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BIO-ARCHITECTS: Designing with Nature's Smallest Tools explores the intersection of architecture and biology, focusing on how nature's smallest organisms and systems can inspire sustainable and innovative design. The book delves into the concept of biomimicry, drawing inspiration from the way microorganisms, plants, and insects interact with their environments to create efficient, adaptive, and resilient structures. It highlights how architects can incorporate these natural principles into their designs to improve sustainability, reduce environmental impact, and create spaces that coexist harmoniously with nature.

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BIO-ARCHITECTS

Designing with Nature's Smallest Tools

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Bio-Architects: Designing with Nature's Smallest Tools

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Chapter - 1
Dairy Product Fortification with Bioactive Nano-formulation

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Chapter - 1

Dairy Product Fortification with Bioactive Nano-formulation

Khadija Nadim, Subhasis Sarkar, Suranjana Sarkar and Bidisha Ghosh

Abstract

Dairy products offer a novel method to addressing the inadequacies by enabling the incorporation of vitamins and minerals into milk and its derivatives. Using a process called fortification, milk and dairy products are made into functional foods that give their consumers the calcium and vitamin D they lack in their diets. Fortification is essential because it enables the deliberate addition of essential nutrients, such as vitamins and minerals, to food products. Dairy products, such as milk, yogurt, and cheese, can be fortified with vitamins, such as A, D, and B12, as well as minerals, such as calcium and iron, to address potential deficiencies in these nutrients. Milk and dairy products provided 9.1% of the total energy supply. Adding vitamin D could be more practical to fortify staple foods like rice, Maida, chapatti, flour, and rice. The addition of fortified dairy products possess nutrient qualities such as calcium and probiotics that could prevent diseases like osteoporosis due to aging. The process of maintaining a coated product or food by covering a bioactive compound (BAC) in liquid, solid, or gaseous forms inside a matrix is known as Nano encapsulation. BACs that are used in meals to promote health, such as vitamins, probiotics, prebiotics, antioxidants, and antimicrobials. This review will emphasize on nano-formulation and how it has been harnessed in the fortification of milk and dairy products.

Keyword: Bioactive compounds (BAC), dairy products, fortification micronutrient, nutrient, and nano-encapsulation

1. Introduction

Over recent years, there has been a growing interest in vitamin D and its supply from the diet, including the possibility of raising its intake through the use of fortified foods (Wierzbicka, E. (2022). Even while other minerals (potassium, zinc, etc.) and vitamins (A, C, and K) also play a role, vitamin D and calcium are crucial dietary components that influence bone mass. In

actuality, calcium homeostasis and absorption are significantly influenced by vitamin D and certain minerals (Goldzman *et al* 2004) The actual daily recommended intake of vitamin D from diet is directly connected to age; it ranges from 5 μg for infants, kids, teens, and adults (including nursing and pregnant women) to 15 μg for those over 65years (Polzonetti et.al 2020).Water-soluble vitamins (B complex and vitamin C) and fat-soluble vitamins (A, D, E, and K) are the two groups of vitamins based on liquid solubility. Since humans are not able to synthesis most vitamins, they must be obtained from diet or dietary supplements. Vitamins are bioactive nutrients that have a number of health-promoting qualities and a significant impact on human development and well-being. Vitamin D deficiency is a public health issue that affects both men and women and has serious financial and personal repercussions. Consumers can preserve and enhance their vitamin D status by increasing their intake of natural or fortified food sources or dietary supplements containing vitamin D.

Milk is the main fortified food in the U.S. and in Canada (Itkonen, S. T. (2018) but the amount of vitamin D added to milk (1 mg/100 g fluid milk) is not adequate to produce the desired increase or maintain circulating 25(OH) D. Vitamin D fortification at higher levels (10 mg/50 g powdered milk) showed significant effects in improving vitamin D status and bone mineralization in older women milk (Polzonetti et.al 2020). The inclusion of different foods (like dairy products) fortified with BACs in people's daily diets, which leads to the creation of foods with novel biological properties and increased consumer acceptance, is currently one of the most important challenges facing food fortification (Dahiya, D. (2023). Due to the possible negative effects of bioactive compounds on the quality of the final product (e.g., sensory properties such as taste, appearance, odor), the need for an effective concentration of these compounds to have positive effects on health is a major challenge (Rashidinejad *et al*., 2016b, Rubio *et al*., 2014). This means that the direct fortifying of different dairy products with bioactives is a complex approach (Rivero-Pino, F. (2023). Dairy products are recognised as the best dietary composition to fulfil children's growth and development requirements and enhance their nutritional status since they are a good or exceptional source of protein, calcium, phosphorus, magnesium, zinc, iodine, potassium, vitamin A, B vitamins, and vitamin D.

They also play a significant role in daily dietary recommendations in the global dietary guidelines (Ding, Y et.al 2022). When exposed to heat, light, moisture, or oxygen during manufacturing or storage, vitamin D is susceptible to deterioration. Foods that undergo thermal processing, such as boiling,

pressure cooking, frying, steaming, baking, and sterilising, can have a substantial impact on their final vitamin D content (Kilic-Akyilmaz *et al* (2022). These factors ultimately affect the actual availability of Vit D to the human body and must be considered while addressing the bioavailability of Vit D present in any food matrix (Maurya, V. K. *et al* (2020). In food enrichment technology, nano encapsulation has enormous promise, particularly for fat-soluble elements. It may be beneficial for lowering vitamin D oxidation, promoting site-specific delivery, and boosting cells' systematic uptake of the nutrient (Nikooyeh *et al* 2022).

1.1 Vitamin D: A Roller Coaster Ride to Its Sources Redefined, the Current Scenario of the Issues and Challenges Faced due to its Deficiency

Within the class of fat-soluble steroidal chemicals, vitamin D comes in two primary forms: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). D3 can be produced by the skin or is received through the diet (natural, fortified foods, supplements, etc.). The best dietary sources of vitamin D3 are oily fish and fish liver oil, however these foods are rarely found in normal diets. Oily fish like eel, salmon, herring, and mackerel have the greatest levels of cholecalciferol (Schmid, A., & Walther, B. (2013). Additional sources of vitamin D that help meet the required daily intake include egg yolks, animal products, and fortified foods, such as some dairy products, particularly yoghurts and fluid milk, as well as some breakfast cereals and fat spreads (Cashman, K. D., & Kiely, M. (2024).Meat and dairy products, which make up a large portion of the average western diet, contribute to total vitamin D intake, but less so than fish. Natural foods are thought to only provide roughly 20% of the daily required amount of vitamin D. Furthermore, cholecalciferol or ergocalciferol added to products through technical procedures is known as fortification, and it can contribute significantly to the availability of vitamin D. The market is seeing a growing number of fortified food products, such as morning cereals that are ready to eat, spreadable fats and margarines, and some dairy products that can help increase dietary consumption of vitamin D. Most of the population’s average daily vitamin D intake is below the recommended dietary standard and does not ensure optimal levels of vitamin D. Given a lack of naturally occurring vitamin D sources in the diet, food fortification and dietary supplements are crucial strategies for ensuring that the general public consumes enough vitamin D. To guarantee that the present vitamin D targets are met, public health policies addressing supplementation, bio fortification, and fortification are required (Wierzbicka, *et al* 2022).

2. Osteoporosis: An Inevitable Curse due to Vitamin D Deficiency

Strategies to lower the risks of osteoporosis and the associated medical expenses are of great interest. An increased risk of fractures is the main effect of osteoporosis, a degenerative disease caused by a decline in bone mineral density (BMD). Due to poor bone mineral density (BMD), millions of people ver 50 in the US are at an increased risk of getting osteoporosis (Li, J. (2024). Patients with osteoporosis are usually treated with bisphosphonates, calcium, and vitamin D (Brouns *et al* 2000). Although calcium and vitamin D intake are crucial, especially for elderly people, diet appears to have a minimal impact on osteoporosis. A higher risk of osteoporosis has been linked to diets low in dairy products (Malmir, H., *et al* (2020).

Studies have indicated that even a slight deficiency in vitamin D may have an adverse impact on children's and adolescents' bone mineral mass. In addition to calcium supplementation, the impact of vitamin D supplementation (200 IU or 400 IU per day) on bone mineral deposition was investigated in 212 teenage girls (mean age 11.4 years).

Considering that most people don't get enough vitamin D from their diet, initiatives to increase vitamin D intake by expanding the variety of foods that can be fortified and the amount of vitamin D added to meals should be considered. Public education, dietary supplementation, and food fortification must all be taken into consideration as viable approaches to raise the population's vitamin D status.

Additionally, adults who want to ensure a sufficient vitamin D status as well as certain risk groups may require additional consideration in terms of addressing low vitamin D status by dietary supplementation, with the aim to reach the target levels of 30 to 50 ng/mL (75 to 125 nmol/L) (Wierzbicka, *et al* 2022).

3. Vitamin D Fortification Strategies

Strategies to enhance the population's vitamin D status include weight loss, increased exposure to UVB radiation from the sun, the production of fortified foods, increased intake of naturally vitamin D-containing foods, and the use of vitamin D supplements. Low-fat milk, spreads, breakfast cereals, and several infantry foods are the most common foods fortified with vitamin D. Conventional vitamin D food fortification involves adding vitamin D to food or using the technique of "bio-addition" to enrich it with vitamin D. The phrase "bio-addition of vitamin D," sometimes referred to as "bio-fortification," refers to a variety of methods for increasing the nutrient's content in food without directly supplying it from outside sources.

3.1 Vitamin D Fortification of Dairy products

Since milk is a staple item that is well-accepted by the public, it has been utilised extensively as a vehicle for vitamin D fortification. With benefits over direct addition and emulsification techniques, encapsulation appears to be a vital tool for designing vitamin D materials with the intended functionality to provide vitamin D through beverages (Wierzbicka, E (2022). Milk is a complete food that provides various nutrients, notably carbohydrates (primarily lactose), proteins, fat, minerals, and vitamins to the ordinary human diet. Together with calcium which have a synergistic effect on human health, are naturally occurring in milk. The parathyroid gland secretes parathyroid hormone in response to a drop in blood levels of ionized calcium. The amount of the inactive form of vitamin D decreases as a result of this hormone's stimulation of the conversion of vitamin D to its active form, calcitriol (1, 25-dihydroxyvitamin D) (Hansdottir, S., W. (2008).Calcium absorption in the intestine is influenced by vitamin D, as calcitriol, and deficiency in vitamin D is linked to reduced absorption of dietary calcium (Polzonetti *et al* 2020). Dairy products and milk are thought to be an appropriate matrix for vitamin D enrichment. Whole milk has naturally occurring vitamin D concentrations between 0.34 to 0.84 IU/g of fat unfortified milk is not a substantial source of this vitamin (Zahedirad, M. *et al*. (2019). One of the most common sources of vitamin D is fortified milk and dairy products. Other alternatives, such bio-fortifying animal-based foods like eggs and fried fish, might also be taken into consideration as strategies to boost the population's consumption of vitamin D. On the other hand, meat ought to be carefully examined as an extra strategy to raise the population's consumption of vitamin D.

3.2 Efficiency of Nano-formulation

Nanotechnology is a rapidly developing field with enormous potential across many industries, including the food, medical, and mechanical fields (Sahoo *et al* 2021). The features, structures, and sizes of nanomaterials are used to classify them. These high surface volume ratio nano materials (Phung, X. *et al* (2003). can exhibit some excellent physiochemical properties in terms of solubility, diffusivity, bioavailability, colour, optics, strength, in toxicity, magnetism, and thermodynamics among other areas (Avella *et al*., 2005). Nowadays, the food industry needs such technology to continue leading the way in the food processing and marketing sectors while producing authentic, tasty, and easily transportable food items. One such technology that has a wide range of applications in food processing is nanotechnology. Nanoparticles are frequently added to food as food additives (Sridhar, A *et al* 2021) to prolong its shelf life by preventing contamination. Food (nano-sized) ingredients,

nutritional supplements (such proteins and antioxidants), and additives (like flavour and colour) can all be nanoencapsulated in food processing to create nanocapsules that can be used in functional foods (Ezhilarasi, P. N., *et al* (2013).

Nanotechnology create customized nutritional diets for various target groups, aging populations, and lifestyles. This technique guarantees the development of tools for nutrition nanotherapy (Sherwani, F. A. (2021). which targets the delivery of nutrients. It can create smart systems that use nanoencapsulation to release nutrients under controlled conditions. It is feasible to create nanoscale enzymatic reactors for the creation of novel products by food fortification (Sastry *et al*., 2013). Widespread interest is being shown in electrospun nanofibers as encapsulating or structuring polymeric films for food packaging (Chung *et al*., 2017).

Conclusion

Even though the body can produce enough vitamin D on its own to meet daily needs, there is a widespread deficit of the nutrient all across the world. Increasing vitamin D consumption ought to be regarded as a top concern for public health. Increase the diversity of your diet, the amount of natural sources (such fatty fish) that you eat, the amount of foods that are supplemented with vitamin D (pay special attention to milk and dairy products), and the amount of foods that are naturally high in vitamin D. Fortification of dairy products with added vitamin D, particularly milk and other dairy products (especially fermented ones), may be a suitable approach to enhance population-level vitamin D status and intake(Wierzbicka *et al* 2022)

Nanotechnology has the potential to significantly advance food science in a wide range of fields, encompassing multiple disciplines and encompassing many facets of food processing. Nanotechnology has a promising role in the field of food technology, with applications ranging from extending product shelf life to improving food quality storage, tracing and monitoring toxins and pollutants, and introducing nutritional or health supplements into the body through food (Maurya, V *et al* 2020).

Conflict of Interest

The authors have no conflict of interest.

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Chapter - 2
Effect of Biofilm in Diabetic Foot Ulcer

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Chapter - 2

Effect of Biofilm in Diabetic Foot Ulcer

Satarupa Das, Addityaa Sinha Roy, Akash Saha and Debjit De

Abstract

The most common fatal consequence in individuals with diabetes mellitus is foot infections. These infections increase mortality and diminish quality of life by causing lower limb amputation. A crucial stage in the pathophysiology of diabetic foot ulcers (DFU) is biofilm formation, which contributes to the lesion's chronicity, the advancement of the disease, the emergence of antibiotic resistance, and the difficulty of treating wound healing. One possible factor contributing to the chronicity of wounds, is the diversity of bacterial populations present in the wounds. Furthermore, the proliferation of the infecting organisms in biofilm presents additional barrier to the healing of chronic wounds. Biofilms, often known as "slime," are microbial communities that adhere to surfaces or the surfaces of other species and envelop themselves in hydrated extracellular polymeric material (EPS). Although EPS's physical and chemical characteristics might change, polysaccharides make up the majority of its composition. Other macromolecules like proteins, DNA, lipids, and even humic compounds are linked to EPS. Diseases associated with biofilms are typically chronic illnesses that progress slowly. They seem to be extremely resistant to antimicrobial therapy and are rarely eliminated by the host immune system.

Keywords: Diabetes mellitus, limb amputation, diabetic foot ulcers, lesion, chronicity, antibiotic resistance, Biofilm, extracellular polymeric material

Introduction

A diabetic foot ulcer (DFU) can occur in 15-25% of people with *diabetes mellitus* in their lifetime. The most frequent, serious, and expensive side effect of *diabetes mellitus* is infection, which carries a significant risk of lower limb amputation-related death and morbidity. The three most frequent causes of diabetes-related amputations are wound infection, inadequate wound healing, and ischemia. In fact, biofilm-infected foot ulcerations precede 80% of lower limb amputations in people with diabetes. Within 18 months, there is a higher

chance of death due to infected wounds. A key factor in the formation of DFU is the host-microorganism interaction. Traditionally, bacteria in DFU are grouped into functionally equivalent pathogroups (FEP), where commensal and pathogenic bacteria co-aggregate in a pathogenic biofilm in a symbiotic manner to sustain a persistent infection [1–8]. The risk of vascular LLA in people with diabetes is expected to be eight times higher in the general population (those over 45) than in people without the disease. In people over 85 years of age, the incidence in men and women is projected to be 15 times and 12 times higher, respectively, than the average incidence rates across every demographic group. Sadly, the COVID-19 pandemic has negatively impacted the way DFU patients receive healthcare. In fact, compared to 2019 statistics, a study conducted in Naples found that individuals with diabetes who were admitted to a Comprehensive Care Center for DFU care had a three times greater chance of amputation. It has been estimated that DFUs cause one leg amputation every 30 seconds globally [9-13].

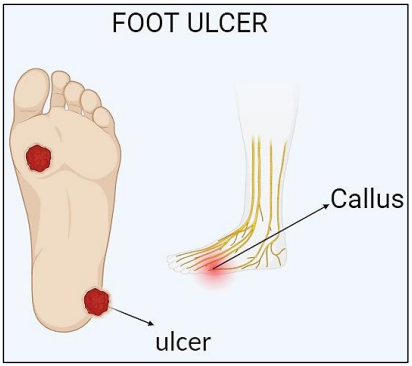

Fig 1: Diabetic Foot Ulcer

Both aerobic and anaerobic types of pathogens, such as *Streptococcus sp.*, Staphylococcus sp., *Pseudomonas aeruginosa*, *Proteobacteria sp.*, and coliform bacteria, can cause these infections. The many microorganisms in DFUs might exist in a sessile or planktonic state. Bacteria that create biofilms are shielded from antibiotics and the host's immune system by the self-produced polymeric matrix that envelops the cells. Thus, even in the face of systemic antibiotic treatment, biofilms in DFUs might have been in charge of the infection's delayed healing and subsequent chronicity [14– 22].

Diabetic Foot Ulcers (DFU)

Diabetes can cause ulcers on the foot, which can be quite painful and often result in amputation of the limb. The management of foot ulcers necessitates early evaluation by experts due to their complex pathogenesis and variable

clinical presentation. The goals of treatments should be to treat peripheral neuropathy, infection, peripheral ischemia, and abnormal pressure loading brought on by restricted joint mobility. Ulcers often progress to chronic wounds even with treatment. Diabetic foot ulcers have been overlooked in healthcare research and planning, and therapeutic practice is based more on perception than scientific truth. Moreover, the various specialties involved in the diseased processes have fragmented and insensitive communication, and the pathological processes themselves are inadequately taught and comprehended. When precise statistics on foot ulcers are lacking, amputation rates are frequently employed as a rough indicator. However, misinformation about amputations can sometimes exist, and definitions might be ambiguous. Amputation serves as a marker for both the state of the illness and its treatment. Many factors influence the choice to operate, and these considerations differ throughout centres and patients. A high rate of amputations may be the consequence of late appearance, high disease prevalence, and insufficient resources, but it may also be an indication of a specific strategy used by local surgeons. Major amputation is frequently the best option to guarantee an early return to a relatively independent life rather than a degrading admission of defeat. On the other hand, a low rate of amputation may indicate improved care, but it may also mask the consequences of an overly conservative approach, which include prolonged incapacity, pain, and death from unhealed ulcers. Diabetes is characterized by a combination of metabolic problems that contribute to peripheral nerve injury. Peripheral experiencing, stimulation of the foot's tiny muscles, and delicate cardiovascular regulation of the pedal circulation are all impacted by the impairment. Lack of protective feeling in sensory neuropathy causes a person to be unaware of potential or existing ulceration. The muscles needed for appropriate foot movement are impacted by motor neuropathy, which changes the stresses propagated while walking and results in reactive swelling of the skin (callus) at abnormally loaded areas. Then, ischemic necrosis of the tissues under the callus causes the skin and subcutaneous tissue to break down, giving rise to a neuropathic ulcer that looks punched out [51].

Formation of Biofilm in DFUs

With 40–60 million affected worldwide, diabetic foot ulcer is a serious medical, social, and financial issue. The primary risk factors for diabetic foot ulcers include advanced age, male gender, Type 2 diabetes, a lower BMI, a longer duration of diabetes, hypertension, diabetic retinopathy, and a history of smoking [23–24]. Planktonic and sessile are the two basic states in which microorganisms can dwell in the environment. Bacteria navigate their

surroundings freely while they are in the planktonic state. Microorganisms in the sessile condition adhere to solid surfaces such as prosthetics or urinary catheters, or more commonly, to one another, forming multicellular aggregates that may initiate the creation of biofilms. It takes several steps to develop a biofilm. It involves the self-production of an extracellular polymeric substance (EPS) matrix that encases diverse colonies of microorganisms (fungi and/or bacteria). EPS gives the ability to stick to biotic or abiotic surfaces and is composed of proteins, glycoproteins, and polysaccharides [25-26].

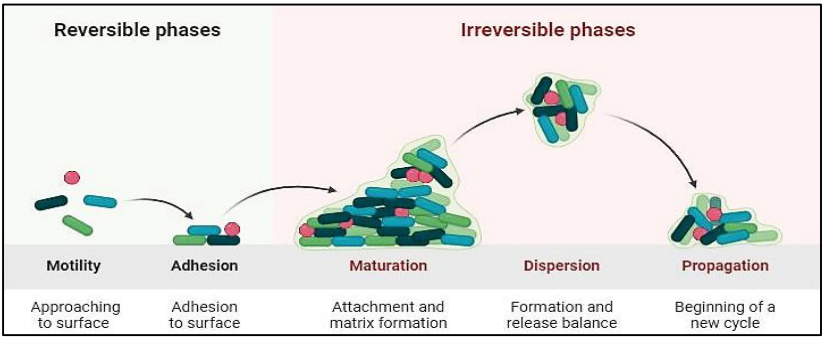

Fig 2: Formation of Biofilm

The most dependable samples for detecting biofilms in interior tissues are biopsy tissues in clinical settings. However, due to contamination from the skin microbiota, the challenge of separating the biofilm from the host epithelium, and the development of anaerobes in the deep tissues, using swabs to gather biofilm samples from the wound surface is seen as an inappropriate technique. A tissue sample from the base of the debrided incision should be investigated when an intermediate or serious soft tissue infection has been observed and a wound is present. The most frequent method for quantifying biofilms in tissue samples is microscopy. The best methods for identifying biofilms in biopsies are fluorescence in situ hybridization (FISH), scanning electron microscopy, and confocal laser scanning microscopy [27].

Role of Biofilm in Pathogenicity

Bacterial pathogenicity and biofilm development are regulated by quorum sensing (QS), an internal communication mechanism that can be developed by biofilm-containing cells. The development of biofilms is influenced by the density of bacteria. It is possible for microbial cells in a biofilm to separate and spread throughout the wound environment. Because of their adaptation within the biofilm, the discharged bacteria's behaviour may differ from that of

the original colonizing bacteria. [25,28-30]. The epithelial barrier's ability to function, the host immune system's control, and defense against invasive pathogenic microbes all depend on the skin microbiota. Following an injury, pathogenic species and skin microbiota may multiply and colonize the wound. Indeed, research has shown that species isolated from long-term wounds coexist with healthy skin. Park *et al*., for instance, examined the microbial community of diabetic foot wound (DFW) tissue in the same patients and compared it to normal foot skin. Comparing normal samples to infected tissue, the authors discovered a greater abundance of *Actinobacteria*, *Staphylococcus, Corynebacterium*, and *Propionibacterium sp*. In contrast, wound tissues showed an approximate proportion of anaerobes (*Bacteroides* and *Enterococcus*) and *Pseudomonas sp.* [31–36]. Anaerobes in wounds have the potential to worsen infection and hinder wound healing. These findings are consistent with previous research, indicating that DFUs have a higher concentration of anaerobic bacteria than other wound types and are linked to polymicrobial colonization. DFU chronicity is caused by polymicrobial communities that can be arranged into biofilms in addition to the production of several virulence factors. The complex communities known as biofilms are made up of microbial cells embedded in an extracellular polymeric substance (EPS) matrix that the bacteria themselves generate. Proteins, lipids, nucleic acids, polysaccharides, and other substances make up this matrix, which gives bacteria the capacity to stick to biotic or abiotic surfaces [37-42]. It is believed that biofilms play a role in about 65% of human bacterial illnesses. In fact, they have been linked to a number of different diseases, including endocarditis, cystic fibrosis, nephrolithiasis, mouth infections, and infections brought on by indwelling medical devices. Furthermore, biofilms are present in both acute and chronic wound infections, providing some physiological and ecological benefits to bacteria. In actuality, bacteria are physically shielded by biofilms from antimicrobial agents (antibiotics and disinfectants) by the complex structure of the biofilm, which lowers the concentration of antimicrobials that are active against the embedded cells. Furthermore, the tight cell-to-cell contact found in biofilms facilitates the horizontal gene transfer of antibiotic-resistant genes [43–50].

