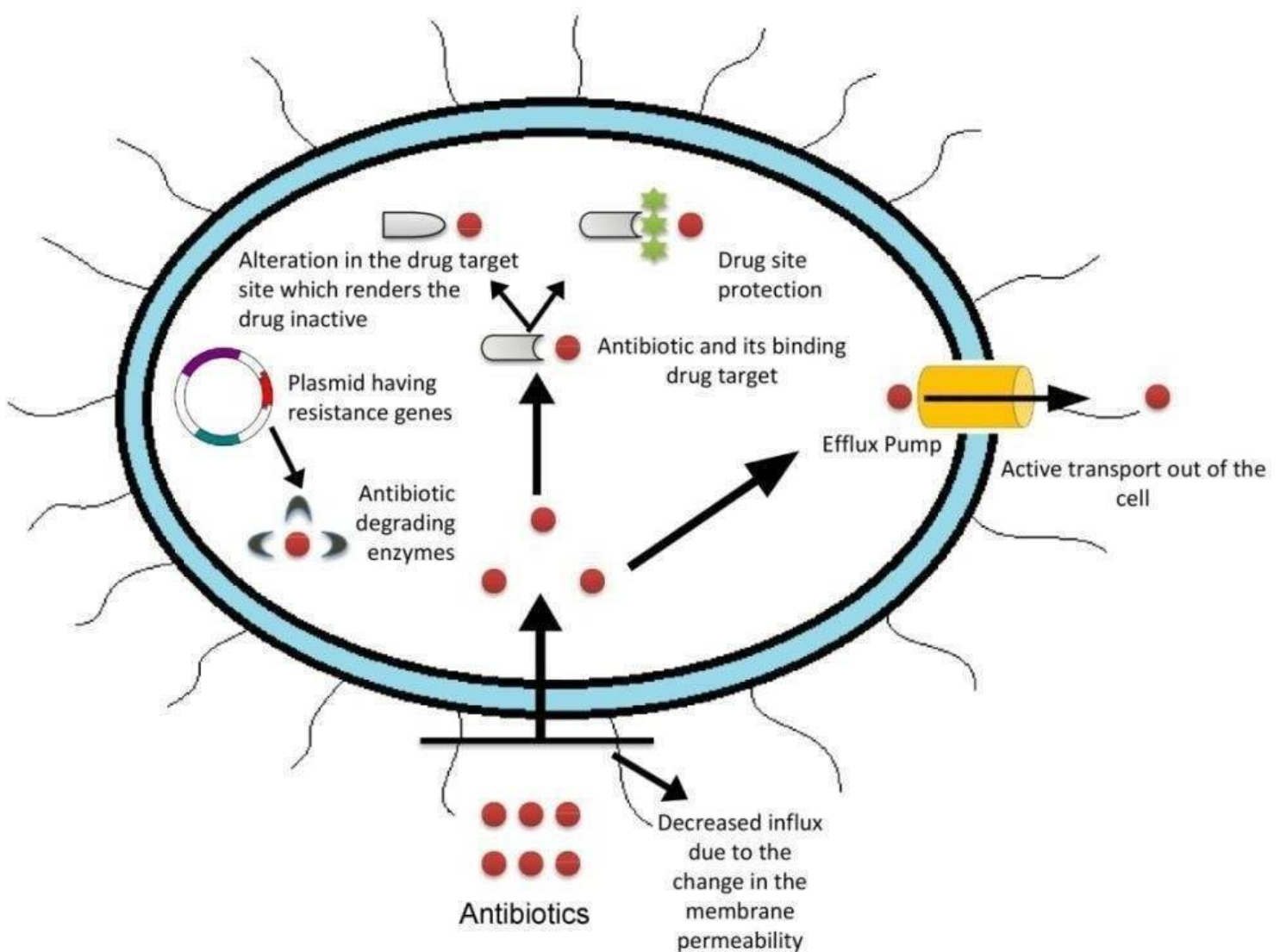


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International Journal of Environment and Health Sciences

From The Editor's Desk...

The year 2020 was a difficult year for the mankind, but at the same time, it made us all realize that the power of unity and discipline is of utmost importance while managing the severest of crises. The COVID-19 pandemic impacted public health and environment alike. However, the year 2021 is being deemed to be the 'Year of Recovery'. All of us need to join hands together to tackle the aftermath of the pandemic and to ensure that we stay firm in our efforts to create a sustainable environment.

Surveys suggest that in order to ensure a better respiratory capacity and overall improved health, the necessity of clean air and pure water needs to be addressed more now than ever before. Perils linked to environmental risk factors have to be managed for a bluer and greener earth. In this outlook, propagating awareness for environmental sustainability has become the need of the hour. Formation of regulatory bodies and authorities to disseminate societal alertness towards environmental safety is on the rise.

With this perspective, the International Journal of Environment and Health Sciences (IJEHS) proposes to provide a reliable platform to discuss technologies and strategies for management of aforesaid environmental matters, especially for the current post-COVID-19 period. IJEHS will be quintessential to academicians, industry professionals and researchers who are actively engaged in the areas of environmental issues and related health effects. We are pleased to inform that ISSN for IJEHS is available as 2582-5283. IJEHS is referenced in Crossref, the official Digital Object Identifier Agency (doi 10.47062). IJEHS is now also indexed in the International Scientific Indexing (ISI).

We invite original research articles, short communications and critical reviews directed towards an academic, clinical and industrial audience. The first section of the journal focuses on burning environmental issues like pollutants and their fate, waste management, resource conservation, remediation technologies, etc. The second section includes all topics relevant to physiological impact of environmental risk factors and application of alternative medicinal approaches as remedial measures. Detailed scope can be found in the home page of the journal (www.stenvironment.org/journals). Notes on development of any novel and validated strategy or tool to address environmental challenges are welcome. Discussion on proceedings of conferences conducted on environmental themes and related health aspects will also be considered. All submissions will be meticulously scrutinized by pioneers in the field to ensure publication of only articles of high quality and relevance. Authors are requested to take special precautions to avert plagiarism and redundancy.

It is high time that we realize the gravity of circumstances and take potent steps to undo the adversities already triggered. In this pursuit, IJEHS expects to be the ideal platform to discuss sustainable ideas and potential solutions. We thank all authors who have contributed to the journal and have consistently been with us in the past year. With this, I wish all our readers a Very Happy New Year, 2021 and I hope our audience and patrons shall come together in this effort to promulgate their part in resurrecting our valuable environment.

Dr. Kshipra Misra
Editor-in-Chief, IJEHS



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A.
Environmental Sciences Section



GREEN HIGHWAYS-A SUSTAINABLE APPROACH

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Abstract

Several infrastructure developmental activities lead to change in the natural environment and sometimes it leads to excessive environmental degradation. Unplanned management of natural resources is already limiting the development in some areas, resulting in growing scale of economic activity and subsequently posing serious challenges for environmental management. Environment- friendly methods and technologies are essential in today's scenario of global issues such as climate change, global warming, energy depletion and other environmental concerns which have led to the emergence of the green concept in India. The policymakers and researchers strongly believe that the increase in green technologies will result in sustainable societies and economic development. Considering economic development and environmental sustainability in roads and the highways sector, it is necessary to adopt the green concept.

The objective of the study is to present the concept of green highways, the available green practices, technical aspects, further benefits and implementation of such practices and the possible challenges in the Indian context. It is an integrated partnership planning program to promote environmental sustainability.

Keywords

Green Highways concept, economic development and sustainability practices.

Introduction

Roads and Highways make a vital contribution to economic growth and development. The road transport sector is providing access to employment, social, health and educational services and act in fighting against poverty. Over the next several years, developing countries will be substantially expanding and restoring their infrastructure networks. Asia is going to construct many new roads and highways to stimulate economic and social development. Every infrastructure project requires a sustainable practice that needs further consideration and consciousness efforts. As part of sustainability practices, the concept of ecofriendly or green highway technologies are to be adopted in the recent world scenario of combating global warming and climate change.

Most of the road construction projects lead to loss of surrounding natural resources due to the construction or

expansion works. Therefore, the restoration of the natural environment in the project area is necessary for considering environmental requirements throughout the stages of planning, designing, and execution. Road construction projects must be met with the dimensions of economic, social and environmental considerations. Green Road construction practices will meet human needs and at the same time minimize the usage of natural resources to support nature for the present and future perspective.

Factors influencing Green Highways

There are certain reasons which can affect the implementation of the concept of green technologies in the construction industry to become an emergence in India. They are:

Unawareness- not aware of nature, its importance and sometimes any knowledge about conservation techniques and the actual process of renewable and non-renewable resources.

Inattentiveness- Non-protecting the environment means that it is given less priority, leading to direct effects on human health and environment. Many construction projects release pollutants and use excessive natural resources, which shows an ineffective way of implementation practices.

Non-Performance activities- Many times road development comes at the cost of disturbance to ecological balance; loss of vegetation is an unavoidable consequence in road construction projects. Lack of Planning and continuous monitoring of environmental components is a major concern in developmental projects.

Pollution burden -All stages of highway projects i.e. construction, maintenance, and operation stage require energy-intensive inputs that are derived from the burning of fossil fuels [1]. This results in the release of a massive amount of greenhouse gases and other ambient air pollutants.

Additional Cost -Traffic Congestion in cities and towns is increasing widespread all over the world. The enormous and growing cost caused by it in terms of loss of time, vehicle operating cost and degradation of environmental quality necessitate to find out ways for sustainable practices.

Concept of Green Highways

The concept of Green Highways is relatively new and is derived from “Sustainable Development principles” which emphasizes the coexistence of global development with the environment and ecology, which is gradually taking hold around the world. The Green highway initiative is an effort to design eco-friendly highways and to promote environmental sustainability throughout the stage of project cycle through integrated partnerships, rewards, and market-based solutions [2].

The green concept was initially implemented in the USA through a public private-partnership known as Green Highway Partnership program[3]. The GHP is a social group and has identified the characteristics of Green Highway as well as a green rating system for roads and Highways. The partnership program aims by providing conditions that are “better than before”. GHP has developed some principles to develop a green highway which are to:

- Achieve goals through voluntary participation and public/private partnerships,
- Utilize market-based approaches and economic incentives,
- Provide communication and support network to avoid duplication and help streamline business practices and processes among those organizations supporting and enabling the “Green Highways” philosophy,

- Promote collaborative approach for conservation and integrated watershed management that leverages efforts of all levels of government and the private sector to maximize benefits
- Promote innovative storm water protection
- Promote use of recycled materials
- Prevent cutting/ felling of avenue trees by adopting various design alternatives wherever necessary and implement the tree transplantation measures to preserve trees
- Recognize and encourage existing environmental stewardship practices among transportation agencies by promoting them among a broader stakeholder universe
- Remove barriers to achieving innovative and positive results
- Leverage transportation and environmental resources (public and private) to multiply benefits and maximize results, and
- Support and stimulate applied research and training to remove barriers identified by partners and stakeholders.

The green highway concept has been used in the following ways (according to the Green Highway Partnership Program- (GHP) [4]:

Watershed driven Storm water Management method - The system holds runoff as well as treats in its natural way by holding it to reach the groundwater table. This technique protects runoff water in the way of treating and holding the water. This system also reduces the pollutants at the site and discharge into the adjacent drain through diverting the storm water runoff to areas where it can penetrate to reach the ground water table.

Resource Conservation Methods - Resource conservation method is the consumption of resources effectively by the method of reuse, recycle and replace the selected products/material or technologies that minimize the overall use or consumption of natural resources.

Societal Benefits - Highways are an important asset for local economies. An aesthetically appealing highway design can attract more business into a community and supply local jobs [5]. The highway design options with safety aspects including provision for animal crossing shall decrease the accident rate.

Pollution free environment - The construction and operation activities involved during any infrastructure projects shall result in the emission of pollutants in the form of greenhouse gases. Monitoring the pollution levels and estimating the level of carbon footprint shall give an overall performance of the green highways.

Table 1: Important techniques considered in each component under GHP

Component	Techniques/methods	Description
Watershed/Storm Water Management Practices	Use of dry swales for stormwater recharge-	A simple drainage channel by selecting carefully, highly permeable soil (usually sandy loam), gravel bed or sandbags for developing small check dams along the roads and highways.
	Use of porous pavements in roadside facilities-	Permeable pavement surface facilities with a stone reservoir underneath that can store surface run-off before infiltrating it into the subsoil, thereby infiltrated directly into the soil, shall be used at parking areas, bus ramps, etc. Such pavements also help in noise reduction in some extend.
	Bio-retention techniques (landscape design system consists of a soil bed planted with suitable vegetation which will remove silt (mud/sediments) and the pollutants from surface water runoff)	Bio-retention is the process of collecting the stormwater run-off to remove contaminants and sedimentation and the treated water is allowed to reach the ground water table. Stormwater is collected into the Bio-retention area which consists of a grass buffer strip, sand bed, ponding area, organic layer, planting soil or plants. It is designed to retain stormwater before it discharges.
	Bio-retention techniques (landscape design system consists of a soil bed planted with suitable vegetation which will remove silt (mud/sediments) and the pollutants from surface water runoff)	Bio-retention is the process of collecting the stormwater run-off to remove contaminants and sedimentation and the treated water is allowed to reach the ground water table. Stormwater is collected into the Bio-retention area which consists of a grass buffer strip, sand bed, ponding area, organic layer, planting soil or plants. It is designed to retain stormwater before it discharges.
Resource Conservation Methods	Tree transplantation technique	It is to preserve the trees within 30 to 90 cm girth size or seven to ten years old trees where the trees to be cut due to laying new roads or road widening activities. The preserved trees shall be transplanted in an open area, where the less density of trees located such as School premises, Govt. building campuses and government open lands, etc. to increase the green cover on waste/open lands.
	The use of renewable energy (Solar lighting system)	To adopt the non-conventional energy sources, streetlights, parking lights, and lighting at junctions, etc. shall be considered.
	Reuse of Fly Ash	Fly ash is a residue generated by burning coal and is available in large quantities next to coal-based power plants. Fly ash poses health risks by way severe respiratory and skin problems and also takes up valuable landfill space. Hence, the use of fly ash in the construction industry is encouraged.
	Reuse of existing pavement material	The possible recycling option for pavement material in India is Hot Recycling. Stretches of existing bituminous pavements (road surface material) that are to be reconstructed, left out due to curve improvements or may get buried under flyovers or existing pavement can be milled off as a reclaimed asphalt pavement (RAP) that can be transported to hot mix plant for recycling.
	Resource-efficient construction technology	Presently most roads have bituminous surface constructed using naturally available road aggregates and bitumen at very high temperatures to produce hot mix asphalt (HMA). Heating of bitumen to very high temperatures is linked to environmental degradation due to air

		pollution on account of an increase in emission of gases into the atmosphere. Low energy mixes such as warm mix asphalt (WMA) prepared and used at much lower temperatures than HMA are being extensively used in western countries to minimize air pollution, energy savings, etc. Production of WMA is at significantly lower the temperature between 100 to 140°C as against HMA which is produced at high temperatures between 150 to 170°C.
Societal Benefits	Safety provisions:	Pedestrian or Cattle crossing along and across the highways are common. Options for the safety of the pedestrian and cattle should be explored, like providing dedicated footpaths cattle underpass, cattle crossing with the provision of rumble strips, cattle crossings with other safety aspects to be considered.
	Solid waste management:	During the planning and design stage the reconnaissance survey should be conducted, based on the site visits locations should be identified for the requirement of solid waste management practices along the highways. Mostly at the settlement locations cleaning requirement due to lack of dust bins and improper maintenance or irregularity in the collection of waste etc., Hence, this practice is must be explored as the cleaning process is as part of sustainable practice and benefits of society.
	Quiet pavements:	Quieter pavement the pavement that produces less noise than another from during the movement of vehicles (traffic). Such pavements can be either asphalt or concrete pavement, but incorporating practices to make them quieter is important. A quieter pavement can be considered at any location subject to any environment and any amount and type of traffic. Furthermore, quieter pavements of both asphalt and concrete can achieve the same level of cost-effectiveness, durability, and safety expected. It requires detailed study and research for further needful.
	Landscaping:	Landscaping along the highways is one of the key enhancement measures that are usually suggested while designing the highways. In general, landscaping shall be proposed along the RoW, parking area, road median, island, junction and oxbow land/incidental spaces. However, its size and extent will depend on the land availability.
Pollution free environment	During Project Construction:	The construction and operation activities involved during any infrastructure projects shall result in the emission of pollutants in the form of greenhouse gases; it should be minimized by using innovative techniques.
	During project Operation:	Gases emitted from the burning of fuel by the traffic plying on the highway after completion of road construction. This shall be avoided by using clean fuels or electric vehicles and efficient traffic measures.

