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Message from Editors

Department of Biotechnology, Jaypee Institute of Information Technology, Noida, UP, India organized 5th International Conference Advances in Biosciences and Biotechnology (ICABB22), themed on Innovations in Life Sciences and Computational Biology. It was held virtually from the campus of JIIT Noida, during 20th-22nd January 2022. It was initially planned in hybrid mode, but due COVID, the conference was shifted to the virtual platform as the pandemic kept all the researchers and scientists at their places.

On this platform, bright minds from all over the world met and shared their researches. 181 abstracts were presented in total, in oral and e-poster forms. 11 eminent speakers from the India and abroad shared their valuable experiences and knowledge with the scientific community. The conference had speakers from reputed organizations and Universities, such as, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare, Govt. of India; University of Paris, France; University of New South Wales, Australia; The University of Newcastle, Australia; Jawaharlal Nehru University, New Delhi, India; University of Delhi, Delhi, India; Indian Institute of Technology Bombay, India; Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy, Govt. of India; CSIR-CIMAP Research Centre, Bangalore, India. It was a perfect platform for gaining and exchanging valuable knowledge from eminent speakers and fellow young scientists, and a paradise for getting inspired from major scientific advancements witnessed by all. This edition of the conference "ICABB22" featured many e-short presentations and oral presentations on the themes of Computational Biology and Applications, Medical and Microbial Biotechnology, Food, Agriculture and Natural Products, Environmental and Industrial Biotechnology, Nanotechnology and Drug Delivery.

The three days of the conference witnessed many ground-breaking ideas from young talented minds. Though, the pandemic kept us apart, the high spirits and enthusiasm during the conference proved that scientific knowledge can be gained and shared irrespective of distances.

The participants were further invited to submit full articles for publication in the journal. 31 articles were selected for publication in two issues. The present issue of the journal includes the selected articles from the broad stream of Microbial and Environmental Biotechnology. It majorly focuses on application of microbes and other technologies for biomedical and environmental concerns. Consequent issue for August, 2022 will carry selected articles from the participants on the area of Biomedical Sciences and Computational Biology.

The organizing team is grateful to the eminent speakers, reviewers, student volunteers, all the presenters and attendees for making this conference a success. We would like to express our gratitude to the peer reviewers for their valuable suggestions, corrections, improvement and constructively critical comments, which helped in increasing the quality of the publications. In the end, we will like to end this note off with a quote, "*Our world is built on biology and once we begin to understand it, it then becomes a technology*" — Ryan Bethencourt.

Prof. Pammi Gauba
Chairperson

Prof. Rachana
Convener

Dr. Shazia Haider
Convener

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Biomedical Waste Production and Its Safe Management During COVID-19 Pandemic in India and Worldwide: Challenges and Management Strategies

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ABSTRACT

Arrival of COVID-19 pandemic has created a great drop down in human health, the country's economy and environmental health, for almost every nation. The sudden outbreak in COVID-19 in India and all over the world has generated massive pollution because of biomedical and plastic waste which has created challenges and is a matter of concern for waste management strategies especially in developing countries. During the first and second wave of COVID-19, hospital discharge such as PPE kit, gloves, antibiotics and unused medicine etc. has created massive environmental pollution. Safe disposal of biomedical and plastic waste is very important otherwise, this will lead to another pandemic i.e., waste pandemic. There is a positive point about this pandemic that it has led to more awareness towards environmental protection. In this situation it has now become more necessary to manage the biomedical waste and plastic pollution to protect our environment. This review article reports, country wise waste production before and during the COVID-19 pandemic, its occurrence and states where it is successfully managed throughout the world. This article also summarizes the regulatory framework for COVID-19-based biomedical waste management strategies in India.

Keywords: COVID-19, Biomedical Waste, Plastic Pollution, Waste Management, Environment.

1. INTRODUCTION

COVID-19 is considered as one of the most dangerous diseases ever to hit the earth. The first COVID-19 incident happened in Wuhan, China in December 2019, and thereafter COVID-19 infection spread to all countries around the world [1,2]. The World Health Organization (WHO) quickly designated it as a "Public Health Emergency of International Concern". Populations all around were affected by the transmission from patients and health-care professionals since the middle of December 2019. This infection was spread through close contact from human-to-human [3]. To control the disease, most of the countries have enacted city-wide lockdowns and quarantine procedures. The governments put in a lot of effort such as detection of positive cases and retrospective investigations of patient groups. As of January 23, the Chinese government imposed a strict lockdown on Wuhan and its adjacent areas. Flights, railroads, and public transportation were suspended, and industries, restaurant and entertainment businesses were also shut down [4]. Early in 27 January 2020, the Indian government also issued a travel alert for visitors and began screening them. In response to the global COVID-19 outbreak, India's Prime Minister declared a Janata curfew on March 22, 2020.

Rising medical technologies and advanced hospital facilities have resulted in an increase in the amount of waste produced by healthcare organizations. All wastes from any medical procedure, research institutions, and laboratories are referred to as "Health Care Waste" or "Bio-Medical Waste" (BMW). The inappropriate management of BMW has resulted in a number of problems such as the spreading of infectious diseases and many types of environmental contamination [5]. Apart from the health concerns, BMW can also release highly toxic gasses and pollutants during their incineration which also have a negative influence on human health and the environment. To address the problems associated with BMW in India, the Bio-Medical Waste (Management and Handling) Rules, 1998 were formulated under the provisions of the Environment (Protection) Act 1986. All persons that generate, collect, receive, store, transport, handle, or dispose of BMW, are subject to these rules. In order to reduce environmental impact, it was amended in 2016 and again in 2018 to improve segregation, transportation, and disposal [6].

COVID-19 epidemic has impacted negatively on human life in all ways such as healthcare system, environment, economy, and society. To control the spread, India took several measures apart from quarantine, they did establishment of quarantine camps, increased the manufacturing of personal protective equipment (PPE) kits, face masks, hand sanitizers, and surface disinfectants. Due to sudden increase of COVID-19 virus, patients and doctors generated high number of BMW [7]. Therefore, highly used PPE kit, its decontamination and recycling are highly required to save the environment. These problems have posed a number of challenges to current BMW legislation and management practices. Plastic and biomedical waste has to be disposed off properly; otherwise, waste will lead to waste pandemic in future [7]. As per WHO report, developing nations have to manage proper collection and disposal of HCW (health care waste) which includes trash generated during human or animal testing, diagnosis, treatment, or vaccinations [10]. In this context, India is segregating and managing waste by BMW Management Rules and policies [11].

2. WORLDWIDE BIOMEDICAL WASTE GENERATION BEFORE AND DURING THE COVID19 PERIOD

It is reported that during the COVID-19 period in Wuhan, China, healthcare waste creation was increased by 60 % [12]. Several studies [13,16] have highlighted the management of BMW during this pandemic as a critical problem, which can enhance the risk of further spread of infections in upcoming days. Moreover, from 2016-2019 PPE kit manufacturing increased by only 6.5 %, while during the pandemic period, WHO reports about 40 % monthly increase in PPE kit production [17]. To address the extremely transmittable nature of COVID-19 virus, an unexpected demand for PPE kits and other types of single-use medical-care equipment has resulted in the huge generation of biomedical waste [17]. During the pandemic, per capita, daily disposal of a single-use face mask generated about 66 kT of Y-BMW (yellow biomedical waste) within a year [18]. Therefore, the world's waste management systems have been disrupted by such a dramatic increase in the volume of waste during the COVID-19 pandemic. As per reported data, biomedical waste production increased six fold (240 tons per day) in Wuhan, China, during the peak of the pandemic. As a result, China's regional governments decided to implement other waste management measures (SCMP 2020). COVID-19 infections were also expected to increase waste generation in the United States from 5 MT/y to 300 MT/y. US authorities also made significant efforts to handle such a massive amount of medical waste [19]. It is well documented that, during this pandemic period, purchases of basic safety equipment, items, and food increased by more than 20% in just one supermarket[19] . In this situation, buying safe goods and their disposal resulted in tons of garbage. Based on the reported data, an average of 2 face masks per inhabitant per day was reported in just 15 African nations. In 2020, the total number of face masks per day around 586,833,053 was reported [20,22].

Initially it was estimated that, Asia, with the world's largest population, will produce the most discarded facemasks per day (1.8 billion), which was then followed by Europe, Africa, Latin America and the Caribbean, North America, and Oceania, with 445, 411, 380, 244, and 22 million facemasks per day, respectively. China, India, the United States, Brazil, Nigeria, and the United Kingdom are estimated to generate at least 702, 386, 219, 140, 75, and 45 million contaminated masks per day if all of their citizens wear and discard a facemask or face shield per day [23,24]. According to Kalina et al., the global monthly consumption of facial masks and medical gloves were roughly 129 billion and 65 billion, respectively, for a population of 7.8 billion people [25]. According to the report of the World Economic Forum (WEF), around 39,500 facial masks, 11,500 medical gloves, 1500 gowns, and 4200 filtering face piece respiratory masks (FFP_3) have been used in the UK during February 2020 [26]. As per reported data given by Freedonia Group, a US-based leading market research group, about 312 % of face shields were required during the pandemic period in the USA [27]. In this time, the demand for gloves was increased by 12.5 % in 2020 in China also. China alone produced and consumed up to 116 million of face masks in February 2020, which was 12 times higher than the January 2020 month [28].

In Bangladesh, a total of 14,500 tons of plastic waste was generated. Along with that, Dhaka alone generated about 3076 tons of biomedical waste during the first month of the COVID-19 outbreak due to utilization of high amounts of medical essentials. In South Korea, about 295 tons of infectious medical waste was generated, in which hospitals contribute around 61%, isolation centers contribute around 34 %, and the community treatment facilities contribute around 5% from February to March 2020 [29].

Moreover, in the Indonesia biomedical waste generation was also increased by about 30 % (10,903 tons to 14,606 tons) during the COVID-19 outbreak from January to April 2020. Similarly, in the Philippines, Vietnam, and Kuala Lumpur in Malaysia

around 16,800, 9600, and 9240 tons of medical waste generation was reported. Thailand reported around 62 % more plastic consumption in April 2020 as compared to the April 2019 which was about 1.5–2 million masks used daily nationwide. In the UK consumption of plastic medical kits was from 7.5 to 12 million. Whereas, France used around 40 million surgical masks weekly and Japan used about 600 million facial masks per day as of April 2020.

As we all know, plastics are widely usable in various industries because of their flexibility, affordability, and durability. Moreover, plastics also have excellent flexibility, strength, water resistance quality, and insulating power [30,31]. Plastics are highly required and essential in the healthcare system because of these qualities, especially for one time use diagnostic kits and medical devices. As per the CPCB 2019 data, India produces roughly 9200 tons of PW (plastic waste) every day, for a total of more than 3.3 million tons per year [32]. Maharashtra, Tamil Nadu, Gujarat, and West Bengal are the top plastic-producing Indian states. As we have already stated, after COVID-19 demand for medical essentials was increased which resulted in an increase in biomedical waste generation. To address the growing amount of plastic production, India's 22 states/union territories (UTs) have completely banned SUP (single use plastic) carry bags, while seven states have just partially prohibited them [32]. However, the fast infection of COVID-19 has made the SUP prohibition difficult. This is considered as a policy compromise due to the pandemic and for human health.

3. BIOMEDICAL WASTE GENERATION IN INDIA BEFORE AND AFTER COVID-19 PANDEMIC

As per the reported data of CPCB 2019, BMW is produced in India at a rate of 619 t/day. Maharashtra, Uttar Pradesh, Karnataka, Tamil Nadu, and Kerala were the top five states that produced around 47 % of BMW [32]. Karnataka produced the highest amount of BMW, which was around 77.5 t/day, while Arunachal Pradesh produced the least, around 0.4 t/day. Delhi produced the most BMW, at 28.8 t/day, while Lakshadweep produced the least amount of waste, at 0.1 t/day [32]. The composition and distribution of BMW in India is highly variable, depending on the variety of factors such as effectiveness of source segregation, healthcare personnel' awareness and availability of waste. According to studies, the large number of COVID-19 patients influences the generation, collection and formation of BMW [33,34]. Indian airports handled around 340 million passengers in 2020, in which 274 million passengers were domestic and 66 million passengers were foreign. All airlines began distributing the PPE kits to passengers and it indicates that air travelers in India used roughly 340 million aprons, face masks, face shields, gloves, and sanitizers. The significant rise in the PPE and medical equipment showed a significant impact on the waste management system which was the result of the COVID-19 outbreak. As per reported data, to manage the spread of the COVID-19 virus, India required around 2.5 million personal protective equipment (PPE) kits every day [29] that contain roughly 20–25 % of plastic in their material composition. This massive amount of plastic will be disposed of in landfills which will cause contamination in the environment [35].

Maharashtra, Tamil Nadu, Delhi, Gujarat, Uttar Pradesh, Kerala, West Bengal, Madhya Pradesh, Haryana, and Punjab were the primary contributors of biomedical waste during the first wave in September 2020. In September 2020, the average biomedical waste generation was about 183 t/day and average generation during the second wave (May 2021) was around 203 t/day. As per CPCB 2020 data, during both waves, Lakshadweep produced the least amount of biomedical waste which was around 10 kg/day [36]. In addition, a number of pharmaceutical companies have successfully launched vaccines on the market to protect COVID-19 virus. As we all know vaccination was required to control the infection caused by COVID-19 virus and Covaxin was created by Bharat Biotech, which was India's first home ground vaccine. In collaboration with Oxford-Astra Zeneca, the Serum Institute of India (SII) launched the Covishield vaccine. In India, the vaccination program began on January 16, 2021. India has now become the world's fastest country to provide 140 million vaccine doses in less than 100 days. Development of vaccines also generates a significant amount of BMW and PW, such as syringes, gloves, and vaccine containers. The vaccination of the entire country is expected to generate approximately 2.78 billion vaccine containers, syringes and gloves. As a result, properly collecting, segregating, and disposing of all waste generated during vaccination is necessary. Because of the growing usage of personal protective equipment, medical equipment, and vaccination the world is on the point of creating a global garbage pandemic unless it is effectively monitored and controlled. Improper management of waste, pose a risk of virus transmission and damage the terrestrial and marine habitats[30]. The lack and gaps in present waste management systems, such as limited manpower and capacity limits, could also contribute to improper garbage processing that pollutes the environment.

According to another report, biomedical waste

generated by COVID-19 increased by more than 13000 kg per day in Kerala during a single week (13–18 May 2020), an increase of 2–2.5 tons per day. According to the National Green Tribunal's order of July 20, India's capacity for incineration of COVID-19 biomedical waste is around 840 metric tons (MT). It is estimated that around 55 % of the country's total incineration capacity is being utilized [37]. Estimates of biomedical production in India ranged from 0.3 kg to 1kg per bed per day [38]. In non-COVID-19 period, Karnataka and Maharashtra produced the highest amount of biomedical waste, with 65 MT and 62 MT every day, respectively. Whereas, in COVID-19 period, in Maharashtra biomedical waste production has reached around 44 MT per day and in Karnataka, it has reached around 19 MT per day. As already discussed, in non-COVID-19 period, Karnataka was the highest producer of biomedical waste which was then followed by Maharashtra, Tamil Nadu and Uttar Pradesh. Goswami et al., 2021, investigated 13 states of India which have been at the top in terms of total COVID-19 positive cases and generation of COVID-19 related biomedical waste [39]. They have reported that Maharashtra is the state which was mostly affected by the current pandemic and it is one of India's top biomedical waste generating states. Annual report of Maharashtra Pollution Control Board (MPCB) (2018–19) stated that, the state has 60,414 healthcare facilities, where 50,440 kg/day of BMW is generated from bedded hospitals, 11,793 kg/day is generated from non-bedded hospitals and 185 kg/day is generated from other sources.

4. 'REGULATORY AGENCIES' ROLE IN BIOMEDICAL WASTE -MANAGEMENT OF COVID19

The World Health Organization (WHO 2020) released advisories that encouraged countries in making proper policy decisions about the safe disposal of HCW [40]. In India, the CPCB is responsible for the creation of BMW (2016) rules. Biomedical rules interventions include an adequate supply of yellow, red, white, and blue bags/containers to all the hospitals and quarantine facilities for onsite segregation and collection of waste. Considering the problem of biomedical waste management during pandemic situations, the Central Pollution Control Board (CPCB) has initially formulated guidelines (Guidelines for Handling, Treatment and Disposal of Waste Generated during Treatment/Diagnosis/Quarantine of COVID-19 Patients). The CPCB issued detailed guidelines for the segregation, collection, storage, transportation, and disposal of biological waste generated in COVID-19 treatment facilities. Operators of Common Bio-Medical Waste Treatment Facilities must ensure regular sanitization of workers participating in the handling and collection of biomedical waste, and they should be equipped with appropriate personal protective equipment," according to the suggestions. Further they have added, "Any medical staff that is exposed to the virus, including ambulance crews and anyone involved in the processing of any COVID-19-infected material, should have a personal protective equipment kit." The National Green Tribunal of India called for more revisions to the rules so that all areas of liquid and solid waste management, such as the disposal of used personal protective equipment, are carefully considered at the individual level (PPE). All administrations and management authorities are required to provide appropriate personal protective equipment (PPE) for the public and to handle biomedical waste in accordance with the requirements [41,42].

On March 18, 2020, the CPCB announced the first policy intervention for the safe handling and disposal of biomedical waste [41,42]. To protect against leaks, these rules recommend utilizing double-layered bags for the collection of waste from quarantine centers. It is also recommended that bins labeled as "COVID-19 trash" should be used. For proper disposal, the trash should be transported to CBMWTFs or C-BMWTS. PPE kits should be provided to all staff handling BMW. Plastic bins, containers, and trolleys used for the transporting or storing purposes of BMW to be disinfected thoroughly with a 1 % of NaOCl solution [41,42]. On April 19, 2020, the second revision of the guidelines was released. On April 18th, 2020, there were over 16,000 COVID-19 cases reported [35]. COVID-19 patients who are unable to use toilets should use incinerable diapers. Yellow containers to be used to store used masks, hats, head covers, plastic coveralls, shoe covers, disposable gowns, and so on. CPCB released the third amended guidelines on June 10, 2020. On June 9th, 2020, the overall number of COVID-19 cases in India reached around 0.25 million [35]. The instructions on the segregation of BMW and other general solid waste (GSW) were given priority in this update due to the growth in GSW creation. In this modification, the use of color-coded containers with foot-driven lids was suggested. Waste such as syringe wrappers, medicines, empty juice or water bottles and fruit peel-offs that have not been contaminated by COVID-19 patient's body be collected and disposed properly as per the rule given by SWM (Solid waste management rules) standards 2016 [43]. Non-disposable products that can be cleaned, handled, and disinfected must be reused to reduce waste. COVID-19 patients' disposable plates, glass, leftover food, used tissues, toiletries, and masks will all be handled as Y-BMW (yellow color waste). These recommendations allow BMW to be disposed of in hazardous waste (HW) incinerators due to the significant amount of Y-BMW generation that exceeds the capabilities of CBMWTFs and C-

BMWTS. CPCB released its fourth modification of guidelines on July 17, 2020. On July 16, 2020, the total number of COVID-19 cases in India exceeded 1 million [35]. GSW and BMW were separated from isolation, quarantine centers, homecare, and HCFs under the terms of this amendment. The collection of GSW should not be done in yellow containers. BMW and GSW should be separated at the point of generation. The general used PPE kit should be stored in separate containers for at least 72 hours before being shredded and disposed of as dry GSW. Another significant policy intervention to track C-BMW management, released the software application known as "COVID19BMW" which tracks the generation, collection and disposal of C-BMW from various homecare centers, sample collection centers, isolation areas, testing areas and other locations as well. This application tracks the amount of waste generated and treated in the various locations. This application allows diverse stakeholders to share their information.

5. CONCLUSION

Since the corona virus outbreaks, there has been a spike in sales for single-use plastics. COVID-19 has resulted in an enormous volume of single –use plastic waste being generated around the world. Due to COVID-19 pandemic, the current global estimate of single –use PPE generated daily is roughly 1.6 million tons/day, which is around 3.4 billion single-use face masks or face shields discarded daily. According to these numbers, the risk of single-use plastic and personal protective equipment (PPE) pollution in the environment is increasing. This has the potential to result in plastic contamination, posing a major threat to human health, the survival of marine species, and pollution of the environment. Not only is there a risk to the environment from improperly disposed non-biodegradable PPE made of plastics, but there are also risks to human health from eating fish, which is a common source of nutrition for many people around the world. As it was already discussed, biomedical waste is a serious health hazard. Biomedical waste that has not been properly disposed of could be a source of various pathogens. COVID-19 is a highly infectious virus that can transmit fast from one person to another via a variety of routes. The risk of infection is very high due to the high transmission rate of the COVID-19 virus, and the demand for medical essentials is also very high, resulting in a lot of biomedical waste in the environment. As a result, the Central Pollution Control Board has produced many guidelines in response to the disease. These regulations are in place to keep medical staff and others involved in its handling and maintenance from ill. The formulation of policies for waste management facilities has been made easier as a result of these standards. If these newly established requirements are properly followed, managing the exponential development in BMW will be easier and safer for the environment and community.

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Therapeutic Peptides for Antimicrobial Resistance

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ABSTRACT

Antimicrobial resistance (AMR) has posed a severe danger to worldwide public health. Numerous antibiotics have become ineffective as bacteria are becoming resistant to drugs, and attention is now turning towards the alternate medicines for treatment of infections. Synthetic chemistry, genetic engineering, and biotechnological advancements have opened up new pathways for the hunt for alternatives for antibiotics. Recent trends show an increase in the utilisation of peptides as therapeutic candidates, owing to their significant benefits over small molecules. These peptides can also be employed as anti-biofilm agents. This review paper discusses therapeutic peptides as antimicrobial agents, their mode of operation and sources through which we get antimicrobial peptides.

Keywords: Antimicrobial Resistance, Antibiotics.

1. INTRODUCTION

AMR is a major cause of worry on the global health landscape and demands immediate action [1], [4]. Most antimicrobial medications' efficacy in the treatment of various microbial problems has recently declined, which might have a severe impact on the outcome of surgery and chemotherapy. This has occurred as a result of the widespread overuse and misapplication of basic antibacterial medicines. According to the World Health Organization (WHO), 4.8 lakh persons get multidrug-resistant for TB each year [5]. AMR has also slowed the treatment of potentially fatal illnesses such as TB, pneumonia, HIV/AIDS, and malaria [6]. This can have serious economic ramifications, since patients having treatment for resistant infections must pay more than patients undergoing treatment for non-resistant diseases owing to the use of more costly medications, a longer length of sickness, and more testing.

To address the problem with AMR species, the world health organization did a collaboration with their external partners and member states and created a report with the title “Antimicrobial resistance: Global report on surveillance 2014” for understanding the AMR species magnitude in the global level. In which it was found that the species namely Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli have extremely high rates of resistance for antimicrobial drugs [7].

In 2015 for the crisis of AMR, a global action plan was adopted by WHO which had 5 objectives those are: “a) To improve awareness and understanding of antimicrobial resistance through effective communication, education and training; b) To strengthen the knowledge and evidence base through surveillance and research; c) To reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures; d) To optimize the use of antimicrobial medicines in human and animal health; e) To develop the economic case for sustainable investment that takes account of the needs of all countries and to increase investment in new medicines, diagnostic tools, vaccines and other interventions” [8] Generally, the developing and the under developing countries faces more problem due to AMR species.

It was found that E. coli from India has become resistant to several drugs such as Cotrimoxazole, Nalidixic and Ampicillin in the year between 2004-07 [9]. In recent years also it was found that E. coli has become resistant to third generations of Fluoroquinolones and cephalosporins. Salmonella typhi and Klebsiella pneumonia has also being found resistant to fluoroquinolones [10].

Many multicellular organisms produce antimicrobial peptides and host-defense peptides (HDPs) as the first line of defence for invasion of pathogens [11], [13] They exhibit a wide range of actions, including antifungal, anti-protistal, anti-plasmodial, antiviral, antibacterial, insecticidal, and spermicidal properties,

as well as immunomodulation [14]. Although the majority these AMPs have net cationic charge but some with negative charge hav also been identified [15]. These are amphiphilic in nature; the hydrophobic site interact with lipids in the bacterial cell membrane and the cationic site interacts with the negatively charged cell surface of bacteria with electrostatic interactions. This causes cell membrane breakdown and, eventually, bacterial death [16], [17]. Since mammal's cells are zwitterionic, they do not interact well with the AMPs having positive charge, making them hazardous to bacteria. In addition to action of the membrane, intracellular targets are also being investigated. There have been reports of microorganisms developing resistance to AMPs [18], [19]. However, the breakdown of the cell membrane of bacteria is too energy-inefficient for it to evolve resistance. The wide range of features and benefits listed above have motivated scientists to investigate AMPs as potential antibiotics [20], [21].

2. SOURCES OF AMP

Around 5000 AMPs have been discovered from natural sources out of which some are from prokaryotes and some are from eukaryotes and rest are synthesized. Generally, if we talk about humans, the tissues which are exposed to the outside pathogens are most likely to produce the AMPs, it works as an innate immune response against fungi, bacteria and viruses [22]. AMP production in humans is generally done by phagocytes, lymphocytes, epithelial cells, lymph nodes etc. In mammals AMP production is also done by infections initiated by bacteria. The LPS (lipopolysaccharides) of the bacteria affects the host cells responses results in the formation of AMPs [23].

3. STRUCTURE OF AMP

Based on the secondary structures of peptides there are four forms of anti-microbial peptides these are α-helix, β-sheet, loop and extended [24]. Most common form among them is alpha-helix in which the two amino acid strands are 0.15 nm apart and the angle between them is 100 degrees [25]. Some examples of alpha-helix are indolicin (coiled and cyclic), magainin and protegrin [26]. Except beta-sheet other three structures can be determined with the help of NMR with detergent micelles, but for the beta-sheets we need aqueous solution along with NMR. The characteristics feature of beta-sheets is that the two strands of amino acids are linked together with a di-sulphide bond. Generally the activity and selectivity of anti-microbial peptides are defined by the various properties of the peptides which are charge, hydrophobic and hydrophilic nature of helix, angle between the helix [25].

4. ISOLATION AND PURIFICATION OF AMPS

Commonly acetic acid with acetone method is used for isolating the AMPs [27]. The AMPs in the sources like plant, tissues are incubated with 10 percent glacial acetic acid at 4 degrees Celsius for a night, after that it is taken out and centrifugation is done, the supernatant formed is taken and is treated with acetone. If the AMPs are there, they will get precipitated [27]. The purification is done with the help of ion-exchange chromatography along with precipitation by ammonium sulphate in which firstly the sample is run with cations or anions exchanging chromatography and later on it is run on SDS-PAGE [28].

5. PEPTIDES AS THERAPEUTICS

Peptides are short amino acid sequences, building blocks of proteins abundantly found in humans. The variety of amino acid sequences that comprise peptides makes them appealing for supramolecular and bioorganic studies. The therapeutic applicability of peptides as medications has been systematically explored throughout the last several decades and based on these research, the exploitability of peptides as pharmaceuticals has become feasible [29]. Peptides were first overlooked as therapeutic agents because they are very susceptible to proteolytic breakdown and having low bioavailability, rendering them inefficient for oral administration.

Novel synthetic techniques, such as peptide-polymer conjugates, recombinant DNA technologies, solid phase, organo-metallic complexes, and liquid phase, are being used to boost productivity while decreasing metabolism [30], [31]. Several peptide-based drugs like Lupron^{TM} from Abbott labs have already entered the market for treating the prostate cancer [32].

5.1. ACTION MECHANISM OF AMPS

The working process of the AMPs is not exactly known yet, their antimicrobial activity needs to be properly understood. Many researches have shown that the AMPs generally target the plasma-membrane with the phenomena called permeation which could result in the disruption of the cell and cause lysis of the cell. Their mode of action depends generally on the structural properties. Unlike traditional antibiotics, which normally work by inhibiting protein synthesis, DNA, RNA synthesis and cell wall production, most AMPs permeabilize microbial membranes, altering potential of the transmembrane and causing cell death

[33]. The most basic models of peptide membrane penetration entail the creation of holes which spans the membrane (barrel-stave pore model), whereas the carpet model is the most widely referenced model of AMP membrane instability [34]. Aside from permeabilization through the membrane, these peptides can cause the neutralisation or destruction of LPS (lipopolysaccharide), which is a major endotoxin that causes infections from the Gram-negative, and hence protect from sepsis [35].

A peptide antibiotic Alamethicin, stimulates creation of distinct pores in the membrane, which resembles a barrel with helical peptide staves (barrel-stave model) [36]. Alamethicin assumes an alpha-helical structure, attaches then mixed and gets inserted into bilayers specially those which are hydrated with the water vapour, as seen from the structural investigations [37].

Partition of the peptides into zwitterionic and acidic membranes, according to experimental data obtained with dermaseptin to exemplify the carpet model [38]. Then these peptides are electrostatically attracted towards the phospholipids having anionic head groups at various places in the membrane's surface making it looks like carpet. The peptides which are self-oriented generate toroidal transitory holes in the plasma-membrane at threshold peptide concentrations, ultimately forming micelles. Melittin, protegrins, and magainins all display toroidal pore model [36], [39]. AMP helices get inserted inside membrane, causing bending of the lipid monolayers across the hole, lining the water core with both lipid head groups and inserted peptides [39]. The interactions of lipids with negative charge and peptides with positively charge forms a toroidal hole [35].

Various models that explain the destabilisation of the membrane includes the molecular shape model, in which the morphology of the membrane and the interaction of the membrane with AMPs are there, then there is detergent model, in which it is shown that if the concentration of AMP is high it results in the membrane disintegration [40], separation of lipid domains by the AMPs by forming a phase boundary and making anionic clusters in the lipid clustering model [41]; to understand the activity of the AMP like how it binds, gets associated or inserted with the membrane and the detailed kinetics behind it is explained by the sinking raft model [42]; and for the prediction of complex function of AMP propensity, amphipathicity, and hydrophobicity for inducing membrane permeabilization is given by the interfacial activity model [43].

5.2. AMPS AS AN ANTI-BIOFILM

Anti-biofilm actions and other combinations of AMPs with antibiotics have been demonstrated in studies. Still, the actual mechanism through which it inhibits the biofilm is not known yet. A plausible mechanism might be that the AMPs along with the antibiotics disrupt the matrix of the biofilm, which results in dispersion of bacterial cells in the biofilms. Another proposed mechanism is the interference with QS(Quorum sensing) and reduction of adhesion between solid surfaces and bacterial cells [44]. Several appealing characteristics of a novel antibiotic class are present in AMPs, including a low incidence of bacterial resistance, a broad spectrum of activity, and a particular method of action that includes pore creation in the plasma-membrane [33].

AMPs are usually stable across a wide range of temperature and pH, which may be advantageous for the synthesis of deliverable products in a scaled-up manner. Because of their unique mechanism of action, having low toxicity for eukaryotic cells which allow them to be used for a broad therapeutic window. Even AMPs having low concentration are reported to have disruptive and inhibitory properties which can destroy the biofilms. They also work together with antibiotics and are active in animal models [45]. As they are found very attracted towards the lipid bi-layer of the bacterial membranes which is negatively charged, the resistance towards them is very rare [23]. Permeabilization and the creation of holes inside cytoplasmic membranes, the most common method, allows AMPs to work on non-growing bacteria or slow-growing bacteria [46]. AMPs also have quicker killing kinetics than most traditional antibiotics [47].

AMPs' additional potential includes the capacity to operate at several phases of biofilm development and by various modes of action, such as QS downregulation [48], destruction of pre-formed biofilms, prevention of biofilm production, and inhibition of adhesion [49]. In addition, for the multi-drug resistant bacteria they are quite active [50]. Although there is a better knowledge of the functions of QS in development of biofilm, there are currently few reports of AMPs having potential for suppressing the systems of quorum-sensing employed by various bacterial pathogens [44].