Microbial Marker for DFU Treatment

An average of 1.52 microorganisms per case were discovered in the current study, which involved the isolation of 157 organisms from 103 patients. This is marginally more than the results of cultures that produced an average of 1.21. Numerous investigations conducted both domestically and overseas have shown that diabetic foot infections are polymicrobial in origin.

The majority of the organisms identified were gram-negative. The majority of the isolated bacteria in the current study were gram-negative *P. aeruginosa* (22%), gram-positive *S. aureus* (19%), and other bacteria such as *K. pneumoniae, E. coli*, and *Proteus sp.* (17%, 18%, and 11%, respectively). [*K. pneumoniae* (18%), *Proteus sp.* (9.3%), *E. coli* (15%), *P. aeruginosa* (19%), and so on], yet in their investigation, *S. aureus* (31%) showed the highest positivity. The most frequent strains were *Proteus sp.* (20.73%), *Klebsiella sp.* (12.35%), and *Pseudomonas sp.* (11.73%) [52–58].

Type of Compound	Effect on Biofilm	Reference
Micasin [isolated from *Microsporum canis]*	Exhibit broad-spectrum antibacterial action by altering protein folding against both *P. aeruginosa* and methicillin-resistant *S. aureus.*	62
Cathelicidins [isolated from the intestinal tissues of *Myxine glutinosa*]	Prevents *Staphylococcus epidermidis* from adhering to artificial surfaces and from forming biofilms.	60
Plectasin [isolated from *Pseudoplectania nigrella*]	The first-ever characterized fungal defensin demonstrated inhibitory efficacy against Gram-positive bacteria, including *S. aureus, C. diphtheria*e, and *S. pyogenes.*	61
Alamethicin [isolated from *T. viridea*]	active against both Gram-positive bacteria such as *E. faecalis, S. hemolyticus*, and *S. aureus* and Gram-negative bacteria such as *E. coli, K. pneumoniae*, and *P. aeruginosa*	59

Conclusion

A big part of why diabetic foot ulcers (DFUs) stay open for a long time is biofilm growth. Pathogens are protected from the host's immune system and antimicrobial treatments by biofilms, which are made up of complex groups of microbes encased in an extracellular matrix. This defence causes infections to last longer, wounds to heal more slowly, and the risk of complications like amputation to rise. Biofilms make the environment more inflammatory, stop granulation tissue from forming, and mess up the body's normal way of healing wounds. It's important to get an early diagnosis and use targeted treatments like debridement, improved antimicrobial strategies, and new medicines like quorum-sensing inhibitors and anti-biofilm agents now that we know what role biofilms play. Future study should focus on making diagnostic tools better at finding biofilms and coming up with specific ways to manage biofilms in DFUs more effectively, which would lower morbidity and improve patient outcomes.

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Chapter - 3
Grape and Its Extracts-Potential Anti-Cancer Agents

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Chapter - 3

Grape and Its Extracts-Potential Anti-Cancer Agents

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Abstract

There is evidence that eating foods high in antioxidants can prevent cancer. The grape (Vitis vinifera), one of the world's largest fruit harvests and most consumed fruit worldwide, is high in antioxidants. Due to its antibacterial, antioxidant, and anti-inflammatory properties, grapes are the classic examples of fruits that are used for both exclusive medicinal and nutritional purposes. Here, the main phenolic antioxidants found in grape skin and seed extracts are described together with their composition and cancer-prevention properties. Antioxidants found in grapes show promise in combating a variety of cancer cells by targeting epidermal growth factor receptor (EGFR) and its auxiliary pathways, preventing COX-2 and prostaglandin overexpression. Grape seed extract (GSE) is mostly composed of polyphenols and phenolic acids, their ingestion is safe and is known to provide numerous and significant health advantages. More than 70% of the phytochemical content of grapes is made up of resveratrol, quercetin, kaempferol, epicatechin, catechin, and anthocyanins (cyanidin and malvidin). The significance of resveratrol in lowering many cancers in humans, such as those of the breast, cervix, uterus, blood, kidney, liver, eye, brain, lung, skin, stomach, colon, head and neck, bone, ovarian, thyroid, prostate, and cervical has been examined. The ongoing research into the synergistic or additive effects of grape-derived compounds underscores their potential in cancer prevention and treatment, also aims to optimize the therapeutic benefits of these compounds and better understand their interactions and mechanisms.

Keywords: Grape, antioxidant activity, phenolic compounds, anticancer effects, grape seed extract, resveratrol, chemoprevention, phytochemicals, cancer treatment

Introduction

Extensive research has been conducted on preventive strategies, particularly those pertaining to diet and lifestyle, in response to the rising

global incidence of cancer. Many studies have demonstrated the substantial influence of dietary decisions on cancer risk, emphasizing the importance of foods high in antioxidants. Antioxidants are substances that aid in the neutralization of free radicals, which are unstable chemicals that have the potential to cause oxidative damage to cells and the emergence of cancer (Rauf A *et al*., 2016). Among various antioxidant-rich foods, grapes (Vitis vinifera) have garnered considerable attention due to their unique phytochemical profile and widespread consumption as fresh fruit (table grape) and processed fruit (wine, grape juice, molasses, and raisins) (Fengmei Zhu, Bin Du & Jun Li *et al*., 2015).

The main components of grape pomace are the seeds, skins, and stems. This fruit is known for its potential health advantages in addition to its cultural and gastronomic value. Studies show that grapes are rich in vitamins, phytochemicals, and important minerals, including polyphenols, which are well-known for having strong antioxidant characteristics (Rengarajan T *et al*., 2016, Singh CK *et al*., 2016). A multitude of health-promoting benefits, including a decreased risk of cancer and cardiovascular disorders, have been linked to the consumption of grapes, wine, and grape juice in several epidemiological studies (Rauf A *et al*., 2016).

The beneficial health effects of the well-known polyphenolic compounds present in grapes, such as resveratrol, quercetin, and anthocyanins, have been the subject of much research. Among them, Resveratrol, a naturally occurring polyphenolic compound has become very popular due to its anticancer potential, first reported in 1997 (Jang *et al*., 1997). Researchers have since focused on this compound due to its potential in facilitating inflammation, tumorigenesis, and cardioprotective effects. Quercetin and anthocyanins have demonstrated promising results in laboratory studies, indicating potential anti-cancer effects through various mechanisms, including the regulation of cellular signalling pathways and reduction of oxidative stress (Khanna, S., & Roy, S. 2012).

Numerous mechanisms underlie the protective properties of antioxidants obtained from grapes against cancer. It is well recognized that these substances target significant biochemical pathways connected to apoptosis, inflammation, and cell proliferation (Zhou K *et al*., 2012). For instance, the inhibition of the epidermal growth factor receptor (EGFR) pathway has been identified as a significant mechanism through which resveratrol and other phenolic compounds can exert anticancer effects. Grape seeds consist of monomeric phenolic chemicals, including (+)-catechins, (-)-epicatechins, and (-)-epicatechin-3-O-gallate, which have antimutagenic and antiviral properties

(Jang JY wt al., 2022). They also contain high amounts of phytopharmaceuticals that have been linked to reduced risk of chronic illnesses. Additionally, the modulation of cyclooxygenase-2 (COX-2) and the reduction of inflammatory mediators further illustrate how grape antioxidants may contribute to cancer prevention (Mancini M *et al*., 2013).

In light of these factors, this paper aims to explore the rich phytochemical composition of grapes, their modes of action as cancer agents, and the implications of ongoing research for public health in light of these aspects. It is possible to enhance the therapeutic potential of antioxidants in cancer prevention and treatment techniques by comprehending the interactions between these components and other dietary elements.

Antioxidant Properties of Grapes

Grapes are celebrated not only for their delightful taste and versatility but also for their remarkable health benefits, focusing on their rich composition of phytochemicals, especially polyphenols. Polyphenols, known for their strong antioxidant properties, help protect cells from oxidative stress, which is linked to diseases like cancer (Mancini M *et al*., 2013), cardiovascular disorders, and neurodegenerative conditions. Therefore, consuming antioxidant-rich foods such as grapes is vital for promoting health and preventing diseases.

Key Phenolic Compounds

The primary phenolic compounds in grapes contribute significantly to their antioxidant capacity and potential health benefits. These compounds include.

Resveratrol: Resveratrol activates multiple systems to produce its chemo protective properties. Apoptosis induction of malignant cells is one of them. It has been demonstrated that resveratrol causes apoptosis and slows the growth of tumours, hence preventing the proliferation of different cancer cell types (Singh CK *et al*., 2016). It achieves this by modulating critical signaling pathways, such as the PI3K/Akt and MAPK pathways, which are essential for cell survival and proliferation. It also has cardio protective benefits by improving endothelial function and lowering oxidative stress in blood vessels.

Quercetin: Quercetin has significant antioxidant activity, effectively decreasing free radicals and oxidative damage. Research has shown that quercetin can inhibit the proliferation of various cancer cells, including those of the breast, colon, and prostate, by inducing cell cycle arrest and apoptosis (Rauf A *et al*., 2016).

Epicatechin and Catechin: These flavan-3-ols compounds exhibit strong antioxidant activity, contributing to the overall health benefits of grapes. They have potential anti-cancer actions via modulating signaling pathways involved in cancer formation and progression (Lucas, I. K., and Kolodziej, H. *et al*., 2015). They have been associated with improved cardiovascular health by enhancing endothelial function and reducing blood pressure.

Anthocyanins (Cyanidin and Malvidin): Anthocyanins not only contribute to the aesthetic appeal of grapes but also offer significant antioxidant properties that help neutralize oxidative stress, further supporting their role in cancer prevention. Anthocyanins have been shown to decrease cancer cell proliferation and induce apoptosis, especially in breast and colon malignancies (Lee, J. H., & Canning, S. A. *et al*., 2013).

Mechanisms of Action

The anticancer properties of antioxidants derived from grapes can be elucidated through a variety of biological mechanisms that target critical pathways involved in cancer development and progression. Understanding such mechanisms offers insight into how grape chemicals may help prevent and treat cancer.

Targeting the Epidermal Growth Factor Receptor

The epidermal growth factor receptor, commonly known as EGFR, plays a pivotal role in regulating various cellular processes, including growth, proliferation and differentiation. Theexcessive production of this receptor can cause uncontrolled cell division and contribute to cancer progression, including breast, lung, and colorectal cancers (Xia EQ *et al*., 2009). Research has shown that certain compounds found in grapes, particularly resveratrol, can effectively inhibit the signaling pathways associated with EGFR. It inhibits the activity of this receptor, reducing cancer cell proliferation and preventing their spread. This technique helps eliminate potentially malignant cells from the body, limiting tumour growth and improving prognosis (Sun, H., & Zhang, X. *et al*., 2015). Furthermore, the inhibition of EGFR signaling can trigger a cascade of downstream effects that promote apoptosis, which is a form of programmed cell death.

Anti-inflammatory Effects

The anti-inflammatory properties of grape-derived antioxidants increase their significance in cancer prevention. Chronic inflammation is known to be linked to tumorigenesis, can promote cancer development by creating a

favorable microenvironment (Aluyen JK *et al*., 2012). By reducing inflammation, grape antioxidants contribute to lower the risk of cancer progression. These chemicals suppress inflammatory cytokines and mediators that contribute to chronic inflammation via many routes. By fostering a less inflammatory environment, grape-derived antioxidants help protect cells from the damaging effects of prolonged inflammation (Khanna, S., & Roy, S. *et al*., 2012), thereby reducing the likelihood of cancerous transformations.

Modulation of Cyclooxygenase-2 and Prostaglandins

Cyclooxygenase-2, often abbreviated as COX-2, is an enzyme that is frequently overexpressed in many tumours. Overexpression causes increased amounts of prostaglandins, which contribute to inflammation and cancer growth (Kumar A *et al*., 2018). Chronic inflammation is widely recognized as a substantial risk factor for the development of different malignancies. In a recent study, researchers used male albino Wistar rats to explore the effect of resveratrol on inflammation, utilizing COX-2 as a biomarker. The results from these studies revealed that resveratrol has an inhibiting effect on inflammation when COX-2 is used as a biomarker [Banerjee, Last Bueso-Ramos, & Aggarwal, 2002]. Antioxidants generated from grapes have the ability to suppress the expression of COX-2, which effectively lowers prostaglandin production. This reduction in inflammatory mediators plays a crucial role in mitigating the inflammatory response associated with tumour growth. By decreasing inflammation, grape antioxidants help create a less favorable environment for cancer cells, ultimately aiding in cancer prevention (Mancini M *et al*., 2013).

Induction of Apoptosis

One of the essential processes that grape compounds can influence is apoptosis, the programmed cell death mechanism that eliminates damaged or potentially harmful cells. Apoptosis in cancer cells can be induced by certain signaling pathways that are activated by compounds present in grapes. This process comprises the regulation of several proteins that are essential for controlling cell death and survival. For instance, resveratrol has been shown to upregulate pro-apoptotic factors while downregulating an apoptotic protein, effectively tipping the balance toward cell death in cancerous cells (Mancini M *et al*., 2013, Singh CK *et al*., 2016). Grape compounds have a crucial role in cancer prevention and treatment efforts by promoting apoptosis, which aids in the elimination of cells that may lead to tumour formation and growth and formation.

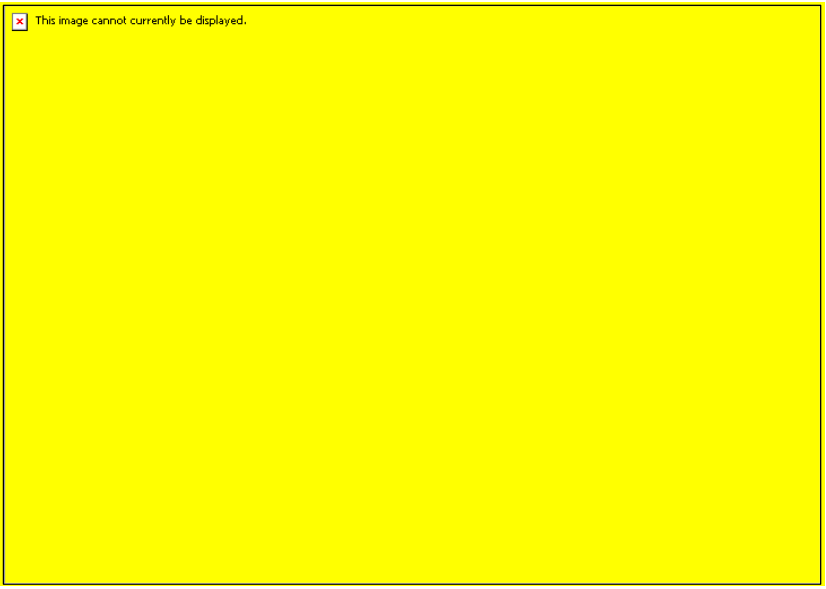

Fig 1: Apoptotic signalling pathway (Zhang, X. L., Yu, H., Xiong, Y. Y., Ma, S. T., Zhao, L., and She, S. F. 2013).

Antiproliferation Mechanisms

Several cancer cell lines have been used to demonstrate the antiproliferative properties ofresveratrol. One study examined the influence of nuclear factor-κB (NF-κB) in the presence of resveratrol in multiple myeloma cell lines. In order to examine the concentration-dependent effects of resveratrol, three cell lines were treated with doses ranging from 12.5 to 200 µmol/l for a full day (Kaur M, *et al*., 2009). It was shown that while a minimum concentration of 100 µmol/l inhibited the active form of NF-κB, a minimum concentration of 50 µmol/l was required to inhibit growth. It has been demonstrated that resveratrol inhibits COX, activating MIC-1 and reducing proliferation (Singh CK *et al*., 2016).

Health Benefits of Grape Seed Extract

Grape seed extract, commonly referred to as GSE, is derived from the seeds of grapes and is renowned for its rich concentration of bioactive compounds. These elements provide GSE its powerful antioxidant and anti-cancer properties, which is why nutritional science and preventive medicine are quite interested in it.

Composition of Grape Seed Extract

The composition of grape seed extract is primarily characterized by its high levels of polyphenols, phenolic and hydroxy-benzoic acids. Stylbenes

(trans-resveratrol) as well, are occasionally found. Two of the key components of GSE include:

Proanthocyanidins: They are a class of flavonoids that are particularly abundant in grape seeds. Strong antioxidant properties are possessed by proanthocyanidins.as a result of which they can scavenge free radicals and mitigate oxidative stress. Studies have shown that these substances can prevent tumour cells from growing in a variety of cancer forms (Fengmei Zhu, Bin Du & Jun Li 2015, Rauf A, *et al*., 2016). They promote apoptosis, the programmed cell death process, which is essential for eliminating potentially harmful cells. Their capacity to alter pathways linked to cancer is aided by their anti-inflammatory properties. Additionally, they may enhance the efficacy of conventional cancer treatments by sensitizing cancer cells to chemotherapy agents (Dinicola S1, Cucina A2).

Phenolic Acids: The presence of different phenolic acids in grape seed extract increases its antioxidant potential. These compounds play a crucial role in protecting cells from oxidative damage and reducing inflammation. Numerous health advantages, including as enhanced metabolic health and cardiovascular protection, have been associated with them (Rengarajan T, *et al*., 2016). In the context of cancer, Phenolic acids may contribute to the overall anti-cancer benefits of GSE by preventing cell proliferation and inducing apoptosis in cancer cells. Their capacity to alter different signaling pathways reinforces their protective function against illnesses linked to oxidative stress.

The Pro-Oxidant Effect of Grape Seed Extract on Cancer Cells

Numerous studies have been conducted on the antioxidant properties of GSE and grape phenolic compounds, primarily procyanidins and resveratrol. GSE has a significant chelating action on transition metal ions, which lowers lipid peroxidation, strong free radical scavenging activity (Rengarajan T, *et al*., 2016), and inhibits ROS-induced DNA damage (Singh CK *et al*., 2016). Through its interaction with the antioxidant response element (ARE) or the electrophile-responsive element (EpRE), GSE-induced antioxidant enzyme production is linked to the activation of the redox-sensitive transcription factor nuclear factor erythroid-2 p45 (NFE2)-related factor (Nrf2) (Aluyen JK, *et al*., 2012, Rauf A *et al*., 2018). In a double-blinded randomized crossover human experiment, dietary supplementation of GSE was demonstrated to improve the glutathione/oxidized glutathione ratio, lower oxidative stress, and increase total antioxidant levels *in vivo* (Fengmei Zhu, Bin Du & Jun Li *et al*., 2015). One of the main factors contributing to chronic degenerative diseases

(diabetes mellitus, cardiovascular disease, cancer) is oxidative stress, which is brought on by increased ROS generation that surpasses the body's antioxidant defences (Zhou K, *et al*., 2012, Dinicola S1). ROS participate in triggering the apoptotic process, as programmed cell death is tightly regulated by the oxidative environment (Zhou K, *et al*., 2012). It is possible to speculate that grape polyphenols are involved in regulating intracellular peroxide production given that GSE has a protective antioxidant effect in normal cells that show deficiencies in glutathione levels or catalase activity (Rauf A, *et al*., 2018). Therefore, as multiple investigations have shown, the anti-oxidant qualities of GSE medication may effectively prevent the development of ROS-dependent illness (Rengarajan T *et al*., 2016).

An Overview of Safety and Toxicity

The safety and toxicity profile of grape seed extract is well-documented, making it a viable option for dietary supplementation aimed at cancer prevention. Given its well-established safety and toxicity profile, grape seed extract is a promising option for dietary supplements aimed to prevent cancer (Zhang, X. L. *et al*., 2013). Clinical trials and observational studies have demonstrated that consistent GSE use can significantly enhance a number of biomarkers linked to cancer risk. For instance, GSE supplementation has been linked to reduction in inflammatory markers, such as cytokines, which play a pivotal role in cancer progression. Furthermore, GSE has been demonstrated to improve general cellular health by reducing markers of oxidative stress, such as malondialdehyde and oxidized low-density lipoprotein (Lucas, I. K., and Kolodziej, H *et al*., 2015).

Resveratrol and Cancer Type

Resveratrol is a polyphenolic chemical that is mostly found in grape skins. Because of its multifaceted properties, which may prevent tumour growth and progression in a variety of cancer types, it has attracted a lot of attention in the field of cancer research. It is a viable candidate for cancer prevention and therapy due to its capacity to affect important cellular processes. The effects of resveratrol on particular cancer types are described in detail below.

Breast Cancer

Studies reveal that resveratrol, particularly in estrogen-dependent breast cancer cells, shows notable anti-cancer properties. The compound has been shown to modulate the activity of estrogen receptors, which play a crucial role in the proliferation of breast cancer cells. Depending on the cellular environment, resveratrol's binding to these receptors can either imitate or inhibit the effects of estrogen (Mancini M *et al*., 2013). In estrogen-sensitive

breast cancer cell lines, this dual action may promote apoptosis and inhibit the development of tumour cells.

In a recent study, Abdel-Latif and colleagues [Abdel-Latif *et al*., 2015] examined how resveratrol affected the herceptin's cytotoxicity profile in the HER-2 receptor-positive and HER-2 receptor-negative breast cancer cell lines, respectively. When compared to single therapies, these researchers discovered that a combination of herceptin and resveratrol led to a notable decrease in the expression of the HER-2 receptor (Rauf A *et al*., 2018). Along with looking at the mechanisms underlying resveratrol's mediated protection against estrogen-induced breast cancer, Singh and colleagues also investigated the impact of resveratrol on inhibition. Along with looking at the mechanisms underlying resveratrol's mediated protection against estrogen-induced breast cancer, Singh and colleagues also investigated the impact of resveratrol on inhibition [Dinicola S1]. Furthermore, the expression of certain genes linked to cell proliferation and survival, such as those involving the phosphoinositide pathway, can be downregulated by resveratrol.

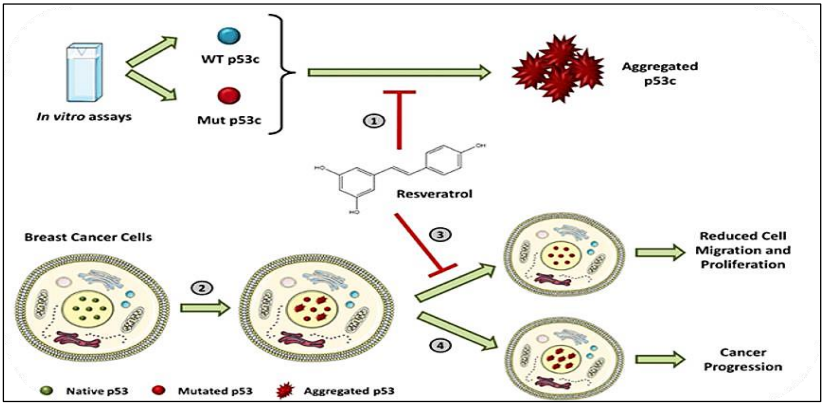

Fig 2: Resveratrol mechanism to inhibit the cancer cells (Dinicola S1, Cucina A2, Antonacci D3 and Bizzarri M4).

