Advances in Chemical Science & Biotechnology for Water Purification, Energy Production and Stress Management

Edited by

Kummari SV Krishna Rao

Faculty of Chemistry, Yogi Vemana University, India

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Preface

In recent years, there has been a substantial advancement in the science and technology due to potential applications of materials for the development of numerous scientific, industrial, ecological, and technological fields. Biology, medicine, pharmacy, agriculture, geology, food industry, and material science are the main fields which have been benefited from the great technological progress developed by chemical science and biotechnology.

The aim of this book is to present the innovative progress performed in the recent years in the field of chemical science and biotechnology with water purification, energy production and stress management applications. Different types of methods and materials are discussed in detail regarding design, fabrication, functionalization, and optimization in order to obtain desired properties. This book, entitled "Advances in Chemical Science & Biotechnology for Water Purification, Energy Production and Stress Management", contain 16 chapters, prepared by outstanding researchers from India, Ecuador, Saudi Arabia and USA.

Chapter 1, *Recent innovations in natural gums based adsorbents for the removal of toxic metal ions* prepared by *Sudha Vani et al.*, reports the updated information on the natural plant gums and their adsorptive capacity towards the toxic metal ions. Further studies of natural plant gums will be helpful for the discovery of possible biomedical devices in the form of hydrogels.

Chapter 2, *Recent advances in effective remediation of groundwater fluoride* prepared by *Sunitha* reports the recent advances and updated information on recent advances in defluoridation techniques. Adsorption, precipitation/coagulation, ion exchange, and membrane processes are the key defluoridation strategies that have been studied with varying degrees of success. Along with this, a variety of chemical, indigenous, and herbal materials are used. Precipitation is the most well-known and commonly used process, particularly at the community level. Adsorption can be carried out with high efficiency and cost effectiveness using locally available adsorbent materials. Ion Exchange extracts up to 90% of fluoride while keeping the water's taste and color. This work will be beneficial for researchers working toward further improvement in defluoridation technologies.

Chapter 3, Landuse and landcover analysis using remote sensing and GIS to study the change impact on water resources in parts of Porumamilla, YSR District, Andhra Pradesh, India prepared by Srinivasa Gowd et al., reports the updated information on the Landuse and Landcover Analysis. This chapter describes the Remote Sensing and GIS approaches which are helpful to analyse the temporal analysis of the Landuse and Landcover of our area of interest.

Chapter 4, *Assessment of heavy metal contamination in soils and groundwater at an industrial area, Bangalore, Karnataka, India* prepared by *Srinivasa Gowd et al.*, reports the updated information on the Heavy metals Analysis of soil and groundwater. This chapter describes that the soil resource of Bommasandra is chemically altering due to heavy traffic on groundwater and excess application of chemical fertilizers in agricultural soils and industrial soils and hence remedial measures at this stage are of utmost importance to safeguard the human population from heavy metal contamination and future health risk.

Chapter 5, Land use and land cover analysis of Mandavi river basin by using RS and GIS of Rayalaseema region, Andhra Pradesh, India prepared by Sudarsana Raju et al., reports the updated information on the Landuse and Landcover Analysis. This chapter describes the Remote Sensing and GIS approaches are helpful to analyses the temporal analysis of the Landuse and Landcover of our area of interest.

Chapter 6, *Advancements in polymer electrolyte membranes for fuel cell and drug delivery applications* prepared by *Sivagangi Reddy et al.*, reports the polymer electrolyte membranes (PEMs) of current interest, due to their physico-chemical interaction with the guest molecules. PEMs are capable to transport or permeate, adsorb and delivery of molecules, ions and other required reagents. This chapter provides basic concepts as well as the progress with regard to PEMs based science and technology of fuel cells and drug delivery.

Chapter 7, Advancement in hole transport materials and device architecture of perovskite solar cells prepared by Susmitha Kalvapalli et al. reports the information on the progression in potential hole transport materials for perovskite solar cells application. This chapter also describes the device architectures and working mechanism of perovskite solar cells, its advancement.

Chapter 8, *Microbial nanowires for bioenergy application* prepared by *Jaffer Mohiddin et al.*, reports the updated information on the microbial nanowires. This chapter also describes its applications in biology. Further studies will be helpful for the discovery of an alternative renewable energy in the form of electrical energy by a group of bacteria, Geobacter of the genus *proteobacteria*

Chapter 9, *Zeolite Remediate: A Multi Featured Green Technology*, prepared by *Raghu Babu* reports uses of zeolites in ore beneficiation of extraction of uranium from uranium plant waste water and from the groundwater around uranium mining area. It is efficiently used in agriculture industry as zeofertilizer and zeo soil conditioner. Also, they can be efficiently used in the mitigation of air pollution. The other very prominent use of zeolite is that it could be in the storage of high-volume gasses of LPG etc., at very low pressures even in the cylinders made of tough plastic.

Chapter 10, *Recent advancement in hyper-interconnected porous carbon materials* prepared by *Reddi Mohan Naidu Kalla* reports on new porous polymers which shows high surface area, modification of various functional groups, carbonization and highly stable materials; owing to these the porous polymer materials used for the huge applications such as gas absorption, energy storage, conversion of epoxide to cyclic carbonates, sensor technology, and various catalysis studies such as esterification's, heterocyclic compounds synthesis and dyes degradations.

Chapter 11, *Biological synthesis of nanoparticles from bacteria, fungi, algae and plants* prepared by *Subhosh Chandra et al.*, reports the latest information on biosynthesis of nanoparticles from bacteria, fungi, algae and plants. In addition, reports nanoparticles as antimicrobials. Advanced studies on nanoparticles characterization and applications of various nanoparticles certainly show the new avenues to the young researchers.

Chapter 12, *Therapeutic applications of Nigella sativa and its metal nanoparticles* prepared by *Anjum et al.* reports the updated information on the biomedical research of black seed plant. This chapter also describes the green synthesis of different metal and metal oxide nanoparticles using kalonji and their biological applications. Further studies will be helpful for the discovery of possible drug candidates using kalonji phytochemicals.

Chapter 13, *Impact of fungicides on soil enzyme activities* prepared by *Srinivasulu et al.* reports the up-to-date information on the soil enzyme activities as influences by fungicides in soils. This chapter also describes the effect of different doses of pesticides on soil enzyme activities in soils.

Chapter 14, *Mechanisms Involved in Microbes and Heavy metal Interactions in Bioremediation* prepared by *Mary Sandeepa* et al., reports the microbial mechanisms, which involve active efflux or sequestration with insoluble compounds or proteins, through which they may detoxify or metabolize the heavy meals like Zn, As, Cd Ni, Pd, Hg. This book chapter explains about the chemistry involved in different metal microbe interaction.

Chapter 15, *Biotechnology: A field of biological advancement with special reference to plant* & *animal agriculture and medicine* prepared by *Ramachandra Reddy* and *Abhijith Venkat*, details the role of biotechnology and its advancement in the fields of agriculture including transgenic plant and animal systems, and medicine. Especially this chapter focused in the areas of transgenic plant production including salinity, drought, disease, insect resistant plants through micropropagation and production of immunological molecules. Biotechnology of farm animals for improved growth, milk production, nutrition enhancement, reproduction and fecundity, environment protection, diseases resistant livestock, enhancement of fiber and hair were also emphasized in this chapter. Plantibodies and other biologically important compounds biological production and gene therapy are some of the medical advances of biotechnology discussed.

Chapter 16, *Proteomics studies in plants under abiotic stress tolerance* prepared by *Riazunnisa* reports updated knowledge on plant stress proteomes, this information is helpful for abiotic stress management in plants. This chapter also describes the critical role of proteins against stress leading to either tolerance or death of a plant.

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Recent Innovations in Natural Gums Based Adsorbents for the Removal of Toxic Metal Ion from Wastewater

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Abstract: Natural gums which are hydrophilic carbohydrate polymers of high molecular weights, generally composed of monosaccharide units joined by glucosidic bonds have been attracting many researchers. These are used in confectionaries, dairy products, beverages, as emulsifier in food products, petroleum and for oil-well-acidizing purpose in the industry. They also have pharmaceutical applications as demulcents, adhesives in pill manufacture and as emulsifying agents. These natural polysaccharides do hold advantages over the synthetic polymers, generally because they are nontoxic, less expensive, and freely available. They are also used for the removal of heavy metals from water. They are readily available, cheap and eco-friendly. The present chapter focuses on the application of gums particularly in the removal of heavy metals from water.

Key words: Natural Gums, Adsorption, Toxic Metal Ions, Green Technologies

1.1. Introduction

A brief introduction to the area of natural gums and their applications in the removal of toxic metal ions is incorporated in this chapter. Sorption with natural gums is considered to be an effective method for the extraction of metals because of the ease of handling, environmental safety, and high selectivity for metals

1.2. Toxic Metal ions

To a large degree, modern industry [electro plating, metal finishing, textile, storage batteries, lead smelting, mining, plating and the ceramic and glass industries] is responsible for contamination of the environment with toxic heavy metals and rare earth elements. Lakes, rivers and oceans are over whelmed with waste matter. Hence toxic heavy metal pollution has become serious [1]. Although various inorganic salts and trace elements are required to maintain normal good health, they can be toxic above threshold levels. The toxic effects of heavy metals present many risks to humans, particularly when present in water and food [2-4]. Even low concentration (ppb) of heavy metals in natural water supplies can have detrimental effects on wildlife and humans [5]. These metals cause various ailments [Table 1.1] such as dehydration, stomach ache, nausea, dizziness, lack of coordination in muscles, destroying the nervous systems of young children, lung irritation, eye irritation, skin rashes, vomiting, abdominal pain, lung insufficiency and liver damage [6-14].

Effective removal of toxic heavy metals from aqueous solution is fundamental to the environment conservation and public health [13]. And the recovery of metal ions from industrial effluents has led many research groups to investigate the possibility of using waste biomaterials for metal uptake [14,15]. Several methods exist to remove toxic metals from aqueous solution such as ion exchange, reverse osmosis, adsorption, complexation and precipitation [16,17]. Adsorption is considered as an effective and economical method for removal of pollutants from waste water [18]. The adsorption capacity of several low-cost-adsorbents has been investigated, mainly using biopolymers, which are obtained from renewable sources and adsorb metallic ions selectively [19].

Detection, analysis, and monitoring of water purity are critical to evaluating toxicity and pollutant levels. Currently, detection schemes require bulky, expensive instrumentation and componentry such as atomic absorption or fluorimetry [20]. Heavy metals are a major concern in potable water testing. Heavy metals are toxic even in low concentrations since they accumulate in living organisms.

Sudha Vani et al.

S.No.	Heavy metals	Toxic effects		
1	Cadmium	Renal failure, human carcinogen, osteomalacia, itai-itai		
		disease, weakens the bone, respiratory disease, gastrointestinal		
		diseases, birth defects, anaemia, proteinuria, high blood		
		pressure, sterility among males, inhibits the calcium control in		
		biological systems, hepatic toxicity and flu disorders.		
2	Chromium (VI)	Renal failure, skin and mucous membrane irritation, eczema,		
		geno toxic, alopecia, allergic dermatitis, hemolysis, lung		
		cancer etc.		
3	Lead	Renal failure, Delayed development in children both mental		
		and physical, affects the sense organs and circulatory system,		
		loss of voluntary muscle function, anemia, vomiting, high		
		blood pressure, nervous system disorders, mental retardation,		
		decreased hemoglobin synthesis, cardiovascular diseases,		
		cancer to the human body, disorders in bone metabolism and		
		reproduction etc.,		
4	Nickel	Anaphylaxis, lung cancer, hair loss, destroys red blood cells,		
		cause liver diseases, nephrotoxic		
5	Copper	Cancer in respiratory tract, lack of blood, gastro intestinal		
		distress, liver and kidney damage.		
6	Mercury	Hypersensitivity, fever, vomiting, neurasthenia, affects		
		joints in the human body, kidney disease, affects the muscle		
		movements, death, unconsciousness, abortion, skin cell death		
		in humans, inflammation of gums, painful extremities,		
		nervousness, affects the vision, memory loss		
7	Zinc	Creates dizziness, corrosive to skin and eye, zincpox, sweet		
		taste, throat dryness, cough, weakness, generalized aching,		
		chills, fever, nausea, vomiting		

 Table 1.1. Toxic effects of some heavy metals.

Biopolymers are non-toxic, selective, efficient and inexpensive and thus highly competitive with ion-exchange resins and activated carbon. The usage of carbohydrates in the adsorption of heavy metal ions from aqueous solutions is greatly increased in recent years. Many biopolymers such as sodium alginate, chitosan, and some fungi are known to bind metal ions strongly and could be used for toxic metal adsorption [21,22]. Alginates are natural polyelectrolytes that comprise a family of polysaccharides which contain 1, 4-linked β-Dmannuronic and α -L-guluronic acid residues arranged in a block wise, non-regular order along the chain. Cation specific affinity of the alginate gel and its fundamental physiochemical and rheological properties are determined by this arrangement. Coordination sites of two homo polymeric guluronic chains are aligned to accommodate divalent cations easily according to the well-known "egg-box model". Some studies showed that mainly ion-exchange is responsible for this, whereas other studies reported the sorption through complexation in addition to the ion-exchange [23-26].

1.3. Natural Gums

Natural gums are produced from woody plants either naturally from exudations are from cracks in the bark or damage to the bark by insects or animals or in seed coatings. Gum flow is also artificially induced by incisions in the bark. Waste products are stored as resins and gums in Sclerenchyma part. Sclerenchyma is a type of permanent tissue in plants that are produced from the matured collenchyma in plants.

1.3.1. Sources: The important gum yielding trees are Acacia nilotica (babul), A catechu(khair), Steruculiaurens(kullu), Anogeissuslatifolia (dhawra), Buteamonosperma (palas), Bauhinia retusa (semal), Lanneacoromandelica (lendia) and Azadirachtaindica (neem). Gums produced commercially mainly include: gum Arabic (Acacia senegal, Figure 1.3), gumghatti, (Anogeissuslatifolia), neem gum (Azadirachtaindica), gum karaya (Sterculia urens; Cochlospermumgossypium), Joel or Jingan gum (Lanneacoromandelica), and Mesquite gum (Prosopisjuliflora).

Natural gums are hydrophilic carbohydrate polymers of high molecular weights, generally composed of monosaccharide units joined by glucosidic bonds [27]. They are generally insoluble in oils or organic solvents such as hydrocarbons, ether, or alcohols. Gums are either water-soluble as in the case of gum arabic or absorb water and swell up or form mucilages by the absorption of large amounts of water (gum tragacanth) or disperse in cold water to give a Sudha Vani et al.

viscous solution or jelly [28]. On hydrolysis they yield arabinose, galactose, mannose and glucuronic acid. Gums are typical products of broadleaved trees and shrubs., The viscous, brittle nodule, which forms, can easily be removed by the hand. Uses of gums for domestic consumption and for sale to earn some cash are very common among the forest dwelling communities, particularly tribes in India. These are used as in confectionaries, dairy products, beverages, as emulsifier in food products, petroleum and for oil-well- acidizing purpose in the industry.

They also have pharmaceutical applications as demulcents, adhesives in pill manufacture and as emulsifying agents. Industrial uses are for adhesives, lithography, paints and inks. The major gum producing states in India are Andhra Pradesh, Madhya Pradesh, Chhattisgarh, Orissa, Maharashtra, Gujarat, Rajasthan, etc. Gum Arabic, Gum Ghatti and Gum Karaya are commercially important gums produced in India. Gums of commercial interest are also obtained from the fruit of the carob (Ceratoniasiliqua), Gum Mesquite (Prosopislatifolia) and Indian Squilled as from Urgineaindica.

1.3.2. Classification

Based on solubility in water gums are classified as : (1) Soluble: Certain gums dissolve in water to form transparent colloidal solution (e.g. Gum Arabic). (2) Insoluble: Gums such as gum tragacanth, gum karaya do not dissolve in water but swell up into sufficient amount to a jellylike mass. However, if sufficient of water is added they yield a thick transparent solution. (3) Partially soluble gums: Partially soluble gums first form a swollen jelly by dispersing in water and become solution on addition of more water. Mogador or Morocco gum (from Acacia gummifera) is an example of partially soluble gum.