6. CONCLUSION

Several efforts are being undertaken for producing AMPs as novel antimicrobials. Still, designing them rationally is challenging due to the peptides' intricate interactions with membranes and with one another. The hydrophobicity, amphipathicity, size, sequence,

helicity, charge and subtended angel between the hydrophilic and hydrophobic surfaces of the helix have all been found as structural factors essential to the microbicidal action of AMPs [45]. Although for the development of therapeutics promising AMPs have advanced to preclinical and clinical stages, still further research is needed for identification of plant based or natural novel AMPs and also ways to enhance their stability, delivery and activity for expanding its range for other clinical application. Further research on the AMPs for destruction of biofilm or prevention from biofilm, might include looking at enzymatic AMPs, including glycoside hydrolase dispersin B and deoxyribonuclease I [51]. Because there is limited evidence on new AMPs with anti-biofilm capabilities, additional research is needed for understanding the specific processes of action, notably the QS biofilm-promoting signals quenching. Broadening the investigations on the AMPs' dispersion effects to include both preformed and forming biofilms is also critical. Synergism research employing AMPs is needed urgently for uncovering effective AMP-drug or AMP-AMP combos to guarantee that these antimicrobials have minimal adverse effects. Commercial-scale manufacturing systems to synthesise AMPs are needed urgently as the cost of production remains a major challenge.

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Prevalence of Rare Disease

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ABSTRACT

Rare disease affects fewer people in the population of specific areas. The rate of occurrence of rare diseases is only one among 1000 of the population. In this review, we focused on rare diseases and their most common types which can be found globally. According to the National Organization of Rare Diseases (NORD), there are around 7000-8000 rare diseases found in the world. In the globe, there are about 300 million people living with one of the rare diseases. There are about 70% of rare diseases are genetically determined. Due to the low number of rare diseases found in the world, people have insufficient awareness. They are not even familiar with the fact that they are suffering from a rare disease. In most cases, it has been observed that the longevity period of patients is very less, due to the high rate of progression of the disease. So, the need for an hour to escalate awareness among people, patients, and clinicians is a must regarding the root cause of rare diseases. Treatment of rare diseases has become a challenge for researchers due to the less availability of patient data. Thus, it's become a challenge to investigate, the diagnosis of disease among cohort patients, accurate knowledge of root cause plus proper affordable medication to specific rare disease patients. Therefore, awareness of rare diseases will make it easier to find more treatments and cures. It will result in hope for millions of sufferers of rare diseases.

Keywords: Rare Disease, Rate of Progression, Longevity, Medication, Treatment.

1. INTRODUCTION

The disease can be defined as any abnormal deviation in the normal functioning of the body which leads to failure in body function. Thus, a number of symptoms and signs appeared in the body of the diseased. These symptoms and signs are different from the normal functioning and metabolism of the body. On the basis of symptoms, a disease is diagnosed. In pathology, there are various basic features of any disease like the cause of the disease and then there is a need to understand the mechanism of development of the disease. After this, there will be some morphological changes in the body. This will lead to consequences that arise in various functions that lead to various alterations in normal functions of the body. After observing morphological characters (physical alterations) and newly generated functions, we can describe various signs and symptoms of a specific disease [1].

Rare disease can be described as a disease that affects less number of populations in the area. The rate of occurrence of rare diseases is only one among 1000 of the population [1]. It varies according to the place, country, and the number of populations. To define rare diseases there were different parameters considered by the **Rare Disease Terminology & Definitions used in the Outcomes Research Working Group.** If 40-50 cases were reported per population of 100,000 then we can term that specific disease a Rare Disease. If we talk about the rate of occurrence of a rare disease in the USA, then according to The Orphan Drug Act (**ODA, 1983**) under US law. It defines as a rare disease as it affects only **200,000** people of the total population of the USA [1]. And if we talk about the European Union, then the number is 5 out of 10,000 people. In B**r**azil, the number will be 65 among the population of 100,000 and thus the number of cases out of the total population varies for different countries. About 85% of rare diseases are caused by genetic mutation and majorly children have more records of rare diseases than adults. It's about 50-75% of the rare genetic disease occur in children. About 35% of children's death occurs due to rare disease within 1 year of a child's development years [2].

According to the Indian Medical Council of Research (ICMR) the rate of occurrence, then it will be less than 1 person among the population of 2500 people. And the Organization for Rare Disease India (ORDI) described the number should be less than 1 among the 5000 people for rare diseases [1].

Rare disease affects around 6% of the population. Genetically determined rare diseases are 70%. According to the recent calculation of the cumulative prevalence at a global level, the Orphanet epidemiology data file shows that in 2017, the rate of prevalence of the rare diseases is about 3.5-5.9%. This data shows that with minimum prevalence there are around 263-446 million people affected by it. Table 1 shows the different types of common and rare diseases found worldwide. Fig.1. represents the classification of rare diseases.

Table 1: Different types of Common and Rare Diseases worldwide

Common Disease	Rare Disease
Communicable Disease	Autoimmune Disease
Innate Disease	Bacterial Infections
Metabolic Disease	Chromosomal Disorders
Environmental Hazards	Rare Cancer
Hereditary Diseases	New-born Disorder
Physiological Diseases	Nervous System Disorder

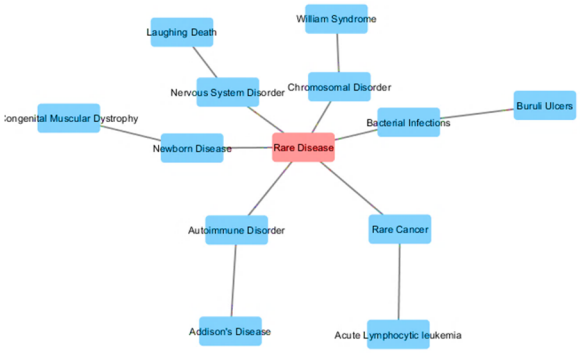

Fig. 1. Classification of Rare Disease

1) Autoimmune disease: Autoimmune disease can define as when the body attacks its own immune system. Generally, we all know that the immune system helps our body from the attack of foreign particles and kills pathogens. But here the immune system fails to distinguish between foreign particles and our body cells and mistakenly starts to kill body cells. Several causes of autoimmune disease are still unknown. But it's assumed that, If a patient is under any treatment, then sometimes many drugs can lead to some adverse effects like manipulating the genes. That results in confusion in the Immune system. The autoimmune disease leads to complete change and damage of tissues. Abnormal growth of organs is another result in patients [3].

Here is the list of several autoimmune rare diseases, for example:

- Addison's disease.
- Autoimmune gastrointestinal Dysmotility (AGID)
- Autoimmune hemolytic anemia (AIHA)
- Autoimmune hepatitis
- Autoimmune lymphoproliferative Syndrome (ALPS).

2) Bacterial Infections: Infections that are associated with bacteria are known as bacterial infections. These infections have a large effect on living organisms. Infections in some cases are mostly spread to children and adults. Scientists work to find specific reasons to find the cause of bacterial infections, but still, research is going on various reasons for causing infections. Here is a list of some bacterial infections and rare diseases. For example: -

- Anthrax, Atypical mycobacteriosis,
- Familial Balantidiasis
- Brucellosis
- Bubonic Plague
- Buruli ulcer

3) Chromosomal Disorders: As the name suggests these kinds of diseases causes due to mutations in chromosomes. That led to changes in their originality and functions in organisms. Due to the loss of information about genes, complete genetic material gets misled. That leads to several chromosomal disorders among living organisms. Progeria is an autosomal dominant condition, where the LMNA gene gets altered. Fig. 2 shows symptoms of Williams' syndrome which mostly occurs in babies and children. It is diagnosed in early childhood. It is caused by the deletion of about 27 genes from the long arm of one of the two chromosome 7s.

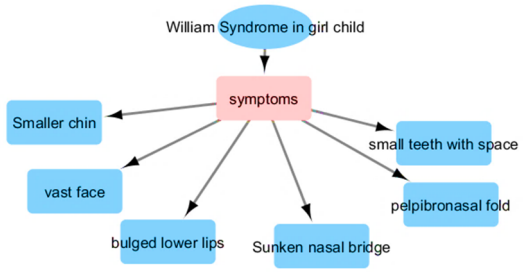

Figure 2: Symptoms of William Syndrome.

Here is the list of several rare genetic diseases:
- 49, XXXXX Syndrome (Pentasomy X)
- 47, XXX Syndrome (TriSomy X)
- 1q Duplication.
- Ring Chromosome 18
- Hutchinson Gilford Progeria Syndrome (HGPS).
- William Syndrome.

4)Rare Cancers: As we know that cancer is a vital disease. It is caused due to unlimited cell divisions in the body. That results in the dysfunction of cells. It leads to the formation of cancer in the body. Mostly these cancers are lethal in condition and lead to the death of a person. Sometimes they seem harmless but lead to lethal effects on the body. For example, in Bednar Tumor a person suffers from pigmentation on the body but with the passage of time, these pigmented spots lead to harm to the lower layer of the skin. And lead to the worst condition of the patient. Here are some examples of rare cancers: -
- Acinic Cell Carcinoma (Acinic cell tumor)
- Acute Lymphocytic leukemia (ALL)
- Aicardi Syndrome (Neurological disorder).
- Bednar Tumor (Pigmented Dermatofibrosarcoa Protuberans)

5)Newborn Disease: These rare diseases most commonly can be found in newborn babies. Mainly these are classified as Genetic disorders which they acquire from their parents. In these cases, newborn children have specific disorders at the time of birth. For example, in congenital muscular dystrophy, a child is born with a nervous system disorder. For Example: -
- Carpenter Syndrome.
- Congenital muscular dystrophy.

6)Nervous System Disease: These disorders mostly damage the functioning part of the brain of living beings. Most of the disorders are related to motor nerves and are rare degenerative brain disorders. In these cases, it's observed that patients are unable to do any brain-related activities. Hallucinations, difficulty in performing any work, and also poor concentration can be seen among patients. Some of the nervous system disorders are: -
- Laughing Death (Kuru)
- Biotinidase Deficiency (Biotin Disorder)
- Cap myopathy
- Cerebella Parenchyma Disorder 3(Autosomal recessive Cerebellar Parenchymal disorder Type 3).

Aquagenic urticaria is a condition in which a person suffers from water allergy. There is another condition in which a person has 2 skeletons. It is difficult to believe that two skeletons exist in a single individual. This condition is known as **FOP** (Fibrodysplasia ossificans Progressive), where connective tissues such as tendons, muscles, and ligaments turn into bone. A new skeleton is formed from the connective tissues. It leads to difficulty in the movement of the body for the patient. In some rare cases, it's observed that the death of a person occurs due to laughing bursts. It is also known as laughing sickness (Kuru). Predormal symptoms are headache and joint pain in the legs. These kinds of conditions can exist in the world. These instances (conditions) are termed rare diseases. As the name suggests these diseases are observed in few people among the population.

2. SOME OF THE RARE DISEASES WHICH ARE COUNTED AMONG THE TOP 10 RARE DISEASES ARE DESCRIBED BELOW

1) Alice in Wonderland Syndrome (AIWS):

This disease is mainly observed in children and the main reason behind the disease is an acute infection. In some cases, it is observed that a child experiences AIWS during Ebola virus infections. In 1952, it was Lipmann who described several patients having hallucinations of alteration in the size of their body parts like hands, and arms. But one thing was common they experienced these hallucinations during a migraine attack [4]. First-time use of this term was by Todd in 1955, so it's also known as **Todd's syndrome**.

1.1 Symptoms of Alice in Wonderland Syndrome:

It's a *neuropsychological condition* that leads to the distortion of perception. He reported 6 cases of AIWS, in which 4 patients were migraineur. They experienced **macroasomatognosia** and **microsomatognosia**. In macroasomatognosia condition a person hallucinates that his/her body parts seem larger than usual. While in microsomatognosia, a person hallucinates the shrinking of his/her body parts more than usual. Other distortions were reported as *visual illusion, auditory illusions*, *somatopsychic duality*, the *feeling of depersonalization, zoopsia,* and *dyschromatopsia*. Since 1955, about 170 cases of AIWS have been reported in the literature. Some authors differentiate AIWS on the basis of hallucination and symptoms they experienced. It's concluded that, in AIWS, there is an imbalance between the low and high-order cortices in the Parietal –Temporal –Occipital (TPO). This section is concerned mainly with somatic sensations, hearing, and vision. So, the perception will be based on information received from three different sensory modalities combined [5]. Fig. 3. represents various

types of hallucinations in AIWS patients.

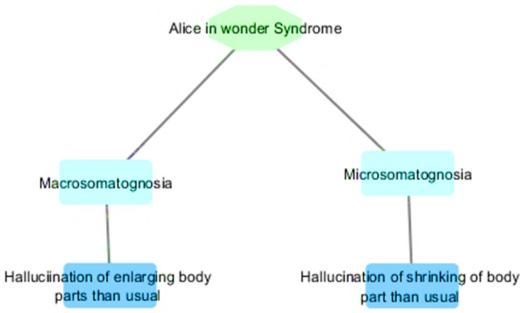

Fig. 3. Representation of various hallucinations of AIWS patients

2) Fibrodysplasia Ossificans Progressiva (FOP)
FOP is a rare connective tissue disorder. FOP is also known as **Munchmeyar disease** and **stone man syndrome**. FOP is an autosomal dominant inheritance, which leads to passing the genetic traits from parents to their children. In some cases, parents without FOP accidentally have FOP offspring. About 50% chances of passing from parents to offspring. This disease is characterized by *ectopic ossification* of ligaments, tendons, muscles, and ligaments. The occurrence rate of FOP is very rare, with only 1 in 2 million people [6].

Thus, the extra formation of bone leads to restricted bone movement. It targets connective tissue as we know connective tissue is biological tissue that tends to work as support and bind together the organ. It causes by a mutation in the gene encoding Activin A receptor type 1/Activin like Kinase 2 (ACVR1 /ALK2) gene. ACVR1 gene encodes ACVR1 protein, this protein is a Bone Morphogenic Protein (BMP) [7,8]. BMP has the ability to form bone and cartilage. But a mutation in the ACVR1 gene leads to the formation of bone. These bones are formed from soft tissue. It causes abnormal formation of extra bone across the tendon, ligament, muscle, ligament, and formation of bridges of bones across the Joints. Thus, the formation of extra bones leads to restriction of movement in the affected areas. The formation of new bones leads to the formation of secondary skeletons [8]. It leads to restriction in body movement.

2.1 Symptoms of FOP:
The onset period of FOP is about the initial years of development about 10 years of a child. During this period of time, painful swellings of soft tissue occur. This swelling of soft tissue occurs spontaneously and can lead to the transformation of skeletal muscle, ligaments, and fascia into heterotopic bone [6]. Symptoms can be seen in childhood and it starts from the neck area. And generally, children have big toes at an early age. A short big toe appears at the time of birth. This abnormal turning of the toe is known as Valgus deviation. Most people develop bumps around their necks. It also leads to abnormalities in the spine in some cases [9].

Statistics: About 2500 cases are noticed of FOP.1-2 in million have FOP.

3) Hutchinson Gilford Progeria Syndrome (HGPS)
HGPS is a rare condition, in which premature symptoms appear in a patient. They have their mean age of living around 13-15 years. There is a gene known as *laminA/C* (*LMNA*). This undergoes splicing, which leads to the main cause of Hutchinson Gilford Progeria Syndrome. This is the main reason of the mutation of the LMNA gene, it leads to improper synthesis and maturation of laminA protein known as progerin. Several kinds of epigenetic alterations can be seen in HGPS. For example: -Histone-covalent modifications, and DNA methylation [10].

Progeria patients mainly have premature aging and they are more prone to other health serious conditions, for example, dislocation of hips, heart-related disorders, and many more. The occurrence rate of Progeria is about 1-2 people per million population. There are around 300-400 children affected with Progeria disease.

3.1) Symptoms of Hutchinson Gilford Progeria Syndrome:
There are various symptoms of children suffering from progeria. For example:

- Loss of fat in the body, which results in a tiny appearance.
- Protruding and bulging eyes.
- Veins are visible on the scalp.
- Loss of hair and premature aging. All these symptoms can be seen in figure 8, which explains the features of an HGPS patient.

3. CONCLUSION

There are about 8000 enormous rare diseases in the world. It is important to find their symptoms, causes, and pathogenicity properly. These findings will lead to awareness of the rare disease. When awareness will be there, then finding their cure and treatment is not so far. Here in the review top, most rare diseases were described and only some amount of work has been done. So, some rare diseases are those whose studies are still going on and yet are to be understood properly.

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A Review on Fermentation of Indigenous Rice Varieties from an Omics Perspective

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ABSTRACT

Fermented foods have been an integral component of the dietary habits of people in most countries, especially where traditional systems of medicine are widely prevalent such as: India, China and most of South East Asia. The fermentation process of the food with its associated microorganisms, changes the texture, nutritional profile, shelf life and organoleptic properties to a large extent. The fermented food also acquires medicinal properties during the process, which can be assessed by observing the secreted bioactive compounds from the microorganisms. Antimicrobial, anticancer, antioxidant and other medicinal characteristics of the fermented food have been attributed to the microorganisms forming part of the fermentation process. Recent studies suggest that the uncultivable microbes have a great role to play in food fermentation, as evidenced by metagenomics and metatranscriptomics approaches. The current review focuses on different varieties of rice and the various traditions followed for their fermentation. Results from modern scientific approaches, profiling the changes occurring during fermentation, the triome component of microorganisms, rice and its secreted components are emphasized upon.

Keywords: Fermented Food, Indigenous Rice, Nutritional Value, Metabolic Activities.

1. INTRODUCTION

Cereal grains such as: rice, wheat, and maize constitute the most significant staple food crops in the world, of which rice varieties are considered as the worldwide stable meal [1,2,3]. Additionally, rice is grown all throughout the world, from the wettest rainforests to the driest deserts. In this regard, rice is the most essential strategic crop for global food and nutrition security. It is a kharif crop that requires a lot of water and temperatures over 25°C along with yearly rainfall of more than 100mm, or an appropriate irrigation system for its proper cultivation [4]. The reported production record states that India and other Asian nations are among the top rice producers [2]. Aside from its socio-economic value, traditional hulled rice grains are extremely beneficial to humankind and their health (Figs 1, 2). In figure 1 and 2 there are some nutritional of traditional raw rice varieties and their applications of rice. It is a great diet for preventing digestive system diseases and hypertension due to its low sugar, gluten-free, low fat, low cholesterol, protein (including all eight necessary amino acids), low salt level, and low fiber content is currently use in traditional rice varieties. Also, certain varieties of rice contain significantly high levels of major (calcium, phosphorus and potassium) and minor (iron, zinc, manganese) nutrients and minerals which assist to nourish the hormonal system, heal wounds, enrich the circulation, and maintain internal water balance, among other things [5].

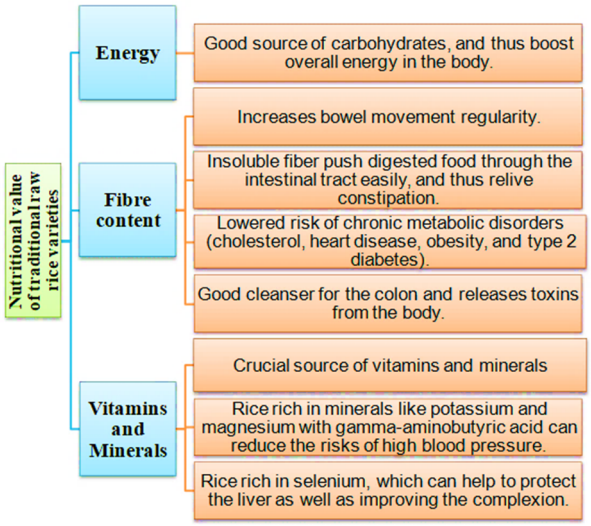

Figure 1: Documented nutritional profile of traditional raw rice varieties [67]

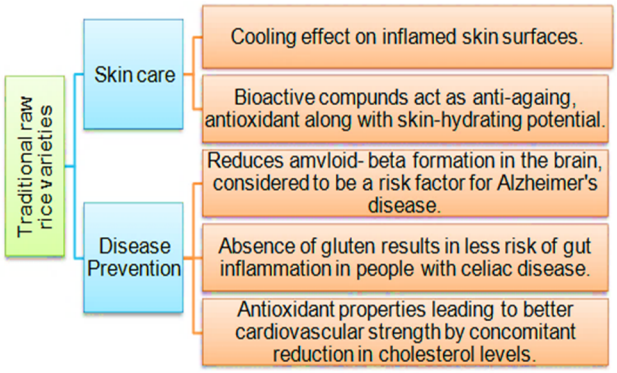

Figure 2: Applications of traditional raw rice varieties [67]

In spite of the advantageous qualities, rice is lacking in a few fundamental components (e.g. fundamental amino acids) and inefficient in giving coordinate advantage, fermentation may be the foremost straightforward and temperate way of progressing their wholesome value, and useful qualities [6,7]. Traditional rice-based meals and drinks have been acknowledged as a global interest in the creation of many vital amino acids, vitamins, minerals, pre-biotic, and pro-biotic organisms throughout the last several decades [8]. The identification and extraction of bioactive compounds, vitamins, and other substances from fermented rice has improved as research and technology has progressed [9]. Fermented foods and drinks, on the other hand, constitute an important component of Indian cultural tradition, even now [10]. These fermentation processes have been created for long-term nutrition and food preservation throughout the history of human civilization [11, 12, 8]. Alcoholic, lactic acid, acetic acid, and alkali fermentation are the four primary fermentation processes. Yeasts are the most common organisms involved in alcohol fermentation, which results in the generation of ethanol. The acetic acid and lactic acid fermentation involves *Acetobacter spp*. and lactic acid bacteria, respectively to convert feedstock. The alkali fermentation is commonly done for fish and seeds, which are commonly used as condiments [13].

2. INDIGENOUS RICE VARIETIES AND SIGNIFICANCE

Plants that are native to a region are considered indigenous. This comprises plants that have been present in a certain location for a long time. Figure 3 shows the indigenous rice varieties grown in India. As an agricultural country, India has cultivated and tested 574 indigenous rice varieties on more than 10,000 farmers' fields, with the help of state agricultural institutes, to provide ecosystem services and minimize vulnerability [14]. Depending on the growth season or environment, each rice type was traditionally employed for particular reasons. Each kind has its own characteristics and health advantages to offer. Traditional rice cultivars have greater nutritional characteristics than developed rice variants. The majority of the data demonstrated that traditional rice cultivars had medicinal and ayurvedic characteristics. These rice types are extremely beneficial in the treatment of heart disease, diabetes, cancer, and other disorders. However, freshly enhanced cultivars lack these favorable characteristics.

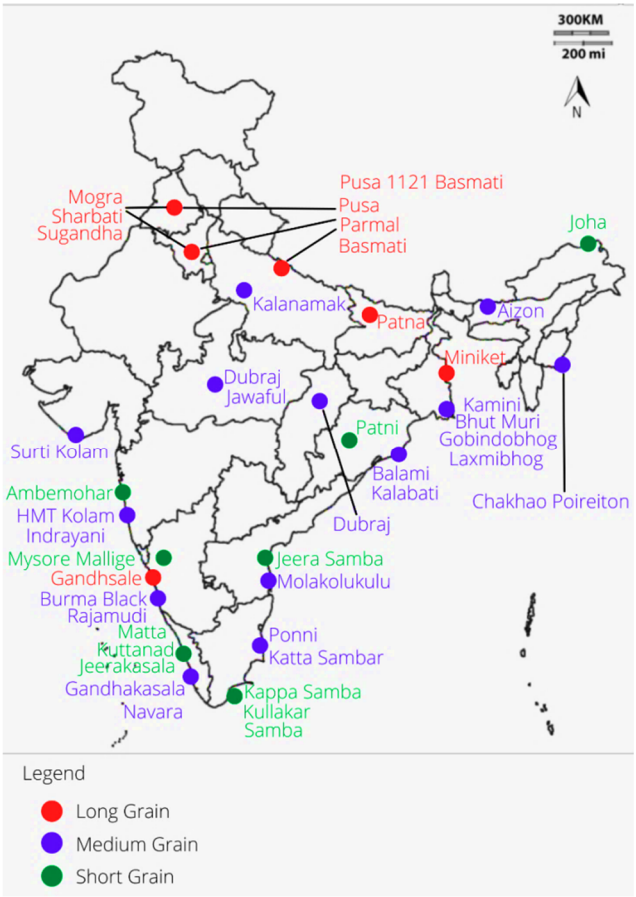

Figure 3: Indigenous rice variety in India [adapted from 68]

Rice variety namely, paalkudaivazhai has earbuds appearance like open umbrellas. Pal kudaivazhai earned its name since it aids breastfeeding women in producing more milk when consumed as kanji. This is advantageous to both moms and children. Table 1 illustrates the medicinal value of various rice varieties in different states. The high fiber content of this rice aids digestion, relieves constipation, and removes toxins from the colon. Rajamudi rice, a Karnataka type, strengthens bones. Zinc, which is abundant in Rajamudi rice, boosts immunity and speeds up the body's recuperation and mending. It

also offers a heart- health advantage and low glycemic index [32, 34, 15]. Traditionally, the most effective treatment for fevers and ulcers was red rice (Raktasali). Additionally, it enhances eyesight, improves voice, boosts sperm count, acts as a diuretic, and is antitoxic. According to Ayurvedic teachings, rice satisfies the majority of the criteria for a wholesome diet, and it is the only cereal consumed whole grain, which is more easily digested than flour. Doctors prescribe Kaluheenati, which has greater fibre content, for managing the poisonous effects of snakebites, hepatitis patients, breastfeeding women, and it is also supposed to improve physical strength and male sexual potency. Pachchaperumal and Madathawalu rice types are high in protein and are commonly used in Ayurvedic treatments to boost the immune system [16]. Brown rice is high in vitamins, including thiamine, riboflavin, and niacin. Whereas, rice varieties developed in modern times are deficient in vitamins and minerals because they are concentrated in the exterior of the seed, which are removed during the polishing or "bleaching" process, whilst the parboiled rice is abundant in these vitamins due to its unique processing [17].

Table 1: Medicinal value of various rice varieties [adapted from 18, 19, 20]

Rice Variety	State	Medicinal value
Njavara	Kerala	Neurological problems, Arthritis
Gathuwanor	Madhya Pradesh	Rheumatism
Nagkesar	Madhya Pradesh	Lung diseases
Karhani	Chhattisgarh and Jharkhand, Madhya Pradesh	Paralysis
Laicha	Chhattisgarh	Skin infection
Karanaga	Not available	Dysentery
Baisoor	Madhya Pradesh	Epilepsy, Headache
Alcha	Madhya Pradesh	Pimples
Kalimooch	Madhya Pradesh (MP), Chhattisgarh and Maharashtra	Skin diseases
Maharaji	Chhattisgarh, Madhya Pradesh	Post-natal tonic for women
Kari Bhatta	Karnataka	Skin infections
Kaluheenati	Sri Lanka	Hepatitis
Karikagga	Karnataka	Cooling effect
Atikaya	Karnataka	Health tonic
Erumakkari	Kerala	Cough
Kaflaya	Himachal Pradesh and Uttar Pradesh	Leucorrhea
Matali, Lal Dhan	Himachal Pradesh and Uttar Pradesh	High blood pressure, fever

3. FERMENTATION

With advancement in time, malnutrition and dietary deficiencies have become prevalent concerns in developing and poor nations. Lower nutrient availability and infectious diseases are the major concerns related to food safety and malnutrition. Simple and cost-effective, and traditionally utilized techniques, based primarily on the fermentation process, are thought to be successful in combating these issues. Fermentation results in better nutritional profile, digestibility, and bioavailability of nutrients, and lowering anti-nutritional elements and extending the product's shelf life and safety at the same time [21, 22].

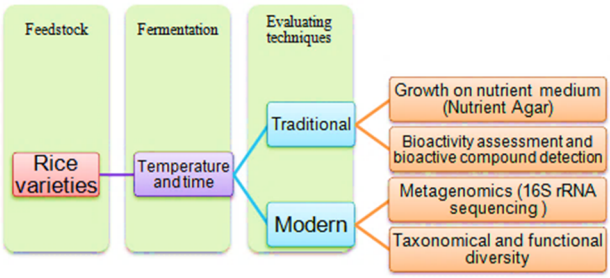

Figure 4: Schematic diagram of traditional fermented rice and techniques

Fermented foods and drinks are still a part of the Indian subcontinent's cultural legacy today. For significant nutrition and food preservation, these procedures have been evolved throughout the history of human civilization. Fermentation is a systemic chemical conversion of organic substances to simpler compounds with non-pathogenic microbial communities [23]. Changing the conditions of fermentation helps to activate the enzymes that already exist, as well as modify the pH, which increases the operation of specific enzymes such amylases, proteases, hemi-cellulases, and phytases. The technical and nutritional impacts of fermented cereal meals were influenced by microbial metabolites and enzymes [25]. Apart from the nutritional aspect of traditional fermented food, they also serve as preventative measures for lifestyle disorders like: hypertension, diabetes, and cardiovascular diseases linked to hypercholesterolemia. It is time to collaborate with various food and beverage production organizations to share people's expertise of food preparation and traditional food commodities with multiple health advantages, which will aid in the introduction of novel items to the market, resulting in a win-win situation [24].

Fermentation of rice can be acidic, alcoholic, or both. The technique begins with rice grain preparation, such as soaking, milling, and boiling, which helps to break the starch framework and solubilizes nutrient components via endo and exocleaving pathways. Furthermore, microbial metabolic activities release carbon dioxide and other gasses, making batter spongy. Time determines the degree of fermentation, which affects the texture, taste, appearance, and perfume of the meal. Microbial fermentation of rice and its by-products is being paid increasing attention in recent times by the scientific community [26].

There are two techniques utilized in food fermentation. Firstly, foods can be fermented naturally; these are called “wild ferments” or “spontaneous ferments”, leaving it to the naturally present microbes in the food to carry out the fermentation process. Sauerkraut, kimchi, and certain fermented soy products are fermented this way [27]. Secondly, foods can be fermented using specific starter cultures, known as “culture-dependent ferments"; kefir, kombucha and natto fall in this category [27]. “Backslopping”, uses a small amount of a previously fermented batch being added to the raw food, such as done in preparation of sourdough bread [27]. Functional microorganisms transform the chemical constituents of raw materials of plant/animal sources during food fermentation thereby enhancing the bioavailability of nutrients, enriching sensory quality of the food, imparting bio-preservative effects and improvement of food safety, degrading toxic components and anti-nutritive factors, producing antioxidant and antimicrobial compounds, stimulating the probiotic functions, and fortifying with some health-promoting bioactive compounds [29].

Culturable and non-culturable microorganisms naturally ferment the majority of global fermented foods and beverages. Traditional food fermentation represents an extremely valuable cultural heritage in most regions, and harbors a huge genetic potential of valuable but hitherto undiscovered strains [28]. Holistic approaches for identification and complete profiling of both culturable and non-culturable microorganisms in global fermented foods are of interest to food microbiologists. The application of culture-independent technique has thrown new light on the diversity of a number of hitherto unknown and non-cultural microorganisms in naturally fermented foods [28].

Microorganisms are found throughout nature, thriving in a vast range of environmental conditions. The majority of them are unculturable or difficult to culture by traditional methods. Metagenomics enables the study of all microorganisms, regardless of whether they can be cultured or not, through the analysis of genomic data obtained directly from an environmental sample, providing knowledge of the species present, and allowing the extraction of information regarding the functionality of microbial communities in their natural habitat. Function-based screenings, following the cloning and expression of metagenomic DNA in a heterologous host, can be applied to the discovery of novel proteins of industrial interest encoded by the genes of previously inaccessible microorganisms [30].

4. MICROBIOLOGY OF FERMENTATION

Fermentation is essentially a technique of obtaining energy for bacteria (who care even less about definitions). After all, microorganisms require energy to carry out activities (such as nutrition transport and biosynthesis), sustain physicochemical integrity (such as ionic and osmotic balance), and grow and reproduce. Given the multitude of bacteria involved in the production of fermented foods, they are all now classified into one of three phyla: Proteobacteria, Firmicutes, or Actinobacteria. Lactic acid bacteria, kind of Gram-positive bacterial communities in the Firmicutes, are the principal organisms employed in the production of fermented foods. The genus Bacillus and *Brevibacterium*, which contain species employed in the production of a few fermented foods, are also included in this phylum [8].

Microorganisms are responsible for the acidity, taste, and texture of fermented foods, including the health advantages that go beyond plain nutrition. Microorganisms could also be found as part of the food's natural microbiota or as a consequence of microorganisms being added as starting cultures in an industrialized food fermentation process. Microbial cultures can also be utilized to create a variety of chemicals (enzymes, tastes, perfumes, and so on) being used as dietary supplements or in situ during food fermentation processes [31,6]. Despite adding beneficial effects during fermentation, microorganisms in food also help prevent many harmful chemicals and microorganisms during the fermentation process. These microorganisms are also responsible for the production of new enzymes that assist with digestion.