Lung and Colon Cancer

There have been protective benefits of resveratrol against oxidative damage in lung and colon problems, which is especially important considering how oxidative stress contributes to the development of cancer (Rauf A *et al*., 20180. Yang *et al*.'s recent study revealed that giving lung cancer A549 cells 25 μmol/L of resveratrol up-regulated 21 long-noncoding RNAs (lncRNAs) and down-regulated 19 lncRNAs. Further investigation by these scientists revealed that the most obviously changed lncRNA, AK001796 was down-regulated in lung cancer cells treated with resveratrol, but overexpressed in

lung cancer tissues and cell lines (Yang *et al*., 2015). Another discovery that in A549 human lung adenocarcinoma epithelial cells, treatment with escalating concentrations (0-175 μM) of resveratrol for 24 hours causes a decrease in cell viability and death by mitochondrial pathway alignment (Lucas and Kolodziej, 2015). Resveratrol effectively reduces the invasiveness of cancerous cells by upregulating antioxidant enzymes and downregulating inflammatory cytokines, thus mitigating oxidative stress and inflammation in lung tissues.

Resveratrol has been demonstrated to affect colon cancer by preventing the development of colon cancer cells and triggering apoptosis. According to research, resveratrol can change the expression of genes related to cell cycle regulation and apoptosis, which reduces cell proliferation. These researchers came to the conclusion that resveratrol upregulates the expression of miR-96 and, by downregulating the expression of Kras, can inhibit the development and progression of colorectal cancers [Saud *et al*., 2014]. Its ability to stop precancerous lesions from forming in the colon highlights its protective effect against colon cancer even more.

Cervical Cancer

According to a study by García-Zepeda and colleagues, resveratrol causes autophagy and apoptosis, which lead to the death of cervical cancer cells. It reduced p53 expression and increased autophagy, or lysosomal permeability. All cell lines treated with resveratrol showed decreased expression of p65, an NF-κB subunit, with the exception of SiHa. These findings suggest that resveratrol induces cell death in cell lines originated from cervical cancer via distinct pathways (García-Zepeda *et al*., 2013). Zhang *et al*. investigated how resveratrol affected the signaling pathways regulated by Notch, Wnt, and STAT3 in various cervical cancer cell lines. They found that adding 100 μM resveratrol to HeLa and SiHa cells caused significant apoptosis and inhibited the three signaling pathways (STAT3, Notch, and Wnt) (Zhang *et al*., 2014). These scientists found that resveratrol suppresses Stat3 phosphorylation, which in turn prevents the growth of new cell (Li *et al*., 2015). Resveratrol causes an increase in Cathepsin L's cytosolic expression and activity, as well as a decrease of lysosomal membrane permeability (LMP). Resveratrol causes apoptosis in HeLa cells by raising the activities of caspase-9 and caspase-3 and decreasing the potential of the mitochondria (Dhandayuthapani *et al*., 2013).

Other Cancers

The potential of resveratrol as a broad-spectrum anticancer agent extends beyond the aforementioned cancer types. Studies have suggested that

resveratrol may have beneficial effects on cancers of the pancreas, skin, stomach, liver, bone, blood, brain, ovary (Rauf A *et al*., 2018). Resveratrol's attractiveness as a dietary supplement for cancer prevention is increased by its capacity to influence several pathways implicated in cancer biology. Research on its effectiveness when combined with conventional medicines is still ongoing, with the goal of maximizing its potential as an adjuvant treatment for different types of cancer (Dhandayuthapani *et al*., 2013).

Conclusion

The exploration of grapes and grape-derived compounds, particularly in relation to their anticancer properties, presents a compelling narrative in the field of nutrition and public health. The numerous health benefits of grapes are being better understood, and it is becoming evident that these fruits can significantly improve general health and prevent cancer. Beyond their delightful taste, grapes are a great source of essential nutrients and bioactive components like vitamins, flavonoids, and polyphenols. These constituents contribute to their strong antioxidant and anti-inflammatory properties, which are crucial in mitigating oxidative stress-an established risk factor for cancer development. Numerous studies have demonstrated the potential of grapes, particularly those containing resveratrol, to promote cancer cell death, inhibit tumour development, and modify critical signaling pathways linked to the advancement of cancer. Due to their accessibility and affordability, grapes are a great option for nutritional approaches, especially in areas where cancer rates are on the rise. Incorporating grapes with other positive habits like regular exercise and stress reduction with a balanced diet can result in a holistic strategy for cancer prevention.

Ongoing Research and Future Directions

Scientific research is being conducted on chemicals derived from grapes, namely their potential as cancer treatments. This section highlights ongoing research initiatives and outlines future directions that may significantly impact cancer prevention and treatment strategies.

Bioavailability Studies: Understanding a grape-derived compound's bioavailability-that is, how well it is absorbed, digested, and utilized by the body-is essential to comprehending its usefulness. Current research is increasingly focusing on the pharmacokinetics of grape polyphenols, including resveratrol and proanthocyanidins. For example, investigations could look into techniques like nano-encapsulation, which might increase resveratrol's solubility and absorption.

Clinical Investigations: To assess the effectiveness of grape extracts and each of its separate components in the prevention and treatment of cancer, rigorous clinical trials are necessary. Subsequent studies ought to concentrate on particular forms of cancer and take into account factors like dosage, duration of supplementation, and timing of administration in relation to conventional therapies. Additionally, they can shed light on potential adverse effects, interactions with medications, and alternative patient populations for care. Additionally, they can shed light on potential adverse effects, interactions with medications, and alternative patient populations for care.

Mechanism of Interaction: Gaining insight into the particular signaling pathways and biological targets that grape antioxidants interact with can lead to the discovery of new therapeutic targets and help develop more effective cancer treatment approaches. Research may examine the impact of resveratrol on the NF-kB pathway, for example, as this system is crucial for both the development of cancer and inflammatory responses.

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Chapter - 4
Unravelling Severe Combined Immunodeficiency (SCID): from Genetic Roots to Lifesaving Treatments

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Chapter - 4

Unravelling Severe Combined Immunodeficiency (SCID): from Genetic Roots to Lifesaving Treatments

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Abstract

Severe Combined Immunodeficiency (SCID) encompasses a spectrum of rare genetic disorders that result in significant impairments in both T-and B-lymphocyte functions, leading to severe immunodeficiency. Babies having SCID are extremely susceptible to potentially fatal diseases due to their underdeveloped immune systems. As per common patho-physiological mechanisms, certain SCID subtypes exhibit both typical as well as unusual (non-immune characteristics, whether present or absent) attributes. These forms can now be divided down to 6 categories-Abnormal cytokine-mediated signalling, pre-T cell receptor problems, elevated lymphocyte cell death, thymus development defects, calcium flux problems, and other processes. This review article here delves into genetic underpinnings, clinical symptoms, diagnostic methodologies, and therapeutic strategies for SCID. Recent advancements in newborn screening, hematopoietic stem cell transplantation (HSCT), and gene therapy have markedly enhanced the prognosis for those affected. HSCT has shown high success rates, especially when performed early. Gene therapy is emerging as a promising alternative, offering potential cures for specific genetic defects. Early detection and immediate intervention are vital for improving patient outcomes. This review paper consolidates recent research findings to provide a comprehensive understanding of SCID and explores future directions in its management to improve the quality of life for individuals with SCID.

Keywords: Severe combined immunodeficiency; t and b-lymphocytes; hematopoietic stem cell transplantation (hsct); gene therapy

Introduction

Severe Combined Immunodeficiency (SCID), also known as Swiss-type agammaglobulinemic, represents a critical area of study within immunology

and genetics due to its severe impact on affected individuals and the potential for life-saving interventions. In rare cases, the immune system may not mature at birth, leading to primary immunodeficiency illness. The most extensively researched PID is severe combined immunodeficiency illness (SCID). It is recognized as a paediatric emergency in children [Justiz-Vaillant, *et al.*, (2023)]. SCID encompasses a heterogeneous group of genetic disorders defined by abnormal generation of functional T and B lymphocytes caused by a variety of genetic abnormalities, resulting in varying clinical manifestations, leading to a severely compromised immune system. SCID is characterized by a faulty antibody response as a result of either direct involvement with B lymphocytes or inappropriate B lymphocyte activation caused by non-functional T helper cells. T, B, and NK (natural killer) lymphocytes share cell lineages, signalling routes, along with metabolic processes. Hence, In SCID, the B lymphocytes as well as Natural Killer cells are also typically significantly impaired. Different kinds of SCID are identified by varying T/B/NK numbers: $T^-B^-NK^-$, $T^-B^+NK^-$, $T^-B^-NK^+$, T^-B^+ NK^+ ('-' means absence or severely reduced counts) [Cossu F. (2010)]. SCID is the most severe type of primary immunodeficiency, and there are at least nine identified genes in which mutations cause SCID. It is also known as bubble boy sickness and bubble baby disease because its victims are particularly susceptible to infectious diseases, and some, such as David Vetter, have gained notoriety for living in a sterile environment. SCID patients are typically impacted by severe bacterial, viral, or fungal infections early in life, and they frequently present with interstitial lung disease, chronic diarrhoea, and failure to thrive. Ear infections, recurrent Pneumocystis jirovecii (formerly carinii) pneumonia, and widespread oral candidiasis are prevalent. This disorder impacts across the acquired immune system and the natural (innate) immune system and can lead to lethal issues, occurring as part of the initial 2 years of birth. Treatment options for SCID have evolved significantly for the last few decades. Hematopoietic stem cell transplantation (HSCT) remains the cornerstone of therapy, offering the potential for a cure by reconstituting the immune system. Gene therapy has emerged as a promising alternative, particularly for patients with specific genetic mutations. Supportive care, including immunoglobulin replacement and antimicrobial prophylaxis, is essential in managing infections until definitive treatment can be administered. In almost 80% of cases, congenital immunodeficiencies are sporadic and there is no known family history of this illness [Cossu F. (2010)]. Studies from newborn screening programs throughout United States over the previous five years points to the true incidence likely to be closer to 1 in 58 000 live births, rather than the initial estimate of 1 in 100 000. [Rivers, L., & Gaspar, H. B. (2015)] However,

this may vary depending on the population studied. The inaugural successful transplantation to cure SCID was executed in 1968. [Gaspar, *et al*., (2013)].

When to suspect SCID?

Identifying SCID early through newborn screening is essential for timely treatment. SCID must be suspected during

Recurrent Infections: Infants with SCID frequently have severe, persistent infections that do not respond to routine therapies. These illnesses may include pneumonia, bronchitis, ear infections, and chronic diarrhoea.

Failure to Thrive: Babies with SCID may not develop or acquire weight as predicted.

Persistent Thrush: Ongoing yeast infections in the mouth or throat (thrush) that do not respond to treatment.

Family History: A family history of immunodeficiency or infant mortality caused by infections can be a strong signal.

Unusual Infections: Infections produced by organisms that do not normally cause disease in healthy people, like Pneumocystis jiroveci pneumonia.

Low Lymphocyte Count: Routine blood tests that reveal a low lymphocyte count can raise the suspicion pertaining to the syndrome.

Genetic Underpinnings along with Immunological Attributes linked to multiple types of SCID Disorder

X-linked SCID (SCID-X1), caused by mutations in the IL2RG gene or other T^-B^+ variants, accounts for 40-50% of all SCID cases. T^-B^-types caused by VDJ recombination abnormalities account for approximately 30%, while adenosine deaminase shortfall accounts for approximately 10-15%. However, these results are derived from a single-centre study in the United States and the European immunodeficiency transplantation database (SCETIDE), and the distribution of genetic abnormalities may be influenced by population characteristics [Buckley, *et al*., (1997)]. In countries with higher levels of parental consanguinity, autosomal recessive disorders could be more common. In a cohort of 117 sufferers got a transplantation since 2000, rate towards molecular based validated gamma chain defects was the same as that for ADA and Rag lacking patients (17%, 19%, and 15%, respectively). Within this group, 20 out of 117 sufferers (17%) were found to not have a genomic analysis at the moment of transplantation. A more thorough evaluation on those patients is underway using next-generation sequencing technology. Omenn's syndrome and later onset combined immunodeficiency

(CID) can be caused by hypomorphic or particular mutations in the genes that cause SCID, including inflammatory symptoms [OMENN G. S. (1965)]. Omenn's syndrome is characterized clinically by inability to grow, immunocompromised diseases, an acute erythrodermatous irritation, and organ enlargement, as well as the presence of self-derived inoperative oligoclonal self-reactive T cells and insufficient B-cell based immune function. Additionally, the majority of sufferers possess elevated IgE counts and hypereosinophilia. Omenn's syndrome, formerly assumed to be caused primarily by hypomorphic mutations in the RAG 1/2, which resulted in incomplete functioning of the enzyme recombinase with formation of particular self-targeting T lymphocyte duplicates [Villa A, *et al*., 1998]. Hypomorphic mutations in several genes can cause clinical and immunological Omenn's syndrome [Wada, T., *et al*., (2008)].

SCID Classification and Features

X-Linked SCID

Features

- **Genetic Cause:** Mutations in the gene that encodes the γc chain of the interleukin (IL)-2 receptor are the cause [Justiz-Vaillant, *et al*., (2023)]. This receptor is required for thymic Treg formation, with the regulating of T-reg balance and inhibition. Infection occurs frequently in the absence of such cells.
- **Inheritance:** Almost 50% of SCID scenarios are X-linked, impacting mostly males, signifying the most prevalent kind of SCID.
- **Immune Deficiency:** T and NK cells are severely deficient, while B cells are non-functional.
- **Clinical Presentation:** Symptoms include severe infections, failure to thrive, and recurrent diarrhoea. **Adenosine Deaminase (ADA) Deficiency**

Features

- **Genetic Cause:** This is the 2nd most common kind of SCID, comprising of 15% of overall scenarios. The disorder is caused by a deficiency of the enzyme adenosine deaminase (ADA), which transforms adenosine to inosine and subsequently deoxyadenosine to deoxyinosine. This causes a buildup of deoxyadenosine within the cell. Deoxyadenosine triphosphate (dATP) is a harmful derivative of deoxyadenosine, particularly damaging to lymphocyte progenitors. [Justiz-Vaillant, *et al*., (2023)].

- **Inheritance:** Autosomal recessive.
- **Immune Deficiency:** Severe immune deficit, including T, B, and NK cells.
- **Clinical Presentation:** Neurological impairments involve auditory and sight problems, problems with cognition, and mobility disabilities.

Deficiency SCID due to lack of RAG-1 and RAG-2

Features

- **Genetic Cause:** This particular form of SCID ranks in the 3rd position as the commonest and is identified by variations in Recombination Activation Genes 1 and 2. These genes are a multiple-subunit system that breaks down dsDNA between the antigen-receptor-coding section & the flanking recombination signal sequence (RSS). This triggers V (D) J recombination, resulting in the jumbling of DNA proteins that encode specific antigens on the surface. In the absence of such enzymes, T cell receptor synthesis collapses, resulting in abnormal T-lymphocytes and infections.
- **Inheritance:** Autosomal recessive.
- **Immune Deficiency:** Lack of T and B cells, but normal NK cells.
- **Clinical Presentation:** Symptoms include severe infections, failure to thrive, and lack of lymphoid tissue.

Insufficiency of IL-7R protein leading to SCID

Features

- **Genetic Cause:** Hepatocyte growth factor and interlukin-7 (IL7) create a heterodimeric complex that stimulates the development of pre-pro B cells. It has also been identified as a cofactor in the T cell receptor beta (V (D) J rearrangement due to T cells synthesis. It ranks 4^{th} position as the commonest SCID variety. Babies suffering this syndrome show fewer to zero T and B lymphocytes. Without T cells, B lymphocytes are unable to conduct somatic hypermutation or class change.
- **Inheritance:** Autosomal recessive.
- **Immune Deficiency:** Severe T cell deficit, but normal B and NK cells.
- **Clinical Presentation:** Clinical Symptoms include severe infections, failure to thrive, and lymphopenia.

Leaky SCID

Features

- **Genetic Cause:** Leaky SCID develops if any infant exhibits indications exactly like a classic SCID. Yet, the T cell count isn't sufficiently reduced to qualify as typical SCID. The term "leaky" refers to a condition when a significant number of T cells "leak" through and show up within patient's bloodstream. Unfortunately, such T cells cannot battle infections. In leaky SCID, T cells over-activate against organs and tissues, leading to autoimmune symptoms and self-attack [Justiz-Vaillant, *et al*., (2023)]. Children with leaky SCID may develop gene mutations similar to those with conventional SCID, such as deficits in RAG-1 and RAG-2 genes. Babies could be wrongly diagnosed, causing the condition and being detected later in life, even as adults. In leaky SCID, gene mutations can result in normal or elevated T cell counts, affecting the immune system.
- **Inheritance:** Inheritance may be autosomal recessive or X-linked.
- **Immune Deficiency:** Partial T cell function, varying B and NK cell deficits.
- **Clinical Presentation:** Mild infections, autoimmune symptoms, and granulomas, unlike usual SCID. Moreover, causing skin irritations, loss of hair, reddish skin, frailty, swelled lymph systems, enlarged liver, enlarged spleen, and diarrhea as well. Thyroid problems and anaemia are some other complexities.

Omenn Syndrome

Features

- **Genetic Cause:** Omenn Syndrome (OS) gets triggered via variations in genes, resulting with large number faulty T cells, without any problems within B or NK cells. A T cell deficiency can severely impact a child's immune system. This is a severe autosomal recessive hereditary T+ or T++ SCID deficit [Justiz-Vaillant, (2023)]. OS may exhibit in form of SCID or exist independently. Genetic mutations in genes such as adenosine deaminase deficiency, RAG1, RAG-2, Artemis, and DNA ligase 4 can lead to OS. OS patients have more deaths compared to typical SCID because of multiple opportunely advantageous ailments. Early microbiological and histological analysis of skin biopsies is crucial for accurate diagnosis.

- **Inheritance:** Autosomal recessive.
- **Immune Deficiency:** Oligoclonal T cells, no B cells, and normal or elevated NK cells.
- **Clinical Presentation:** Clinical signs include erythroderma, hepatosplenomegaly, lymphadenopathy, and severe infections.

Lack of CD3 Complex Component-SCID

Features

- **Genetic Cause:** The CD3 complex serves for T cell pan marker. The CD3 complex is necessary for cell signalling or transmission towards nucleus that begins with antigen interaction. It's due to presence of numerous alpha, beta, gamma, delta, epsilon, and zeta transmembrane chains which signal downstream to the nucleus, enabling cytokine synthesis and discharge. The three subtypes are CD3D, CD3E, and CD247/CD3Z. Mutations in CD3-encoding genes cause damage to T cells.
- **Inheritance:** Autosomal recessive.
- **Immune Deficiency:** Severe T cell deficit, but normal B and NK cells.
- **Clinical Presentation:** Clinical Symptoms include severe infections, failure to thrive, and lymphopenia.

JAK3 Deficiency SCID

Features

- **Genetic Cause:** The Janus kinase 3 gene, also known as the interleukin 2 receptor gene (IL2RG), increases the proliferation of T lymphocytes and natural killer cells. Patients with JAK3 deficient SCID exhibit characteristics comparable to those with lymphopenia. JAK3 is not found on the X chromosome, therefore it can affect both male and female new-borns.
- **Inheritance:** Autosomal recessive.
- **Immune Deficiency:** T and NK cells are severely deficient, while B cells are non-functional.
- **Clinical Presentation:** Clinical Symptoms include severe infections, failure to thrive, and recurrent diarrhoea.

Other Mechanisms Responsible behind SCID

Coronin-1A Defect: Homozygous missense mutation in the Coronin-1A (CORO1A) gene of a mice exhibit high T cell lymphopenia resulting in uncovering Coronin-1A shortage in a 13-month-old girl with $ART^{-}B^{+}NK^{+}$ SCID, due to deletion of the full CORO1A gene [Cossu F. (2010)]. On one allele (600 kbs deletion in 16p11.2) and a dinucleotide deletion, leading to frameshift & premature termination of the other allele. Coronin-1A is highly expressed in T cells, and its deficiency results in overabundance of F-actinin within cortical thymocytes, significantly reduced cell mobility, intrathymic retention of single positive $CD4^{+}CD8^{-}$or $CD4^{-}CD8^{+}$ mature T lymphocytes, and serious peripheral T lymphopenia.

MHC Class II (MHCII) Defect: The genes CIITA, RFXANK, RFX5, and RFXAP encode 4 factors regulating the promoters and transcription of the HLA DR, DP, and DQ cluster, which is located at 6p21.3; their mutations result in lacking Major histocompatibility complex class II (MHCII) molecules, those gets normally expressed on the cell surface by thymic epithelial cells, activated T lymphocytes, and cells (B lymphocytes, dendritic cells, monocytes/macrophages) that present antigens to $CD4^{+}$ T lymphocytes. HSCT in young children (<2 years) should be conducted with an HLA-identical sibling or the best compatible donor due to a high prevalence of acute GvHD and underlying viral infections [Picard, C., & Fischer, A. (2010)].

CHH (Cartilage Hair Hypoplasia): Mutations in the RMRP (ribonuclease mitochondrial RNA processing) gene, which encodes the 267-nucleotide-long RNA component of the mitochondrial RNA-processing endonuclease (a multiprotein RNA complex with at least ten different proteins), result in defects in ribosomal RNA processing, mitochondrial and cellular replication, and a heterogeneous phenotype [Notarangelo, *et al.*, (2008)].

Hoyeraal-Hreidarsson Syndrome (HHS): Hoyeraal-Hreidarsson syndrome (HHS) is a severe infantile version of dyskeratosis congenita that is characterized by telomerase defects, $T^{+}B^{-}NK^{-}$SCID, and cerebellar hypoplasia.

Hereditary Folate Malabsorption (HFM): Hereditary folate malabsorption (HFM) is caused by proton-coupled folate transporter (PCFT) gene abnormalities, resulting in folate malabsorption in the duodenum and upper jejunum and depletion of transplacental reserves by 3-4 months of life.

ZAP-70 Deficiency: Combined with lack of CD8 protein results in such SCID condition, where CD4+ T cells don't respond to TCR-mediated stimuli

in vivo. CD3 gamma subunit proteins are typically detected in unusual sufferers.

Nezelof's Syndrome: Representing immunodeficiency with immunoglobulins being present after the age of five.

- **Griscelli's Syndrome:** Causes fine silvery hair, enlarged liver, and lymphadenopathy in individuals.

Microorganisms Affecting SCID Patients

Viruses

Cytomegalovirus: Immunosuppression-related infectious disease., such as CMV, Epstein-Barr virus, adenovirus, enterovirus, herpes simplex virus, respiratory syncytial virus, rotavirus, and parainfluenza virus, causing serious disseminated problems in SCID patients, fatal in case of no detection or untreated [Cirillo E., *et al*., (2015)]. It is reported of Cytomegalovirus (CMV) of being pumped out from breastmilk, hence breastfeeding isn't recommended in case of SCID sufferers unless the mother shows up CMV-antibody to be negative [Kumrah R., *et al*., (2019)].

Adenovirus: Adenovirus infection can cause ophthalmic, respiratory, gastrointestinal, or hepatic disorders in immunocompetent people, and it is usually moderate and self-limiting [Lion T. 2014]. However, in SCID patients, adenovirus can cause severe and persistent viral pneumonia, bronchiolitis, hepatitis, or gastroenteritis, all of which can be fatal [Echavarría M. (2008)].

