocean's dynamic and challenging environment. This article explores the overall view about mammals and their crucial role in ocean ecosystems and the urgent need for their conservation and also examines the diverse array of marine mammals, including whales, dolphins, seals, and sea otters, highlighting their significance in maintaining marine biodiversity and ecosystem balance. It delves into the myriad threats facing these majestic creatures, such as habitat degradation, pollution, climate change, and overfishing, which jeopardize their survival. By analyzing current conservation efforts and innovative approaches, the article emphasizes the importance of global collaboration and sustainable practices in safeguarding marine mammal populations. Furthermore, it underscores the interconnectedness between marine mammal conservation and human well-being, emphasizing the socio-economic benefits derived from healthy marine ecosystems. Ultimately, "Guardians of the Deep" advocates for increased awareness, research, and conservation action to ensure the continued existence of these iconic guardians of the seas for generations to come.

Keywords: Mammals, Guardians, ocean, sea lions, seals

Introduction

Marine mammals are aquatic animals that live in and around marine environments, such as the ocean. They consist of creatures like polar bears, seals, whales, manatees, and sea otters. The only thing that unites this informal community is their dependency on maritime environments for survival and food. (Britannica, 1997). The way that different species of marine mammals adapt to an aquatic lifestyle differs greatly. Due to their complete aquatic nature, sirenians and cetaceans are both required aquatic inhabitants. Being semiaquatic, seals and sea lions spend most of their time in the water but must come ashore for mating, reproducing, and molting, among other essential tasks. On the other hand, polar bears and otters are significantly less suited to live in water. Marine mammals' diets vary greatly as well; some eat zooplankton, while others eat fish, squid, shellfish, or

sea grass, and a few consume other mammals. (Britannica, 1997) While marine mammals are few when compared to land mammals, their contributions to various ecosystems are significant, particularly in terms of marine ecosystem maintenance, which includes processes such as prey population regulation. This involvement in ecosystem maintenance makes them particularly important, as 23% of marine mammal species are currently threatened.

Diversity

- 130 Marine mammal species have been recognized worldwide. (Jefferson et al., 2011).
- According, to Jeyabaskaran and Vivekanandan 2013, the Indian seas support 26 species of marine mammal families Delphinidae, Physeteridae, Kogiidae, Ziphiidae, Phocoenidae, and Platanistidae including 25 cetacean species, five Baleen whales and the rest are odontocetes.

Distribution

- Distribution of mammals are widely distributed throughout the globe, but their distribution is patchy and coincides with the productivity of the oceans. (Rice,1998)
- Species richness peaks at around 40° latitude, both north and south. The total species range is highly variable for marine mammal species.
- Abundance and species richness are high on the South Sri Lanka coast followed by the Southeastern Arabian Sea (off Kerala – Karnataka). Marine mammals are not randomly distributed in the world's oceans. In the Arctic region, Polar Bears are present, and in the Pacific Coast Sea Otters are distributed widely, Additionally, Dugongs highly live on the West Indian coasts.

Adaptations

- Diving behavior (nostrils and blowholes)
- Modified forelimbs
- Thick corneas and produce viscous mucus
- To counteract drag, mammals have pointed, spindle-like shapes (bubble layer)
- Buoyancy (upward force, density of the animal)- blubber layer instead of swim bladder
- Lungs and other air-filled sinuses

Feeding ecology

- Baleen whales are batch feeders, taking in large amounts of prey and filtering them from the waters with the fringes on the inside of their baleen plates.
- The pinnipeds generally feed on fishes and squids although some take primarily invertebrates such as krill.
- The sirenians are all herbivores and therefore do not need to be particularly fast to catch prey.
- The sea otter feeds mostly on invertebrates, such as crabs and sea urchins. Otters often use rocks as tools to break apart the hard shells of their prey.

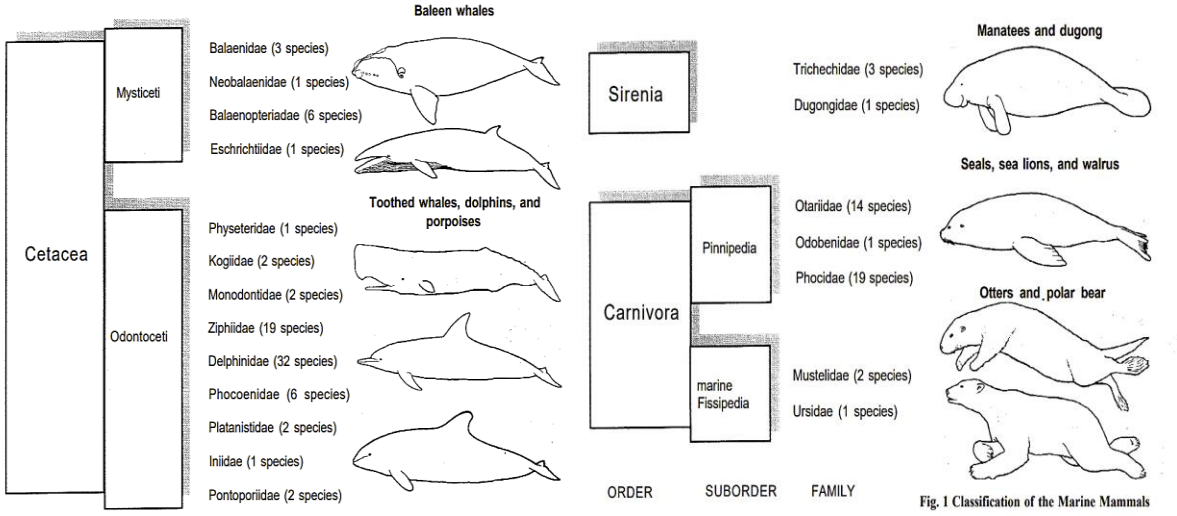

Fig. 1 Classification of the Marine Mammals

Source: Marine Mammals of the World, FAO. 1993.320. p. 587

Strandings

Strandings may be small-scale events involving single animals, or larger-scale events involving dozens of animals ("mass stranding"). Strandings may be routine, caused by commonly seen injuries or diseases, caused by less common circumstances ("unusual mortality event").