5. CULTIVABLE MICROORGANISMS

The traditional phenotypic identification methods rely entirely on culture-dependent procedures alone

for cultivable microbial communities in culture media, neglecting other unknown uncultivable bacteria that may play large or small roles in the fermentation process. Table 2 gives a thorough grasp of the cultivable microorganisms as well as, the benefits they bring. The kind of microorganism participating in the fermentation influences the nature of fermented foods. Acidic fermented foods have several advantages, including resistance to deterioration by bacteria and their toxins, longer storage period and moisture, and improved flavour and nutritional value [35].

Table 2: Cultivable microorganisms and their benefits

Fermented food	Cultural Conditions and Nutritional benefit	Micro-Organisms detect	Reference
Dhokla	32°C for 15 h. Antioxidant Property	*Leuconostoc mesenteroides, Lactobacillus fermentum* and *Pediococcus pentosaceus*	[32]
Idli + Xylooligosaccharides (XOC)	Room Temperature for 4-18 hours Prebiotic potential	*Lactobacillus fermentum* and *Pediococcus cerevisiae*	[33]
Rice beer	4 °C for 7-10 days Antioxidant Property	*Paracoccus, Enterococcus, Olivibacter* and *Cellulosimicrobium*	[34]
Jeung-pyun	35 °C for 6.5 h	*Lactobacillus plantarum, Lb. pentosus,* and *Lb. brevis.*	[35]
Chicha	30-35 ºC for 36 h Gastrointestinal microbiota	*Bifidobacterium* and *Propioniobacterium*	[37]
Haria	37 ºC for 4 days (anaerobic condition) Probiotic Activity and antioxidant activity	*Lactobacillus fermentum* and *Bifidobacterium sp.*	[38,36]
Fermented brown rice	37 ºC for 48 hours Antioxidant activity	*Limosilactobacillusreuteri AKT1, Limosilactobacillus fermentum AKT2*	[40]
Chyang	Anaerobic fermentation Amylolytic activity		[39]
Poko	30 ºC for 5 days	*Saccharomyces cerevisiae, Candida versatilis, Lactobacillus spp, Pediococcus spp* and *Rhizopus spp*	[41]
Manapu	30 °C for 5-7 days	*Bacillus spp*	[41]
Mana	30 °C for 9-10 days	*Bacillus spp*	[41]
Koji	15 days Antioxidant Property	*Aspergillus candidus*	[58]

The study of the microbiology and metabolic characteristics of rice during spontaneous fermentation shows that to best define the microbiota associated with the fermented food such as Chicha, traditional culturing approaches must be supplemented with culture-independent ones [37].

The antioxidants present in traditional Dhokla are known to both serve as preservatives and supply essential antioxidants in vivo. Many studies have shown the beneficial effects of antioxidants against oxidative stress-induced degenerative and age-related diseases, as well as cancer and aging [32].

Rice beer is traditionally prepared and consumed by various ethnic populations in Southeast Asian countries. In Asia and Africa, the cereals rice, corn, wheat, millet, sorghum etc. constitute more than 80% of the diet. Digestibility and nutritional value of a complex form of cereal depend on its processing as feed. Fermentation of cereals reduces anti-nutritional factors and provides more macro- and micronutrients. In cereal-base fermentation, the saccharolytic activity of microbes converts the complex starch into simple sugars which in turn are converted into organic acids and alcohol. It is a rich source of nutrients which makes it a natural antioxidant. Rice beer is known for its wide array of medicinal properties as well as health benefits [34].

The effect of Xylo-oligosaccharides (XOS) addition on batter fermentation and its quality attributes of idli, a cereal–legume-based Indian traditional fermented food. It has been found that, addition of XOS improved the rate of fermentation and reduced the fermentation period when compared to the conventional way of batter fermentation [33].

6. NUTRIGENOMICS

Nutrition has a strong impact on human health. The recent trends in genomics studies have provided a clue to understanding how nutrients affect the genome and can enhance health in various ways. Personalized nutrition profiles or Nutrigenomics looks at ways to prevent, or delay diseases, and suggest the best modes of nutrition based on the personal gene profile [69, 70]. Various Nutrigenomics-based studies have shown that brown rice provides for their better health (Table 3).

Table 3: Mechanism of Nutrigenomics signaling pathway of brown rice (adapted from 72)

Property of Nutrigenomic	Signaling Pathway	References
Antioxidant	Nuclear factor beta (NF-K) Mitogen activated protein kinase (p38 MAPK)	[72, 73, 74, 75]
Anti hypocholesterolaemia	ATP binding cassette (ABCA) 1 AKT and apolipoprotein	[72, 76, 77]
Anti hyperglycemia	Suppression of fbp and pck genes	[72, 74]

7. METAGENOMICS

Direct DNA extraction from fermented food samples, generally known as culture-independent approaches, is now widely employed in food science to evaluate both cultivable and non-cultivable microbial communities in fermented foods. Culture-independent approaches originally debuted in the field of food microbiology towards the mid-1990s, and they have since been widely used. These approaches don't really require culture and instead use genetic information (DNA and RNA) for identifying and locating changes in the major populations in a given environment. A Polymerase Chain Reaction (PCR)- Denaturing Gradient Gel Electrophoresis (DGGE) evaluation is the most prevalent culture-independent technology for isolating microorganisms from fermented foods [31].

Culture independent technology capable of providing quick and enormous information on both the structure and variation of the microbial population. The primary metabolites produced during the breakdown of resistant-to-digestion food components entering the large intestine, serving as a source of energy and nutrition [44]. The table 4 below illustrates the broad impact of modern technologies on bacterial community profiling. The zymotic fluid of Chinese rice wine was found to represent a bacterial environment where each species decomposed distinct substrates and created diverse chemicals, according to taxonomic metagenomic investigations [43]. *L. brevis* induced unsuccessful fermenting in beer and red wine, and the bacterium generated chemicals that resulted in objectionable aromas and a bitter taste. *L. plantarum*, on the other hand, seems to be a contributing element to the high quality of automated wines, as this bacterium can undertake malolactic fermentation. The functional evaluations revealed several potentially crucial features for wine fermentation, assisting the brewery in improving its automated procedures. A study of rice wine found that enzymes involved in the synthesis of monophenols, such as 4-coumaric acid, ferulic acid, and proto catechuic acid, are derived mostly from the raw materials rather than from microbial metabolism [45].

The indigenous microbial diversity in various sources of local beverages could be a rich resource for oenological research. The first culture-independent study of starter microbiota was reported in the traditional Vietnamese alcohol fermentation starters (banh men) through Polymerase Chain Reaction (PCR)-based Denaturing Gradient Gel Electrophoresis (DGGE). The main difference between traditional and industrial starters is that traditional starters have a higher resilience over an industrial one [47].

Fermented foods are a significant part of the daily diet of many people both in the Li populations and around the world. During the production of a fermented product, microorganisms transform raw material into a product with an increased value, generally by extending the shelf life of the raw materials and increasing the nutritional value of the product by improving the production of organoleptic attributes. Metagenomic approach has enabled exploration of microbial compositions in a range of traditional fermented foods while bypassing the need for cultivation, allowing the identification of a vast array of microorganisms never previously isolated in culture. Hence, by combining new technologies like next generation sequencing and metagenomics, with conventional techniques like microbial culturing and q-PCR, we present an in-depth profiling and characterization of the microbiome.

The DNA molecule has a variable half-life dependent on many factors including the biological activity of the matrix in which it is present. Functional properties of the fermented food microbiota can also be investigated by metagenomics. Indeed, functional diversity can be explored through genetic screening of genes of interest. Metagenomics is potentially a tool of choice to assess the survival of pathogens, toxinogens or spoilage species over fermented food elaboration process. A possible limit of the metagenomics approach is its failure to detect the less numerous microorganisms in an environment contain a dominant microbiota. Metatranscriptomic analysis of fermented food microbiota still presents technical locks. Metagenomics data include a huge proportion of genes encoding classical cell functions with little interest for food microbiota [48].

Table 4: Illustrate the broad impact of modern technologies on bacterial community profiling.

Rice variety	Taxonomical diversity	Functional diversity	Reference
Tapuy	*Acetobacter ascendens*, *Bacillus aquimaris*	Potential probiotic	[42]
Xaj-pitha (rice wine)	*Rhizopus delemar, Mucor circinelloides, and Aspergillus sp*	Flavour	[47]
Chinese Rice Wine	*L. plantarum, L. curvatu, L. brevis*	Synthesis of biotin, malolactic fermentation and production of short-chain fatty acid.	[43]
Gluten-free rice	*Clostridiaceae, Ruminococcaceae*, *Lachnospiraceae* and *Streptococcaceae*	Human digestive function, antioxidant property	[44]
Huangjiu (Rice wine)	*Saccharomyces, Aspergillus, Saccharopolyspora*, *Staphylococcus, Lactobacillus*, and *Lactococcus*	Flavor formation	[45]
Yucha	*Lactococcus, Enterococcus, Vibrio, Weissella, Pediococcus, Enterobacter, Salinivibrio*, *Acinetobacter, Macrococcus, Kluyvera* and *Clostridium*	Energy production and conversion, Amino acid transport and metabolism	[46]
Zha-chili	*Lactobacillus, Pediococcus* and *Leuconostoc* including *Lactobacillus acetotolerans, Lactobacillus plantarum*,	Peptidase	[50]

8. BIOACTIVE COMPONENTS

So far, it has been established that fermentation might lead to various bioactive compounds that could have potential benefit to human mankind and industry as well. A recent study confirmed health benefits are required to investigate this nutrition rice fermented meal into a functional one [9]. The herbal starter-based rice fermented product was high in lactic acid bacteria, *Bifidobacterium sp*., yeast, and other microorganisms, as well as oligosaccharides, unsaturated fatty acids, and amino acids. Traditional fermented rice beer has a variety of beneficial substances, including malto-oligosaccharides which are low in calories, limit the growth of intestine pathogenic microbes, and are extremely nourishing for newborns and the elderly [7]. Furthermore, pyranose derivatives, as well as phenolic and flavanol group of chemicals, are accumulated, which contributes to enhanced antioxidant, and immune-stimulatory properties. Enzymatically enacted Chinese rice wine seemed to have a significantly faster and more efficient fermentation rate, as well as more flavor precursors, notably decreasing sugars and amino acids [51]. However, no effect of enzymatic process on the flavors of finished rice wine during fermentation has yet been observed.

Enzymatically extruded Chinese rice wine had a substantially better fermentation rate and efficiency, as well as more taste precursors, particularly reducing sugars and amino acids. However, no influence of enzymatic extrusion on the tastes of the finished rice wine or those formed during the fermentation process has yet been discovered. The esterification of alcohols with fatty acids or the production of alcohol acetyl transferase employing higher alcohols and acetyl-CoA as substrates produces the greatest category of volatile chemicals in Chinese rice wine [51]. Table 5 illustrates the various types of rice variety with their applications and bioactive components.

Rice straw and husk are key biomass sources for biorefining around the world. Rice husk exhibits significantly more post-hydrothermal pre-treatment recalcitrance than rice straw, owing to high quantities of lignin and silica. During hydrothermal pre-treatment, it also produces larger quantities of fermentation inhibitors [53].

Table 5: Bioactive Components of Rice Variety and Their Application

Rice variety	Fermentation	Bioactive compound and Application	Reference
Rice bran	30 °C for 48 hours	Phenolic compounds from lignocellulosic; Prevent chronic diseases and oxidative reactions	[52]
Rice bran	32 °C for 12 days	α-tocopherol and γ-oryzanol; Antioxidant activity	[54]
Rice Wine	30 °C for 5 days for chief fermentation and 15 days at 15 °C for post fermentation	2-heptanol, 1-octen-3-ol, ethyl 4-hydroxybenzoate, methylpentyl 2-propenoate, γ -hexalactone, and 4-vinylguaiacol; Flavor compounds	[51]
Rice wine	28 °C for 12 days	Alcohols, esters, and aromatic compounds; Enzymatic extrusion	[55]
Rice Bran		Gamma-oryzanol, phenolic acids, phytic acid, β-sitosterol and vitamin E; Antioxidant activity	[56]
khanomjeen	24- 48 h	2-methylpropanoic acid, 3-methylbutanoic acid, diacetyl, ethyl acetate, ethyl valerate; Oxidative degradation of lipids	[57]
Koji	15 days	Linoleic acid; Antioxidant property	[58]
Chinese Rice wine (Huangjiu)	30 °C for 96 h (as the main fermentation), then incubated at 18 °C for 3- 4h (as post-fermentation)	2-methylpropanol, 3-methylbutanol and 2-phenylethyl alcohol; Amylase, glucoamylase and acid protease	[59]
White and Brown Rice	30 °C for 4 days	γ-lactones, 4-hydroxydecanoic acid, on the formation of γ-decalactone, ricinoleic acid, fatty acids; Organoleptic properties	[60]
Khoa-Mak	30 °C for 72 hours	Monascus pigments; Stronger antioxidant activity	[61]
Glutinous rice (khao – Mak)	Room temperature for 7 days	α -aminobutyric acid; High antioxidant activity	[62, 63]
Brown rice	45 h	GABA, tryptophan, coumaric acid, L-ascorbic acid, linoleic acid, β-carotenol, eugenol, 6-gingerol; Antioxidant activity	[66]
Rice husk and rice straw		Lignocellulosic; Bioethanol production	[53]
Red-Mold Rice		Azaphilonoid Pigment; Anti-Tumor-Initiating	[65]
Brown Rice	48 h at 37 °C	Gamma-aminobutyric acid, coumarin, cinnamic acid, butanoic acid, ascorbic acid, nicotinic acid, and stearic acid; Cardiovascular diseases, diabetes, and stress-related disorders (anxiety/depression)	[40]
Rice koji	30°C and 85% humidity for 14 days.	α-amylase, aminopeptidase, amyloglucosidase, lactase, and lipase; Antioxidant activity, skin-lighting and preventing enzymatic browning	[66]

9. CONCLUSION

The various studies presented emphasize that considerably more scientific information is required to fully understand the fermentation of rice, the microorganisms involved and the bioactive compounds they produce, since comprehensive studies utilizing omics platforms are far less in proportion to the traditional rice varieties

documented. These studies can bring more perfection to the process, especially when adapted to industrial scale production and bring in economic and health benefits. Currently, here we limited studies and data on metagenomics and Metatranscriptomic on traditional fermented rice from India. We require these studies so that the roles of uncultivable microorganisms in rice fermentation are firmly established. These can provide valuable IPR to supplement the Geographical Indication tags already given to many traditional varieties.

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Review Analysis of Multidrug Antimicrobial Resistance Facilitation via Bacterial Biofilm Formation

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ABSTRACT

Pathogenic microbes are adapting strategies to escape from the effect of antibiotics. They are developing changes in their physiology and in genetic material over subsequent generations leading to their tolerance towards antimicrobial agents. Antimicrobial resistance is becoming a global threat in the health industry and can take us back to the dark age. The underline reasons for this resistance can be outlined as to increase in world population, variations by people's migrations, extensive use of antibiotics, and unhygienic conditions. The failure to develop or discover new antibiotics, as well as the indiscriminate use of antibiotics, are predisposing factors for the evolution of antibiotic resistance. It has resulted in a scarcity of treatment choices, particularly for some of the most severe infections. Many bacteria have multidrug resistance patterns that are difficult to treat and, in some cases, untreatable with standard antibiotics. As a result, there is now a scarcity of effective medicines and efficient preventative measures for a variety of harmful bacteria. Furthermore, because there are few new antibiotics being discovered at the moment, we must create unique therapeutic alternatives and alternative antimicrobial medicines to combat MDR bacteria. The production of biofilms is one of the mechanisms involved in multidrug resistance, and it can make infection control difficult. In any biofilm, micro-organism can suspend their activities till it gets a favourable environment and those microbes which burst out from biofilms, have tolerance for inhibitors. This tolerance to inhibitors is known to be acquired while growing in biofilms, as with time, as the cells reproduce, the genetic makeup also changes through mutations or by plasmid conjugation. Tolerance to antimicrobial compounds is calculated by a meta-analysis of literature data. In this writeup, we are going to discuss in detail about the formation of biofilms, factors leading to resistance and anti-biofilm agents.

Keywords: Antimicrobial Resistance, Biofilms, EPS, Quorum Sensing, Antibiotics.

1. INTRODUCTION

Antibiotic resistance is well-known, as one of the defining mechanisms for resistance within a bacterial film is the ability to coordinate gene expression and mediate bacterial communication [1]. Surgical implants, fuel production, papermaking, metallurgy, and food service all have economic and environmental concerns due to the microbes' biofilm resilience. Bacterial biofilms have developed antibiotic resistance and are linked to a variety of chronic illnesses [1].

1.1. ANTIBIOTICS AND THEIR FUNCTIONS

Antibiotics are antimicrobial substances that obstruct the growth and replication of a bacteria, these are the drugs which are used to treat the diseases caused by microbial pathogens [2]. They work by blocking essential processes in the bacterial cells which then helps our immune system to confront the bacterial cell [2]. Most of the Antibiotics do not affect the human body and so they can be ingested safely as a medicine [3]. Once the antibiotic medicine is swallowed, it gets absorbed in the body like other nutrients, It flows throughout the body reaching the target area where the pathogen is causing infection [3]. Antibiotics that affect an ample range of bacterias are known as broad-spectrum antibiotics whereas, the antibiotics that affect only a handful number of bacterias are known as narrow-spectrum antibiotics [3].

1.2. MECHANISM OF ACTION OF ANTIBIOTICS

Different types of antibiotics work in different ways. Beta-lactam antibiotics target cell wall in bacteria and degrade the peptidoglycan layer leading to lysing of the cell wall [4]. Glycopeptides inhibit the synthesis of cell walls [4]. Tetracyclines work upon the 16s r-

RNA sequences that are conserved and inhibit protein synthesis. Quinolones interferes with nucleic acid synthesis [5].

1.3. MODE OF ADMINISTRATION

Most of the antibiotics are oral antibiotics but in severe cases, antibiotics are inserted through syringes directly into the blood or applied to the body physically [6]. Most of the antibiotics cure bacterial infections, but there are some antibiotics that work against fungal infections too but antibiotics do not cure viral infections at all [6].

1.4. ANTIBIOTICS AND THEIR SIDE EFFECTS

Antibiotics like other drugs can also cause side effects, Common side effects of antibiotics are: stomach ache and problems like diarrhea and intestinal infections and oral thrush infections and allergic reactions [6].

2. DETAILED STRUCTURE OF BIOFILMS AND ITS MECHANISM OF FUNCTION

2.1. BIOFILM FORMATION

Biofilm formation is a highly complex process, in which microorganism cells transform from planktonic to the sessile mode of growth [7].

The genesis of Biofilm has the following important steps [a] attachment initially to a surface [b] formation of micro-colony [c] three-dimensional structure formation [d] biofilm formation, maturation and detachment [dispersal] [7]. When a bacterium cell reaches to near some surface/support so close that its motion is slowed down, it makes a reversible connection with the surface and/or with the already adhered other microbes to the surface [7]. Expression of certain genes in extra polysaccharide or EPS regulates the formation of microcolony and promotes cell division [8]. Three-Dimensional Structure Formation and Maturation After the micro-colony formation stage of biofilm, expression of certain biofilm-related genes take place [8].

2.2. COMPOSITION OF ECM

Extracellular DNA [eDNA]is constituted of chromosomal DNA, that is released into the extracellular milieu ECM through cell lysis though dedicated secretion systems, or membrane vesicles [9]. Thus, anchoring the eDNA to the cell surface by DNA-binding proteins is a widespread mechanism for biofilm formation that may also facilitate multispecies biofilms [9]. Indeed, eDNA is used for the initiation of biofilm formation in many pathogenic bacteria, including Gram-positive and Gram-negative negative bacteria and mycobacteria [9]. This will influence the accessibility of these substances to particular niches inside the biofilm, and determines, amongst others, the variation in antibiotic susceptibility of cells within biofilms [9].

ECM is a dynamic 3-D network of molecules that gives cells and tissues structural support. Collagens, elastin, laminins, and tenascins, as well as proteoglycans and glycosaminoglycans, hyaluronan, and their cell receptors such as CD44 and integrins, which are responsible for cell adhesion, form a well-organized functional network with important functions in health and illness [10]. Enzymes like matrix metalloproteinases and particular glycosidases like heparanase and hyaluronidases, on the other hand, help to reconstruct the matrix and have an impact on human health [10]. Matrix components have an impact on a variety of cell processes and functions, including cell proliferation and survival, migration, differentiation, autophagy, angiogenesis, and immune control [10].

Several functions have been attributed to the ECM, based on its extraordinary capacity to establish intermolecular interactions between its components, and surface-exposed structures of the cells, biotic and abiotic substrata, and many environmental molecules [9]. Various proteins can be implicated in these interactions, such as autotransporters, lipoproteins or two-partner secretion protein A of Gram-negative bacteria, and cell wall-associated proteins in Gram-positive bacteria and fungi [9]. In addition, ECM retains several other substances, for instance, nutrients, energy sources, antibiotics, antibiotic-degrading enzymes, and molecules released by cell lysis, thereby constituting a recycling unit [9].

2.3. HETEROGENEOUS NATURE OF BIOFILMS

Biofilms typically contain diverse and active cells [11]. As a result, a continuum of quasi-independent bacterial populations with different phenotypes [i.e., susceptible, resistant, and tolerant cells] may co-evolve in biofilm, resulting in differences in the spatiotemporal organisation of cell death and antibiotic tolerance development within biofilms [11]. However, when the biofilm's layers increase, a multitude of nutrients and oxygen gradients emerge, resulting indiversity of phenotypic and metabolism [11]. This leads to difficulty in antibiotic penetration and its effect as antibacterial activity [11].

2.4. QUORUM SENSING IN BACTERIA

Quorum sensing allows bacteria to communicate with one another throughout the biofilm building process [12]. Quorum sensing controls the metabolic activity of planktonic cells and can lead to the production of microbial biofilms and enhanced pathogenicity [12]. Cell-to-cell signaling has recently been demonstrated to play a role in cell attachment and detachment from biofilms showed that certain dental plaque bacteria can modulate expression of the genes encoding fimbrial expression [fimA] in *Porphyromonas gingivalis* would not attach to *Streptococcus cristatis* biofilms grown on glass slides [12]. *P. gingivalis*, on the other hand, readily attached to *S. gordonii. S. cristatus* cell free extract substantially affected expression of fimA in *P. gingivalis*, as determined by using a reporter system. S. cristatus is able to modulate *P. gingivalis* fimA expression and prevent its attachment to the biofilm [13].

Davies et al. showed that two different cell-to-cell signaling systems in *P. aeruginosa*, lasR-lasI and rhlR-rhlI, were involved in biofilm formation [13]. At sufficient population densities, these signals are required for activation of genes involved in biofilm differentiation [13]. Mutants unable to produce both signals [double mutant] were able to pro duke a biofilm, but unlike the wild type, their biofilms were much thinner, cells were more densely packed, and the typical biofilm architecture was lacking [13]. In addition, these mutant biofilms were much more easily removed from surfaces by a surfactant treatment [13]. Addition of homoserine lactone to the medium containing the mutant biofilms resulted in biofilms similar to the wild type with respect to structure and thickness [13]. Stickler et al. also detected acylated homoserine lactone signals homoserine lactone signals in biofilms of gram-negative bacteria on urethral catheters [14]. Yung-Hua et al. showed that induction of genetic competence [enabling the uptake and incorporation of exogenous DNA by transformation] is also mediated by quorum sensing in S. mutans. Transformational frequencies were 10–600 times higher in biofilms than planktonic cells [15].

3. RESISTANCE AGAINST ANTIBIOTICS DUE TO BACTERIAL BIOFILMS

Antibiotic resistance is a phase when the antibiotics can not kill or stop pathogenic bacteria, The germ continues to grow [16]. After being exposed to the antibiotics the bacteria become resistant to the antibiotics as the genes mutate [16]. Bacteria can be provided with biofilm-specific properties that limit drug distribution and efficacy [17]. In order for antimicrobials to work in biofilm-forming microorganisms, a number of factors need to be overcome: increased antibiotic resistance, increased cell proliferation, and high molecular density of the cell [17].

A bacteria with antibiotic resistance can also spread the resistance through contact with another bacterial cell [17]. Bacteria stick on the surface and grow as an antibiotic-resistant biofilm [17]. Reduced resistance to antibiotics contributes to the survival of biofilm infections, such as infections associated with implants [17].

Insufficient penetration of the antibiotic into the biofilm, inhibition of nutrients and slow growth, reversible stress responses and persistent cell formation are considered contributing causes of diseases [17][18]. Genetic and biochemical details of these biofilm protective layers, all genes and genetic products that contribute to this resistance, may be targeted for the development of covalent agents [17][18].

3.1. MECHANISM OF ANTIMICROBIAL-RESISTANCE IN BACTERIA

Bacteria can show resistance to antibiotics through a variety of mechanisms, which can be divided into intrinsic and extrinsic or acquired mechanisms.

For intrinsic mechanisms, the difference in cell wall structure between Gram-positive and Gram-negative organisms plays a significant role [19] The bacteria limit the susceptibility to a number of different antibiotics and make many traditional antibiotics ineffective against gram-negative cells [19]. It has been shown that the influence of this permeability barrier, at which the permeabilization of *Escherichia coli*, outer membrane, significantly increases the sensitivity to antibiotics [19]. It is also conveyed through active export; the insertion inactivation of pumps for the delivery of multiple drugs, which help determine the initial level of drug accumulation in the cell [e.AcrAB-TolC], also increases susceptibility to antibiotics [20].

Acquired resistances can result from several mechanisms and include those that can be inherited through vertical transmission of genetic material or through plasmid-mediated horizontal transmission [21]. The acquired resistance mechanisms can be roughly divided into three different classes [21]. First, bacteria can mutate to change structure to protect [21]. There are numerous examples of this, such as fluoroquinolone antibiotics that target DNA gyrase in

gram-negative cells, and mutation or decoration of the target site decreases drug binding and creates resistance [21]. Second, the bacteria can produce enzymes that can inactivate or modify the antibiotic, rendering it ineffective. Extended spectrum β-lactamases are an important example of this [21]. CTX-M enzymes are one of the most commonly isolated ESBLs found in gram-negative species and reduce the potency of penicillins, cephalosporins, and aztreonam worldwide [21]. Third, bacteria can prevent entry. Important examples include antibiotics that achieve their goal by upregulating normal cell outflow or reducing cell membrane permeability by suppressing porin production; MaxAB-OprM in *Pseudomonas aeruginosa* or AcrAB-TolC in *E. coli* are important examples of efflux pumps that can export multiple drugs [21].

These antibiotic-resistant bacterias spread their genes forming a new antibiotic-resistant cell through the vertical transfer of genes [22]. This resistance leads to chronic diseases in biofilm communities, this resistance in biofilm communities is dissimilar to that of the planktonic cell, the cells of Biofilms shield microorganisms from variation in pH, osmotic potential, nutritional requirements, wear and tear but also prevent bacterial biofilm communities from accessing antibiotics and immune cells in the host [22]. As a result, the biofilm matrix provides extra resistance to bacterium, which may not only withstand hard conditions but also resist antibiotics, which can lead to infections by hazardous insects [22].

3.2. MAJOR FACTORS CAUSING RESISTANCE IN THE BACTERIA TO THE ANTIBIOTICS

Biofilm resistance to antibiotics depends on several factors, including physical, physiological, and gene related factors. Therefore, this multifactorial nature of biofilm development and drug tolerance poses significant challenges for the use of conventional antimicrobial agents [25].

The microorganisms in a biofilm are resistant because of the following suggested factors:[25]

1] Polymer matrix, which can limit the diffusion of antibiotics.

Extra-cellular polymeric substances [EPS] form a matrix, protecting bacteria from contact with antibiotics, and avoiding antibacterial drug penetration at higher bactericidal concentrations [26].

2] Interaction of antibiotics with a polymer matrix reduces their activity.

Bacteria inside biofilm implement different strategies inorder to save itself from hostile situations. Interaction of antibiotics with biofilm matrix can retard and affect their reactivity and activity. Creating Genetically resistant and tough cells tolerant to various antibiotics along with slower growth rate where antibiotics are not effective [26].

3] Resistance caused by enzymes such as β-lactamase.

The distribution barrier is created by the biofilm matrix due to the presence of β - lactamase which dissolves into the cell or membrane space follicle [23].

4] Changes in metabolic activity within the biofilm.

Bacteria cells with-in biofilms are responsible to generate persister cells who are intert metabolically. It is one of the mechanisms it uses to gain the ability to survive in high concentration of antibiotics [27].

5] Genetic Changes in the target cells or masking of the target sites,

A huge number of bacterial cells in the matrix makes it too dense. This leads to very close overlapping contact between them, enabling them to breed and exchange resistant genes, finally leading to the generation of an entire resistant community [28].

6]Extrusion of antibiotics by efflux pumps.

The development of resistant mechanisms allows genes to generate enzymes that inactivate the effect of antibiotics or use efflux pumps to remove antibiotics [29][30].

7]The presence of the outer membrane structure as in Gram-negative bacteria.

Glycocalyx uses electrostatic binding forces, van der Waals forces, and hydrogen to maintain the adhesion and adhesion of biofilms in solids and the maturation of biofilms [1].

Antibiotic resistance and biofilm bacterial viability rely on these pathways [25]. Bacteria in biofilms have various antibiotic resistance mechanisms and utilise diverse natural or inherent resistance mechanisms [25]. Bacteria inside biofilms implement different strategies in order to save themselves from hostile situations. Interaction of antibiotics with biofilm matrix can retard and affect their reactivity and activity. Creating Genetically resistant and tough cells tolerant to various antibiotics along with slower growth rate where antibiotics are not effective [25]. Biofilm-forming bacteria have a high rate of mutation, which allows them to evolve resistant mechanisms, which in turn allows their genes to generate enzymes that inactivate antibiotics or expel them via efflux pumps [25]. Bacteria in biofilms form metabolically

inert persistent cells [25]. This is one of their methods for evading drugs and surviving in high antibiotic concentrations [31]. The bacterial population is dense, and horizontal transmission of resistance and virulence genes is effective [31]. The bacterial population is dense, and horizontal transmission of resistance and virulence genes is effective [31]. The matrix contains an excessive number of bacteria, resulting in intimate contact between them, allowing them to exchange resistant genes and, eventually, the entire population to acquire this resistant gene [31]. As a result, antibiotic resistance is mostly designed by the genetic diversification of bacteria in biofilms [31].

3.3. COMPOSITION OF GLYCOCALYX/ CAPSULE

The glycocalyx is an important component of biofilms, their size ranges from 0.2 to 1.0 μm and is reported in Gram-positive and Gram-negative viruses [32][33]. Glycocalyx uses electrostatic binding forces, van der Waals forces, and hydrogen to maintain adhesion and adhesion of biofilms in solids and maturation of biofilms [34][35]. The composition of the sugar coating is controlled by the growth of biofilms that support the evolution of bacteria and survive in the most unpopular environment to proliferate [1]. The composition of biofilm tablets, such as glycoproteins and polysaccharides, is affected in many natural environments [36]. Antimicrobial resistance to antibiotics and other antimicrobial ingredients is enhanced by a sugar-coated substrate. Substrate adsorption sites reduce the transport of biocides and act as exoenzyme compounds [10]. The Exo enzyme provides a substrate of biodegradable metabolites, reducing the activity of certain antibodies and the activity of specific drugs [37][38].

In *Pseudomonas aeruginosa*, pure oxygen conditions under administered ciprofloxacin and tobramycin antibiotics, lead to bacterial biofilm destruction [39][40]. The reduction of oxygen-availability promoted antibiotic resistance [39][40]. Therefore it was concluded Bacterial biofilm increases resistance levels against antibiotics under anaerobic condition through expressing specific-genes [39][40].

3.4. ENZYME-MEDIATED RESISTANCE

Enzymatic reduction resistance of heavy metals such as mercury, antimony, nickel, cadmium, arsenic, cobalt, zinc, lead, copper, chromium is performed by certain bacterial species [41]. These enzymes, beta-lactamase that provide resistance to biofilm [42]. Detoxification happens due to enzymatic reduction of heavy metal and ions resistance-genes. The presence of heavy metals induced the broader spectrum of resistant phenotype [42].

When an Antibiotic fails to kill or control the pathogenic bacteria any other unconventional drug is not used, these are preserved for use in certain circumstances only to avoid the growth of antibiotic resistance [7].