Rotavirus: Largest reason behind gastroenteritis within children, and to be vaccinated provides primary method to stay prevented [Chiu M., *et al*., (2019)]. Yet, the vaccines made up of living rotavirus strains shows to induce serious diarrhoea in babies with SCID, therefore must not be used [Patel N. C., *et al*., (2010)]. Epstein-Barr virus infects more than 95% of the human population during a certain time of life, yet they show typically zero symptoms. Adolescents with illnesses showing symptoms can develop infectious mononucleosis, which is represented by fever, sore throat, enlargement of spleen, and lymphadenopathy. Because the virus primarily targets B cells, SCID individuals with defective or missing B cells are more likely to develop EBV-associated lymphomas due to chronic viremia and lymphoproliferation [Cohen J. I. (2015)].

Parvovirus: Parvovirus B-19, not a rare infection in swiftly dividing erythroid progenitor cells, and babies are the primary victim to be get infected. Immunocompetent host infections may be of zero symptoms or symptomatic, may cause erythema infectiosum, arthropathy, anaemia, thrombocytopenia,

hepatitis, and myocarditis. In immunocompromised hosts, Parvovirus B-19 infection causes chronic red cell aplasia, acute lymphoblastic leukaemia (ALL), and virus-associated hemophagocytic syndrome (VAHS) [Heegaard, E. D., Brown, K. E., (2002)].

Varicella-Zoster Virus: Varicella-zoster virus (VZV) infection is mainly acquired through pulmonary inoculation and causes latency throughout life in the sensory ganglia of immunocompetent patients [Gershon AA, *et al*., (2015)]. Immunocompromised patients being in a higher probability of consequences including as reactivation, herpes zoster, retinal necrosis, and death. Worldwide immunization with live VZV vaccines has reduced many of the complications of VZV infection; nevertheless, vaccination in SCID individuals linked to widespread infection, particularly vaccine-associated pneumonia, and hence must not be used.

Bacteria

Recurrent sinopulmonary infections are common in primary immunodeficiencies like SCID and can lead to serious consequences such as lung abscess, empyema, and pneumatocele. Pneumonia is caused by bacteria such as Staphylococcus aureus, Pseudomonas spp., Mycobacterium bovis, and other atypical mycobacteria. Clinical imaging is a valuable diagnosing tool for serious lung infections in children with PIDs for frequently lacking thymic shadow [Wu, E.Y., *et al*., (2016)].

Gram-positive bacteria, such Staphylococcus aureus, and Gram-negative bacteria, like Klebsiella pneumoniae, Pseudomonas aeruginosa, Burkholderia, and Chryseobacterium, are frequently implicated. SCID patients who don't have immunoglobulins remains always in danger of repeated infections with encapsulated bacteria [Aguilar C, *et al*., 2014].

Skin sepsis in Omenn syndrome may result from bacterial colonization by *Staphylococcus aureus*, *Streptococcus pyogenes*, enterococcus, and Gram-negative bacteria such as Pseudomonas species [Cutts, *et al*., (2021)]. Bacterial infections can cause recurring and potentially fatal skin abscesses, folliculitis, impetigo, and furunculosis. In the absence of curative treatment, survival often lasts many months after delivery.

Fungi

Patients with SCID are more likely to develop disseminated fungal infections, the most common of which are caused by invasive *Candida albicans* and *Aspergillus*. *Acremonium* and *Pichia* are two other unusual bacteria linked to SCID syndrome. *Candida albicans* skin colonization,

oropharynx, and gut is characterized by constant oral thrush or diaper dermatitis, which progresses upto broad skin engagement [Antachopoulos C, *et al*., 2007]. The definitive treatment for SCID is hematopoietic stem cell transplantation, while fluconazole (3 mg/kg OD) is given in form of a prophylactic against candidiasis, successfully tolerated by patients.

In immune-compromised children, invasive aspergillosis (IA) can be fatal. Infection is usually acquired in the community or else through nosocomial illnesses induced due to exposure to hospital construction, renovation, and air-conditioning systems. Bronchopneumonia is the most prevalent manifestation of Aspergillosis infection in patients with SCID and other main immunodeficiency's [Kobayashi, S., *et al*., (2007)]. Other clinical signs of invasive aspergillosis shows lung infarction, pulmonary thrombosis, and pleural effusions.

Cryptococcus neoformans causes cryptococcosis, a kind of subacute or chronic systemic mycosis. *Cryptococcus neoformans* is an opportunistic fungus that affects immunocompromised people. In patients with SCID, the respiratory tract is the main point to enter and shows being lethal due to overwhelming pneumonia. *Cryptococcus neoformans* was discovered in the skin lesions of a SCID sufferer who came with a maculopapular rash and lobar consolidation. The treatment proved resistant to medicinal intervention yet sensitive to HSCT [Alsum, Z., *et al*., (2012)].

Parasites

Parasitic infections are the leading cause of gastrointestinal sickness in people with SCID. The most prevalent protozoans infecting SCID patients are *Giardia duodenalis*, *Giradia intestinalis* (*Giardia lamblia*), and *Cryptosporidium* spp. Other parasites implicated include *Schistosoma* species, *Blastocystis hominis*, *Fasciola* species, and *Trichostrongylus* species [Parvaneh, L., *et al*., (2019)].

The gastrointestinal (GI) tract is the largest lymphoid organ in the body [McCabe R. P., (2002)]. GI signs are the 2nd most prevalent manifestation of primary immunodeficiency diseases (PID), following pulmonary disease. Gastrointestinal issues such as chronic diarrhoea, malabsorption, and abdominal pain affect up to half of people with primary immunodeficiencies. *Giardia intestinalis* is a zoonotic protozoan parasite that normally living in small intestines of humans and other animals. Immunocompetent people may experience asymptomatic or mild diarrhoea, whereas immunocompromised patients may experience severe and persistent diarrhoea as well as malabsorption.

Cryptosporidium species, particularly *C. parvum*, can induce serious and acute enteropathy, producing proinflammatory cytokines like interleukin-8 (IL-8) in intestinal epithelial cells of people with PIDs. Disseminated cryptosporidiosis may cause biliary tract disease, pancreatitis, lung disease, and stunted growth in SCID individuals. SCID sufferers have had disseminated cryptosporidiosis, which can lead to overwhelming sepsis and even death. Although the International Agency for Research on Cancer (IARC) didn't classify protozoans as carcinogens in humans, *Cryptosporidium* has been linked to colonic adenocarcinoma in SCID mice; thus, physicians must be aware of this potential issue, and infection in SCID patients should be treated promptly.

Immunization with the vaccinia virus and bacille Calmette-Guérin (BCG) vaccine is extensively used in numerous countries, although it has been shown to worsen variables in SCID patients. These vaccines can cause disseminated, deadly illness and should not be given to persons with SCID [Kim, H. Y., *et al*., (2017)].

Precision Treatment of Severe Combined Immunodeficiency

Hematopoietic Stem Cell Transplantation (HSCT)

Hematopoietic stem cell transplantation (HSCT), preferred potentially curative treatment for SCID. Though HSCT saves lives, it only partially restores immunity because recovery is a dynamic process. SCID sufferers who had done transplantations before the age of 3.5 months showed best surviving rates [Hardin, O., *et al*., (2022)]. Early transplantation provides the greatest outcomes for SCID babies. Infants diagnosed with SCID are frequently treated with hematopoietic stem cell treatment (HSCT), also known as bone marrow transplantation. It's a difficult medical task that requires a significant amount of time and preparation. After an appropriate donor has been located, hematopoietic stem cells are extracted, administering into SCID newborns. Hematopoietic stem cells are immature and eventually develop into red blood cells, white blood cells, and platelets. Such cells subsequently grow over time, and immunity is gradually acquired; demonstrated by the survival rate of the process, which ranges from 70-95% [Mosaad Y. M. (2014)] [Hawley R. G., *et al*., (2006)].

Donor grafts can be obtained from bone marrow and mobilized peripheral blood from related or unrelated donors, as well as umbilical cord samples from unrelated neonates. To allow the donor stem cells to undergo normal haematopoiesis, chemotherapy is typically used to kill native bone marrow and make room for donor cells to engraft. Myeloablative conditioning

generally results in the highest levels of donor engraftment, and while prior myeloablative regimens produced significant morbidity and mortality, current reduced intense regimens are far less toxic [Gennery A. R., *et al*., (2010)]. The conditioning procedure also causes an extraordinarily vulnerable time in which the recipient bone marrow has been damaged but donor stem cells have not yet begun to create functional cells, making the transplant recipient particularly susceptible to infection. After engraftment, interactions between the graft and the host (GvHD) might arise, which can have serious implications. More typically, it produces persistent inflammation, necessitating immunosuppression with corticosteroids, which can lead to linked problems such as poor growth, osteopenia, and susceptibility to infection, particularly if used for an extended period.

In some cases of SCID, HSCT can be performed without any chemotherapy. X-SCID and ADA-SCID patients, in particular, benefit from unconditioned transplants from matched family donors and experience strong immunological recovery [Hassan A, *et al*., (2012)]. In other SCID forms, unconditioned transplants are associated with inadequate immune reconstitution, which can be problematic in the long run.

The first transplant for SCID was performed in 1968 [Kenny A. B., & Hitzig W. H. (1979)]. Concentrating HSCTs in a few centers of expertise and pooling data can lead to safer procedures. The European cohort includes over 1500 patients who underwent HSCT for PID. This allows centers of excellence to share lessons learned and analyse big data sets to generate best practice guidelines (European Bone Marrow Transplant Guidelines).

Factors affecting the outcome of hematopoietic stem cell transplantation (HSCT) [Cuvelier GD, *et al*., (2009)]:

Age and Clinical Condition at Diagnosis and throughout HSCT: There is growing evidence that diagnosis and HSCT are most effective if done during first few months of life (1-4 months), before clinical presentation with infections and failure to thrive; of course, this emphasizes the importance of population-based newborn screening for SCID, which allows for the early diagnosis of asymptomatic newborns.

Hematopoietic Stem Cell Donor: Unfortunately, only a small percentage of SCID infants have an HLA-matched sibling, which is obviously the ideal donor; for infants without a matched related donor, alternative donors include an HLA-matched unrelated volunteer or an HLA-matched unrelated banked cord blood unit, or, if none of these are available in a short period of time, rigorously T-depleted haploidentical related (mother or father) bone marrow or peripheral blood stem cells (PBSC).

No Conditioning Versus Conditioning Regimen, and SCID Genotype/Phenotype: HSCT engraftment without pretransplant conditioning regimen is theoretically possible in most SCID infants, especially if NK^-SCID (e.g., ADA-SCID, common γc defect, JAK3 defect). However, donor B cell reconstitution is frequently defective, resulting in persistent B cell deficiency that requires long-term immunoglobulin replacement therapy. New, now-in-use cytoreductive nonmyeloablative regimens, particularly those containing fludarabine, appear safe and cause less toxicity, as well as presumably fewer long-term effects on growth and the endocrine system; thus, they are likely to be recommended, if possible, in all of these very young (few months of age) children [Veys P. (2010)].

Gene Therapy (GT)

ADA-SCID was the first molecularly diagnosed SCID in 1972 [Giblett, E. R., *et al*., (1972)], and gene therapy was initially implemented to treat it in the early 1990s [Blaese, R. M., *et al*., (1995)]. However, Gene therapy can be an effective treatment, especially for X-linked SCID. During this procedure, stem cells are extracted from the patient's bone marrow, the normal gene is inserted via a carrier known as a vector, and the restored cells are returned. Early attempts to cure X-linked SCID with gene therapy were successful in restoring T cell function in children, but about 25% of them got leukaemia two to five years later [Kohn, D. B., *et al*., (2003)]. The vectors used in gene therapy were proposed to incorrectly activate genes that control cell formation, resulting in leukaemia. Early gene therapy with the initial design of retroviral vectors for X-SCID had several significant side consequences, including an increased incidence of lymphoproliferative diseases [Kohn D. B. (2010)]. Twenty patients with X-SCID received gene therapy in Paris and London between 1999 and 2006, and their T cells recovered successfully. However, five developed leukaemia after receiving gene therapy, with four children going into remission and one dying [Fischer, A., *et al*., (2010)]. The identification of the deleterious consequences resulted in the extensive characterization of retroviral vector integration profiles, and a new generation of self-inactivating and lentiviral vectors were developed to address these problems. Modern gene therapy approaches frequently involve modified vectors, which are more efficient and have fewer possible problems.

Artemis SCID gene therapy is now available to infants diagnosed with X-linked SCID. Here, hematopoietic stem cells with mutant gene are collected from bone marrow or blood. Next it is transported to the lab, where "correct" copies are created. This copy is now injected into a deactivated virus, which effectively enters hematopoietic stem cells. After the virus penetrates the cell,

the patient's regular HSC mixes with the new copy and is allowed to become hematopoietic stem cells. This improved form of the cell can now be separated and stored at cryogenic temperatures. The infant is then treated with conditioning, which might take the form of chemotherapy or immunosuppressive drugs where the newborn is given a simple IV injection to allow corrected hematopoietic stem cells to disseminate throughout their body [Booth, N. A., *et al*., (2022)].

Enzyme Replacement Therapy (ERT)

ADA deficient sufferers don't have the essential enzyme adenosine deaminase; thus, newborns receive a weekly intramuscular injection of elapegademase, which contains adenosine deaminase. Though easy and painless the surgery appears, it is not a permanent cure of SCID; rather, it is a stopgap measure before a much more permanent procedure, such as HSCT or gene therapy [Basheer, F., *et al*., (2022)]. Demonstration that employing enzyme replacement therapy prior to stem cell therapy can boost and raise the number of T cells was done, resulting in a lower incidence of infection until a definite procedure is adopted.

Treatment of Infections

Though Stem Cell Therapy (SCT) being final treatment for SCID, reverse isolation, which involves keeping and protecting the patient in a protected environment, avoiding live immunizations, therapeutic use of immunoglobulins, and early preventive use of antimicrobials, can help manage infections. Early preventive antibiotic therapy is commonly utilized in SCID treatment to decrease incidence and severity of infections, particularly bacterial sinopulmonary infections. Prophylactic antiviral and antifungal medication are also recommended in SCID. Over the last two decades, new antifungal drugs have been produced, including lipid formulations of amphotericin B (liposomal amphotericin B), new azoles (voriconazole, posaconazole, and isavuconazole), and echinocandins (micafungin, caspofungin, and anidulafungin) [Ledoux, M. P., *et al*., (2020)].

Newborn Screening for Severe Combined Immunodeficiency

The goal of newborn screening is to uncover inborn disorders early on, so that rapid treatment can prevent death or severe damage. The first heritable immune disorders to be screened for in newborns are severe combined immunodeficiency (SCID), which is caused by abnormalities in any of a wide range of gene products required for the development of adaptive immunity supplied by T and B cells. In 2008, Wisconsin began newborn screening for severe combined immunodeficiency (SCID) with assays to identify T-cell

receptor excision circles (TRECs), and SCID was added to the national recommended standard panel for newborn screened illnesses in 2010. In most situations, population-based screening is the only way to discover SCID before infections begin, as more than 80% do not have a positive family history [Chan, K., *et al*., (2005)] [Chan, A., *et al*., (2011)]. T-cell receptor excision circles (TRECs), a biomarker for T lymphopoiesis [Douek, D. C., *et al*., (1998)], can be quantified using polymerase chain reaction (PCR) with DNA extracted from infant dried blood spots collected for newborn screening. TRECs were missing from dried blood spots of apparently healthy babies who were later diagnosed with SCID. After confirming the utility of the TREC test, adapting it for pilot newborn screening programs in Wisconsin and Massachusetts, and conducting an evidence-based review, the US Department of Health and Human Services Secretary recommended in 2010 that SCID be added to the Uniform Screening Panel for all newborns, with related T-cell deficiencies added to the list of secondary targets.

11 Screening Programs in the United States [Kwan, A., *et al*., (2014)]

All SCID newborn screening programs operating as of July 31, 2013, were invited, and 11 provided data for this analysis, with the accrual dates listed below:

California (August 16, 2010, to May 31, 2013), Colorado (February 1, 2012, to March 31, 2013), Connecticut (October 1, 2011, to May 1, 2013), Delaware (July 6, 2012, to June 30, 2013), Massachusetts (February 1, 2009, to January 31, 2013), Michigan (October 1, 2011, to March 31, 2013), Mississippi (January 1, 2012, to December 31, 2012), New York (September 29, 2010, to September 28, 2012), Texas (December 1, 2012, to May 31, 2013), Wisconsin (January 1, 2008, to December 31, 2012), and the Navajo Nation spanning parts of Arizona, New Mexico, and Utah, where health care is provided through the Navajo Area Indian Health Service (February 1, 2012, to June 30, 2013)

	California	Colorado	Connecticut	Delaware	Massachusetts	Michigan	Mississippi	Navajo Nation	New York	Texas	Wisconsin	Total
Duration of screening included, mo	34	13	19	12	48	18	12	17	24	6	60	
Infants screened, No.[a]	1 384 606	70 989	57 136	11 202	293 371	162 528	37 613	3498	485 912	183 191	340 037	3 030 083
Flow cytometry referrals,[b] No. (%) [95% CI][c]	206 (14.9) [12-17]	10 (14.1) [5.4-23]	22 (38.5) [22-55]	9 (80.3) [28-133]	63 (21.5) [16-27]	114 (70.1) [57-83]	5 (13.3) [1.6-25]	1 (28.6)	478 (98.4) [90-107]	249 (135.9) [119-153]	108 (31.8) [26-38]	1265 (41.8) [39-44]
SCID cases	23	1	3	1	4	2	1	1	10	2	4	52
SCID incidence	1/60 000	1/71 000	1/19 000[d]	1/11 000[d]	1/73 000	1/81 000	1/38 000	1/3500[d]	1/49 000	1/92 000	1/85 000	1/58 000 [1/46 000-1/80 000]
SCID cases per 100 000 screened, No. [95% CI][c]	1.7 [1.0-2.3]	1.4 [0.3-5.2]	5.2 [1.9-15]	8.9 [2.2-49]	1.4 [0.4-3.5]	1.2 [0.4-4.4]	2.7 [0.6-5]	29 [6.9-159]	2.0 [0.8-3.3]	1.1 [0.3-3.9]	1.2 [0.3-3.0]	1.72 [1.3-2.2]
SCID infant survival, No./Total No. (%) [95% CI][c,e]	21/23 (91) [83-100]	1/1 (100)	3/3 (100)	1/1 (100)	4/4 (100)	1/2 (50)	0/1	1/1 (100)	9/10 (90) [70-100]	0/2	4/4 (100)	45/52[f] (86) [79-98]

Abbreviation: SCID, severe combined immunodeficiency.

[a]Includes 175 nonviable infants (<0.006% from all programs) who had a nonnormal TREC result, but died before further testing could be done; although causes of these deaths were not available and it is theoretically possible that some of these infants had SCID, SCID is not known to lead to death in the newborn period.

[b]Per 100 000 screened. Referral criteria for flow cytometry varied according to individual program algorithms.

[c]Confidence intervals derived from normal approximation of binomial data or inversion of cumulative binomial distribution, as appropriate. No CI given where numbers were too small.

[d]Navajo SCID incidence was an outlier, consistent with the known founder mutation of this population. Because of the low numbers screened, apparent higher incidences in Connecticut and Delaware were not significantly different (P >.05) from the other states.

[e]Survival percentage CI for California and New York is the exact 1-sided binomial CI; total survival percentage CI is 2-sided.

[f]of the 7 deceased infants, 1 each in Mississippi, New York, and Texas died prior to receiving immune restoring therapy because of complications present before referral for hematopoietic cell transplantation; the remaining 4 died after transplantation.

This study collected data on 30, 30,083 newborns from 11 programs. Nonparticipating programs claimed insufficient data, a lack of workers to collect data, and privacy issues. California, with nearly three years of

screening and 12.5% of all US births, contributed 46%, followed by New York, with 16% after two years. Wisconsin and Massachusetts, with lower yearly birth rates but longer program durations, contributed 11% and 10%, respectively. There were 52 SCID cases (42 with normal SCID, 9 with leaky SCID, and 1 with Omenn Syndrome), with an overall incidence of 1 in 58 000 births (95% confidence interval, 1/46 000-1/80 000)

Conclusion

Severe Combined Immunodeficiency (SCID) is a significant, life-threatening illness that requires prompt diagnosis and treatment to avoid deadly consequences. The introduction of newborn screening programs has been critical in identifying afflicted infants at birth, allowing for prompt treatment. Hematopoietic stem cell transplantation (HSCT) remains a cornerstone therapy, with the potential to cure many patients. Furthermore, advances in gene therapy have created new therapeutic options, giving hope to patients with genetic mutations that were previously thought to be incurable. The ongoing improvement of these therapeutic techniques has resulted in significantly improved survival rates and quality of life for SCID patients. Ongoing research and therapeutic trials are critical for gaining a better understanding of the genetic and molecular foundations of SCID. These initiatives are critical for improving existing medicines and establishing new therapeutic approaches. As our knowledge grows, so does our ability to better manage this complicated condition, with the ultimate goal of curing or even preventing SCID.

Conflict of Interest

The authors declare no conflict of interest.

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Chapter - 5
Impact of Marine Contamination on Human Health

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Chapter - 5

Impact of Marine Contamination on Human Health

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Abstract

The main environmental factor contributing to disease in the world today is pollution, which is undesirable waste that humans release into the air, water, and land. An estimated nine million premature deaths annually, significant financial losses, the depletion of human capital, and ecological degradation are all caused by it. Ocean pollution is a significant part of global pollution, but it is not well understood and managed. It seriously jeopardizes human health and welfare. We are still learning about the type and scope of these effects. The majority of nations have inadequate controls over the pervasive and increasingly severe ocean pollution. It is a complex mixture of petroleum, plastics, manufactured chemicals, pesticides, fertilizers, and pharmaceutical chemicals, runoff from agriculture, sewage, and urban and industrial wastes. It also contains toxic metals. The majority, over 80%, comes from sources on land. Rivers, runoff, atmospheric deposition, and direct discharges are the ways it gets to the seas. Marine ecosystems are negatively impacted by ocean pollution in a number of ways, and these effects are made worse by global climate change. Pollutants derived from petroleum inhibit the production of oxygen through photosynthesis in marine microorganisms. Artificial chemicals, such as flame retardants, phthalates, bisphenol A, and perfluorinated compounds-many of which are discharged into the ocean from plastic waste-can interfere with endocrine signaling, lower male fertility, harm the nervous system, and raise the risk of cancer. Fish and shellfish become accumulated with the powerful toxins produced by harmful algal blooms (HABs). These poisons can quickly result in death and serious neurological impairment when consumed. Respiratory illnesses can also be brought on by HAB toxic elements that enter the air. Many advantages arise from preventing ocean pollution. In addition to improving human health and wellbeing, it strengthens economies, draws more tourists, and aids in the recovery of fisheries. Through it, the Sustainable Development Goals (SDG) are advanced. They will provide centuries of benefits.

Keywords: Ocean pollution, toxic metals, male fertility, cancer, harmful algal blooms (HABs).

Overview

The oceans are enormous. They contain 97% of the world's water, make up more than 70% of its surface, have some of the planet's most diverse habitats, and support the economies of countries all over the world (Cunha *et al*. 2021). Small marine animals are a major source of atmospheric oxygen (Cerezo *et al*. 2015). The oceans moderate global warming and maintain environmental equilibrium by absorbing more than 90% of the excess heat introduced into the current state of the planet and almost 33% of carbon dioxide emissions (Gruber *et al*. 2019).

The seas are fundamental for human wellbeing and prosperity (Rockstrom *et al*. 2009). They supply millions with food, millions with a means of subsistence, and several vital medications (Ercolano *et al*. 2019). They are a source of happiness, beauty, calm, and leisure and have historic cultural importance. For the health and welfare of individuals living on small island nations, the oceans are especially vital (The Lancet. 2019). Coastal regions, particularly those in the Global South, and the upper Arctic. The condition of the oceans affects these populations' very survival. Even with their enormous size, the seas are threatened, and the primary cause of this vulnerability is human activity (Whitmee *et al*. 2015).