Source: Ashoka Highway Research Centre-Research Activities, Nasik and Gujarat State Highways Project-II Report.

Present Practices on Green Concept

The initiation of green technologies is increasing slowly in India and other countries. Most of the Nations have already started with viable options. Now it is time to turn and follow the environment- friendly construction methods:

The Government of India and the Ministry of Environment, Forest and Climate Change has initiated the promotion of green technologies by framing various legislations and policies as statutory requirements and environmental clearances for highway projects.

The Ministry of Road Transport and Highways (MoRTH), Govt. of India launched a National Green Highway (Plantation, Transplantation, Beautification, and Maintenance) Policy, 2015 under National Green Highway Mission (NGHM). NGHM is a primary Authority to oversee the construction activities and focuses on inclusive growth and continuous innovation to make the project viable as well as beneficial to the environment [6].

India Roads Congress (IRC) published various manuals on Green Highways construction practices such as manuals for

environmental clearance procedures for highway projects, usage of eco-friendly material in highway construction, etc. [7].

The National Highway Authority of India (NHAI) planned to construct environment-friendly highways to reduce construction related carbon footprint. The authority also desires to explore carbon reduction measures, overestimation of carbon credits, and develop a rating system for the national highways on their performance on environmental friendliness.

- India's first smart and green highway constructed near Delhi Eastern Peripheral Expressway, which was inaugurated recently in 2018.
- Another study carried out by NHAI for Lucknow-Muzaffar Green National Highway states that the concept of green highways aims for environmental mainstreaming and stewardship in all aspects of the highway project cycle.
- Research study has been carried out by CRRI-Central Road Research Institute, New Delhi, introducing about plastic waste technology particularly in mixing with bitumen for road construction; India already began deploying this technology with the use of plastic waste for road construction particularly in bitumen on a 1,000 km stretch in Bangalore.
- Assam State initiated the best use of green technology of road construction methods using cold bitumen emulsions, which takes less time and fuel consumption, and has been approved by IIT-Guwahati and the Central Road Research Institute (CRRI) as ideal for the weather conditions of Assam.

Other countries have also initiated similar projects by addressing the concept of green highways and bridges through an international network:

- The Department of Commerce and Energy, along with the Washington Department of Commerce and Transportation planned to develop electric cars for the West Coast Green highway, a 1350-mile strip starting from Mexico and ending in Canada.
- Switzerland has built vegetated overpasses called as green bridges, eco ducts.
- Germany has developed the landscape ecology where adjacent land use and land conservation as mitigation for highway development.
- Highway England department spends the money by setting aside the budget for a green retrofit. Fund allocation included for the improvement of air quality, cycling access, and the environment and ecology, other funds for innovation, housing and growth.

- Washington Department of Transportation started to promote the use of clean fuels and electric vehicles at the West Coast Green Highway, with this initiative, increasing the market demand for high efficiency, low carbon-emitting vehicles to reduce the transportation sector impact on the environment and dependency on foreign oil.
- Finland proposed world's first green highway by building a "carbon-neutral highway" that would include charging stations and biofuel stations.

However, it needs further involvement and innovation towards the implementation of green highways in a sustainable manner. Most of the State level projects follow only the traditional way of construction activities as of now. It is necessary to turn out with the more encouraging way of doing with market-driven approaches of sustainable engineering designs of roadway construction practices.

Benefits of Green Highways

Green Highways will be benefitted particularly in terms of reduction of carbon emissions, societal benefits, and improvement in health aspects. The technologies of green infrastructure are known to potentially replenish the ground water by triggering natural infiltration capabilities of the earth's surface runoff. This technology prevents transportation of pollutants to nearby waterbodies by infiltrating them at the source of generation itself.

The main aim of green highway approach is carbon sequestration by plantation and using soil techniques. Carbon sequestration is the process of capturing and eliminating carbon dioxide from the atmosphere by using geoengineering techniques and by natural process such as photosynthesis, etc. The concrete structures, emissions from vehicles, industries, air conditioners are contributing to the global warming and urban heat in addition to the forest displacement. Green infrastructure is the best option to combat the urban heat and energy demand.

The existence of trees & vegetation in an area enables removal of certain pollutants (majority of particulate matters) from the atmosphere through their leaf and canopy arrangement. If trees are planted widely in the entire habited areas, trees & plants can maintain cool temperature, create the peace-loving atmosphere through lowering down the ground-level concentrations of ozone and GHG emissions. Green infrastructure including vegetation & green space elevates the mental health which also actually leads to economic benefits.

Implementation Challenges in Indian Context

Implementation of Green Highways is initiated in various states of India; however, implementation practices are observed basically on a pilot basis study by choosing one component or based on selection criteria. The reason for adopting the pilot study could be the non-availability of practical experiences and not any further research and

development in highway construction projects. Few challenges or difficulties[8] in adopting the green highway concept fully as identified by literature study are categorized in the three aspects as follows:

Technical and Physical Barriers:

- High costs involved in removal of existing infrastructure/materials and installing new surfaces or replacement with ecofriendly material (E.g.: porous pavements)
- Limited studies and access to necessary materials (E.g.: usage of Fly ash, coco pit, etc.)
- Non-availability of land width along the roads and highways or at parking lots to install bio-swales or even rainwater harvesting structures or discharge pits, limiting local governments and public involvement choices of technology.
- Climate-resilient measures also present unique challenges to implementation
- Researchers are trying to prove benefits for the green highway. Further research on green initiatives will help in providing better guidelines or technologies that will be most appropriate for the different climatic zones.

Regulatory Challenges

- National government agencies are taking an active role in promoting the use of green transportation infrastructure, but paradoxically, those are not stringent to reach the local entities.
- The state and central Governments have been strong believers for green infrastructure projects; however, those are non-restrictive to implement.
- The program should be directed on a regional or state level, and regional project executives should be the decision makers over defining a green infrastructure technology.
- Local government's regulations or resolutions should restrict the approvals which discourage the use of innovative or ecofriendly way of technologies.

Social or cultural barriers

- The project executives have a tendency to not take risk in infrastructure projects and hence unwilling to adopt new technologies which may be considered as an experimental or unproven technology due to the assumption of high cost, non-reliability, high maintenance cost, or are unaware about the best products to use.
- Due to non-adoptability of green technologies leads to a shortage of trained contractors who can design and implement the integrated systems, which makes it more difficult and costly.
- Many companies, non-profits, and industry organizations are into development of programs to

promote environmental-friendly methods in construction technologies; however, it varies with the success levels.

- Demonstrating the market-driven techniques in infrastructure project are more attractive to consumers and an effective means of encouraging implementation as part of corporate citizenship programs.
- There is no particular research and development of green technologies and limited studies/resources for implementing practically.
- Overall, the green infrastructure is not a well-known term outside the environmental field, hence it can be used as the term of reuse or recycling methods in construction methodology to minimize the impact on environment.

Survey conducted

As a preliminary step, a quantitative research has been started by selecting survey method through questionnaire with an objective to get to know the awareness and importance of green highways among the people of India and to identify the challenges and gaps towards implementation. The questionnaire was finalized and circulated to all the stakeholders and road users, interacting with different departments. 125 out of 200 responses were received from various stakeholders belonging to different field of exposures, including government and private agencies, construction industries and academic institutions. The quick compilation was done with the responses received from the survey through Google forms. It was noted that 90 percent of the responses are in favor of the eco-friendly and sustainable way of construction management and 33 percent of responses revealed their opinion that due to lack of practical knowledge and lack of technology and research studies, implementation of a sustainable way of construction management is difficult. Only 22 percent of respondents have shown their response that they need more clarity on climate change and global warming and also expressed that more awareness among the people is important about material consumption and conservation of natural resources. On the basis of this survey authors are moving forward for both qualitative and quantitative research on study on green highways.

Summary and Conclusion

Green Highway Partnership (GHP) program has been launched with the aim of achieving a safe and efficient transportation system with an environmental stewardship and sustainability. The entire human community is moving towards greener practices. Green highway is a promising concept that involve socially desirable, economically feasible and ecologically viable practice to contribute to curb the global warming and environmental pollution. Applying all green technologies considered under GHP would not be possible to every highway construction project, each project highway will be unique to the extent of environmental impacts that can vary from project to project on basis of

climatic conditions. The analysis of the project area of environmental perimeters, social benefits are most important in the project planning stage.

This study will be one of the reference documents for highway concessions that may foresee, prevent and overcome possible difficulties for the issues relating to the development of green highway. This paper reviewed the literature on one of the highway constructions practices as a pilot green highway concept and other relevant publications from India and other countries to brief about the Green Highway Concept and to provide a clear view on implementation practices and challenges. The review of the literature study perceived the importance of green highways and sustainability practices with the concept of GHP that should begin from project preparation and its execution and maintenance of the entire project cycle.

Green infrastructure contributes a significant role in mitigating the impacts on the natural environment. Mandatory for green construction methodology is being noticed, explored, and realized for future sustainability. The study involves the contribution towards innovative eco-friendly techniques that are available and practically used in different countries or states and its applicability of selected component, at relevant project cycle that must be developed for sustainable highways.

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CLIMATE RISING AND FALLING STATE OF HYDROSPHERIC OXYGEN LEVEL: A GRIM GLOBAL CONCERN

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Abstract

Though humans' basic needs are prioritized first, health and environment quality are equally important. Environmental issues are based on a variety of factors. One of these is the rising temperature and consequent decrease in oxygen levels in water bodies such as lakes, oceans, and seas. The right amount of oxygen is essential for life on Earth because it serves as a lifeline for living organisms; it could jeopardize marine ecosystems, shifting habitat conditions, human health, and the environment as a whole. The data for this study were primarily gathered from secondary sources such as books, government offices, research articles, and websites published at various times. According to research, the primary cause of marine oxygen loss is human-caused global warming. Human beings also play a role in coastal regions by depositing wastes in the water bodies, moreover it is difficult to avoid this completely. Most significant drops in oxygen levels have been noticed in the equator and the Arctic Ocean. The spatial distribution and pattern of low or no oxygen across the globe is the focus of this review article. It also goes over the reasons for unequal oxygen loss in different parts of the world.

Keywords

Climate rising, Lakes, Oceans, Oxygen Loss, Seas

Introduction

According to the most recent and comprehensive research of Oxygen Changes in the World's Oceans, total global oxygen concentration has decreased by 2% on an average between 1960 and 2010. (Laffoley and Baxter, 2019). Climate change is considered to be a key cause to this "deoxygenation," since it affects the quantity of oxygen that seawater can contain as well as the circulation patterns that deliver oxygen-rich water to deeper oceans. There are pockets of little or no oxygen all around the planet, including sections of the tropical oceans off the coasts of California, Peru, and Namibia, as well as the subterranean waters of the Arabian Sea. Most marine life will perish since the oxygen levels in these places are so low. Nitrous oxide (N₂O), a strong greenhouse gas, can be released in low oxygen zones (Schmidtko S, Stramma L, Visbeck M., 2017). This paper aims to demonstrate how the rising temperature of the climate affects the level of oxygen in the hydrosphere.

The world is now more than 1°C warmer than before industrialization, and it is on track to be 3 degrees warmer in the near future. Compared to natural processes that affect climates, such as solar fluctuation and volcanic eruptions, human actions, primarily the burning of fossil fuels and deforestation, have contributed to climate change over the last 50 years.

According to NASA and the National Oceanic and Atmospheric Administration's alarming new analysis, the amount of heat trapped by the Earth has doubled in just 15 years. According to researchers, it's a massive amount of energy that's already having far-reaching effects.

According to NASA scientist Norman Loeb, the planet is absorbing too much energy, which will result in higher temperatures and more melting of snow and sea ice, as well as a rise in sea level. Scientists discovered that the Earth is

gaining more energy than it should, causing the planet to heat up even more. Around 90% of the excess energy generated by this imbalance ends up in the ocean. Warming ocean temperatures also cause acidification and oxygen depletion, which hurts fish and other marine biodiversity (Loeb N, Lyman *et. al.*, 2012).

According to the researchers, human-induced greenhouse gas emissions are certainly a part of the cause of this energy imbalance. As the global temperature rises, the amount of water vapour in the atmosphere rises and raises the temperature even more. Melting snowpack and sea ice, which act as natural solar reflectors, are also diminishing due to climate change (Rachel Ramirez, June 18, 2021, CNN). Norman Loeb opines that the rate that we're seeing this energy imbalance should subside in the coming decades, otherwise, more alarming climate changes will be witnessed.

Objectives of the study

One of the goals of this research is to gain a better understanding of how rising temperatures affect the amount of oxygen in the hydrosphere and to examine how oxygen is lost in the oceans, seas, and lakes. In addition, the impact of oxygen loss on marine ecology will also be assessed. Furthermore, mitigation measures are taken into account in order to predict future trends in oxygen levels.

Methodology

The research was carried out using secondary data gathered from a variety of sources i.e., published books as well as unpublished soft copies obtained via the internet and software. Also, several articles published in various national and international journals for this purpose were consulted.

Background Literature

It is important for all higher forms of marine life to have access to oxygen, even though it accounts for just approximately 0.6 percent of the total amount of oxygen in the atmosphere. Furthermore, the respiration of organisms uses oxygen on a continual basis virtually everywhere in the water. An average of 2% decrease in oxygen concentration in the worldwide seas has been observed since 1960, according to the most current and thorough investigation of oxygen variations in global oceans. The amount of deoxygenation varies significantly across the seas, according to the study. The equator and the Arctic have seen the most significant drops in oxygen levels.

Marine oxygen depletion is believed to be mostly driven by human-induced global warming, according to recent studies. Humans also have a role in coastal regions by giving nutrients to the oceans, however it is difficult to distinguish between the many mechanisms at action.

The ocean and its dissolved oxygen concentration are both affected by global warming in a variety of ways. The solubility of oxygen in water is one of the effects of this compound, among others. The amount of gas that can dissolve in warm water is proportional to the amount of gas

that can dissolve in cold water. The upper few hundred meters of the ocean's surface, which have only recently come into contact with the atmosphere, have been the primary target of this process up until now. It has been estimated that this phenomenon is responsible for up to 20% of total marine oxygen loss to date, with around 50% of that loss occurring in the upper 1,000 meters of the ocean.