Recent strandings in India

- Jan 24, 2015- blue whale stranded along the shallow waters of the Arabian Sea (Maharashtra)
- Jan 2016 – mass stranding of pilot whales in Manapad
- Feb 2016- stranding of a sperm whale in Odisha
- April 2018- Bryde's whale along the coast of Maharashtra

Threats to marine mammals

1. ***Habitat Loss and Degradation***: Destruction and degradation of marine habitats, such as coastal areas, feeding grounds, and breeding sites, due to coastal development, pollution, and climate change, can significantly impact marine mammals. Loss of habitat can disrupt their feeding, breeding, and migration patterns, leading to population declines. Killer whales - are more vulnerable to disturbance from vessels when they are feeding, rather than when resting, traveling, or socializing. This leads to the need for protected area management strategies should target feeding "hotspots," thus prioritizing the protection of habitat used for the behavior in which a species is most vulnerable to anthropogenic disturbance (Ashe et al., 2010).
2. ***Pollution*:** Marine mammals are exposed to various pollutants, including oil spills, plastic debris, heavy metals, and chemical contaminants from industrial and agricultural runoff. These pollutants can accumulate in their bodies, leading to health issues such as reproductive problems, immune suppression, and organ damage.
3. ***Climate Change***: Climate change poses significant threats to marine mammals through various mechanisms, including sea level rise, ocean acidification, changes in temperature and currents, and alterations in prey availability. These changes can affect marine mammal habitats, prey distribution, and reproductive success, leading to population declines and shifts in species distributions.
4. ***Overfishing and Bycatch***: Overfishing and bycatch in commercial fishing operations pose significant threats to marine mammal populations. By-catch is the incidental capture of non-target species in fisheries it affects all cetaceans, both small and big, in all habitat types. However, smaller cetaceans and pinnipeds are most vulnerable as their size means that escape once they are entangled is highly unlikely and they frequently drown.
5. ***Noise Pollution*:** Anthropogenic noise from shipping, seismic surveys, naval exercises, and offshore construction activities can disrupt the communication, navigation, and feeding behaviors of marine mammals. Prolonged exposure to high levels of noise pollution can cause stress, hearing loss, and interference with vital activities, ultimately impacting their survival.
6. ***Entanglement in Marine Debris***: Marine mammals, such as whales, dolphins, and seals, are at risk of becoming entangled in fishing gear, such as nets, lines, and traps, as well as other marine debris like plastic bags and ropes. Entanglement can cause injuries, infections, or even death by restricting movement, impairing feeding, and increasing vulnerability to predators.

7. ***Illegal Wildlife Trade and Harvesting***: Some marine mammals, such as whales, seals, and walruses, are targeted for their meat, blubber, fur, or other body parts in illegal wildlife trade and harvesting activities. Overexploitation for commercial purposes can lead to population declines and threaten the survival of vulnerable species.

8. ***Collision with Vessels***: Marine mammals are at risk of colliding with vessels, including ships, boats, and watercraft, especially in areas of high maritime traffic. Collisions can cause injuries or fatalities, particularly for large species like whales, and may also result in damage to vessels. Vessel strikes cause death for several marine mammals, especially whales. In particular, fast commercial vessels such as container ships can cause major injuries or death when they collide with marine mammals. Collisions occur both with large commercial vessels and recreational boats and cause injury to whales or smaller cetaceans. The critically endangered northern right whale is particularly affected by vessel strikes. Boats traveling at speeds over 13 knots are more likely to kill cetaceans

9. ***Exploitation****:* Hunted by coastal aboriginal humans historically for food, Fur, oil, and other resources. Blue whales, and the North Pacific right whale, are much lower compared to their pre-exploited levels. The FAO estimated that 70% of the world's commercial fish stocks are fully or over-exploited and severely depleted.

Marine mammal conservation is urgently needed for several compelling reasons:

1. ***Biodiversity Preservation***: Marine mammals play crucial roles as they are the "Keystone" species. Their presence helps maintain the balance and health of marine ecosystems. Conserving marine mammals contributes to preserving overall biodiversity and ecosystem stability.

2. ***Economic Importance***: Marine mammals support important economic activities such as ecotourism, recreational whale watching, and fishing industries. Healthy populations of marine mammals attract tourists and generate revenue for local communities. Conservation efforts ensure the sustainability of these economic activities in the long term.

3. ***Cultural Significance***: Many indigenous cultures around the world have deep cultural and spiritual connections to marine mammals. These species often hold symbolic significance and are integral to traditional practices, folklore, and cultural identity. Conserving marine mammals is essential for honoring and respecting indigenous traditions and heritage.

4. ***Scientific Research***: Marine mammals serve as valuable subjects for scientific research, providing insights into marine ecology, behavior, physiology, and

evolution. Studying marine mammals helps scientists understand the complex dynamics of marine ecosystems and informs conservation strategies. Preserving these species ensures continued opportunities for scientific discovery and knowledge advancement.

5. ***Climate Change Indicators***: Marine mammals are sensitive to environmental changes, making them valuable indicators of ecosystem health and climate change impacts. Monitoring marine mammal populations provides early warning signs of ecosystem disturbances and helps assess the effectiveness of climate change mitigation measures. Protecting marine mammals contributes to broader efforts to address climate change and environmental degradation.

6. ***Interconnectedness with Human Health***: The health of marine ecosystems, including marine mammals, is closely linked to human well-being. Marine mammals can serve as sentinels for ocean health, signaling potential risks to human health from pollution, contaminants, and disease transmission. By conserving marine mammals, we protect not only their populations but also human health and livelihoods.

Preserving Marine Mammals for the Future: The Suggestions are,

- Strictly banning the capture of these animals, both intentionally and unintentionally
- Replacing destructive gears such as set gill nets.
- Popularising the usage of acoustically opaque gill nets in localities where these mammals feed and/or breed and establishing marine parks and sanctuaries
- Accurate reporting of all the landings and stranding along with relevant details on the identity and vital biological data to research organizations,
- Strengthening research on the behavior and biology of marine mammals and extension
- Formulation of bilateral/multilateral cooperation among the concerned countries for their conservation as well as the provision of adequate funds for the same
- Setting up a "National Marine Environment Protection and Resources Conservation Authority", with wide and constructive legislative powers to protect, conserve and audit the marine ecosystems and their resources.

Given the urgency and complexity of the threats facing marine mammals, concerted efforts are needed from governments, conservation organizations, researchers, industries, and local communities to implement effective conservation measures, mitigate human impacts, and safeguard the future of these iconic and ecologically important species.

References

Ashe, E., D. P. Noren, and R. Williams. 2010. "Animal behavior and marine protected areas: incorporating behavioral data into the selection of marine protected areas for an endangered killer whale population." *Animal Conservation* 13, no. 2: 196-203.

Britannica CD (1997) “The Indian Ocean”. In: Britannica CD, Version 97, *Encyclopaedia Britannica, Inc.*

Jeyabaskaran, R. and Vivekanandan, E (2013) Marine mammals and fisheries interactions in Indian seas. In: Regional Symposium on Ecosystem Approaches to Marine Fisheries & Biodiversity, October 27-30, 2013, Kochi.

Jefferson, T. A., Webber, M. A., & Pitman, R. L. (2011). Marine mammals of the world: a comprehensive guide to their identification. Elsevier.

Rice, D.W. 1998., *Marine mammals of the world*. NOAA Technical Report NMFSSSRF-711, 15pp

ANESTRUS AND ITS THERAPEUTIC MANAGEMENT IN CATTLE AND BUFFALORAJ DESAI,

Shweta P Khatri, Meet Desai and Ketan Desai

Introduction

Anestrus is one of the most commonly occurring reproductive problems in cattle and buffalo in India, affecting livestock productivity and economics to a great extent. The problem is more severe in sub urban and rural areas of the country. It is a functional disorder of the reproductive cycle which is characterized by absence of overt signs of estrus manifested either dueto lack of expression of estrus or failure of its detection. Anestrus is observed in post pubertal heifers, during pregnancy, lactation and in early postpartum period in adult animals. The condition may be associated with uterine pathology such as pyometra, fetal resorption, maceration and mummification. Anestrus is a multi-causative factors associated problem but its occurrence signalsthe inadequate nutrition, environmental stress, uterine pathology and improper managemental practices. Diagnosis of the condition is based on the exploration of the different causative factors responsible for it. Though many therapeutic agents (hormonal and non-hormonal) have been usedbut as such there is no single panacea to correct it.