Natural gums can be classified according to their origin. They can also be classified as uncharged or ionic polymers (polyelectrolyte).

- Natural gums obtained from seaweeds: Polyelectrolytes: Agar, Alginic acid and Sodium alginate, Carrageenan
- (2) Natural gums obtained from non-marine botanical resources:
 - a) Polyelectrolytes: Gum arabic, Gum tragacanth, Karaya gum
 - b) Uncharged Guar gum, Locust bean gum, Glucomannan, Tara gum
- (3) Natural gums produced by bacterial fermentation:
 - (a) Polyelectrolytes: Gellan gum
 - (b) Uncharged: Xanthan gum Agar

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1.3.2.1. Agar

Agar or agar-agar is a jelly-like substance, obtained from algae. Agar forms the supporting structure in the cell walls of certain species of algae (Gelidiumamansii), and which is released on boiling. These algae are known as a garophytes, and belong to the Rhodophyta (red algae) phylum. Agar is actually the resulting mixture of two components: the linear polysaccharide agarose (70%), and a heterogeneous mixture of smaller molecules called a garopectin (30%). Agar-agar is a natural vegetable gelatin counterpart available as dried strips or in powdered form alginic acid & sodium alginate an anionic polysaccharide distributed widely in the cell walls of brown algae, where through binding with water it forms a viscous gum empirical formula is $NaC_6H_7O_6$.

1.3.2.2. Carrageenan

Carrageenans are a family of linear sulfated polysaccharides that are extracted from red edible seaweeds. They are widely used in the food industry, for their gelling, thickening, and stabilizing properties. Their main application is in dairy and meat products, due to their strong binding to food proteins. There are three main varieties of carrageenan, which differ in their degree of sulfation. Kappa-carrageenan has one sulfate group per disaccharide, iota-carrageenan has two, and lambda-carrageenan has three.

1.3.2.3. Gum Arabic

Gum Arabic, (Acacia gum), is a natural gum consisting of the hardened sap of various species of the acacia tree. gumarabic is predominantly collected from two related species, namely Acaciasenegal and Acaciaseyal. Gum arabic is a complex mixture of glycoproteins and polysaccharides. It is the original source of the sugars arabinose and ribose, both of which were first discovered and isolated from it, and are named after it. Gum arabic is used primarily in the food industry as a stabilizer and it is edible.

1.3.2.4. Gum tragacanth

Gum tragacanth is a viscous, odourless, tasteless, water-soluble mixture of polysaccharides. Obtained from sap that is drained from the root of the plant (goat's thorn and locoweed) and dried. used in foods as an emulsifier, thickener, stabilizer, and texturant additive. A vegetable gum produced as an exudate by trees of the genus Sterculia.

1.3.2.5. Gum karaya

Gum karaya is an acid polysaccharide composed of the sugars galactose, rhamnose and galacturonic acid. It is used as a thickener and emulsifier in foods. Also used to adulterate Gum

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tragacanth due to their similar physical characteristics. The primary structure has been shown to be composed of D-glucuronic acid, D-galacturonicacid,D-galactose and L-rhamnose, in proportions differing according to the quality, type of gum and origin . It forms extremely strong adhesives with small amount of water. It is widely used in pharmaceutical and dental adhesive preparations. It is nontoxic and is generally considered not to be metabolized in humans. It is used in several industries due to its low solubility in water and low cost. It has been used in printing and textile industry, in pharmaceutical and medicinal preparations such as cosmetics, lozenges, jellies, emulsions, lotions, sprays, pastes and laxatives and to control diarrhea. Gum Karaya from Sterculiaurens, S. villosa (India), S. setigera (Africa), which provides the raw material for xatives and laxatives.

1.3.2.6. Guar gum

Guar gum is obtained from guar beans which has thickening and stabilizing properties useful in the food industry. The guar seeds are dehusked, milled and screened to obtain the guar gum. a polysaccharide composed of the sugars galactose and mannose. Used in baked goods, dairy products, condiments, soups, instant oatmeal, sweet desserts etc.

1.3.2.7. Locust bean gum

A galactomannan vegetable gum extracted from the seeds of the carob tree. It is used as a thickening agent and a gelling agent in food industry. occurs as a white to yellow-white powder. consists chiefly of high-molecular-weight polysaccharides, composed of galactose and mannose units combined through glycosidic linkages. The bean, when made into powder, is sweet-with a flavour similar to chocolate.

1.3.2.8. Glucomannan

Glucomannan is a water-soluble polysaccharide that is considered a dietary fiber. The component sugars are β -(1 \rightarrow 4)-linked D-mannose and D-glucose in a ratio of 1.6:1. It is a hemicellulose component in the cell walls of some plant species. Glucomannan is a food additive used as an emulsifier and thickener.

1.3.2.9. Tara gum

Tara gum is a white, nearly odourless powder that is produced by separating and grinding the endosperm of *T. spinosa* seeds. Tara gum consists of a linear main chain of (1-4)- β -D-

mannopyranose units attached by (1-6) linkages with α -D-galactopyranose units. Used in the preparation of confectionery foods, such as ice cream.

1.3.2.10. Gellan gum

Gellan gum is a watersoluble anionic polysaccharide produced by the bacterium Sphingomonaselodea. Consists of two residues of D-glucose and one of each residue of Lrhamnose and D-glucuronic acid. It is widely used as a thickener, emulsifier, and stabilizer.

1.3.2.11. Xanthan gum

Xanthan gum is a powerful thickening agent, and also has uses as a stabilizer to prevent ingredients from separating. It can be produced from a range of simple sugars (glucose, sucrose, or lactose) using a fermentation process. Xanthan gum derives its name from the strain of bacteria used during the fermentation process, Xanthomonascampestris used in salad dressings and sauces, ice creams etc.

1.3.2.12. Gum Ghatti

Gum ghatti (Anogeissuslatifolia) or Indian gum is a complex non-starch polysaccharide. It has been widely employed in food, pharmaceuticals, paper and other industries cation and thickening primarily due to its excellent emulsifying property. It is an amorphous, translucent exudate of the Anogeissuslatifolia tree of the Combretaceae family. The tree is quite large and is found in the dry, deciduous forests of India. Gum Ghatii has been used in a range of emulsions, suspensions and as a sizing agent in the paper industry. It is used to emulsify petroleum and non-petroleum waxes to form liquid and wax paste and wide use in the paper industry as emulsions, which coatings and as moisture barriers. Oil-well acidizing is another use for gum ghatti. The gum is moistened with water insoluble non-aqueous liquid that is inert both to gum and to the acid solution. This gum has unique commercial position because of its viscosity, intermediate between those of gum Arabic and gum karaya. When used in bulk laxatives, gum karaya is usually processed so that it is 8 to 30 mesh in size. These coarse gum particles absorb water and swell to 60 to 100 times their original volume, forming a discontinuous type of mucilage. This type of mucilage is very effective as a laxative. Another important pharmaceutical application of gum karaya is as a denature adhesive. The powder gum is usually dusted onto dental plate, and it swells when it touches the moist surface of the mouth. The rapid swelling of the gum particles, their relative insolubility, and their unusual resistance to bacterial and enzymatic breakdown make the gum suitable for this use. Sudha Vani et al.

Natural gums have pharmaceutical applications as demulcents, adhesives in pill manufacture and as emulsifying agents. Their use as biodegradable polymeric materials to deliver bioactive agents has been hampered by the synthetic materials. These natural polysaccharides do hold advantages over the synthetic polymers, generally because they are nontoxic, less expensive, and freely available. Natural gums can also be modified to have tailor-made materials for drug delivery systems and thus can compete with the synthetic biodegradable excipients available in the market.

Various plant seeds and gums are also used for the removal of heavy metals from water. They have great potential in removal of heavy metals due to their biosorption properties. They are readily available, cheap and eco-friendly. They remove both suspended and dissolved matters simultaneously. [29].

1.3.3. Gum based composites for adsorption studies:

The stem of the Moringaoleifera tree exudes a gum which is initially white in colour but changes to reddish brown or brownish black on exposure to sunlight. It is sparingly soluble in water but swells in contact with water giving a highly viscous solution. It is a polyuronide consisting of arabinose, galactose and glucuronic acid in the proportion of 10:7:2; rhamnose is present in traces [Figure 1.1] [31-35]. The characterisation studies of Moringaoleifera gum confirmed that, the gum was found to be hygroscopic and organoleptically acceptable. The pH was found to be 5.77.

Various parts of Moringaoleifera, i.e. fruit, seed, leaf, flower, bark and root, have been used extensively in traditional medicine. One of these usages is the removal of heavy metal toxicity. Besides, this plant is reported to have various chemical constituents that have the biological activities including the depletion of metal concentration and the antioxidant properties that are potential to reduce the oxidative stress induced by heavy metal [30]. The Moringaoleifera seed polyelectrolytes can be recommended to be a good coagulant for turbidity and heavy metal removal [31,32]. Moringaoleifera seed have the ability to chelate the 4-Nitroaniline (4-NA), a toxic, mutagenic and carcinogenic aromatic nitro compound [33]. 71.42 and 66.67 (mg g⁻¹) for the adsorption of metal ions in order of Co(II) > Zn(II) > Cr(III) > Cd(II). The experimental results demonstrate that the above selective order of adsorption capacity is due to formation of stable chelating ring between the bidentate amidoxime ligand and metal ion [Figure 1.3].



Figure 1.1. Polysaccharide structure of *Moringaoleifera*gum.



Figure 1.2. Illustration of synthetic procedure for preparation of ITG-g-PAO and mechanism of metal ion adsorption [36]. [Courtesy of Arameh Masoumi, Mousa Ghaemy, (2014). Removal of metal ions from water using nanohydrogel tragacanth gum-g-polyamidoxime: Isotherm and kinetic study Carbohydrate Polymers 108: 206-215.]



Figure 1.3. Schematic diagram showing the cross-linking of a heavy metal ion within MOGC composite coagulant [37]. [Courtesy of Ravikumar K & Udayakumar J (2019) Moringa oleifera gum composite a novel material for heavy metals removal, International Journal of Environmental Analytical Chemistry.1-41]

Ravikumar and Sheeja, (2013) conducted the analysis of the heavy metals cadmium, copper, chromium and lead, before and after treatment of water with Moringaoleifera seed coagulant. The results showed that the percentage removal by Moringa seeds were 95% for copper, 93% for lead, 76% for cadmium and 70% for chromium. This is due to the presence of some functional group such as hydroxyl, carboxyl amines, phenolic, methoxyl, hydroxyl aliphatic groups [4]. Ravikumar K and udayakumar investigated the chances of making sustainable materials and processes with composite coagulants and adsorbents made of clay and Moringaoleifera. They have removed heavy metals by coagulo-adsorption and double filtration processes. Arameh Masoumi and Mousa prepared new biosorbent by grafting polyacrylonitrile onto Iranian tragacanth gum (ITG), a naturally and abundantly available polysaccharide, and subsequent amidoximation in the presence of hydroxylamine hydrochloride. This nano-hydrogel with amidoxime functional groups [C(NH₂) NOH], named polyamidoxime-g-tragacanth (ITG-g-PAO), was characterized and used for the removal of metal ions from aqueous solution. Functional groups $[C(NH_2) NOH]$ help for the removal of metal ions from aqueous solutions [Figure 1.2]. The maximum adsorption capacities (Q_m) of ITG-g-PAO as obtained from Langmuir adsorption isotherm were found to be 100.0, 76.92,

Gum kondagogu (GK) modified magnetic iron oxide nanoparticles were synthesized, characterized and successfully exploited for the removal of hazardous metal cations by P. Saravananetal [46]. The maximum decontamination of toxic metal cations removed by the GK-MNP was observed at pH5.0 \pm 0.1. GK modified magnetic iron oxide nanoparticles (MNP) have been prepared by co- precipitating Fe²⁺ and Fe³⁺ ions using ammonia solution in presence of GK bio macromolecules. The removal efficiencies for a variety of metal cations by the GK-MNP were determined quantitatively in the order: Cd²⁺>Cu²⁺>Pb²⁺>Ni²⁺>Zn²⁺>Hg²⁺ at a pH of 5.0 \pm 0.1 and at a temperature of 30.0 \pm 1.0 °C. A maximum of 106.8 mg/g and a minimum of 35.07 mg/g adsorption capacities were observed for the Cd²⁺ and Hg²⁺ ions, respectively using the Langmuir isotherm model. This study demonstrates that the biosorption (%), adsorption capacity (mg/g) and equilibrium biosorption time of native gum can be effectively improved by incorporating iron oxide nanoparticles in the gum network.

Magnetic nanocomposites based on natural biomacromolecules such as gum Arabic and chitosan have been successfully demonstrated as bio-sorbents for toxic metal ions [47,48]. Along these lines, herein we explore the biosorbent capabilities of GK-a novel biopolymer derived from Cochlosper mumgossypium tree [49,50]. Extensive research work carried out by P. Saravanan group exemplifies the salient morphological, physico-chemical, structural, rheological, pharmaceutical and emulsifying properties of this gum [51-54]. This gum is rich in acidic sugar residues such as, galacturonic and glucuronic acids, which accounts for 52% of the total carbohydrates, rest being accounted by neutral sugars such as glucose, galactose, rhamnose and arabinose. In addition, GK also contains tannins, proteins and minerals. Based on the spectroscopic characterization, the probable structural feature assigned to GK was $(1\rightarrow 2)\beta$ -D-Galp, $(1\rightarrow 6)$ - β -D-Galp, $(1\rightarrow 4)\beta$ -D-GlcpA,4-O-Me- α -D-GlcpA, $(1\rightarrow 2)\alpha$ -L-Rha and $(1\rightarrow 4)\alpha$ -D-GalpA.

Besides, it was shown that the native gum itself can be used as a potential biosorbent for removal of toxic metal contaminants. In order to further improve their adsorption capacity and enhance the separation rate, here in authors propose to modify the native gum in the form of magnetic nano-adsorbent by functionalizing the gum network with the iron oxide nanoparticles.

Guar gum is a polysaccharide composed of galactomannan units. Each unit of galactomannan consists of two units of β -D-mannopyranosyl joined via 1,4-linkage; with one unit of α -D-**Sudha Vani et al.**

galacto pyranosyl residue joined by 1-6-linkage as side chains. Hydrogel of guar gum has been used to remove Cu⁺² in aqueous solution. Some workers modified guar gum with multiwalled carbon nanotubes, magnetic iron oxide nanoparticles, methylmethycrylate, polyacrylamide or silica for the removal of toxic dyes (neutral red and methylene blue), Cr⁺⁴ and Cd⁺² from aqueous solution. It was observed that nano identities are also toxic to our environment; leading to chances of further environmental contamination due mainly to difficulty in their separation. It was, therefore, considered worthwhile to incorporate nano ZnO particles with guar gum, which may be easily separated after adsorption process.

Tabrez A. Khan et al. reported the adsorption efficiency of guar gum-nano zinc oxide (GG/nZnO) biocomposite, dynamics and thermodynamics of the uptake of Cr⁺⁴ ions from aqueous solution. The low contact time (50 min.) and adsorbent dose (1.0 g/L) together with high removal efficiency (98.63%) indicated that GG/nZnO biocomposite is a useful adsorbent for rapid and effective removal of Cr^{+4} from aqueous solution. At pH 7, 98.63% Cr(VI) was removed with a contact time of 50 min. and an adsorbent dose 1.0 g/L. The maximum adsorption capacity calculated from Langmuir adsorption isotherm was found to be 55.56 mg/g at 303 K. The adsorption data best followed the Langmuir and Freundlich isotherm models. The pseudo-second order kinetic model described the data better than pseudo-first order, which is evident from the high correlation coefficient values and close agreement between experimental and calculated qe values. Thermodynamic parameters (ΔG° and ΔH°) suggested the adsorption process to be spontaneous and endothermic. The guar gum-nano zinc oxide (GG/nZnO) biocomposite is inexpensive and advantageous for the removal of Cr⁺⁶ from aqueous solution due to its small adsorption time, good adsorption capacity and ability to work at pH 7; a natural pH of most water bodies. This elucidates the practical applicability of this adsorbent in the removal of Cr^{+6} from natural waterbodies.