3.5. HETEROGENEITY IN METABOLISM OF BIOFILMS

Cells inside biofilms are not homogeneous, they fluctuate in their genotypes, aggregates, stress reaction, metabolic pathways and natural conditions inside the biofilm [1]. In the biofilm bacterial group may exist as a monolayer or can extend up to a few layers framing a thick coat [1]. Because of the presence of water channels, supplements focus stays unpredictable in biofilms, and the reason for substrate move inside layers of biofilm isn't because of dissemination alone yet additionally by convective fluid exchange [23]. As the biofilm becomes denser and more defined, the inclination of porosity, metabolically dynamic biomass and oxygen diffusivity turns out to be more prominent [23].

An investigation of oxygen profile inspecting in biofilms uncovers that oxygen can't infiltrate proficiently through water channels inside biofilms along these lines, clarifying the endurance of anaerobic microbes in the circulated air through medium [24]. Another purpose behind the low dissemination of oxygen is that cells in the highest layer of biofilm effectively breathes the oxygen which happens in single-species biofilm but is tougher to reach to deeper layers of multi-layered biofilm [24]. Thus, a hereditary variety that emerges in a biofilm through transformation/variety heterogeneity, can be added to the way that sub-populace emerges in one type of biofilms [24].

4. TREATMENT OF BIOFILMS

Here target therapies for biofilm infection are discussed in detail. The major types of treatment of biofilms are:

- Phage Therapy
- Quorum sensing inhibitors
- Antibiotics Combination
- Antimicrobial Peptides
- Biosurfactants

4.1. PHAGE THERAPY

Use of phage treatments are potential biofilm killing

mechanisms [31]. Phage-Therapy targets lytic-phages by formerly killing their bacteria-hosts. However, they do not have integrases enzyme and some other required enzymes means for horizontal-gene transfer [31]. For penetration and biofilm-distruption by phage, we can get specific treatment considering specific characterstic properties of phage that control their spread, penetration and diffusion [31]. *Pseudomonas-aeruginosa*-biofilm has shown reduced biofilm-viscocity post bacteriophage treatment of viral enzymes [43].

This problem can be treated with a phage cocktail. If the target is a single species, the target-only phage serves as a spectator, but because biofilms are generally a multicultural society, cocktails successfully transform biofilm [43]. A popular feature of the phage cocktail is that when a particular phage is activated against a particular phage, the target is untrue and can prevent the growth of parasite resistant bacteria contained in this cocktail [43]. In addition, the phage can increase the destruction by using the interactive power in the cocktail.

4.2. QUORUM SENSING INHIBITORS

The notion of quorum sensing controls gene activity in accordance with oscillations in cell population density [44]. Bacterium related to Quorum sensing produces autoinducers which are the biochemical signal compounds, the proportion of which increases with cell density. [44]. The complexity of removing microflora, as well as the rise in resistance to antibiotics, demands the search for novel approaches to battle undesired microbes [44]. Concentrating on the Quorum Sensor module is a viable method [45]. There are several substances in the surroundings that interfere with microbial transmission [45]. Macromolecular Quorum dampening enzymes and microparticulate are the two types on which the substances have been classified on the basis of their respective molecular mass and chemical composition. [45].The quorum-sensing mechanism in bacterium is dependent somewhat on the creation, secretion, and sensing of external biochemical signal molecules known as autoinducers. [45]. Numerous species of harmful bacteria may adjust to new surroundings by modulating the genes involved for the synthesis of biofilms, virulence genes, antibiotics, or the transfer of genetic information in the process of transformation or when they are conjugated. [45].

4.3. BIOSURFACTANTS

They impede biofilm formation by altering the adhesion coefficient owing to restricted cell hydrophobicity and membraneous rupture, restricting constrained e- transport reactions and so reducing energy requirements in bacterial cells [46]. Different classes of biosurfactants are produced by the different available microorganisms that have bactericidal, antibiofilm, and fungicidal [46]. *Pediococcus acidilactici* and *Lactobacillus Plantarum* biosurfactants quorum detection signal-molecules [46]. According to one research, they have limited the development of the S. aureus colony by altering the transcription of biofilm-associated genes dltB, icaA, and cidA.[46]. Biosurfactant compounds could be used on implantable devices such as urinary catheters and osteo implants to inhibit infectious microorganisms from building biofilms without using artificial drugs[46]. Sophorolipids and Rhamnolipids are hypothesized to be effective biofilm antagonists for both gram-positive as well as negative bacterium[46]. Few investigations revealed that *Lacto acidophilus* biofilm biosurfactants reduced the development of *P.vulgaris* and *S.aureus* biofilms in polydimethylsiloxane implants [47]. Rhamnosus-derived biosurfactants promote plasmolysis by modifying membraneous integrity, allowing compounds to be employed as anti-biofilm reagents in silicone devices and voice prosthesis in the surgical intervention [47]. Biosurfactants' anti-biofilm action can be considerably enhanced when combined with caprylic acid, which inhibits the biofilm development of *Pseudomonas aeruginosa, Escherichia coli,* and *Bacillus subtilis.* Amphotericin B and fluconazole work in combination to inhibit the production of biofilm communities and preexisting biofilms of *Candida albicans*, while irritants such as Sodium dodecyl-sulfate resulted in the breakdown of *Pseudomonas aeruginosa* biofilms [47].

4.4. ANTIMICROBIAL PEPTIDES

Antibacterial Peptides [AMP] are intrinsic immunologic molecular signals with a broad AMR [48]. Antimicrobial peptides modulate the inflammatory reaction by acting on the epithelium and proinflammatory cells, culminating in cytokine production, proliferation and differentiation, angiogenesis, healing, and chemotactic [48]. AMP has been utilized to inhibit the production and elimination of advanced biofilms [48]. Naturally derived or synthetically made AMP have been shown to protect microbial colonization of substrates, destroy pathogens in biofilms, and alter the composition of biofilms [48]. Anti-biofilm mechanisms include [1] altering or degrading the membrane permeability of inner linings in biofilms; [2] disrupting bacterial signaling systems; [3] degradation of the biopolymer

and the biofilm composite; [4] hindering the alerting strategies to stop severe bacterial reaction; and [5] beneath of genes that code for biofilm production and enhancer-binding transport [45]. Numerous syntheses have the ability to rapidly degrade existing *Pseudomonas aeruginosa* biofilms [46]. Although the process of Antibacterial Peptides of biofilm disintegration is unknown, the fast death of cells trapped in biofilms might imply that they are starting to break down bacterial barriers [46]. Ampicillin, -lactams, polymyxin E, doxycycline, daptomycin, azithromycin, ciprofloxacin, and clarithromycin have all demonstrated substantial synergistic action with Antibacterial Peptides [46].

It is generally accepted that AMPs target the cytoplasmic membrane primarily through permeation and cell lysis activities [47]. Studies have shown that the mode of action of AMPs is specifically based on their structural properties such as sequence, size, cationic nature, hydrophobicity and amphipath city [47]. Unlike traditional antibiotics, which inhibit the synthesis of cell wall or DNA, RNA and protein synthesis, most AMPs permeabilize microbial membranes, affecting transmembrane potential and causing cell death [49]. The barrel-stave pore model, while the carpet model is the most frequently cited model of membrane destabilization by AMP [49]. In addition to membrane permeabilization, AMP can lead to the neutralization or disaggregation of lipopolysaccharide [LPS], a primary endotoxin that is responsible for gram-negative infections and which together provide protection against sepsis [49].

4.5. COMBINATION OF ANTIBIOTICS

Antibiotic combinations are emerging as a treatment option in the treatment of biofilm-related diseases [50]. Coristine has been used as the backbone of an integrated film-based therapy. Corischin based compounds have been shown to be effective against the biofilm *Pseudomonas aeruginosa*, effective in removing antibodies and preventing the emergence of drug-resistant antibodies [50]. For instance, aminoglycoside is widely used to treat *Pseudomonas aeruginosa.* Several aminoglycoside compounds exhibit antimicrobial activity on the membrane [11]. The combination of meropenem and tobramycin provides an accelerating effect [11]. Efficient destruction of biofilm cells in the upper layer of biofilm. Gentamicin and ciprofloxacin are strongly synthesized against the *Pseudomonas aeruginosa* biofilm using clinical drugs [11]. Fosfomycin and tobramycin have also been shown to have an interaction effect with *Pseudomonas aeruginosa*, which differentiates cystic fibrosis under conditions that mimic micro aerobic or anaerobic properties [11]. Finally, a combination of several antibiotics may prevent the emergence of biofilm-resistant mutations, but has been shown to be fully effective in overcoming existing resistance and cell destruction mechanisms [1].

5. CONCLUSION

The ability of microbes to mutate and adapt to environmental signals has resulted in a medical condition as they have developed resistance to most, if not all, commercialized antibiotics. Biofilm development is an old leading cause of bacterial adaptation that adds significantly to the problem owing to its resistance to intervention. Undoubtedly, biofilms are the source of significant illness and mortality. As previously noted, biofilm intransigence involves a variety of processes, involving metabolism diversity, stressors, active efflux modulation, antibiotic retention and deactivation inside the ECM, interbacterial communication, enhanced changeability, and genetic information interchange. Most of these variables have been uncovered, notably in *Pseudomonas aeruginosa* strains. The uniqueness and complexity of the revealed processes, however, suggest that they should be studied in other bacteria as well. A much greater challenging, yet crucial, would have to investigate biofilms in real epidemics, where complex microbial communities are common and a range of ecological circumstances, such as innate immunity or antibiotic penetration in tissues, are prominent. Addressing the processes that mediate recalcitrance will undoubtedly inform treatment methods to effectively combat biofilm infections. It should be supplemented with approaches for quick biofilm infection identification and in-vivo biofilm biology and composition characterization. Moreover, the provision of a spectrum of biofilm-inhibiting and dissipating drugs will help in the selection of appropriate therapy approaches for particular biofilm-associated infections.

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E-waste: A Concern

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ABSTRACT

Dependency on gadgets has elevated the usage of digital products and lead to the serious problem of E-waste generation. E-waste is now one of the major concerns in term of its management and pollution caused. E-waste includes all the discarded electronic products like refrigerators, television, air conditioner, laptops, personal computers etc. These products contain heavy metals (Pb, Sb, As, Cd, Ni, Hg, Cr, Cu, Zn), plastics and polychlorinated biphenyls (PCB). Presence of these components in E-waste is a matter of concern. They are non-biodegradable in nature and need a proper mechanism for disposal. Various management policies are already existing in both developing and developed countries to treat this waste but there is no implementation of these protocols for the management of E-waste, globally. Improper handling of this waste results in the transfer of heavy metals from this waste into the environment which is very harmful for the food chain. This review discusses the definition of E-waste, its world-wide scenario and problems related to its management.

Keywords: E-waste, Heavy Metals, Soil, Impact, Management.

1. INTRODUCTION

Safe environment is the major concern around the globe. Anthropogenic and natural source of pollutants are both equally responsible for the contamination of environment. One of the most harmful sources of Environment pollution is E-waste or Electronic waste. Major participant of E-waste are the developed countries but with time, developing countries like India and China are also participating equally [1]. E-waste is the matter of major concern because it contains various heavy metals and other harmful component which are non- biodegradable in nature and can have detrimental effect on complete ecosystem [2].

The word E-waste is an informal term used to indicate unused or discarded electronic products. Electronic waste is created when an electronic equipment is discarded after it become non-operational or non-workable. According to E-waste management rules,2016 published by Government of India in Ministry of Environment, Forest & Climate Change this waste includes discarded monitor, mobile phones, chargers, motherboards, headphones, televisions, printers and other appliances and all separate components attached with them like wires, cables, batteries, circuit boards etc.

Advancement in the world of technology has led to all the companies, institutes, private and government working bodies turning their manual paper work into digital work which resulted in exponential increase in the graph of global marketing of Electrical and Electronic Equipment (EEE). Short life span of gadgets, low recycling of E-waste [3], trend of having more than one or two electronic devices and continuous upgradation of technology influences consumers to change their gadgets more often in order to keep up with technology [4], are all causing continuous increase in market demand of EEE and this is the main reason why countries with the highest Gross Domestic Product (GDP) generate higher rate of E-waste. [5]

China, USA, Japan, India, Germany, Brazil, Russia, France, Indonesia and Italy are the leading electronic waste producing countries. Currently, our country secures 3rd position in E-waste generation next to China and USA which are on first and second place, respectively. Graph of India's E-waste generation is continuously rising from one financial year to other and current scenario suggests that it will continue rising over the next few years [6].

Developed countries have facilities, funds, and most importantly they have technology for treating E-waste properly but developing countries does not have appropriate funds and regulations for the management of E-waste, which is one of the biggest reasons which makes E-waste a harmful pollutant of the

Environment. In developing countries like India major portion of E-waste is handled by group of people who are totally unaware of the composition of E-waste and the its harmful effects. E-waste is hazardous until or unless not treated in a right manner, otherwise it can be a most profitable business opportunity, as it contains abundant source of valuable metals like gold, which can be recycled and reused from this waste if recovered in an authorized manner and brought back to production cycle.

2. E-WASTE

Matter E-waste or Electronic waste is the discarded electronic appliances that has out reached its working period. *M.Aboughaly et al.* [5] define WEEE as damaged equipment which are made up of Electric circuits and electromagnetic fields, which are dumped due to its inability to deliver up to mark performance. Different International treaties define E-waste differently, according to European Union WEEE Directives (EU), E- waste is a waste generated from household appliances and other electronic equipment and Basel Convention define E-waste as objects which requires provisions of National Law for disposal. And according to MoEF& CC, India, definition of E-waste is, completely or partially discarded equipment by consumer or bulk consumers, which are also rejected by recyclers or cannot repair further. Step Initiative (Solving the E-waste problem), mentioned that EEE cover all the goods or appliances which run on batteries and depend on electricity.

E-waste is composed of various harmful and hazardous substances which cannot degrade naturally and require proper method for treatment or handling, this makes E-waste a hot topic of discussion in various developing and in some developed countries. Maximum portion of it is composed of metals and plastic, which can be reused in manufacturing new electronic equipments and this can be considered as alternative resource for many commercially used metals [7]. If treated appropriately, it can be the biggest business opportunity and also contribute in economic growth of the country [8] but at the same time its improper handling is also a biggest warning to the environment. Chemically it is constructed of various metals and non-metals which make its configuration and handling different and difficult from other solid municipal waste. Therefore, recycling or handling of E-waste is a challenge for waste management organizations.

3. CATEGORIES OF E-WASTE

There is a large diversity in EEE (Electronic and Electrical Equipment) products present in the retail world. So, a proper framework is needed to group the E-waste into different categories. Classification of E-waste will benefit in addressing the E-waste challenges. It will help in segregation of large and environmentally harmful electronic waste (E-waste) and will be useful in providing and compiling E-waste data statistically in almost all the proposed measurement and framework. For statistical purpose, E-waste Statistics Guidelines on "Classification Reporting and Indicators - Second edition" has classified EEE products into 54 different categories. This stratification of EEE products into different categories is referred as the UNU- Keys [12]. According to the Global E-waste Monitor report 2020, on the basis of Waste Management characteristics these 54 EEE products are narrowed to six general categories that are mentioned in Table 1 [10], [28].

4. COMPOSITION OF E-WASTE

E-waste is a diverse mixture of more than 1000 of totally different substances out of which some are hazardous to the health of individuals and to the environment. 60% of E-waste is comprises of different type of metals, plastics (30%) of the portion and rest include the other hazardous pollutants, according to Kumar et al. [13]. Quantitatively E-waste contains plastic, refractory oxides and metals in the ratio of 30:30:40. Andrade et al. [9], Mentioned that E-waste is a heterogenous mixture of toxic and non-toxic substances and it is composed of Ferrous metals, Non-Ferrous metals, Plastic, Glass, Printed Circuit boards (PCB), ceramic, rubber, etc. and out of these categories ferrous metals cover approximately half of the composition of E-waste. Yamane et al. [20], categorized E-waste into different classes i.e., heavy metals, halogens, radioactive elements, etc., heavy metals like Cadmium, Lead, Nickel, Cobalt, Zinc, Chromium, Arsenic are characterized as toxic components of E-waste and if not treated right can have a detrimental effect on the health of organisms as well as on the Environment. Along with these harmful metals some valuable metals like gold, silver, platinum and copper are also present in this waste, which can be retrieved from and their depletion can be prevented. Their origin and their impact on health and environment are mentioned in Table2.

5. GLOBAL SCENARIO

According to the Global E-waste Monitor 2020 report, globally E-waste is increasing rapidly at the rate of almost 2Mt per year and it is expected that it will exceed 74 Mt in 2030 and the major reason behind this is the increase in the demand of electronic products as

Table 1: Categories of E-waste

Category	Example	Share in E-waste generation (%)
Temperature Exchange Equipment	Cooling and freezing Equipment like-Air conditioner and refrigerator	23
Screen and Monitor	All the equipments which are having screens like- All type of computers and television	11
Lamps	Equipment having fluorescent lights, LED lamps etc.	2
Large Equipment	Large household and commercially used machines come under this category. Like- Washing Machine, Printing Press etc.	26
Small Equipment	Small household and commercially used machines. Like- Calculators, Radio sets, Camera etc.	30
Small IT and Telecommunication	Equipment like Mobile phone, GPS devices, iPad, printers etc.	7

well as their short life span and less options for repair. [30] All the seven continents of the world contribute in the generation of E-waste. According to the Global E-waste Monitor 2020 report, in 2019, Asia ranks number one in the generation of E-waste and generated 24.9 Mt of E-waste followed by America and Europe which generated 13.1 Mt. and 12 Mt. respectively where as in terms of per person Europe stands first worldwide by generating 16.2 kg per capita of E-waste followed by Oceania that produced 16.1 kg per capita. Countries like Canada, United States, United Kingdom, China, France and Brazil are also fighting with the concept of improper handling of E-waste. In United States of America, E-waste covers almost 3% portion of the total municipal solid waste and for this it has acquired EPR strategy, USEPA in 2011 collaborated with various official agencies but now according to USEPA 2016, there are no federal system for the management of E-waste in USA. Out of total E-waste, approximately 50-70% of E-waste is transported to various developing countries [16] and only 25% of E- waste is recycled domestically (USEPA,2016). Similarly, United Kingdom is one of the highly developed and economically developed country of the world due to which usage of electronic product is much high over there, which ultimately make E-waste as a fastest growing waste of U.K. Similar to other countriesU.K also transport its E-waste to developing countries in Asia and Africa and domestically recycle approximately 17% of total generated E-waste [16]. U.K has legislature for controlling E-waste from 2002, which is known as EU (E-waste Directive to regulate E-waste), under which responsibility for handling this waste is given to private operators [17], in spite of having legislation E-waste from this country travels to the developing countries. Along with U.K, France is also generating E-waste in high amount. According to Ongondo et al. [18], generation rate of E-waste of France in year 2014, was 17-23 kg per inhabitant. E- waste recycling management system already existed in France from 2005, but only around 38% of total E- waste generated from market undergo formal sectors, rest reached to the informal sectors, as informal traders of E-waste recycling are dominant in France, these sectors recycle this waste in an inappropriate using environmentally unfriendly methods. Apart for all these countries and states, China is also one of the famous Asian countries, which is struggling with the improper recycling of E-waste and receiving most of the E-waste from developed countries. World's 70% of E-waste is received by Guiyu, a town in China. In China, only 25% of E-waste is recycled under the supervision of authorized sectors and rest in informal

recycling sectors. Many studies have reported that very high concentration of heavy metals is present in ground and river sediments in Guiyu [19]. Brazil is also a leading country in the generation of E-waste, population of Brazil is very high and the craze of using developed technology in this country is also very high, approximately 200 million people frames the complete population of Brazil and it was estimated that 7 Kg E-waste per capita is produced by Brazil and it also receive E-waste across the boundaries [26]. In Brazil domestic waste is considered under one of the government policies i.e., Brazilian National Policy for Solid Waste (NPSW). But according to Blade et al. [11] only Brazil from Latin America not yet been able to imposed legislations over its E-waste.

In 2002, Basal convention came into action to address E-waste, and which looked into E-waste eco- friendly management practices to prevent illegal traffic of E-waste to developing countries and better management of E-waste globally in spite of the formulation of Basal convention for ensuring that environmental problems are not going to be exported overseas [23], developed countries recycle as many e-waste they can and transport rest of the waste to the developing countries [21] which are already struggling with their own E-waste management practices due to lack of knowledge and funds, like Asian countries (India, China, Pakistan, Bangladesh, Sri Lanka, Bhutan and Nepal) and African countries (Ghana, Nigeria, Kenya). All this transported E-waste is received by Informal sectors of these developing countries without any maintaining any records and legal documentation [13]. In exchange of E-waste, developing countries gets payment. Sometimes developing countries that cannot afford the manufacture of new electronic equipment accept this waste in the form of donation also from developed countries [12]. United Nations Environmental Programme (UNEP 2016) also stated that only 10% of E-waste is properly recycled by developed countries and rest is transported to above mentioned developing countries [1].

6. SCENARIO OF E-WASTE IN INDIA

As per the reports by CPCB (Central Pollution Control Board) in the year report of 2019-2020, it has been reported that India generated 1,014,961 tonnes of E-waste in 2019-2020, which is 32% more than the E-waste reported in the CPCB report of 2017-18 i.e., 771,215 tonnes. According to Federica et al [31], E-waste accumulates nearly three times faster than other waste. E-waste regulations in India came to effect from May 2012, with additional amendments from 2016. Regulations came in 2012 acknowledged that the major issue regarding E-waste management recycling is the poor drafting of information on the rate of generation of E-waste in a particular country [7]. For this particular purpose the regulation 2012, assigned a task to SPCB (State Pollution control Board) to release state wise E-waste inventories, but this is still under process.

In South Asia, India is the only fortunate country to have all the required legal instruments and E- waste management since 2011 but due to the poor system of inventory management and violation of rules more than 90% of total E-waste generated in India is still in the hands of unauthorized dealers and only 10% of E-waste is handled by authorized sectors in an environment friendly manner. In the month of July, 2021, DPCC (Delhi Pollution Control Committee) conducted an inspection of the CPCB authorized E-waste collection centres, which are registered under EPR (Extended Procedure Responsibility) and found that 72 authorized dealers were not obeying the guidelines given by CPCB, DPCC clearly mentioned that disobedience of E-waste management rules is very high in the capital, state board of others states i.e., Madhya Pradesh, Maharashtra and Orissa also reported the same problem regarding the violation of E-waste management rules. India has various authorized dealers which deals with E-waste in an appropriate manner but fails to do it to their approved capabilities, due to lack of information and funds from government.

Consumers and producers of E-waste in developing countries are unaware with the procedure of returning of damaged electronic products or equipments according to regulations made by the government, they usually give their E-waste to unauthorized scrap dealers which come to their door step to collect this waste, especially owner of household E-waste who are totally unaware with the fate of this waste. India has perfectly managed informal system scrap dealers in place due to which they are still handling approximately 95% of E-waste generated in India. Therefore, informal sectors with the help of these scrap dealers make it more convenient for consumer and producers to get rid of this waste. Informal sectors are increasing day by day with the help of unemployed population as these sectors provide livelihood to many people who are complete unaware with the consequences of working in this sector. Easy to understand system design and flexibility of unauthorized sector the advantages of this sector. The process of recycling of E-waste by informal sectors is inappropriate but their role in collection and transportation of E-waste is recognizable with respect to the management of the country's total E-waste.

The work of recycling E-waste is complicated as it requires proper understanding of resource recovery from waste. There should be skilled workers to handle such a harmful stream of waste and most importantly recyclers involved in this occupation must have gone through the environmental policies in order to save the environment from the later consequences [27] and these are the main things which are completely lacking in this sector and make this system an inappropriate one for the recycling of E-waste.

7. MAJOR ENVIRONMENT AND HEALTH ISSUES

Various studies have found that increase in inappropriate handling of E-waste is directly proportional to the increase in number of adverse health effects. The fate of E-waste after collection by dumpster is either goes to landfills or handed over to unauthorized dealers. If this waste goes to landfills then, the major component of E-waste i.e., heavy metals which are non-biodegradable in nature can accumulate in the soil for a longer period of time and can totally damage the fertility of the soil or damage this soil for life. Even though, heavy metals play major role in the fertility of soil but on crossing the threshold limit heavy metals can become toxic to soil as well as can have adverse effect on the same land for a very longer span of time [13]. Heavy metals and flame retardants from E-waste can leak through the soil and reach groundwater level and also contaminate the same. Contamination of soil and ground water surely reaches the crops which are planted near to it or crops which may be planted in this contaminated area in the future. Figure .1 [15,22]. Among all the living organism's life processes of plants are affected the most as elevated level of these pollutants have a very negative effect on physiological activities of plants and heavy metals disturb the process of respiration, photosynthesis, electron transport chain and cell division in them. Heavy metal toxicity can also damage the structure of the plant cell due to oxidative stress which directly affect the metabolism and growth of plants. Once E-waste finds its way to the soil it automatically finds its way to reach each and every component of the environment. Removal of heavy metal contaminants from the biological tissues is challenging and has become matter of utmost concern.[15]

As per the reports by Global E-waste Monitor 2020, E-waste has become one of the major contributors of rising Global warming. Around 98 million tonnes of carbon dioxide equivalents were released into the environment by the crude recycling of undocumented house hold E-waste. Burning of the waste transmits cyanogenetic exhaust and mixes various harmful gases into the air. This directly gets inhaled by the local residents and labours involved in the field, and worsens the health condition. It is reported that 12.9 million of female workers and approximately 18 million of children between the age group of 5 to 15 years are also actively engaged in informal sectors for E-waste recycling. Pregnant women engaged in this work are directly harming their health and developing risk for their future child as there is a chance of passing some hazardous chemicals from pregnant mother to unborn child in her womb which can adversely affect the growth of unborn child and can even cause damage to their DNA, resulting in a life time damage to the child's health Report 2020. Population exposed to these harmful substances regularly complains about headache, chest pain, weakness, dizziness and breathing problems. Oral ingestion of heavy metal contaminated food is a major pathway which is responsible for the transfer of heavy metals from the environment to the human body. [25] Some of the major impacts of heavy metals and other toxic substance present in the E-waste which can harm environment and living organism are mentioned in Table 2 [13,24].

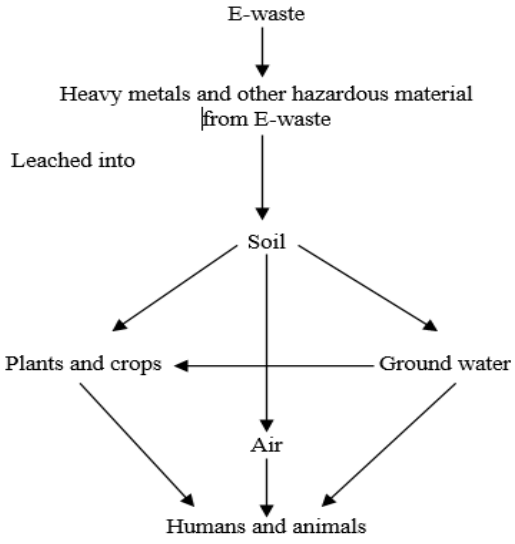

Fig. 1: Flow chart depicting movement of hazardous component of E-waste into the environment

Table 2: Sources of generation of E-waste and their impact on Environment and Human Beings

Source of E-waste	Element from E-waste	Impact on Environment and Human beings	References
CRT screens, batteries, Glass panel, monitor	Pb	Disturbance in nervous system. Loss of appetite. Can affect multiple organs like- Kidney, Reproductive system and gastrointestinal system. If reaches ground water level can release toxic phosphor.	Kumar et al 2018 Kapahi et al 2019 Mariappean et al 2015
Lithium Batteries	Li	Pollute environment by producing volatile fumes.	Kapahi et al 2019
Rechargeable NiCd batteries	Ni	Allergic reaction	Kumar et al 2018
Fluorescent lamps, alkaline batteries and switches, also present in thermostat, sensors	Hg	Have acute toxic effect on health. Cause problems related to skin, inhalation and ingestion. Can damage brain and kidney.	Kapahi et al 2019 Mariappean et al 2015
Cooling element insulation foam	Chlorofluorocarbon	Its burning can cause toxic emission.	Kumar et al
Fluorescent coating	Infrequent earth elements	Cause problem to skin.	Kumar et al. 2018
Light emanating diode	Gallium arsenide	Harmful to health of Human beings.	Kumar et al. 2018
Interior of a CRTs	Zinc Sulphide	If inhaled and can cause lung related disease.	Kumar et al. 2018
Toner cartridges for laser printer	Toner dust	Risky if inhaled and can cause irritation in Respiratory tract.	Mariappean et al. 2015
Condensers, Transformers	PCBs	Very harmful and cause various health issues like cancer, damage immune system and nervous system. Use of PCB during production of E-waste cause heavy pollution.	Kumar et al. 2018
Thermal power plants	As	It has carcinogenic and cardiovascular effect and neurobehavioral disorder.	Kapahi et al. 2019 Dotaniva et al. 2015
In the production of batteries, semiconductors chip and infra-red detectors	Cd	Its exposure leads to lung and stomach cancer. May cause renal injury. Genetic disorders. Acute inhalation problem.	Kapahi et al. 2019
Untreated steel plant	Cr	Responsible for various health problems like kidney damage, asthma, cardiovascular, renal, neurological disorder etc.	Kapahi et al. 2019 Mariappean et al. 2015

8. CONCLUSION

This review discusses the definition of E-waste given by various agencies and worldwide management of E-waste. In spite of having rules and regulations in almost all parts of the world, it has been seen that proper implementation of these rules are lacking in both developed and developing countries, which results in improper handling of E-waste and leads to contamination of environment. The transportation of E-waste from developed countries to developing countries is increasing the level of improper handling in these recipient countries. Therefore, it is very important to implement eco-friendly technology with high annual recycling capacity for proper management of E-waste in India as well as other countries. Proper implementation of rules and regulations can save environment from further damage.

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Bio-Mining: A Sustainable Method for Metal Extraction

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ABSTRACT

Mining sector is said to be one of the most major forms of environmental pollution because of the type of processes involved in the conventional mining. Mining industry has been disturbing and destroying the ecosystem and wildlife in many possible ways. So, a major challenge for the mining industries is to supply metal and minerals without further degradation of the ecosystem. With this growing concern, eco-friendly method of using microorganisms holds promises for mining work. Use of organisms for the extraction of metal has been observed since the 18th century and this method of metal extraction is now being termed as "bio-mining". The present review article discusses the methodology involved and the use of bio-mining for the benefit of the environment. The process of bio-mining can be achieved by two different procedures. The first way is bioleaching in which metal is directly extracted from its constituent ore and the second way is, bio-oxidation in which the metal is made more accessible in the ore so that the extraction becomes easier by the use of conventional mining techniques. A wide diversity of microorganisms such as T. ferrooxidans and L. ferrooxidans, are used in the above-mentioned processes. The commonly extracted metals are copper, gold, nickel etc. The use of bio-mining can further be extended to reclaim the land already degraded by the conventional mining methods. It can also be employed for handling e-waste which is further elaborated in the article. Bio-mining being both eco-friendly and economic has led to its worldwide acceptance.

Keywords: Bio-Mining, Bioleaching, Micro-Organisms, Bioremediation.

1. INTRODUCTION

Mining *is the process of extraction of economically valuable minerals from the surface of the earth as well as the sea*. Mining has been conducted since prehistoric times according to archaeological discoveries. In the Neolithic period, mining techniques were as simple as using hands, shafts or stone tools. Eventually, fire was begun to be used for clearing tunnels and to reach great depth in less time. Great progress in mining techniques was made in the late middle ages when explosives came into use in order to clear out large rocks. The earliest used explosive was black powder (it is the first explosive to be used in history, mainly composed of potassium nitrate, charcoal and Sulphur)[1] which was then replaced by dynamites in the 19th century. Along with this, advancement in motorized mining tools was also seen. Drills, lifts, and steam powered pumps decreased the cost and time of extraction and increased the efficiency of miners to break big rocks. Industrial revolution stimulated the improvement in mining equipment and technology.

At present the process of mining can be divided into various steps which logically together form a life cycle. But all these different phases, starting from the first step to the last, are associated with a number of environmental effects.

1.1. ENVIROMENTAL CONSEQUENCES OF CONVENTIOANL MINING METHODS

Mining sector is said to be one of the most major causes for environmental pollution. They are:

Soil Pollution

Surface mining is highly destructive but still mining industries prefer to go for surface mining because of its less labor requirement and more yield than underground mining. During surface mining topsoil is cleared from the area of mining causing destruction of landscape, wildlife habitats, forests etc. Majorly, metallic ores are obscured beneath a cover of soil known as 'waste rock' that has to be removed in order to grant way into the deposit of the metal ore. This quantity of overburden produced by mining is enormous. All this waste contains extensively elevated levels of poisonous elements which get accumulated on the site in the form of masses, on the

plane, backfill in open depths, or inside the mine underneath.