Furthermore, worldwide ocean circulation patterns are altered as a result of warming, with the mixing of oxygen-rich surface waters with oxygen-poor deeper water being affected. It also has an impact on the consumption of marine oxygen because it alters the pace at which organisms metabolize and respire, among other things.

Finally, when it comes to nutrient availability and production in the upper ocean, warming has an indirect influence on downward export of organic matter accessible for respiration across the ocean (Oschlies, A. *et al.*, 2018).

Climate Induced Changes

When academics and other social scientists began researching globalization in the late 1980s and early 1990s, climate change became widely recognized (Alam, 2021). Climate change is one of the most serious environmental threats humanity has ever faced. There is more to it than just hot air and melting ice. It has far-reaching consequences that extend beyond where people live and where food is grown. People with more knowledge are better able to assess the risks posed by climate change and have less perceived ambiguity about it, which influences their pro-environmental behavior (Alam *et. al.*, 2021). During the twentieth century, water temperatures in several lake habitats around the world rose (Austin & Colman, 2008; Schneider & Hook, 2010). Deeper water levels in lakes change less frequently than surface waters (Pilla, 2021). The ecological health of lakes and reservoirs around the world is still threatened by low dissolved oxygen levels. Additionally, climate-induced changes in lake stratification and mixing constitute an anthropogenic hazard, resulting in reduced deep-water oxygen levels as a result of increased nutrient loads. (Saros, 2012; Savin, 1977). Temperature increase as a result of global warming is the major cause of decreasing oxygen levels in the atmosphere, as warmer water cannot store as much oxygen. Furthermore, as summer temperatures rise, the top layer of lakes becomes hotter and less thicker than the water below, causing plummeting, mixing, and reduced oxygen delivery to the depths. According to scientists, oxygen levels in deep seas have decreased by 19%, while surface levels have decreased by 5% (2002, Portner).

Oxygen levels have increased at the surface of some lakes. Warmer temperatures, on the other hand, are more likely to cause algal blooms, which can produce harmful chemicals (Hallegraeff, 2003; Karlson *et al.* 2021). According to a recent research, the world's lakes are being suffocated by the climate catastrophe. According to experts, climate rising is triggering oxygen levels in lakes to drop, resulting in the

extinction of species and the jeopardization of drinking water supplies. According to a study, oxygen levels in lakes have decreased three to nine times faster in the last 40 years. Lake Ammersee is one of Germany's climate-vulnerable lakes (Kraemer, et al., 2021; Woolway, 2021).

"All complex life relies on oxygen, and as oxygen levels fall, many different species' habitats are significantly reduced" (Jane et al., 2021). Since 1970, the average animal population has decreased by 84 percent. One of the reasons, in addition to global warming and pollution, is the excessive use of water for farming. Freshwater ecosystems' fish, insects, birds, and mammals have already been severely harmed.

Despite the fact that the majority of the lakes studied were in temperate zones, particularly in Europe and the United States, there were a few records from higher latitudes, closer to the poles, as well as tropical lakes in Africa. The study found that, regardless of location, oxygen levels were decreasing (National Research Council, & Climate Research Committee, 1996).

Surface water oxygen levels were rising in nearly a fifth of the lakes studied, and nearly all of them were polluted. This is a sign of widespread algal blooms, according to Rose: "We couldn't tell for sure without taxonomic data, but nothing else we know of can explain this pattern."

Even maintaining the status quo, as global temperatures continue to rise, lowering lake oxygen levels necessitate cleaning up freshwater bodies. According to Rose, a clean-up at Oneida Lake in New York resulted in improved water clarity, which allowed for increased photosynthesis from oxygen-producing algae (Dale et al., 2006).

According to the scientists' best-case scenario, just 10% of the examined species would be endangered during the next 80 years if the global temperature climbed by 1.5 °C. Researchers determined that if global warming exceeds pre-industrial temperatures by 4-5 °C by 2100, 60 percent of the fish species tested will be unable to live in their current habitats (7.2 °F). A study of over 700 freshwater and saltwater fish species examined how changing water temperatures impact water oxygen levels, placing embryos and pregnant fish at risk of being killed or severely injured (Dahlke *et al.*, 2018).

Even under this scenario, the Atlantic Cod, Swordfish, Pacific Salmon, Alaska Pollock, and Pacific Cod that are used to produce frozen fish sticks, which are both economically and environmentally valuable, are still in danger of being depleted. A 10 percent loss in species can have substantial consequences for the entire ecosystem "stem," making it difficult to estimate the consequences of a 10 percent loss in species. Take, for example, the North Sea, where we anticipate that the temperature will have increased to the point that Atlantic Cod would be unable to spawn by the end of the century. Flemming T. Dahlke and colleagues from the

Alfred-Wegener Institute, Helmholtz Centre for Polar and Marine Research, University of Bremen, Germany, explained that because it is such a significant predator, removing it from the system has a significant impact on the ecosystem as a whole as well as all the processes and interactions between species (Dahlke, *et al.*, 2018).

When the temperature rises, fish use more energy and demand more oxygen. The heat, on the other hand, reduces the amount of oxygen available. Embryos, which cannot regulate their oxygen levels, and spawning fish, which require more oxygen to produce progeny, are particularly vulnerable in these conditions.

Dahlke noted that this estimate is conservative because it excludes other climate-related issues that could harm marine life, such as ocean acidification, which could exacerbate the effects on vulnerable species.

"Some tropical fish are already living in zones where their maximum tolerance has been reached, in places where the temperature has reached 40 degrees," Portner says (Prof Hans-Otto Portner, a climatologist of the Alfred-Wegener-Institute in Bremerhaven, Germany) He went on to say, "Humans are pushing the planet's temperature range out of a livable range, and we're losing good habitat as a result. It is worthwhile to contribute to the 1.5 °C target." (Portner, 2002).

Appropriate Ocean Surveillance

Ocean oxygen depletion is being widely recognized as a serious danger to marine ecosystems and changing habitat conditions in many regions of the world's oceans, particularly in the Pacific. Increased likelihood of deoxygenation feedbacks on climate, including the generation of strong greenhouse gases such as N₂O and methane in low-oxygen circumstances is associated with a warmer temperature. As a result, it is important to resolve the disparity between observations and models, which is eventually necessary for trustworthy future forecasts. To overcome these gaps, we propose that more extensive and internationally coordinated ocean observations be conducted. In addition, multidisciplinary process studies are required to better understand the delicate balance between oxygenation and oxygen consumption in the seas which are always changing. Another advantage of improving the models in terms of the ocean oxygen budget is that oxygen is a good parameter for calibrating models that compute the ocean's CO₂ uptake, which is another benefit of improving the models in terms of the ocean oxygen budget. We would also acquire a better knowledge of the carbon cycle as a result of this experiment (Oschlies, A. *et al.*, 2018).

Conclusion

To avoid the worsening ramifications of climate change, an alternative arrangement is required to maintain the rising and falling state of hydrospheric oxygen levels. Many innovators are introducing technology or business models that produce

less carbon than current methods, such as leather made from mushrooms instead of animal hide, electric cars instead of fossil fuels, etc. In addition, scholars are looking into new technologies to reabsorb carbon that has already been released into the atmosphere in order to find new ways to combat climate change from a different angle. These methods have many potential benefits, but they also have a lot of challenges right now.

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ASSESSMENT OF GROUNDWATER QUALITY FOR DRINKING PURPOSE IN BARWALA TOWN AND ITS SURROUNDINGS, PANCHKULA DISTRICT, HARYANA, INDIA

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Abstract

Water is important for drinking, agriculture and industrial purposes. Water quality plays vital role in its utilization for different purposes. Water quality for drinking purpose should be as per BIS drinking water standards (IS 10500:2012). Barwala town in Panchkula district, Haryana, India has been selected in the present study for groundwater quality assessment for drinking purpose. In the study area six groundwater samples were collected in the month of June 2019 in plastic 250 ml bottles. Geo-coordinates of the sample locations were noted with mobile GPS. Groundwater samples were analysed using Field Water Testing Kit prepared by Tamil Nadu Water Supply and Drainage Board (TWAD) Chennai for ten chemical parameters-pH, hardness, chloride, fluoride, iron, ammonia, nitrite, nitrate, phosphate and residual chlorine. In the study area pH ranges from 7 to 8, hardness ranges from 150 mg/l to 400 mg/l, chloride ranges from 100 mg/l to 150 mg/l, fluoride ranges from 0.5 mg/l to 2 mg/l, iron ranges from 0 mg/l to 5.0 mg/l, ammonia ranges from 0.5 mg/l to 5.0 mg/l, nitrite ranges from 0.2 mg/l to 1 mg/l, nitrate ranges from 45 mg/l to 150 mg/l, phosphate ranges from 0.5 mg/l to 1.0 mg/l and residual chlorine ranges from 0 mg/l to 0.5 mg/l. In the study area groundwater quality is non-potable in five groundwater samples and potable in one groundwater sample. The study is highly useful for monitoring of groundwater quality for drinking purpose in the study area.

Keywords

Groundwater, quality, drinking, Barwala, Panchkula, Haryana.

Introduction

Water is important for drinking, agriculture and industrial purposes. Availability of good quality groundwater plays vital role in developmental activities. But the present developmental activities adversely affected the surface and groundwater quality as well as quantity. In urban areas sewerage and solid waste are mainly responsible for polluting the groundwater quality. Many workers have done work on groundwater quality assessment for drinking purpose in urban and rural areas. Some important studies are reported by Agrawal (2009), Ana *et al.* (2018), Balakrishnan, *et al.* (2011), Das and Nag (2015), Durgadevagi, *et al.* (2016), Hussain and Prasad (2013). Jeihouni, *et al.* (2014), Mahadevaswamy, *et al.* (2011), Okoye, *et al.* (2016), Pandian and Jeyachandran (2014), Patel and Dhiman (2011), Rajesh, (2016), Sarkar, *et al.* (2012), Satyanarayana, *et al.* (2013), Saxena and Saxena

(2015), Sengupta and Dalwani (2008), Shahida and Ummatul (2015), Sheikh and Kumari (2017), Sinha, *et al.* (2018), Subramani, *et al.* (2012), Thomas *et al.* (2015), Topper and Horn (2011), Vashisth (2017).

Study Area

The study area Barwala Town and its surroundings covers 7921850.61 m² area and lies between the latitude 30°34'33.72" N- 30°32'25.01" N and longitude 76°55'37.74"E-76°57'22.05" E (Figure1). Barwalais a sub-tehsil in the Panchkula District of Haryana State. Barwala is located 20 km towards south from Panchkula the district headquarters. The total geographical area of the town is 439 hectares and population 8,307 and about 1,569 houses. Barwala as a block consists of 35 gram panchayats and 10 block sameeties.

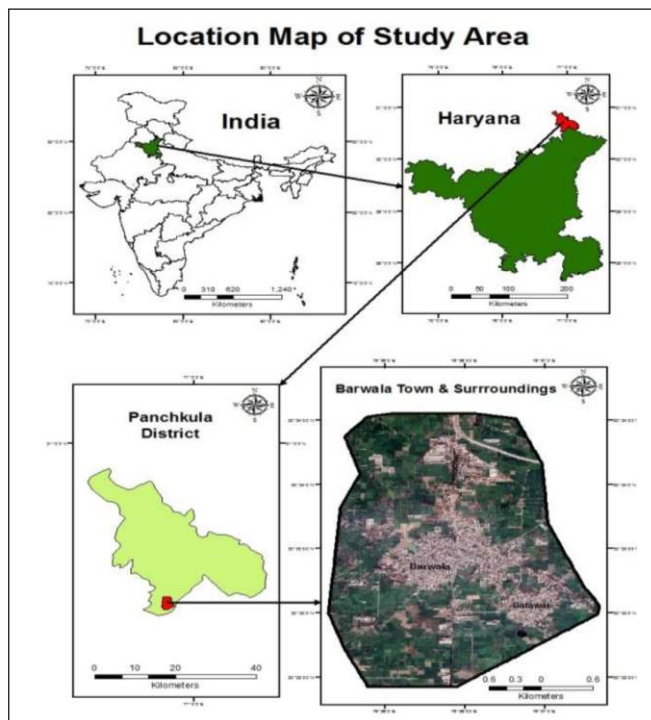


Figure 1: Location map of the study area.

Objective

The main objective of the study was to assess groundwater quality for drinking purpose in the study area.

Materials and Methods

Six groundwater samples were collected during field visit in the month of June 2019 in plastic 250 ml bottles (Table 1). Geo-coordinates of groundwater sample locations were noted using mobile GPS. Chemical analysis of groundwater samples was done using Tamil Nadu Water Supply and Drainage Board (TWAD), Chennai - prepared Field Water Testing Kit for ten chemical parameters-pH, hardness, chloride, fluoride, iron, ammonia, nitrite, nitrate, phosphate and residual chlorine (Table 2). The groundwater samples analysis results have been categorized as desirable, permissible and non-potable on the basis of BIS Drinking Water Standards (IS 10500:2012) (Table 3).

Results and Discussion

pH

In the study area pH varies from 7 to 8. pH is desirable in all the six groundwatersamples-Shiv Colony-S1, Barwala (8.0), Near Bhareli Road, Barwala (7.0), Shiv Colony-S2, Barwala (7.5), Power House Colony-S1, Batawar, Near Bhagwanpur Road, Batawar (7.5) and Power House Colony-S2, Batawar (7.0) (Figure 2).

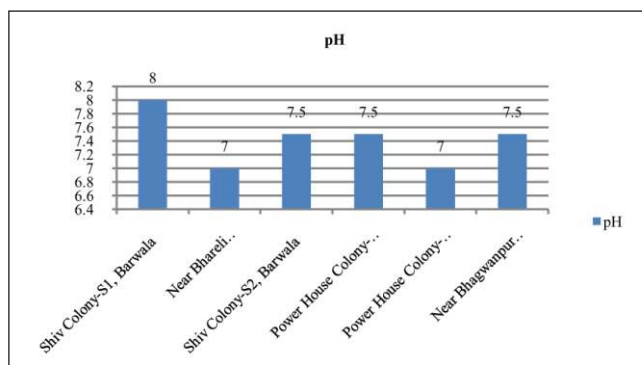


Figure 2: pH in groundwater samples.

Table 1: Groundwater sample locations in the study area.

S. No.	Sample Location	Source	Latitude	Longitude
1.	Shiv Colony-S1, Barwala	Hand Pump	30.556763°	76.943313°
2.	Near Bhareli Road, Barwala	Hand Pump	30.554332°	76.938308°
3.	Shiv Colony-S2, Barwala	Tube Well	30.557629°	76.942901°
4.	Power House Colony-S1, Batawar	Tube Well	30.557288°	76.947365°
5.	Near Bhagwanpur Road, Batawar	Tube Well	30.552843°	76.946186°
6.	Power House Colony-S2, Batawar	Hand Pump	30.557135°	76.947375°

Table 2: Results of chemical analysis of groundwater samples.