Physiological Anestrus

Animals remain anestrus during certain physiological stages which does not related to infertility viz., before puberty, during pregnancy, lactation and early postpartum period. Accordingly, physiological anestrus has been classified into pre–pubertal, gestational, lactational and post- partum anestrus.

Pathological Causes Of Anestrus

Certain pathological conditions i.e. Persistent Corpus Luteum, Silent Estrus, ovarian agenesis, dysgenesis or derangement of follicular–luteal dynamics leads to anestrus causing infertility and pose a herd problem. Such conditions may be congenital or acquired.

Treatment

Anestrus can be treated according to their cause, however; there is no single panacea to correct it.Various therapeutic agents including hormonal and non–hormonal compounds have been used extensively for the restoration of cyclicity in anestrus cattle and buffalo by several workers with varying degree of success. In order to ensure effective treatment, the health and nutritional status of the animals must be in good conditions. Besides deworming, the supplementation of vitamins, minerals and antioxidants in feed are useful to improve health status of the animals.

Non Hormonal Treatments

Plant Based Heat Inducers

Plants have been used for the treatment of animals since long back. Plants synthesize varieties of phytochemicals such as alkaloids, glycosides, terpenes and tannins (secondary metabolites) as a part of their normal metabolic activity and many of these have therapeutic actions when consumed by animals. Many plants are rich source of vitamins and minerals whereas some have estrogenic property which is useful in restoration of cyclicity in anestrus animals. Almost all the parts of plantsuch as seeds, berries, roots, leaves, bark and flowers have been used as therapeutic agents either directly (crude drugs) or their active principles, after separation though various chemical process. Many plants such as *Murraya koenigii* (curry leaves), *Nigella sativa* (kalonji), *Abroma augusta* (Ulatkambal), *Saraca asoca* (Ashoka), *Trigonella foenum–graecum* (Methi), *Bambusa aruninacea*, *Carica papaya*, *Asparagus recemosus*, *Leptadenia reticulate*, *Courupita guianesis*, *Pergulacia daemia*, *Semecarpus anacardium cucumber*, *and jute plants* either alone or in combinations have been fed to treat the anestrus animals with variable response on induction of estrus.

Indigenous herbal preparations such as Prajana HS (Indian Herbs), Janova (Dabur), Sajani (Sarabhai), Heat up (Century) Heat raj (Ranjan), Fertivet (Ar Ex Labs) and Aloes compounds (Alarsar) are commercially available and effective in restoration cyclicity with good success rates.These formulations are potent combinations of herbs, formulated to induce ovarian activity.

Lugol's Iodine

Lugol's iodine treatment is cheaper and effective means of management of anestrus but response has been variable (45 to 91.7%) among cattle and buffaloes. Lugol's iodine solution (5%) has traditionally been used as a cervical paint. It is presumed that painting of Lugol's iodine on posterior part of the cervix causes local irritation and brings about reflux stimulation at anterior pituitary for secretion of gonadotrophins and consequently cyclicity. Lugol's iodine is an irritatingsolution and intrauterine infusion of Lugol's solution (0.5 to 1.0%) causes hyperemia (enhanced circulation) of uterine mucosa resulting into degree of iodine absorption from uterus. The absorbed iodine probably increases the metabolic rate of body through stimulating the thyroid hormone secretion. Another probable mechanism of intrauterine use of Lugol's iodine is that it acts as chemical curator (due to its irritating nature) and replaces the uterine mucosa with new tissue. Thenewly formed tissues of endometrium release luteolytic factors (PGF2α) that reaches to the corpusluteum via utero–ovarian pathway and causes luteolysis. Thus, it initiates the estrus cycle, if anestrus is due to PCL.

Hormonal Treatments

Estrogens Based Treatment

Exogenous administration of estrogen produce estrus signs in anestrus animals with or without concurrent ovulation. In presence of dominant follicle, estrogen administration results in expression of estrus and ovulation because of its positive feedback effect over pituitary for LH surge. For this reason, it has been used to induce ovulation and to reduce postpartum anestrus period. Conversely, estrogen induces anovulatory estrus in absence of dominant follicle. Estrogenshave also been shown to cause luteolysis in ruminants probably through stimulating the prostaglandin secretion from endometrium as well decreasing the level of circulating LH. One ortwo doses of intramuscular injections estradiol (3–10mg) or estrone (5–15mg) at three days

interval can be used to regresses the retained corpus luteum associated with pyometra, mummification and mucometra.

Progesterone Based Treatment

Exogenous administration of progesterone mimics the luteal phase of the estrus cycle by exerting negative feedback effect over hypothalamus and pituitary for LH release. Upon withdrawal of progesterone, the normal follicular phase of the cycle is stimulated. However, for such treatment seem to be effective, abrupt decrease in progesterone level is required at the end of treatment. Intravaginal progesterone releasing devices such as PRID (progesterone–releasing intravaginal device), CIDR (controlled internal drug release) and CueMate are effective in restoration of cyclicity in anestrus animals. Ear implants (Crestar and Synchromate–B) also produce required abrupt decrease in progesterone concentration at the end of treatment. Progesterone therapy aloneis not particularly effective for the treatment of anovulatory anestrus; hence other hormones have been incorporated in most of the progesterone based therapy.

Gonadotropic Releasing Hormone (GnRH) Based Treatment

The single intramuscular injection of GnRH analogue (10 to 20µg Buserelin) has been used effective in induction of estrus and concurrent ovulation with variable response (45.5 to 87.5%) within 4–22 days. The variable response may be due to differential action of GnRH on different stages of follicular development. It induces ovulation, if mature follicle is present at the time of administration by inducing the LH surge. However, single injection of GnRH is not always effective in deep anestrus animals. On the contrary, it stimulates emergence of new follicular wave through enhanced secretion of FSH, thus effective in long term. Pulsatile/intermittent injections ofsmall dose of GnRH (at every 2 hours, intravenously) has been tried in order to induce LH pulses,however, intermittent injection make this technique impractical.

To achieve better response, GnRH has been combined with other drugs such as phosphorus injection (Tonophosphan), prostaglandin, estradiol and progesterone. The

Ovsynch protocol or GPG regimen (GnRH–PG–GnRH), used to synchronize ovulations in dairy cows has been widelyused to treat anestrus cattle and buffaloes and results are also promising. Under this protocol firstinjection of GnRH (at day 0) induces ovulation, if dominant follicle is present and if not luteinizeswith emergence of new follicular wave 1 to 2 days later, PGF2α injection given on day 7 regress the CL formed in response to first injection of GnRH and second injection GnRH on day 9 induces ovulation of new dominant follicle subsequently, all the treated animals are inseminated within 16–20 hours of second injection of GnRH.