Contamination of heavy metals in soil at the place of waste disposal and its surroundings is the most prominent side-effect of conventional mining. This metal contamination of soil's environment and its potential threat to global and marine environment and health of humans is considered to be the major responsibility challenging today's scientists. The metals in earth are not innately lethal if it is present in low concentration but now, we can see there is an escalating occurrence of metal pollution from the generated waste. The amount of Metal present in the soil is somewhat affected by the reaction that takes place in between the components of metal and soil. The examples of metals that are often seen as contaminants are Pb, Cr, Cd, Cr, Zn, and Hg.

Noise Pollution

Noise pollution is also associated with various activities done during different processes of mining which includes clatter from machine engines, sound during load and unloads of material, generation of power, and many others [2]. Collective sounds from all the actions mentioned above have a direct effect on the animals and the people living close by. The various types of mining equipment generate vibrations of high frequency, specially blasting which is taken as to be the chief source. They can affect the steadiness of constructed structures and houses of individuals living near the area of mining

Air and Water Pollution

For mining a large area of earth is dug and a significant amount of rocks are crushed and moved. This causes a large release of earthly particles into the air, increasing the particulate matter in the surroundings. Not only this, some of the toxic substances from mine tailing can enter into the air. This can have direct health effects on people working on the site or living nearby. A similar example is seen in Arizona where in Navajo reservation lands due to uranium mining major health effects were seen on people such as tuberculosis and lung cancer [3].

Conventional mining requires a large amount of water for extraction and processing of ore. This requirement for water is meet by the local rivers or underground water. This not only depletes the water supplies but also contaminates the water making it unfit for consumption. For example, depletion of underground water is seen in Santa Cruz River basin in Arizona because of the nearby Cu mining [4].

Habitat Loss

A direct impact of mining is seen on the local bio-diversity of the area, starting from the removal of the vegetation from the site of mining to the rehabilitation of animals. Many animals and birds lose their habitat, fishes are killed because of toxic water, and areas are cleared leading to large deforestation. Mining negatively affects the ecosystem, degrading the quality of living.

Hazardous Waste

Mining produces a large amount of toxic waste and by-products. Managing these is one of the major problems, usually the waste is either filled back or is dumped in land-fills. This is not only causing problems at the site but also where the waste is disposed of. The issue increases if the waste is radioactive it has to be treated properly before it is discarded.

So, a major challenge for the mining industries is to supply metal and minerals without further degradation of the ecosystem. With this growing concern options for an eco-friendly way of mining using microorganisms like bacteria holds promises. Various eco-friendly techniques have come into picture such as:

Biosorption is another technique that uses of bacteria, fungi, or algae as adsorbents for the recovery of metals. The major advantages of biosorption are: Firstly, it is highly effective in reducing the metal content. Secondly, it uses biosorvents that are not much expensive.

Bio hydrometallurgy is another promising and eco-friendly technique that exploit microbiological processes for recovery of metal.

Bio-mining: Mining industries wisely use the natural ability of microorganisms to digest, absorb and to refine ores for extraction of metals.

2. BIO-MINING

"Bio-mining is an increasingly applied biotechnological procedure for processing of ores in the mining industry (bio hydrometallurgy)."[2]. Bio-mining is a process of extracting metals of economic interest like gold from its ores using micro-organism. Most common metals that can be bio mined are Cu, U, Ni and Au. They are majorly applied in extracting metals from the sulphide ore.

2.1. HISTORY OF BIOMINING

The use of microorganisms for the extraction of metal from its constituent mineral is fundamentally the utilization of properties of different bio-life for an eco-friendly extraction. For numerous decades microbes have been utilized for the withdrawal of overwhelming metals from the core of earth. The utilization of Bio-mining has been seen for a long time in spite of the fact that individuals of that period didn't know about the association of organisms. The utilization of organisms to concentrate copper has its foundations somewhere down in times long past that is around 18th century. The first microorganism utilized in the assembly of metals was given by roman author Gaius Plinius Secundus (23-79 A.D.). The German doctor and mineralogist Georgius Agricola (1494–1555) in his work "de re metallica " mentioned the recuperation of copper by draining minerals containing copper. Rio Tinto mines in south-western Spain are also known as the supporter of bio-hydrometallurgy. Rio Tinto mines have been abused since pre-Roman occasions for the metals like Cu, Ag, and Au. Bioleaching at the Rio Tinto mines apparently began in the 1890s. The river Tinto also known as the red river is named so because of the red colored water obtained due the higher concentration of iron. The dissolved iron is found because of the microbes [5]. This river is known for its non-drinkable water and the lack of fish in it.

2.2. PROCESS OF BIO-MINING

Profitable metals are normally found in strong structures. A very few microorganisms can oxidize these metals effectively, and enable them to dissolve in water. It is utilized for metals that can be all the more effectively recuperated from the strong rocks. This process is known as bio-leaching. Bio-leaching is hence the process which helps in extraction of metal by making them water soluble. An alternate bio-digging method is utilized for metals that can't be broken down into water soluble form by the microorganisms, in this method organisms, separate the encompassing minerals, making it simpler to recoup the metal of intrigue straightforwardly from the stone. This type of extraction is known as bio-oxidation [6]. Organisms are particularly great at oxidizing sulfidic minerals like iron and copper [6]. Different metals, similar to gold, can't be disintegrated straightforwardly by this microbial procedure. In both the procedures, the microbial response occurs in any place, where organisms, rocks, and basic supplements are accessible.

2.2.1. TYPES OF PROCESSES USED IN BIO-MINING

The two different types of processes used in bio-mining, are:

- Irrigation type**:** it involves seeping down of leaching solution through the squashed mineral that can be stacked in segments, load, or dumps. Example: in-situ draining
- *Stirred tank type:* in this, the process takes place in a tank that is continuously stirred.

Bio-mining process can take from days to months thus making this process a bit slow. Dump and load type of biomining is known to be the most used and most settled bio-mining procedures, yet the utilization of blended tank draining is used increasingly for the minerals that are impervious to filtering, including some copper sulfides such as chalcopyrite [7].

In loads of ground method, the second-rate minerals are ground into powder and heaped in a watered open-air area. The stacks are then treated with an acidic fluid that contains a small amount of the required bacterial populace (some normally existing inside the mineral). The fluids with the metals removed are then pumped into another area where metal is recovered.

3. BIO-LEACHING

Bioleaching is known as the alteration of solid insoluble metals (mostly sulfide) into a form that is easily soluble in water (mostly sulfates) with the help of microorganisms. Bioleaching is also regarded as a “Green Technology” [8]. A large number of metals like the extraction of Copper, Gold, Uranium, Nickel, Cobalt etc. can be viably extracted from their respective ores by bio leaching method. It will turn out to be much progressively significant in future years, as it maintains a strategic use of energy and thus reduces the total cost

3.1. HISTORY OF BIOLEACHING

Just like biomining bioleaching also have a vast history, where the process was being used by miners without the actual knowledge of the chemistry behind it. What miners simply observed that watering the sulphidic ore of copper will eventually lead to illusion of copper which further can be reobtained from the solution. Factually, bioleaching is testified to be used since 20-70 AD, in countries like China, India and Spain it is known to be used for as long as 2000 years [9]. The very first evidence of presence of microbes was noticed in 1947 in a coal deposit, the microbes where *Thiobacillus ferrooxidans* which were later called as *acidothiobacillus* [10]. It was after that

acidiothiobacillus were started to be used for research purposes and later on for commercial benefits also. Followed by it, in 1950 dump leaching for copper was done in Bingham Canyon. A boost to bioleaching was seen and different types of bioleaching were seen like heap and in-situ leaching for copper was done. For example in-situ leaching of uranium in Canada, heap leaching of gold in Nevada [9].

3.2. MECHANISM OF BIOLEACHING

The procedure of bioleaching is some way or another totally dependent on the movement of *T. ferrooxidans, L. ferrooxidans* and *T. thiooxidans* which can easily convert the intensely insoluble metallic sulfides to water solvent metal sulfates through the procedure of bio-draining. The organic change of metals by microorganisms may happen by direct or indirect bio-leaching [11,12]

3.2.1. DIRECT BACTERIAL FILTERING

In this sort of filtering, there is an immediate contact between the bacterial cell and the outside of mineral sulfide and the oxidation to sulfate happen by a few enzymatically catalyzed advances

For example: In this process, pyrite is oxidized to iron(III) [13] sulfate according to the following reactions:

$$4FeS_2 + 14O_2 + 4H_2O \xrightarrow{\text{bacteria}} 4FeSO_4 + 4H_2SO4$$

$$4FeS_2 + O_2 + 2H_2SO_4 \xrightarrow{\text{bacteria}} 4Fe(SO_4)_3 + 4H_2SO4$$

The following reaction shows the direct bio-leaching of pyrites:

$$4FeS_2 + 15O_2 + 2H_2O \xrightarrow{\text{bacteria}} 4Fe(SO_4)_3 + 4H_2SO4$$

The general equation for the direct bacterial leaching can be as follows:

$$MeS + 2O_2 \xrightarrow{\text{bacteria}} MeSO_4$$

Where MeS is metal sulphide

3.2.2. INDIRECT BACTERIAL LEACHING

In this type of leaching organisms are not in straight contact with minerals in fact leaching agents are produced by bio-organisms which oxidize them [13]

For example:

$$MeS + Fe(SO_4)_3 \longrightarrow MeSO_4 + FeSO_4 + S0$$

To maintain the correct level of Fe inside the solution the oxidation should take place in an environment having a pH below five. The rising level of ferrous ions are readily deoxidized to ferric ion by *T.ferrooxidans* or *L.ferrooxidans* which can then again be involved in the process of oxidation. The bacterium in indirect leaching only acts as a catalyst as it accelerates the re-oxidization of ferrous ions which in reality is a very slow process. According to Lacey and Lowsen bacterial oxidation of ferrous ions increase 10^5- 10^6 times in pH 2-3.

3.3. LEACHING PROCESSES

Three strategies have been monetarily utilized for extraction of metals from extra evaluation minerals.

- *In situ* filtering or arrangement mining
- Dump filtering
- Heap filtering

All the previously mentioned strategies have been utilized since most recent four decades due to their focal points like low-value, appropriateness for various kinds of mines and extra evaluation metals and natural worthiness.

***In-situ* leaching**

The mineral ore at it's place of origin is exposed to leaching. Surface blasting of rock is done just to increase permeability of water. Thereafter, water rich *Thiobacillus* is pumped into the ores using drilled passage. As the acidic water leaks through the stone, it gathers at the base. The water is pumped again from which mineral is extracted. The water is used again after the generation of bacteria. Use of in situ filtering relies upon the geographical and land highlights of the mineral body, and furthermore on the hydrological and mechanical properties of the host stone [14].

Dump leaching

The mineral is at long last ground and dumped in huge loads down a mountainside. The waste dumped at mining site can't be monetarily handled utilizing any old mining strategy. In this manner dump filtering strategy is utilized in which the mineral is then exposed to persistent sprinkling of water containing the ideal microorganism. The water gets gathered at the base which is then used to concentrate metal. The water can be reused for recovery of bacteria. Since the bio-oxidation of sulfides is exothermic, temperatures of the request of 60-80^{o}C can be accomplished inside the landfill along these lines Presence of a few thermophilic autotrophs which can endure higher temperatures has been accounted [15].

Heap leaching

This type of leaching is the most known and used for the mining of metals, particularly utilised for copper.

Heaps of poor quality minerals or waste materials are organized after they are crushed. The piles are close to a couple of meters high by a couple of meters wide in order to enable the oxygen to diffuse to all pieces of the load. The heap is then sprayed with solution containing a fraction of bacterial population. The run-off is collected from which the metal is then extracted [16].

4. BIO-DIVERSITY OF BIO-MINING MICROBES

A wide variety of micro-organism are used in biomining. They are classified into different classes based on the temperature they work best in as shown in the table below:

Table 3.1: Classes of microbes used in bio-mining based on temperature

Classification	Temperature. (^{0}C)
Psychrophile	20
Mesophille	35-42
Moderate thermophile	45-50
Extreme thermophile	65-80

4.1. MESOPHILLES

They are ones that show growth at an optimum temperature of 25-40 degree Celsius and do not show any growth above 45 degree Celsius [17]

Acidithiobacillus

Bacteria of the genera *Acidithiobacillus* possess the following characteristics:

- They are generally strict aerobes and can be both obligate and facultative chemolithotrophs. They can be mixotrophs also.
- ***Acidithiobacillus*** can cultivate in solution having pH ranging from 0.5 to 10.
- They are mesophiles having optimum temperature of 30°C. However, they can grow in wide temperature range between 2 to 37°C.

The five main species of this genus are:

- *Acidithiobacillus thioparus,*
- *Acidithiobacillus dentrificans,*
- *Acidithiobacillus thiooxidans,*
- *Acidithiobacillus intermedius*
- *Acidithiobacillus ferrooxidans.*

Example

At. ferrooxidans

At one point it was viewed as the most significant microorganism utilized in biomining forms that work at a temperature of 40°C or less. Be that as it may, anyway *At. ferrooxidansis* not favored now in circumstances in which the ferric iron concentration is a lot higher than the ferrous iron (high redox potential). *At. ferrooxidansis* still considered to be one of the best among other bacteria in dump and stack filtering conditions extraordinarily in uranium and copper oxide/sulfide draining [18]. *At. ferrooxidans* shows rapid growth in comparison with numerous other bio-mining microbes and is commonly supported inside the pH range of 0–2.5, giving that the proportion of solvent ferrous iron to ferric iron is high [19].

Thermophiles

Thermophilic iron-oxidizing bacterium is further classified into moderate and extreme thermophile.

Moderate Thermophiles

The optimum temperature for development and leaching out of metal for such thermophiles is from 40 to 45°C [20].

Example

Sulfobacillus thermosulphidooxidans is a gram positive, non-motile, spore shaping eubacterium.

Extreme Thermophile

The ideal temperature for development and metal draining for such thermophiles is between 65 to 85°C. They all are oxygen consuming, extraordinary thermophilic and acidophilic microorganisms that can oxidize ferrous particles, basic sulfur and sulfide mineral [20]. There ideal temperature for development is somewhere in the range of 55 and 90°C, ideally it is 70-75°C. They can develop between the pH of range 1 to 5, with ideal development around pH 3.0 [20].

5. CURRENT SCENARIO

In present day scenario microbes have been used to extract metals in very efficient way such as:

Urban Bio-Mining (*bioremediation of industrial waste*)

Demand for metals is increasing with the rapid industrialization, but the reserves of high-grade ores are being diminished [21] Metals are used for industrial purposes as well as in large number of gadgets used in day to day life. These days, metal-bearing buildups are being created in immense sums and this amount is expanding because of increment in populace just as the enhancement of the utilizations of metals. All the metal used these days is obtained by the process of primary mining technique and processing of very large quantities of rocks and metalliferous minerals which are finite and unequally

distributed in the world.

When electrical and electronic hardware reach a point where they can no longer be used are named as waste electrical and electronic equipment (WEEE) whose proper disposal is a serious global concern [22]. About 50 million tons of WEEE is generated globally [23]. About 30% of metallic and 70% of non-metallic substance can be removed from the printed circuit boards (PCBs).

The percentage of metal present in a PCB depends upon the origin of the material (type and make of instrument) and are mostly present in the following percentages: 10–27% Cu, 8–38% Fe, 2–19% Al, 0.3–2% Ni, 1–3%, Pb, 200–3000 ppm Ag, 20–500 ppm Au, 10–200 ppm Pd, etc [24].

In request to concentrate metals from WEEEs by bioleaching, much consideration have been centered around PCBs. Acidophilic autotrophic, acidophilic heterotrophic, or canogenic heterotrophic microscopic organisms have been utilized to recoup different metals from PCBs.

The disposal of this unsafe modern waste additionally brings about defilement of both land and ground water, and causes genuine ecological harm thus all this waste must be treated to moderate its natural effect. And yet, these solid wastes, (for example, electronic piece material and burning fly fiery remains) with high metal qualities, can be utilized as an auxiliary mineral for metal recovery and reusing. Bio urban mining of auxiliary crude materials is not exclusively used to enhance the supply of metals and materials but along with it, it helps in decreasing the demand for essential mineral assets. The utilization of microbes in the form of bio-catalysts in bio- urban mining would be an extraordinary answer for detoxification of risky squanders too recoveries of metal from waste

USE OF URBAN BIO-MINING IS SPACE

If we talk about space missions mainly consist of metallic components, from the spacecraft to electronics. But more metal add more mass, and electronics have the additional problem of a limited lifespan. Thus, current mission architectures must look for alternative. In space the metal is directly discarded but on earth, metals are first recycled. But the current processes are toxic and environmentally hazardous. So a solution is proposed to recycle the junk metal in non-toxic environment and also enhancing the mission success by reducing up-mass; that is the sent electronics will be used as feedstock to make fresh electronic components, by the process of 'urban bio-mining. Here astronauts' uses specific microbes to leach out the metal from the old, not-in-use metal equipment already present on the space craft.

The main objective of this projects to take first step towards the recycling of IC chip and reprinting then , using the abilities of micro-organisms to reduce mass and toxic waste. This elemental material bio-mined by engineered micro-organisms is printed using plasma printer which uses martian atmospheric gases to generate plasma [25].

6. CONCLUSION AND FUTURE PROSPECT

The process of Conventional mining more or less dependent upon the application of toxic substances accompanied by high temperature causing environmental destruction. With increasing awareness on the environmental effects of primary mining and to satisfy the growing need of metal resources have prompted the humans to switch to a cleaner and safer method for the extraction of metals. In this global drive towards green solution bio-mining turned about be an attractive alternative to overcome the ill effects of conventional mining and to extract metal from low grade ores.

Bio-mining has an edge over conventional mining in many things such as:

- Considerably green, low gas emission
- Require less labour
- Less operating and capital cost
- No external fuel required
- No toxic waste generated
- Low maintenance requirement

Therefore the use of bio-mining is growing rapidly and many more countries are adapting this technique. But it do have its own set of disadvantages like it is limited to only a few number of ores and extraction of metal by this technique can be a bit slow. But the advantages over power the disadvantages thus making it the most suitable technique for now.

With advancements in genomics, proteomics, transcriptomics, metabolomics (26) scientists are able to study and explore more about microbes. There are various other microbes that are yet to be put into use for their desirable properties. Genome sequencing will also play a very important role in modifying the micro-organisms as per the requirement, these genetically modified microbes will make the process of bio-mining much more efficient and effective.

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Hexavalent Chromium Induced Toxicity and Bioremediation Strategies: Current Research and Future Directions

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ABSTRACT

Chromium is an element found in the earth's crust and is of great importance in the current industrial scenario, but poses an overall threat to the environment. IARC (International Agency for Research on Cancer) has classified it as a potent carcinogen. Its environmental behavior is determined by its valence states, Cr(III) and Cr(VI), which are relatively stable and largely dominant. Cr(III) is a trace metal and holds an important role in glucose and lipid metabolism in the body, whereas Cr(VI) is considered a potent carcinogen. However, microbial bioremediation strategies that involve the conversion of Cr(VI) to Cr(III) via chromium reductase secretion and phytoremediation by sequestering it in plant aerial parts have a high potential for removing chromium pollution from the environment. This review provides detailed insights about the presence of chromium in the environment, its speciation, toxicity in plants and humans, and remediation measures.

Keywords: Chromium Contamination, Bioremediation, Phytoremediation, Toxicity, Speciation.

1. INTRODUCTION

Damage to the environment has been inflicted by the overexploitation of natural resources. Water and soil have been contaminated with noxious heavy metals, including chromium (Cr), lead (Pb), arsenic (As), cadmium (Cd), etc., which have significant harm to microorganisms and plants in both the earth's fauna as well as harm to humans via the food chain [1-2]. Hexavalent chromium contamination in the environment is prevalent due to its widespread use in a variety of industrial applications such as metallurgy, electroplating, leather tanning, paint and dye making, stainless steel manufacturing, and pulp and paper industries [3]. Cr(VI) is responsible for soil deterioration, disruption of microbial activity, decreased plant productivity and has adverse impacts on human health. In the modern periodic table, Group VI-B contains the transition metal chromium, with oxidation numbers ranging from Cr(II) to Cr(VI). Trivalent Cr(III) and hexavalent Cr(VI) are the most common forms in nature and exhibit distinct biochemical and physicochemical properties [4]. The most prevalent naturally occurring state is Cr(III), which in soil and water forms complexes with organic compounds. It exists as chromic oxides (Cr_2O_3), hydroxides $Cr(OH_3)$ and sulphates ($Cr_2(SO_4)_3.12(H_2O)$ [5]. Cr(VI) has a high potential to oxidize and is the most toxic form of chromium. It has higher mobility than Cr(III) due to its frequent binding with oxygen in the form of chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) ions. Cr(VI) is more water soluble and thus has a higher bioavailability than the trivalent form [6]. The World Health Organization (WHO) and Agency for Toxic Substances and Disease Registry have well documented the health impacts related to chromium. The United States Environmental Protection Agency (USEPA) has also listed Cr(VI) as one of 17 chemicals that have a high impact on human health [7]. Hexavalent chromium species Cr(VI) species namely Cr_2O^{2-}, $Cr_2O_7^{2-}$ and CrO_4^{2-} are highly bioavailable and mobile forms in aqueous environment. Due to their mutagenic and carcinogenic properties, these forms are considered the most lethal [8]. Cr(VI) crosses cell membranes due to high redox potential, causing disruption in cellular and molecular components. This results in DNA modification in plants and animals, protein degradation, and membrane disruption [9]. It also has noxious effects on kidneys and liver functions as well as the development of epigastric pain and allergic reactions in the stomach, causing ulceration and hemorrhage [10]. Figure 1 illustrates chromium pollution in the environment and its toxicity impacts. Therefore, to address the issue of Cr(VI) contamination in the environment, a variety of remediation methods have been developed. In general, the methods can be classified as physical, chemical, or

biological. Bioremediation using plants and microbes has grown rapidly in recent years and is now considered the most viable option for environmental management. In comparison to physical and chemical methods, bioremediation to deal with Cr(VI) proves to be convenient to operate, cost-effective and eco-friendly.

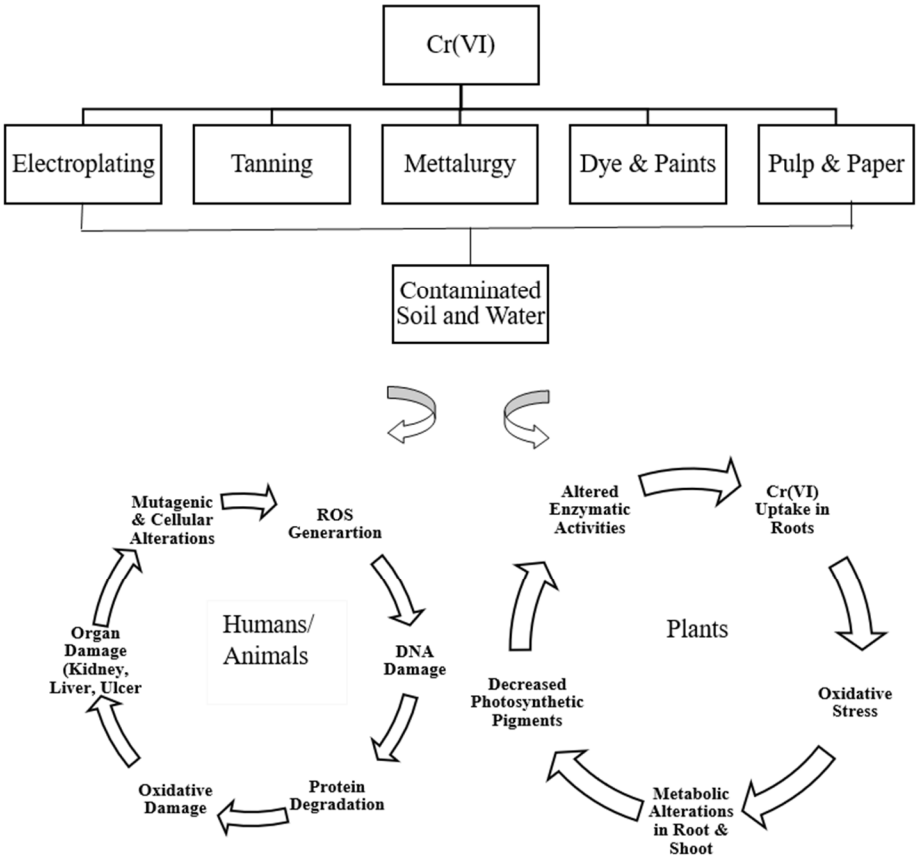

Fig. 1: Cr(VI) Accumulation in Environment and Toxicity Impact [11-13]

2. CHROMIUM TOXICITY IN PLANTS

Accumulation of Cr(VI) in plants results in the overproduction of reactive oxygen species (ROS) as a byproduct of various biochemical reactions in mitochondria, peroxisomes, and chloroplast [14]. Stress caused by the excessive ROS accumulation hinders the overall plant's growth and development [15]. Hydrogen peroxide (H_2O_2), superoxide anion ($O2^-$), peroxyl (RO^-), hydroxyl ion (HO^-), singlet oxygen ($^1O_2$), alkoxyl (RO^-), and organic hydroperoxide (ROOH) are some of the ROS expressed in plants [16-17]. ROS levels in plants are regulated by a variety of mechanisms in plants, including biosynthesis of reactive oxygen species, enzymatic scavenging, and other non-enzymatic mechanisms [18]. Physiological, biochemical, and molecular alterations in the plant cell by excessive ROS accumulation are believed to be provoked by the direct interaction and inhibition of enzymes, lipids, proteins, and genetic material (DNA/RNA) which results in suppression of cell division and programmed cell death, which occurs via direct interaction of Cr(VI) with the membrane or by ROS generation and accumulation [19-21].

Plants have a well-organized and defined antioxidant enzyme defense mechanism to combat heavy metal stress and to scavenge ROS [18]. To minimize oxidative stress, plants use antioxidant enzymes viz. catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxide (POX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione S transferase (GST), etc. [22-23]. Aside

from changes in enzymatic activity, Cr(VI)-induced oxidative stress in plants causes lipid peroxidation, which includes membrane lipid disruption and proteins that cause cell damage [24]. Studies on malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS), a product of lipid peroxidation after induction of heavy metal stress, are reported in many studies [18]. Cr(VI) causes genotoxicity, which includes chromosomal fragmentation and bridging, DNA damage, an increase in tail DNA percentage, tail length, and tail moments, methylation changes, mutation, chromosomal aberrations, micronucleus alterations, genomic instability, DNA crosslinks and breakage, replication dysfunction, etc. These changes have been reported in several plant species viz., *Glycine max*, *Vicia faba, Allium cepa, Hordeum vulgare, Vicia sativa, Zea mays*, and *Arabidopsis thaliana* [25-35]. In plants, Cr(VI) toxicity causes cytotoxicity as well as ultrastructure alterations, necrosis, cellular damage, and electron-dense material accumulation in subcellular compartments [25]. The absorption of micronutrients is affected by Cr(VI) cytotoxicity, which leads to the cessation of cell cycle arrest and cell death. It is also responsible for the formation of swollen guard cells and alters permeability, decreased stomatal aperture, and altered osmotic pressure of the membrane, thereby causing stomatal abnormalities [36-37]. This alters photosynthesis, transpiration, and respiration rates [38]. As a result, alteration in photosynthetic pigment (chlorophyll (chl) a, b, & total) occurs. Ultrastructure studies on Cr(VI) toxicity have revealed spherical and contracted chloroplasts and plastids, underdeveloped mitochondria, deformed cristae, and mitochondrial outer membrane rupture [39-40]. Underdeveloped nucleus, damaged nuclear membrane, distorted thylakoid, altered thylakoid orientation, increased plastoglobuli, large starch grains and swelled chloroplasts are reported in the leaves and roots of *Nicotiana tabacum*, altered mitochondria with reduced internal cristae, distorted thylakoid in *Taraxacum officinale*, swollen chloroplast, chloroplast envelope breakage, mitochondrial vacuolization in the leaves of *Potamogeton cripus* are reported due to Cr(VI) induced toxicity [41-43]. Plant defense mechanisms restrict the accumulation of pollutants in the fewer sensitive organelles to protect the highly sensitive organelles [44]. The first line of cellular defense against Cr(VI) toxicity is the precipitation of electron-dense granules in subcellular compartments and the cell membrane [45-46]. Photosynthesis and photosynthetic mechanisms are also disturbed as a result of chromium toxicity, resulting in reduced plant growth and yield. It mainly influences plastids structure, photosynthetic phosphorylation, electron transport chain, and CO_2 fixation [47-49]. Chromium interaction with plants causes chloroplast membrane distortion, decreases volume and autofluorescence, and affects light-dark reactions [50]. Cr(VI)-induced redox changes in iron and copper carriers, as well as Cr-Cytochrome binding, inhibit the electron transport chain [51]. ROS leads to degradation of antenna proteins, substitution of magnesium ion with H^+ ion, and distortion of the thylakoid membrane. Cr(VI) induces degradation of δ-aminolaevulinic acid (5ALA) dehydratase (enzyme involved in chlorophyll biosynthesis), and its ability to compete for Magnesium and iron translocation to leaves contribute to photosynthetic rate & pigments [17][52]. Toxicity due to chromium contamination in plants is summarized in Table 1.

3. CHROMIUM TOXICITY IN ANIMALS

Hexavalent chromium is highly oxidizable and is easily absorbed by the cells via nonspecific anion carrier proteins. Its uptake is the combination of saturable transport and passive diffusion. Anion exchanger 1AE [1] transports chromate, sulphate and phosphate ions through the anion channel [79]. It may be converted rapidly to trivalent form once it enters the cell, resulting in the formation of Cr intermediates which are highly reactive and ROS which impairs cellular function and promote apoptosis [80]. This makes it mutagenic and carcinogenic to humans [81]. High oxidative stress, chromosome breakage, formation of DNA adducts by Cr(VI) leads to cellular and genotoxicity. Cr(VI) being a skin sensitizer may easily penetrate and cause contact dermatitis and inflammation [82]. NF-κB, AKT, MAPK signaling cascades in keratocytes are stimulated by ROS overproduction due to chromium toxicity and forms TNF-α and IL-1α [83]. Chromium intoxication causes nephrotoxicity following its discharge by kidney causing proximal tubule damage [84]. The basis of carcinogenesis of Cr(VI) is the reduction of hexavalent form to trivalent form Cr(III) via Cr(V) and Cr(IV) interaction with DNA [85]. Formation of Cr(III)-mediated DNA-protein cross-links causes oxidative DNA damage and mutation and initiates carcinogenesis [81]. This also alters cell signaling pathways, causing alterations in gene regulation. As a result of Cr(VI) induces genotoxicity, DNA-Cr adducts are formed. Small Cr-DNA adducts are the most common genetic lesions in mammalian cells caused by chromium toxicity, and they are responsible for mutagenic damage caused by Cr(VI) reduction with cysteine and ascorbate [81][86]. Binary adducts

account for 50%-75% of them and are considered to be weakly mutagenic [87-88]. Apart from this, ternary adducts (crosslinks) viz., glutathione-Cr-DNA, cysteine-Cr-DNA, histidine-Cr-DNA, and ascorbate-Cr-DNA complexes are the predominant form of Cr-DNA adducts and include trivalent chromium bridging DNA and L-Cr-DNA [87]. Ascorbate-Cr-DNA crosslinks are the most potent premutagenic Cr-DNA modifications [89]. Hexavalent chromium promotes and reduces cell viability and is also responsible for cellular and genomic DNA fragmentation, membrane damage and lactate dehydrogenase leakage, intracellular oxidized states, activation of protein kinase-C, apoptotic and necrotic cell death [90]. Increased chances of centrosome amplification and the possibility of spindle assembly checkpoint bypass occur due to DNA strand breakage and subsequent improper repair, which ultimately results in chromosomal instability, neoplastic transformation and cancer [91-92]. Cr(VI) compounds are confirmed to be carcinogenic based on the epidemiological and experimental investigations [93].