S. No.	Sample Location	pH	Hardness (mg/l)	Chloride (mg/l)	Fluoride (mg/l)	Iron (mg/l)	Ammonia (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)	Phosphate (mg/l)	Residual Chlorine (mg/l)
1.	Shiv Colony-S1, Barwala	8.0	250	150	0.5	1.0	2.0	0.5	100	1.0	0.2
2.	Near Bhareli Road, Barwala	7.0	400	100	1.0	2.0	5.0	0.2	150	0.5	0.5
3.	Shiv Colony-S2, Barwala	7.5	400	150	1.0	5.0	0.5	0.2	45	0.5	0.2
4.	Power House Colony-S1, Batawar	7.5	150	150	1.0	0.3	5.0	1.0	100	0.5	0.2
5.	Near Bhagwanpur Road, Batawar	7.5	310	150	1.0	0	0.5	0.2	45	1.0	0
6.	Power House Colony-S2, Batawar	7.0	300	100	0.5	0.3	5.0	0.5	100	1.0	0.2

Table 3: BIS drinking water standards (IS 10500:2012)

S. No.	Constituent	Potable		Non-Potable
		Desirable	Permissible	
1.	pH	6.5 to 8.5	-	<6.5 to >8.5
2.	Total Hardness (mg/l)	<200	200-600	>600
3.	Chloride (mg/l)	<250	250-1000	>1000
4.	Fluoride (mg/l)	<1.0	1.0-1.5	>1.5
5.	Iron (mg/l)	<0.3	-	>0.3
6.	Ammonia (mg/l)	<0.5	-	>0.5
7.	Nitrite (mg/l)	<1.0	-	>1.0
8.	Nitrate (mg/l)	<45	-	>45
9.	Phosphate (mg/l)	<1.0	-	>1.0
10.	Residual Chlorine (mg/l)	<0.2	0.2-1	>1.0

Hardness

In the study area, hardness ranges from 150 mg/l to 400 mg/l. Hardness is desirable in groundwater sample at Power House Colony-S1, Batawar (150 mg/L) and permissible in groundwater samples at Shiv Colony-S1, Barwala (250 mg/l), Near Bharel Road, Barwala (400 mg/l), Shiv Colony-S2, Barwala (400 mg/l), Near Primary Health Centre, Barwala (360 mg/l), Near Bhagwanpur Road, Batawar (310 mg/l), Power House Colony-S2, Batawar (300 mg/l) (Figure 3).

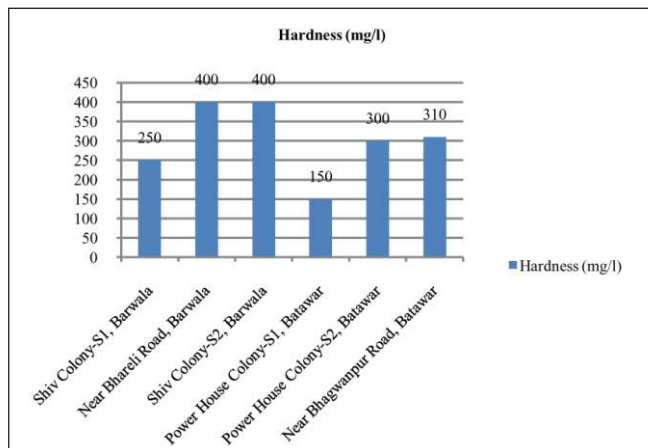


Figure 3: Hardness in groundwater samples.

Chloride

In the study area, chloride ranges from 100 mg/l to 250 mg/l. Chloride is desirable in all the six groundwater samples - Shiv Colony-S1, Barwala (150 mg/l), Near Bhareli Road, Barwala (100 mg/l), Shiv Colony-2, Barwala (150 mg/l), Near Bhagwanpur Road, Batawar (150 mg/l), Power House Colony-S2, Batawar (100 mg/l), Power House Colony-S1, Batawar (150 mg/l) (Figure 4).

Fluoride

In the study area, fluoride ranges from 0.5 mg/l to 1 mg/l. Fluoride is desirable in all the six groundwater samples at Shiv Colony-S1, Barwala (0.5 mg/l), Near Bhareli Road,

Barwala (1.0 mg/l), Shiv Colony-S2, Barwala (1.0 mg/l), Power House Colony-S1, Batawar (1.0 mg/l), Near Bhagwanpur Road, Batawar (1.0 mg/l), Power House Colony-S2, Batawar (0.5 mg/l) (Figure 5).

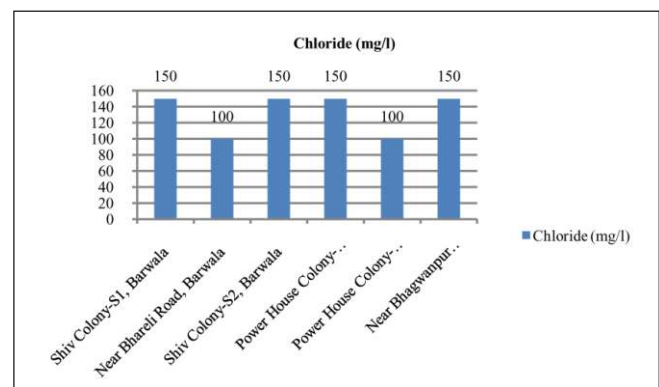


Figure 4: Chloride in groundwater samples.

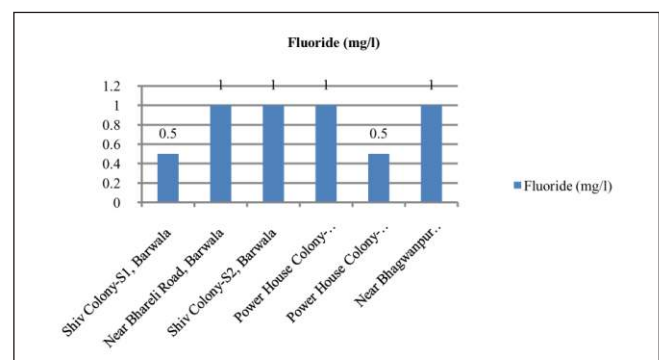


Figure 5: Fluoride in groundwater samples.

Iron

In the study area, iron ranges from 0 mg/l to 5.0 mg/l. Iron is desirable in groundwater samples at Power House Colony-S1, Batawar (0.3 mg/l), Near Bhagwanpur Road, Batawar (0 mg/l), Power House Colony-S2, Batawar (0.3 mg/l) and non-potable in groundwater samples at Shiv Colony-S1, Barwala

(1.0 mg/l), Near Bhareli Road, Barwala (2.0 mg/l), Shiv Colony-S2, Barwala (5.0 mg/l) (Figure 6).

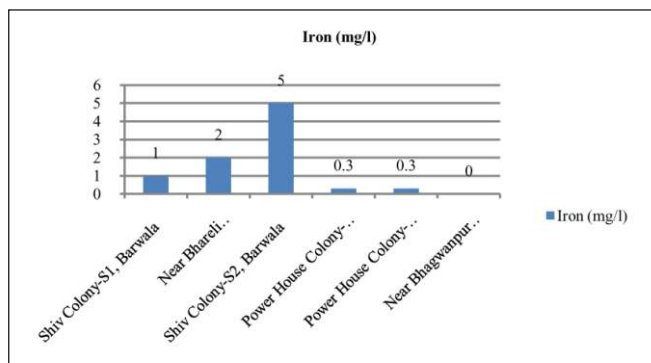


Figure 6: Iron in groundwater samples.

Ammonia

In the study area, ammonia ranges from 0.5 mg/l to 5.0 mg/l. Ammonia is desirable in groundwater samples at Shiv Colony-S2, Barwala (0.5 mg/l), Near Bhagwanpur Road, Batawar (0.5 mg/l) and non-portable in groundwater samples at Shiv Colony-S1, Barwala (2.0 mg/l), Near Bhareli Road, Barwala (5.0 mg/l), Power House Colony-S1, Batawar (5.0 mg/l), Power House Colony-S2, Batawar (5.0 mg/l) (Figure 7).

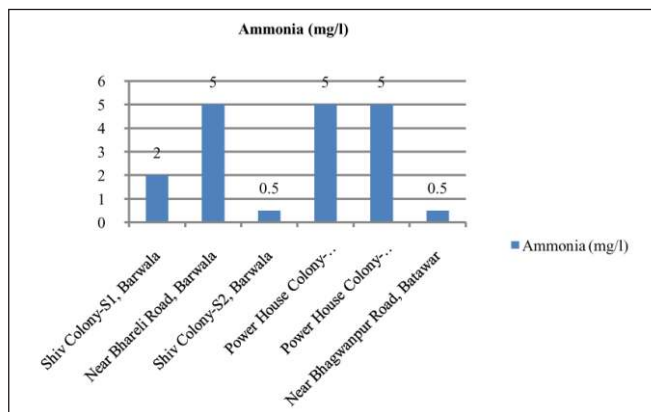


Figure 7: Ammonia in groundwater samples.

Nitrite

In the study area, nitrite ranges from 0.2 mg/l to 1 mg/l. Nitrite is desirable in all the six groundwater samples - Shiv Colony-S1, Barwala (0.5 mg/l), Near Bhareli Road, Barwala (0.2 mg/l), Shiv Colony-S2, Barwala (0.2 mg/l), Power House Colony-S1, Batawar (1.0 mg/l), Near Bhagwanpur Road, Batawar (0.2 mg/l) and Power House Colony-S2, Batawar (0.5 mg/l) (Fig.8).

Nitrate

In the study area, nitrate ranges from 20 mg/l to 150 mg/l. Nitrate is desirable in groundwater samples at Shiv Colony-S2, Barwala Town (45 mg/l), Near Bhagwanpur Road, Batawar (45 mg/l) and non-portable in groundwater samples at Shiv Colony-S1, Barwala (100 mg/l), Near Bhareli Road, Barwala (150 mg/l), Power House Colony-S1, Batawar (100 mg/l) and Power House Colony-S2, Batawar (100 mg/l) (Figure 9).

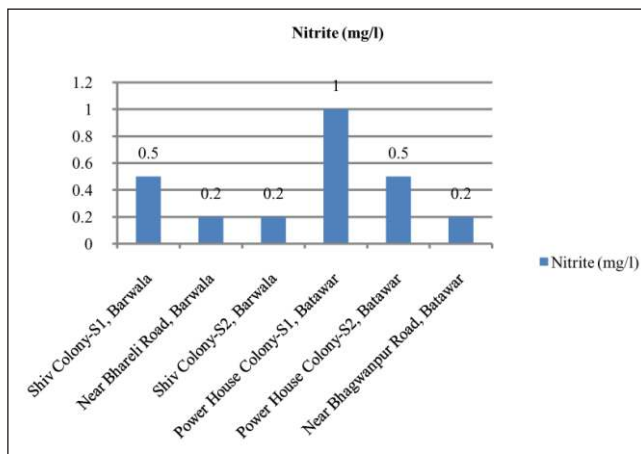


Figure 8: Nitrite in groundwater samples.

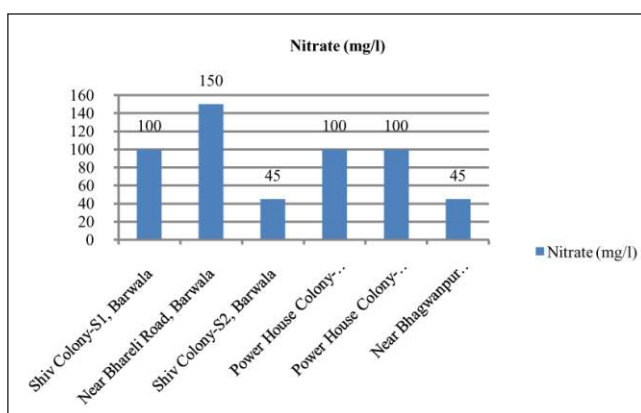


Figure 9: Nitrate in groundwater samples.

Phosphate

In the study area, phosphate ranges from 0.5 mg/l to 1.0 mg/l. Phosphate is desirable in all the six groundwater samples - Shiv Colony-S1, Barwala (1.0 mg/l), Near Bhareli Road, Barwala (0.5 mg/l), Shiv Colony-S2, Barwala (0.5 mg/l), Power House Colony-S1, Batawar (0.5 mg/l), Near Bhagwanpur Road, Batawar (1.0 mg/l), Power House Colony-S2, Batawar (1.0 mg/l) (Figure 10).

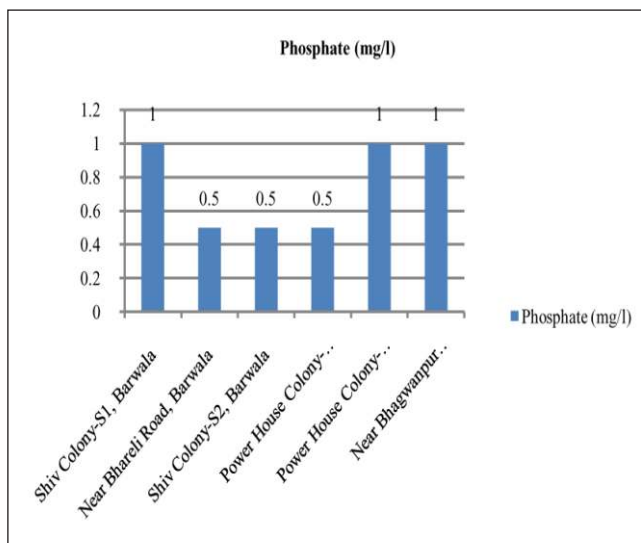


Figure 10: Phosphate in groundwater samples.

Residual Chlorine

In the study area, residual chlorine ranges from 0 mg/l to 0.5 mg/l. Residual Chlorine is desirable in five groundwater samples- Shiv Colony-S1, Barwala (0.2 mg/l), Shiv Colony-S2, Barwala (0.2 mg/l), Power House Colony-S1, Batawar (0.2 mg/l), Near Bhagwanpur Road, Batawar (0 mg/l), Power House Colony-S2, Batawar (0.2 mg/l) and permissible in Near Bhareli Road, Barwala (0.5 mg/l) groundwater sample (Figure 11).

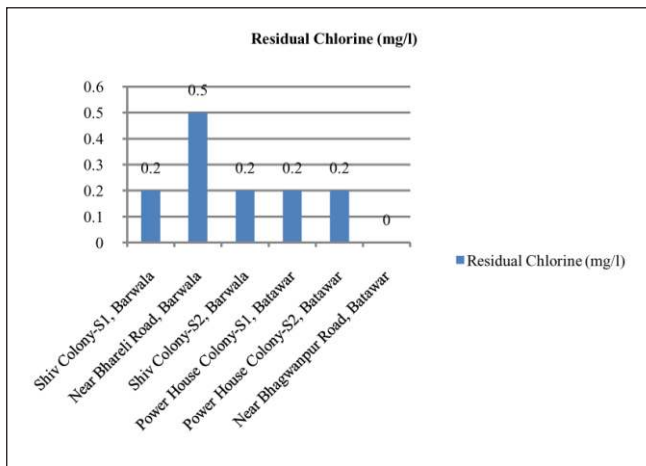


Figure 11: Residual Chlorine in groundwater samples.

Groundwater Quality At Sample Sites

Shiv Colony-S1, Barwala

In groundwater sample at Shiv Colony-S1, Barwala pH (desirable), hardness (permissible), chloride (desirable), fluoride (desirable), iron (non-potable), ammonia (non-potable), nitrite (desirable), nitrate (non-potable), phosphate (desirable) and residual chlorine (desirable) (Figure 12). Overall the groundwater is non-potable due to high iron, ammonia and nitrate.