Rising sea surface temperatures, melting glaciers, and the migration of pathogenic bacteria and hazardous algae species into previously uncontaminated seas are all results of climate change and other human-caused environmental changes. For 600 million people globally, living within 10 meters of sea level, rising seas and more intense coastal storms pose a threat. Increased atmospheric CO_2 concentrations have acidified the oceans, destroying coral reefs, hindering the growth of oysters and other shellfish, and dissolving microbes that hold calcium at the base of the food chain (Hönisch *et al*. 2012).

Oxygen levels in the seas are declining. The amount of fish in stocks is dropping. The seafloors are under danger due to proposed deep underwater metal mining, oil exploration, automated trawling, and dredging (Cheung *et al*. 2016). One of the existential concerns of themodern era is pollution, which is undesirable and frequently dangerous waste material that humans discharge into the environment (Landrigan *et al*. 2018).

Until recently, the global health agenda generally ignored pollution, and it was also disregarded in plans for worldwide development. Too long ago, the

burning of fossil fuels, particularly coal, was considered as the engine of economic expansion, and pollution was seen as an inevitable cost of industrial advancement. This perspective dates back to the 19th and 20th centuries. That pollution is unavoidable and that reducing it will destroy employment and weaken economies is no longer true, though. Numerous nations who have significantly decreased pollution and more than quadrupled their GDPs over the last 50 years have disproved it. Since affordable, renewable energy sources are becoming more widely available and green chemistry is progressing, it is no longer relevant (Swinehart *et al*. 2019).

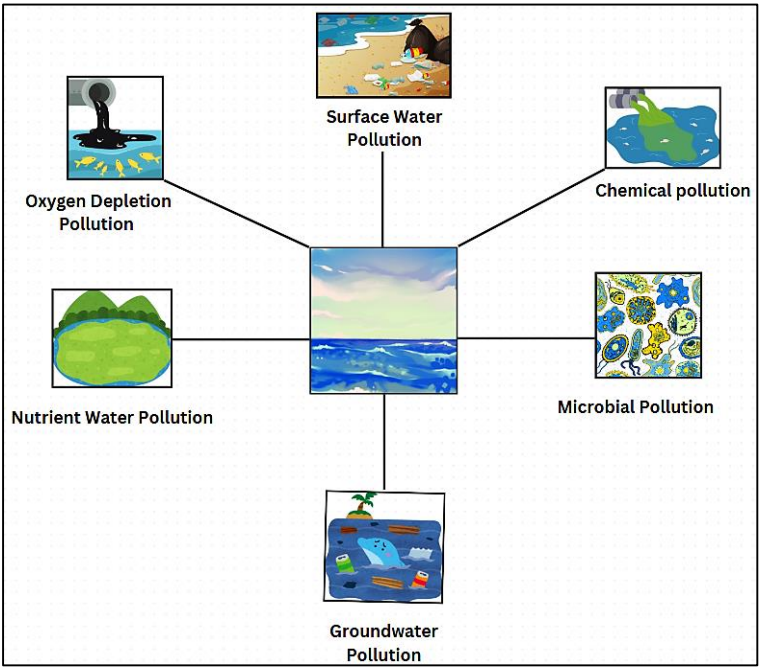

Fig 1: Types of Water Pollution

Strategies

This study is made up of a number of point-cantered studies that basically look at flow data on all sea contamination, including its sources, size, geographic distribution, and populations most at risk, as well as its estimated and known consequences on human health. It looks at how strong the evidence is that toxins have a detrimental impact on wellbeing. We take into account the possible health effects of individual contaminants as well as the astounding mixtures of man-made toxins and organic contaminants found in today's oceans. The relationships and synergistic forces between pollution,

environmental change, and sea fermentation are examined. Due to the fact that the effects of contamination are highly concentrated in low-income countries in the Global South, small island nations, and indigenous communities in the far north, we examine directly how saltwater contamination affects these vulnerable people. Finally, we discuss how sea contamination might be predicted and managed, and we provide contextual evaluations of the advancements in contamination control (Fleming *et al*. 2019).

Discoveries

The Current State of Marine Pollution

It is impossible to avoid saltwater contamination, which is getting worse and spreading geographically. In addition to plastic garbage, gasoline-based pollutants, deadly metals, manufactured synthetic compounds, pharmaceuticals, pesticides, and a toxic mixture of nitrogen, phosphorus, compost, and sewage, sea contamination is a complex and constantly shifting mixture of synthetic and organic components. Approximately 80% of sea contamination comes from land-based sources, with the remaining 20% coming from emissions from marine traffic, seaward contemporary activities, and adrift rubbish collection. Generally speaking, contamination is severe near coasts and in narrows, harbors, and estuaries where riverine contamination, horticulture spillage, contemporary delivery, and wastewater releases result in terrible inshore pollution. Along the banks of rapidly becoming non-industrialized countries in the Worldwide South, one can observe some of the worst sea contamination in the world (Wheeler *et al*. 2014).

In 96% of the Baltic Ocean, 91% of the Dark Ocean, 87% of the Mediterranean Ocean, and 75% of the North-East Atlantic Sea, contamination by dangerous metals, contemporary synthetic materials, and plastic waste is at problem levels, according to the European Climate Organization (EEA). The global threat of plastic trash contamination has grown. Rapid industrialization, the continued growth in the production and delivery of synthetics and plastics into the environment, the emergence of artificially heightened agribusiness, the massive influx of strong and fluid waste into streams, harbors, and estuaries, and the lack of feedstock material reuse are the main causes of sea contamination (Berdalet *et al*. 2015).

Ocean pollution can be attributed to.

- The two primary sources of mercury pollution in the ocean are coal combustion and gold mining (Outridge *et al*. 2018).

- Inadequate controls on chemical releases and exponential growth in chemical production are the main reasons why manufactured chemicals pollute the ocean (Landrigan *et al*. 2011).
- Marine pollution from plastic waste reflects the massive increase in plastic production worldwide, which now exceeds 420 million tons annually (E. P. R. O. 2019).
- Uncontrolled population growth and economic expansion have led to sewage, industrial discharges, and agricultural runoff polluting inshore waters along the world's coasts (Hugo *et al*. 2011).
- Pathogenic bacteria, viruses, and parasites grow, eutrophication occurs, and harmful algal blooms (HABs), often referred to as "red tides," "brown tides," and "green tides," occur more frequently and with greater severity. Some of these HABs emit potent poisons that cause disease (Small *et al*. 2003).

Even though the extent of ocean pollution is enormous, and its effects on human and ecosystem health are becoming increasingly recognized, there are still many unanswered questions regarding the sources of pollution, the levels of pollution in various sea regions, the sizes of high-risk populations, the extent of human exposure, and the severity of health effects. The effects of ocean pollution on human health and well-being are understated as a result of these gaps, and the extent to which ocean pollution contributes to the worldwide burden of disease cannot yet be accurately measured (Whiteford *et al*. 2013).

Pollution, Ocean Acidification, Global Warming, and Climate Change

Ocean temperatures have gradually increased since the 1970s in tandem with changes in the global climate. Of the excess heat released into the climate system, they have absorbed over 90% of it. Sea surface temperatures are increasing by 0.13°C every ten years on average. Marine heatwaves have become more than twice as frequent (Cai *et al*. 2009).

Increases in the frequency and severity of extreme weather events like heat waves, floods, and powerful hurricanes, as well as modifications to large-scale planetary phenomena like El Niño events and the Indian Ocean Dipole, are additional effects of climate change on the oceans (Cook *et al*. 2016).

Natural marine pathogens, including Vibrio species, including the cholera-causing Vibrio cholerae, are becoming more abundant and occupying larger geographic areas as a result of rising sea surface temperatures and growing ocean pollution. Increases in the incidence of diseases linked to

Vibrio and the transfer of these infections to previously unaffected areas are the expected outcomes. Particularly at risk are low-income nations with extensive coastal development and inefficient sanitation systems brought on by natural catastrophes, civil unrest, conflict, sea level rise, and coastal overdevelopment (Escobar *et al*. 2015).

Similarly, ocean pollution, sea surface warming, and climate change seem to be contributing to an increase in the frequency, intensity, and worldwide geographic reach of harmful algal blooms (HABs). As coastal waters warm, ocean stratification shifts, current changes, nutrient upwelling shifts, land runoff, and micronutrient availability shift, certain harmful algae species are shifting poleward. The development of HABs in previously unaffected areas and the exposure of previously unexposed individuals in the circumpolar regions to HAB toxins are the expected outcomes (Wells *et al*. 2015).

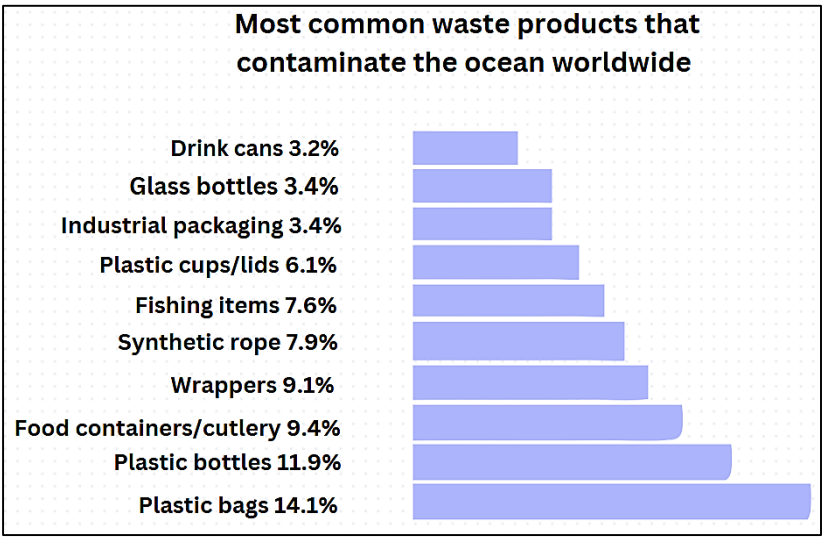

Fig 2: Oceans contaminating common waste products

Ocean Pollution's Effect on Human Health

Impact of Methylmercury Pollution on the Heart

Blood and tissue samples with elevated methylmercury contents are linked to a higher risk of cardiovascular disease, acute coronary episodes, and coronary heart disease. In 2000, it was determined by the US National Research Council that aberrant cardiac function and changes in blood pressure are caused by methylmercury build-up in the heart (Virtanen *et al*. 2005). These conclusions have been supported by further research. A 2011 US EPA

expert panel found a direct correlation between methylmercury and acute myocardial infarction and elevated cardiovascular risk factors, including atherosclerosis, oxidative stress, reduced heart rate variability, and to some extent, hypertension. Similarly, a 2017 systematic review discovered that methylmercury has a long-lasting variety of effects on cardiac parasympathetic function, increasing the risk of hypertension, myocardial infarction, and death via increasing the creation of free radicals. These findings have been validated by additional study (Sørensen *et al*. 1999).

Rapid Deterioration of Neurocognitive Function in Adult Methylmercury Exposure

Adult exposures to methylmercury may also impair brain function, according to recent research. For example, in a cross-sectional study of 129 men and women in six towns on the Cuiaba River in Brazil, lower motor speed, manual dexterity, and concentration were linked to higher hair mercury concentrations. Aspects of memory and language learning were also compromised. As mercury contents in hair rose, so did the severity of these consequences. Adult methylmercury exposure impairs attention span, fine motor skills, and verbal memory. These effects are comparable to those previously documented in children exposed during pregnancy, although they seem to happen at much larger exposure levels (Yokoo *et al*. 2003).

The Neurotoxic Effects of Methylmercury

The organ in the human body most susceptible to methylmercury exposure is the brain. The nine months of pregnancy and the first few years of postnatal life are when this vulnerability is most pronounced because of the rapid expansion of the brain (Grandjean *et al*. 1997). Methylmercury exposure during early human development does not seem to have a safe threshold (Debes *et al*. 2016). The Faroe Islands have conducted prospective epidemiological cohort studies that show that children exposed to methylmercury during pregnancy have lower language abilities, motor function, attention span, memory, and other brain processes. Following up with these kids until they are 22 years old shows that these deficiencies continue and seem to be irreversible (Boucher *et al*. 2012).

Methylmercury exposure in early life has been linked to slowed processing of visual information, decreased IQ, diminished comprehension and perceptual reasoning, impaired memory, shortened attention span, and an increased risk of attention deficit/hyperactivity disorder (ADHD), according to a similar study done in Nunavik on the development of children at the age of 11. Children who were exposed to high levels of methylmercury during

pregnancy also showed neurobehavioral abnormalities, according to other prospective studies (Boucher *et al*. 2012). As the human brain continues to develop during this time, exposure to mercury later in childhood and adolescence can potentially result in harm. Certain individuals may be more susceptible to methylmercury due to genetic causes (Julvez *et al*. 2019).

The Global Burden of Disease and the Role of Marine Mercury Pollution

Estimates of the global burden of disease (GBD) caused by ocean mercury pollution have started to be made. According to a recent estimate, prenatal exposures to methylmercury from the mothers' intake of mercury-contaminated seafood during pregnancy cause between 317,000 and 637,000 kids born in the United States each year to have cognitive function deficits. The extent of these losses varies from 0.2 to 5.13 IQ points, contingent on the degree of exposure. Additionally, these researchers discovered that extra cases of mental retardation (IQ < 70) are linked to population-wide IQ declines brought on by extensive exposure to methylmercury, accounting for 3.2% (range: 0.2–5.4%) of all cases of mental retardation in the US (Trasande *et al*. 2005).

Impact of Ocean Plastic Pollution on Human Health

Human health hazards from marine microplastics are still poorly known, and there is a great deal of uncertainty about their possible hazard. According to a recent analysis by the European Academies of Science's SAPEA, there is now "no evidence of widespread risk to human health" from marine plastic pollution (Wright *et al*. 2017).

The estimate may alter, the paper says, as new information becomes available and the number of plastic debris dumped into the oceans keeps growing. Taking preventative measures is necessary to safeguard human health from the possible risks posed by marine plastic. There is enough information to support immediate action to stop the ongoing release of plastic garbage into the oceans, even though our understanding of the health risks is still lacking (Wyles *et al*. 2016).

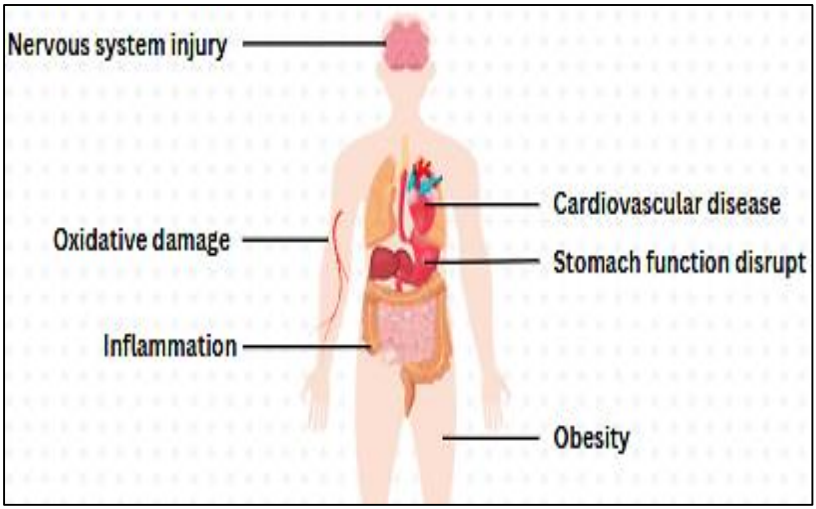

Fig 3: Regions at risk for micro plastic damage

Achievements in Ocean Pollution Control and Prevention

According to the 2018 Lancet Commission on Pollution and Health, a significant amount of pollution may be reduced, and diseases linked to pollution can be avoided. The majority of high-income nations and a growing number of middle-income nations have reduced their most egregious pollution by passing environmental laws and creating regulatory frameworks, according to the Commission. These laws are supported by rigorous regulation and have a scientific foundation. They are based on the "polluter-pays principle," have defined goals and timelines, and receive sufficient funding. These nations now enjoy better health, longer lifespans, and cleaner air and fresh water. It was determined by the Lancet Commission that pollution control is "a winnable battle" (Landrigan *et al.* 2018)

The methods for preventing and controlling ocean pollution are starting to be applied to the management of freshwater and air pollution. The understanding that 80 percent of ocean pollution originates from land-based sources has been essential to the success of these initiatives. In light of this, effective initiatives to prevent marine pollution have located, targeted, and decreased emissions from significant land-based polluters. Multi-scale monitoring that monitors pollution discharges, analyses the amounts of pollutants in the seas and marine biota, and evaluates human exposures and health consequences has served as their guide. They have had the support of rigorous enforcement. Making their data, strategies, and success reports publicly available on open-source platforms has allowed them to interact with the public and civil society (Teplitski *et al*. 2009).

These tactics are starting to show results. Agricultural runoff has been lessened, plastic pollution has decreased, sewage has been more efficiently treated, and industrial discharges have decreased in some places, as detailed in the case studies that follow. Polluted harbors have been cleaned, estuaries have been revitalized, shellfish beds and aquaculture operations have been protected, fish stocks have recovered, coral reefs have been restored, coastal contamination has decreased, levels of toxic chemicals in marine organisms have decreased, and the frequency and severity of HABs have decreased. Broader prevention is achievable, as evidenced by the accomplishments made thus far in controlling ocean pollution (Lamb *et al*. 2017).

Conclusion

Ocean pollution is an international issue. It transcends national borders and has several origins. In most nations, it is poorly controlled and getting worse. Originates from land-based sources in excess of 80%. Ocean degradation is most evidently caused by plastic debris, which has rightfully garnered a lot of attention. It kills fish, whales, dolphins, and seabirds. Numerous harmful and cancer-causing compounds are present in the fibers, microparticles, and nanoparticles of plastic that are broken down by it. After being absorbed by fish and shellfish, these chemical-laden particles make their way up the marine food chain and may eventually be eaten by people. Only now is the threat they pose to human health being evaluated. Ocean pollution also includes mercury from small-scale gold mining and coal combustion, petroleum discharges from pipeline leaks and oil spills, persistent organic pollutants like PCBs and DDT, thousands of manufactured chemicals with unknown toxicity, pesticides, nitrogen, and phosphorus from agricultural runoff and animal waste, and sewage discharges that contain multiple microbial contaminants. Together with sea surface warming and ocean acidification, ocean pollution causes coral reef degradation, the spread of illnesses that can be fatal, and an increase in the frequency and severity of HABs.

Author Contributions

Acquisition and interpretation of data is done by Diptika Dey and Rupam Sengupta. Conception,design and revising of the article are done by Rupesh Dutta Banik.

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Chapter - 6
An Initiative to Qualitative Detect of Micro-Plastics in Water Bodies of Semi Urban Area

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Chapter - 6

An Initiative to Qualitative Detect of Micro-Plastics in Water Bodies of Semi Urban Area

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Abstract

Initiating the qualitative detection of micro plastics in stagnant water bodies within semi-urban areas is essential for protecting both the environment and human health. Semi-urban areas often face a mix of urban waste management challenges and rural practices, leading to higher risks of micro plastic contamination in water bodies. Stagnant water bodies in these regions are frequently used for irrigation, livestock, and sometimes household purposes, making contamination a direct threat to the local population. Micro plastics can degrade water quality and harm aquatic organisms, which can have a cascading effect on food security and ecosystem health. Moreover, these pollutants can enter the food chain, posing potential health risks to humans, including through consumption of contaminated water or crops irrigated with polluted water. This initiative also fosters community awareness, encouraging better waste management practices and proactive environmental protection. In this article we have reported an easy detection process of micro plastic contamination in stagnant water bodies though citizen sciences approach.

Keywords: Micro plastics pollution, water bodies, citizen science.

Introduction

Microplastics, tiny plastic particles less than 5mm in diameter, have emerged as a significant environmental concern due to their widespread presence in aquatic ecosystems. These particles originate from various sources, including the breakdown of larger plastic debris, personal care products, and industrial processes. Microplastics can be ingested by marine organisms, potentially entering the food chain and impacting both wildlife and human health. Given the growing awareness of the detrimental effects of plastic pollution, there is an urgent need to develop reliable methods for extracting and analysing microplastics from water bodies. Micro-plastic is

formed when plastic is broken or break-down into small units ranging between 5mm to 3mm this small particles present in large quantises in the water bodies is a major cause of the water ecosystem as the presence of the microplastic in water bodies cause cancer, heart disease, inflammatory bowels mammalian reproductive problems, stomach cancer. Microplastic is a major cancer-causing agent which have major health problems in organisms. This microplastic is collected in this water body's trough a number of sources and a number of ways as a result of industries, lack of waste management, textiles, city dust, etc. The presence of microplastic is seemingly everywhere from the deepest of oceans to rivers flowing from mountains. Which showcase the spread of the micro plastic pollution. Microplastic enters in this waterbody through various ways like release of untreated or partially treated waste, urban rundown and most importantly direct disposal of microplastics waste. A huge variety of animals which includes planktons, large fish consume microplastic in their digestive tract. This consumption of microplastic can lead to variety of problem like Toxic chemical indigestion, reducing the nutrients intake and physical intake leads to physical blockage of their digestive tract. It's also takes a long time to get dispose or degrade in nature because of resistance to natural degrading agents and its compound structure, which causes it to further breakdown. This issue and has profound ecological impact. It's also the leading cause for other environment pollutants such as persistent organic pollution. When enter in the body these contaminated particles cause bioaccumulation of harmful substance within the tissue, causing health issues like cardiovascular and respiratory and ingestion issues. In recent years microplastic is been a concerning issue due to its increased demand and wide spread use in multiple fields has led to inceased pollution as a result and has been causing pollutions all over the globe. This microplastic get collected in water bodies by many ways but some of the most common ways were found out to be firstly waste disposed by industries or factories directly without any treatment been directly been release in water bodies as a cheap and easy disposal way foe waste disposal another common way turns out to be household waste disposal such as plastic water bottle, styrophome, polythene bags, etc and In the last 75 years the plastic production has increased dramatically from 1.5 million tonnes to 322 million tonnes per year globally and an estimated of 4 million tonnes of plastic is predicted to have entered the marine environment from land-based sources in 2010 alone (Jambeck *et al.*, 2014). One of the major pollution it's responsible to cause is water pollution in particular. As its tendency to easily breakdown into small particles and enter the natural habitat of the aquatic ecosystem and then making its way to the food chain through which either directly by the water or by means of the food

chain its ulimatelly even effecting us humans as plastic has been found to be one of the most common cancer causing agent. Microplastic debris is widespread, impinging upon the poles to gain a clearer understanding of microplastic availability to marine life, and thus of risks posed to the health of benthic communities and associated ecological processes, it is important to obtain accurate measures of microplastic abundance in sediments. Indeed, a recent review highlighted the difficulties in developing a global picture of benthic microplastic prevalence due to the lack of reliable microplastic abundance measurements. The decanting of floating plastic whilst simultaneously avoiding disruption of the settled sediment poses a challenge, typically yielding low extraction efficiencies and hence requiring repeat extractions.(Zhou, Y. et.al.,2020)

Methods

Collection Sample: Different samples were collected from different locations by pouring water from the water bodies through a 5mm sieve and concentrating it through a 200 micrometer filtrate funnel. The materials retained on 5mm were remove or discarded. The materials from 200 micrometre sieve were collected, next the sample is rinsed with distilled water to remove salt. (Pratap, J.C et. al., 2019) Pass water sample 20 lit through 05mm seave (Purpose: Removal of Debris).Then the sample with distilled water was taken in the collection bottle.