Gum kondagogu is a branched and acidic polysaccharide obtained as a mixed calcium, magnesium, sodium and potassium salt. It has a high content of uronic acid and medium molecular mass. The morphological, physicochemical, structural and rheological properties of gum kondagogu have been established in the recent past. The toxicological evaluation of gum kondagogu had established that, this gum was non-toxic and has potential application as a food additive. The pharmaceutical application of gum kondagogu were reported recently. Solution and conformational properties of gum kondagogu has also been investigated. Non-food applications of gum kondagogu are yet to be explored as a natural

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biosorbents. Even though, many plant-derived materials have earlier been explored as biosorbents, the natural exudate gums are yet to be evaluated for their potential in biosorption of toxic heavy metals, contaminating the environment. Gum kondagogu is an acidic gum with high content uronic acid and the major functional groups identified in the gum are hydroxyl, acetyl, carbonyl and carboxylic groups. The zeta potential of native gum was determined to be 23.4 mV, indicating that it contains negatively charged groups [55-57]. Previously, it was reported that the major binding groups responsible for metal biosorption are hydroxyl, carboxyl, carbonyl, thiol, amine, phosphonate and phosphodiester. Interestingly, gum kondagogu fulfils the requirements as a biosorbents as it contains many of the functional groups identified earlier that are involved in metal binding. Thus, this gum has a potential application as a matrix for toxic metal biosorption from aqueous media.

Native sago starch, isolated from sago palm, draws innumerable attention as a promising natural polymer. As such, this polysaccharide was chosen as it is one of the rich sources for low-cost sorbent that also biodegradable. CMSS have been constantly used as a natural polymer in hydrogel synthesis due to the advantages of low rates of retrogradation, increase thermal stability and hydrophilic functional group [58,59]. However, despite the favourable key structure of carboxylic COO⁻ groups that can be bind to divalent metal ions, yet CMSS has low mechanical strength and instability in the gel form [60]. A chemically crosslinked hydrogel is crucial for forming a permanent gel with high porosity which plays prominent roles to enhance pollutant sorption [61].

Amyrah Auni Keirudin et al. synthesized CMSS (carboxymethyl sago starch)-based hydrogel by crosslinking with citric acid via esterification and then applied as a metal sorbent to overcome excessive heavy metal pollution [Figure 1.4]. The sorption capacity of Pb²⁺, Cu²⁺, Ni²⁺and Zn²⁺ onto CMSS/CA hydrogel were 64.48, 36.56, 16.21, 18.45 mg/g, respectively. Their experiments demonstrated that CMSS/CA hydrogel has high selectivity towards Pb²⁺ in both non-competitive and competitive conditions. As such, the usage of low-cost and biosorbent like CMSS/CA hydrogel is crucial for industries as it brings benefits in terms of operation cost and environmentally friendly [61].

Also, the sorption behaviour of CMSS/CA hydrogel was discussed with kinetics, isotherm and thermodynamics studies. Moreover, the selectivity of sorbent towards different heavy metal ions and desorption of heavy metal ion were also studied.

The sorption of heavy metal ions depends on the nature of charges of hydrogel since it involved ionic interaction between the negative charge from the carboxylate group of -COONa in hydrogel and the positive charge of divalent metal ions [62]. Figure 1.5 describes the proposed mechanism of ionic interaction for the sorption of metal ions on the active sites of the CMSS/CA hydrogel.

At pH 1, the sorption capacity of CMSS/CA hydrogel on Pb²⁺, Ni²⁺ and Zn²⁺ was zero, while the sorption capacity of Cu²⁺ was only 1.8 mg/g. At low pH, the concentration of H⁺ was high and led to active sites of -COONa in carboxymethyl protonated into COOH and generated competition between H⁺ and metal ions for the sorption sites. Therefore, the reduction of ligands for metal ions binding caused inhibition in metal ion sorption by the hydrogel [62]. Therefore, the sorption capacities of the CMSS/CA hydrogel at pH 1 and 2 were low. As the pH increased from pH 2 to 5, all the heavy metals showed a similar trend, where the sorption capacity gradually increased. At this stage, the concentration of hydrogen ions was low and led to carboxyl groups deprotonated into carboxylate ion (COO⁻). Hence, the ligand sites were available and the heavy metal ions no longer required to compete with H⁺ ions to form bonds with carboxylate ions in hydrogel.

By comparing the sorption capacity [Table 1.2] of CMSS/CA hydrogel with the maximum sorption capacity of the previous studies, the CMSS/CA hydrogel noticeably shows high sorption efficiency towards the Pb^{2+} . Thus, the CMSS/CA hydrogel can be considered as a good alternative to be employed as bio-sorbent to remove heavy metal ions in wastewater treatment.

As such, the removal percentage of Cu^{2+} was higher compared to Ni^{2+} and Zn^{2+} due to complexes that were formed between copper and the ligands which is most stable in accordance with Irving-Wiliam's series. The Irving-Wiliams series refer to the stability of complexes formed by first-row transition metal ions which suggest the order: $Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$ (Table 1.2). On the other hand, the selectivity study showed that Pb^{2+} was the most selective metal ion owing to a higher tendency to lose its electron and binds with active sites of the CMSS/CA hydrogel compared to other metal ions. TTPCA-TKP resin has a structure which facilitates the adsorption of heavy metals because it has C=O carbonyl groups, C-H stretching, O-H stretching, and -OH groups. So the resin has demonstrated outstanding removal capabilities for heavy metal ions from effluent by chelating, cross-linking and cation exchange **Sudha Vani et al. 15**



Figure 1.4. Schematic diagram describing the preparation steps for the synthesis of CMSS/CA hydrogel [60]. [Courtesy of Sri Norleha Basri, Norhazlin Zainuddin , Kamaruddin Hashim ,Nor AzahYus of (2016) Preparation and characterization of irradiated carboxymethyl sago starch-acid hydrogel and its application as metal scavenger in aqueous solution. Carbohydrate Polymers,138:34-40]



Figure 1.5. Proposed mechanism of ionic interaction between the active site of CMSS/CA hydrogel with metal ions [61]. [Courtesy of Keirudin, Amyrah & Zainuddin, Norhazlin & Yusof, Nor. (2020). Crosslinked Carboxymethyl Sago Starch/Citric Acid Hydrogel for Sorption of Pb²⁺, Cu²⁺, Ni²⁺ and Zn²⁺ from Aqueous Solution, Polymers. 12 : 2465]

Sorbent	Heavy Metal Ion	Sorption Capacity (mg/g)
Gellan Gum/Graphene Oxide	Zn ²⁺	120.48
Porous starch xanthate	Pb ²⁺	107.36
Porous starch citrate	Pb ²⁺	48.30
Carboxymethyl Cellulose/sodium	Pb ²⁺	0.0036
styrene	Cu^{2+}	0.0252
sulfonate	Ni ²⁺	0.0267
	Zn^{2+}	0.0303
Crosslinked carboxymethyl corn starch	Pb ²⁺	76.53
Carboxy methyl sago starch-lactic	Pb ²⁺	56.08
acid	Cu ²⁺	17.19
Crosslinked carboxymethyl konjac	Cu ²⁺	25.50
glucomannan	Pb^{2+}	29.20
Oil palm bio-waste/Chitosan/ multi- walled carbon nanotubes (MWCNTs)/ Polyvinyl alcohol	Pb ²⁺	30.01
CMSS/CA	Pb ²⁺	64.48
	Cu^{2+}	36.56
	Ni ²⁺	16.21
	Zn^{2+}	18.45

Table 1.2. Comparison studies on the sorption of the heavy metal ions.

Meenakshi Choudhary et al. have synthesised chemically modified TKP tamarind resin and applied for the removal of heavy metal ions from effluent stream (Scheme 1.1.). Proline group has been incorporated into TKP (tamarind kernel powder) with the help of cyanuric chloride in the presence of dioxane medium. It serves the purpose of both flocculants as well as selective ion binders for heavy metals.

TTPCA-TKP resin was successful in reducing the toxic metal ion concentration to the safer limit. This resin had good removal efficiency for lead and iron ions. The polysaccharide based. The TKP-silica nanohybrid material was synthesized through TKP templated base catalyzed polymerization of TEOS. Due to the formation of nanostructures, a large number of free sorbent sites are available at the hybrid which makes the material efficient in Hg(II) binding.

Guar gum is obtained from the beans of the Cyamopsis tetragonoloba plant. Chemically, it is an exopolysaccharide composed of galactose and mannose. Guar gum has been used as an adsorbent for Hg, Cr, and Pb (Pandey & Ramontja) ions removal. Anushree Srivastava explored the utility of guar gum for the removal of fluoride from aqueous solution. The removal capacity of guar gum powder fluoride was found to be 98% with 71.2 mg/g at optimal experimental condition. The negative values of ΔG° and ΔH° indicate the spontaneous and exothermic nature of adsorption respectively. ΔH° value suggests that the adsorption process occurred due to chemisorption. The negative value of ΔS° suggests the decrease in randomness at the solid-liquid interface. Guar gum is eco-friendly low-cost biosorbent that has been used for removal of other metals as well as dye from solution. But Anushree Srivastava used guar gum based hydrogel beads for removal of fluoride.



Scheme 1.1. Schematic representation of Hg(II) sorption by the nanohybrids (A) TH; (B) TH1 [63]. [Courtesy of Vandana Singh & Pramendra Kumar (2011) Design of Nanostructured Tamarind Seed Kernel Polysaccharide-Silica Hybrids for Mercury (II) Removal, Separation Science and Technology, 46:5, 825-838].

1.4. Conclusion

As natural gums and gum composites are eco-friendly, cost effective and nontoxic they can be used for application in macro level treatment systems. A lot could be achieved if the potentials locked up in these plant materials are harnessed by further research, and used to tackle the problem of heavy metals in water. Future studies however required not only for effective removal of toxic heavy metals, but also dyes from aqueous solutions to public health.

Conflict of Interest

The authors declare that they have no conflicts.

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Recent advances in Effective Remediation of Groundwater Fluoride

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Abstract: Fluoride is generally referred to as a double-edged sword as dental caries are related to dental caries, but enormous intake causes dental and skeletal fluorosis. Fluoride in ground water comes due to dissolution of natural minerals in the rocks, soils into which water interacts. Toxic fluoride concentration in drinking water is noted in several areas around the world and millions of people depend on groundwater with range of concentrations exceeding the World Health Organization (WHO) guideline level of 1.5 mg/L. In India, 65 million individuals are believed to be affected by endemic fluorosis. With a proposal to resolve the problem, the Rajiv Gandhi National Drinking Water Mission has been established to monitor fluorosis. Nalgonda technique, Prasanti activated alumina technology, UNICEF in India using defluoridation based on households have been developed. A simple and economical domestic defluoridation process was developed by various researchers on the basis of findings and comprehensive investigations. This paper seeks with recent techniques on defluoridation studies using traditional and unconventional materials and to compile the various pros and cons of these defluoridation techniques, including prashanti activated alumina technology, membrane method, water treatment residues (WTR), Fe₃O₄/Al₂O₃ nanoparticles adsorption using red mud, clay, bauxite, clay, Mentha longifolia.

Key Words: Emerging trends, Defluoridation, Ground water, Fluorosis, India

2.1. Introduction

Entry to healthy drinking water is a fundamental human right and one of the most important indicators of development. Groundwater is the most valuable natural resource and national asset, and it has taken a predominant role in the overall water resource. Groundwater is significant and invariable sources of drinking water around the globe [1]. It is estimated that 1.5 billion people across the world rely on groundwater for drinking and irrigation. Groundwater provides about 80% of all agricultural water used for domestic purposes in India, with about 39 million hectares irrigated by 45 percent of all agricultural water. Groundwater quality data demonstrates rock geological history, groundwater recharge, discharge, climate, movement, storage. Groundwater is India's main water supply for domestic, irrigation, and industrial uses. Groundwater is an effective tool for determining pollution levels and plays a pivotal role in many aspects of the food chain [2-4].

Due to a shortage of surface water in India, most of people rely on groundwater for a variety of purposes, including drinking, domestic, irrigation, and industrial. Due to this, the availability of safe drinking water is essential for human development [5]. Groundwater is the ultimate natural resource and a vital national commodity, and it has taken on a significant role in the overall water resource. However, rapid urbanization and industrialization have resulted in environmental pollution and health effects due to increasing population, new technical advances, and the depletion of groundwater resources. Owing to a lack of effective waste management methods, many anthropogenic activities and their byproducts have contaminated surface and subsurface water. Water quality is influenced by effluents from industries, agriculture, and horticulture transportation. Hence, the quality of drinking water is critical in a variety of health concerns.

Contamination of Fluoride in ground water has been an important public health concern around the world for the past two decades. Because of their importance, such work has been taken up all over the world in arid and semi-arid regions. Greater fluoride concentration in groundwater is one of the main health consequences in several countries. Fluoride levels are higher in greater than 23 countries around the world, especially in places like India, Eastern Africa, Central Africa, China, and South America [6-9]. Numerous groundwater quality related work involving fluoride have been conducted in India, especially in arid and semi-arid regions [10-12]. Groundwater is majorly used for drinking and agricultural practices in South India's semi-arid zone, and several quality studies have been conducted [13-27]. The concentration of fluoride is governed by a variety of factors, such as availability of fluoride-bearing rocks, rock composition, the mechanism of rock water interaction, evaporation, evapotranspiration, the appearance of aluminium fluoride ions, beryllium, ferric acid, and a series of mixed fluoride hydroxide cofactors [28]. Fluorides are found in three major forms: fluorspar or calcium fluoride (CaF₂), apatite or rock phosphate (Ca₃F(PO₄)₃), and cryolite (Na₃AlF₆)[10], with higher fluoride dosages (1.5 mg/L) and fluoride concentrations (>3.0 mg/L) required for normal bone mineralization and dental enamel formation [13].

Fluorine is the lightest of the halogens and the 13th most abundant naturally occurring element in the Earth's crust. Since it is the crucial electronegative and reactive of all other elements, Elemental fluorine is only present as fluoride mineral complexes in nature. Fluorides make up 0.06-0.08% of the Earth's surface, but they have a low average abundance (300 mg kg-1); [6]. Moreover, unlike any of the other halogens, the bulk of fluoride concentration in the Earth's crust comes from lithology, with just a limited amount originating from other sources like air, seawater, and other anthropogenic practices [10]. Fluorine is both the most electronegative and reactive of all known elements: electronegativity 4.0; It has a 626 g/g abundance in the continental crust [21]. Fluorine is rarely occur as free in nature, but it is commonly found as the fluoride ion in minerals (F⁻). The presence of fluorine in groundwater has sparked global interest owing to its major effect on human physiological conditions [14]. Fluorine is found in natural waters primarily as the free fluoride ion, while fluoride complexes of Al, Be, B, and Si can also be found under certain prevailing conditions. Fluoride ingestion into the human body from potable water at a concentration of 1 mg/l facilitates bone growth and arrests dental caries. The World Health Organization [5] framed a maximum tolerance limit for fluoride level in drinking water at 1.5 mg/l.

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2.1.1. Public Health Importance

Fluoride not only has remarkable chemical properties, and also has physiological properties that are important to human and animal health. Fluoride ions can inhibit or activate enzymatic processes even at very low concentrations. Phosphatase, lipase, and urease enzymes are all inhibited by it. Fluoride has been shown to interact with thyroid function (Teotia et al, 1972). On the other hand, it is found to be useful in the natural phase of mineralisation of hard tissues, such as bones, also in trace quantities. Fluoride has been shown to interact with thyroid function (Teotia et al, 1972). On the other hand, it is found to be useful in the natural phase of mineralisation of hard tissues, such as bones, also in trace quantities. Fluoride in drinking water up to 1.0 mg/l is advantage to children's health because it facilitates the production of cariesresistant dental enamel. Fluoride in drinking water above 1.0 mg/l has been shown to be detrimental to human and animal physiology, aside from a few beneficial effects. The toxic effects on teeth include chalky white patches, pitting, and chipped off tips, as well as premature tooth loss (Fig:2.1). Skeletal defects and joint pain occur at concentrations greater than 2-3 mg/l. Ingestion of the ion for an extended period of time causes spinal weakness and kyphosis (Stooping forward). Symptoms of dental and bone fluorosis include being unable to squat and being bedridden for the remainder of one's life (Jolly et al, 1970). In addition to humans, cattle are affected by fluoride ion ingestion. The seriousness of the fluorosis issue is exacerbated by the fact that skeletal changes are almost often permanent and the condition is incurable.