Table 1: Chromium induced Antioxidant Enzyme Modulation, Lipid Peroxidation and Altered Photosynthetic Activity

Species Name	Enzymatic Activity	Lipid Peroxidation	Photosynthetic Parameters	References
Triticum aestivum	CAT, APX	MDA	Reduces PSII activity, PSII heterogenicity,	[53]
Hordeum vulgare	CAT, SOD, POD, APX	MDA	Reduced Chl a, b & total, and carotenoid	[53-54]
Oryza sativa	SOD, POD, CAT, APX, GR	MDA, TBARS	Reduced Chlorophyll a, b, total and Carotenoids, Reduced F_v/F_m	[55-57]
Brassica napus	CAT, SOD, POD, APX	TBARS	Ruptured thylakoid membrane	[58-59]
Arabidopsis thaliana	CAT, SOD, POD	MDA	Damaged mitochondria & plastid, Reduced Chlorophyll a, b, total	[18][60-61]
Vigna radiate	CAT, GPX	MDA	Reduced Chl total	[62-63]
Raphanus sativus	CAT, SOD, POD	MDA	Reduced total Chl	[64-65]
Sorghum bicolor	CAT, SOD, APX, GR, GST	MDA	Reduced chlorophyll content	[66-67]
Brassica oleracea	CAT, SOD, POD	MDA	Reduced chlorophyll a, b & total and carotenoid	[68-70]
Amaranthus viridis	CAT, SOD POD, GST	MDA	Inhibition of photochemistry of PSII	[71-72]
Pisum sativum	CAT	MDA	Reduced chlorophyll, alteration on PSI & PSII activity	[73-75]
Allium cepa	CAT, SOD, GPX	MDA	Reduced total chlorophyll	[28][76]
Brassica rapa	SOD, APX	MDA	Reduced chlorophyll a, b & total and carotenoid	[77-78]

In most studies, a positive correlation between the duration of exposure to chromium and deaths by lung cancer was found. Acute Cr(VI) toxicity is associated with respiratory allergies, gastrointestinal bleeding, skin cancer and ulcers, infertility, and other symptoms. Ingestion of high doses of chromium

causes cardiovascular, gastrointestinal, renal, hepatic, and neurological fatalities [94]. Upon dichromate compound ingestion, caustic burns of the stomach and duodenum and gastrointestinal hemorrhage were noted [95]. In a study, passage of chromium from mother to child via placenta and increased birth and developmental defects have been studied and reported informally near the areas of poor chromite mining, chrome plating, and leather tanning [96]. Workers exposed to Cr(VI) had higher levels of low molecular weight urinary proteins, 2-microglobulin, and tubular antigens, indicating early kidney damage. Exposure to 4µg m^3 Cr(VI) led to lowest-observed-adverse-effect-level (LOAEL) [97]. People exposed to less than 2µg m^3 Cr(VI) developed smeary, crusty and atrophied septum mucosa; at concentration 2-200 µgm^3, nasal irritation, mucosal ulceration and atrophy and septum perforation were observed [96].

4. BIOLOGICAL REMEDIATION STRATEGIES

Biological remediation is an effective alternative to remediating and restoring chromium-contaminated sites. Use of microbes (bacteria, yeast, fungi); bioremediation and use of plants (phytoremediation) proves to be efficient, eco-friendly and cost effective strategies to render soil free from Cr(VI) [98]. Alteration of redox reactions and solubility through the manipulation of a variety of biotic and abiotic factors or by various complexation reactions is the basis of bioremediation [99]. Various strains of bacteria, fungi, algae, and yeast have been used to convert Cr(VI) to a non-hazardous form. Cr(VI) is reduced to Cr(III) owing to the biosorption abilities of microbes. Bacterial strains such as Micrococcus, Enterobacter, Pseudomonas, Bacillus, etc. reduce hexavalent chromium to trivalent form [100]. Spirogyra and *Chlorella vulgaris* are the reported algal strains for biosorption. The Cr-degradative nature of various fungal strains such as *Rhizopus arrhizus, Rhizopus oryzae, Penicillium chrysogenum*, and *Gloeophyllum sepiarium* has been reported [101]. Yeast such as; *Yarrowiali polytica, Pichia guilliermondii* are known for their potential in biosorption and ion exchange mechanisms [102]. A list of potential microbial species used for microbial remediation of chromium is presented in Table 2.

Table 2: Potential microbial species for bioremediation

Microbial Species	Temperature (^{0}C)/pH	Remediation (%)	Reference
Cellulosimicrobium funkei ARS	35/7	100	[103]
Pseudomonas aeruginosa	30/8	66.7	[104]
Bacillus amyloliquefaciens CSBT9	35/7	100	[105]
Enterobacter cloacae CTWI-06	37/7	94	[106]
Bacillus subtilis PAW3	35/6	100	[107]
Staphylococcus aureus K1	35/8	99	[108]
Bacillus strain TCL	40/7	97	[109]
Spirodela polyrrhiza	33/5	99.3	[110]
Halomonas aquamarine	28/6.5	91.2	[111]
Pannonibacter indicus	25/10	99.8	[112]
Pseudomonas putida	37/8.3	62	[113]
Shewanella loihica PV-4	25/7.4	91.2	[114]
Anthrobacter viscosus	28/4	17	[115]
Oceanobacillus oncorhynchi W4	30/9	74.2	[116]

Besides microbial remediation, phytoremediation is also an efficient and eco-friendly way to remediate heavy metals form soil and water. Phytoaccumulation, phytodegradation, phytovolatilization, rhizofiltration,

rhizodegradation, phytostabalisation are the mechanisms used by plants (terrestrial and aquatic) in phytoremediation. Phytoextraction is the estimation of the plant's capacity to remediate heavy metals and is done on the basis of bioconcentration factor (BCF) and translocation factor (TF). BCF is the metal concentration from soil to aerial plant parts to soil and TF is the metal concentration from root to shoot [117]. Phytodegradation is also called phyto-transformation. This involves the secretion of exudates and enzymes such as dehalogenase, nitro reductase, laccase, oxygenase, etc., which catalyse the degradation and mineralization of pollutants into less toxic substances [118]. Phytostabilization reduces the mobility of chromium in soil, which is done by plant root exudates and bio enzymes. In addition, this can be combined with lime, organic carbon, or phosphates to precipitate, stabilize, and immobilize Cr(VI) [119]. Rhizofiltration involves adsorption and uptake of pollutants into the root and the removal of heavy metal from the rhizosphere. It is believed that rhizofiltration effectively remediates Cr(VI) from the soil. During this, the root's exudates alter the nearing environment by a change in pH, which helps in the precipitation of chromium and decreases its mobility. Brown mustard (*Brassica juncea*), black mustard (*Brassica nigrus*), and sunflower (*Helianthus annus*) have been successively used for rhizofiltration due to the presence of long fibrous roots [120-121]. Partitioning of pollutants into the air spaces inside the plant tissues from where they successively diffuse into the air is involved in Phytovolatilization. High evapotranspiration rate of certain plants aids in phytovolatilization and is particularly used for volatile organic compounds like methyl-Hg and Se (selenium), where HgO and $[Se(CH)_3]$ Dimethyl selenide are end compounds [122]. A list of potential plant species used for phytoremediation of chromium is presented in Table 3.

Table 3: Potential plant species for phytoremediation

Pant Species	Incubation Period (Days)	Remediation (%)	Reference
Cicer arietinum L.	30	50	[123]
Mahalaxmi rice	120	17.9	[106]
Tagetes erecta (L.)	35	94	[124]
Helianthus annus	56	97	[125]
Cyperus papyrus	72	73.77	[126]
Pennisetum purpureum	56	66.8	[127]
Eichhornia crassipes	15	99.5	[128]
Cyperus kylinga	30	60	[129]
Ailanthus altrissima	90	56	[130]
Pluchea indica	30	100	[131]
Nopalea cochenillifera	16	100	[132]
Phragmites australis	90	54	[130]
Polygonum coccineum	72	76.37	[126]

5. CONCLUSION FUTURE PROSPECTS

Various industrial and anthropogenic activities contaminate the environment and lead to Cr(VI) pollution. Its high mobility and solubility make it a potent carcinogen and mutagen. Heavy Cr(VI) exposure in plants mediates ROS production and alters various enzymatic processes, resulting in cellular, subcellular, and genotoxic effects that affect overall metabolism, development, and yield. On the other hand, consuming crops intoxicated with chromium causes adverse effects on human health and leads to cancer and other serious health aberrations. At the source of generation, physico-chemical and biological remediation strategies are helpful in reducing the chromium load from the environment. Bioremediation of Cr(VI) using plants and microbes and a combination of both incorporating it with nanotechnology may be the cost-effective, eco-friendly and sustainable strategy for the restoration of chromium contaminated sites.

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Presence of Antibiotics in Environment and its Impact on Living Organisms

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ABSTRACT

Antibiotic discoveries have bought a rapid innovation in the health sector and their usage has revolutionized modern way of living. In addition to uses like treatment of infectious diseases in humans and animals, they are also widely used in agriculture, aquaculture, bee keeping, and livestock as growth promoters. They are used in livestock farming to increase meat production by preventing the outbreaks of infections and diseases. In spite of various benefits, regular release of antibiotics into the environment has become an alarming concern. As they have adverse impact on living organisms. The antibiotic contamination or pollution has been extensively detected in plants, humans, and animals. It affects the overall growth and functioning of the plants, inhibits the growth of soil microorganisms, and develops the antibiotic resistance in human and animal. The major contributors of antibiotic contamination in the environment are Wastewater Treatment Plants (WWTPs), through them release of veterinary antibiotics takes place in unmetabolised form, which easily gets accumulated in the environment. The increased and persistent usage of antibiotics is a major global concern. Therefore, Antibiotic remediation measures can solve the problem up to great extent. Phytoremediation is an alternative for different conventional strategies. It is a cheaper, eco-friendly, feasible, and sustainable method. This review focuses on the sources of antibiotic, its impact on living organisms, and antibiotic resistance.

Keywords: Antibiotics, Feasible, Phytoremediation, Sustainable, Veterinary.

1. INTRODUCTION

Antibiotics are one of the vital therapeutic revelations of the 20th century. The first use of antibiotics as medicine started with synthetic antibiotics derived from dyes. In 1909, the activity of the first synthetic antibiotic arsenic-based drug named Salvarsan was discovered by Paul Ehrlich in cooperation with Alfred Bertheim and Sahachiro Hata [1]. Later in 1928, the 'accidental' identification by Alexander Fleming of Staphylococci growth inhibition around mold colonies in petri dishes, forgotten during a holiday time at his laboratory which led to the discovery of penicillin [2].

Antibiotic usage has changed the pattern of living. They are used in the treatment and prevention of bacterial infections. They help in surgical procedures by preventing infection of incisions [3].

Antibiotics are complex molecules with different functional groups attached in their chemical structures [4]. They are chemotherapeutic agents which inhibit or kill the growth of micro-organisms. The antibiotics are classified on basis of their mechanism of action, chemical structure, action spectrum, and route of administration [5]. Based on this classification the most commonly groups are: beta-lactams, sulfonamides, monobactams, carbapenems, aminoglycosides, fluoroquinolones, tetracycline, and chloramphenicol etc. An antibiotic interferes with the survival of bacteria through a particular mode of action for example, inhibition of cell wall synthesis, protein synthesis, and nucleic acids synthesis in the targeted cell, alteration of cell membranes, competitive antagonism and antimetabolite activity.

They are therefore efficiently used for treatment of various human and veterinary diseases. In livestock, the antibiotics are vastly used as therapeutic agents. They are used to control the infectious diseases and hence increase the meat production by overall promoting the animal growth [6].The antibiotics are usually added in the feed of cattle, pigs, poultry, pets and fish. A significant amount of undigested antibiotic is released into the environment via excretion through animals. It further reaches into the environment through various sources such as municipal wastewater effluents, animal manure,

sewage sludge, and biosolids that are frequently used for irrigation of the agricultural lands [4] as shown in (Figure 1).

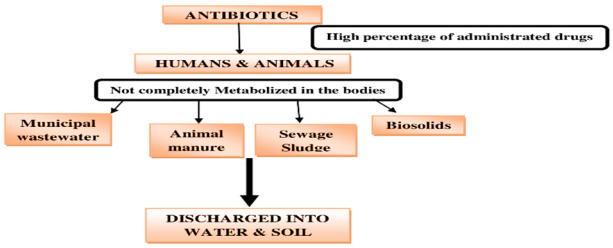

Fig. 1: Flow chart depicting the movement of antibiotics into the environment

2. PRESENCE OF ANTIBIOTIC IN THE ENVIRONMENT

With the increased use of the antibiotics, the amount of antibiotics entering into the environment is also increasing. In (Table1) the maximum reported concentrations of major antibiotics in manure, sewage, and soil has been represented. Antibiotics like Ciprofloxacin, Enrofloxacin, Chlortetracycline, Norfloxacin and Oxytetracycline are majorly reported to be found in the environment [4]. It has been seen that the main pathway for aquatic contamination by human pharmaceuticals is through sewage treatment plants. These treatment plants receive wastewater from various households and hospitals. In a study, the analysis of pharmaceuticals in the effluent from wastewater treatment plant situated near Hyderabad, India was performed. It was observed that various 11 drugs were detected at levels >100 µg/L [8]. Out of all 11 active pharmaceuticals the highest concentration of fluoroquinolones was found, specifically of ciprofloxacin ranging from 28,000- 31,000 µg/L, which was greater than the maximal therapeutic human plasma level [5][8].

In another study reported by Diwan et al. the presence of antibiotics was investigated in water sample collected from two hospitals in Ujjain district of Madhya Pradesh, India. The samples were collected from various sources such as, incoming safe water sources, exiting water, and groundwater sources near/in hospital area [9]. It was observed that there was no presence of antibiotics either in incoming safe water or groundwater sources near hospital. Out of 13 targeted antibiotics only 8 were detected in the analyzed wastewater samples. The most commonly detected antibiotic was Ciprofloxacin and its presence was found in all the samples from both the hospitals. The concentration of ciprofloxacin ranged up to 31,000 μg^{-19}. This leads to its accumulation in the environment due to incomplete removal of antibiotics from effluent wastewater [9]. Incessant discharge of antibiotics into the natural environment and their hazardous impact on the living organisms has led to development of antibiotic resistance, which is an alarming concern.

Table 1: Maximum reported antibiotic concentrations detected in manure, sewage and soil [4]

Class	Antibiotic	Concen-tration	References
Manure (µg/kg)			
Fluoroquinolones	Ciprofloxacin	45,000	Zhao et al., 2010
	Norfloxacin	225,000	
Sulfonamides	Sulfadiazine	91,000	Martinez-Carballo et al.,2007
Tetracyclines	Chlortetracycline	764,000	Masse et al., 2014
	Oxytetracycline	354,000	Chen et al., 2012
	Tetracycline	98,000	Pan et al., 2011
Sewage Sludge (µg/kg dry weight)			
Fluoroquinolones	Ciprofloxacin	426 (8,905)	Li et al.,2013; Lillenberg et al.,2010
Sulfonamides	Sulfadimethoxine	0-20 (22.7)	Li et al.,2013; Lillenberg et al.,2010
Tetracyclines	-	8,326	Cheng et al., 2014
Soil (µg/kg)			
Fluoroquinolones	Ciprofloxacin	5,600	Van Doorslaer et al., 2014; Pan and Chu et al. 2017; Karci and Balcioglu et al., 2009
	Norfloxacin	2,160	
	Enrofloxacin	1,347.6	
Sulfonamides	Sulfadiazine	85.5	Dolliver et al., 2007; Carter et al., 2014; Pan and Chu, 2017
	Sulfamethazine	200-25,000	
Tetracyclines	Chlortetracycline	12,900	Hamscher et al.,2002; Lukaszewiez et al., 2018; Karci and Balcioglu, 2009; Pan and Chu et al. 2017; Liu et al., 2016; Tasho and Cho, 2016
	Oxytetracycline	50,000	
	Tetracycline	2,683	

2.1. IN WATER

Residues of antibiotics and antibiotic resistant bacteria enters the aquatic environment such as in rivers, streams, lakes, seawater, and groundwater through multiple pathways like wastewater treatment plants, sewage sludge, hospital waste and agricultural waste. They enter into the water cycle when excreted by humans and animals in a partially metabolized form or when water run-off from agricultural fields having manure & slurry prepared from the live

stocks. When the expired drugs are not disposed carefully, and are thrown into toilets or dustbins lead their way in a Wastewater Treatment Plants (WWTPs). Thus, this leads to further long chain of contamination. In a study performed objectives investigated were: -the occurrence of antibiotic residues and antibiotic resistant *E. coli* in water, and quality of water and sediments of the Kshipra river, India for over three years during various seasons [11]. It was observed that antibiotics like sulfamethoxazole, ofloxacin, norfloxacin, and metronidazole were present in the river water. Out of these; sulfamethoxazole was most frequently detected antibiotic in the river water constantly irrespective of season.

2.2. IN SOIL

Introduction of antibiotics into soil takes place through wastewater, sewage sludge, and manure application. The continuous application of manure has led to increased accumulation of antibiotics in the soil. It is considered as the major source for the presence of the pharmaceutical antibiotics into land. The livestock are given antibiotics in their feed which when not completely metabolized is released by the animals. This waste is used as manure and applied on large scale in agricultural fields.

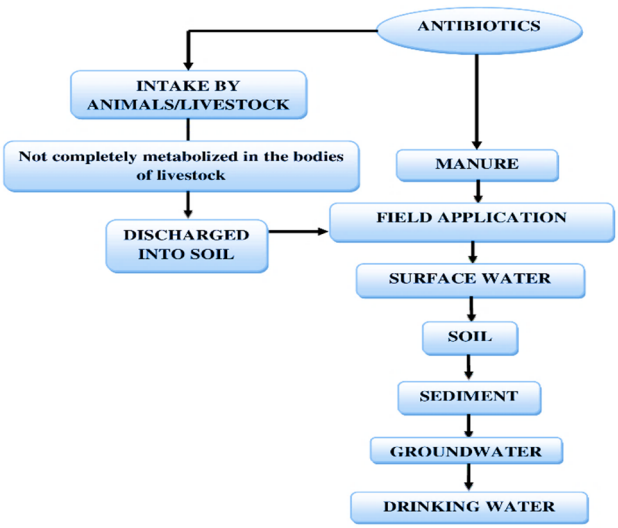

Fig. 2: Presence of antibiotics in soil and water from livestock

2.3. IN FOOD

Animals are fed antibiotics for growth promotion, treatment of disease, prevention from various infections & diseases. A major amount of antibiotic residues is present in meat products, which causes antibiotic resistance in humans. Presence of antibiotics has been detected in pork, chicken, and beef. Antibiotics like sulfonamides and tetracyclines were found at higher concentration. Antibiotics very easily enter into aquaculture through veterinary pharmaceuticals. In countries like China, Vietnam, India, and Italy antibiotic residues have been detected in fishes and shrimps [12]. Deposition of antibiotics residues are also found in dairy products such as in eggs and milk. In various studies conducted in Asia on different food products, the presence of antibiotics such as fluoroquinolones and sulfonamides was observed in raw milk samples, similarly the concentration of about 8589 µg/kg and 808 µg/kg of chlortetracycline was observed in egg white and egg yolk respectively and also highest concentration of enrofloxacin 1485 µg/kg was observed in chicken eggs. As, the antibiotics are being fed to livestock which results in high amount of antibiotic concentration in the manure. This causes antibiotic residues to get accumulated in the crops, vegetables and grains. Presence of antibiotic residues was also found in vegetables in concentration ranging between 0.02-0.85 µg/kg, which was irrigated by pharmaceutical wastewater [12].

2.4. IN PLANTS

The presence of antibiotics in water and soil has built pathway to enter into the biota. Biosolids application into the agricultural field causes contamination of surface water and ground water. The crops, aquatic plants, and animals readily take up the antibiotics present in the environment. Antibiotics presence in the plants and aquatic animals has created a challenge for the standards of food safety. The unmetabolised or untreated antibiotic contaminants are usually taken up by the plants through absorption by root surfaces, root uptake, translocation, and animal intake. In a study conducted during winter season, the high levels of Oxytetracycline, tetracycline, and chlortetracycline within range of 78-330, 1.9-5.6, and 92-481 µg/kg accumulated in coriander leaves [5]. Similarly, sulfadoxine, sulfachloropyridazine, chloramphenicol, and sulfamethoxazole got accumulated in the range of 0.2-0.6, 0.1-0.5, 8-30, and 0.9-2.7 µg/kg respectively in radish leaves [5].

2.5. IN ANIMALS

The food animals such as goat, pigs, chicken, ducks, and dairy product cattle are given antibiotics mainly for three reasons such as for treatment, for boosting up the growth, and as metaphylaxis in order to keep the clinically fit animals healthy from any unexpected outbreak of a disease. In studies

conducted in India, it was found that samples collected from various farms were positive for *Salmonella*. All the isolates of *Salmonella* were resistant to Oxytetracycline. Similarly, in an another study of a meat shop it was found that around 96% of chicken samples had *S.aureus* in them, and all of the samples were sensitive to ciprofloxacin, doxycycline and cloxacillin, and mostly resistant to tetracycline [13].

3. IMPACT OF ANTIBIOTICS ON LIVING ORGANISMS

Antibiotics have impacted living organisms such as plants, humans, and animals alike. Few antibiotics which are used majorly are tetracycline, fluoroquinolones, sulfadimethoxine, ciprofloxacin, and erythromycin. all these cause toxic effects on living organisms as per ways discussed below.

3.1. IMPACT ON PLANTS

In soil, antibiotics along with micro and macro nutrients get adsorbed and ultimately affect the overall growth and functioning of the plants. When antibiotics get into the cultivable land, they might actually effect growth and development of plants, along with soil microbial activity. In a study performed, two different classes of antibiotics were selected and their effects on plant growth and soil microbial activity were analyzed. The selected antibiotics for study were tetracycline, chlortetracycline, and tylosin; sulfonamides, sulfamethoxazole, and sulfamethazine. Study was performed on varying plant species like sweet oat, rice, and cucumber collected from different side. In the seed germination test, it was observed that rice was most sensitive to sulfamethoxazole with the effective concentration 10 value of 0.1 mg/L. All the three plant seeds germination was inhibited by chlortetracycline and tetracycline with effective concentration (EC50) values <300 mg/L [14]. Similarly, sulfamethoxazole and sulfamethazine too inhibited seed germination of the three plants with the EC50 values for the two sulfonamides being < 100 mg/L [14]. Among all the three plant species, the most sensitive to all the six antibiotics was sweet oat with varying toxicity values. In the plant growth test, only sulfonamides class (sulfamethoxazole and sulfamethazine) strongly inhibited the rice growth in soil. Presence of antibiotic reduces photosynthesis, growth (root and shoot), inhibition of seed germination, DNA synthesis, and negatively influences the overall plant morphology [5].

Table 2: Impact of Antibiotics on plants [1] [3] [10]

Antibiotic	Impact
Tetracycline	Inhibits the Seed Germination Affects: Chloroplastic & Mitochondrial protein synthesis[1]
Fluoroquinolones	Inhibition of DNA Synthesis Negative effects on plant morphology & Photosynthesis Affects: Chloroplastic & Mitochondrial protein synthesis[1]
Ciprofloxacin	Decreases photosynthesis & overall growth
Chlortetracycline Sulphamethoxazole Sulfamethazine	Inhibits the Seed Germination[3]
Sulphadimethoxine	Reduces the growth of roots, hypocotyls, and leaves in plants of *Panicum miliaceum*, *Pisum sativum* and *Zeamays*[10]

3.2. IMPACT ON SOIL MICROBIAL POPULATIONS

Soil microbes play an important role in maintaining the quality of soil. They inhibit the growth of pathogens by acting as biological control agents. Due to the presence of antibiotics in soil the growth of soil microorganisms gets inhibited. (Table 3)

In a study similar to that performed on plants, six antibiotics were selected and their effects on soil microbial and enzyme activity were evaluated on varying plant species like sweet oat, rice, and cucumber. The antibiotics like tetracycline and tylosin showed little effects on soil microbial respiration. Whereas sulfonamides showed a significant amount of decrease in soil respiration in span of first four days. It was also observed that as the concentration of sulfamethoxazole increased in the soil, the soil respiration decreased simultaneously. The study explained that antibiotics addition in the soil at the concentration (1-300mg/Kg) will have an impact on soil phosphatase activity ($p<0.05$). When the Effective Concentration 10 values were calculated for all the six antibiotics, it was observed that it ranged from 1 mg/kg for sulfamethazine to 406 mg/kg for tetracycline [14]. In another study effects of ciprofloxacin antibiotic were studied thoroughly on soil microbial communities and it was observed that in the first 25 days of experiments there was reduction in soil microbial activity. The exposed concentration of ciprofloxacin ranged from 0.2 to 20 mg kg^{-1} [15].

Table 3: Impact of antibiotics on soil microbial functioning [4], [16-19]

ANTIBIOTIC	IMPACT
Sulfadiazine	Decrease in Soil Respiration (SR)
Sulfamethazine	Decrease in Soil Respiration (SR)
Sulfadimethoxine	Inhibition of Soil Nitrification (at high doses)
Sulfadiazine	Inhibition of Denitrification (at low doses)
Oxytetracycline	Block/Inhibition of Fe(III) Reduction
Sulfadiazine	Block/Inhibition of Fe(III) Reduction
Oxytetracycline	Inhibition of dehydrogenases (DHA)
Sulfadiazine	Inhibition of DHAs and phosphatases (PHOSs)
Sulfamethazine	Inhibition of urease (URE) and DHAs
Tetracycline	Inhibition of DHAs and URE
Chlortetracycline	Inhibition of PHOSs

3.3. IMPACT ON ANIMALS

As per ReAct report India comes under top five countries with the largest shares of global antimicrobial consumption in food-animal production is the fourth largest consumer of antibiotics in food animals [23]. The recent study said that by 2030 the use of antibiotics in animal feed will increase by 82% in India [23]. Van Boeckel et al. had predicted use of antibiotics will grow, making India their fourth-largest consumer in food animals by 2030. Antibiotics such as tetracycline, ciprofloxacin, and doxycycline, penicillin and quinolone are commonly used for growth promotion in poultry. Sahu and Saxena reported that antibiotic residues were found in chicken meat which was for human consumption [24]. It was found that of the 70 chicken meat samples tested, 40% contained antibiotic residues. The most common antibiotic detected are shown in (Figure 3)

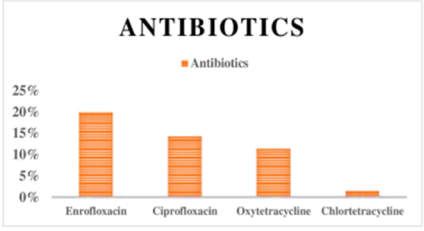

Fig. 3: Most common antibiotics detection in meat samples. [24]

Veterinary antibiotics are intended to influence microorganisms found in animals. They easily get eliminated in the environment in their active form or as by-products. This further contaminates the environment, and adversely affects the aquatic biota, terrestrial biota, and humans through consumption of contaminated food acquired from aquaculture. To show the negative impacts of the antibiotics, few studies have been performed to check the toxicity with the help of various test organisms. Reproduction of aquatic organisms, such as *Artemia sp.* and *Daphnia magna* was affected adversely when they were exposed to the antibiotic toxicity. Reproductive effect in any organism causes a considerable amount of damage to that particular population of the organism and hence will lead to the misbalance in the tropic chain. In another study, toxicity of acetaminophen and lincomycin were observed on fish *Oryzias latipes*. It was found that when fish was exposed to 95 mgL^{-1} of acetaminophen and 0.42 mg L^{-1} of lincomycin, a significant reduction in survival was observed.

3.4. IMPACT ON HUMANS

Antibiotic contamination in the environment through livestock or hospital wastes leads to its entrance into human body. Exposure for the long term via drinking water, food, or consumer goods, causes accumulation in human body. It has been calculated that around more than half of the U.S. population has been exposed to triclosan (an antimicrobial used in soap and clothes) or other antibiotics through consumer goods [25]. Each year around 25,000 European people die due to the direct result of resistant infection [27]. It has been observed that post-antibiotic era, there is an exponential increase in antibiotic resistance in the environment and humans. The spread of resistance in healthcare settings and in the community threatens the enormous gains made by the availability of antibiotic therapy [21]. Antibiotic-resistant bacteria infection can lead to serious illness, increased mortality rates, and an increased risk of complications and hospital admission.

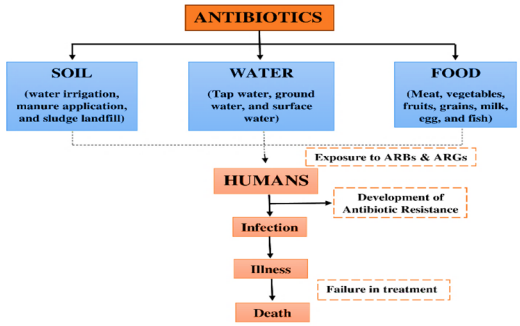

Fig. 4: Impact on Human

4. ANTIBIOTIC RESISTANCE

Over the past few years, India has arisen as a worldwide hotspot for antibiotic resistance, with rising rates to most antibiotics in prevalent pathogens and increasing number of treatment failures.

When the Antibiotics are discharged into the environment, they lead to the formation of resistance and even multiple resistances in organisms. Antibiotic resistance (ABR) is the capability of some bacteria to defend themselves against the action or effects of an antibiotic.

Numerous classes of antimicrobials that are utilized for humans are likewise being utilized in food animals. Apart from the utilization of these prescribed medicines for treatment of animals, numerous food-animal makers additionally use them to promote growth or for regular disease prevention. Such extensive utilization of antibiotics speeds up the evolution of antibiotic resistant bacteria. Farm and slaughterhouse laborers, and veterinarians, who come in close contact with affected animals, are additionally in danger of transferring such resistant bacteria on to other people.

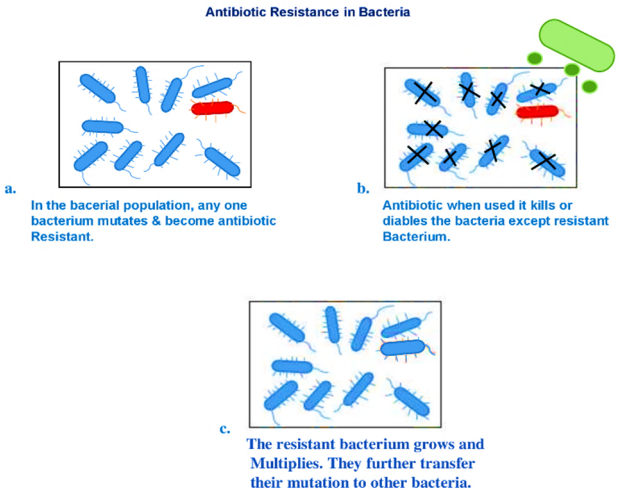

Fig. 5: Antibiotics Resistance

The pathway through which the development of antibiotic resistance in humans and animals takes place is, when animals are fed antibiotics for a long duration, they keep hold of bacteria which are resistant to antibiotics [22]. They feel physical stress for example, in the movement [21]. Proliferation of the bacteria takes place inside the animal. Due to the interaction among other animals, the resistant bacteria get transmitted and thus form a colonization of antibiotic resistant bacteria. They get released in the feces in an unmetabolised form and come in contact with humans either in farms or slaughter houses. When an infected individual is admitted to a hospital, it further transmits to other patients through environment or health worker hands. Colonization in other patients produces bacteria with multi-drug resistance [21] [22].

5. CONCLUSION

Antibiotic contamination in the environment will increase more drastically in the future due to its enhanced consumption all around the world. They easily get accumulated in the environment when used for prolonged durations and further cause negative effects on the living organisms, whether it is plants, humans or animals.

The main common cause for antibiotic contamination is the WWTPs (Wastewater Treatment Plants). They are responsible for the transportation of antibiotics to soil, surface water, groundwater, drinking water, plants, and aquatic animals. Pharmaceutical/ Drug manufacturing plants and hospital effluents are also one of the sources of the contamination in the soil. Land application of the manure, made from the livestock which has been fed antibiotics for prevention from diseases and growth promotion etc. is also one of the sources of soil contamination by antibiotics. This further hampers the whole food chain and causes antibiotic resistance in the animals and humans.