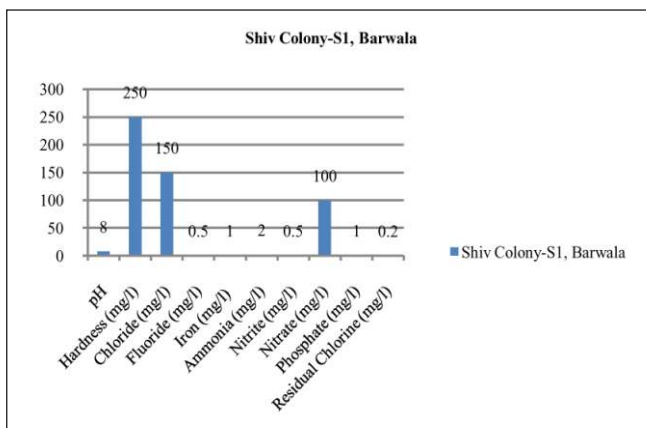


Figure 12: Groundwater quality at Shiv Colony-S1, Barwala.

Shiv Colony-S2, Barwala

In groundwater sample at Shiv Colony-S2, Barwala pH (desirable), hardness (permissible), chloride (desirable), fluoride (desirable), iron (non-potable), ammonia (desirable), nitrite (desirable), nitrate (desirable), phosphate (desirable)

and residual chlorine (desirable) (Figure 13). Overall the groundwater is non-potable due to high iron in the groundwater.

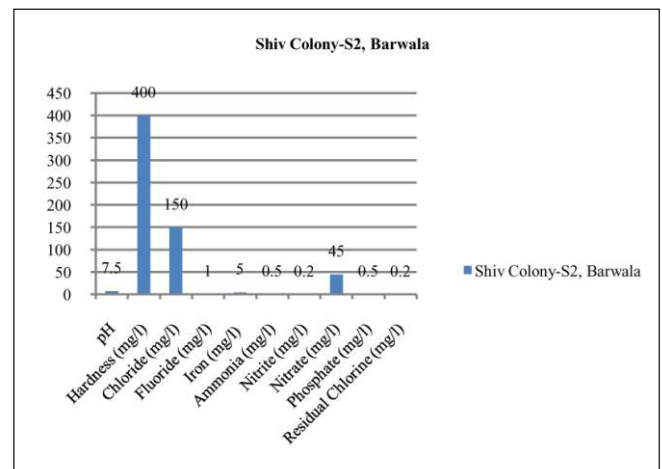


Figure 13: Groundwater quality at Shiv Colony-S2, Barwala.

Near Bhareli Road, Barwala

In groundwater sample at Near Bhareli Road, Barwala pH (desirable), hardness (permissible), chloride (desirable), fluoride (desirable), iron (non-potable), ammonia (non-potable), nitrite (desirable), nitrate (non-potable), phosphate (desirable) and residual chlorine (desirable) (Figure 14). Overall the groundwater is non-potable due to high iron, ammonia and nitrate.

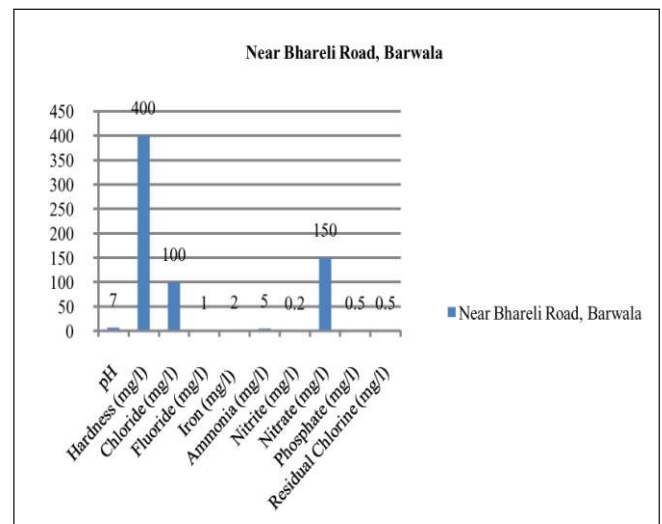


Figure 14: Groundwater quality at Near Bhareli Road, Barwala.

Power House Colony-S1, Batawar

In groundwater sample at Power House Colony-S1, Batawar pH (desirable), hardness (desirable), chloride (desirable), fluoride (desirable), iron (desirable), ammonia (non-potable), nitrite (desirable), nitrate (non-potable), phosphate (desirable) and residual chlorine (desirable) (Figure 15). Overall the groundwater is non-potable due to high ammonia and nitrate.

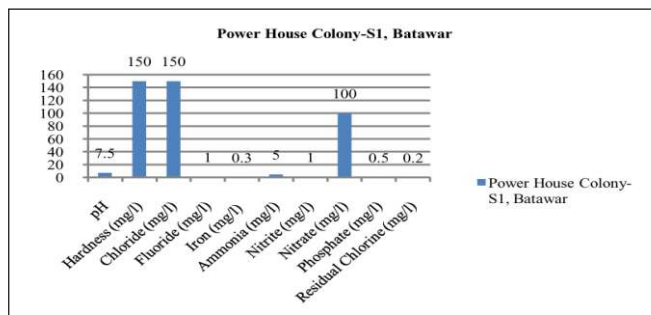


Figure 15: Groundwater quality at Power House Colony-S1, Batawar.

Power House Colony-S2, Batawar

In groundwater sample at Power House Colony-S2, Batawar pH (desirable), hardness (permissible), chloride (desirable), fluoride (desirable), iron (desirable), ammonia (non-potable), nitrite (desirable), nitrate (non-potable), phosphate (desirable) and residual chlorine (desirable) (Figure 16). Overall the groundwater is non-potable due to high ammonia and nitrate.

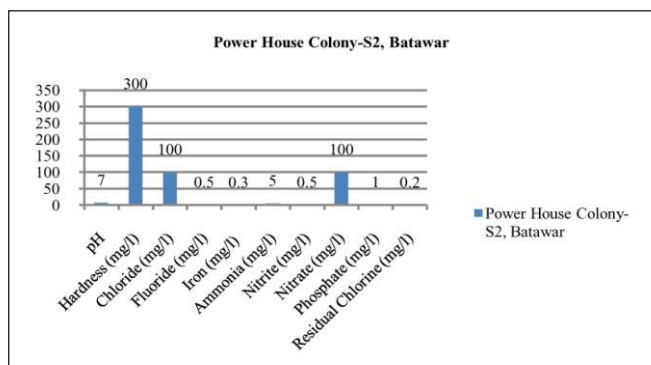


Figure 16: Groundwater quality at Power House Colony-S2, Batawar.

Near Bhagwanpur Road, Batawar

In groundwater sample at Near Bhagwanpur Road, Batawar pH (desirable), hardness (permissible), chloride (desirable), fluoride (desirable), iron (desirable), ammonia (desirable), nitrite (desirable), nitrate (desirable), phosphate (desirable) and residual chlorine (desirable) (Figure 17). Overall the groundwater is drinkable because all the analysed chemical parameters are under potable category of BIS drinking water standards (IS 10500:2012).

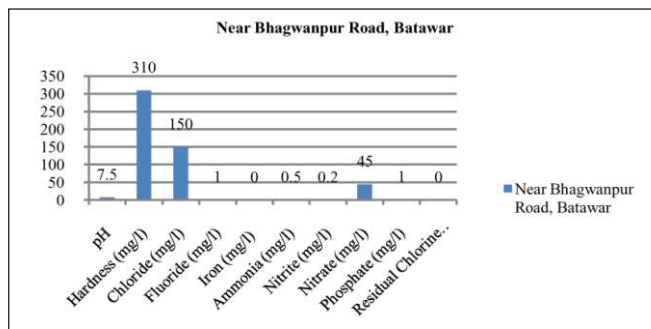


Figure 17: Groundwater quality at Near Bhagwanpur Road, Batawar.

CONCLUSIONS

In the study area pH ranges from 7 to 8 and desirable for drinking purpose in all the six groundwater samples. Hardness ranges from 150 mg/l to 400 mg/l and desirable in one groundwater sample and permissible in groundwater samples at Shiv Colony-S1, Barwala (250 mg/l), Near Bharel Road, Barwala (400 mg/l), Shiv Colony-S2, Barwala (400 mg/l), Near Primary Health Centre, Barwala (360 mg/l), Near Bhagwanpur Road, Batawar (310 mg/l), Power House Colony-S2, Batawar (300 mg/l). Chloride ranges from 100 mg/l to 150 mg/l and desirable in all the six groundwater samples. Fluoride ranges from 0.5 mg/l to 2 mg/l and desirable in all the six groundwater samples. Iron ranges from 0 mg/l to 5.0 mg/l and desirable in three groundwater samples and non-potable in groundwater samples at Shiv Colony-S1, Barwala (1.0 mg/l), Near Bhareli Road, Barwala (2.0 mg/l), Shiv Colony-S2, Barwala (5.0 mg/l), Near Primary Health Centre, Barwala (3.0 mg/l). Ammonia ranges from 0.5 mg/l to 5.0 mg/l and desirable in two groundwater samples and non-potable in groundwater samples at Shiv Colony-S1, Barwala (2.0 mg/l), Near Bhareli Road, Barwala (5.0 mg/l), Power House Colony-S1, Batawar (5.0 mg/l), Power House Colony-S2, Batawar (5.0 mg/l). Nitrite ranges from 0.2 mg/l to 1 mg/l and desirable in all the six groundwater samples. Nitrate ranges from 45 mg/l to 150 mg/l and desirable in two groundwater samples and non-portable in groundwater samples at Shiv Colony-S1, Barwala (100 mg/l), Near Bhareli Road, Barwala (150 mg/l), Power House Colony-S1, Batawar (100 mg/l) and Power House Colony-S2, Batawar (100 mg/l). Phosphate ranges from 0.5 mg/l to 1.0 mg/l and desirable in all the six groundwater samples. Residual chlorine ranges from 0 mg/l to 0.5 mg/l and desirable in five groundwater samples and permissible in Near Bhareli Road, Barwala (0.5 mg/l) groundwater sample. Shiv Colony-S1, Barwala groundwater is non-potable due to high iron, ammonia and nitrate, Shiv Colony-S2, Barwala groundwater is non-potable due to high iron in the groundwater, Near Bhareli Road, Barwala groundwater is non-potable due to high iron, ammonia and nitrate, Power House Colony-S1, Batawar groundwater is non-potable due to high ammonia and nitrate, Power House Colony-S2, Batawar groundwater is non-potable due to high ammonia and nitrate, Near Bhagwanpur Road, Batawar groundwater is drinkable because all the analysed chemical parameters are under potable category.

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B.
Health Sciences Section



THE EMERGING WORLD OF ANTIBIOTICS AND THE TERROR OF ANTIBIOTIC RESISTANT MICROORGANISMS: A REVIEW

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Abstract

In today's time many of the pathogenic bacterial strains have started showing resistance to antibiotics which is one of the biggest health challenges faced. This rising popularity gained by pathogens has a propensity to cause infection in people at any phase of life, in healthcare, veterinary, and cultivation industries, enlisting to primary public health challenges, most countries are going to encounter. Antibiotics used inappropriately in human, animals, food, agricultural arenas have caused a rather catastrophic dilemma. Antibiotic resistance results when the bacteria can resist the action of antibiotics and continue causing infection. Both gram-positive and gram-negative resistant bacteria have been deemed serious and urgent threats as they have evolved to develop resistance mechanisms, as a result, the organisms continue to grow and cause infection, even in the presence of antibiotics. In April 2014, World Health Organization (WHO) published the first global report on surveillance of AMR, illustrates the degree of this antibiotic resistance in various parts of the world and also the existence of large breaks in the prevailing investigation, there fore enumerating to be one of the gravest global public health threats in the world.

Keywords

Antibiotics, antibiotic Resistance, pathogenic bacteria, public health challenge, infection

Introduction

Antibiotics are drugs that are used to destroy or inhibit the growth of bacterial cells. Antibiotics are either bacteriostatic or bactericidal in nature and help the body's immune system to eradicate them[1]. These drugs inhibit the formation of basic genetic material such as the DNA, RNA and hereby inhibiting the protein synthesis too, and this is carried out by many specific actions such as their membrane synthesizing agent.[2] The bactericidal antibiotic is those classes of drugs that kill the bacteria by either interfering with its cell wall synthesis or by inhibiting one of its cell components during replication thus killing it. The bacteriostatic antibiotics are that class that inhibits the further growth of the bacterial infection.

Mechanism of action of drugs

The bacteria are targeted by the antibiotics which prevent them from their cell wall and thereby causing cell death. This is because of the presence of a thick peptidoglycan which is present in most of the bacterial cells. The linear strands of

peptidoglycan undergo cross-linking due to transglycosidases, and the peptide chains extend from the sugars present in the polymers, forming the cross-links joining one peptide chain to another.^[5] These sugars are specific derivatives of carbohydrates forming the inside layer of the peptidoglycan layer. The sugar components of the layer contain residues of β -(1,4) linked NAM (N-Acetyl Muramic Acid) and NAG (N-AcetylGlucosamine) arranged alternatively [3]. These sugars on either side of the layer are joined together with pentapeptides which are cross-linked by the peptide bond that holds them together and thus strengthening the cell wall. These are the antibiotics whose molecular structure consists of a beta-lactam ring. There are various derivatives of beta lactam antibiotics such as penicillin (penams), cephalosporins (cephems), monobactams, carbapenems[4] and carbacephems.[5] These are the most widely used antibiotics whose action against the bacterial cell is carried out by inhibiting the cell wall synthesis. Protein Synthesis Inhibitors such as tetracycline, erythromycin, chloramphenicol, and aminoglycosides are

the majorly used antibiotics. These antibiotics work as protein-synthesis inhibitors interfering with the cell synthesis and thus inhibiting protein formation. Antibiotics such as erythromycin inhibit the process of protein synthesis by attaching itself to the 23 S ribosomal subunit of the 50 S unit and hence prevents the assembly of the various subunits of 50 S ribosomal unit. Erythromycin, clarithromycin and roxithromycin are the antibiotics that inhibit extension at the transpeptidation stage of synthesis by preventing the 50 Spolypeptide export tunnel.[3]

Replication of all living forms, including bacteria takes place by the replication of the genetic material that is DNA. There is a class of antibiotics that interferes with the replication of DNA by either binding itself to directly one of these or by inhibiting the components of the cell involved in the process of replication and hence the survival. Examples: quinolones, metronidazole, and rifampicin[6]. Quinolones are a primary group of antibiotics that inhibit the process of DNA synthesis by attacking the topoisomerase, the enzyme commonly responsible for the super coiling of the ds DNA strand, most frequently Topoisomerase II. The topoisomerase II is an instrumental enzyme used in the process involved in the mechanism of DNA replication as it cuts the double stranded DNA, relaxes the super coils and then rejoins them together at the cut ends. Therefore, permitting the super coiled strand of DNA to be replicated or even transcribed when they are separate.[7]

Folic acid is a vitamin that is required to make nucleotides and many amino acids. In the absence of an important metabolite like this, the cell would fail to make DNA, RNA and most of the proteins. Bacteria make their own folic acid which is used in many enzymes and many metabolites are formed too. There are certain drugs that inhibit the process of folic acid synthesis that are bacteriostatic because the bacteria cannot reproduce without sufficient folic acid to make DNA, RNA or proteins and broad spectrum as they are effective against various types of bacteria. Different sulpha drugs with the combination of trimethoprim helps in inhibiting different enzymes participating in the mechanism of formation of folic acid. Sulfonamides are competitive inhibitors of dihydrofolate synthesis. Trimethoprim is responsible for inhibiting the action of the enzyme dihydrofolate reductase. When trimethoprim and this enzyme are used together can be used to cure a wide range of susceptible bacterial infections (gram negative and gram positive).[8]

Antibiotic Resistance: a concern

There has been an increase in antibiotic use over the past years, however, the discovery of the various antibiotics has got slow because of the increased resistance of the pathogens against the existing antibiotics. Due to this, the antibiotic which has got resistant to the pathogen/disease is not able to combat the same. The arising resistance of the various microbes including bacteria, viruses, etc. is due to the mutations in their genome that have caused its evolution. This

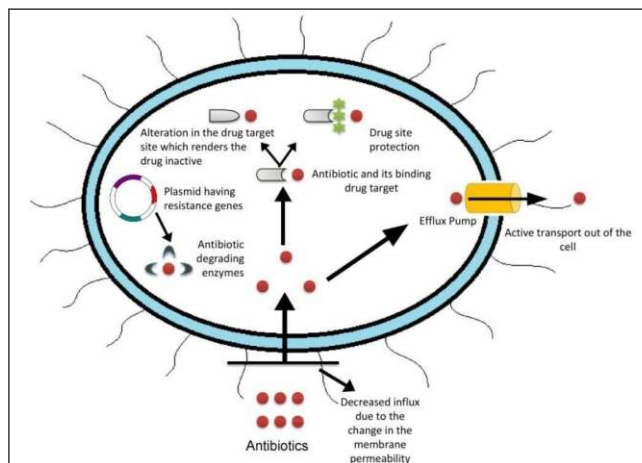


Figure 1: Showing different resistance mechanisms against antibiotics.

resistance renders our drugs ineffective hence threatening the most significant advances in medical history. Antimicrobial resistance is posing a serious global threat to the environment, humans and animals.