Table 1: Water Sample Table (West Bengal)

Sample No.	Location	District	Water Source	Comment
Sample 1	22°55'54.2"N 88°27'55.9"E	North 24 parganas	pond	This pond is locally used for washing and discarding
Sample 2	22°56'21.4"N 88°27'13.0"E	North 24 parganas	Lake	Locale garbage and sewage water is added
Sample 3	22°55'52.4"N 88°27'47.6"E	North 24 parganas	pond	This pond is locally used for washing and
Sample 4	22°55'58.7"N 88°27'58.0"E	North 24 parganas	Mothura jhill	Fishing
Sample 5	22°55'53.7"N 88°27'40.5"E	North 24 parganas	pond	Locale garbage and sewage water is added
Sample 6	22°57'22.8"N 88°24'45.4"E	North 24 parganas	Rani rashmoni ghat	Swimming, Animal cleaning ,Fishing, etc.

Filtration: In the lab the water sample from the container/Collection bottle is passed through a plankton net. After the water has been passed, the sample collected in the net is transferred to a sample beaker by placing the net over the beaker and gently striking the net from the other side. The sample is collected in the beaker.

Plant Material Digestion: 20 ml of 30% hydrogen peroxide is added to the sample and heated at 75°C for 30 minutes in a conical flask. (Jambeck, J.R et. al., 2014)

Animal Material Digestion: KOH solution is added at 50°C for 1 hour. Polyethylene, polypropylene, polystyrene, polyvinyl chloride, and nylon remain intact. Cellulose acetate is hindered, and glass fibers are resistant to treatment. (Mukhanov, V. S. *et al*., 2019)

Overflow Method: After the sample is taken out, a setup is made by placing a small beaker of approximately 100 ml inside a larger beaker of 1000 ml. The sample is placed in the smaller beaker. 100 ml of NaCl solution is prepared and gently poured into the smaller beaker to produce an overflow. The excess water from the larger beaker is removed along with some water from the upper surface of the smaller beaker. (Jambeck, J.R et. al., 2014) (Vega-Herrera, An et. al., 2022)

Filtration: A setup is established using a pump filter, and a circular piece of Whatman paper is cut and placed in the filter of the setup. The sample is gently poured into the setup, and the pump is turned on for 20 to 30 minutes until all the sample is filtered. (Zhou, Y. *et al*., 2020) Once filtered, the Whatman paper is gently removed by disassembling the setup and placed in a Petri dish. The Petri dish is then placed in a hot air oven at 75°C for 30 minutes to remove all moisture and allow it to dry. Once dried, the Petri dish is taken out, and the surface of the paper is scraped with the help of a blade. The sample is then collected for FTIR analysis. (Rathore, C et. al., 2023)

Result and Discussion

Determination of micro plastic in stagnant water bodies: After the sample was treated with the Plant and Animal digestion respectively the sample was filtrated through the wattmen paper through the set-up. The wattmen paper was observed to have deep patch in the portion were the sample was been filtrated. After drying the paper in the Hot-Air oven small shinny particles were been observed on the surface of the wattmen paper which appears similar and may be plastic shards.

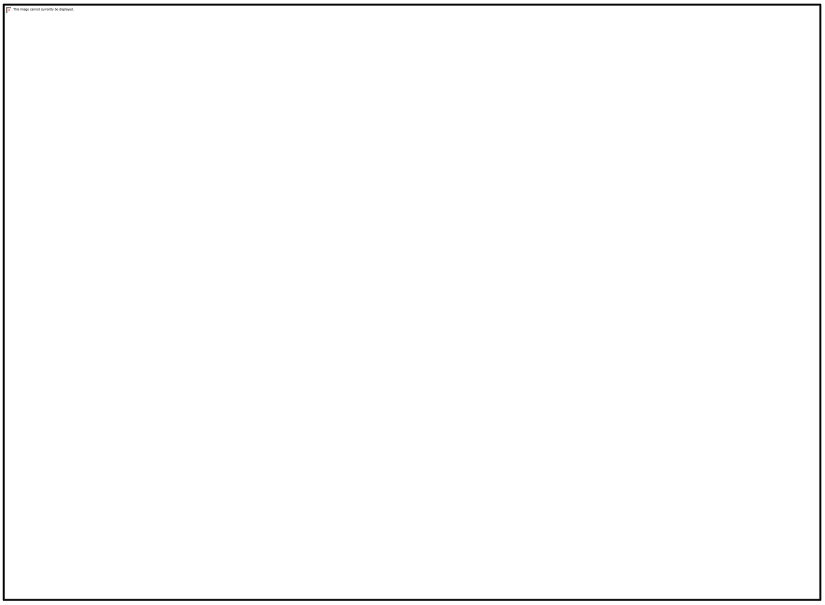

Fig 1: Microplastic detection through FTIR 1A, 1B. FTIR graph of Control vs Sample containing filter paper. Enlarged area of graph 1A, 1B in 1C, 1D with peak identification for LDPE, PET respectively.

The sample (Figure 1) collected from a small stagnant water source (> approx. radius 30ft) at (22°55'54.2"N 88°27'55.9"E) after digestion and filtration the sample were tested under the FTIR has shown peaks at 3673,2650,1471, 719 which matches with peaks of plastic PET, LLDPE, LDPE, LLDPE respectively. All location shows presence of LLDPE and LDPE whereas PET found in few cases. This indicated possible presence of the mentioned micro plastic may be present in the water bodies.

Determination of micro plastic in stagnant large water bodies (Figure 2)(< approx. radius 100ft): The sample collected from a stagnant large water source at (22°55'58.7"N 88°27'58.0"E) after digestion and filtration the sample were tested under the FTIR has shown peaks at 3700, 3650, 2956, 1471, 1094 which matches with peaks of plastic LLDPE(C=H), LDPE, LLDPE(C-H) respectively. Denoting that the mentioned micro plastic may be present in the water bodies.

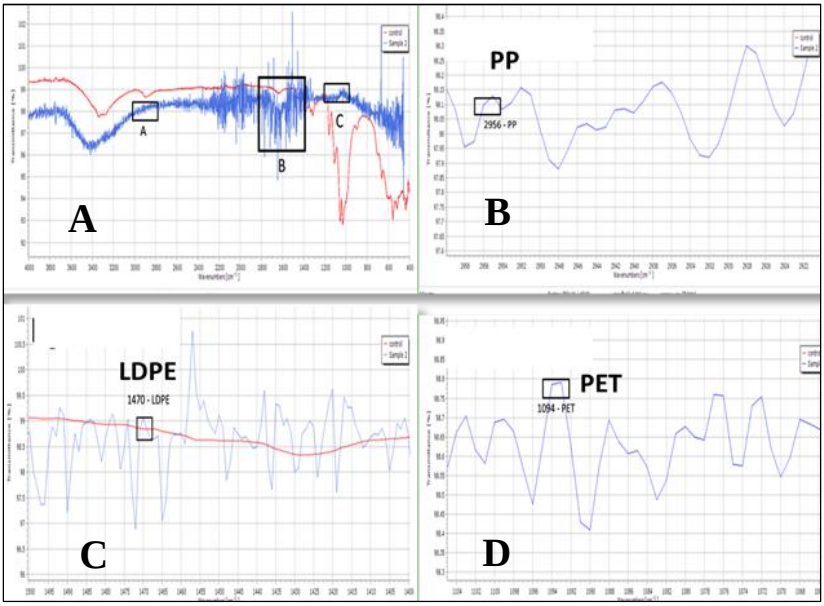

Fig 2: Microplastic detection through FTIR A. FTIR graph of Control vs Sample containing filter paper. Enalrged area of graph 2 in 2A, 2B, 2C with peak identification for PP, LDPE, PET respectively.

Table 2: Detection of Plastic type Via Via Peak Analysis

Sample	Peak range	Micro plastic	Reference
Sample 1	2915	LLDPE	(Rathore, C et. al., 2023
	1471	LDPE	(Rathore, C et. al., 2023
	719	LLDPE	(Rathore, C et. al., 2023
Sample 2	1471	LDPE	(Rathore, C et. al., 2023
	1094	PET	(Rathore, C et. al., 2023
	2956	PP	(Rathore, C et. al., 2023
Sample 3	1315	LDPE	(Rathore, C et. al., 2023
Sample 4	3650	PP	(Rathore, C et. al., 2023
	3700	PP	(Rathore, C et. al., 2023
Sample 5	3650	PP	(Rathore, C et. al., 2023
Sample 6	3700	PP	(Rathore, C et. al., 2023
	3650	PP	(Rathore, C et. al., 2023

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Chapter - 7
Targeting Pathogens with Predators: Advances in *Bdellovibrio*-Based Treatment Strategies

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Chapter - 7

Targeting Pathogens with Predators: Advances in *Bdellovibrio*-Based Treatment Strategies

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Abstract

Bdellovibrio-based treatments are emerging as a novel approach to managing bacterial infections. *Bdellovibrio* and similar organisms (BALOs) are predatory bacteria that target and consume Gram-negative bacteria, undergoing a lifecycle that includes invasion, replication inside, and destruction of their prey. This predatory behavior can effectively reduce harmful bacteria populations, positioning *Bdellovibrio* as a potential biocontrol agent. Studies have demonstrated that *Bdellovibrio* species can decrease bacterial loads in various settings, such as medical, agricultural, and aquaculture environments, without negatively impacting beneficial microbes or the host organism. The promise of *Bdellovibrio*-based therapies lies in their ability to target antibiotic-resistant bacteria, presenting a new solution amidst the growing issue of antimicrobial resistance. While clinical applications are still in the experimental phase, preliminary studies in animal models have shown both safety and efficacy. Continued research is essential to elucidate *Bdellovibrio*-host interactions, refine delivery methods, and meet regulatory standards for therapeutic use. Successful development of *Bdellovibrio*-based treatments could significantly alter our strategies for managing infectious diseases and promoting environmental health.

Keywords: Antimicrobial resistance, bacterial infections, *bdellovibrio*, biocontrol agent, predatory bacteria

Introduction

Predation plays a vital role in maintaining ecological balance across all ecosystems, involving interactions where organisms hunt and consume others. This process is not limited to large animals but also includes microorganisms (Johnke *et al*., 2020). Among these, some bacteria are predatory, feeding on other bacteria. These predatory bacteria fall into two categories: obligate predators, which need to consume other bacteria to survive, and facultative

predators, which can switch to other food sources if prey is unavailable. Obligate predatory bacteria are primarily found in specific groups of α-proteobacteria and δ-proteobacteria, collectively known as *Bdellovibrio* and like organisms (BALOs) (Paix *et al*., 2019). Despite being discovered nearly 60 years ago (Stolp and Starr, 1963), many aspects of their molecular mechanisms and the overall importance of bacterial predation remain under-researched. However, interest in these bacteria has grown recently due to rising concerns about antibiotic resistance and environmental health. Predatory bacteria have the potential to be used as natural antibiotics, for water purification, pest control, and other biotechnological applications.

Bdellovibrio bacteriovorus is one of the most thoroughly studied BALOs and serves as a model for understanding bacterial predation. Identified in the 1960s, this small, highly mobile bacterium invades and consumes harmful bacteria from the inside. It can target several pathogens, including those resistant to antibiotics, such as E. coli and Salmonella. Other BALOs, like *Bdellovibrio* exovorus and Micavibrio aeruginosavorus, attack prey from the outside. The rise of antibiotic-resistant bacteria presents a formidable challenge to global healthcare, driving the need for innovative treatment strategies. Conventional antibiotics are becoming increasingly ineffective, prompting researchers to explore alternative methods. One such promising approach is the use of predatory bacteria as biocontrol agents. In particular, *Bdellovibrio* and like organisms (BALOs) have shown significant potential due to their unique ability to prey on Gram-negative bacteria (Bratanis *et al*., 2020). *Bdellovibrio* bacteriovorus, a key member of this group, follows a predatory lifecycle that includes the invasion of prey bacteria, replication within them, and ultimately lysing the host cells. This natural predation offers a novel method to reduce harmful bacterial populations, potentially overcoming some limitations of traditional antibiotics, such as resistance development and negative impacts on beneficial microbiota. Recent research has advanced our understanding of *Bdellovibrio*'s biology, genetics, and ecological interactions, leading to innovative therapeutic strategies. Studies have demonstrated *Bdellovibrio*'s effectiveness in controlling infections caused by multidrug-resistant bacteria in both laboratory and animal models. Additionally, *Bdellovibrio*'s non-toxicity to human cells suggests it could be a safe treatment option (Cavallo *et al*., 2021).

This review aims to provide an in-depth look at the progress in *Bdellovibrio*-based treatments. We will examine the biological mechanisms of *Bdellovibrio* predation, recent developments in its therapeutic application, and discuss the challenges and future prospects of this emerging field. By

exploring these aspects, we hope to underscore the potential of *Bdellovibrio* as a novel solution to combating antibiotic-resistant bacterial infections.

Life Cycle of *Bdellovibrio*

The life cycle of *Bdellovibrio* bacteriovorus involves several stages as it hunts, invades, and consumes other bacteria, particularly Gram-negative bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* species. The life cycle has been described in detail below (Makowski *et al*., 2020).

Search and Attachment

Bdellovibrio bacteriovorus is a highly motile bacterium, swimming quickly to locate prey. When it encounters a suitable Gram-negative bacterium, such as *Escherichia coli* or *Pseudomonas aeruginosa*, it attaches to the prey's outer membrane.

Invasion

After attachment, *Bdellovibrio* creates a small hole in the prey's outer membrane. It then enters the periplasmic space, which is the area between the prey's outer and inner membranes.

Establishment

Inside the periplasmic space, *Bdellovibrio* rounds up and transforms the prey cell into a structure called a Bdelloplast. The bdelloplast provides a protective environment for *Bdellovibrio* to grow and develop.

Feeding and Growth

Bdellovibrio secretes enzymes to break down the prey's cellular components, absorbing the released nutrients to grow and replicate. It elongates and undergoes multiple cell divisions within the Bdelloplast.

Lysis and Release

After consuming all available nutrients from the prey, *Bdellovibrio* exits the Bdelloplast. The prey cell bursts open (lyses), releasing the newly formed *Bdellovibrio* cells into the environment. These new *Bdellovibrio* cells are then free to find and attack new prey, such as *Salmonella* species, continuing the cycle.

***Bdellovibrio* Based Therapeutics**

Treating Infection

Bdellovibrio offers a novel approach to treating infections caused by

bacteria resistant to traditional antibiotics. Unlike conventional antibiotics, which target specific bacterial processes or structures, *Bdellovibrio* directly preys on bacteria. It enters the periplasmic space of Gram-negative bacteria and consumes the internal resources, leading to the bacteria's death. This predatory strategy effectively bypasses the typical resistance mechanisms that bacteria develop, such as modifications to drug targets or efflux pumps that expel antibiotics from within the cell. Additionally, *Bdellovibrio* targets specific pathogens while leaving beneficial bacteria largely unaffected, reducing harm to the host's microbiome. Its method of action also reduces the risk of resistance development against itself. *Bdellovibrio* can be used alongside traditional antibiotics to decrease bacterial populations, potentially making the antibiotics more effective. It has shown promise in treating chronic infections and wounds, especially those caused by multi-resistant bacterial strains. *Bdellovibrio* presents a valuable alternative for managing infections, particularly those resistant to conventional treatments, by offering a mechanism that circumvents standard antibiotic resistance pathways.

According to the study by Tajabadi *et al*., 2023, evaluated the effectiveness of *Bdellovibrio* bacteriovorus HD100 in treating burn wounds infected with *Pseudomonas aeruginosa* using a mouse model. The study compared two treatments: the antibiotic meropenem and *Bdellovibrio*. Both treatments resulted in faster healing of the burn wounds compared to a control group. Histopathological analysis confirmed that both treatments accelerated the healing process. Additionally, quantitative reverse transcription PCR (qRT-PCR) was used to measure the levels of several genes related to inflammation and healing, including tnf-α, pdgf, tgf-β1, ifn-γ, vegf, and col1. The findings showed that *Bdellovibrio* not only reduced the duration of inflammation but also improved the healing process. Although both treatments affected gene expression similarly, pdgf was notably upregulated in both cases. *Bdellovibrio* was effective in controlling *Pseudomonas aeruginosa* infections in burn wounds and enhancing the healing process.

In study by Romanowski *et al*., 2024, researchers investigated whether predatory bacteria could help manage eye infections caused by resistant Gram-negative bacteria, specifically in cases of endophthalmitis. They used a rabbit model to test two types of predatory bacteria: *Bdellovibrio bacteriovorus* and *Micavibrio aeruginosavorus*. The findings revealed that both predatory bacteria effectively reduced the growth of *Pseudomonas aeruginosa* and *Serratia marcescens* in the eye. However, they did not significantly decrease the levels of Staphylococcus aureus, as this type of bacteria is not effectively targeted by these predators. Furthermore, *Bdellovibrio bacteriovorus* that was

inactivated by UV light could not stop *Pseudomonas aeruginosa* from growing, indicating that active predation is essential for controlling bacterial growth, rather than relying solely on an immune response. The study highlights that while predatory bacteria can control certain Gram-negative bacteria in the eye, their effectiveness varies depending on the type of bacteria and requires them to be active.

In a similar study by Romanowski *et al*., 2023 revealed that *Bdellovibrio bacteriovorus* can significantly reduce the severity of eye infections caused by *Pseudomonas aeruginosa*. When rabbits were infected with the wild-type strain of *Pseudomonas aeruginosa* (PA14), 54% of the corneas developed perforations. However, when *Bdellovibrio bacteriovorus* was co-injected, the incidence of corneal perforations dropped to just 4%. Additionally, *Bdellovibrio bacteriovorus* was found to reduce the growth of the wild-type Pseudomonas aeruginosa by a factor of seven. In contrast, a mutant version of *Pseudomonas aeruginosa* with a defect in bacterial communication (ΔlasR) showed less growth compared to the wild-type but was not significantly affected by *Bdellovibrio bacteriovorus*. These findings suggest that *Bdellovibrio bacteriovorus* is effective in diminishing the impact of *Pseudomonas aeruginosa* infections and preventing corneal damage, with bacterial communication mechanisms playing a crucial role in the infection's severity.

Atterbury *et al*., 2011 assessed the use of *Bdellovibrio bacteriovorus*, a bacterium known for preying on Gram-negative bacteria, to treat infections in poultry. The results indicated that administering Bdellovibrio to healthy birds changed the diversity of their gut bacteria but did not negatively affect their growth or general health. Additionally, there was no sign of *Bdellovibrio* contaminating the drinking water or feces. In birds previously infected with a *Salmonella enterica* strain that colonizes the gut, *Bdellovibrio* significantly reduced the Salmonella count and improved the condition of the cecum, suggesting decreased inflammation. These findings demonstrate that *Bdellovibrio bacteriovorus* can effectively manage Salmonella infections in poultry and may offer potential as a treatment for similar infections in other animals and humans.

Work by Silva *et al*., 2023 revealed that *Bdellovibrio bacteriovorus* HD100 treatment led to less bone loss in rats with experimental periodontitis (EP) compared to those not treated. This treatment also increased certain immune signals (MCP-1, RANTES, IL-10, and TGF-β) and decreased TNF-α levels. There were no significant changes in other immune signals (IL-1β, IL-6, and M-CSF) between treated and untreated rats. Rats receiving

Bdellovibrio alone showed higher levels of IL-6, TNF-α, RANTES, and MCP-1 than control rats. Additionally, the *Bdellovibrio*-treated rats had more of specific protective proteins (BD-1, BD-2, and BD-3) in their tissues, indicating an improved immune response. However, no notable differences were observed in other immune markers between the treated and untreated groups. In summary, *Bdellovibrio bacteriovorus* HD100 was effective in reducing bone loss, enhancing immune defenses, and altering immune signal levels in rats with EP.

Cui *et al*., 2021 demonstrated that cryomicroneedles (cryoMNs) are effective for delivering *Bdellovibrio bacteriovorus* to treat bacterial infections in the eye. These cryoMNs are created by freezing *Bdellovibrio* with a cryoprotectant inside a microneedle template, preserving over 80% of the bacteria's viability for long-term storage. In laboratory tests and a rodent eye infection model, the cryoMNs were able to significantly inhibit the growth of Gram-negative bacteria. The treatment reduced the infection by nearly six times over 2.5 days, with no significant adverse effects on corneal thickness or structure. This approach provides a safe and effective method for delivering new antimicrobial agents to the eye, offering promising prospects for treating ocular surface disorders.

Cancer Therapy

Bdellovibrio bacteriovorus, known for its ability to prey on Gram-negative bacteria, is not typically associated with cancer treatment. However, innovative research is investigating its potential use in this field. One promising application is in managing infections that often affect cancer patients, particularly those undergoing immunosuppressive treatments. These patients are at a higher risk for antibiotic-resistant bacterial infections. By controlling these infections, *Bdellovibrio* could help reduce complications during cancer therapy. Additionally, some tumors harbor bacterial biofilms. *Bdellovibrio's* ability to disrupt these biofilms could decrease the likelihood of secondary infections and inflammation, making the tumor environment less conducive to bacterial growth (Dashiff and Kadouri, 2011).

Furthermore, *Bdellovibrio* might enhance the effectiveness of conventional cancer treatments. While it does not directly attack cancer cells, its role in reducing bacterial populations and biofilms can support the efficacy of chemotherapy and radiotherapy. Exploring indirect benefits, such as the modulation of the immune system by *Bdellovibrio*, could also offer new therapeutic insights. Genetic engineering presents another exciting avenue, where *Bdellovibrio* could be modified to deliver anti-cancer agents directly to

tumors. Developing targeted delivery systems using *Bdellovibrio's* natural predatory behavior could facilitate the direct release of therapeutic agents into the tumor microenvironment. Moreover, *Bdellovibrio* could be utilized to alter the gut microbiome, which is increasingly recognized for its role in cancer progression and treatment response (Willis *et al*., 2016; Gupta *et al*., 2016). By fostering a healthier microbiome, *Bdellovibrio* might contribute to a more favorable environment for cancer therapy.

Probiotic Therapy

Probiotic therapy with *Bdellovibrio bacteriovorus* is an innovative method that utilizes the bacterium's predatory characteristics to promote health. Unlike conventional probiotics, which typically involve beneficial bacteria like *Lactobacillus* or *Bifidobacterium*, *Bdellovibrio* specifically targets harmful Gram-negative bacteria. This bacterium works by invading and consuming these pathogens, thereby decreasing their numbers. Additionally, *Bdellovibrio* can disrupt bacterial biofilms-protective barriers that many pathogens create to evade the immune system and antibiotics. By dismantling these biofilms, *Bdellovibrio* makes the bacteria more vulnerable to treatments and immune responses (Iebba *et al*., 2013).

Bdellovibrio has the potential to enhance gut health by addressing infections and restoring microbial balance in the digestive tract. It could be particularly effective for managing conditions such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). Moreover, *Bdellovibrio* might serve as a preventive treatment for high-risk groups, such as patients in hospitals or individuals with weakened immune systems, by reducing the incidence of bacterial infections. It could also be applied to decontaminate medical surfaces, helping to limit the spread of harmful bacteria (Bonfiglio *et al*. 2020).

Research indicates that *Bdellovibrio* does not damage human cells, highlighting its safety as a therapeutic option. *Bdellovibrio bacteriovorus* in probiotic therapy presents a promising approach for controlling bacterial infections, supporting immune health, and improving the efficacy of standard treatments. Ongoing research could further establish its role as a valuable tool in probiotic therapy.