Prostaglandin Based Treatment

Prostaglandin (PGF2α) is the treatment of choice for persistent corpus luteum and sub estrus. Natural or synthetic analogue of PGF2α as a single dose has been used with a reasonable degree of success for management of silent estrus in cattle and buffaloes. It should be born in mind that PGF2α is only effective between days 6–16 of the cycle and in the presence of active corpus luteum. An intramuscular injection of 25mg (total dose) of natural PGF2α or 250 to 500 micrograms of synthetic ones is required to regress the CL in both cattle and buffaloes. However,a lower dose of PGF2α (5mg) are also effective to regress the CL through intra–vulvo–submucosal.

USE OF GRAPHENE FOR IMPROVING SOIL PROPERTIES

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Abstract

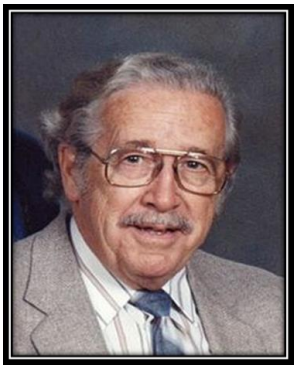
The escalating global population drives a heightened demand for food production, placing immense pressure on soil, a primary nutrient source for crops. However, rampant fertilizer misuse and poor management practices degrade soil physicochemical properties. To address these challenges, scientists turn to nanomaterials, notably graphene. Graphene, a 2D carbon lattice with diverse dimensional applications, revolutionizes soil enhancement. Its integration augments nutrient availability, soil structure, and microbial vitality while reducing micronutrient diffusion. Notably, graphene fosters bacterial proliferation and improves soil porosity and drainage. This super material, lighter than hair yet stronger than steel, embodies a pivotal role in 21st-century agricultural advancements, offering sustainable solutions for global food security.

Introduction

In recent years, with the increasing global population, tremendously increases the demand for food production and soil is considered as major reservoir of nutrients which supplies to crops for their growth and development and enhances the food production to meet the demand increasing population but due to overuse of fertilizers and mismanagement practices lead to deteriorate the soil physicochemical properties. Hence to overcome these problems many scientists were worked on nano materials and one of the most promising material was Graphene.

Graphene is a flat monolayer of carbon atoms tightly packed into a two-dimensional (2D) honeycomb lattice has molecular bond length of 0.142 nm, and is a basic building block for graphitic materials of all other dimensionalities, It can be wrapped up into 0D fullerenes, rolled into 1D nanotubes or stacked into 3D graphite and is widely used for describing properties of various carbon-based materials. Alessandrino *et al*.(2023).

History



The theory of graphene was first explored by P. R. Wallace in 1947 as a starting point and the term graphene first appeared in 1987. Graphene is a combination of "graphite" and the suffix -ene, named by Hanns-Peter Boehm. Scientist unknowingly produced GRAPHENE in small quantities for centuries from graphite until it was rediscovered, isolated and characterized in 2004 by Andre Geim and Konstantin Novoselov at the University of Manchester. In 2010, six years after discovery, they were awarded with Nobel Prize in Physics for ground breaking graphene experiments and defined Graphene is a crystalline allotrope of carbon with 2-dimensional properties having high optical transparency, thermal conductivity, mechanical strength, specific surface area, electron mobility, hence used in different fields.

Properties of graphene

The properties of graphene are unique due to its all carbon structure and nanoscale geometry. Zhang *et al*.(2014).

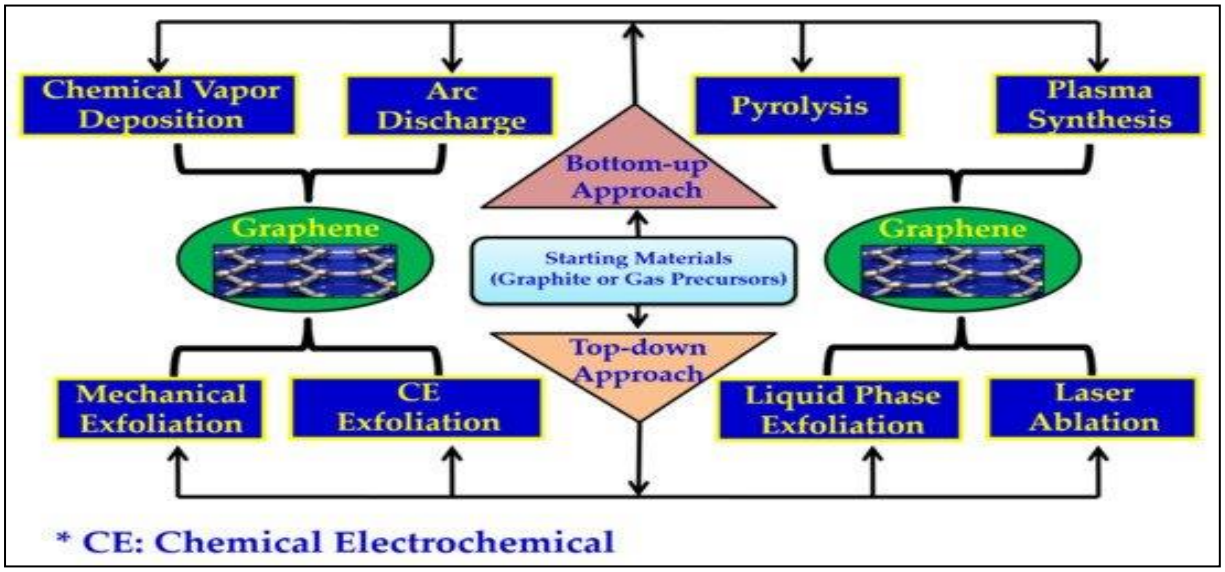
Electronic Properties: graphene has a delocalized pi-electron system across the entirety of its surface, the movement of electrons is very fluid. The graphene system also exhibits no band gap, due to overlapped pi-electrons, allowing for an easy movement of electrons without the need to input energy into the system. The electronic mobility of graphene is very high and the electrons act like photons, with respect to their movement capabilities. The electrons are also able to move sub-micrometer distances without scattering.

Thermal Properties: The repeating structure of graphene makes it an ideal material to conduct heat in plane. Inter plane conductivity is problematic and typically other nanomaterials such as CNTs are added to boost inter plane conductivity. The regular structure allows the movement of phonons through the material without impediment at any point along the surface. Graphene can exhibit two types of thermal conductivity- in-plane and inter-plane. The in-plane conductivity of a single-layered sheet is 3000-5000 W m-1 K-1, but the cross-plane conductivity can be as low as 6 W m-1 K-1, due to the weak inter-plane van der Waals forces. The specific heat capacity for graphene has never been directly measured, but the specific heat of the electronic gas in graphene has been estimated to be around 2.6 μ J g-1 K-1 at 5 K.

Mechanical Strength: Graphene is one of the strongest materials ever discovered with a tensile strength of 1.3 x 1011 Pa. In addition to having an un rivaled strength, it is also very lightweight (0.77 mgm-2). The mechanical strength of graphene is unmatched and as such can significantly enhance strength in many composite materials.

Flexibility/Elasticity: The repeating sp2 hybridized backbone of graphene molecules allow for flexibility, as there is rotation around some of the bonds, whilst still providing enough rigidity and stability that the molecule can withstand changes in conformation and support other ions. This is a very desirable property as there are not many molecules that can be flexible and supportive at the same time. In terms of its elasticity, graphene has found to have a spring constant between 1-5 Nm-1, with a Young's modulus of 0.5 TPa.