Figure 2.1: Dental Fluorosis

2.2. Methods

2.2.1. Fluoride Ion Selective Electrode

The use of an ion selective electrode for fluorides is the noted popular electro analytical approach in determining fluoride ions in water solutions. The fluoride selective electrode is extremely sensitive, with a temperature range of 0 to 50°C. In commercial use, electrodes with standard homogenic membranes made from fluoride lantana (LaF3), which were first denoted by Frant and Ross, are commonly applied for potentiometric analysis of fluoride ions (Frant and Ross, 1966; Frant, 1994).

2.2.2. Theoretical structure

The potentiometric approach is focused on possible measurements to determine a galvanic element's electromotive power. Ion selective electrodes (ISEs), that are capable of rapid and selective measurements of analyte levels are almost exclusively used in direct potentiometric determinations.

2.2.3 Materials and Processes

NaF dried for 1 hour at 100°C. The composition of liquid NaF is uncertain. Fluoride ISE with KCl (7.55 g) and Ag/AgCl or SCE for reference electrode. A multimeter or pH meter is required that can show mV potentials is needed (Figure. 2.2).



Figure. 2.2: Ion selective electrode (Orion 4 star ion meter, Model: pH/ISE).

The fluoride electrode is used to perform a simple, inexpensive, and accurate potentiometric determination of fluoride content in solutions. The use of TISAB buffer eliminates any negative interfering effects that could affect the process of determination. The fluoride content of samples is measured using direct potentiometry and, in the case of extremely low values, the normal addition process. When it came to determining fluoride ions in water, Method ISP proved to be more efficient, fast, and reliable. It also has an advantage over other analytical methods in that it is non-destructive and allows samples to be used for other purposes. The most popular and convenient methods of fluoride and chloride ion determination tend to be electro analytical methods based on potentiometry with ion-selective electrodes. Because of their high selectivity, precision, and low detection limits, fluoride selective electrodes can be used to assess fluoride concentrations in drinking water. The study's benefits include a fast analysis time, the elimination of sample pretreatment, the measurement system's simplicity, and a relatively low instrument cost.

2.3. Technologies of Defluoridation

To comprehend the environmental effects of altering fluorine mechanisms, there is an emerging trend to first comprehend fluorine's natural cycling. This section summarizes about this element, its environmental mechanisms, actions of its diverse types. Although this topic seems to be unrelated to fluoridating a public water source, it will provide insight into the chemistry and reactivity of fluorine in natural environments, as well as possible reactions with pipes and additives in the water supply. It also considers what happens to the element after it is released into the environment through wastewater treatment plant effluent. Fluorine (F) is a halogen with an atomic mass of 18.998, an atomic number of 9, and a valence of one. In its pure form, it is a diatomic gas (F₂). Fluorine is the most electronegative element known, and it is a very strong oxidizer in its gaseous form, F_2 . Fluorine occurs in nature as fluoride ion due to its strong oxidizing ability (F). The most popular and environmentally reactive ionic form is this one. Fluorine's natural concentration in the earth's crust varies between 0.06 to 0.09 percent by weight. Despite the fact that fluorine is a necessary element, Fluorine is an important ingredient for mammals, but at high concentrations, it is poisonous to both animals and plants. The purpose of fluoridation is to add fluorine in its ionic (fluoride) form to water sources. To accomplish this, a variety of compounds are used, all of which dissociate into their respective ionic forms and

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enter equilibrium with the chemistry of the receiving water. Fluoride flows at a rate of 3.7 million metric tons per year from continental drainages to the oceans in the environment. Mining extracts 2 million tons per year, while combustion of fossil fuels and industrial pollution release 0.022 million tons (as HF, SiF₄, and metal fluorides) into the atmosphere. Dust blown by the wind and volcanic emissions are also sources of pollution in the atmosphere (Gaciri and Daries, 1993). Fluorine precipitates in marine sediments that could be exposed over geologic time, allowing fluorine to be returned to continental landmasses. Fluorine from the atmosphere is mainly recycled to the earth through precipitation.

The aim of fluoride removal was to treat contaminated water and lower the fluoride content to safe levels. Defluoridation is a notable process of removing fluoride ions from potable water. The various methods of extracting excess fluoride from water that have been attempted can be divided into four categories as follows: a) methods of adsorption, b) ion exchange mechanisms, c) methods of precipitation, and d) miscellaneous methods. Some defluoridation techniques were developed to arrest fluoride content in water using adsorbents such as sunflower plant dry powder, phytomass steam, neem bark powder, activated carbon, cotton jute, bagasse ash, burnt bone powder, phosphate treated saw dust, bone char, and others. Defluoridation was also achieved using the Nalgonda procedure, process of activated alumina, and the ion exchange process. Moreover these techniques are not commonly used due to their high cost, low performance, or inapplicability on a large scale. Because of the ever-increasing population and the increased demand for safe drinking water, the advent of treatment of harmful components, particularly fluoride in fluid solution, has become vital source of concern for them since then. Fluoride exposure originated by natural, anthropogenic practices the fluid framework, also it causes a number of diseases such as joint pain, malignant development, weak bones, and damage to the cerebrum.

The current research focuses on various fluoride removal mechanisms, viz., adsorption, ion exchange, membrane filtration, and electrocoagulation, that are used to remove fluoride from fluid media. However, using various techniques like ion exchange, layer filtration, and electrocoagulation, evacuation productivity was increased to 90–95 percent, 99 percent, and 85.5 percent, respectively. Adsorption has been broadly investigated as an extremely viable and

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emergent technique because of easy operation, minimal effort, fair efficiency. However, using various techniques such as ion exchange, layer filtration, and electrocoagulation, evacuation productivity was increased to 90-95 percent, 99 percent, and 85.5 percent, respectively. The findings of this study clearly show that each process has discovered a new mechanism for the remediation of harmful fluoride particles [38].

Because of increased awareness and demand for potable water to address the problems of the developing population, especially in developing countries like India, fluoride remediation from drinking water has become an undeniably notable issue of open concern. This paper provides an overview of recent advancements in defluoridation techniques, as well as a discussion of the issues and research opportunities in the vital area of fluoride removal. X-Ray diffraction (XRD), field emission scanning electron microscopy (FESEM), energy-dispersive X-ray spectroscopy (EDX), transmission electron microscopy, Fourier transform infrared spectroscopy (FTIR)., inductively coupled plasma mass spectrometry, atomic absorption spectrometry, and inductively coupled plasma-optical emission spectroscopy are the spectroscopic methods used in this study to produce adsorbents/adsorption devices [39].



2.3.1. Prasanti Technology Applying Activated Alumina

Figure 2.3: Nalgonda Technique

Prasanti's activated alumina fluoride removal technology is currently applied for water defluoridation in Indian villages [40]. It was established due to research and development V. Sunitha

activities at the Satya Sai Higher Learning University's Department of Bioscience at Prasanti Nilayam, Puttaparthi (Anantapur District) of Andhra Pradesh, South India (Figure 2.3).

2.3.2 Membrane technology

Despite the fact that many traditional water purification methods are currently being used to resolve the issue of groundwater pollution, none of them has been found to be a user-friendly or cost-effective solution due to some or other limitations, and none of them has a payback period of zero or very long.

2.3.3. The membrane mechanism of reverse osmosis (RO)

Over the last decade, the RO membrane system has developed itself as a notable method of supplying safe drinking water without the problems associated with other conventional methods [41]. RO is a physical process for removing contaminants by adding pressure to the feed water and guiding it with a semi-permeable membrane. The mechanism is the polar opposite of natural osmosis since pressure is generated to the concentrated side of the membrane, which overcomes the usual osmotic pressure.

Numerous studies [42-46] have demonstrated fluoride removal efficiencies of up to 98 percent using membrane processes. This property of nano-filtration in comparison to reverse diffusion can have certain advantages, and experimental and theoretical studies are devoted to a material retention mechanism to facilitate the production and selection of targeted membranes as well as condition optimization [47]. As the pH of the water is increased from 5.5-7, the fluoride removal in the reverse osmosis phase has been stated to range from 45-90 percent. The economics of a plan is often evaluated in particular circumstances. There are some disadvantages to this process, such as the extraction of all water ions, but it is very good for defluoridation. Although certain minerals are required for optimal growth after treatment, remineralization is needed.

2.3.4. Residues from the Treatment of Water (WTR)

WTR has been shown to be an adsorbent for the repair of many anions found in water and soil (perchlorate, selenium, and arsenic) and may be able to extract fluoride from polluted water. In

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order to meet the drinking water criteria for fluoride removal capacity, safe reuse of publicly accessible waste from drinking water treatment plants, namely WTR, has been used. Precision surface region, Fourier transform infrared (FT-IR), scanning electron microscopy, and X-ray powder diffraction have all been used to characterize WTRR (XRD). To determine the best adsorption strength, batch adsorption experiments were conducted using WTR dosage, contact time, agitation velocity, initial levels of fluoride concentration, temperature, specific area and water pH. The metal hydroxyl and metal oxide groups generated to the elimination of fluoride, according to the FT-IR and XRD experiments. The use of WTR will also help with defluoridation and provide a means of reuse for the treatment of drinking water waste [48].

2.3.5. Fe₃O₄/Al₂O₃ Nanoparticles:

Using Fe_3O_4 and Al_2O_3 NPs that were green synthesised with jojoba defatted meal extract as a reducing agent, a low-cost defluoridation technique was developed (Sonu kumara et al., 2017). These NPs were impregnated into polyurethane foam (PUF) and used to make tea infusion bags.

The Al₂O₃ NPs-PUF showed a higher water defluoridation capacity of 43.47 mg g⁻¹ of F compared to 34.48 mg g1 of F with Fe₃O₄ NPs-PUF. The F was 1.5 mg L1 below the appropriate amount before the synthesised Al₂O₃-PUF infusion bags were used. The F was 1.5 mg L1 below the appropriate amount before the synthesised Al₂O₃-PUF infusion bags were used. The effect of various parameters like pH, contact time, PUF scale, and initial F concentration has been verified through sorption experiments. A mixture of FESEM, EDX, XRD, and FTIR techniques is used to describe the adsorbent's different properties. The advantages of the established method described in this study are high F removal capability, comprehensibility, portability, sustainable, viable making this approach perspective for constrained environments, mainly in rural areas with high F groundwater pollution [49].

Different groups investigated the optimal adsorption potential of different materials under similar conditions. Under similar conditions, the adsorption potential of carbon nanotubes, typical soil, $g-Al_2O_3$, and activated carbon follows the order CNT > soil > $g-Al_2O_3$ > activated carbon; while SWCNs, had a higher fluoride removal efficiency than MWCNs, which was also very high in optimal conditions with two forms of fine powder and 150 mesh activated alumina. Under

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similar conditions, the adsorption potential of alumina-infused carbon nanotubes was observed to be 13.5 times that of AIC-300 (4 times) g-Al₂O₃ and IRA-410 polymeric resin. Under ideal conditions, the overall adsorption potential of charcoals was discovered to be bone char > coal charcoal > wood charcoal > carbon black > petroleum coke in the same order (Figure 2.4.).



Figure 2.4. Comparison of carbon-based materials adsorption ability for water defluoridation.

2.3.6. Techniques for Adsorption

This technique is based on the adsorption of fluoride ions to the surface of an active agent. In the process of adsorption, raw water is allowed to pass through a series of bed containing defluoridating material. Physical, chemical, or ion exchange processes are used to keep fluoride in the content. After a certain period of action, the adsorbent becomes saturated and needs regeneration [50].

2.3.6.1. Activated alumina

Low-temperature dehydration (300-600°C) of aluminium hydroxides creates activated alumina (Al_2O_3) , which has been applied for defluoridation since 1934. Fluoride is extracted by a ligand exchange reaction on the surface of activated alumina. Alumina (aluminum oxide, Al_2O_2) is nearly insoluble in water. The solubility is determined by previous heat treatment method. Various activated grades of alumina are available at a low cost in the United States. The porosity and surface area of the alumina determine the suitability of the defluoridation grade [51].

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2.3.6.2. Alumina Plus Manganese Dioxide.

Manganese dioxide is a major insoluble sorbent that is non-hazardous. It is not used explicitly because, in comparison to the current work with dispersion over disposed earthenware, it may be needed in greater quantities (DEW). Alumina and manganese dioxide are combined. Manganese dioxide is an essential sorbent that is either insoluble or toxic. It is not allowed to be used because, in comparison to the current work with dispersion over disposed earthenware, it may be needed in larger quantities (DEW). Alumina and Manganese Dioxide combined. Manganese dioxide is an integral sorbent that is neither soluble nor toxic. It is not legal to use because, in comparison to current work with dispersion over disposed earthenware, it comparison to current work with dispersion over disposed earthenware, it can be needed in larger quantities (DEW). Alumina and Manganese Dioxide combined. Manganese dioxide is an integral sorbent that is neither soluble nor toxic. It is not legal to use because, in comparison to current work with dispersion over disposed earthenware, it may be needed in larger quantities (DEW). Alumina with a MnO₂ coating [52-54] MnO₂ in bentonite clay [55] Enabled carbon with MnO₂ coating [56-57] Many studies have found KMnO₄-coated activated carbon [58] and hydrated oxides of manganese ores to be relevant to fluoride removal. The removal of DEW fluoride at a pH of 7.15 with 0.025 percent MnO₂ was critical. Rinsing the spent sorbent with 1.5 M NaOH was found to be successful [59], suggesting that the reaction used to remove fluoride is reversible.

2.3.6.3. Bauxite

Widely available geomaterials which have been researched in recent years in order to determine an efficient solution for extracting high water fluoride content [60]. Bauxite is a readily available mineral that mainly contains alumina, iron, silica, and titanium oxides. As indicated by [54], several similar studies have revealed the removal of Cr(VI) and As(III). F was removed from aqueous solutions using thermally activated titania rich bauxite. Previous studies have shown that heat-activated bauxite has the potential to remove anionic pollutants including Cr(VI) and As(III)[61]. A bauxite sample from India's Eastern Ghats (Orissa) was used to defluoridate synthetic and fluoride-bearing groundwater obtained from fluoride-endemic villages near Tarabalu, Atri, and Taptapani hot springs of Orissa [62-63]. As bauxite is a prevalent mineral in several parts of the world, defluoridation can be a quick, reliable, and low-cost process. Bauxite is a strong material with a lot of mechanical power, and it's abundant, so it's a good choice for fluoride removal over other adsorbents. Finally, fluoride sorption processes on bauxite are a composite process that involves intra-particle diffusion, physisorption, and chemisorption [64]. The high alumina content bauxite tested for this study denoted a promising property as a low-cost and active adsorbent for the

Defluoridation. According to the findings, bauxite is a readily accessible material that can be used without the need for pre-activation, which is needed for alumina and other adsorbents. Based on factors like the active phase of modified bauxite, treatment system, pH balance, and initial concentration, several processes have been implicated in the process of fluoride sorption on bauxite, including ligand exchange, ion exchange, intra-particle diffusion, cation exchange and surface complexation [65].

2.3.6.4. Red mud (RM)

The solid waste residue produced during the caustic digestion of bauxite ores during the manufacture of alumina is known as red mud (RM). With a pH of 10-12.5, red mud is a highly soluble waste material. Tiny fragments of aluminum, iron, silicon, titanium oxides, and hydroxides make up the majority of red mud. It has a profound effect on waste disposal owing to its alkaline nature, chemical and mineralogical species, which poses a major challenge when alumina industries are developed. The application of red mud in the treatment of soil polluted by groundwater would be extremely beneficial [63]. It's also worth noting that red mud contains a lot of metal particles and organic substances that are released under unusual circumstances. However, it is important to note that red mud contains some metal ions as well as organic matter, all of which can be released under some conditions.