Current Status

Bdellovibrio bacteriovorus therapy is a developing area with significant promise, though it remains largely in the experimental stages. This bacterium, recognized for its predatory behavior against Gram-negative pathogens, is being investigated as a potential alternative to traditional antibiotics,

especially for tackling antibiotic-resistant infections. Research has shown that *Bdellovibrio* can effectively target and reduce harmful bacteria and disrupt biofilms in laboratory and animal models. However, human clinical trials are still in the early stages, and these are crucial for determining its safety, effectiveness, and practical use in treating various conditions, including chronic infections and potentially as a probiotic therapy for digestive issues. There are several challenges to overcome, such as achieving regulatory approval, developing reliable delivery methods, and ensuring the safety of *Bdellovibrio* in clinical settings. Ongoing research aims to enhance its predatory capabilities through genetic modifications, refine delivery techniques, and complete comprehensive clinical evaluations.

Conclusion

The future of *Bdellovibrio bacteriovorus* therapy looks very promising and offers significant potential for advancing medical treatments and addressing pressing global health issues. Research is anticipated to focus on enhancing the genetic modification of *Bdellovibrio* to boost its targeting precision and therapeutic efficacy. This could involve engineering the bacterium to produce specific antimicrobial agents or to more effectively identify and attack particular bacterial strains. Innovations in delivery systems, such as advanced microneedles and other targeted methods, will be essential to ensure accurate application and sustained levels of the therapy.

Clinical trials will be crucial for determining the safety, effectiveness, and optimal application of *Bdellovibrio* in human medicine. Successful trials could pave the way for regulatory approval, making it a viable option for treating infections, especially those resistant to traditional antibiotics. Additionally, integrating *Bdellovibrio* with other treatments, such as antibiotics or probiotics, may improve treatment outcomes and offer a more comprehensive approach to managing multidrug-resistant infections.

Moreover, *Bdellovibrio* could have broader applications beyond infection treatment, including environmental decontamination, food safety, and potentially even cancer therapy. Its ability to disrupt bacterial biofilms and target specific pathogens makes it a versatile tool with many potential uses. Ensuring its safety and effectiveness, particularly regarding its impact on human cells and beneficial microbiota, will be vital for its successful implementation. If proven effective, *Bdellovibrio* therapy could play a significant role in combating antibiotic resistance and providing new treatment options.

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Chapter - 8
Recent Advances in Biofilm-Mediated Wastewater Treatment: A Concise Review

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Chapter - 8

Recent Advances in Biofilm-Mediated Wastewater Treatment: A Concise Review

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Abstract

The amount of pollution produced by companies has grown along with global development. Numerous pollutants are conventionally removed from wastewater using physical, biological, or chemical techniques. A number of phenomena that affect the quality of water, such as eutrophication, decreases amount of dissolved oxygen, and subsequent accumulation of toxins. As a result, removing organic contaminants from wastewater becomes essential. Recent reports focuses on bioremediation as an environmentally friendly approach to remove hazardous contaminants for environmental sustainability and safety. Using microbial communities like algae, fungus, and bacteria, bioremediation has been utilized to remove, neutralize, and mineralize a variety of inorganic and organic pollutants from wastewater and polluted settings. Biofilm-mediated remediation has been an increasing domain of interest to challenge water pollution. Biofilm-forming bacteria can compete for resources, exhibit a higher tolerance to pollutants, and provide a protective habitat for cells. They have an impressive survival rate under harsh environmental conditions. Biofilm communities are suitable for the sorption and metabolism of heavy metals and organic pollutants. Because of their excellent capacity to eliminate contaminants, they have been utilized for wastewater treatment for the past several years. In this review, we primarily unearthed the underlying mechanism of biofilm formation with concomitant applications in reclamation of wastewater.

Keywords: Bioremediation, biofilm, heavy metals, waste water

Introduction

Despite making up more than 70% of the earth, just 3% of it is suitable for human consumption; the other 97% is made up of saltwater (M. Ahamed *et al*., 2019). The shortage of water affects nearly four billion individuals globally for minimum a month of the course of the year (Dongare *et al*., 2017).

One of the main issues facing contemporary civilization is access to safe and clean water since freshwater resources are becoming increasingly scarce due to a growing mismatch between supply and demand. Wastewater from diverse industrial and urban operations creates a severe hazard to ecosystems and the health of people since it includes compounds that are sensitive and toxic. Numerous ecosystems are contaminated when wastewaters comprising organic and inorganic contaminants from different industries are released into the environment. Therefore, prompt treatment of urban and industrial wastewater is a crucial area of study for the creation of technologies that will raise the quality of wastewater and satisfy environmental regulations (Tripathi *et al*., 2010). To address the ecological problems brought on by contaminated water, a number of scientists have focused their attention on creating innovative methods for treating polluted water (Xu *et al*., 2018). Physical procedures such as adsorption, coagulation, filtration via membranes and chemical techniques including ozonation, biological methods and oxidation have been established thus far to decrease wastewater discharge and manage pollutant hazards (Sharma *et al*., 2016). However, there is a significant energy need and operating cost associated with the chemical and physical processes. For environmental restoration, the use of biological agents—such as fungus, bacteria, and plants-is a low-cost, environmentally beneficial substitute for the presently employed chemical and physical methods. There are several ways to use bacteria for treating wastewater, including either deceased or being alive, suspended or embedded (Zhang *et al*., 2022). Bioremediation has become recognized as an environmentally acceptable technology for the removal of harmful chemicals for environmental safety and preservation due to the operational, budgetary, and start-up limits of some typical wastewater treatment plants (Prasad *et al*., 2012). As part of the bioremediation process, microbial populations, such as fungus, algae and microbes, are often neutralized, mineralized, decomposed, and frequently remove a variety of inorganic and organic pollutants from polluted environments and wastewater (Miranda *et al*., 2017). One popular remediation technique is biological remediation, which uses a wide range of bio-inoculants' metabolic capacities to improve and lessen environmental contaminants. It is safe for the environment, compatible, affordable, and effective.

Biofilm

A biofilm is an organized collection of bacterial organisms that have been immobilized in a synthetic matrix produced by extracellular polymeric substances (EPS)-producing microbes. EPS offers defence against

environmental threats, predatory protozoa, and contaminants on both biotic and abiotic surfaces. EPS is made up of lipids, proteins, polysaccharides, nucleic acids and surfactants (A. Maurya *et al*., 2020). Water, which makes up 97% of biofilms, is an essential component and significantly affects the movement of nutrients inside the biofilm matrix (Flemming *et al*., 2016). The thickness of the biofilm layer is around ten to thirty mm, whereas the matrix of EPS layer ranges from 0.2 to 1.0 mm. The majority of EPS (65–95%) and bacteria make up the remaining portion of biofilms; the Fourier Transform Infrared (FTIR) spectra show that proteins and polysaccharides make up the majority of biofilms (Mosharaf *et al*., 2018). Bacterial strain, nutritional availability, and environmental growth conditions all have a major role in determining the composition of EPS. Several methods have been identified as an affordable and sustainable alternative to environmental clean-up, including biofilm-mediated remediation. Microbial communities provide great potential for the restoration of any harmed ecosystem due to their extraordinary adaptability, usefulness, genetic variety, and cellular simplicity. Native microbial communities and microorganisms can use biomineralization, biotransformation, biosorption, and bioaccumulation to detoxify hazardous metal pollution. One of the several ecological remediation techniques, When it comes to the removal or refining of pollutants, biofilm-mediated remediation has been regarded as a reliable, efficient, and well-managed substitute for the use of planktonic microorganisms. Presently, it is employed as a novel remediation substitute for the biodegradation of environmental pollutants (Sandhya *et al*., 2022). Biofilm-based memory methods are less costly and easier to utilize than chemical and physical processes. The price of extracting components from waste is also included in the case of physiochemical methods. The bulk of research on clean-up using biofilms, however, have depended on civilizations of a single species, even though a wide range of bacteria are present in almost all biofilm ecosystems that exist which are connected with effective ecological remediation (A. Maurya *et al*., 2020). Considering that biopolymer is the main component of biofilm that are highly biocompatible and biodegradable, they may find use in the biomedical field, tissue engineering, food business & medicines. It is a a clean energy source since their basic ingredients are readily available, renewable, and produced in large quantities (Vinod *et al*., 2020).

Biofilm Formation

The process of creating and developing biofilms is intriguingly complicated and involves altered genetic genotype expression, transmission and physiology caused by signal molecules. Biofilms may form on both biotic

and abiotic surfaces in most humid conditions. It was shown that macromolecular accumulation is facilitated by moderate rhamnolipid and calcium ion concentrations, which has important implications for the regulation of film production (Huang *et al*., 2018). The phrase "nonselective adhesion" refers to the way in which bacteria attach themselves to transporters and other microbes via high-affinity adhesion features or extra surface structures (like filaments or pili); specific adherence is the term used to describe a specific linking proteins in the outer layer of bacteria that causes sticking to occur (Verstraeten *et al*., 2008). Most crucial elements in the formation and maturity of biofilms are the proliferation and accumulation of microorganisms. The declining biofilm state is the outcome of the biofilm thickening and nutrient transfer being hindered as the biofilm system works, which lowers biofilm activity and treatment effectiveness. The dissociation of old film caused by sloughing and erosion is necessary for the biofilm to begin to reactivate. The process of developing a biofilm comprises many key stages that have been identified (Huang *et al*., 2014).

Initial attachment first attachment happens when a substance's surface is exposed to aqueous liquids like blood or water and is then covered with polymers made of the medium. The conditioning layer is a naturally occurring layer that begins to form minutes after disclosure and continues to grow over several hours. The conditioning film cells cling quickly and effectively to hydrophobic, nonpolar surfaces like plastics. Additionally, early adhesion and colonization increase with surface roughness. This is as a result of larger surface areas on rougher surfaces (Donlan *et al*., 2002). In order to have an impact in the developing biofilm, planktonic microorganisms naturally adhere to this conditioning layer (Jensen *et al*., 2008).

Irreversible attachment the second step, Unchangeable Bonding when the microbes begin to produce additional polymeric material, the second stage, irreversible attachment (Flemming *et al*., 2007). Bacteria in the film are attached to the surface by the polyhydroxyl groups in EPS by hydrogen bonding (Kjelleberg *et al*., 2007). The bacteria are unable to escape from the outer layer at this time. It has been discovered that developed biofilms stay motionless until the end of their growth cycle (Kolari *et al*., 2001).

The Matrix of Cells

The EPS is composed of a wide variety of proteins, glycolipids, glycoproteins, and extracellular DNA. Highly hydrated biopolymers found in the extracellular polymeric material provide a matrix that holds water and holds the biofilm together. Nutrients are needed for biofilm organisms, and this matrix works with the surroundings to supply them. Micro-colonies form inside the EPS during the development's maturation phases. Operating as

pathways for oxygen and nutrients to pass and support the biofilm (Flemming *et al*., 2007).

Developing Maturity. The main objective of the biofilm in its mature phases is its three-dimensional development. This is done in addition to gathering trash from the nearby area, such as sand, and recruiting fresh plank tonic microorganisms. The biofilm also grows throughout these phases as a result of frequent reproduction taking place in the micro-colonies.

Dispersion. The last stage of development is called dispersal, or cell shedding. This stage might be referred to as "expansion". Although a lot of bacteria propagate passively from biofilms, sessile, matrix-encased cells of biofilm can also transform into free-swimming plank tonic microorganisms, leading to vigorous dispersion events (Webb *et al*., 2007), Quorum sensing, sometimes referred to as communication between cells, is necessary for biofilm propagation (Donlan *et al*.,2002). Only when communities of bacteria are able to recognize that their population is sufficient to produce the majority of QS can they employ it for dispersion activation.

Biofilm Reactor and Technology

Reactors for biofilms are classified according to the number of phases they include (gas, liquid, and solid) and whether or not the biofilm is connected to a transporter that is mobile or stationary inside the bioreactor. Additionally, they are divided into seven basic types based on the way that electron donors or acceptors are used.

1. Two-stage Methodology

a) Delivering biofilm-encrusted transporter and bulk water. When a bioreactor is used for denitrification, for example, Water is circulated through the fluidized bed biofilm reactor (FBBR), which serves as both an electron donor and an acceptor of electrons.

b) Because the electron source and acceptor are situated in the biofilm reactor, water flows past it. The transporter material filled with biofilm and water in general.

c) A described biofilm-loaded anode and a characterized cathode are water-filled space divided by a layer of proton transport barrier. As in a biofilm-based microbial fuel cell, the membrane has the source of electrons on one side and the acceptor of electrons on the other.

2. Triple-Phase Methodology

a) Stationary biofilm-loaded carrier, general water and air. Upward and

downward movement of air and water is critical for biofilm in the following phase, including TF.

b) A biofilm-loaded transporter, water in its bulk & air are all fixed place. Gas bubbles in the biofilm reactor circulate the water. Polystyrene beads are semi-fixed, while gravel is a fixed media, like biologically activated filters.

c) Bulk water, biofilm-loaded transporters, & air are transported. There is water in the bioreactor. Gas bubbles, such as those appears in an MBBR (aerobic moving bed biofilm reactor), are used to inject air.

d) A microporous hollow fiber membrane, such as the membrane biofilm reactor (MBfR) that circulates water and gas through its surface to the biofilm.

Because biofilms are present in both natural and artificial systems, they can be beneficial in the treatment of waste and municipal water. Suspended growth reactors and biofilm can produce uniform results for carbon oxidation, denitrification, desulfurization, and nitrification. Additionally, a variety of oxidizing contaminants have been treated using biofilm reactors. In both activated wastewater and biofilm systems, the same bacteria are in charge of the metabolic procedures, and they react in the same way to the surrounding variables (pH, temperature, macronutrient availability) [Henze *et al*., 2008].

Treatment of Waste Water through Biofilms

Biofilm systems provide a number of benefits over suspended growth systems when it comes to wastewater treatment. Reduced sludge production because of shorter hydraulic retention times, longer residence times for biomass, minimal space requirements, resilience to environmental changes, slower microbial development, and enhanced absorption of refractory materials, high concentrations of active biomass are some advantages of biofilm treatment methods (Brar *et al*., 2006). Additionally, biofilm approaches allow for more accurate control over response rates and population dynamics (Lazarova *et al*., 2000). The membrane immobilized cell reactor, airlift reactor, fluidized bed biofilm reactor, granular filter, high rate plastic media filter, trickling filter, and rotating biological reactor are a few of the biofilm reactor topologies used in wastewater treatment (Lazarova *et al*., 2000). Generally, consideration is given to the state of the base element when attempting to differentiate between moving and fixed bed operations. Systems with fixed beds are those in which the biofilm forms on stationary medium, such sponges, fragmented carriers, membranes, plastic particles, or pebbles (Lazarova *et al*., 2000). The fluid movement in the medium provides oxygen

and nutrients to the microorganisms. Moving bed systems are defined as any biofilm activity involving a continuously mobile media that is maintained by high air, mechanical stirring, or water velocity (Rodgers *et al*., 2003). It is important to take into account the biofilm carrier media's density, porosity, size, and erosion resistance (Christensson *et al*., 2004). Enhanced biological activity with a reduced reactor capacity can be facilitated by employing a material with a considerable specific area of surface (m2/m3). Reactor backwashing, which alters flow velocity or stirring strength, is typically used to control the thickness of the biofilm (Rodgers *et al*., 2003). Besides primary, secondary, and tertiary effluent treatment, biofilm methods have been effectively used to remediate industrial wastewater. Biofilms use removal strategies such as bioaccumulation and bioaugmentation to clean wastewater (Singh *et al*., 2006), biomineralization, biosorption, and biological degradation (Singh *et al*., 2006). Organic solvents and heavy metals can be efficiently bioabsorbed by components of the biofilm matrix (Guibaud *et al*., 2006). Reactors that use specific strains or naturally occurring microbial flora to remove carbon tetrachloride have been documented in the literature (Jin *et al*., 1998), along with systems that use mixed pharmaceutical industry waste (Rosen *et al*., 1998), pyrene and phenanthrene (Eriksson *et al*., 2002), alkanes (Yamaguchi *et al*., 1999), and the chlorophenols (Zilouei *et al*., 2006). These compounds are found in wastewater at significantly higher concentrations than the minimal amount required for both human and animal health. Biofilm reactors are crucial to environmental biotechnology, despite the fact that not all elements of their construction and functioning are well understood, biofilm reactors have been around since the early days of contemporary water treatment. Researchers employed continually dispersed sewage flow across a fixed bed (Corbett *et al*., 1903), it used a constant drip flow to medicate a medium coated in biofilm (Stoddart *et al*., 1911). The TF technique is said to have been created by these accounts. Over a century has passed since these reports were published, and in that time, effective developments in biofilm design, mathematical modelling, and scholarly understanding have given rise to modern and emerging biofilm reactors that are beneficial for applying initial-based management and design techniques to traditional biofilm reactors as well as preliminary-based design methodologies. Common compartments in all biofilm reactors enhance mass transfer and biochemical conversion. Biofilm reactors include five major compartments.

i) A wastewater or effluent distribution system.

ii) A containment structure.

iii) A biofilm carrier.

iv) A water or effluent collecting system.

v) A mixing system.

The common components of biofilm reactors are described in response to several biofilm reactors that are commercially available. There have been five documented biofilm reactors: the BAF, FBBR, MBBR, RBC, and TF.

Table-Bioremediation of pollutants from wastewater using biofilms

Type of waste water	Biofilms	Pollutants	Reference
wastewater that is salinized	MBR	COD and ammonium	(Yang *et al*., 2018)
Municipal sewage	MBBR	4-Naproxen, diclofenac, 17b-estradiol, and nonylphenol	(Abtahi *et al*., 2018)
Wastewater collected from petroleum storage facilities that was tainted with aromatic hydrocarbons	*Chlorella vulgaris*	Taking nutrients away	(Gao *et al*., 2015)
Municipal sewage	Microalgal biofilm	Nitrogen and Phosphorus	(Shayan *et al*., 2016)

Future Prospective

Further study is also needed, for example, to restore old biofilm utilizing affordable and environmentally friendly regulatory methods. Furthermore, People's interest in state-of-the-art biofilm reaction technologies and principles is increasing as the demand for cleaner energy sources and pollution elimination grows, using in situ monitoring methods and chemical biology technology to determine the properties of biofilm from a more microscopic viewpoint, as well as particular and useful ways to reduce the time that biofilm forms in refractive processes. The use of this method for treating industrial wastewater is constrained by the lack of techno-economic data on biofilm sustainability, dependability, and system performance. Additionally, several components of wastewater discharge, such as suspended particles, hinder the biofilm's ability to function by preventing substrate and oxygen from diffusing into the biofilm.

Conclusion

As the quantity of harmful xenobiotics in wastewater has increased, many bioremediation techniques have been developed to get rid of them. Over the last several years, biofilm-mediated reduction of contaminants from wastewater has been utilized extensively as one of the prospective strategies. Under this method, quorum sensing facilitates the flow of metabolites, genetic

material, and signaling molecules between bacterial cells of different strains. Understanding the variety of bacteria in biofilms through the use of metagenomic techniques. Globally, the application of microbiome-based bioremediation methods is rising significantly and the production of genetically engineered biofilm consequently boosts the removal of contaminants. Quorum sensing technology is used in businesses to filter contaminants out of wastewater. It controls the production of biofilm, EPS, and biosurfactants, which may effectively remove heavy metals and organic compounds from wastewater. Quorum sensing and quenching in wastewater treatment facilities are in charge of biofouling, granulation, aggregation, colonization, organic material metabolism, and nutrient removal. In summary, this strategy would provide a crucial knowledge of how many physiological, environmental, and mutational variables impact the propensity to form biofilms, as well as how these elements consistently interact with one another. It is necessary to encourage studies on various types of biofilm for waste water management.

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Chapter - 9
Role of Brain Derived Neurotrophic Factor (BDNF) Polymorphism (rs6265) on Alzheimer's disease

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Chapter - 9

Role of Brain Derived Neurotrophic Factor (BDNF) Polymorphism (rs6265) on Alzheimer's disease

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The most prevalent kind of dementia is Alzheimer's disease which is multifactorial. Pathological aspects of Alzheimer's disease include the presence of neurofibrillary tangles, amyloid plaques and neuronal loss. BDNF plays a critical role in Alzheimer's disease, through encouraging neuronal survival, sustaining synaptic plasticity, and preserving cognitive functions. Differential severity and progression of Alzheimer's disease may be caused by genetic variation in the Brain Derived Neurotrophic Factor (BDNF) gene. We looked at the BDNF rs6265 Val66Met polymorphism in AD patients to see if there was any relationship between this polymorphism and progression of the disease.

Patients were selected from the Bangur Institute of Neurosciences' Cognitive Disorder Clinic based on clinical and neuroimaging results (brain MRI or CT scan), as well as family member's medical history.

For this study, a total of 60 patients and 50 normal controls were enrolled. Out of these, twenty-two patients have a strong family history. With the patients' consent, 5ml venous blood samples were used to isolate DNA. Identification of BDNF alleles were done by Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism. For sixty patients, six-month follow-up data was available. In our study, we found that the BDNF polymorphism proportion for Alzheimer's disease patients was GG 47%, GA 35%, and AA 18%, whereas for control subjects, it was 84% GG and 16% GA. There were more male AD patients than female patients (37 males against 23 females). We have found that age of disease onset, disease severity, and disease progression are related to at least one an allele in the BDNF gene.

Strong family history of the patient further confirmed the findings. These results suggest that BDNF rs6265 polymorphism plays a crucial part in early-onset AD with more unusual clinical characteristics.

Chapter - 10
Heavy Metal Contamination and Remediation: A Critical Review

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Chapter - 10

Heavy Metal Contamination and Remediation: A Critical Review

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Abstract

Heavy metal contamination poses a significant threat to environmental and human health. The release of toxic metals like lead, mercury, arsenic, and cadmium into soil, water, and Air has severe ecological and health impacts. This review article discusses the sources, effects, and Remediation strategies for heavy metal contamination. We examine the conventional and Innovative methods for heavy metal removal, including phytoremediation, bioremediation, Chemical precipitation, and advanced oxidation processes. The advantages and limitations of each Approach are critically evaluated. Furthermore, we highlight the need for sustainable and cost-Effective solutions to mitigate heavy metal pollution. Future research directions and policy Implications for effective heavy metal remediation and management are also discussed. This Review aims to provide a comprehensive understanding of heavy metal contamination and Remediation, contributing to the development of efficient strategies for environmental protection and public health safety.

Keywords: Remediation, contamination, heavy metal, pollution etc.

Introduction

The rapid industrialization and overpopulation have led to a significant increase in xenobiotic components, including metals, in the environment, which can lead to human and environmental health issues. Heavy metals are a group of metals and metalloids with an atomic density over 4000 kg/m3. They are toxic to humans even at low concentrations. Examples include copper, cadmium, zinc, chromium, arsenic, boron, cobalt, titanium, strontium, tin, vanadium, nickel, molybdenum, mercury, and lead. These heavy metals are essential for plant growth but can be harmful when their concentrations exceed permissible limits [3]. Other heavy metals, such as lead, mercury, cadmium, and arsenic, are not essential for plant and animal growth. Soil pollution from

heavy metals can occur from industrial wastewater, sewage sludge, fertilizers, treated wastewater use in land applications, and soil mineral weathering [2]. Heavy metals, even at low concentrations, affect plants, soil, humans, plants, and animals due to their toxic nature. Air, water, and land pollution can significantly impact the quality of soil and water resources, while soil pollution occurs when pollutants like heavy metals, hydrocarbons, and pesticides are released or disposed of. Water pollution can contribute to soil pollution and vice versa, and harmful substances from wastewater can permeate into the soil from water bodies, posing serious consequences on human health and the environment. Wastewater often contains toxins such as cleansers, pesticides, prepared nourishment, drugs, and organic/inorganic toxins, such as heavy metals and organic/inorganic salts. The improper disposal of wastewater into the environment contaminates the soil, and there is an urgent need for these metals to be separated from wastewater to make it reusable [1]. Traditional innovations for effective remediation include substance precipitation, surface adsorption, film filtration, particle trade, photocatalysis, and flocculation. However, the most capable method to remove heavy metals from wastewater is adsorption on nanomaterials, as other methods do not adequately remove such heavy metals. It is crucial to deploy advanced and site-specific remediation technologies that can effectively and safely remediate soils polluted by heavy metals. In recent decades, different soil remediation technologies have been implemented, focusing on decreasing the maximum and/or bioavailable heavy metal concentrations in the soil and their possible mechanisms in the food supply chain. However, these methods are expensive, environmentally harmful, and time-consuming, making soil clean-up a difficult job [9, 10].