SYNTHESIS OF GRAPHENE:

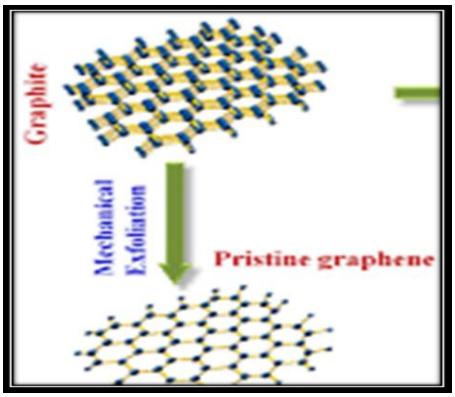


Graphene synthesis means any process of fabricating or extracting graphene from graphite. The method to be chosen is governed by the desired size, quantity, and purity. Synthesis technique contributes to the structure and properties of graphene produced. There are variations of graphene layers from different techniques such as a single layer, double layer, or multiple layers, and they have different applications in various fields of science and technology.

Graphene synthesised by two approaches: Top-Down and Bottom-Up approaches.

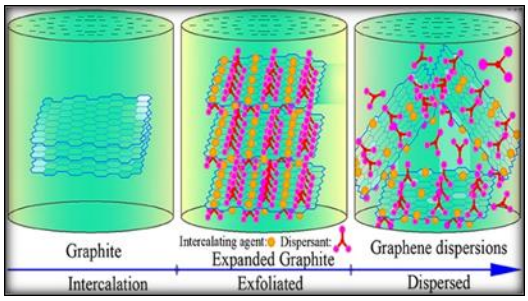
Top-Down approach

1. **Mechanical exfoliation:** It is the distortion of weak van der Waals force holding carbon–carbon atom together and graphite was subjected for varying temperature and pressure, then peeled off the graphite, layer by layer using scotch tape and distorted layer was for subjected for the sonication.



Mechanical exfoliation

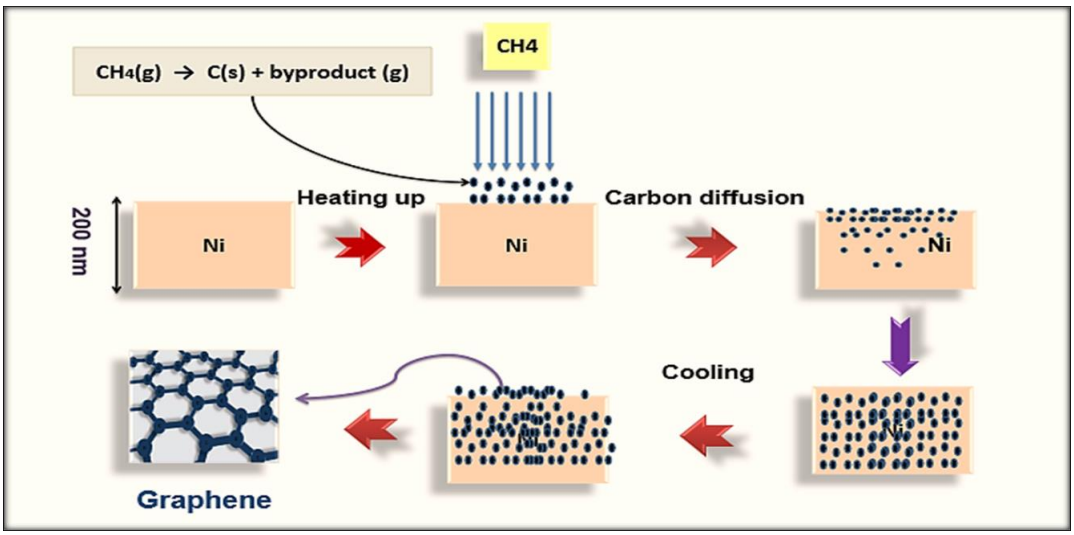
2. **Chemical exfoliation:** Graphite was added with potassium as a intercalating agent to expand the graphite and added with dispersing agent (ethanol) to disperse the expanded graphite and subjected for the sonication to separate the monolayer of graphene.



Chemical exfoliation

Bottom-Up approach

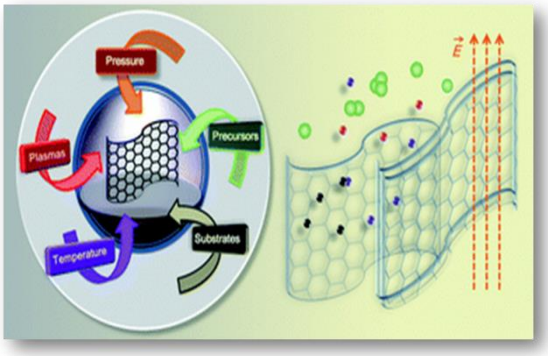
1. **Thermal Chemical Vapour deposition:** Synthesis of graphene through thermal chemical vapor deposition (CVD) has been quite new. The first report on planar few layer graphene (PFLG), synthesized by CVD, was found in 2006. In this work, a natural, eco-friendly, low cost precursor, camphor, was used to synthesize graphene on Ni foils. Camphor was first evaporated at 180°C and then pyrolyzed, in another chamber of the CVD furnace, at 700 to 850°C, using argon as the carrier gas. Upon natural cooling to room temperature, few-layer graphene sheets were observed on the Ni foils. Graphene, thus produced, was found to have multiple folds (in HRTEM images) and estimated to have approximately 35 layers of graphene sheets. This study opened up a new processing route for graphene synthesis, though several issues like controlling the number of layers, minimizing the folds *etc*, were yet to be solved.



In another approach, 1 to 2 nm thick graphene sheet was reported to be grown on Ni substrate by thermal CVD, while the same treatment failed to synthesize graphene on Si. The process used a precursor gas mixture of H_2 and CH_4 (92:8 ratio), a total gas pressure of 80 Torr and was activated by DC discharge. Nanometer thick (confirmed by Auger Electron Spectroscopy) graphitic films were found to have atomically smooth micrometer size regions, separated by ridges. While the ridge formation was proposed to be due to difference in thermal expansion coefficients of Ni and graphite, the nucleation process was attributed to heteroepitaxial growth of graphene on Ni. Then three to four layer graphene formation on polycrystalline Ni foils (of 500 μm thickness), through thermal CVD process. A precursor gas mixture of CH_4, H_2 and Ar (0.15:1:2 ratio), at a total flow rate of 315 sccm, was used for the synthesis process, allowing 20 min at 1000°C for the synthesis process. HRTEM and Raman spectroscopic analyses confirmed formation of graphene on Ni under moderate cooling rates only, while high and low cooling rates were found to be detrimental to graphene synthesis process. This difference in graphene formation was attributed to solubility of C in Ni and the kinetics of C segregation. Ni has a good solubility for carbon atoms. At slow cooling rate, C atoms get sufficient time to diffuse into bulk Ni and no segregation is found on the surface. At a moderate cooling rate, C atoms segregate and forms graphene, while at a higher rate also C atoms segregate out of Ni, but form a less crystalline, defective graphitic structure.