Antibiotic Resistant Microorganisms

- Antibiotic resistant *Gonorrhea* strain
- Metronidazole resistant *Clostridium difficile*
- Methicillin resistant *Staphylococcus aureus*
- Vancomycin-resistant *Enterococci* (VRE)
- Extended spectrum beta-lactamases (ESBL)
- Penicillin resistant *Streptococcus pneumoniae* (PRSP)
- Vancomycin resistant *Staphylococcus aureus* (VRSA)
- Multidrug resistant Tuberculosis (MDR-TB)
- Multidrug resistant *Staphylococcus pneumoniae* (MDRSP)

Antibiotic resistant *Gonorrhea* strain

Gonorrhea is an infection which is transmitted via sex and spread by the bacteria *Neisseria gonorrhoea*. A person is infected with gonorrhea by openings, the bacterial strain entering the body through mouth, anus, penis or vagina. The most common way a woman is infected is by her cervix and the man is mostly infected by the urethra, the tube that carries the urine from the urinary bladder to outside the body.[9]

Mechanism of antibiotic resistance in *Gonococcus*

Over the past few years, the gonococci bacterial strain has developed tolerance against the action of antibiotic drugs due to the process of mutations and/or gene (whole or parts) acquisition occurring spontaneously, are efficiently chosen because of antibiotic burden in patients and, in the population and generally.[10] On a global scale, this bacteria *N. gonorrhoeae* has shown resistance to almost all significant antimicrobials, including penicillin, quinolones, sulfonamides, macrolides and tetracyclines.[11] This rise of resistance in this bacteria is done by point mutation and also

horizontal gene transfer even with other *Neisseria* species. These resistance mechanisms which have been acquired include the alteration in the drug binding site, increased in activation of antibiotics, decreased concentration of drugs. For example, when there is a mutation in the *penA* gene which is known for encoding the cell wall protein penicillin binding proteins 2, confers lesser sensitivity to the beta-lactam drugs [12][13]. The gonococcus strain has known to develop most of the physiological methods of resistance to many antibiotics which are advised during its treatment, e.g., (i) target modification or protection that reduces affinity for the antimicrobials, (ii) modifying or damaging the antimicrobials by enzymatic mechanisms, (iii) increased efflux of antimicrobials, and (iv) decreased influx of antimicrobials. Many genetic determinants responsible for AMR is situated on the chromosomes at the *bla_{TEM}* gene [14][15] and the *tetM* gene [16]. These genes *bla_{TEM}* and *tetM* are responsible for causing high resistance to penicillin and tetracycline correspondingly, and this resistance is suspected to be plasmidborne. Gonococci builds its resistance to antibiotics by the process of transfer of genes (transformation and following recombination in to the genome) or by means of mutations at specific sites. Introduction of antibiotics to gonococci or other *Neisseria* spp. for the treatment of gonorrhoea or other infections can pick out for strains which inhibit the mechanism of antibiotics. [12]

Metronidazole resistant *Clostridium difficile*

Clostridium difficile is a Gram positive anaerobe causing *Clostridium difficile* infection (CDI) and disrupting the intestinal environment [17]. The most common way of transmitting CDI is by spores and has the capability of causing severe damage to the colon and can be fatal. There has been an increase in the number of deaths by CDI over the past few years and poses an economic burden as well. [18] According to the data given by Centre of Disease Control (CDC), in United States, this infection is responsible for over 29,000 deaths each year with more than 400,000 people getting infected, posing a economic burden of about \$1 billion in additional medical budgetary costs. [19] For the first line of treatment, the antibiotics to be used are metronidazole and vancomycin. Most of the CDI's are susceptible to either of the drugs [20]. The other antibiotics used are erythromycin, clindamycin, penicillins, lincomycin, tetracyclines, aminoglycosides, cephalosporins, and fluoroquinolones, which have been used to treat bacterial infections. [21][22] Metronidazole (MTZ) is a nitroaromatic prodrug with a relatively lower molecular weight and can easily diffuse across the membrane of aerobes and anaerobes as well. [23][23] It has developed multi resistance mechanisms which aids in antimicrobial resistance in *Clostridium difficile*. These resistance mechanisms include the mobile genetic elements (MGEs), genes causing rising resistance in bacterial chromosome, modifications concerning the targets of antimicrobials and/or in absorption ways in *C. difficile*, and in the development of biofilm. [19] Different phenomenon such as conjugation, transduction, or transformation of MEG's

specifically transposons within different *C. difficile* strains and other bacterial cells help in acquiring antimicrobial resistance. [21]

A mechanism mediating antibiotic resistance in *C. difficile* includes modifications in the antimicrobial targets and/or in the absorption pathways. Data has been suggested that the rising resistance against Vancomycin might be because of alteration in amino acid constituting the proteins in peptidoglycan biosynthesis like MurG [24] Some different influences like selective pressure due to exposure to antibiotics in the environment make modifications in the objective sites of antimicrobials and/or in the absorption paths in *C. difficile*. For example, due to this method of selective pressure rifampin, rifampin and rifaximin class of antibiotics, have stopped working against *C. difficile* as they have gained the ability to induce mutations in the sub-unit of the *rpoB* gene, that translates a RNA polymerase of the bacterial strain [25][26]

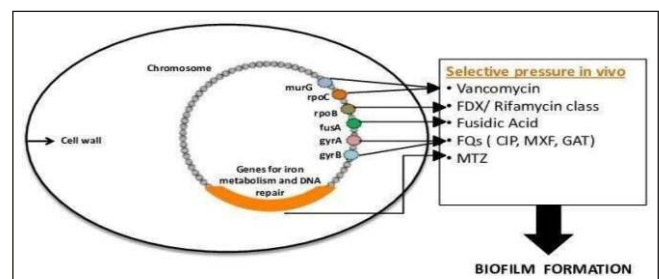


Figure 2: Selective pressure in vivo leading to alterations in antibiotic targets and causes antibiotic resistance. The resistance to the antibiotics also stimulates the bio-film formation. Bio-formation via different mechanisms further contributes to antimicrobial resistance in *Clostridium difficile*. CFs, cephalosporins; CHL, chloramphenicol; CIP, ciprofloxacin; CRO, ceftriaxone; CTT, cefotetan; CTX, cefotaxime; FOX, cefoxitin; FQs, fluoroquinolones; GAT, gatifloxacin; LZD, linezolid; MLSB, macrolide-lincosamide-streptogramin B; MTZ, metronidazole; MXF, moxifloxacin; PBPs, penicillin-binding proteins; TET, tetracycline; VAN, vancomycin.

Formation of biofilm has been found out to be one of the most important factors contributing to antibiotic resistance. The process involving the formation of biofilm involves a dense multi component matrix of many layers comprising of DNA, proteins, and polysaccharides. [27] It is evident that biofilms have the ability to defend infective bacteria from adverse stresses which may include antimicrobials and hence promote its survival and virulence. [27]

The mechanism of action of Metronidazole is carried out by its reduction by the pyruvate: ferredoxin oxidoreductase which is commonly present in facultative anaerobic bacteria, and are responsible for alteration in its chemical structure. Pyruvate: ferredoxin oxidoreductase normally triggers the formation of ATP through the process of oxidative decarboxylation of pyruvate. The nitro group in metronidazole while present in the cell acts as an acceptor of electrons which

come together and then transfers to the hydrogen ions in the mentioned cycle. The conversion of metronidazole during reduced form is responsible for the creation of a concentration difference that takes up more drugs and stimulates the development of intermediary compounds and free radicals that are lethal to the cell.[23][28][29]

Many studies on the antibiotic resistance in isolates of *C. difficile* from Europe, Asia and North America has reported an increase in resistance to metronidazole as well as to moxifloxacin and clindamycin.[30]

Methicillin resistant *Staphylococcus aureus*

Staphylococcus aureus is a Gram positive bacteria and a usual member of the microbiota of the human body as it is present in the nose of nearly 30% and on the skin of about 20% of the healthy adults. The strength of the ability of aureus to cause infection varies from mild to life threatening. *S. aureus* is known to cause wide range of skin infections and also affects the soft tissues, bones, joints, or it may even cause infection in the prosthetic devices.[31] According to the data taken from the Emerging Infections Program (EIP) MRSA population surveillance(2005–2016) and the Premier and Cerner Electronic Health Record databases (2012–2017) there is an estimated of 119,247 *S. aureus* blood stream diseases, with 19832 connected deaths. Although the number of people getting infections has reduced from 2015, there has been a significant morbidity rate with the number of people catching the infections.[32]

The increased resistance in *S. aureus* is primarily due to increased consumption of antibiotics like methicillin and other beta-lactams. Resistance in MRSA is primarily interceded by *mecA* that encodes for a penicillin binding protein PBP-2a that is resistant to the mechanism of action by β -lactams intrinsically[33][34]. When MRSA encounters antibiotics like methicillin, flucloxacillin, dicloxacillin, nafcillin inactivates the four PBPs present which have a higher-binding-affinity. However, PBP-2a shows a lower binding capacity for methicillin, and then these PBPs, allows the bacterial cell to proliferate in the presence of these antibacterial medicines. PBP 2a is functionally effective in

the presence of the different concentrations of β -lactam antibiotics that stops most endogenous PBPenzymes. Then this further takes over for its roles used in the production of cell walls and allows progress in the presence of the β -lactam inhibitors.[35] There are other genes that supervise the phenotype which are methicillin resistant and the PBP-2 a production. *MecR1* and *MecI* are genes which are situated upstream of the gene *mecA* which are responsible for controlling the PBP-2a expression[36]

Vancomycin-resistant Enterococci (VRE)

Enterococcus is a Gram-positive and anaerobic bacteria which is a common part of the intestinal flora. There are nearly more than 17 different strains but *Enterococcus faecium* and *Enterococcus faecalis* are the most common. Overtime enterococcus has developed a resistance mechanism against a strong antibiotic that was used as a first line therapy to treat it in the first place. A characteristic property of the genus *Enterococcus* is that it confirms resistance to a some amount of antibiotic drugs, even though a few varieties (e.g., *E. faecium*) are further vitally resilient than other species [37]. VRE infections are more difficult to treat than other infections with enterococci, because fewer antibiotics can kill the bacteria [38]. In the past few years, enterococcus not only has been resistant to only vancomycin but also to many beta-lactams and also aminoglycosides[39]. This has led to them becoming the new superbugs which are resistant to many antibiotics, making it difficult to find a cure. As recognized in the early 1950's, when enterococcus was treated with penicillin, the response rate was lower than expected. Later, it was found that most enterococci are resistant to activity of β -lactam and glycopeptide antibiotics inhibiting different bacterial strains[40]. Also, several isolates of *E. faecium* are extremely resistant to penicillins, hence their PBP's have low binding affinity for penicillins.[41]

There are different types of Enterococci resistant to vancomycin that have been categorized based on their phenotypes and genotypes, as summarized in the table 1. [42] It was later that vancomycin was proved to be a strong

Table 1: Properties of phenotypes of some enterococci resistant to glycopeptides in many reported isolates.[42][43]

Variable	VanA	VanB	VanC	VanD	VanE
Vancomycin MIC (μ g/mL)	64->1000	4-1024	2-32	64-256	16
Teicoplanin MIC (μ g/mL)	16-512	≤ 0.5	≤ 0.5	4-32	0.5
Most frequent Enterococcus spp.	<i>E. faecium</i> <i>E. faecalis</i>	<i>E. faecium</i> <i>E. faecalis</i>	<i>E. gallinarum</i> <i>E. casseliflavus</i>	<i>E. faecium</i>	<i>E. faecium</i>
Genetic Determinant van	A cluster; acquired	vanB cluster; acquired	vanC1, vanC2 cluster; intrinsic	vanC2 cluster;	vanE cluster; acquired
Transferability	Yes	Yes	No	ND	ND

antibiotic and can be used to treat this infection. Vancomycin is an antibiotic of the class glycopeptides that functions when it attaches to the terminal ^D-Ala-^D-Ala. This terminal molecule is a part of the pentapeptide section of the *N*-acetylglucosamine (NAG)-*N*-acetylmuramic acid (NAM) peptidoglycan (PG) cell wall precursor.[44]

It has been nearly 30 years that vancomycin has been used and without any emergence of marked resistance. [45] However, later resistant isolates were found in England, then in France, followed by Eastern countries of United States.[46] Resistance shown against the glycopeptide antibiotics in *Enterococcus* spp. is controlled by the operon (Van) that is the Vancomycin Resistant operon. The Van operon may possibly be transferred on the plasmid, either chromosomally or extrachromosomally. The Van operon comprises of a response regulator that is *vanS-vanR*; a ^D-lactate dehydrogenase gene, *vanH*; a ^D-Ala-^D-Ala dipeptidase gene, *vanX*; and a type of variable ligase which has 9 variant genes (*vanA*, *vanB*, *vanC*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM*, and *vanN*)[47]

Extended spectrum beta-lactamases (ESBL)

Beta-lactams are the class of antibiotics which destroy the bacterial cells by acting on their cell wall. It consists of penicillins, carbapenems, monobactams, cephalosporins. The use of beta-lactam antimicrobials is very common to treat bacterial infections mostly caused by gram negative bacteria worldwide.[48]

The beta-lactams function by inhibiting the final phase in the process for the formation of peptidoglycan which is carried out by acylation of the transpeptidase. The acylation of these transpeptidases forms cross-links in the peptides producing the peptidoglycan. PBP's are the objective site to where the beta-lactams bind, furthermore this method of binding thus interferes with the last transpeptidation process which causes failure of sustainability and thus death via process like self-digestion.[49]

The Gram negative bacteria has been exposed to the beta – lactams persistently causing them to develop resistance by forming an enzyme by mutation called as the beta lactamases that renders the antibiotics in active and broadening their cope of survival.[50] There has been a surprising rise in the number of beta lactamases and the number has reached 200[51].