This review examines the various technologies currently available for heavy metal remediation in terms of mechanisms, advantages, drawbacks, applicability, and costs. It also examines the methods for treating heavy metal-contaminated soils using physicochemical, biological, and techniques that combine these approaches, as well as the potential toxicity of metals after treatment, the interaction of metals with soil constituents, the difficulties of using this modification for site remediation, and potential opportunities. The study explores the origins and risks of heavy metals in the soil and addresses methods and influencers for microbial remediation, offering avaluable guide for restoring the health of the soil ecosystem. The data used to determine the most useful remedial technology for the treatment of contaminated soils will be of great use to industrial site owners, farmers, and urban gardeners.

Impact on Soil and Plants

Heavy metals are dangerous contaminants that can easily aggregate in soils, dust, and water, posing serious environmental impacts. They can be transferred to surrounding water environments, causing hazardous effects on agricultural products and food accumulation [4]. Remediation methods for extracting heavy metals involve converting them into various forms, with exchangeable metals and aqueous solvents being suitable for plants due to their toxic conditions. Soil contamination by heavy metals has occurred through man-made activities at millions of sites with 20 million hectares of land [5]. This pollution is of crucial importance due to the potential consequences on human and environmental life due to rapid industrialization and urbanization. Cultivation through industrial effluents has indirectly contributed to contaminated agricultural land but also indirectly contributed to crop growth. The evaluation of remediation strategies used to clean up polluted soil found that the efficiency of these procedures was below expectations. The multiple technical and economical components of remediation make it difficult, making typical soil remediation methods ineffective on polluted areas. Heavy metal removal from polluted soil is an expensive and time-consuming technique, and the additional pollutants released into the environment by these clean-up approaches are limited [11].

Heavy metals contribute to soil pollution, impacting food generation, quality, and well-being. Some heavy metals are poisonous to plants even at low concentrations, while others accumulate in plant tissues to moderately abnormal states without noticeable side effects or decreased yield. Growing plants in contaminated areas changes their metabolism, physiological, and biochemical means, leading to metal accumulation, lower biomass generation, and reduced growth. Research on the toxicity of heavy metals in plants must consider the perpetual impacts caused by contaminated soils and their sub-atomic behaviors [7].

The harmfulness of a heavy metal depends on its oxidation state, with Cr (VI) being the most poisonous type and often related to oxygen. Cr (III) is less portable and less harmful, and is more prevalent in organic matter in soil and aquatic systems [31, 32]. The ability of heavy metals to survive in the soil in the form bioavailable to roots is affected by their adsorption, desorption, and complexation in the soil matrix, which are strongly affected by soil composition, pH, and structure. Heavy metal poisonous quality is species-dependent, with metal-tolerant plants and hyper accumulators having barrier mechanisms to avoid harm caused by heavy metal-initiated pressure [8].

Impact on Humans

Heavy metals, such as copper, cadmium, and zinc, are commonly used in various industries and can lead to various health issues. Exposure to high levels of these heavy metals can result in gastrointestinal and renal toxicity, cardiovascular issues, tumors, hematic, melancholy, tubular and glomerular dysfunctions, and osteoporosis. New born children, kids, and young people are particularly susceptible to these harmful effects, leading to formative difficulties and low insight. The majority of heavy metal contaminations come from Industrial wastewater, including mining, pharmaceuticals, electroplating, rubber and plastics, metal finishing, tanneries, organic chemicals, pesticides, and timber and wood items. These heavy metals are transported by runoff water and degrade water sources due to industrial activities. Every living organism, including plants, animals, and microorganisms, relies on water for life. Toxic metals can attach to the surface of microorganisms through bioaccumulation activities and can be chemically changed as they digest food materials. Copper is an essential element for human health, essential for haemoglobin production in red platelets, and is found in enzymes that transport oxygen. It also functions as a micronutrient for humans. However, high doses of copper can be dangerous to living organisms, entering the body through food, residue, and water. The lack of copper can cause anemia, bone and cardiovascular issues, weakening in mental and sensory systems, defective keratinization of hair, decreased levels of synapses, dopamine, and norephedrine and imperfect myelination in the brain stem and spinal line [38, 39, and 40].

The food chain is a major pathway for human exposure to soil pollution, with heavy metals being a significant concern due to their potential health risks in humans and animals. These heavy metals are poisonous and can cause serious harm even at low concentrations. The exposure of heavy metals to food items like juices, beverages, and wines can lead to health issues such as cancer, developmental retardation, kidney damage, immunological impact, endocrine disruption, and neurological impacts.

Ingestion of copper-polluted water or foods can cause intense gastrointestinal side effects, and high amounts of copper salt can cause queasiness and intense gastric pain. In severe cases, copper exposure can lead to suicide attempts. Ingestion of excessive copper can result in seizures, laziness, and cerebral pain, with subsequent side effects including epigastric pain, gastrointestinal bleeding, diarrheal, vomiting, tachycardia, haematuria, respiratory problems, haemolytic anaemia, liver damage, kidney damage, and death. Wilson's disease patients may have increased levels of copper in their

liver during childhood, which typically develop between the ages of 6 and 40 years and can lead to liver failure if untreated [19].

Cadmium (Cd) is a widely used metal in various industries, including batteries, ceramics, electronics, and textiles. It is found in air, water, and soil near industrial sources, particularly nonferrous mining and metal handling activities. Cd is a highly harmful metal that can accumulate in the human body and cause irreversible harm to various biological systems even at very low concentrations. It is more effectively retained in aviation routes than in the gastrointestinal tract. Tobacco smoking is a common source of cadmium in non-contaminated areas, with one cigarette containing around 1 to 2 go Cd, depending on the type, brand, and production area. The human body is bound to metallophones, with over half of Cd in the human body accumulating in the liver and kidney due to their ability to orchestrate metallophone. Cd can be disposed of through urine, but the amount of Cd discharged daily in urine is extremely limited [16].

Zinc (Zn) is generally uncommon in nature but has a long history of use due to its availability in restricted deposits and ease of extraction from ores. It is mined and delivered in more than 30 countries and ranks fourth among the metals worldwide in annual usage. The principal ore, ZnS, is available worldwide alongside lead deposits and oxidizes quickly to produce various optional minerals, such as ZnCO3. Trace metals like Ga, Ge, and Cd related to ZnS minerals are separated during extraction operations. Most zinc-lead deposits are located in strata-bound stores in carbonate rocks and are usually found along and inside dolomite and calcite minerals. The most industrial use of Zn comes from its chemical and metallurgical characteristics. The biggest application of Zn is in galvanizing iron and steel items, which gives a corrosion-resistant coating. These items are used in construction, siding, apparatus housings, office hardware, heating andventilation conduits, and vehicle and building enterprises for roofing, vehicle door boards, and underbody parts. Zinc is substituted by copper and plastic items in residential plumbing systems. New combinations, such as zinc-aluminum alloy, have been created as protective coatings. Zinc oxide is also used for cosmetics, photocopy paper, agricultural products, paints, and medicinal products. Zn dust, a finely isolated type of the metal, is used in separating silver and gold from the cyanide solution, coloring textile materials, printing, and fat purification. Climate-safe paints made from zinc oxide and zinc dust provide a strong and durable coating on exterior surfaces. Zn is generally a basic micronutrient and less dangerous metal.

However, an extreme level of Zn consumption, including breathing Zn vapors and eating Zn-defiled foods and water, can cause system dysfunctions, causing growth and proliferation disabilities. Zinc is released into the environment mainly due to industrial operations such as steel manufacturing, mining, coal burning, and waste burning. Zinc poisoning can result in acute symptoms such as diarrhea, liver failure, bloody urine, icterus, kidney failure, stomach cramps, abdominal cramps, epigastric pain, nausea, and vomiting, as well as chronic symptoms such as pancreatic damage, anemia, and lower levels of high-density lipoprotein cholesterol. The BIS recommends a permissible level of zinc in drinking water of 5 mg/L [26, 27, and 28].

Impact on Aquatic Environments

Heavy metals release into aquatic systems can result in physical, chemical, and biological processes. These effects can be classified into two general categories: the effects of heavy metals on the environment and the effects of the environment on the heavy metals. The first classification depends on natural conditions, such as diversity, density, species composition, and community structure. The nature and degree of change depend on the concentration of heavy metal species in the water and dregs. The second classification stresses that conditions in receiving waters may prompt an adjustment in the speciation and harmfulness of heavy metals. These conditions include differential contribution of anthropogenic and geochemical material, nature of industrial effluents, and suspended solids and concentration of chelators. The aquatic environment is described by longitudinal varieties in colloidal particles, suspended solids, and natural/synthetic ligands, and vertical varieties in redox conditions, level of mixing, and densities of living life forms. The effects of heavy metals on aquatic plants are profoundly variable [23, 24, and 25].

While characteristic responses, such as diminished diversity and density of populations, mostly occur in exceedingly defiled zones, more conflicting impacts occur in intolerably or softly contaminated regions. The population responses to toxic heavy metals are impacted by the varieties in common natural parameters, such as light and temperature. The release of heavy metals can also create changes in physical conditions in the receiving aquatic environment, such as changes in water pH, organic content of the substrate, and size of particles in water. Plants in aquatic systems react to these problems by a decline in density, diversity, and composition of species [6].

Plants are sessile life forms that must adapt to different soil compositions for their living and reproduction. Many metals share their basic lethality

mechanisms, and plants manage these metals through general removal routes. The effects due to metal toxicity are made more complex by competition, as elevated amounts of one metal may imbalance the removal and movement of others, leading to toxicity behavior. The Increase' number of targets for the toxicity of heavy metals implies that negative impacts tend to be seen in cells exposed at first (cells responsible for metal uptake). Heavy metals interfere with ionic homeostasis and the activity of enzymes, and these impacts are evident in physiological processes such as germination, development, photosynthesis, plant water balance, essential digestion, and multiplication. In conclusion, heavy metal pollution has significant physical, chemical, and biological effects on aquatic ecosystems.

Treatment of These Toxic Metals and Future Aspects

Heavy metals pose serious health risks to humans and other living creatures due to their bioaccumulation in human food chains and increased concentration in aquatic systems. These heavy metals can be found in industrial wastewater, agricultural runoff, household, and commercial applications. Various treatment methods are available for the removal of toxic heavy metals from water/wastewater, including chemical precipitation, chemical coagulation and flocculation, electrochemical methods, membrane filtration, ion exchange, bioremediation, and adsorption [14].

Chemical precipitation is widely used for the removal of heavy metals from wastewater due to its affordability and ease of operation. The pH of the wastewater is adjusted to the basic conditions, and the precipitating agent reacts with the heavy metal ions to form insoluble precipitates. These precipitates can be separated through sedimentation or filtration operations. Traditional chemical precipitation processes include sulfide precipitation and hydroxide precipitation. Hydroxide precipitation is the most widely used chemical precipitation technique due to its low cost, simplicity, and easy control of pH.

However, hydroxide precipitation has limitations in industrial applications due to the production of large volumes of low-density sludges, which create dewatering and disposal issues. Some metal hydroxides are amphoteric, and mixed metals can cause issues when the ideal pH for one metal may return another metal into the solution. Additionally, complexing agents in wastewater can restrain metal hydroxide precipitation.

Sulfide precipitation is also a successful procedure for treating harmful heavy metals due to its lower solubilities and non-amphoteric nature. It can achieve high levels of metal removal over a wide pH range and exhibits

preferable thickening and dewatering qualities over hydroxide sludges. However, there are potential perils associated with sulfide precipitation, such as the increase of dangerous hydrogen sulfide vapour in acidic conditions and the need for a large amount of sludge generation, increased sludge disposal costs, slower metal precipitation, poor settling, and long-term ecological effects of sludge disposal [20,21].

Coagulation/flocculation is another method for treating heavy metal-contaminated wastewater. It destabilizes colloidal particles using a chemical coagulant and results in sedimentation. The process involves destabilizing the unstable particles into massive floccules, which are then settled in the sedimentation tank. Many coagulants, such as alum, ferric chloride, and ferrous sulfate, are commonly used in traditional wastewater treatment technologies [29]. Coagulation is a crucial technique for wastewater treatment, but it cannot completely remove heavy metals from wastewater. Macromolecule flocculants have been effective in removing heavy metals from wastewater, but they have limitations, such as high operational costs due to excessive chemical utilization.

Electrochemical methods are highly effective wastewater treatment methods, particularly for the removal of heavy metal ions from industrial wastewater. These methods involve the recovery ofheavy metals in the elemental metallic state using anodic and cathodic reactions in the electrochemical cell. However, these methods require significant capital investment and more power supply, which limits their wide industrial applications. Due to stringent environmental rules and regulations, electrochemical methods have attracted many scientists and researchers to work in this research field [17, 18].

Electrocoagulation is a widely used method for the removal of heavy metals from wastewater, involving the production of coagulants in situ by dissolving iron or aluminium ions from iron or aluminium electrodes. This process produces hydrogen gas, which is used to float the flocculated particles from the water. It has been applied for the removal of heavy metals, dyes, fluorides, nitrates, sulfates, pharmaceuticals, and phenolic compounds from wastewater. Electrodeposition is another widely used method for the recovery of toxic metal ions from wastewater, with advantages such as the purest form of metal, low operating costs, and no problems in sludge disposal. Electro flotation is a solid-liquid separation process that floats toxic heavy metals to the water surface by small bubbles of oxygen and hydrogen gases produced from water electrolysis [33].

Membrane filtration treatment methods have shown excellent results for the removal of heavy metals from wastewater. Membranes are complex structures with dynamic elements on the nanometer scale. The recent reverse osmosis system uses homogeneous polymer thin films bolstered by a permeable support structure. The main advantage of membrane filtration is higher removal efficiency, lesser space requirement, and easier operation [34, 35].

Reverse osmosis consists of a semi-permeable membrane that allows water to pass through it and rejects toxic heavy metals from the wastewater. However, it has limitations such as handling rejection, membrane fouling, and high power cost. Ultrafiltration operates at low transmembrane pressures for the recovery of dissolved and colloidal solids, and effective removal of heavy metal ions has been achieved through polymer enhanced ultrafiltration and micellar enhanced ultrafiltration methods [15].

Nanofiltration is the intermediate operation between reverse osmosis and ultrafiltration, offering high efficiency, lower energy requirements, reliability, and easy operation. Electrodialysis is the type of membrane operation for the separation of ions from one solution to another solution using charged ion exchange membranes under an electric field. The anion-exchange and cation-exchangemembranes are the two types used in applications. Despite their success in removing toxic heavy metals from wastewater, membrane treatment methods face challenges such as complexity, higher cost, membrane fouling, and lower permeate flux [36, 37].

Adsorption is a widely used and efficient treatment method for the removal of heavy metals from wastewater. It involves a solid-liquid mass transfer operation, where the heavy metal (adsorbate) migrates from the wastewater to a solid surface (adsorbent) and is bonded due to chemical or physical adsorption. The process can be reversible, allowing the adsorbent to be regenerated using proper desorption methods. Activated carbon (AC) is a popular adsorbent for removing heavy metals from wastewater due to its higher surface area and affinity towards heavy metals. AC is typically produced for treating organic pollutants but can also be used for wastewater treatment. There are two types of commercially produced activated carbons: H type (activated at high temperature and removes H+ ions) and L type (oxidized at low temperature and removes OH-ions). GAC is mainly used in batch and column mode for heavy metal removal from wastewater. However, PAC may not completely recover the adsorbent for regeneration [12, 13].

The important surface properties of AC, such as surface area, functional groups, surface charge, size, and porosity, are crucial for the removal of heavy metals from wastewater. However, the application of AC is restricted due to its high cost and limited regeneration behavior. To overcome this issue, waste materials from agricultural operations can act as an alternative material to AC for heavy metal-contaminated wastewater treatment. These materials offer advantages over conventional materials, including lower costs, higher efficiency, higher adsorption capacity, easy operation, no sludge production, and the possibility of regeneration and recovery of attached metals from the spent adsorbent.

Role of Microbes in Heavy Metal Treatment

Algae, unicellular or multicellular organisms found in terrestrial, fresh, and marine environments, have been effectively utilized in the removal of organic and inorganic pollutants from wastewater. Algae cell walls consist of functional groups such as amino, hydroxyl, carboxyl, and sulfate, which act as the main binding sites for the removal of heavy metals from wastewater. The properties of the algae, characteristics of the heavy metals, and wastewater characteristics mainly affect the removal of heavy metals by the algae. Some major types of algae, including green microalgae (in freshwater) and macroalgae (marine green, red, and brown), have been successfully applied for the removal of different kinds of heavy metals from wastewater. Heavy metals generally enter the cell wall of the algae (rapid process) followed by entering the inside of the cell (slow process). Some green microalgae species, such as *Chlorella Vulgaris, Chlorella miniata, Chlamydomonas reinhardtii, Cladonia rangiformis, Fucus spiralis, Laminaria Hyperborea, Sargassum filipendula, Sargassum wightii, Sphaeroplea, Turbinaria conoides,* Spirulina platensis, etc., have been successfully adopted for the removal of toxic heavy metals from the wastewater [40].

Bacteria(*Escherichia coli, Bacillus cereus, Pseudomonas fluorescens, Bacillus cereus, Bacillus firmus, Kocuria rhizophila, Bacillus licheniformis, Micrococcus luteus, Cupriavidus metallidurans, Staphylococcus xylosus, Bacillus megaterium, Enterobacter cloacae, Streptomyces rimosus, Pantoea agglomerans, Salmonella typhi, Pseudomonas aeruginosa, Ochrobactrum intermedium, Desulfovibrio desulfuricans)* and fungi *(Aspergillus niger, Aspergillus sydoni, Ganoderma lucidum, Cephalosporium aphidicola, Pleurotus sapidus, Tolypocladium inflatum, Neurospora crassa, Trichoderma longibrachiatum, Saccharomyces cerevisiae, Mucar rouxii, Penicillium janthinellum, Rhizopus arrhizus, Rhizopus oryzae, Saccharomyces cerevisiae,*

Lepiota hystrix, Aspergillus brasiliensis, Aspergillus flavus, Penicillium cirtinum, Penicillium oxalicum, Aspergillus terreus, Pleurotus platypus, Phanerochaete chrysosporium, Penicillium citrinum, etc) species have also been effectively utilized for the treatment of heavy metal-contaminated wastewater.

Bacteria are single-cell organisms with a membrane-bounded nucleus or organelle similar to chloroplasts and mitochondria, while fungi are eukaryotic organisms with tubular fibers called hyphae, which have adequate functional groups to remove heavy metals from wastewater. Most biomasses have the capacity to remove heavy metals from wastewater, but they cannot act as alternative materials in real industrial wastewater treatment. Biosorption is the passive uptake of heavy metal ions by microorganisms, which occurs on the cell wall and can be carried out by fragments of cells or dead biomass. This process involves several processes, including electrostatic interaction, ion exchange, precipitation, the redox process, and surface complexation. Biosorption can occur through fragments of cells and tissues, dead biomass, or living cells as passive uptake via surface complexation onto the cell wall and other outer layers. Bioaccumulation is the active uptake of heavy metal ions across the cell membrane into the cytoplasm through the cell metabolic cycle. This process is dependent on various physical, chemical, and biological mechanisms, including intracellular and extracellular processes. Organisms that accumulate heavy metals must have a tolerance to one or more metals at higher concentrations and exhibit enhanced transformational abilities to change toxic chemicals to harmless forms [41]. Metal uptake mechanisms by various biosorbents depend on the cellular surface of microbes, as well as the exchange of metal ions and complex formations with the metal ions on the reactive chemical sites of the cell surface. Cell wall components vary among different microorganisms, with Gram-positive bacteria having a peptidoglycan layer and Gram-negative bacteria having a polyelectrolyte cell wall. Fungi have rigid cell walls made up of chitin, inorganic ions, lipids, nitrogen-containing polysaccharides, polyphosphates, and proteins. They can tolerate and detoxify metal ions by active uptake, extracellular and intracellular precipitation, and valence transformation. Algae have cell walls composed of cellulose, sulfonated polysaccharides, and other binding sites. Non-essential metal uptake usually consists of transporters committed to the acquisition of vital organic and inorganic ions. Microorganisms can secrete metal-binding metabolites, produce extracellular polymeric substances, and biofilms [42, 43].

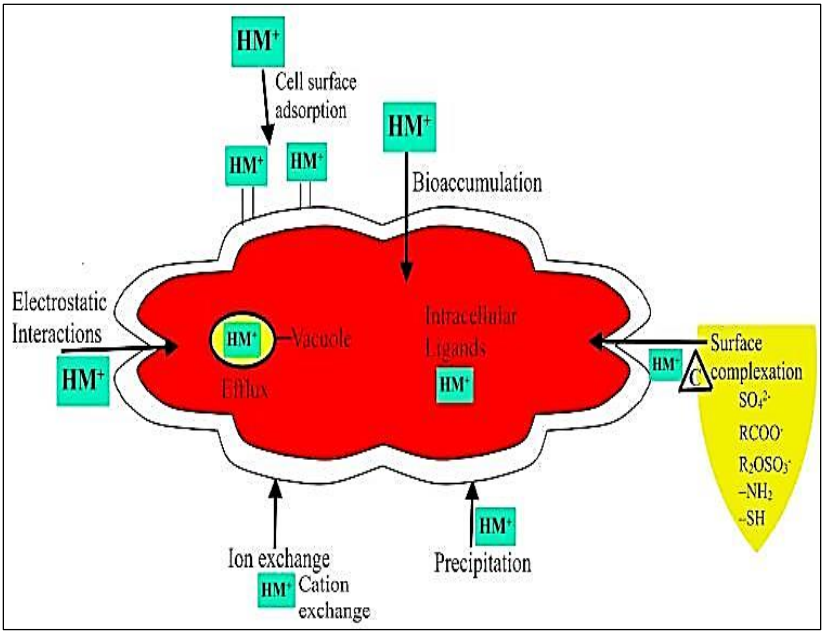

Fig: Mechanisms of heavy metal uptake by microorganisms
Source: https://images.app.goo.gl/6WPebHgidSxZYvqM9

Conclusion

The importance of low-cost adsorbents for the removal of toxic metals from wastewater has been highlighted, with various industrial and agricultural wastes studied extensively. However, the efficiency of remediation strategies used to clean up polluted soil is below expectations due to the multiple technical and economical components of remediation. Heavy metal removal from polluted soil is expensive, time-consuming, and limited in the release of extra pollutants into the environment. Biosorption is a promising technology that exchanges metal species in aqueous medium for a counter ion attached to biomass. The promising potential of biosorption technology depends on the efficiency of various microbial and plant-based biomass. Active binding sites on the peptidoglycan and polysaccharides components of the cell wall of biomasses contribute to higher metal uptake. Despite the market demand for cheaper and greener treatment technologies, biosorption technology has limited industrial adoption even when used in conjunction with other conventional treatment approaches. The presence of multi-functional groups on the biomass surface is responsible for its non-selectivity to a particular metal ion, which is a major hindrance to its commercialization. Future research directions for biosorption technology involve the identification and

designing of better and more selective biosorbents, more development of biosorption models and mechanisms, and further assessments of the costs of development.

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