2. **Plasma Enhanced Chemical Vapour Technique:** Vertically oriented graphene (VG) nano sheets have attracted growing interest for a wide range of applications, from energy storage, catalysis and field emission to gas sensing, due to their unique orientation, exposed sharp edges, non-stacking morphology, and huge surface-to-

volume ratio. Plasma-enhanced chemical vapour deposition (PECVD) has emerged as a key method for VG synthesis Interest in synthesizing graphene through plasma enhanced chemical vapour deposition (PECVD) is contemporary to that of exfoliation, the process used Si wafer, Ni, W, Mo and some other metal sheets as substrates and a gas mixture of CH_4 and H_2, these precursor gases were sprayed at the pressure of 12 Pa, 900W power and maintained 680^0C. The gases gets dissociate into carbon and hydrogen lead to diffusion of the C and diffused on the substrate material and segregate themselves by forming molecular bond among themselves.



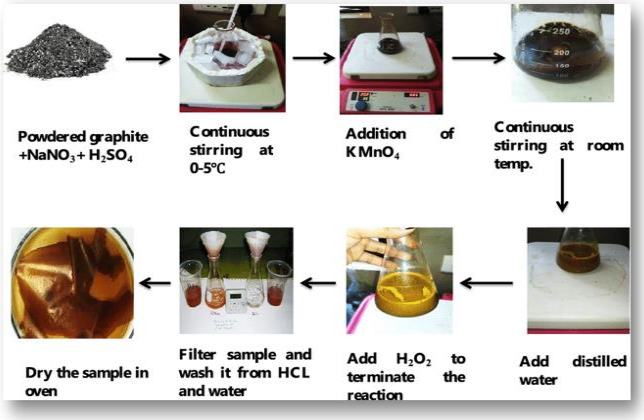
Synthesis of multilayer graphene nanoflake films (MGNF) on Si substrates, through microwave PECVD (MW-PECVD). Graphene produced in this method had highly graphitized knife-edge structure, with 2 to 3 nm thickness at the sharp edges. Graphene sheets were roughly vertical to the substrate (Si) and reported to show excellent bio-sensing capability (for dopamine). The report also claimed a very high growth rate of graphene, 1.6 µm min^{-1}, which was 10 times faster than other processes. In a very similar way, Yuan et al. have synthesized high quality graphene sheets, 1 to 3 layers thick, on stainless steel substrate at 500°C, by microwave PECVD. The process used a gas mixture of CH_4 and H_2 (1:9 ratio, at a total pressure of 30 Torr and 200 sccm flow rate) and microwave power of 1200W. Graphene, produced in this method, was found to show better crystallinity, than any other method.

Other different methods are also used for the synthesis of the graphene and its derivatives like graphene oxide, reduced graphene oxide, graphene nanotubes and fullerene *etc.*

Graphene oxide

Graphene oxide (GO) is a product of graphene obtained by oxidizing graphene. It has a single monomolecular layer containing oxygen functionalities such as carboxyl, carbonyl, epoxide, or hydroxyl groups. The stable dispersion of GO in polar solvents combined with its high specific surface area and high density of oxygen functional groups makes it an ideal platform for a wide variety of chemical functionalization. Therefore, GO is widely considered as the starting material to synthesise novel GN-based materials. The separate layers of graphene in graphite are held together by van der Waals forces. Graphene oxide is dispersible in water and other organic solvents like ethanol, 1-propanol,

acetone, methanol, ethylene glycol, pyridine, etc. as well as in different matrixes. This property of GO was due to the presence of the oxygen functionalities.



GO are synthesized mostly based on widely reported Hummers method in which graphite is oxidized by a solution of potassium permanganate in hydrogen tetraoxosulfate I,e., powdered graphite was mixed with sodium nitrate, nitric acid and sulphuric acid and continuously stirred at 0-50C in ice bath and added with potassium permanganate at room temperature and continuously stirred which was added with distilled water for the dilution, then added with H2O2, to terminate the reaction by removing KMn04 in which colour changed from dark green to yellow colour and filter the solution by washing with HCl and distilled water, where colour turns to brown and dry the filterate.

Reduced graphene oxide

There are many methods to reduce graphene oxide (GO) into reduced graphene oxide (rGO), but most fall into three main categories: chemical reduction, thermal reduction and electrochemical reduction. The other methods include hydrazine vapor treatment, annealing, laser and microwave reduction. The reduction process is vital to producing rGO, as it determines how consistent the rGO structure is with the GO precursor. Many commercial producers of Graphene Nanoplatelets are in fact providing a product similar to industrial scale rGO as their GNP product. However this method differs from the rGO most people refer to which is a higher quality research product used for nano enabled devices.

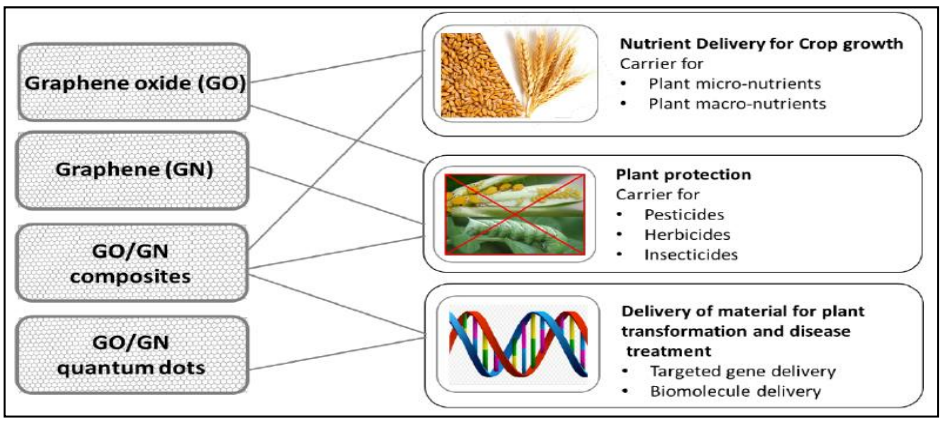
Thermal reduction produces rGO with a high surface area that is close to the surface area of pristine graphene. However, the intense heating process causes a high-pressure build-up of carbon dioxide which causes structural damage to the graphene layers. Electrochemical reduction shows the best results in terms of production and quality. The rGO produced is consistent with that of pristine graphene. During the electrochemical

process, the substrates (generally ITO or glass) are coated with a layer of GO and a current is passed through the material (via electrodes at either end of the substrate). rGO produced by this method have shown to have a high carbon to oxygen ratio and have exhibited conductivity comparable to that of silver. The process also benefits from no toxic waste.