The enzymes, beta-lactamases, break down the antibiotic by opening the beta-lactam ring and hence it becomes inactive. The two other enzymes which show a familiar action are of the class plasmid mediated beta-lactamases are TEM-1 and TEM-2. They are present in gram-negative bacteria including Enterobacteriaceae, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*[52]. ESBL is simply transferred among members of *Enterobacteriaceae* because it is plasmid mediated. The distribution of this resistance applies to beta-lactams and also to other frequently utilized antibiotics such as fluoroquinolones, amino glycosides, and sulphonamides.[53]

These enzymes hydrolyze the penicillins and some of the narrow spectrum cephalosporins.

Penicillin resistant *Streptococcus pneumoniae* (PRSP)

There has been an increased resistance in bacteria which has become non susceptible to antibiotics especially penicillin over the past few years. The studies show that there has been a significant rise in the number of penicillin resistant *Streptococcus pneumoniae* in entire Asia as well as the United States. A gradual increase in the intermediate resistance to penicillin (IRP) has been documented in India since 1995. [54][55]

It has been observed that the affinity of the Penicillin Binding Proteins (PBP's) of the gram negative bacteria to bind with the antibiotics have drastically gone down. The isolates were examined from different parts of the world has demonstrated that the decrease in affinity of these PBPs with the antibiotics is resistance mechanism developed by the gram negative bacteria.[56]

The primary resistance mechanism in *Streptococcus pneumoniae* is the alteration in the enzyme targets that are the penicillin-binding proteins (PBPs) for the beta-lactams. There has been observed mutation in the enzyme targets (PBPs) that prove the reduced binding interaction of the beta-lactams to the target sites. However, mutations at points were chosen in the research laboratory, clinical isolates of *Streptococcus pneumoniae* exhibit a structure like mosaic of the altered genes of PBP, the consequence of interspecies gene transfer and events of recombination.[57]

The method of beta-lactam resistance of *S. pneumoniae* includes genetic mutations that modify penicillin-binding protein structure, following a decreased affinity for all beta-lactam antibiotics.[58]

A study conducted by Reynolds *et al.* showed that, certain antibiotic-resistant pneumococcal pneumonia, resistance caused 32,398 additional outpatient visits and 19,336 extra hospitalizations. This led to \$91 million (4%) indirect medical costs and \$233 million (5%) in total costs.[59]

Vancomycin resistant *Staphylococcus aureus* (VRSA)

Vancomycin resistant *Staphylococcus aureus* are the strains of *S. aureus* that have gradually developed resistance against the glycopeptides, especially vancomycin. There were experiments conducted to study about the vancomycin resistant genes and it was observed that *E. faecalis* can transfer its vancomycin resistant genes to *S. aureus* by gene transfer.[60]

There are VRSA strains that have the transposon Tn1546, which is developed vancomycin-resistant *Enterococcus faecalis*. This bacterial strain has been suspected to modify the structure of cell wall and its metabolic processes.[61] The newly discovered VRSA from isolates with a vancomycin MIC $\geq 100\mu\text{g/ml}$ were discovered in wounds of

diabetic patients co-infected by *E. faecalis* and MRSA resistant species. Irrespective of their fundamental variations, the biochemical processes of VISA and VRSA strains has indicated to reveal several shared features.[61]

Vancomycin functions by attaching itself permanently to the terminal ^D-alanyl-^D-alanine of disaccharide-pentapeptide precursors in cell wall, and thus hindering assembly of the bacterial cellwall. Enterococci has evolved to develop resistance to vancomycin by way of substituting lactate instead of the terminalalanine, that has a significantly decreased affinity for vancomycin.[62]

Many researches of this coagulase-negative staphylococci resistant to vancomycin have demonstrated that these modified cell wall precursors are produced. This is formed, but small quantities as to be included for the degree of resistance detected. [63].

There has been a hypothesis that is yet to be proven, that says these modified cross-links may hinder vancomycin binding to peptides acting as targets.[64]

Multi drug resistant Tuberculosis (MDR-TB)

Multidrug-resistant TB (MDR-TB) is TB that does not act in response to at least isoniazid and rifampicin, the 2 most powerful anti-TB drugs. In 2012, there were approximately 450,000 new cases and 170,000 deaths because of MDR-TB.[65]

India has highest burden of Tuberculosis in the world.[66] Figures published by the Ministry of Health in India says that nearly 3 million new cases of TB occur each year in the country.[66]

Tubercle bacilli has a surprisingly high proportion of chromosomal mutation that shows resistance to antibiotics. Latest molecular analysis of some MDRTB strains reckons that the MDR phenotype is a consequence of successive accumulation of individual mutations in distinctgenes, not by novel methods due to single mutagenic events.[67]

The primary explanation for resistance to antibiotics in MTB is the emerging of different mutations in variety of genes encoding for targets to which drug binds or enzymes responsible got activation of drugs. These mutations occur principally in the manner of SNPs, insertions or deletions and to a less significant extent, significant deletions.[68]

The mechanism of antibiotic resistance in TB happens via two mechanisms: (i) resistance which occurs primarily is the transmitted antibiotic resistance takes place when the resistant strains are communicated to a new host, and (ii) the resistance that occurs secondarily or developed resistance to drugs, this happens via gaining of drug resistance mutations to single or supplementary drugs. [69][70].

Multi drug resistant *Staphylococcus Pneumoniae* (MDRSP)

The staphylococcal infections has caused a major concern by showing various antibiotic resistance medication of

infections particularly of methicillin-resistant *S. aureus* (MRSA). This has arisen due to the wide spread usage of antimicrobial agents, combined with the spread of an significant percentage of the organism by person-to-person contacts.[71]

Conclusion

This is an undeniable fact that the problem of antibiotic resistance has a severe impact on the global health and the economy of the world altogether. This problem cannot be attained by the combined efforts of the government, the medical professionals and most importantly the public. Before the government talks about the science behind the resistance, it is necessary to create awareness among the public by effective communications, discussions, seminars etc., discussing the harmful effects it has on the society. The concept of properly taking antibiotics and in proper dosage should be inculcated and the harmful effects of resistance to the body should be taught among the people. Apart from the groundwork and interaction with the public, the researchers should take up more research work in the field of AMR, finding the ways with which one can know and develop strategies to combat AMR.

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A Comprehensive Review on *Ganoderma lucidum* derived Bioactive peptide Ling Zhi-8

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Abstract

Ganoderma lucidum (GL) is a medicinal mushroom which is highly appreciated in Traditional Chinese Medicine and has also been extensively studied in the contemporary world. Scientific investigations have established various medicinal and nutraceutical activities of this medicinal mushroom. The fungus is known to be a repository of numerous bioactive metabolites including terpenoids, polysaccharides, proteins and peptides, nucleotides, fatty acids, steroids, vitamins and minerals. Among these biologically active compounds, the most researched class of compounds are terpenoids and polysaccharides. Consequently, several review articles describing the pharmacological properties of terpenoids and polysaccharides derived from GL are available. Amidst the proteins isolated from GL, Ling Zhi-8 (LZ-8) is the most studied protein. However, no review is available to the best of our knowledge, although there are numerous studies pertaining to the bioactivities of this protein and its recombinant form. The protein LZ-8 has been reported to exhibit immunomodulatory, mitogenic, anti-tumor, anti-cancer, anti-diabetic and anti-osteoporotic activities. Thus, in this article we intend to summarize the research conducted so far regarding the therapeutic effects and mechanisms of action of LZ-8 protein isolated from GL and also its recombinant form. This shall enable a comprehensive review on the research conducted regarding LZ-8 protein until now.

Keywords

Ganoderma lucidum, fungal immunomodulatory proteins, medicinal mushrooms, Ling Zhi-8, immunomodulation, mitogen.

Introduction

Since time memorial, medicinal mushrooms have been advantageous to humankind due to their enormous medicinal and nutraceutical benefits. They are recognized as a good source of several biologically active metabolites and hence, are used as health supplements and cosmetics. A medicinal mushroom called as “*Ling Zhi*”, “*Reishi*” or *Ganoderma lucidum* (GL), which is well documented in Traditional Chinese Medicine and Japanese Medicine, has been extensively studied pertaining to its secondary metabolites and their bioactivities. According to the literature survey, GL extracts, fractions and various bioactive compounds isolated from it have demonstrated numerous pharmacological properties including antiangiogenic, anticancer, anti-inflammatory, antimicrobial, antioxidative, antitumor, immunomodulatory and neuroprotective activities [1, 2, 3].

The genus *Ganoderma* consists of approximately 400 different types of bioactive constituents and about 279

secondary bioactive metabolites such as terpenoids, polysaccharides, proteins and peptides, nucleotides, enzymes, fatty acids, steroids, vitamins and minerals have been reported from GL [1, 4]. Among these, the most studied class of metabolites derived from GL are polysaccharides and terpenoids. Consequently, several review articles describing the pharmacological properties of terpenoids and polysaccharides derived from GL are available.

Nevertheless, recently studies on peptides isolated from GL has commenced by several researcher groups. Amidst the proteins isolated from GL, Ling Zhi-8 (LZ-8) is the most studied protein. However, no review is available to the best of our knowledge, although there are numerous studies pertaining to the bioactivities of this protein and its recombinant form. The protein LZ-8 has been reported to exhibit immunomodulatory, mitogenic, anti-tumor, anti-cancer, anti-diabetic and anti-osteoporotic activities [2].

Thus, in this article we intend to summarize the research conducted so far regarding the therapeutic effects and mechanisms of action of LZ-8 protein isolated from GL and also its recombinant form. This shall enable a comprehensive review on the research conducted regarding LZ-8 protein until now.

Ling Zhi-8 (Lz-8)

Fungi are considered a rich source of proteins [5] and consist of over 30% of the mushroom dry weight [6]. At present, proteins namely, Ling Zhi-8 (LZ-8), Ganodermin, Lzp-1, Lzp-2 and Lzp-3 and several others have been isolated from *Ganoderma lucidum* (GL) and characterized using electrophoretic and chromatographic techniques [7]. In the sections below, we have described the various biological activities of GL derived protein LZ-8 and associated mechanisms.

Structural and Physicochemical Characteristics of Ling Zhi-8

Ling Zhi-8 (LZ-8) or FIP-glu belongs to the family of fungal immunomodulatory protein (FIP).

To date, LZ-8 (FIP-glu), FIP-gts, FIP-fve, FIP-vvo, FIP-gja, FIP-gmi, FIP-gsi and FIP-tve have been identified from *Ganoderma lucidum*, *Ganoderma tsugae*, *Flammulina velutipes*, *Volvariella volvacea*, *Ganoderma japonicum*, *Ganoderma microsporum*, *Ganoderma sinensis* and *Trametes versicolor* [8]. These FIPs are small molecule proteins with wide-ranging bioactivities such as anti-allergy, antitumor, ability to stimulate immune cells to produce a variety of cytokines, etc. [8, 9]. Ling Zhi-8 (LZ-8) is known to be the first protein isolated from the mycelium culture of *Ganoderma lucidum* by Kino et al [10]. The protein was purified by using two chromatographic techniques sequentially. At first, by gel filtration chromatography using Sephadex G-75 column (5 X 100 cm), previously equilibrated with 10mM Tris-HCl buffer (pH 8.0). Afterwards, ion-exchange chromatography was employed by absorbing the most active fraction onto a DEAE-Sephadex A-25 column (2.6 X 40 cm), previously equilibrated with 10mM Tris-HCl buffer (pH 8.0). The authors reported the molecular weight of the purified protein, i.e., LZ-8 to be 13kDa as determined by Tricine-sodium dodecyl surface-PAGE electrophoresis with an isoelectric point of 4.4. LZ-8 was found to contain only two sugars- mannose and hexosamine which constituted 0.6% of its sugar content. It comprised of relatively large amounts of asparagine (or aspartic acid) and valine. Half-cysteine, histidine, methionine, and hydroxyproline were not detected in the protein [10].

The native form of LZ-8 has a molecular mass of 24kDa is a homodimer of the LZ-8 polypeptide. Each polypeptide chain consists of 110 amino acid residues, and has molecular mass of 12,420 Da which includes the amino-end acetyl group also. Within the structure of LZ-8, there is no consensus sequence Asn-X-Ser or Thr as an attachment site for an Asn-linked oligosaccharide chain. This corresponds to the very low carbohydrate content of LZ-8 as shown previously [10].

Furthermore, the LZ-8 polypeptide chain exhibits significant similarity both in its sequence and in its predicted secondary structure to the variable region of immunoglobulin heavy chain (I_{gV_H}) [11].

Mitogenic activity of Ling Zhi-8

Recent findings have revealed that a homodimeric structure is necessary for the immunomodulatory ability of FIPs. Dimerization is the key process through which FIPs bind to their cell surface receptors and exert their activity. This fact has been revealed by a recent study, wherein a structure-based multiple alignment of LZ-8 and an FIP from *Volvariella volvacea* was performed. During this study, the electrostatic potential of their protein surfaces was compared and a model summarizing the unique electrostatic interaction in LZ-8 dimerization was developed. The results highlighted the fact that dimerization of native LZ-8 or rLZ-8 plays a crucial role in inducing the expression of IL-2. The interleukin, IL-2 is responsible for the regulation of certain autoimmune processes and in the generation and function of regulatory T-cells [9].

Previously also, LZ-8 has been reported to demonstrate mitogenic activity on human peripheral blood lymphocytes (PBL) by acting as a potent T-cell activator and mediating its effects by upregulating IL-2 and intercellular adhesion molecule-1 (ICAM-1) expression along with an increased production of IFN- γ TNF α , and IL- β molecules, which are known to be associated with regulation of ICAM-1 expression [12].

The intercellular adhesion molecule-1 is a transmembrane glycoprotein belonging to the immunoglobulin (Ig) superfamily. It is expressed by several cell types including endothelial cells, fibroblasts, and leucocytes. ICAM-1 plays crucial roles in adhesion of cells, trans-endothelial migration of leucocytes to sites of inflammation and lymphocyte activation [13]. Further, Bao et al., performed electrostatic potential and virtual amino acid mutation analyses which indicated that L10, W12 and D45 are the key amino acid residues responsible for the high immunomodulatory activity of LZ-8. Hence, structural and physicochemical properties are major factors affecting the biological activities of FIPs [9].

It was in the year 1989, when Kino et al., first examined the hemagglutination activity of LZ-8 using human and sheep red blood cells (RBCs). The assay revealed that LZ-8 could not agglutinate human RBCs, but, the protein agglutinated sheep RBCs. The difference in the hemagglutination activity of LZ-8 towards human and sheep RBCs was unexplainable by the authors. Furthermore, their study confirmed that LZ-8 is not a lectin.

The same group also studied the mitogenic activity and immunomodulatory activity of LZ-8. The mitogenic activity was assessed by determining effect of LZ-8 on [3 H] thymidine uptake by cultured spleen cells alone or in the presence of

LPS or Con A. The results indicated an additive effect of LZ-8 and LPS at low concentrations (0.13-3.13 microgram/ml) on [³H] thymidine uptake and hence, it was inferred that the target cell of LZ-8 might be different from that of LPS; which is known to activate B-lymphocytes [10]. LZ-8 has also shown its mitogenic activity towards human peripheral mononuclear cell and mouse splenocytes. However, the mitogenic effect of LZ-8 obligated the presence of monocytes [14].