2.3.6.5. Clay minerals

Clay minerals are the chief natural adsorbents due to their small particle sizes, lamellar structures, and negatively charged surfaces. They are strong cation adsorbents with a broad specific surface region because of these characteristics. Adsorbents made of fired clays are inexpensive to use since they are easy to prepare and regenerate (firing temperature below 1000 °C). Clay and several clay minerals can thus be useful adsorbents in the purification of the environment [66-67]. The fluoride binding effect of basic pottery has been studied by a number of researchers. Fluoride binding capacity in 80 mg/kg clayware has been documented, and results have demonstrated that fluoride absorption in clayware is slow and finite [66]. Mud pots' key benefits are their cost-effectiveness and their ability to be affordable in rural areas [67].

2.3.7. Sorption study

Adsorption experiments are used to obtain rate and equilibrium data using the batch technique. The tests are performed by mixing a 10 g/L adsorbent dosage of 50 mL of aqueous solution with a known fluoride ion concentration and shaking the samples at a rate of 200 strokes/min with a shaking tool. By adding 0.5N HNO₃/0.1M NaOH to fluoride-containing samples, the pH is maintained at the optimal level (5-6). All of the measurements are performed at room temperature (29 0.5 $^{\circ}$ C). The absorption of fluoride ions by the adsorbent material at their maximum pH values appears to be very rapid, according to preliminary investigations. Within the first hour of interaction with fluoride ions at a concentration of 10 mg/L and an adsorbent dose of 10 mg/L for treated bio-sorbents, 80 percent of the adsorption usually occurs [16].

2.3.7.1. Mechanism of Sorption

The Freundlich and Langmuir models are associated with fluoride ion removal sorption data [68]. Experiments on the adsorption capacity of Mentha longifolia (Mint) from aqueous solutions were studied at room temperature. The effects of many major parameters on adsorbent materials, like pH, contact duration, adsorbent quantity and particle size, and concentration of absorption fluoride ions, was investigated from a kinetic standpoint. The rate and equilibrium results are obtained using a batch technique in adsorption experiments. The sorption properties were calculated using the Freundlich and Langmuir isotherms. Mentha longifolia (Mint) has been shown to be efficient in extracting fluoride from groundwater samples [16].

2.4. Conclusion

Fluoride-polluted ground water is causing health issues in India. In this paper, we discussed different strategies for addressing the issue of higher fluoride levels in drinking water, including RO, nanofiltration, biopolymers, and others. Application of membrane technique is a low-cost and straightforward method for removing fluoride. Enabled alumina removal of fluoride is a tried-and-true treatment method. Because of the solubility of aluminum, immoderate application of aluminum salts generates sludge and has serious health consequences. The membrane technique is a low-cost and straightforward method for removing fluoride for removing fluoride. Fluoride removal by activated alumina is a tried-and-true treatment method. Ion Exchange extracts up to 90% of fluoride thus leaving the water's taste and color unchanged. However, higher costs are a

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drawback, and treated water with a low pH and greater chloride content. The WHO and the USEPA guidelines enabled alumina adsorption to be one of the most effective fluoride removal technologies available. Attempts have been made as in the past to make modified alumina by infusing with metal oxides that have proven to be of significant defluoridation capacity. However, the ability to completely remove carbon-based fluoride technologies is still limited, and numerous experiments have been performed to alter the surface of defluoridation-enhancing carbon-based adsorbents. Moreover, some drawbacks have been recorded, including difficulties with regeneration and poor performance at high fluoride concentrations. Defluoridation was shown to be an inexpensive defluoridation technique when applied with Fe₃O₄ and Al₂O₃ NPs synthesized green using jojoba defatted meal extract as a reduction agent. The benefits of the proven technique described in this study include higher F removal capability, sensibility, portability, eco-friendly, viable, and hence making this strategy more fetching for resourcelimited environments, particularly in rural areas where high groundwater fluoride pollution causes health issues. Biosorbents, especially chitosan, provide satisfactory results in terms of fluoride removal after modifications. Locally available adsorbent materials with good performance with cost effectiveness can be used for adsorption. This technique makes use of herbal and indigenous items that are both cost-effective and readily available. These products are acceptable for use in Indian conditions. With the presence of sulphate, phosphate, and bicarbonate, the process is pH-dependent and leads to ionic rivalry. The disposal of fluorideladen sludge is a downside. Membrane processes are extremely effective and do not require the use of chemicals. It can function over a large pH spectrum and has little interaction with other ions. It does, however, necessitate higher costs and skilled labor. For water with high salinity, this method might not be appropriate. The ability to extract fluoride varies depending on a number of chemicals, geographical, and economic conditions unique to the region, resulting in actual installations that vary from generalizations. Any approaches that are effective in one field cannot be effective in other areas. As a result, all technology that will be used should be treated well before being deployed in the field.

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Landuse and Landcover Analysis using Remote Sensing and GIS to study the Change Impact on Water Resources in Parts of Porumamilla, YSR District, Andhra Pradesh, India

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Abstract: The use of multi-temporal satellite imaging helps to understand landscape dynamism through digital shift detection techniques. Integrated approach based on advanced applications of remote sensing and geographical information systems (GIS) lends itself as an efficient and effective result-oriented method for studying the development and management of water resources. Land use change pattern reveals the rate of change of groundwater recharge. It is necessary to identify the land use change in the past and present accessible land use, and its allocated and potential changes are major rudiments for planning and management. The current study shows the space and time dynamics of land utilization/decoration in the area of Porumamilla, Kadapa District, Andhra Pradesh. Landsat satellite imagery was obtained by earth explorer site for two separate periods of time, including Landsat Themetic Mapper (TM), quantifying changes in the field of research 2008 to 2018 over the 10 years. Methodology for supervised classification was used in ERDAS 2014 for maximum probability technologies. The photographs in the field of research were grouped into five classes: forestry, agriculture and wastelands, built-up, waterbodies. The findings show that over the past decade, land and build-up was increased by 3.48% (12.82 km²) and by 4.03% (14.86 km²), while forestry, woodland and waterways were reduced by 6.81% (25.1 km²) and 3.72% (13.7 km²) and 1.9% (7 km²), respectively.

Key Words: Land use / Land cover, Remote Sensing, GIS, Erdas Imagine, Waterbodies.

3.1. Introduction

Land use / land cover are multiple technologies, sometimes interchangeable [1]. Land cover related to the physical features of the surface of the earth, including the arrangement of plants, water, soil and the soil's other physical properties. While land use refers to the way people and their environments use land, typically focus on the practical role of land in commercial activities. Land use / land cover is the product of environmental and socio-economic forces and their time and space utilization by humans [2]. LULC of the territory, which involves an increase in urbanization requirements and leads to an increase in the number of residents in gifts. Changes in land cover for land use do not necessarily mean land degradation. However, many models change land use due to various social causes, leading to changes in land cover that affect the biodiversity of budgets [3]. LULC of the region, which means that urbanizing needs are growing and that the number of inhabitants is rising with respect to gifts. Changes in ground cover do not inherently mean degradation of LULC change. Many models, however, alter land use for social factors, leading to changes in land cover which have an effect on budget biodiversity [4,5].

Water and radiation emissions and other processes that link impact to the atmosphere and on the biosphere [6,7,8]. The discovery of changes in LULC is very important for a better understanding of the dynamics of the landscape during a period of sustainable management. Changes in land use / land cover are common and accelerate the process, Human activity and natural phenomena are essential for a correct management of the territory and for the decision-making process. Satellite data today for local resources are very useful and relevant for testing changes in LULC [9]. The modern field of sensor technology includes air and satellite systems that repeatedly collect physical data at a rapid rate with GIS, helping us to analyze data generated by different spatial models, which optimize the entire planning process. Implementation of vulnerable data remotely makes it possible to study land cover changes in less time, at lower cost and with greater accuracy. The Landsat-TM image represents valuable and undisturbed land records over the last three decades [10].

The LULC change has become an integral part of the current strategy for natural resource management and the study of environmental change. Increasing the concept of vegetation mapping significantly increases the search for changes, thereby ensuring accurate assessment of the distribution and health of forests, grasslands and agricultural resources.

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The increasing demand for new agricultural land is generally met by surrounding resources, such as forest areas / dominated by heavy areas, such practices can be minimized through land intensification (by increasing Rabi, Kharif and Zaid harvests) so as to meet increased demand for more land for agriculture. The use of remote sensing data allows to study land cover changes for a shorter time, others as a result of land transformation [11]. Since the land use / land cover, two are intimately linked and mutually exclusive, they can be exchanged, since the former is based on land cover and related data. Changes in land use and land cover are native and site specificity becomes gradual, in a way that often escapes our attention. Collectively, however, they add one of the most important aspects of global environmental change.

These thematic guides provide for the introduction of these changes as well as direct the reader through important research topics on land use and land cover: deforestation, desertification, biodiversity loss, land cover and water cycle and urbanization. A total of three thematic maps, such as location, drainage and land use maps and terrestrial coverage, is based on an image acquisition study. The model of land use and land cover compatible with a broad category, agricultural land, forest massifs, water bodies and land that is not cultivated [12]. In this area of education the main natural resource is the forest. Due to human activity, the area under the forest is reduced. Similarly, the land used for cultivation is also declining. But at the same time, the land under the built area is increasing. More recently, the function of building contractors and people in the real estate industry has caused a serious disaster to burn and farm land. This is a destructive situation with land management. Under these circumstances, exploration of the discovery of land-based change is essential to understanding the current situation and planning future generations.

3.2. Study Area

The proposed study area covers the Porumamilla Mandal, Kadapa District, Andhra Pradesh, India. The study area is located in the Survey of India Toposheet Nos: 57 J/13, 57 J/14 and 57 N/2 on 1:50,000 scale and lies between North longitudes 78⁰55' 0'' to 79⁰10' 30" and East latitudes 14⁰ 50' 0" to 15⁰ 10' 0" (Figure 3.1). The study area comprises a total geographical area of 368.65 sq. km. A popular investigation soil survey of Kadapa district exhibits the occurrence of exclusive soil sequence and their associations in the study area.

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Figure 3.1: Location map of the study area.

3.2.1. LULC preparation

Landsat imagery at a resolution of 30 m of 2007-08 and 2017-18 were used for LULC classification. The satellite data covering the study area were obtained earth explorer site. These datasets were imported in ERDAS Imagine version 2014, image pre-processing software to create a FCC. Layer stack options in the image rendering tool box are used to generate the FCC for the area under consideration. The pre configuration of satellite images is performed in the isolation study area of the two images, indicating the geographic area of the study region map as AOI [12]. The methodology for LULU preparation shown in the Figure 3.2.

The LULC classification of such area resulted in program brought with supervised grading with the maximum probability algorithm approach. The underlying theory shows that for all groups and *Srinivasa Gowd et al.* 49

for the standard division of input bands these probabilities are similar [13]. This method, however, involves a long calculation based on the usual distribution of data in each input bar and appears to circumvent signatures. In the study area, five LULC forms were defined according to the classification of signatures they are (i) Forest (ii) Agricultural land (iii) Wastelands (iv) Builtup lands (v) Waterbodies.



Figure 3.2 : Methodology adopted in the study.

3.3. Results And Discussion

3.3.1. Analysis of LULC

Techniques for remote sensing are important tools for detecting shifts in certain time regions. In analysis, the selection of an effective method for detecting changes is important to detect qualitative changes in the regions in order to distinguish remotely sensed data and areas of study.

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The categories Land use / Land cover in the field of research were mapped using Landsat theme mapper for LULC classification at a resolution of 30 m between 2008 and 2018. After a detailed field inspection, the map was completed [14,15,16]. The details were visually interpreted. The research areas include agricultural land include agriculture land, built-up land, barren / Waste land, vegetation / scrub and water bodies for the separate LULC classes [17]. diagrammatically displayed in figures, the identification of shift of the study region. Table 3.1. Figure. 3.3. & 3.4 reflects land use/cover and land use/cover shifts in specific categories of land use. Figure 3.5 demonstrates the percentage difference in LULC graphic representation in 2008–2018.

3.3.2. Agriculture Land

Agriculture land with planted orchards and plantations. The research area has reduced the overall agriculture land from 167.40 sq. km to 142.30 sq. km (45.41 percent to 38.60 percent). Crop fields are under cultivation farm lands. In the region analyzed, the crop fields are wet and dry. Food crops like rice, paddy, groundnut, and vegetables require wet cultivation etc. Dry cultivation includes Bengal gram, redgram and groundnut, etc .

3.3.3. Forest

Land consisting of dense and thick treeline. Forests identified and differ in scale by their red to dark green tones. With a smooth texture, they have an irregular shape. In the North West and North East sections of the sample area, the woods are located. In a decade, the gross vegetation area has fallen from 89.64 km^2 to 75.94 km^2 (24.32 percent to 20.6 percent). The field covering the analysis primarily includes the thick and mixed timber. The relative abundance of shrubs in this group is dominated by small trees. The vegetation / scrub is labelled with a green hue, smooth texture on satellite photos. This is due to decay, deforestation or thorny bush. Such regions are determined by the relationship between yellow color, association with uplands and irregular shape. The scrub land in the north and east of the study region.

3.3.4. Built up Land

Built up land consists of intensive regions with a substantial portion of the terrain occupied by structures and a gross built-up area of 7.52 sq. km to 22.38 sq.km (2.04 percent to 6.07 percent).

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Figure 3.3: Land use / Land cover status of the Study Area in 2008.



Figure 3.4: Land use / Land cover status of the Study Area in 2018.



Figure 3.5: Graphical representation of land use/land cover changes in percent during 2008-2018.

Table 5.1: Area and amount of change in different land use/cover categories in the study areaduring 2008-2018.

Land use / Land cover Categories	2008		2018	
	Sq.km	%	Sq.km	%
Barren/Waste/Rocky Lands	64.04	17.4	76.86	20.8
Water bodies	22.19	6.02	15.19	4.12
Agriculture Fallow Lands	17.85	4.84	35.98	9.76
Agriculture Irrigated Lands	167.4	45.4	142.3	38.6
Forest	89.64	24.3	75.94	20.6
Built-up Lands	7.52	2.04	22.38	6.07

cities, towns, villages, industry complexes and organizations are included in this group. Eedalaplli, Markapuram, Chintarapalle, Kalavakatta, Ramiredddykunta, Vasudevapuram, Akkalreddypalle, Venkatapuram, and Nagalakuntla are the main towns or villages in the study district. In the study area, transport facilities are highways. The roads between Porumamilla and Vijayawada, Porumamilla and Nellore are present in the region.

3.3.5. Waterbodies

Natural and human water systems include water ponds, channels, streams, canals and reservoirs. On the satellite image, the water's feature looks dark. Deep and deep waters of the body are dark grey. Tanks with square, rectangular and blue color tones are distinguished by vineyards. In the vegetation field, the small canal is visible. Much of the tanks are centralized in the middle of the region explored and scattered with more dry tanks in the eastern portion. The water sources of the city have dropped from 22.19 km² to 15.19 km² (from 6.02 percent to 4.12 percent).

3.3.6. Wastelands

Land not tolerant of vegetation is classified as stormy fields or waste lands. Included in this group are rocky barren, salts influenced, scrub-free land, sandy area, sheet rocks and rocky areas. The physical properties of soil, temperature, plaster and local environmental conditions are responsible for the development of these fields. The field of research for stony/waste soils rose by 64,04 sq. km to 76.86 sq. km in the research field and in the south-west portion of the zone (17.37% to 20.85%).