The impact of antibiotics has become a major concern. It has impacted the whole flora-fauna negatively by affecting the overall growth of plants, and making the animals and humans antibiotic resistance. Antibiotic resistance within humans has become a very critical issue. Gradually it will impact the whole community and ultimately making them MDR (Multi Drug Resistance). Removal of antibiotics from environment is not considered to be so easy. In order to tackle the increasing contamination various elimination pathways like (Sorption, Photo degradation, Biodegradation, and Oxidation) were applied into new techniques like Chlorination, Ozonation, Photolysis, and Phytoremediation. Out of all methods, Phytoremediation is considered as one of the prominent ways to overcome the antibiotic contamination. It is very cost effective as it does not require any chemicals or heavy equipment. It's natural and environment friendly way of treating the polluted sites. Continuous efforts should be performed in order to explore and utilize this technology.

6. ACKNOWLEDGEMENT

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Bacteriophages: The Bacteria-Devouring Viruses as Promising Healthcare Agents

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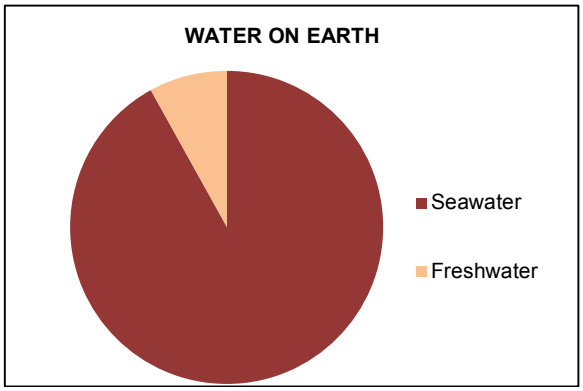
ABSTRACT

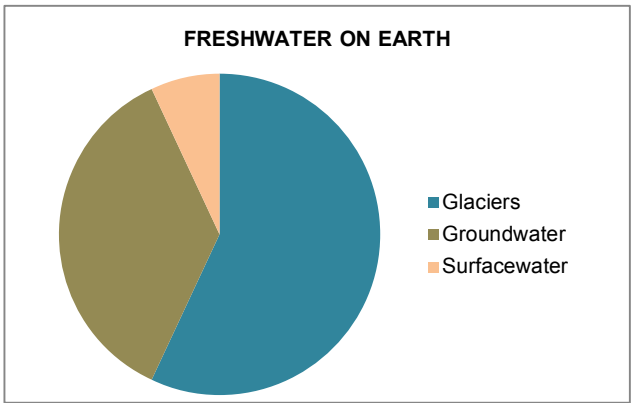
Water is the fundamental need of all living organisms on Earth. Due to human activities, water pollution due to the presence of pathogenic bacteria (*E. coli, Pseudomonas, Salmonella* and others) results in gastro-intestinal illness, skin, ear, respiratory, eye, neurologic and wound infections. With evolution of bacterial strains, efficacy of antibiotics has been decreasing due to antibiotic resistance. Notably, literature survey carried out suggests that phage therapy can be an alternative and promising method to target pathogenic microorganisms. Continuous efforts are being carried out to understand the science behind phage therapy as a therapeutic tool. In the current study, a broad overview of bacteriophages is focused upon; further genomic sequences of specific coliphages have been mined from literature, compared for similarities using phylogenetic tools and analyzed.

Keywords: Water, Bacteriophage, Antibiotics, Phage Therapy, Phylogenetic Analysis.

1. INTRODUCTION

Water is an essential component of all living species' growth and development on this planet. It includes 1% of the free water accessible for human consumption, which is capable of supporting a varied range of biological groups as shown in Figure 1. As the human population grows, so does the demand for safe drinking water. India possesses 4% of freshwater resources in the world [1]. Rivers, streams, lakes, pools, and springs, as well as artificial canals and ponds containing freshwater, brackish water, or groundwater, are all part of these systems. These water systems have specific physico-chemical and biological properties, along with unique ecosystems. These are also sources of fresh water for future use at a low cost to reduce demand on the water distribution system [2].





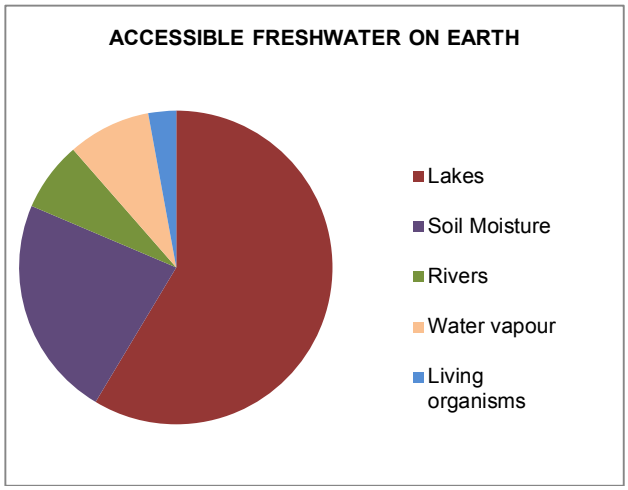


Figure 1. Different water sources on Earth [1]

India consists of 4% of the world's freshwater and 16% of the world's people. Nearly 70% of the water is polluted. About 600 million Indians are under excessive water stress, that is much more than 40% of the available surface water being consumed each year. Every year, some 200,000 people die as a result of a lack of potable water [3]. By 2022, there are 21 cities of India may run out of groundwater, including Delhi, Bengaluru, Chennai, and Hyderabad, impacting one third of the population. Water consumption is expected to be twice as high as water supply by 2030. Currently, Asia is home to around 73 percent of those impacted due to water scarcity by 2050. [4]

Several standstill waterways (ponds, reservoirs, tanks) and floating waterways make up surface water (rivers, canals). The rivers were the lifeblood of India and were vital to its survival. Surface water covers an area of almost 7 million hectares in Figure 2.

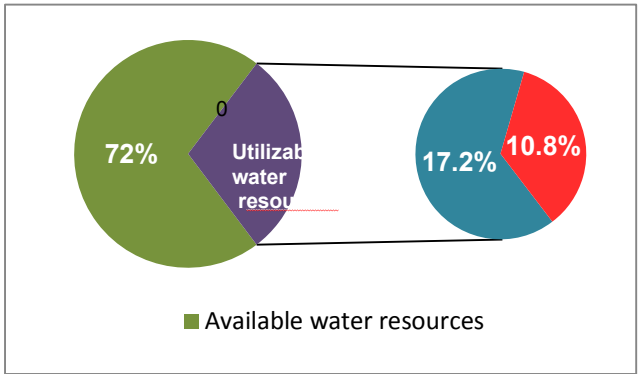


Figure 2. Fresh water resources in India [5]

Numerous international agencies, including World health organization (WHO), Environmental Protection Agency (EPA), International Organization for Standardization (ISO), and others, have developed simple, efficient, and reliable methods for evaluating water quality in order to prevent disease outbreaks. For prevention of diseases, antibiotics are given, with the time microorganisms developed resistance among them. As a result, medical therapies such as antimicrobial medications or antibiotics have become ineffective, raising illness risk and mortality among the population. The Antimicrobial Resistance (AMR) leads to loss of productivity through to lengthier hospital stays and the need for more costly and clinical treatment [6].

Bacteriophages or phages are showing promise as therapeutic agents and constitute an alternative and effective approach for eradicating pathogenic bacteria. They are thought to lyse or eliminate one or more bacteria under the ideal situation.

2. SOURCES

2.1. WATER RESOURCES AND MICROBIAL COMMUNITIES

India is abundant and diverse in natural resources, particularly water, which benefit a variety of environments. These wetlands provide a variety of ecological services and benefits, but they are under significant stress as a result of rapid society, development, and agricultural intensification, as evidenced by the shrinking of their area and a decrease in the hydro - geological, economical, and environmental work. Microorganisms, as well as prokaryotes such as bacteria, protozoa, and algae, and eukaryotes such as fungus, have a numeric and synthetic impact on all water reservoirs. At the microbiological level, the impact of environmental pollution with human activities and also, the habitat changes on water sources are the most evident and cause the most worry. Although the change has been beneficial to humans' short-term aims and needs in certain cases, it has also been destructive (such as water sources contaminated with industrial effluents) since people have utilized [7].

Bacteria: These are the physiologically and biologically most diverse groups, including synthetic autotrophy and photo autotrophy as sources of energy. Surface water microbes are a diverse group of prokaryotes that differ in surface morphology, physiology, and ecologic preferences due to the wide range of habitats they inhabit, which include tidal flats, wet grounds, and water bodies with sulphur compounds such as lakes, ponds, and rivers [7, 8]. Many pathogenic microorganisms were discovered in waterbodies, as well as bacteria (such as *Shigella, E. coli, Vibrio*, and *Salmonella*).

Algae: Freshwater algae range in size from micro to macro organisms that can be seen with the naked eye and appear to be plants. Green algae (Chlorophyta), red algae (Rhodophyta), blue-green algae (Cyanobacteria), and diatoms (Bacillariophyta) are the four principal kinds of algae found in inland water bodies [9].

Diatoms: Microbial clumps and slime like coatings on the reservoir rock are commonly linked with these forms of algae. On riverside materials, such as *Gomphoneis* produces wide, brightening pales.

Fungi: The most of fungi are thought to be terrestrial, with only a few being aquatic. They are thought to have evolved from the terrestrial ecology, where organic waste decays and poses a significant risk of causing illness in living species. As a result, these microorganisms aid in the preservation of

freshwater reservoir quality [10]. *Penicillium citrinum, Alternaria alternata*, and *Fusarium spp*. are prominent fungus found in natural water bodies.

2.2. SOIL AND SEDIMENT AS BACTERIOPHAGES RESERVOIR

The agricultural soil separates into various layers and microbiomes that provides niches for bacteria-bacteriophage complexes. Other than this, geographical diversification, soil's significant changes, influences from plant root microbiome, fungal microbiome, mineral, and nutritional state, make it a rich source of bacteriophages with a wide range of variety. Sediments consist of organic and inorganic deposits found in aquatic habitats, and accumulate various bacteriophages, allowing them to be isolated at greater densities. For bacteriophages with great density and variety, marine sediments are also significant biological habitats. Bacteriophages have been found in several severe soil conditions, such as deserts. The desert habitat, which is characterized by intense heat, a dry sand surface with little moisture, and excessive UV radiation, experiences significant temperature fluctuations throughout the day. Bacteriophages with various morphologies, genetic composition, physicochemical characteristics have also been isolated and described from desert soils. They have also been found in cold habitats such as permafrost ground, where their impact on microbial and community dynamics has been studied [11].

3. DETECTION OF BACTERIOPHAGES IN WATER BODIES

Rivers and lakes are India's second most significant source of waterways as a surface water, capable of supplying vast amounts of water for human existence in the area. However, Indian wetlands (lentic and lotic water systems) are under extreme stress, just like every other region on the planet, due to unsustainable human demands. As a result of rising urbanisation and industrialisation, these lentic water systems become more prone to changing watershed patterns, worsening the amount and characteristics of freshwater water available, as well as microbiological diversification [12]. Despite interpretations of phage densities and virophages in the environments, yet no sequences are found of genetic information of groundwater phages. There is just one publication on subsurface phages, Anath and Lana against *Bacillus* and *Pseudomonas* [13], that contains their sequenced genome and molecular phylogenetic connection, according to investigations conducted all over the world. There is a lack of agreement that somehow this study has a very narrow scope, and this also confirms that groundwater systems are a poorly understood, wide and varied biotope.

Another study found that bacteria developed resistance to phages as a result of mutations or modifications in phage receptors, or as a result of lower sensitivity to the phage, possibly as a result of resistance development as a result of failed treatment, which has become a significant reason of phage therapy failure [14]. During the current study's literature assessment, it became clear that there is a lack of knowledge about bacteriophage behavior and function in the near surroundings for identification and implementation to humans and other animal therapeutics.

Bacteriophages fed on the infective microorganisms, reducing the quantity of health-damaging germs. Based on these observations, the current work recognizes the relevance of bacteriophage identification and characterization against *E. coli*, the most virulent pathogenic bacterium known. *E. coli* phages can be found in a variety of places, including water, animal excrement, and plant excretion.

As indicated in Table 1, 20 virulent phages were chosen for this work based on *E. coli* reference data as the target bacteria of only desirable sources of water. Coliforms act as fecal indicator bacteria, which degrade quickly, according to several impaired testing. According to these findings, it is possible to predict that the proportion of those microbe companies would change as polluted water gets older [15].

Coliphages fulfill a number of the requirements for a perfect indicator of faecal contamination of water:

- Presence in large numbers in the environment
- Easily detectable and countable using basic procedures
- Low-cost estimation procedure
- Responsiveness to and against external influences in order to comply with infective microorganisms.

Table 1: Bacteriophages in selected water bodies

S. No	Phage	Source	Accessionno.	References
1	*Escherichia* phage vB_EcoM_PhAPEC2	River Water	NC_024794	[14]
2	*Escherichia* phage vB_EcoM_PhAPEC5	River Water	NC_024786	[14]
3	*Escherichia* phage vB_EcoM_PhAPEC7	River Water	NC_024790	[14]
4	*Escherichia* phage vB_EcoM_PhAPEC9	River Water	NA	[14]
5	*Escherichia* Phage PGN829.1	SewageWater	MH733496	[16]
6	*Escherichia* virus myPSH2311	Sewage Water	MG976803	[17]
7	*Enterobacter* virus myPSH1140	Sewage Water	NC_055739	[17]
8	vB_EcoP-EP335 (EP335)	Sewage Water	MG748548. 1	[18]
9	Phages vB_EcoM-EP75 (EP75)	Sewage Water	MG748547. 1	[18]
10	*Escherichia* phage PhaxI	Sewage Water	JN673056.1	[19]
11	*E. coli* PTCC 1399 and *E. coli* SBSWF27	River Water	NA	[20]
12	*Escherichia* phage myPSH1131	River Water	MG983840. 1	NCBI
13	*Escherichia* phage vB_EcoP_WFI101126	Sewage Water	MK373770. 1	NCBI
14	Phage vB_EcoP_SU10	Sewage Water	KM044272	[21]
15	*Escherichia* phage ECML-4	Fresh and Salt Water sample	JX128257	[22]
16	Anath and Lana	Ground Water	MG983742. 1, MK473373. 1	[13]
17	Phage 5P_1	Ground Water	MK113949	[23]
18	Phage 5P_2	Ground Water	MK113950	[23]
19	Phage 5P_3	Ground Water	MK113951	[23]
20	PRD1	Ground Water	NC_001421	[24]
21	*Enterobacter* phage PG7	Fish and Pond Water	NC_023561	NCBI
22	*Escherichia* phage PGN6866	Sewage Water	MT127620	NCBI
23	*Escherichia* phage ES17	Raw Sewage	MN508615	[25]

S. No	Phage	Source	Accessionno.	References
24	*Escherichia* phage MN05	Surface Water	MT129655	NCBI
25	*Escherichia* phage MN03	Surface Water	MT129653	NCBI
26	*Escherichia* phage Paul	Fresh Water	MN045231	[26]
27	*Enterobacter* phage BA14	Sewage Water	NC_011040.1	[27]
28	*Escherichia* phage GA2A	Speed River	NC_031943.1	NCBI
29	*Escherichia* phage HZ2R8	Pig farm sewage water	NC_047923.1	NCBI
30	*Enterobacter* phage IME11	Sewage Water	NC_019423.1	[28]
31	*Escherichia* phage IMM-002	River Water	NC_048071.1	NCBI
32	*Escherichia* phage LL11	Sewage Water	NC_048064.1	[29]
33	*Escherichia* phage LL2	Sewage Water	NC_048063.1	NCBI
34	*Escherichia* phage Lidtsur	Sewage Water	NC_048177.1	NCBI
35	*Escherichia* phage Minorna	River Water	NC_048172.1	[30]
36	*Escherichia* phage PGN590	Sewage Water	NC_049830.1	NCBI
37	*Escherichia* phage vB_EcoP_PTXU04	Sewage Water	NC_048193.1	NCBI
38	*Escherichia* phage Pollock	Sewage Water	NC_027381.1	[31]
39	*Escherichia* phage SECphi27	Sewage Water	NC_047938.1	NCBI
40	Stx2 converting phage II DNA	Sewage Water	NC_004914.3	[32]
41	*Escherichia* phage VB_EcoS-Golestan	Sewage Water	NC_042084.1	NCBI
42	*Escherichia* phage YZ1	Sewage Water	NC_047927.1	NCBI
43	*Escherichia* phage ZG49	Hospital Sewage	NC_047777.1	NCBI
44	*Escherichia* phage aalborv	Sewage Water	NC_049829.1	NCBI
45	*Escherichia* phage atuna	Sewage Water	NC_049819.1	NCBI
46	*Escherichia* phage damhaus	Sewage Water	NC_049827.1	NCBI
47	*Escherichia* phage flopper	Sewage Water	NC_048845.1	NCBI

S. No	Phage	Source	Accessionno.	References
48	*Escherichia* phage grams	Sewage Water	NC_049825.1	NCBI
49	*Escherichia* phage haarsle	Sewage Water	NC_049828.1	NCBI
50	*Escherichia* phage herni	Sewage Water	NC_049823.1	NCBI

The order Caudovirales, which includes dsDNA phages and ssDNA phages with or without a tail, contains the bulk of phages. Siphoviridae, Myoviridae, and Podoviridae are the three families that make up the Caudovirales [14]. Compound microscopy is presently a rapidly expanding field of study, with countless applications in chemical research and biology. Transmission electron microscopes (TEM) and scanning electron microscopes (SEM) are the two most common varieties (SEM). The latter tactics have limited influence on phage investigations, but TEM might be highly important in virology as a whole. Several phages are examined as per their source having the same protein complex of the major capsid protein, which itself is expressed in nearly each phage since some morphologies contribute when they infects the bacteria. The bacteriophages identified had different morphologies from one investigation to the next. As a result, the head and tail structures of coliphages from water supply vary. Some phages have a broad head and a short tail, whereas others lack a tail and have a cylindrical head. More structural proteins should be investigated in order to fully comprehend the morphology.

Bacteriophages are the viruses that consume bacteria and destroy them. They are made up of DNA or RNA that is encased in a protein capsid that interfaces with bacteria's specific cell surface receptors and hence they infects the host particularly by hijacking the host's cellular machinery [33]. They are found everywhere in the environment and are thought to be the most abundant organic activator on the planet. In terms of size, shape, and genetic organization, they are incredibly different. All, however, have a genome encased in a shell of capsid proteins on phages, which safeguard the genetic information before releasing it into the next host cell. Hundreds of phages, some of which look as "heads," "legs," and "tails," have been verified using TEM/SEM. Considering their physicality, phages are immobile and depends on chance to accomplish their objectives. Phages, like other viruses, are quite species-specific in terms of their bacterium, and they usually target a certain host species or even certain lines inside a host. Once a phage has attached to a vulnerable species, it targets one of the two reproduction pathways: lytic or lysogenic. The lytic route is the process by which phage DNA is coupled to the receptors located on the surface and injected into the host, where it produces enzymes or proteins that breakdown the host DNA. Furthermore, it makes new viral particles with its host cell machinery and releases 100-200 new phages into the surroundings. In the lysogenic cycle, phage DNA attacks the host organism and uses the integrase enzyme to integrate into the host DNA, resulting in a prophage. As a result, the prophage DNA replicates and splits with the host DNA, resulting in prophage replication [34].

Phages have the ability to be utilized as diagnostic instruments and as a monitor for proteins and antibodies in addition to kill microorganisms in water. Bacteriophages can also be used to trace pollution sources or as a water quality indicator. In the petroleum and agricultural industries, they are also called biocontrol agents. Furthermore, because of their selectivity in interacting with bacterial surface receptors and thereby infecting specific bacteria, phage therapy is used instead of antibacterial drugs. Phages are quite effective in immediately lysing the host bacteria.

3.1. CLASSIFICATION OF BACTERIOPHAGES

Phages are a varied group of organisms. They are made up of ss DNA or ss RNA, as well as ds DNA or ds RNA. D' Herelle made the first bacteriophage discovery in 1917. Ruska discovered the viral nature of bacteriophages in 1943. Lowff, Horne, and Tournier suggested a morphological and nucleic acid-based categorization scheme for bacteriophages in 1962.

The categorization is significant for phylogenetic research since it aids in comparison and understanding of the connection between phage groups, which is necessary for the discovery of novel phages [35], as shown in Table 2 below. Regardless,

phages are still being studied, and because there is a categorization gap that has yet to be filled due to a lack of genomic study, the following data may appear fragmented.

Table 2: Classification of phage families [35]

Family	DNA/RNA
Caudovirales	ds DNA, without envelope
Myoviridae (T4)	Contractile tail
Siphoviridae (λ)	Long tail, non-contractile
Podoviridae (T7)	Tail short
Microviridae (ΦX174)	ss DNA, 27nm, 12 knoblike capsomeres
Tectiviridae (PRD1)	ds DNA, vesicle of lipid, fake-tail, 60 nanometer
Corticoviridae (PM2)	ds DNA, complex capsids, lipids, 63 nm
Leviviridae (MS2)	ss RNA, 23 nm, like polio virus
Cystoviridae (Φ6)	ds RNA, fragmented, envelope of lipid, 70 to 80nanometer
Inoviridae (Fd)	ss DNA, fibres or rod shaped, 7 nanometer
Plasmaviridae (MVL2)	ds DNA, envelope of lipid, without capsid, 80 nanometer

4. METHODS OF DETECTION

4.1. CONVENTIONAL METHODS

Scientists found distinct spaces on bacterial lawns very clearly on the spots of bacterial presence in the form of plaques after the discovery of bacteriophages in 1915. As a result, the bacteriophages were discovered using two standard methods: a spot test and a double agar overlay plaque assay. The double agar overlay assay method, which allows phages to localize in a specific ecosystem on 2 layers of agar, one layer is hard that is on the bottom of the plate or for supporting bacterial growth and the other layer is soft that is on the top of the plate for the interaction of phage-host, that likely to result in a bacterial lawn on which phages bind in the production of plaques [36, 37]. As a result, they are preferred for enumeration. Furthermore, these approaches may produce false-positive results since bacterial lawn clearance may occur attributed to the existence of other bactericidal chemicals. As a result, more research is needed to validate the inhibitory zone.

4.2. MOLECULAR TECHNIQUES

4.2.1. FLOW CYTOMETRY

A fluorescent dye is used to label viral particles, which are then driven via a capillary. The particles move through the capillary's small diameter, enabling dispersion of lights from every particle to be detected. Because the procedure is both quick and thorough, it is commonly used. Based on the fluorescent signal, various viruses show differences in a mixed sample may be distinguished because of their emission and side scatter dispersal, according to a fundamental research [37].

Other studies have measured enzyme production from bacterial cells as a result of phage-induced cell lysis to identify phage multiplication indirectly. Intracellular enzymes such as adenylate kinase and adenosine 5'-triphosphate (ATP), galactosidase used to quantify *E. coli* phage infection. As soon as given substrate is disintegrated, enzyme production is found by bioluminescence. Also, even when starting with a little quantity of phage, these assays are very sensitive, giving a visible signal in a short period (3 hrs). Such approaches are capable of providing high throughput to operate with any phage, and that each species of bacteria may require optimization of enzyme and substrate [38].

4.2.2. NANOSIGHT

Based on dynamic scattering of light by laser-illuminated optical microscopy, a laser-based approach developed by NanoSight Limited permits real-time viewing and enumeration of viral particles in a matter of minutes. The requirement for considerably large sample concentration and a clear liquid, which is difficult to acquire from complex materials like dirt and faeces, are disadvantages [37].

4.2.3. QUANTITATIVE PCR (QPCR)

Polymerase chain reaction (PCR) is a quick and reliable way to confirm the occurrence of phages are quicker than plaque tests. In all qPCR systems, there are two primary chemistries employed. In qPCR, the intercalating fluorescent dyes are used to measure the amount of polymerized DNA product. As the nucleic acids are amplified, fluorescence is detected. Firstly, intercalating fluorescent DNA dyes are used, then, probes are used, then, fluorescent dye and a quencher molecule are attached to a short fragment of DNA. For measurement of double-stranded DNA, intercalating dyes are less sensitive, but fluorescently labelled probes are more exact. M13 and T7 phages were identified by qPCR technology [37].

4.2.4. DROPLET DIGITAL PCR (DDPCR)

Sample consists of a hydrophobic material to generate a water-oil emulsion, and in each droplet, the reaction occurs at the same time in droplet digital PCR (ddPCR). A fluorescence detector is used to count the droplets in the mixture that carries the amplification. Because this is proportional to the quantity of template DNA in the sample, it may be used to determine the beginning concentration without the need of external standards. In comparison to DLA, ddPCR detects higher number of phages that infects the *Pseudomonas syringae* that is a plant pathogen. Before DNase treatment, the variance might be reduced to 3 to 4 times greater before interfering viral capsids [37].

5. HUMAN HEALTH AND PHAGE THERAPY

Antibiotics have been discovered in 1928 and have since been used to treat a variety of serious diseases, saving millions of lives. However, due to overuse of antibiotics, bacteria have evolved and developed multi-antibiotic resistance genes. As a result, common infections have become more severe, resulting in an anticipated 4.95 million fatalities in 2019. Antibiotic-resistant bacterial illnesses killed at least 1.2 million people in New Delhi in 2019. [39] By 2050, it is anticipated that roughly 10 million individuals would have died [40]. From sequenced bacterial genomic data, more than 400 distinct kinds and 20,000 possible multi-drug resistant genes have been predicted [41].

5.1. HISTORY OF PHAGE THERAPY

To avert this, new and effective antibacterial molecules must be found and developed quickly. Phages are widely used in the world but the various researches are underexplored with respect to the spread of Antibiotic resistant genes that expand the bacteria. Humans have learnt to know and identify them. Félix d'Hérelle, who isolated them in the faeces of receiving medical treatment of dysentery patients in 1917, is responsible for their medicinal uses, which were first noted in 1915 by Frederick Twort (1915). D' Hérelle used his discovery right away to treat patients with bacterial illnesses and claimed significant success [42].

Phage therapy grew fast in Europe and the United States, even phages are being developed by numerous American pharmaceutical firms, but these were rejected in these regions. At the same time, the sulfonamides as the strong antimicrobial agents and also, antibiotics were developed and widely used in Germany and United States. Widely used first antibiotic Penicillin has shown huge reduction in infectious diseases. These sulfo-drugs and antibiotics have shown great achievement in the field of medicines. Simultaneously, the tremendous rejection for phages were faced by medical community. After many attempts, there were some initiates taken on phages and became successful in 1990 to 2000 by developing 2 anti-listeria phages which were approved in 2007 and phages were found to be protected in agri-food sector also. In France and Belgium, therapeutic uses of phages for human diseases with the successful results have to be seen in increasing manner [42].

5.2. CURRENT STATUS OF PHAGES

As a result, phage treatment has only been used in a few nations in Eastern Europe, where researches have shown that phages may effectively cure specific illnesses with no negative side effects. Clear effectiveness data from randomised controlled clinical studies is necessary to prove phage treatment as a viable alternative to antibiotics. To address this issue, a growing clinical trial have been conducted in recent years, while only a couple have been completed in which there are few successful case studies of phage therapy treatment. One of the cases of USA, a 26-year-old female of suffering from cystic fibrosis infected by multidrug-resistant (MDR) *Pseudomonas aeruginosa* and phage route administration through intravenously that rapidly show the clinical success by no recurrent pneumonia within 100 days of follow up. Another case where phage administration (oral and inhaled), a female of 17 years infected by MDR *Archomobacter xylosoxidans* caused cystic fibrosis and given clinical success of dyspnoea resolved, reduction in cough and improvement in function of lungs from 54% to 84% by taking 12 months treatment. One more case of USA where a 76-year-old man was infected by *Pseudomonas aeruginosa* that caused prosthetic vascular graft infection with associated fistula and phage therapy was given local (mediastinal fistula) which showed success by not showing any sign of recurrence after 18 months [38].

Following the positive findings, the majority of requests from hospitals and patients from all over the globe were received, necessitating the formation of a phage bank with distinctly defined phages for international access.

5.3. REGULATORY CHALLENGES IN PHAGE THERAPY

5.3.1. QUALITY AND SAFETY REQUIREMENTS

To prepare and manufacture the phages, it is very important to have the quality and safety of the phages. The traditional prolonged medical product research and licencing procedure is not used for sustainable phage products. There is a need for an adaptable framework, with realistic manufacturing, quality, and safety standards, which helps in faster delivery of phage treatment products for "personalised therapy" or public health or medical situations. However, there are no standard guidelines for manufacturing of phages. To fix this concern, a group of phage researchers made a framework of quality and safety standards for long-term phage treatment products [43, 38].

Some of them are all vital equipment and technical devices must be recognised and verified, examined on a regular basis, and kept in good working order according to the manufacturer's specifications because of the physic-chemical properties viz; temperature, pressure, particle counts and some at biological levels by microbial contamination, also the corrective action is needed, to detect system failures and errors and making sure that these key factors are kept in under acceptable parameters throughout all times [43].

5.3.2. STABILITY OF PHAGE PREPARATION

For stability in phages is the most important requirement for phage therapy. Phages should be stored or protected against high temperatures, light, evaporation and contamination. The stability of phages depends on the different types of requirement of phages. Some phages require to maintain their titer for some days, some phages require for years. Some phages may hold on at 4°C, room temperature, some can be destroyed at -20°C due to crystal of ice crystals. So, it becomes difficult to define for all phages. Therefore, storage and preservation that leads to particles in the same form to maintain its stability, it is not defined yet for all the phages according to individual requirements. There are several methods to maintain the phage stability that includes freeze-drying, lyophilization, spray-drying, emulsion, and polymerization techniques, also the encapsulation techniques [44].

5.3.3. PHAGES AGAINST BIOFILMS

Biofilms, as these are microbial assemblages encased in polymeric materials, have been linked to a number of severe and challenging to cure infectious disorders. The exopolymeric matrix components inactivates the drug and makes biofilm cells significantly more resistant to antibiotics. Biofilms shield cells from phage predation and promote the spread of phage-resistant phenotypes, potentially limiting phage treatment against biofilms (Figure 3). There are only 2 cases of phage therapy in Europe having successful results against the biofilms formed of *Pseudomonas aeruginosa* and *Escherichia coli*. Also, phage therapy against biofilms is not successful in some reports as there is no clear explanation to this issue.

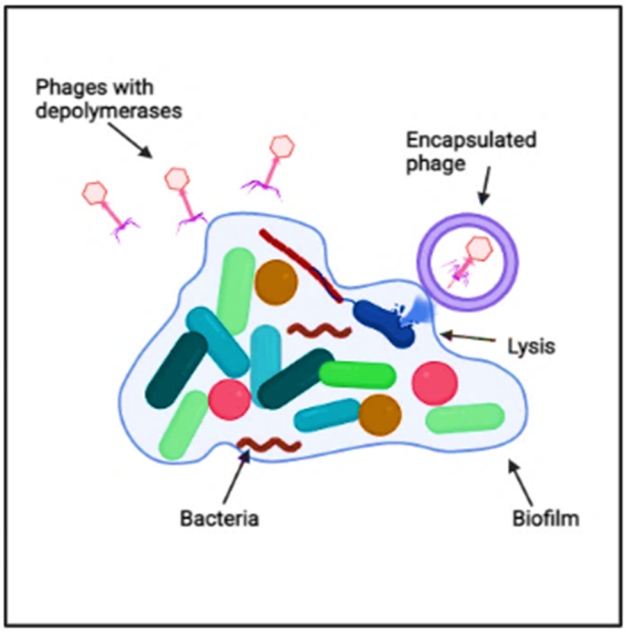


Figure 3: Delivery of Phages against Biofilm

Usually, to infect biofilms, phages secret some degrading enzymes and also, with some mechanical dispersion which helps in penetrating the inner surface of biofilms matrix leading to increased therapeutic efficacy. But biofilms are organized in this way that they have numerous nutrient availability, definite motility with complete metabolic state which affects the phages to treat infectious disease. They can regrow after some hours of phage interaction. In the traditional approach, the wells are infected with a bacterial cell culture and then drained and cleaned at various time periods to eliminate planktonic cells and let sessile bacteria to develop. In several investigations, phages have been shown to be able to target the susceptible host in a biofilm even while a non-susceptible strain is present. As a result, phages' ability to suppress biofilm infections is undeniable. The first chemistry

of phages and the biofilms interactions suggests further study for biofilm related illnesses by the successful phage treatment [45].