Application of Graphene and its derivatives in Agriculture. May *et al*.(2021)

- **GN-based materials with potential application for delivery of genetic material for plant transformation:** GN-based materials have been used as an appropriate candidate for gene delivery due to their high loading capacity, tailorable surface chemistry and increased gene transfection. GN-based materials have been extensively used as gene transformers, and to decrease their toxicity towards different cells and to obtain a positively charged surface for proper electrostatic interaction with anionic oligonucleotides different surface modifications were employed using polymers such as chitosan, polyethyleneimine (PEI) and polyamidoamine (PAMAM). PAMAM functionalized GN with oleic acid had good biocompatibility and improved gene transfection efficiency, up to 13 times than ultrasonicated GN PEI-functionalized GO has been used for siRNA (short interfering RNA) and gene delivery showing significantly low toxicity with high transfection efficiency.
- **GN-based materials as pesticide carriers:** High specificity of GN-based materials to sorb pesticide species with their insensitivity to pH change, in addition to their antibacterial properties, makes them very attractive for pesticide removal compared to other carbon-based materials
- **Nutrient delivery for plant balanced and sustained crop nutrition:** GN-based composites have shown a higher adsorption capacity towards metallic ions because the immobilization of N and P on the GN sheets prevents sheet agglomeration, thus reducing any losses in surface area and adsorption capacity and also acts as a good carrier of micronutrients in the soil and also in plant system.
- **GN-based materials as coatings to slow down the release of nutrients:** Graphene-based materials have been used as a barrier coating to protect different substrates against corrosion due to their impermeable 2D structure, which provides a barrier to reactive gases, liquids, acids and salts. GN-based materials as reinforcement to enhance fertilizer physical properties.
- **GN-based sensors for applications in agriculture:** GN-based materials have been extensively used as reinforcement materials to enhance the mechanical properties of different composites, including cement and polymers due to its high Young's modulus of 1 TPa and yield strength of 130 GPa. In addition, 2D GN sheets are flexible with a very high theoretical specific surface area (2630 m2/g) that enhances

the interfacial interaction between them and the added composites, which makes them a favourable candidate to be used as a replacement for traditional reinforcements.



Application of Graphene and its derivatives in other fields: Wonbong *et al.*(2010)

In other fields graphene and its derivatives were used as major component of:

- **Sensors:** The ideal sensor is able to detect minute changes in its surrounding environment. Due to the planar and consistent arrangement of atoms in a graphene sheet, every atom within the sheet is exposed to the surrounding environment.
- **Batteries:** Graphene can be incorporated into both the anode and the cathode in various battery systems to increase the efficiency of the battery and improve the charge/discharge cycle rate. The excellent electrical conductivity, surface area and dispersibility of graphene enhances the beneficial properties present in many traditional inorganic-based electrodes.
- **Electron Emission Displays:** Graphene is an ideal material for use in electron emission displays as it exhibits a high aspect ratio and the dangling bonds at either end of the sheet allow for efficient electron tunneling. The linear disperisty that the graphene surface provides produces massless Dirac Fermions. When exposed to an electric field, the field emission liberated electrons avoid all back-scattering because their escape velocity is independent to their energy.

Conclusion

The incorporation of graphene into soil positively affects nutrient availability, soil aggregate structure, and microbial activity. Graphene-based fertilizer shows lower micronutrient diffusion compared to others. Bacterial growth increases with graphene at 10 and 100 mg/kg after 4 days. It notably enhances drainage porosity and the van Genuchten parameter. Graphene and its derivatives significantly improve soil physicochemical and biological properties, including aggregate structure, water retention,

bulk density, porosity, nutrient availability, and microbial activity. As a super material lighter than hair yet stronger than steel, graphene emerges as a key player in 21st-century advancements.

References

Alessandrino, L., PavlakiS, C., ColombanI, N., Mastrocicco, M. and Aschonitis, V., 2023, Effects of graphene on soil water-retention curve, van genuchten parameters, and soil pore size distribution - a comparison with traditional soil conditioners. *Water*, 15(7): 1297.

May, A., Coelho, L. F., Silva, E. H. F. M., Viana, R., da S., Vieira Junior, N. A., And Ferreira, W. P. M., 2021, Graphene: A new technology for agriculture. *Res. Soc. Dev.*, pp. 2525-3409.

Wonbong Choi, Indranil Lahiri, Raghunandan Seelaboyina and Yong Soo Kang., 2010, Synthesis of Graphene and Its Applications: A Review. *Crit. Rev. Solid State Mater. Sci.*, **35**(1): 52-71.

Zhang, M., Gao, B., Chen, J., LI, Y., Creamer, A. E. and Chen, H., 2014, Slow-release fertilizer encapsulated by graphene oxide films. *J. Chem. Eng.* pp. 1-24.

APPLICATION OF WIRELESS COMMUNICATION DEVICES IN AGRICULTURE FARM

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Introduction

Agriculture is the backbone of the Indian economy and its share in GDP of the country is approximately 18.3 per cent (Ministry of Statistics & Programme Implementation (MoSPI), 2022-23). Most of Indian population lives in rural areas and the people directly and indirectly depend on agriculture for their livelihood. But the scenario is now changed because the youth of India is not interested to make their career in agriculture resulted in migration of people from rural areas to urban areas to search their job in other sectors for their livelihood. The reasons behind this migration of youth are low profitability from agriculture, more costly inputs, and conventional methods of cultivation, lack of skill, low mechanization levels, social prestige, and full of drudgery. Farm mechanization has been only reduced energy requirement in agriculture; however, monitoring is done through human intervention. Automation in agriculture operation is one of them which will solve the most of the problem in agriculture operation as mentioned above and also attract the youth of India to make their career in agriculture because automation enhances the farmer's profitability by saving costly input by precisely placing the inputs, reducing the labour requirement for completing the agricultural task timely and provides safety and comfort during the work.

Wireless communication

Wireless communication is the communication between two devices through the air without any wire or any conductors by using electromagnetic waves like Infrared (IR), Radio Frequency (RF), Satellite, etc. Nowadays, wireless communication is becoming a part of life and so many devices are being used in our life such as Smartphones, Tabs, Computers, Bluetooth, printers, etc.

Types of Wireless Communication

Different types of wireless communication devices are available in the market and users select them according to their requirements and their suitability for remote communication between two devices. The following are the wireless communication devices:

a) Mobile
b) Codeless Telephone
c) Zigbee Wireless Technology
d) Global Positioning System (GPS)
e) Wireless Fidelity (Wi-Fi)
f) Satellite Television
g) Bluetooth
h) Long Range (LoRa)

The remote area where wired communication is not possible and not easy, in that situation only wireless communication is possible choice. Now a day's wireless communication are adopted because it is independent of wire, flexibility in nature, global coverage, and easily connected with other devices.

Zigbee Wireless Technology

The Zigbee is wireless communication technology designed for low-power, short-range applications like home automation, industrial control systems, and sensor networks (Fig.1). It operates on the 2.4 GHz and 900 MHz frequency bands and is popular for its low energy consumption, reliability, and ability to support a large number of devices in a mesh network.

Fig. 1. Zigbee Module

Global Positioning System (GPS)

The GPS is not a wireless technology in itself but a satellite-based navigation system. It uses a network of satellites orbiting the Earth to provide precise location and time information to GPS-enabled devices. These devices, such as smartphones and GPS receivers, receive signals from multiple GPS satellites to triangulate their exact position on the Earth's surface.

The GPS operates wirelessly, as it communicates with satellites via radio waves (Fig.2). However, GPS is primarily a one-way communication system from satellite to the receiving devices, and it does not transmit data wirelessly to other devices or networks. It is used for accurately positioning geographic coordinates, adding in navigation, tracking assets, and various location–based applications.

Fig.2. NEO-6M GPS Module

Wireless Fidelity (Wi-Fi)

The Wi-Fi is widely used for wireless communication technology that enables devices to connect to the internet and communicate with each other over a local area network (LAN). It is a key technology for wireless networking and internet access in homes, business, public places, and various other institutions.