3.4. Conclusions

The identification of the field analyzed indicates that numerous satellite temporal images play an important role in the calculation of spatial and seasonal patterns that fail to allow traditional mapping. The research indicates that agriculture is the main land use of the study area. The area under waste land has increased by 3.48% (12.82 sq.km) due to deforestation work during 2008 to 2018. The second major category of land in the study area is agriculture which was decreased by 6.81% (25.1 sq.km) due to conversion invegetation, barren land and built-up land. The third major category of land in the study area is water bodies was decreased by 1.9% (7 sq.km). During the study period (i.e., 2008 to 2018), Built-up land has been increased by 4.03% (14.86 sq.km) due to

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conversion into urbanization and industrial areas. Thus, the research now under consideration demonstrates that remote sensing and GIS are important time analytical and quantifying technology in order to attempt spatial phenomena through traditional mapping. Changes are made possible in a less time, cheaper and more reliable way by these innovations.

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Assessment of heavy metal contamination in soils and groundwater at an industrial area, Bangalore, Karnataka, India

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Abstract: Much concern over soil pollution of heavy metals because of accelerated industrialization and urbanization has been addressed in recent years. The present research was conducted to examine the dispensation of potentially dangerous elements (PHEs) (Cr, Cu, Ni, Pb, and Zn) concentrations in soils of the Bommasandra Industrial Area on the Varanasi – Kanyakumari National Highway (Longitude 77°40' to 77°45' and 12°45' to 12° 50' N Latitude), south-east of Bangalore City to determine the level of chemical contamination. Geologically, peninsular gneisses, close-pet granites, lateritic hills, and some basic dykes with undulating terrain are beset by the study area. The semi-arid type is the climate of the study area. Two hundred soil samples were collected from the Bommasandra industrial areas and tested for heavy metals using a Philips Magi X PRO-PW 2440 X-ray fluorescence spectrometer. According to the data, the soil in the region is heavily polluted with heavy metals such as Chromium (Cr) ranges from 11.8 to 335 mg/kg (average of 136 mg/kg), Cu from 10 to 221 mg/kg (average of 77 mg/kg), Pb from 0 to 397 mg/kg (average of 21 mg/kg), Ni from 0 to 308 mg/kg (average of 98 mg/kg), and Zn from 11 to 435 mg/kg (average of 78 mg/kg). The distribution and association of heavy metals in soils was explored, as well as potential remedial steps. These findings are significant for the development of appropriate management strategies through the study of various remediation methods to reduce point and non-point pollution sources.

Key words: Heavy metals; Urbanization; Industrialization; Remediation; Bommasandra.

4.1. Introduction

The region is home to many factories dealing with metal alloys, plastic products, pesticides, the bulk pharmacy industry, food manufacturing, etc. Such sectors could be divided into small and medium-scale enterprises. Most of these factories were discovered to leak their manure directly into nearby trenches and waterways, and solid waste is dumped indiscriminately on exposed fields, along roads, and in lakes. Significant amounts of inorganic and organic compounds, as well as their byproducts, can be found in industrial waste. Many enterprises are in the minor industry and be without sewage pipes [1, 4, 5]. Even now, most of them proscription have sufficient wastewater treatment facilities and dump industrial waste into smooth channels and waterways, reasoning tremendous water and soil pollution. Indiscriminate disposal of residential and industrial waste in the country has become a routine procedure in whatever the quantities of radioactive components in soil and water have significantly surpassed the allowable constraints. Consequently, Kommasandra Lake is joined by heavily colored and hazardous chemical waste. The extent of pollution has become so serious that the climate has been unsuitable for human activity in some areas. The persistent dumping of effluent has resulted in groundwater pollution being common. Few bore-wells, accompanied by translucent oil film buoyant aloft, are heavily polluted.



Figure 4.1. Sample location map of the Study area

4.1.1 Climate

The area under scrutiny is semi-arid. This region normally accommodates its primary period of rainfall from pre-monsoon conventional rains during May, but its occurrence is erratic. The region gets a total of 859 mm of rain each year from the south-west and north-eastern monsoons. Maximum rainfall happens during the southwestern monsoon season, which lasts from June to September. During the northeast monsoon season, the strength and volume of rainfall are variable (November & December). The main dry season lasts from January to May. Rainfall will fall, however, owing to the retreating monsoon climate and cyclonic storms that normally originate in the Bay of Bengal. It gets unbearably hot in the summer and comfortably chilly in the winter. The average temperature rises to 38 degrees Celsius. Temperatures increase slowly from January to the maximum in May, then quickly fall from June to the lowest in November and December. The high temperature in this area produces significant evapotranspiration losses from surface and subsurface water sources and with the arrival of summer, freshwater supplies are exhausted.

4.1.2 Topography and Drainage

South of Bangalore City, on the Varanasi-Kanyakumari National Highway, is the Bommasandra Industrial Development Area (NH44). The extent of the area within the topographic watershed of the agricultural area of Bommasandra and the adjacent residential areas is seen in Figure 4.1, with the agricultural sector situated to the northwest in the highest elevated areas, the area slopes gently towards the south-east. The highest elevation recorded is 900 m above MSL in the sample region, and the lowest is 850 m above MSL.

4.1.3 Soil, Geology and Hydrogeology

The earth layer is the rest of the weathered granite's soil. The soil consists of brown-reddish sand with varying silt materials. The soil is relatively permeable, and the river rate can retain a good deal of precipitation except for more severe rainfall and erosion. Bommasandra study area consisted of peninsular gneisses, closepet granites, lateritic hills, and some basic dykes of undulating terrain. The granites occur in the gneissic rocks as intrusive and differ in color, texture, and structures. Numerous sets of basic and acidic intrusions traverse the area. Dolerite dykes, pegmatite, and some quartz gravel are the intrusions. Dolerite dykes create groundwater tapping problems as they obstruct water movement, while pegmatite veins are favorable for tapping groundwater.

A large portion of the study region is covered by peninsular gneiss and is highly migmatic in nature. Their structure varies from tonalite and trondhjemite to Granodiorite and has a texture that is fine to medium-grained. A general trend of NNW SSE and NW SE with dips from 60 to 80 to the east is shown by peninsular gneiss. In color, they are fine to medium-grained and generally grey. In some places, alternate bands of felsic and mafic minerals are regularly banded, and the banding is irregular in places. They are joined; there are sheet joints almost parallel to the surface of the ground.

In and around Bangalore, four types of gneisses are grouped: (i) Banded Gneisses, (ii) Granitic Gneisses, (iii) Gneissic Granites, and (iv) Granites and Granodiorites. The first three groups are part of the gneissic complex and have an intrusive association with older schists, while granites and Granodiorites are part of younger granites close to each other. These are affected by a several basic dykes and veins of pegmatite.

In the study area, banded gneisses consisting of several varieties with different colors, structure, texture, and mineral composition are present and display well-defined banding in many locations. Granitic gneiss is the key rock formation in the study area. Mainly of migmatitic type, the granitic gneisses are highly banded varying in composition from granite to diorite. Under a mantle of soil and river alluvium of varying thickness, the rocks in the remaining portion of the area lie. Granites occur only in small portions of the study basin and the surrounding gneissic plains have a definite physiographic identity.

Three lakes are part of the study area. Kommasandra Lake, Chandapur Lake, Lake Kittiganhalli. These lakes are interlinked by small streams. The groundwater in the Archaean granites occurs in the weathered and eroded areas beneath the water table and semi-confined environments. In these stones, the occurrence and flow of groundwater are dominantly regulated by the weathering depth and broken areas. These stones have negligible primary porosity but are made with porosity and permeability due to secondary porosity through deep fractures and weathering, which locally shape potential aquifers. The water levels fluctuate annually in almost all of the bore wells. The mean depth of groundwater is roughly 8m. Water levels typically rise in the post-monsoon season with the water table fluctuating between 1 and 15 meters, and water levels generally decrease during the pre-monsoon season and the water table fluctuates between 10 and 25 Mts. Borewells range from 40 to 200 Mts in diameter.

4.2. Methodology

4.2.1 Field Investigations and Sample Preparation

The sampling depth selected is about 10 and 15 cm below the surface and 25 cm deep, to avoid effect from various arbitrary surface conditions as waste and humus, and to maintain normal onsite soil. The samples were ground in the agate swing mill in order to make the sample consistent and to achieve accurate analytical results as the surface layer would represent the bulk sample. The sample weighing is accomplished by using analytical balance using collapsible aluminum cups with a precision of as low as 0.0002 g and pressed pellets are packed. These cups are filled with boric acid, with 1 gm of the sample on the boric acid and are pressed under a hydraulic press of 20 tons.

4.2.2. Analytical procedures

A Philips Magi X PRO model PW 2440 XRF tube with Rh 4 kW tube was used to evaluate the elemental composition. With the PW2440, "The elements of interest can be screened with a very delicate and thorough determination of main and trace elements (Si, Al, Na, Mg, Ca, Fe, P, S, As, Ba, Co, Cd, Cu, Mo, Ni, Pb, Rb, Sr, V, Zn ,and Zr)". Magix PRO is a sequential instrument with a single goniometer-based measurement channel spanning the entire elemental channel. Comparison of foreign soil Samples from the U.S. Geological Survey, Canadian Geological Survey (So-1, So-2, So-3, So-4), France International Working Group, and the National Geophysical Research Institute, India (NGRI-D & NGRI-U) were used to plan the main and trace element calibration curves and to verify the accuracy of analytical components [15].

4.3. Results and discussion

4.3.1. Total heavy metal concentrations

The analytical analyses of the water samples and the heavy metal data overview figures are presented in Table 1 and Table 2, respectively. Heavy metals with enrichment amount that surpass the usually anticipated water distribution pose questions about the suitability of drinking and growing crops [2,7,8,10,14,16,22,28]. In certain pockets, very close to factories, elevated amounts of these elements are found, suggesting that industrial effluent may be the source of these elements.

Heavy Metal	Soil Quality Guidelines, Residential (SQGL)	Concentrations	Soils
Cr	64	Mean	136
		Min	BDL
		Max	335
Cu	63	Mean	77
		Min	10
		Max	221
Ni	50	Mean	98
		Min	BDL
		Max	308
Pb	140	Mean	21
		Min	BDL
		Max	397
Zn	200	Mean	78
		Min	11
		Max	435

Table 4.1. Total heavy metal	concentrations in soil.
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Table 4.2. Total heavy metal concentrations in groundwater.

	Indian Drinking Water limit ISI	Concentrations	Ground Water
		Mean	1
Cd	3	Min	0.1
		Max	6
Cr	50	Mean	18
		Min	32.7
		Max	131
Cu	2000	Mean	26.5
		Min	1.3
		Max	259
Ni	20	Mean	32
		Min	0.1
		Max	311
Pb	10	Mean	35
		Min	2.2
		Max	153
Zn	3000	Mean	394
		Min	31
		Max	3034



Figure 4.2. Distribution of Chromium (ppm) in the soil of Bommasandra Industrial area.

capacity and biological membrane permeability [3]. Cr concentrations in the sampling region ranged from 32.7 to 131 g/L, with an average of 18 g/L (Fig. 2) and were slightly higher in areas occupied by granitic rocks (Fig. 2) [11, 13,25]. The normal Cr range in water is 100 g/L [27]. Since the nearby rocks are mainly granite, with Cr concentrations often below 50 mg/kg, extracting certain large Cr levels from the rocks is not feasible [12]. As a consequence, the roots of Cr tend to be anthropogenic, arising in modern tannery factories that utilize Cr and its variants. Cr is a trace element that is needed for lipid and protein metabolism as well as maintaining a normal glucose tolerance factor. Large Cr doses damage the liver and kidneys, and chromate dust is carcinogenic [24].

4.3.3. Copper (Cu)

The study area found strong copper values. The spectrum of field water tests is $1.3-259 \mu g/L$, averaging 26.5 $\mu g/L$. (Figure 4.3). Recommended threshold value in water is 30 $\mu g/L$. Cu deposition in water can be attributable to rubber, blast furnace, steel, and agrochemical applications in agro-based industry. However, Cu is characterized by so-called point emission sources, like uncontrolled waste dumps, active or expected. These locations suggest significant water-pollution risks [16,23]. Only the quantities of metals leached to solemn/gravel substratum

touch are dispensed from groundwater threat components in this area into the aquifer, eventually contaminating it. Contamination transmission and its dispersion in the aquifer is a very long process, and changes in metal composition in the upper layers of the aquifer do not occur in a short time. However, the narrower column depth, the higher the risk. Cu overdoses may also trigger neurological problems, increased blood pressure, liver and kidney failure [17,21].

4.3.4. Nickel

The Nickel (Ni) concentration in the sample area ranges from 0.1 to 311 μ g/L, with an average of 32 μ g/L. In water, the usual threshold value recommended is 20 μ g/L [27] (Figure 4.4). Water supplies of Ni include water polluted by urban sewage sludge, municipal sewage treatment system wastewater, and groundwater adjacent to landfill sites. More Ni contributes to water in industries such as ceramics, steel and alloys, electroplating and refractory. As confirmed in this report, anthropogenic causes, such as fuel combustion or waste released from factories where Ni is extracted from ore, may have some impact on increased concentrations of Ni.



Figure 4.3. Distribution of Copper (ppm) in the soil of Bommasandra Industrial area.



Figure 4.4. Distribution of Ni (ppm) in the soil of Bommasandra Industrial area.

The spatial distribution of Ni in the sample area is seen by the geochemical map in Fig.4. Skin allergies, particularly dermatitis, are the most frequent adverse effects of Ni toxicity. Among the symptoms identified among staff chronically exposed to Ni compounds in the study region are rhinitis, nasal sinusitis, and nasal mucosal injury. In mammals, the lung is the target organ for Ni toxicity.

4.3.5. Lead (Pb)

The total lead content in the research area's water samples is 35 μ g/L and ranges from 2.2 to 153 μ g/L. (Figure 4.5). The Pb created from smelting, exhaust gases from motor vehicles, and Pb pipe corrosion. Poisoning has sometimes been triggered by Pb salts used in the ceramics industry, which is situated in the study area. Pb solubility is mainly regulated by PbCO₃ and low alkalinity, with higher Pb concentrations in low-pH water. Due to concerns of systemic toxicity, the average recommended WHO concentration for Pb has recently decreased from 50 μ g/L to 10 μ g/L, whereas its concentration in natural waters is seldom exceeded. Pb is a chronic toxin that causes fatigue, irritability, anemia, behavioral changes and intellectual capacity loss in infected patients. Long-

term exposure to lead or its salts (particularly soluble salts or a powerful oxidant called PbO2) can influence both the nervous system and the kidneys.



Distribution of Pb (ppm) in soil in Bommasandra Industrial Area

Figure 4.5. Distribution of Lead (ppm) in the soil of Bommasandra Industrial area.

4.3.6. Zinc

Zinc refers to a group of trace metals potentially most harmful to the biosphere. Industry and usage of liquid waste, composted materials and agrochemicals such as fertilizers and pesticides in cultivation are key pollution causes [22]. The sample field averages $394 \ \mu g/L$ of zinc, varying from 31 to $3034 \ \mu g/L$ (Figure 4.6). The standard water threshold value is $300 \ \mu g/L$ [9] and only six tests revealed a zinc content below the normal threshold value. In India, 47 percent of soils are deficient in zinc, but the high concentration of Zn is found near industrial areas, reflecting an anthropogenic and non-natural source [6]. Also, it is quite unlikely that extracting 3947.9 $\mu g/L$ of Zn from acidic rocks in the sampling region was feasible. Higher Zn pollution causes hematological diseases, worsening mammalian metabolism.



Figure 4.6. Distribution of Zinc (ppm) in the soil of Bommasandra Industrial area.

4.3.7 Results and Risk assessment:

In comparison to the SQGL values, both statistical analysis and the geographical distribution of the concentrations are used to assess possible contamination. To differentiate between source and occurrence, graphs demonstrate some of the relationships between different heavy metals. The graphs show how the concentrations differ depending on the area.



Figure 4.7. Relationship between Copper vs Ni concentration.

Cu contamination is also greater in the region, with more than half of the samples exceeding the SQGL value of 63 mg/kg in the area. The contamination of Ni exceeds more than 70% of the area. Ni's SQGL value is 50 mg/kg (Figure 4.7).