Immunomodulatory activity of Ling Zhi-8

The immunomodulatory activity of LZ-8 was first investigated by determining its effect on systemic anaphylaxis in Carworth Farm Webster (CFW) mice. Treatment of mice with LZ-8 suppressed BSA-induced anaphylaxis completely. Moreover, the treatment with LZ-8 (twice weekly) of CFW mice before sensitization with BSA via any systemic route, prevented anaphylaxis. This suggested that the LZ-8 is an immunosuppressant which acts by inhibiting antibody production.

The immunomodulatory activity of LZ-8 was further confirmed by Arthus reaction. In the Arthus reaction, the extent of immediate reaction at the intradermal site of antigen administration depends on the quantity of existing antibodies producing following sensitization. A reduction in the number of Arthus reaction-positive mice was observed from 90 to 40%. This led to the conclusion that *in vivo*, LZ-8 reduces antibody production but does not block it, totally [10]. Another group also observed that the immunosuppressive activities of LZ-8 caused by the blocking of antigen-specific antibody production. In this study, the *in vivo* effect of LZ-8 on antibody production using the hepatitis B surface antigen (HBs Ag) in mice was conducted. LZ-8 exhibited mitogenic activity *in vitro* towards spleen cells of C57BL/10 (B10) and C57BL/10BR (B10BR). The intraperitoneal administration of LZ-8 twice weekly into the mice (8 and 12 mg/kg) significantly suppressed antibody production to HBs Ag (83.3- 96.8%), whereas, B10 and B10BR mice produced anti-HBs Ag antibody by the twice sensitization of the antigen [15].

Anti-diabetic activity of Ling Zhi-8

Diabetes is a disease that involves immune responses. LZ-8 has also demonstrated an important role in preventing insulinitis as observed in non-obese diabetic (NOD) mouse through its immunomodulatory action. The protein was found to reduce lymphocyte infiltration, augmented antibody detection of insulin in beta cells and reduced plasma glucose concentration in NOD mice. T cell subset population analysis revealed that LZ-8 regulated subsets of immune cells [16]. Furthermore, the immunosuppressive effect of LZ-8 has also been demonstrated *in vitro* in a human mixed lymphocyte culture (MLC) performed in the absence of monocytes, using purified T-cells and Epstein Bar Virus (EBV)-transformed allogeneic B-cells. This group also studied the plausible suppressive effects of LZ-8 in two different models of allogeneic tissue transplantation- allografted mouse skin

model and transplanted allogeneic pancreatic rat islets. Administration of LZ-8 in allografted mouse skin model caused an increase in survival time and had a substantial effect on cellular immunity. LZ-8 facilitated delay in the rejection process in the transplanted allografted pancreatic rat islets model [14].

Hepatoprotective activity of Ling Zhi-8

Besides, mitogenic and immunomodulatory effects, LZ-8 has also demonstrated hepatoprotective properties also, against tetrachloride carbon (CCl₄)-stimulated hepatic injury when administration orally to rats [17]. Moreover, a study has revealed potent wound healing activity of LZ-8 against the electrosurgical induced liver injury. As suggested by the authors, the effect was due to the inhibition of NF- κ B and caspase-3 expressions by LZ-8. NF- κ B is reported to be an important link between hepatic injury, fibrosis, and even hepatocellular carcinoma [18].

Anti-inflammatory activity of Ling Zhi-8

The protein LZ-8 has shown its anti-inflammatory activity for modulating *in vitro* immune responses involved in neural inflammation in murine microglial BV-2 cells. BV-2 is a type of microglial cell derived from C57/BL6 murine. In this study, LZ-8 regulated the LPS-activated immune responses of BV-2 cells by reducing the production of pro-inflammatory mediators, comprising NO, PGE₂, IL-6 and, expression of iNOS and COX-2, via suppression of TLR4-mediated NF- κ B signaling [19].

Anticancer activity of Ling Zhi-8

The protein, LZ-8 exhibits anti-cancer progression and metastasis activity. Recently, it has been reported that LZ-8 produced changes in the proteomic profile (21 proteins) of tumor lesions. In particular, three heat shock proteins (HSPs), namely, HSP60, 70 and 90, were significantly downregulated in tumor lesions of Lewis lung carcinoma 1 (LLC1)-bearing mouse administered with LZ-8 in comparison to control (PBS; phosphate buffered saline). Furthermore, LZ-8 effectively inhibited cell migration and decreased cell viability of LLC1 cells [20]. Earlier, the same group had demonstrated that recombinant LZ-8 reduces the tumor progression in lung cancer LLC1 cell-bearing mouse [21].

Recombinant LZ-8 Protein (rLZ-8)

It became evident from various researches that LZ-8 has potential immunomodulatory and mitogenic activities. However, the isolation and purification of LZ-8 from *Ganoderma lucidum* mycelia was a constraint in the research pertaining to LZ-8. This is because isolation of the native LZ-8 from *Ganoderma lucidum* mycelia and its purification is an expensive and laborious process [22, 23]. Therefore, production of recombinant LZ-8 at high-level was opted to circumvent these complications. Expression vectors such as *E. coli* [22] and *Pichia pastoris* [24] have been used to generate rLZ-8 with better performance in terms of immunomodulatory and mitogenic activities. Recombinant protein production is a beneficial mean to avoid

contamination from cellular proteins and facilitates continuous cultures for downstream processing [23].

The production of LZ-8 into several expression vectors, subsequently led to numerous scientific studies using recombinant LZ-8 (rLZ-8) for elucidating the potential role under several pathological conditions and better understanding of various pharmacological activities possessed by rLZ-8; which has demonstrated similar biological effects as its native form, i.e., LZ-8. Some of these conducted using rLZ-8 are detailed in the following sections.

Antitumor activity of rLZ-8

Wu et al in 2011, elucidated that rLZ-8 increased G1 arrest and, activated p53 and p21 expressions in A549 human lung cancer cells. Moreover, administration of rLZ-8 in mice transplanted with Lewis lung carcinoma cells resulted in ribosomal stress via inhibition of precursor ribosomal RNA synthesis and reduced polysome formation in A549 cells. This further caused an increasing binding of ribosomal protein S7 to MDM2 and a decreased interaction between MDM2 and p53. It is well known that various stress can activate p53 through disruption of p53-MDM2 interaction. In response to ribosomal stress, several RPs, including L5, L11, L23 and S7, can bind to MDM2 and block MDM2-mediated p53 ubiquitination and degradation. This results in p53-dependent cell cycle arrest and/or apoptosis. Thus, the current study highlighted the antitumor activity of rLZ-8, which inhibited lung cancer growth *in vitro* and *in vivo* [25].

In another study, the underlying mechanism for tumor metastasis suppression activity and increased survival rate in Lewis lung carcinoma cell-bearing mice of rLZ-8 was explained. Mechanistically, rLZ-8 induced focal adhesion kinase (FAK) inactivation which downregulated Slug by enhancing ubiquitination proteasome pathway (UPP)-mediated degradation of Slug. Consequently, E-cadherin expression was enhanced and cancer cell mobility was repressed. Nevertheless, MDM2-shRNA obliterated rLZ-8-enhanced Slug degradation. rLZ-8 may be useful as a chemotherapeutic agent for treating lung cancer. EMT process is an important process in tumor metastasis and Slug is a transcription factor that represses E-cadherin transcription and is a crucial event in EMT and tumor metastasis [21].

In the following year, the same group established that rLZ-8 induced cell cycle arrest and apoptosis by downregulating the expression of wild-type and mutated epidermal growth factor receptor (EGFR) and inhibiting EGFR downstream effectors, AKT and ERK-1/2 in lung cancer cells. The progression of malignant lung tumor is associated with activation of mutation in EGFR. During the study, it was also observed that binding of rLZ-8 to EGFR resulted in induction of EGFR autophosphorylation and triggered ubiquitination by stimulating EGFR/Cbl complexes formation. Thereby, causing degradation of EGFR [26]. Later, *in vitro* growth arrest and apoptotic function of rLZ-8 (FIP-glu) and FIP-

SN15 in human glioblastoma U-251 MG cells was also reported. FIP-SN15 is the recombinant DNA sequence generated by DNA shuffling technology between FIP-glu and FIP-gsi [8].

Progressively, the adjuvanticity of LZ-8 for HER-2/neu DNA vaccine against p185^{neu} expressing tumor murine bladder tumor cell line (MBT-2) in mice has also been demonstrated. HER-2/neu (p185^{neu}) is a transmembrane tyrosine kinase receptor and have been associated with tumor progression in certain neoplasms [27]. In this study, rLZ-8 stimulated mouse bone marrow-derived dendritic cells (DCs) via toll-like receptor 4 (TLR4) and augmented the ability of DCs to induce antigen-specific T-cell activation *in vitro* and *in vivo* (in a subunit vaccine model). The antitumor effect of DNA vaccine against MBT-2 tumor in mice was improved with cotreatment with rLZ-8. The mechanism for this amplified antitumor activity was due to the enhancement of vaccine-induced T-helper cells (Th1) and cytotoxic T lymphocytes (CTL) responses. Thus, this work emphasized an important application of rLZ-8 as a promising adjuvant for enhancing the therapeutic efficacy of DNA vaccines against tumors [28].

Antidiabetic activity of rLZ-8

In a recent study, anti-diabetic activity of rLZ-8 instreptozocin (STZ)-induced diabetic rats was described and the underlying mechanism was explained as well. Administration of rLZ-8 into STZ-induced diabetic rats for 3 months alleviated the clinical symptoms of type 1 diabetes (T1D) and dose-dependently diminished blood glucose, blood lipid and hemoglobin A1c (HbA1c) levels. There was a temporary but prominent elevation in insulin levels which protected against STZ-induced pancreatic tissue injury. Moreover, rLZ-8 significantly lowered TNF- α and IL-1 β levels and raised IL-10 levels in serum and pancreas. Furthermore, rLZ-8 treatment resulted in substantial increment in regulatory T-cells (Tregs) number and upregulated Fork head box P3 (Foxp3) expression thereby, reinstating the balance between inflammatory and anti-inflammatory cytokines [29]. The Tregs have been proven to maintain immune homeostasis and play a critical role in tissue regeneration after injury [30].

Similar effects of rLZ-8 were observed during a study, wherein, efficacy of rLZ-8 in curbing pulmonary inflammation was observed in OVA-induced asthmatic mice. In this study also, rLZ-8 remarkably downregulated Th17 cells and upregulated Foxp3⁺ regulatory T (Treg) cells. In bronchoalveolar lavage fluid (BALF), IL-17A level was decreased whereas, IL-10 level was increased. At the same time in the lung tissue, ROR γ t mRNA expression was diminished and Foxp3 mRNA level improved. Both, in murine lung tissue as well as cultured T-cells, rLZ-8 repressed signaling pathways of STAT3 as well as NF- κ B (P100/P52). Thus, rLZ-8 reduced pulmonary inflammation [31].

Therapeutic effects of rLZ-8 against tumor chemotherapy induced-neutropenia and leukemia

The recombinant protein has also been evaluated for its protective effects against cyclophosphamide induced-neutropenia [32] and leukopenia [33] in order to elucidate its potential to prevention of neutropenia and leukemia during tumor chemotherapy. Neutropenia is a clinical condition with an abnormally low levels of neutrophils in the blood and is associated with chemotherapy. In the study led by Lei et al., it was demonstrated that rLZ-8 promoted the differentiation of bone marrow hematopoietic stem cells (HSCs) into granulocytes by binding to colony stimulating factor 1 receptor. It also stimulated the mobilization of HSCs and the release of neutrophils from the bone marrow to peripheral blood by regulating the CXCR4-SDF1 axis [32].

The study pertaining to the therapeutic effect of rLZ-8 on mouse models of cyclophosphamide-induced leukopenia was designed in two ways- single-phase and multi-phase administration methods. In both the models, rLZ-8 treatment resulted in increment in the levels of neutrophils, lymphocytes and monocytes. Moreover, it elevated CD4+ T-cells percentage and also raised secretion of IL-3 and IL-4. Conclusively, the study established the fact that treatment with rLZ-8 was advantageous in improving immune dysfunction and immune system imbalance [33].

Anti-osteoporotic activity of rLZ-8

Current studies have also established the potential role of rLZ-8 in improving the clinical and pathological conditions associated with osteoporosis. In a study the efficacy of rLZ-8 was examined on osteoclast *in vitro* and bone resorption *in vivo*. Under *in vitro* conditions, receptor activator of nuclear factor kappa-B (RANK) ligand induced RAW 264.7 cells were allowed to differentiate into osteoclastic cells. These cells were then treated with various doses of rLZ-8 for seven days and differences in osteoclastic differentiation, apoptosis rate and gene expression were measured with respect to the control. Three dimensional-structured illumination microscopy analysis revealed that rLZ-8 entered and got accumulated gradually into the cytoplasm of RAW264.7 cells but very little quantity escaped into the nucleus.

Additionally, under *in vivo* conditions, retinoic acid was administered to female rats for 14 consecutive days to develop osteopenia changes. Simultaneously, different doses of rLZ-8 were administered to rats treated with retinoic acid to observe changes of bone mineral density, biochemical parameters and organ weight ratio. The results of the study suggested that rLZ-8 inhibited osteoclastic differentiation and promoted osteoclastic apoptosis by regulating RANK- tumor necrosis factor receptor-associated factor 6 (TRAF6) - c-Jun N-terminal kinase (JNK) signaling pathway. Thus, administration with rLZ-8 reversed loss of bone mass and improved ALP activity in osteoporotic rats. Low-to high-dose rLZ-8 treatments displayed little toxic effects on rat organs. Overall, the data advocated anti-osteoporotic effect of rLZ-8 [34].

Furthermore, Yang et al, have also reported therapeutic effects of rLZ-8 in glucocorticoids-induced osteoporosis (GIOP) rat model. The glucocorticoid dexamethasone (DEX) was injected intramuscularly into Wistar rats. During the study, it was found that rLZ-8 could prevent bone loss and improved structural deterioration in femurs of GIOP rats. This observation was confirmed by H&E staining performed for analyzing the amount and morphology of trabeculae. Furthermore, Western blot analysis of OPG and RANKL expression levels in femurs showed that rLZ-8 could increase OPG/RANKL ratio which consequently delayed the process of osteoclastogenesis. Thus, the results of this study emphasized on the fact that rLZ-8 could be developed as an anti-osteoporosis drug for both prevention and treatment of osteoporosis [35].

Conclusion

Ling Zhi-8 is a valuable fungal immunomodulatory protein both in its native (LZ-8) and recombinant form (rLZ-8). It has already established its potential role in ameliorating several disease and clinical conditions. Furthermore, the protein has demonstrated no or minimal toxicity as per reports until now. It is therefore feasible to employ LZ-8 and/or rLZ-8 as a potent drug for the prevention and treatment of diseases, especially those involving immunological responses such as cancer, autoimmune diseases, osteoporosis, diabetes, etc.

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SAVE THE ENVIRONMENT (STE) was founded and registered on 19 November 1990. In 1992 with the collaboration of WWF (India), the organization started working to combat arsenic poisoning problem of water in the arsenic prone areas of West Bengal. Since then STE has been involved in various projects related to combat arsenic problem in India.

Our Vision

To protect present and future generations from various environmental hazards.

Our Mission

To create awareness and motivation among rural communities & provide cost effective, energy efficient & environment friendly technologies.

Our Activities

Conducting interactive sessions, workshops/ seminars, awareness programs, field operations through projects, science fairs, posters & quiz competitions.

**Please join us and become part of our family
by enrolling yourself as Life Member of STE Family**

**Mail us at
info@stenvironment.org
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