5.4. EMERGING APPROACHES

The phages combination with different agents helps in targeting the biofilm communities. There are different approaches to control the bacterial infections. One of them is the combination of phages and antibiotics that have shown the great results in effecting the planktonic cells, also resulted in the elimination of biofilms with a great efficacy (Figure 4). The combination of phages and antibiotics does not always result in biofilm eradication. While a number of researchers have studied at the effects of phage-antibiotic therapy, only few have devised a systematic method to investigate he bacterial response to these agents. For ex, some phages are identified that targets the multi-drug efflux pumps that bacteria use which is outer surface proteins. Bacteria become resistant to the phages only if they modify their multi-drug efflux pump by increasing their susceptibility to particular antibiotic classes. Another approach is the combination of phages and enzymes and many other components that help to destroy the biofilm matrix and boost phage activity. For ex; the combination of phage with DNAse enzymes that eradicate the DNA material of biofilm matrix [38].

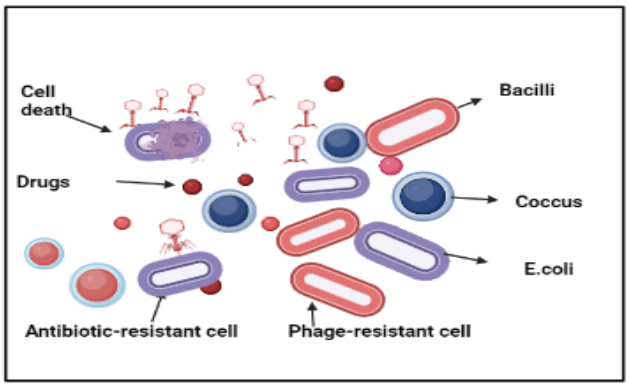


Figure 4. Emerging approaches in phage therapy [adapted from (45)]

Many studies have sought to generate innovative modified phages using genetic and chemical techniques in order to better heal illnesses. Phages are genetically engineered as gene mutation, gene replacement, transgenic genes through the different molecular techniques to enhance the bactericidal efficacy. For phage therapy, strictly lytic phages are preferred over temperate phages due to its strong virulent factor to the bacterial host. However, some efficient approaches come across to improve the efficiency of temperate phages by engineering its phage genomes which aim to cause bacterial cell death same as lytic phages. However, the majority of phage genes have unexplored functions and hence, it is implicated in previously unanticipated negative occurrences [45].

6. CHARACTERIZATION OF BACTERIOPHAGES

The current research outlines the genomic comparability with chosen phages and the phylogenetic studies of selecting protein (Major head/capsid protein) in NCBI-BLAST, led to build up the tree using the neighbor-joining technique utilizing Mega-11 software. Evolutionary relationships of taxa by the Bootstrap consensus tree method with 1000 replicates also, the scale length given is 0.50. As per the different water sources, the constructed phylogram derived following the aforementioned methods revealed a comparable identity in proportion. However, some distinctive patterns were seen in the similarities of phages from specific sources as shown in Figure 5.

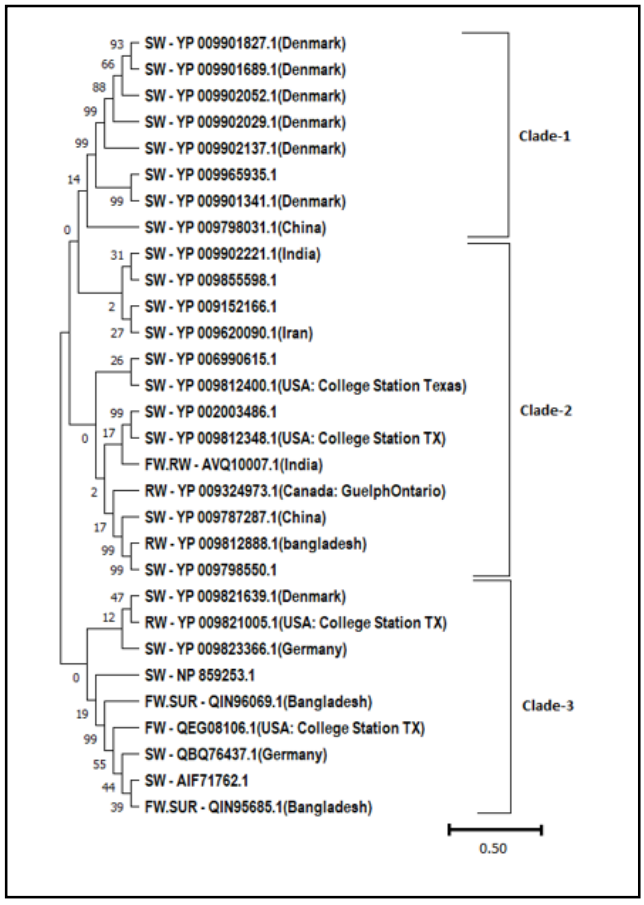


Figure 5: The Neighbor-Joining approach was used to estimate the evolutionary origins.

Branches that refer to divisions that have been replicated in fewer than 50% of bootstrap replicates have been disintegrated. The percentage of phylogenetic tree wherein the related taxa grouped together in the bootstrap method. Poisson correction technique helped in calculating the evolutionary divergence between taxas. On every sequence, all unclear locations were deleted. The total number of positions in the selected method was 894 through MEGA11.

The phylogenetic tree shown here, which was created using a near-joining method, depicts the similarity of bacteriophages from surface water (FW-SUR), river water (FW-RW), sewage water (SW), fresh water (FW) through time. In 1987, Saitou and Nei devised the Neighbor-Joining (NJ) tree deduction approach. The technique relies on distance-based tactics, which are commonly used to build evolutionary trees. The evolutionary tree is built using a matrix of pairwise evolutionary separations between the provided categories.

There are three clades showing in this phylogenetic tree. The first clade showing higher confidence level that supports the similarity in these phages that belongs to sewage water of Denmark area. More than 90 bootstrap values are strongly supported for evolutionary analysis for similarity. Higher the bootstrap value, higher the confidence in similarity.

In the second clade, source of water is same of most phages i.e; sewage water showing very low confidence value i.e; 31 and 27 which are poorly supported and some showing great confidence level of 99 with same source of water and also, with different source of water (river water and sewage water) showing 99 bootstrap value which is highly supported for the similarity of phages.

The third cluster showing poor bootstrap values below 50 which are not being considered for evolutionary analysis even with same source of water (freshwater surface water) and same area (Bangladesh) showing great divergence. Every phylogenetic tree has the scale bar that estimates the sequence divergence in the cladogram, so this scale bar indicates number of substitution per site. The scale length is compared to length in phlyogenetic tree. The 0.50 scale length means that there are 50 base differences for every 100 bases. More study is needed, however, to have a better knowledge of bacteriophages evolution.

7. CONCLUSION

Enteric illnesses have been treated with various bacteriophages that destroy distinct bacterial strains. As they devour the specific bacteria, they demonstrated their bactericidal powers. In Georgia, phage treatment was studied and these phages were utilized to treat a variety of bacterial disorders. Many people die each year as a result of *E. coli* and other microbial strains that have developed drug resistance to antimicrobials as microorganisms change to every iteration. As a result, living creatures have to develop alongside bacterial strains in order to remove microbial species. The presence of *E. coli* at a certain proportion in an aquatic environment might be a biological signal of contamination in the water. As a result, phages can be chosen over antibiotics due to their great selectivity, as well as their long-term viability and high effectiveness, with no resistance development in non-targeted microbial isolates. Though bacterial hosts can evolve and become resistant, the phages also co-evolve, minimizing the chances of bacterial pathogens becoming more dangerous. In order to improve the use of bacteriophages in therapeutic applications, ongoing attempts to evaluate phage behavior and hence the science underpinning phage treatment is required. Regulatory approvals are also required to bring phage therapy into the mainstream treatment methods.

8. REFERENCES

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Bioremediation of Nickel Using Nickel Resistant Bacteria

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ABSTRACT

Heavy metals are introduced into the environment through anthropogenic processes. These heavy metals have become a global problem because they adversely impact human health. Bioremediation is used to remediate the toxic heavy metals from the environment by microorganisms or its enzymes. Bioremediation is preferred over physicochemical methods because most heavy metal salts are water-soluble and cannot be separated by physical separation methods. Also, physicochemical methods are ineffective and expensive when the concentration of heavy metals is very low. Bioremediation includes sustainable remediation technologies to rectify and re-establish the natural condition of soil and helps in getting rid of heavy metal pollution. Therefore, Bioremediation is preferred as it is highly efficient, cost effective and eco-friendly. Nickel is carcinogenic to humans, which causes systemic manifestations including lung cancer. This review aims to investigate the Ni resistant bacteria and its efficacy in bioremediation. Bacteria reduce metals as a detoxification mechanism. They use mechanisms like ATP binding Cassette transporters efflux system, biomass formation etc. Bacteria can significantly remove 70-85% nickel content from the soils after bioremediation process. Bacteria can physically remove considerable amounts of positively charged cationic metals through bioaccumulation or biosorption. At low concentrations, bacteria take up the available metal more quickly. However, metal ions diffuse to the biomass surface by intraparticle diffusion at high concentrations. Additionally, Ni is demonstrated as chemoattractant to bacterial cells under stressful conditions. For future research, more bacterial species can be explored for enhancing the current methods for bioremediation of heavy metals.

Keywords: Bioremediation, Carcinogenic, Chemoattractant, Detoxification, Nickel.

1. INTRODUCTION

Heavy metals are naturally occurring elements. They are introduced into the environment via many natural and anthropogenic process such as rapid industrialization, agricultural processes etc. These heavy metals are toxic at low concentrations and at higher concentrations, they could become poisonous [1].

Due to non-biodegradability of heavy metals, it becomes hard to remove them from contaminated biological tissues. Some of the heavy metals are necessary for the survival of living organisms in small amount such as cobalt (Co), copper (Cu), iron (Fe), manganese (Mn) and molybdenum (Mo). These are dangerous because they tend to bioaccumulate in the surrounding. Hg, As, Zn, Cd, Ur, Se, Ag, Au and Ni are hazardous heavy metals that adversely affects the crop production, human health and quality of the soil. These pollutants also cause many life-threatening diseases like cancer, Alzheimer's disease, atherosclerosis, Parkinson's disease, etc in humans [2]. In Plant cells, these causes high metal toxicity inhibits cytoplasmic enzymes and causes damage to cell structures which directly affects plant growth and metabolism [3].

2. NICKEL

Nickel is a member of group X of the periodic table and is the 24th most copious element found on Earth.

2.1. SOURCES OF NICKEL

In environment, Nickel is found in soils in lower concentrations, but the majority of Ni is present in greater part of the environment through anthropogenic processes such as usage in fertilizers, smelting, pesticides, sludges, and wastewaters. Ni is used in various industries such as metallurgical, food processing and electroplating as well as it is use as a catalyst and for the production of Ni-Cd batteries.

Nickel is consumed through various food items such as Hazelnuts, cocoa, almonds, dates, figs, pineapple, plums, raspberries, oats, brown rice, beans, cabbage, lettuce, lentils, peas, spinach, cabbage, peanuts,

baking powder. It is majorly found in seafood such as shrimps, oysters, crab and salmon.

Nickel is also present in packaged food items like whole grain bread, dark chocolates and soya products. Various items used on daily basis also involves nickel such as inexpensive jewellery, cosmetics, cell phones, eyeglass frames, paper clips, braces, stainless steel articles, zippers, snap buttons, belt buckles, armaments, alloy [4].

2.2. IMPACTS OF NICKEL

It causes risk of environmental pollution, endangering the living beings and ecological systems globally as it cannot be broken down naturally. It causes headaches, gastrointestinal manifestations, respiratory Manifestations, Contact dermatitis, lung fibrosis, cardiovascular diseases, lung cancer, nasal cancer, epigenetic effects [5].

Ni is termed hazardous for human health, as it can damage the central nervous system and result in respiratory disorders. Furthermore, Ni and its related compounds has been categorized as group 1 and 2B carcinogens by the **International Agency for Research on Cancer (IARC)** [4]. Permissible limit of Ni in drinking water is 0.02 mg/L and Permissible limit of Ni in industrial waste is 1.0 mg/L.

2.3. REMOVAL OF NICKEL

High concentrations of Ni can be removed by both physical and chemical methods. The major treatment used for heavy metal degradation include evaporative recovery, coagulation, electrodialysis, floatation, chemical precipitation, flocculation, ion exchange, nanofiltration, reverse osmosis, ultrafiltration etc. Also, physio-chemical methods such as extraction, immobilization, stabilization, soil washing, etc. These methods are generally expensive and less effective for lower concentrations.

Biological methods are preferred over physio-chemical methods as they are more successful at efficient removal. Also, it is cheap, efficient, and eco-friendly method for the removal of heavy metals from contaminated environments.

3. BIOREMEDIATION

Bioremediation is a process which deals with the use of microorganisms like bacteria for the removal of contaminants, toxins and pollutants from the environment.

Depending on the place where Bioremediation is performed, it is categorized into 2 ways: In situ Bioremediation and Ex situ Bioremediation. Bioremediation done at the same site using biological systems is called In situ Bioremediation. Bioremediation done at some other place from the original site is called Ex situ Bioremediation. Bioremediation can be performed in two ways: Bioaugmentation and Bio-simulation. Bioaugmentation- Bioremediation which involves microorganisms for the removal of toxic heavy metals is known as bioaugmentation. Bio-simulation- Bioremediation which involves growth limiting nutrients like phosphorus, nitrogen, oxygen which stimulate the existing bacteria to degrade the hazardous and toxic contaminants is known as bio-simulation [6].

4. BIOREMEDIATION OF NICKEL USING BACTERIA

Many living organisms have evolved to survive in high concentrations of heavy metals under intense conditions. They take up the heavy metal and releases them into the environment in less toxic form. The requirement of Ni for essential processes have been adapted by many microorganisms like bacteria, thus developing resistance mechanisms in aiding the high concentrations of Ni inside the bacterial cell as well as the surrounding environment. The cell wall of bacteria acquires a negative charge that facilitates its interaction with positively charged heavy metal ions. This results in the efficient transition of these ions through the cell membrane.

Different bacteria use different methods for the treatment of heavy metals such as efflux system, biomass formation, operon system etc.

In *Cupriavidus* (Ralstonia) *metallidurans* CH34, one such resistance mechanism is seen [7]. *Cupriavidus metallidurans* CH34 is used as a model organism for heavy metal detoxification and for biotechnological purposes.

Nickel resistance in these bacteria is generally mediated by **Efflux pumps**. It has presence of cnrCBA efflux pump encoded by the cnrYHXCBAT gene system. In this mechanism nickel enters the periplasm, cnrY and cnrC are the promoter genes which initiate transcription at the cnr. Products of three genes cnrYXH regulate expression of whole gene cluster. cnrH activates cnrCBA expression. cnrX and cnrY are membrane-bound proteins, which function as anti-sigma factors. cnrCBA is activated in micromolar concentrations of Ni and then encodes highly efficient pump [8]. It lowers intracellular

concentration by pumping out Ni cations from cytoplasm [9].

5. CASE STUDY OF NICKEL BIOREMEDIATION FROM CONTAMINATED INDUSTRIAL EFFLUENTS

Scientists performed various experiments to investigate efficient role of nickel resistant bacteria with or without nickel in nickel bioremediation. They collected samples from several discharge points of different steel industries. Some of the bacteria on which research was performed are *Enterobacter asburiae KUNi5, Bacillus altitudinis* and *Rhizobium*. In *Enterobacter asburiae* KUNi5- Growth retardation and extended lag phase were found with increasing Ni concentration in the medium [10]. In *Bacillus altitudinis*, Ni uptake was facilitated by an ATP binding Cassette transporters efflux system [9]. *Rhizobium* can physically remove considerable amounts of positively charged cationic metals from solution through either bioaccumulation or biosorption [11].

Ni act as a stressful condition for the bacterial cells. The intracellular adsorption and accumulation increased with increase in time, concentration of nickel was less in the surrounding indicating accumulation of nickel inside the bacterial cell.

6. DISCUSSION

Heavy metals are main reason of pollution, as they are toxic substances that tend to accumulate over time in the surrounding. This review paper focuses on the efficient role of Ni-resistant bacteria for Ni bioremediation. Bacterial growth is dependent upon optimal temperatures, pH, and other parameters, which can cease or decline if they are allowed to grow at other conditions [12]. Most bacteria are mesophilic in nature i.e they function over a moderate range of temperatures that can be easily applied for optimum growth. The bacterial resistance is an adaptive mechanism to ensure survival under stress conditions and high metal toxicity.

ABC transporter family regulates the entry of Ni. In the presence of Ni, reduced growth of cells was observed, indicating that the bacterial cells were in a stressful environment [13]. The uptake of Ni by killed bacterial cells was observed that the dead (killed) cells were not able to uptake Ni from the medium, but due to the availability of bio sorbent, adsorption was observed. The microorganisms such as bacteria attain the help of various motility patterns in order to survive in various stressful conditions in their surroundings.

7. CONCLUSION

This study was performed for the isolation and characterization of Ni-resistant bacteria from the Ni-polluted industrial effluents, which was studied for its bioremediation potential against Ni. The molecular mechanisms of the bacteria in removing Ni from polluted industrial effluents can be helpful in gaining a better knowledge about the resistance and efflux systems that are responsible for bacterial resistance against Ni. The movement of bacterial cells was facilitated towards Ni in a stressful environment [14]. The size of the bacterial cells was changed after the biosorption of Ni ions, as the cells were observed to be bigger and swollen in size, indicating intracellular accumulation of Ni. These properties were helpful in elucidating the efficient role of Nickel resistant bacteria in Ni biosorption. The future of bioremediation as a technology depends on understanding the biotic and abiotic factors influencing the natural selection of organisms capable of metabolizing pollutants. More research is required to fully understand the metabolic mechanisms of different microbes used in bioremediation so as to ascertain their effectiveness.

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Study of Rotifers of Hasanparthy Lake in Relation To Physico-Chemical Properties of Warangal District, Telangana

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ABSTRACT

Rotifers are one of the most important components in zooplankton community. They play a crucial role in interlinking food chain in the aquatic ecosystem. The purpose of this work was to collect and identify the rotifers up to species level; to find physic-chemical parameters of water and their effects on rotifer population. In the present study were conducted from June 2016 to 2017 May in Hasanparthy Lake to investigate rotifer population and their relationship with physic-chemical parameters such as Water temperature [25-00^{0}C-30.02^{0}C], pH[8.05-8.75],DO[5.00-9.51mg/l],TDS[270.0-380.2mg/l], TH [90.5-200.5mg/l],TA[102.5-190.6mg/1], CL[40.55-86.50mg/1], Ca[19.50-29.60mg/l],Mg[20.65-43.50mg/l],BOD [1.5-4.8mg/1], NO_3 [0.06-9.55mg/l], PO_4[0.15-0.45mg/l], and NH_3[0.01-0.05ppm].In total 38 species were identified. Percentage of rotifer families are Brachionidae[27%], Lecanidae[21%], Lepadellidae[13%], Euchlanidae[11%], Testudinellidae[8%], Trichotridae, Aspalanchidae, Notommatidae and Fillinidae [5%]. It is concluded that most of the physic-chemical and biological parameters in the Hasanparthy freshwater lake showed a monthly pattern from the observations, it is observed that the rotifers showed a greater abundance. The rich variety of rotifers indicates the trophic status of the water body as well as its nature for the survival of fishes.

Keywords: Rotifera, Physic-Chemical Parameters, Hasanparthy Lake.

1. INTRODUCTION

Rotifers are the most important soft-bodied metazoans (invertebrates) among the plankton. The name "rotifer" is derived from the Latin word meaning "wheel bearer". The rapid movement of cilia makes the rotifers appear to whirl like a wheel [1]. an integral and important component of aquatic food-webs, have been documented from a wide variety of inland aquatic biotopes of India for more than a century, but there is currently limited information on their diversity in the Indian floodplain lakes in general [2-3].It is one of the opinion of many researchers that the rotifer species composition and their abundance can be used as indicators of trophic status [4-5].Most of the rotifers are round 200-500 micrometer along [6].Plank tonic rotifers have a very short life cycle under favorable conditions of temperature, food and photoperiod. Since the rotifers have short reproductive stages they increase in abundance rapidly under favorable environmental conditions [7]. Some factors affecting the succession of rotifers have been extensively studied which include physic-chemical parameters, food resources, competitors and predators [8].

2. MATERIALS AND METHODS

The study was carried out at monthly intervals from June 2016 to May 2017.Qualitative plankton samples were collected seasonally from different parts of Hasanparthy Lake by a nylon blot plankton net [Mesh size:25µm]. The collected samples were transferred to a clean plastic container of 100ml capacity and preserved in 4% neutralized formaldehyde solution. Identification of rotifer species was done with the aid of standard literature [9-11].In water samples were collected in clean plastic containers [1 liter] for estimation of the physical and chemical parameters like Water Temperature, P^H, Dissolved Oxygen [DO], Total Dissolved Solids [TDS],Total Hardness [TH], Total Alkalinity[TA], Calcium[Ca], Magnesium[Mg],Biological Oxygen Demand [BOD], Chloride [Cl)] Phosphates [PO_4],Nitrates[NO_3] and Ammonia[NH_3] were analyzed by using standard method [12] and Dissolved Oxygen content was estimated through Wrinkle's method [13].

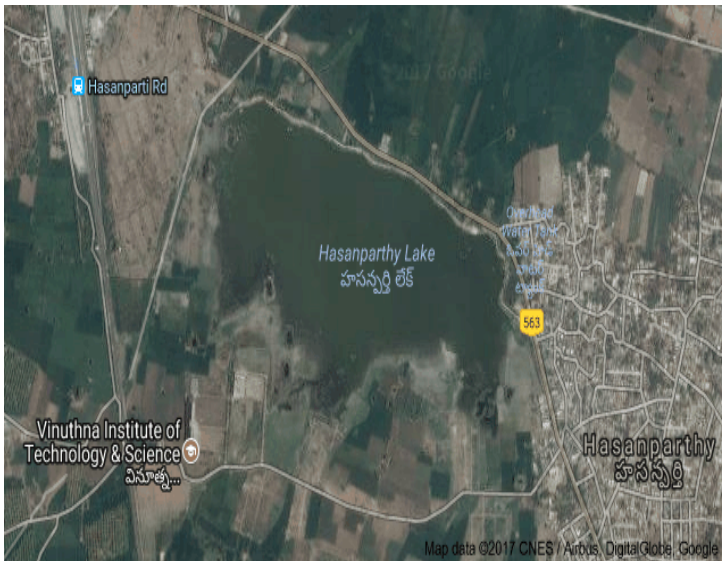

Fig. 1: Shows the Satellite Image of Hasanparthy Lake

3. RESULTS AND DISSCUTION

In the present study physic-chemical analysis of lake shows [Table-1] that water temperature range between 25.00°C-30.02°C. Maximum in May and Minimum in July. [14] Temperature of water elevate the metabolic activity of an organisms. It influences the growth and distribution of plankton. The pH value was [8.05-8.55]. Maximum in June and Minimum in December. Dissolved Oxygen is a sole of the physic-chemical parameters of the water which need to keep the organisms alive and health of the water body of ecosystem [15]. Dissolved Oxygen value was [5.00-9.85 mg/lit]. Maximum in September, Minimum in October. The high content of the dissolved oxygen is an indication of the healthy system [16]. Total Dissolved Solids value was [270.0-380.2mg/l]. Maximum in July, Minimum in March. Total Hardness value was [90.5-200.5mg/lit]. Maximum in March, Minimum in June. Total Alkalinity value was [102.5-190.6mg/lit]. Maximum in April, Minimum in December. Similar results by [17] at Nagaram Tank of Warangal. Chlorides value was [40.55-86.50mg/lit]. Maximum in October, Minimum in September. Calcium value was [19.50-29.60mg/lit]. Maximum in September, Minimum in December. Magnesium value was [20.65-43.50mg/lit]. Maximum in July, Minimum in December. The ionic components like Chloride, Calcium and Magnesium are also in the permissible limits. Biological Oxygen Demand value was [1.5-4.8mg/lit]. Maximum in May, Minimum in November. Nitrates value was [0.06-9.55mg/lit]. Maximum in February, Minimum in August. Phosphates value was [0.15-0.45mg/lit]. Maximum in September, Minimum in November. Ammonia value was [0.01-0.05mg/lit]. Maximum in May, Minimum in September. Nitrates and ammonia concentrations are slightly raised during monsoon season.

In the aquatic ecosystem plankton play critical role not only in converting plant food to animal food but also serves as source of food for their organisms [18]. Rotifers are one of the most important components in zooplankton community. They play a crucial role interlinking food chain in the aquatic ecosystem. They are considered to be one of the most sensitive indicators of water quality [19-20]. It is of opinion of many researchers that the rotifer species composition and their abundance can be used as indicators of trophic status [5],[21-22]. Rotifers [rota means wheel and fera means to bear] derive their name from a specific structure called Corona. It is a ciliated organ. Due to beating of cilia, it gives appearance of a rotating wheel [23]. Rotifers are pseudocoelomate, bilateral, triploblastic and eutelic. They range in size from200 to 500µm [24]. *Brachionus* is dominant group among Rotifera. The present study was conducted that the various zooplankton composition. In total rotifer 38 species were identified belonging to 16 genera [Table-2, 3]. The species of Brachionus and Lecane are more dominant than the species of other rotifer genera. Similar observations are [2],[25-26]. About 2030 species of rotifers are reported worldwide [27-29]. [30] reported 360 species from Indian region. The studies on taxonomic and limnological aspects of the rotifers of Andhra Pradesh made several authors such as [7],[31-39]. Percentage rotifer families are Brachionidae[27%], Lecanidae[21%],

Lepadellidae[13%],Euchlanidae(11%),Testudinellidae and Fillinidae [5%].
e[8%],Trichotridae, Aspalanchidae, Notommatidae

Table1: Physico-chemical parameters of Hasanparthy Lake 2016-2017

PARAMETERS	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY
TEM	29.01	25.00	28.02	29.45	29.16	28.00	29.02	27.50	28.50	29.00	29.50	30.02
PH	8.55	8.51	8.15	8.30	8.65	8.52	8.05	8.42	8.50	8.45	8.30	8.40
DO	8.40	8.35	8.69	9.85	5.00	7.02	8.85	7.00	8.05	8.85	9.51	8.75
TDS	350.5	380.2	330.5	360.5	301.7	315.6	330.2	300.7	308.9	270.0	302.5	300.6
TH	90.5	160.6	160.8	155.0	142.4	160.5	185.5	195.5	160.1	200.5	198.5	180.2
TA	150.0	160.5	160.9	155.5	190.0	180.0	102.5	173.0	172.5	171.0	190.6	165.5
Cl	60.55	68.00	59.25	40.55	86.50	61.80	55.00	61.50	66.90	55.80	60.02	60.50
Ca	25.00	20.20	24.60	29.60	29.55	29.50	19.50	22.60	28.90	26.55	23.40	28.15
Mg	40.45	43.50	38.51	41.20	39.65	34.55	20.65	35.30	32.60	30.80	28.15	29.10
BOD	2.0	2.5	1.8	2.9	3.2	1.5	3.3	1.6	2.6	3.7	4.2	4.8
NO_3	0.11	0.25	0.06	0.16	2.33	1.55	5.80	6.55	9.55	0.33	2.70	3.00
PO_{4-}	0.35	0.40	0.20	0.45	0.32	0.15	0.20	0.17	0.28	0.35	0.22	0.23
NH_3	0.02	0.03	0.02	0.01	0.02	0.03	0.04	0.02	0.02	0.02	0.02	0.05

Phylum: Rotifera
Class: Eurotatoria
Subclass: Monogononta
Order: Plomia

Table2: Rotifer species recorded in Hasanparthy Lake, Warangal

S.No	Family	S.No	Species
I	**Brachionidae**	1	*Anuraeopsis fissa Gosse,1851*
		2	*Brachionus Calyciforus Pallas,1776*
		3	*Brachionus bidentatus Anderson,1889*
		4	*Brachionus angularis Gosse,1851*
		5	*Brachionus diversicornis Daday 1883*
		6	*Brachionus caudtus Barrios&Daday,1894*
		7	*Brachionus rubens Ehrenberg,1838*
		8	*Brachionus falcatus Zacharias,1898*
		9	*Plationus patulus Muller,1786*
		10	*Keratella tropica Apstein,1907*
II	**Euchlanidae**	11	*Diplois daviesie Gosse,1886*
		12	*Dipleuchlanis propatula Gosse,1886*
		13	*Euchlanis dilatata Ehrenberg,1832*
		14	*Tripleuchlanis plicata Levander,1894*
III	**Trichotriidae**	15	*Trichotria tetractis Ehrenberg,1832*
		16	*Macrochaetus daneeli Koste& Shei,1983*

S.No	Family	S.No	Species
IV	**Lepadellidae**	17	*Colurella obtuse Gosse,1886*
		18	*Lepadella (Heterolepadella) (Ehrenbergii perty,1850)*
		19	*Lepadella (Lepadella)ovalis Muller,1786*
		20	*Lepadella dactyliseta stenroos,1898*
		21	*Lepadella ehrenbergi Perty,1850*
V	**Lecanidae**	22	*Lecane lunaris Ehrenberg,1832*
		23	*Lecane bulla Gosse,1851*
		24	*Lecane curvicornis Murray,1983*
		25	*Lecane leontina Turner,1892*
		26	*Lecane luna Muller,1776*
		27	*Lecane hamata Stokes,1896*
		28	*Lecane papuana Murray,1913*
		29	*Lecane quadridentata Ehrenberg,1830*
VI	**Notommatidae**	30	*Cephalodella gibba Ehrengerg,1830*
		31	*Cephalodella forficula Ehrengerg,1830*
VII	**Aspalanchidae**	32	*Aspalanchna priodonta Gosse,1850*
		33	*Aspalanchnaintermedia Hudson,1886*
VIII	**Testudinellidae**	34	*Testudinellina patna Hermann,1783*
		35	*Testudinellina mucronata Gosse,1886*
		36	*Testudinellina parva Ternetz,1892*
IX	**Fillinidae**	37	*Filinia longisepta Ehrenberg,1834*
		38	*Filinia opoliensis Zacharias,1898*

Table3: Showing the number of Species and genera

ROTIFERA			
S. No.	**Family**	**Species**	**Genera**
1	Brachionoidae	10	4
2	Euchlanidae	4	3
3	Trichotridae	2	2
4	Lepadellidae	5	2
5	Lecanidae	8	1
6	Notommatidae	2	1
7	Aspalanchidae	2	1
8	Testudinellidae	3	1
9	Fillinidae	2	1

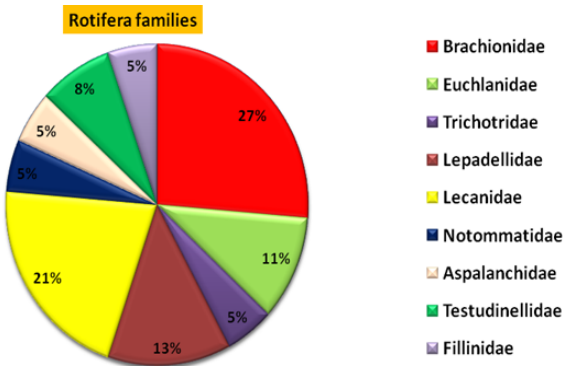

Fig-2: Percentage of Rotifera Families

4. CONCLUSION

The present study is an attempt fauna component of a lake which is utilized by the local community, and the results show greater diversity of invertebrate planktonic faunal groups, especially the rotifers. It is concluded that most of the physic-chemical and biological parameters in the Hasanparthy freshwater lake showed a monthly pattern from the observations. Water temperature [25-00^{0}C-30.02^{0}C], PH[8.05-8.75], DO[5.00-9.51mg/l], TDS[270.0 -380.2mg/l], TH[90.5-200.5mg/l], TA[102.5-190.6mg/1], CL[40.55-86.50mg/1], Ca[19.50-29.60mg/l], Mg[20.65-43.50mg/l], BOD[1.5-4.8mg/1], No_3[0.06-9.55mg/l], Po4[0.15-0.45mg/l] and NH3[0.01-0.05ppm]. In total 38 species were identified. Percentage of rotifer families are Brachionidae[27%], Lecanidae [21%], Lepadellidae[13%], Euchlanidae[11%], Testudinellidae[8%], Trichotridae, Aspalanchidae, Notommatidae and Fillinidae [5%]. It is observed that the rotifers showed a greater abundance. The rich variety of rotifers indicates the trophic status of the water body as well as its nature for the survival of fishes.

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NOTES

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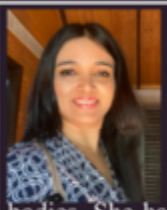

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