Bluetooth

The Bluetooth device is commonly used in various agricultural operations to enable wireless communication and data exchange between different devices and equipment. Bluetooth device is utilized in following agriculture operations:

a) Data Collection and Farm Monitoring
b) Precision Agriculture
c) Livestock Monitoring
d) Equipment Control
e) Inventory Management
f) Work safety
g) Livestock health monitoring

The Bluetooth technology has become an essential part of smart agriculture and a special type of Bluetooth device is shown in Fig. 3. It improves the farmer's efficiency, minimizes the wastage of resources, and improves overall productivity and sustainability of their agricultural operations as well as increases the farmer's profitability.

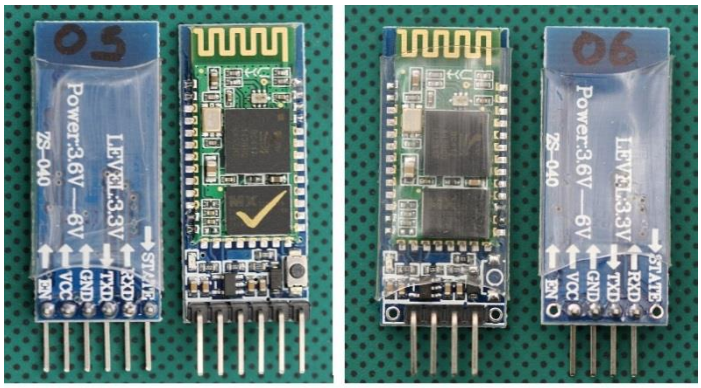

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Fig. 3. Arduino Bluetooth Device

Long Range (LoRa)

The LoRa is designed for wireless communication technology with low-power consumption, and long-range communication in the Internet of Things (IoT) as well as other applications. It is known for its ability to provide long-range coverage with low power consumption, making it suitable for battery-operated devices and applications. Some important points regarding LoRa wireless communication are as follows:

1. Modulation Technique
2. Long Range
3. Lower Power Consumption
4. Low Data Rate
5. License Free Band
6. Scalability
7. Open Standard
8. Applications
9. LoRa WAN and
10. Security

The LoRa has gained popularity in the IoT space due to its unique characteristics of long-range communication, low power consumption, and scalability (Fig.4). It offers a cost-effective solution for a wide range of IoT applications, especially for remote monitoring and control over extended distances.

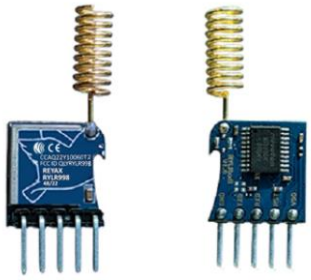

Fig.4. LoRa RYLR998 Antenna Transceiver Module

Role of Automation System

Automation is defined as the series of operations that are carried out without the intervention of human being. In Indian Farms, most of the operation was done manually due to which agriculture operation was full of drudgery and not accurately placed agricultural inputs like seeds, fertilizer, pesticides, etc. at the desired place. Because of this production and productivity of crops were found to be very low causes farmer's profitability decreases resulted in left the agricultural as an occupation and migrated to the other sectors. The farm labor availability during peak time is very low because of labor migration, farmers adopted new automation technology to overcome the lack of labor at peak time of agricultural operation. Automation was introduced in agriculture and took the places of labor and all operation which was done by manual labour, are now done by machine and computers.

Tractor is the main prime movers for agricultural implement and equipment. They are being used 200 years ago in agricultural operations like tillage, leveling, sowing, and haulage operation etc. The tractor was operated manually; however, today's tractor is being controlled remotely through sensors, microcontroller, and actuator, which actuate the various systems (steering, braking, and transmission and lighting systems) in the field.

The application of pesticides and insecticides on crop canopy is automated i.e. sensor first detects/ identify the crop canopy leaf and sends the signal in the form of electric i.e. voltage to the microcontroller after that microcontroller manipulates this signal and sends this signal to drivers and then drivers to actuating devices like a motor which operates the pump resulted in water droplets coming outs from the nozzles and go to the targeted area and deposited there.

The farmers easily remotely control the agricultural machines (Tractor, combine, pumping sets, etc.) which operate on the Farm and controlled from the office or home through cloud communication by simply using a tablet/laptop or mobile. This technology is helpful to those farmers who have no time for going to farm and also attracts the youth of India.

Benefits of Automation in Agriculture

- Reduce cost of operation
- Improve accuracy in agriculture input placement
- Improve operational productivity
- Enhance labour comfort and safety
- Decrease the agriculture accidentals risks
- Increase production and productivity crop and
- Increase the farmer's profitability

Monitoring the Agricultural Farm

Farmers need to monitor the farm activities like agricultural machine operations, irrigation system, weather conditions of farms, growth stages of crops, attack of insect and pest on crops, the water requirement of crops, and weeds competition with crops. Monitoring modules provide reminders to the farmers through alarms or message before coming of any adverse conation in the farm. Above all these activities are easy for farmers to control remotely through sensors, microcontrollers, and actuators through the internet of things from their own home. There is a need to monitor their behavior and performance to improve reliability, efficiency, and sustainability (Izza and Robandi, 2016),

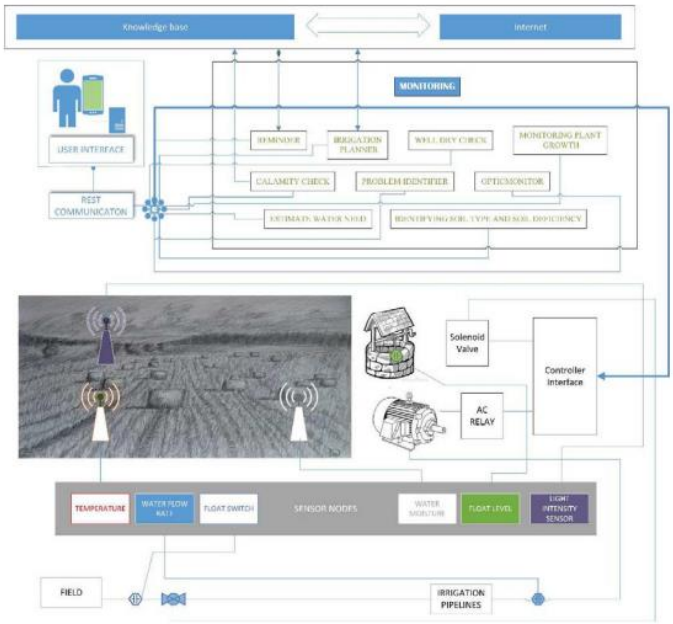

Fig.5. Agricultural farm monitoring

References

Yuniahastuti, I. T; Izza, A; and Robandi, I. 2016. Load Frequency Control (LFC) of Micro-hydro Power Plant with Capacitive Energy Storage (CES) using Bat Algorithm (BA). *International Seminar on Application for Technology of Information and Communication: 147-151*.

Anonymous 2022. Farming automation system and benefited. https://greenly irrigations systems.com/agriculture-farming-automation-system- retrieved on 6/8/2023.

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