4.3.8 Groundwater Investigations:

When illustrating and discussing the collected groundwater data in the Bommasandra Industrial area where the contamination is taking place. The samples were collected from 50 Bore wells, 4Hand pumps, 3 Dug wells which includes the residential area.



Figure 4.8. Relation between Cadmium vs Copper and Copper vs Zinc.

The actual contamination will be less continuous and more dispersed altogether. In the graph, the Cd vs Cu is shown in Figure 4.8. Cd is a small area of contamination, only 7% of groundwater samples exceed the limit of Indian Drinking Water (ISI), and the remaining area is within the permissible limit. Within the permissible limit, Cu is in the groundwater. Cu v Zn indicates a trend line of up to 0.5. There is also Zn contamination within the permissible limit, except that 2% of the area is above the permissible limit [18,19,20,26].

The drinking water limit is not exceeded by all the Cu data and the mean values for the As, Cd, Cr, Cu (Figures 4.9-4.12). The mean concentrations of Ni, Pb, Zn (Figures 4.13,-4.15) are above the drinking water limit in the Bommasandra Industrial Area. The maximum values show very high concentrations for some of the wells. Even if only some of the contaminated industrial wells are not used for drinking water, the general contamination of the discharge effluents into the streams and lakes will contribute significantly. Show interesting findings compared to this research in the table, even if the wells are not recognized. The levels of Ni and Pb are greatly elevated. Since contamination is low, most groundwater areas are below the permissible limit [29].



Figure 4.9. Distribution of Arsenic (ppb) in the Groundwater of Bommasandra industrial Area



Figure 4.10. Distribution of Cadmium (ppb) in the Groundwater of Bommasandra Industrial Area



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Figure 4.12. Distribution of Copper (ppb) in the Groundwater of Bommasandra Industrial Area.



Figure 4.13. Distribution of Ni (ppb) in the Groundwater of Bommasandra Industrial Area.



Figure 4.14. Distribution of Lead (ppb) in the Groundwater of Bommasandra Industrial Area.



Figure 4.15. Distribution of Zinc (ppb) in the Groundwater of Bommasandra Industrial Area.

4.4. Conclusions

According to the study, the soil and groundwater in the study region is substantially tainted by Cd, Cr, Cu, Ni, Pb, and Zn, which could pose a range of health risks. The observed levels of total metal toxicity in each of the samples exceeded international threshold values. High Cr values confirm that the contamination is caused by toxic waste. Ni and Zn exhibited a powerful anthropogenic effect. However, anthropogenic feedback was thought to be the anomalous Ni concentrations near the highway and industry. Copper is differentiated by a broadly distributed anthropogenic effect. This is especially valid for unregulated waste dumps and industrial liquid waste. The large concentrations of Cd and Zn detected in the study area's water often suggest that the pollution of these heavy metals in the area is largely induced by factories in the area, either by the disposal of toxic waste or the release to the ground of untreated effluents contaminating the bodies of water and streams present in the study area. It is necessary to build a pipeline in the region that will move the effluent to a more appropriate treatment plant. Water in the region must be remedied in compliance with environmental safety standards, and there is a requirement for frequent testing of radioactive metal enrichment in the area.

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CHAPTER No. 4

Carbon and its compounds

HOTS: (High Order Thinking Skill) Questions with Answers:

1.An organic compound X with a molecular formula C_2H_6O undergoes oxidation with in presence of alkaline KMnO₄ to form a compound Y. X on heating in presence of Conc. H_2SO_4 at 443K gives Z.which on reaction with H_2O in presence of H_2SO_4 gives back `X.` `Z` reacts with Br_2 (aq) and decolorizes it. Identify X, Y, & Z.and write the reactions involved.

2. An organic compound 'A' is widely used as a preservative in pickles and has a molecular formula $C_2H_2O_2$. This compound reacts with ethanol to form a sweet smelling

compound 'B.

(i) Identify the compound 'A'

(ii) Write the chemical equation for its reaction with ethanol to form compound 'B'.

(iii) How can we get compound 'A' back from 'B'?

(iv) Name the process and write corresponding chemical equation.

(v) Which gas is produced when compound 'A' reacts with washing soda? Write the chemical equation.

3. Hydrocarbon `X` and `Y` having molecular formulae C_3H_8 and C_3H_6 respectively. Both are burnt in different spatula on the bunsen flame. Indicate the color of the flame produced by `X` and `Y`. Identify `X` and `Y`. Write the structural formulae.

4. A compound `X` has molecular formula C_4H_{10} . It undergoes substitution reaction readily than addition reaction. It burns with blue flame and is present in LPG. Identify `X` and give the balanced equation for its combustion and substitution reaction with Cl_2 in presence of sunlight.

5. 'A' compound works well with hard water. It is used for making shampoos & products for cleaning clothes. A is not 100% biodegradable and causes water pollution. 'B' does not work well with hard water. It is 100% biodegradable and does not create water pollution. Identify A & B.

6. An organic compound P with molecular formula C2H6Ois an active ingredient of all alcoholic drinks. It is also used in medicines such as tincture iodine, cough syrups. Identify `P`. Drop a small piece of sodium into the test tube containing `P`.A new compound `Q` is formed with the evaluation of colorless and odorless gas Name the gas evolved and compound `Q` write the chemical reaction.

7. A cyclic compound `X` has molecular formula C6H6. It is unsaturated and burns with sooty flame. Identify `X` and write its structural formula. Will it decolorize bromine water or not and why?

8. An organic compounds `A` is a constituent of antifreeze and has the molecular formula C_2H_6O . upon reaction with alkaline KMnO₄, the compound `A` is oxidized to

another `B` with formula $C_2H_6O_2$. Identify the compound A` and `B`. Write the chemical equation for the reaction which leads to the formulation of `B`

9. Two compounds `X` and `Y` have the same formula $C_2H_4O_2$. One of them reacts with sodium metal to liberate H_2 and CO_2 with NaHCO₃. Second one does not reacts with Na metal and NaHCO₃ but undergo hydrolysis with NaOH to form salt of carboxylic acid and compound `Z` which is called wood spirit. Identify `X`, `Y`, and `Z` and write chemical equation for the reaction involved.

10. A compound `X` with molecular formula C_2H_4 burns with a sooty flame. It decolourise bromine water. Identify `X`. Will it dissolve in water or not? Will it conduct electricity in aq. Solution? Will it have high melting point or low melting point?

Answers

Ans I.

 $CH_{3}-CH_{2}OH \xrightarrow{Alkaline KMnO_{4} + Heat} CH_{3}COOH$ $X \qquad Y$ $CH_{3}-CH_{2}OH \xrightarrow{Hot conc.} H_{2}SO_{4} \rightarrow CH_{2} = CH_{2} + H_{2}O$ Z

Ans 2.

 $\begin{array}{cccc} CH_{3}-COOH \ + \ CH_{3}-CH_{2}OH & \stackrel{Acid}{\longrightarrow} CH_{3}-CH_{3}-CH_{2}-CH_{3} \\ & & O \\ \hline \hline & & O \\ \hline & & O \\ \hline & & O \\ \hline \hline & & O \\ \hline & & O \\ \hline & & O \\ \hline \hline \hline & & O \\ \hline \hline & & O \\ \hline \hline \hline & & O \\ \hline \hline \hline \hline & & O \\ \hline \hline \hline \hline &$

A B (iii)Esters react in the presence of an acid or a base to give back the alcohol and carboxylic acid.

 $(iv) CH_{3}COOC_{2}H_{5} \xrightarrow{NaOH} C_{2}H_{5}OH + CH_{3}COOH$

(v) CO2

$$\rm 2CH_{g}COOH + Na_{2}CO_{g} \rightarrow \rm 2CH_{g}COONa + H_{2}O + CO_{2}$$





Ans 4: $C_{4}H_{10} + 13/2 O2 \longrightarrow 4CO_{2} + 5 H_{2}O$ X (Butane) $C_{4}H_{10} + Cl_{2} \longrightarrow C_{4}H_{9}Cl + HCl$ Ans 5. A is between 8. D is seen

Ans 5 A is detergent & B is soap.

Ans 6:

Ans 7:

 $2Na + 2CH_{3}CH_{2}OH \rightarrow 2CH_{3}CH_{2}O^{-}Na^{+} + H_{2}$ (Sodium ethoxide) 'P' 'Q' H - C - H H - C - H H - C - H H - C - H H - C - H H - C - H H - C - H H - C - H

It does not decolorize bromine water because it does not undergo addition reaction.

Ans 8:

$$\begin{array}{c} CH_{3}-CH_{2}OH \xrightarrow{Alkaline KMnO_{4} + Heat} CH_{3}COOH \\ \hline Or acldified K_{2}Cr_{2}O_{7} + Heat \\ \hline `A` `B` \end{array}$$

Ans 9:

$$\begin{array}{c} CH_{3}COOH + NaHCO_{3} \rightarrow CH_{3}COONa + H_{2}O + CO_{2} \\ & `X` \\ CH_{3}COOH + Na \longrightarrow CH_{3}COONa + H_{2} \\ HCOOCH_{3} + NaOH \longrightarrow HCOONa + CH_{3}OH \\ & `Y` & `Z` \end{array}$$

Ans 10:

`X` is ethene. It will neither dissolve in water nor conduct electricity because it is a covalent compound. It has low melting point.

PRACTICE QUESTIONS

- 1. Write the formula for 'ethanoic acid'and name the functional group present in it.
- 2. Allotropy is a property shown by which class:-Substances, elements, compounds, mixture.
- 3. What is the use of oxyacetylene flame?

- 4. Name the gas which is formed by decomposition of plants and animal matter in marshy areas?
- 5. Mention the name of the by product of soap industry/
- 6. Write the molecular formula and structures of benzene.
- 7. Match the following:-
 - 1. Ethane Used in anti freeze solution.
 - 2. Ethanol fruity smell.
 - 3. Ester fossil fuel.
- 8. Write two uses of fullerenes.
- 9. Complete and balance following equation:-
- a) $CH_2=CH_2+H_2$ heat, Ni

b) CH₄+O₂ →

10. Two alkanes A and B have 4 and 6 carbon atoms respectively in their molecule. In which physical state will they occur at room temp?

- 1) Give a test that can be used to differentiate chemically between butter and cooking oil.
- 2) How will you distinguish between ethanol and ethanoic acid by a suitable chemical test? Write chemical reactions involved.
- 3) Complete the following reactions :-
 - 1) $CH_3COOH + NaHCO_3 \longrightarrow conc.H_2SO_4$
 - 2) HCOOH + CH₃OH
 - 3) $CH_3COOC_2H_5 + H_2O$ —

4) Name the compound $CH_3COOC_2H_5$.Name the acid and alcohol from which it is made .write equation.

Land Use and Land Cover Analysis of Mandavi River Basin by Using RS and GIS of Rayalaseema Region, Andhra Pradesh, India

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Abstract: Watershed's assessment, development and maintenance provide existing and historical land use/cover data and change data. To track land use/cover shifts in the Mandavi river basin, Kadapa district, Andhra Pradesh, India with Landsat satellite imaging for the years 2006 to 2018, we follow monitored classification using maximum probability with ERDAS imagine. These satellite data was used in ERDAS 2014 to research land use/coverage. Six types may be identified: property, built-up land, forest, irrigated land, river and water bodies. Data from 2006 and 2018 indicate that the region of fallow land rose by approximately 0.84% (12.30 km²) and 2.92% (42.82 km²), while land, farmland, river and aquifer areas declined between 1.86% (27.30 km²), 1,34% (19.66 km²), 0.26% (3.87 km²) and 0.29% (4.28 km²) respectively. This evaluation demonstrated that the average precision for 2006 photos and 2018 images were 86.62% and 91.85%. In 2006, Kappa values of 0.8343, 0.8987 and 0.9032 for separate photos were observed. These values suggest that the classification features are appropriate.

Key words: LULC, Remote Sensing, GIS, Kappa Coefficient, Satellite data

5.1. Introduction

Land cover applies to the underlying layers of the Earth's surface that can comprise of numerous separate components such as water, grass, frost, and desert as well as the immediate sub surfaces, including soil, topography, groundwater, and biota [1,2,3,4]. Hydrologic elements affected by land rainfall, streamflow, groundwater recharge, are directly and Evapotranspiration. Human behavior is the main cause of LEC; moreover, climatic change is projected to intensify and accelerate LEC, as well as increase the weakness of species and cultural capital [5]. Water balance and water flow paths are evolving with regard to urbanization, irrigation, erosion, and everyday activities of humans [6]. Hydrological systems can be rather complex and are highly affected by nature and land use (Wang et al., 2007). Human effect on environment is by pollution of heat-trapping greenhouse gases from energy, manufacturing, farming, and other practices. One cannot determine environmental change by gazing at LULC, (climate change) [7,8]. The effects of Lyme disease on the climate have become more relevant concerns in worldwide transition science. Human activities affect the majority of the shifts on the surface of the planet. Changes in the earth's surface can influence energy balance, climate, and geochemical fluxes, and these changes will eventually affect the socioeconomic activities and sustainability of natural capital [9,10]. The global population has been growing steadily, with the increased demand on the natural resources of the world. The dynamic mechanism of LU/LCC is significant, with its social and environmental contextualization at various spatiotemporal scales. LULCC's use on a broader scale may be a valuable method in the protection of our natural capital [11,12,13,15,16]. The awareness between LU/LCC, socio-environmental and ecological processes has improved over the earlier few decades [17]. To consider the spatial-temporal complexities of existence in various ecosystems, we need a deeper grasp of landscape transition [18]. Analysis delivered the spatialtemporal LU/LCC from 1910 to the present.

5.2. Study Area

The present research area is located in south western part of Cuddapah super group of rocks in Kadapa and a little bit of the area covered in Chittoor district (Figure 5.1). Geographically, it is located in semi-arid region, lies between 13°51'25" and 14°18'43"N latitudes and 78°34'00" and 79°1'00"E longitudes and is included in the survey of India toposheets 57J11, 57J12, 57J15, 57J16, 57K09, 57K13, 57N04 having 1:50,000 scale and covers an area of 1464.95 sq.km. Geologically, the study area is predominantly underlain by crystalline rocks of Archean age consisting of Granite, Granodiorite, Granite gneiss, Migmatite, basic and acidic intrusive rocks

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wherein primary porosity is nil hence runoff is more. Dendritic to sub dendritic drainage pattern present and annual rainfall is 686.62 mm/yr. The annual minimum, maximum and average temperature of the study area stands at 20°C, 45.5°C, and 32.75°C, respectively.

5.2.1 Materials and methods

Land use and land cover (LU/LC) analysis was carried out with two methods initially, detailed land use and land cover mapping was carried out through visual interpretation technique using landsat 8 image in the ArcGIS. After that land use and land cover change detections analysis was carried out through supervised classification for that a series of Landsat satellite images were used.

The methodology includes data collection, pre-processing, analysis, quality checking and results (Figure 5.2). Data that is collected from various sources, 1:50,000 scale toposheet data obtained from Survey of India (SOI) and processed in Arc GIS10.4. From the toposheet basin boundary and various thematic layers, for example, tanks, roads, village locations, etc., are digitized and updated to the recent satellite image. Landsat data were getting from the earth explorer (Table 5.1). Satellite image resolution of 30 m of 2006 and 2018 (path/row 143/50) were used for land use/cover change analysis and surface water dynamics. NOAA daily temperature and Evapotranspiration data were got from NOAA Earth System Research laboratory [19] respectively, consequently processed, analyzed in Arc GIS 10.4. Then land use, land cover analysis, Landsat data sets were stored in ERDAS Imagine 2014 and generate a false-color composite (FCC). The supervised classification method with maximum likelihood classification (MLC) algorithm, is mostly accepted classification method used in RS data. Hence we follow supervised classification with MLC methodology using ERDAS Imagine software. Consequently, NDVI (Normalized difference vegetation index) algorithms were used for vegetation analyses. Surface water features obtained by using modified NDWI {ie., (Green-SWIR)/ (Green+SWIR)} why because McFeeters' Normalized difference water index {ie., (Green-NIR)/(Green+NIR)} is unable to separate built-up features from water features [19,20] hence, Xu's modified normalized difference water index is used to extraction of surface water features in the present study. For improving the quality of the maps, ground verification was done with German GPS (Global positioning system) for doubtful areas and miss-classified areas were corrected. Without accuracy assessment, the quality of maps would not be fulfilled. Hence accuracy assessment has been carried out for classified images [21].



Figure 5.1. Study area: Mandavi River Basin.



Figure 5.2. Methodology for monitoring of surface water dynamics.

5.3. Results and Discussion

5.3.1. Supervised classification

Supervised classification is well known, and mostly acceptable classification algorithm used for image classification [22]. Hence supervised classification with the MLC algorithm in ERDAS Imagine software used to evaluate land use/cover change between 2006 and 2018 (Figure 5.3). In this connection LU/LC was classified into six categories in the present study area viz., (i) Agricultural land (ii) built-up land (iii) fallow land (iv) forest land (v) river (vi) water body and with their respective percent areas were shown in the (Figure 5.4).

The LU/LC data reveal that in 2006, about 19.55% (286.37 sq. km) agricultural lands, 0.58% (8.49 sq. km) under built-up land, 56.28% (824.41sq. km) under fallow land, 22.04% (322.92sq. km) under forest land, 0.92% (13.49sq. km) under riverbed and 0.63% (9.25 sq. km) under the water body. And during 2018, about 17.68% (259.06 sq. km) agricultural land denoted that 1.86% of the area has been decreased because of less availability of water in that season i.e., 0.34% (4.97 sq. km) under the water body which means 0.29 percent of surface water is decreased. As well as 1.42% (20.79 sq. km) about built-up lands indicated that 0.84 percent increased, 59.20 % (867.23 sq. km) under fallow land point out that 2.92 percent increased and 20.70 % (303.25 sq. km) under forest land signifies the 1.34 percent decrease when comparing to 2006 LU/LC respectively (Table 5.2 & Figure 5.5).

Year	Satellite sensor	Remote Sensing data date of acquisition (Path/row: 143/50)
2016	LISS IV	21 February
2006	Landsat TM	21 st January 14 th February, 03 rd April and 21 st May
2017	Landsat-8 OLI	13 December
2018	Landsat-8 OLI	 14 January, 30 January, 03rd March, 06 May

 Table .1 satellite data used.



Figure 5.3. Land use and land cover map of the study area

Table 2. Land use and land cover geographical area	a
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LU/LC	Area in sq. km	
Agricultural double-crop	229	
area	227	
Agricultural Crop Land	291	
Agricultural fallow land	297	
Agricultural Plantations	17	
Forest plantations	12	
Forest area	365	
Water bodies	30	
Built-Up Rural	18	
River/Stream	16	
Built-Up Urban	4	
Wastelands	186	



Figure 5.4. Land use land cover of 2006 and 2018.



Figure 5.5. PI diagrams showing the percentage of land use land cover.



Figure 5.6. Land use/cover changes during the 2018 and 2006.

5.3.2. Normalized difference vegetation index (NDVI)

Where, NIR and RED bands are the amount of light reflected by growing vegetation recorded in the satellite sensor (23). Green vegetation has more visible light absorption and high NIR reflectance, which gives in high, +ve NDVI values. Dry vegetation, water, snow, clouds, and soil take up significantly more of NIR resulting in lower NDVI values (24). In the current study, vegetation index was analyzed during the 2006, 2017-18 (Figure 5.7). Vegetation change monitored using ERDAS and GIS software. The result showed that high (17.13 sq. km) and low (5.54sq. km) vegetation index area observed in 2006 and high (27.25 sq. km) and low (5.60 sq. km) vegetation index area in 2017-18 respectively. The pie diagram shows the percentage of vegetation covered during 2006, 2017-18 as shown in (Figure 5.8).

5.3.3. Modified normalized difference water index (MNDWI)

Surface water shrinkage has become a global environmental challenge. To overcome those related struggles, it is necessary for the monitoring of surface water in the catchment scale. RS and GIS both are powerful tools for monitoring, assessment, and management of



CHAPTER 5: Land Use and Land Cover Analysis of Mandavi River Basin by Using RS And GIS

Figure 5.7. Normalized difference vegetation index (NDVI), temporal changes for the period of the 2006-18.



Figure 5.8. PI Diagram showing the percentage of land use land cover during the 2006, 2017.

environmental-related studies, particularly in the field of natural surface water resources studies such as tanks, rivers, lakes, etc. [25]. Due to its remarkable properties like high spectral and temporal resolution a series of cloud-free Landsat satellite images are used to obtain natural surface water features that give accurate data to detect changes in the extent of surface water bodies.

Surface water features obtained by using Xu's modified NDWI {ie., (Green-SWIR) / (Green+SWIR)} why because McFeeters' Normalized difference water index {ie., (Green-NIR) / (Green+NIR)} could not separate built-up land, vegetation and soil noise from water features. Mostly current study area does not receive rainfall in winter and summer (January to May) seasons so that this time period cloud-free satellite data has been chosen for observation of surface water changes with respect to temperature and ET.

The result reveals that the surface water body area has been decreased from 21.24sq. km to 2.39 sq. km during the 21 January 2006 to 21 May 2006 (Figure 5.9 &11) and from 19.81sq. km to 5.38 sq. km during the 13 Dec 2017 to 05 May 2018 (Figure 5.10 & 11), hence temperature and ET were key parameters for surface water degradation. The daily temperature trend line showed that (Figure 5.12 &13), it is increased gradually from Jan to May in 2006. From Dec 2017 to 31 May 2018 the temperature trend line is steadily increasing and in four points on the graph temperature curve has been decreased below the trend line. With 2006, 2017-18 Evapotranspiration is increasing along with temperature [26,27] (Figure 5.14 & 15).

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Figure 5.9. (a) (i) Geo referenced SOI toposheet (1:50,000 scale); Temporal Landsat TM satellite images (21 Jan 2006, 14 Feb 2006, 03 April 2006, 21 May 2006) with false-color composite (RGB: 432); (b)(i) Manually digitized toposheet tanks; MNDWI calculated from TM bands 2 and 5 showing water bodies.



Figure 5.10. Temporal Landsat TM satellite images (13 Dec2017, 14 Jan 2018, 30 Jan 2018, 03 March 2018, 06 May 2018) with false-color composite (RGB: 543); (B) MNDWI calculated from TM bands 3 and 6 showing water bodies.

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Figure 5.11. Temporal water bodies geographical area during the 2006 and 2018. And both years the trend lines are gradually decreased.



Figure 5.12. Temperature values have been increasing gradually, minimum, maximum, average temperature values representing in graph during the 01 January 2006 to 21 May 2006.



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Figure 5.13. Monthly evapotranspiration during the January to May 2006.



Figure 5.14. Temperature values have been increasing gradually, minimum, maximum, average temperature values representing in graph during the 01 December 2017 to 06 May 2018.

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Figure 5.15. Monthly evapotranspiration during the December 2017 to May 2018.

5.4. Conclusion

In the present study geospatial technologies were adopted along with simple, accurate and efficient heuristic approaches like MNDWI, NDVI and supervised classification with maximum likelihood methodologies were applied to monitor LU/LC changes during 2006, 2017-18. The current study reveals that the majority of land use in the study area is fallow land because of insufficient water resources. Agricultural land was reduced by 1.86% (27.30 sq. km), built-up land increased by 0.84% (12.30 sq. km), forest land was reduced by 1.34% (19.66 sq. km) and river and water body has decreased 0.26% (3.87 sq. km), 0.29% (4.28 sq. km) respectively. In 2006, high (1.49%) and low (0.37%) vegetation indexes were observed on 21st Jan and 03 April respectively. Similarly, in 2017-18, high (1.86%) and low (0.38) vegetation indexes were observed on 13 December 2017 and 30 January 2018 respectively. Evapotranspiration is one of the key parameters which were indirectly influenced on surface water; the range of ET in 2006 is 99.82 mm (high) in March and 55.47 mm (low) in February while ET in 2017-18 is 103.90 mm (High) in May 2018 and 6.0 mm (low) in Dec2017. The trends of temperature and ET were correlated with each other and trends of the area of surface water bodies in 2006 and 2017-18 were inversely proportional to the temperature and ET hence RS and geospatial techniques are very useful for spatial non-spatial data analysis.

5.5. References

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Polymer Electrolyte Membranes for Fuel Cell and Drug Delivery Applications

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Abstract: Polymer membranes are emerging substrates for industrial applications like power solutions, toxic metal ion removal and drug delivery technologies. Among all types of membranes polymer electrolyte membranes (PEMs) are current interest, due to their physicochemical interaction with the guest molecules. PEMs are capable to transport or permeate, adsorb and delivery of molecules, ions and other required reagents. This chapter provides basic concepts as well as the progress with regard to PEMs based science and technology of fuel cells and drug delivery.

Key words: Polymer Electrolyte Membranes, Fuel Cell, Drug Delivery

6.1. Introduction

The composition and structure of the constituent materials in a membrane is the utmost important factor in any technology-based membranes [1, 2]. The optimization of membrane performance is very crucial in multicomponent systems, because not all the simple materials either natural or synthesized, typically meet all conditions necessary for a fully capable process [3, 4]. Therefore, it is essential to combine various components to custom new features that can lead to achieving optimum membrane capacity and performance [5]. The performance of the membrane can be optimized by varying the composition of constituent component materials is a general practice that has been employed in the past decade [6-9]. Innovative combinations, knowledge of structure-property relations, and simple fabrication methodologies will need to be integrated to achieve functional products that are excellent in performance.

The presence of reactive functional groups in some polymers like PVA, pectin, and chitosan provides a wide opportunity for chemical modification, which affords an extensive range of derivatives [10-14]. These polymer derivatives have been designed to improve specific properties of native polymer to achieve prerequisite qualities. Various synthetic strategies for the functionalization of these polymers have been extensively reviewed elsewhere [15-19]. Blending of polymers is one of the most promising approach in polymer technology, to get an optimized performance of the fabricated materials, which is due to the fact that resulting blend lock up the attractive features of individual component, while reducing their defective qualities. It is quite interesting to combine different polymers to achieve optimal performance by using structure-property relations.

Generally, synthetic polymers play dominating role in polymer membrane technology, but recently, their acceptance has waned due to their non- bio degradability and non-bio compatibility [20-23]. Though, the polysaccharide-based materials are becoming interesting due to their superior qualities like environmental benignity and economic viability [24-27]. The use of polysaccharides such as chitosan, pectin, sodium alginate and cellulose has supplanted traditional synthetic polymers in energy and biomedical fields [25-28].

However, the strict environmental regulations pave a way to use of biopolymers as a first choice to meet stringent requirements to replace the synthetic polymers. Recently various scientists reported simple biopolymers derived from plant or animal-based materials with a clear and distinct structure. Alginate, pectin, chitosan, and cellulose are well known examples of biopolymers derived from biological resources like plants and animals. Which have been using in different areas of polymer technologies like fuel cell technology, biomedical technology, and food & packing technology [29-32]. To accomplish required functional properties, the polymers should be with effective responsivity to either of internal or external stimuli that is caused by a change in physicochemical microstructure. Recently, large amount of research is undergoing to improve the membrane processing capacity and operation by using novel and effective biopolymers [5].

Chitosan is a natural poly amine polymer derived by the partial de-acetylation of chitin called poly (acetyl-glucosamine) and it contains a large number of $(1 \rightarrow 4)$ linked 2-amino-2-deoxy-D-glucose units [33]. Cs is very good applications in various applications like FCs, membrane separations, pharmaceutical and biomedical, and environmental applications due to the following characteristics [34-38]. Cs is a second most abundant natural polymer on the earth, and it is economically low cost. The characteristics like (a) film-forming property; (b) the structurally modifiable amino and hydroxyl functional groups; and (c) good alcohol resistance are very advantageous for PEMs. It has large number of inherent properties like good biocompatibility, bio degradability, antimicrobial activity against abroad spectrum of microorganism, and mucoadhesive properties. Which makes Cs as excellent candidate in pharmaceutical and biomedical applications. Being a poly amine, chitosan is unique among biopolymers by its high affinity for heavy metal ions and highly conductive for protons in acidic medium. Contemporary investigation on Cs enabled the development of variety functional materials for potential applications in fuel cells, food packing, antimicrobial, tissue engineering, pharmaceutics, and the adsorption of toxic metal ions and dyes etc. [39]. In this regard, chitosan has been blended with numerous other polymers e.g., PVA, poly lactic acid (PLA), polycaprolactone (PC), pectin, alginates, and collagen to make blend materials [40-42].

Pectin (PC) is a natural anionic polysaccharide derived mainly from fruit peals of citrus family. Structurally pectins are a group of polysaccharides, majorly containing poly galacturonates linked

by $(1\rightarrow 4)$ glycosidic bonds [23, 43, 44,]. The materials based on pectin are highly attractive for research and industrial application due to sustainability, nontoxicity, biocompatibility, and biodegradability [23, 45]. Pectins have wide spread applications in the field of food processing, cosmetics, membrane technology, pharmaceutical and biomedical applications [23, 45, 46]. Alginates are natural polyelectrolytes which is block co polymer of β -D-mannuronic and α -Lguluronic acid residues linked through $(1\rightarrow 4)$ glycosydic bond. The hydronium ion affinity, fundamental physio-chemical and rheological properties of the alginate membranes are determined by the organization of repeating groups along the chain [47, 48].

Since its discovery in 1924, PVA has become one of the extremely studied polymers in the literature. PVA is a water-soluble polymer, that is widely used in variety of applications since their easy of materialization, outstanding chemical resistance and mechanical properties, along with exceptional film forming, emulsifying and adhesive properties. PVA is a unique material, having semi-crystallinity, even in atactic form, in spite of the lack of stereo regularity [49-51]. In aqueous solutions, it forms aggregates of hydrogen bonded PVA molecules, when polymer concentration more than 1% PVA solution. Which leads to formation of crystalline regions in the membranes [51]. Moreover, owing to the presence of hydroxyl groups on PVA chain, it can be modified into multifunctional, multi-vinyl macromers through substitution by a variety of substituents [52-55]. Recently, He et al. developed a novel multi-layered and multifunctional wound dressing based on PVA foam to control wound infection and hemostasis. Additionally, it can provide the real time monitoring of the infection cure. The fabricated wound dressings are promising option for future health care sector [56]. Wang et al. prepared phosphonated PrEMs based on PVA using organo phosphonic acid as a proton source, by solution casting method. These optimized PVA membranes show very low methanol permeability (10⁻⁷-10⁻⁶ cm².s⁻¹) at room temperature, and are having comparable proton conductivity with the nation-117 membrane [57].

6.2. Polymeric materials for FCs

6.2.1. Principle of fuel cells

According Wikipedia, "A fuel cell is an electrochemical cell that converts the chemical energy of a fuel (often hydrogen) and an oxidizing agent (often oxygen) into electricity through a pair of

redox reactions". In most of the respects it resembles the batteries, but it can provide electricity over a much longer period of time.

The ever first PEMFCs were developed in early 1950's for Gemini space missions by the General Electric Company (GEC) [58]. Then, the DuPont de Nemours Company invented a perfluorinated sulfonic acid ionomer called Nafion®2 in the year 1962. Finally, GEC laid down a break-through foundation in 1966 by using the same membrane. Further research leads to invention of low platinum loading electrodes and thin film electrodes in the later period up to this millennium.

The PEMFCs use hydrogen as a fuel and oxygen as an oxidant. Generally, oxygen is supplied either in pure form or as an air, based on the requirements and based on type of application. On the surface of anode, the hydrogen molecules split into protons and electrons, out of which the protons travel through the PEM to the cathode, although the electrons are transported via external circuit in the form of load to the cathode. By using the load, the cathode combines oxygen, and protons to form water.

6.2.2. Proton Exchange Membrane PrEM Fuel Cell

The FCs in which proton is transferred from anode to cathode through the PEM are called PrEMFCs, and they play a major role in renewable energy sector. The PrEMFCs can be divided into different types based on the fuel used in them.1) hydrogen fuel cell 2) direct methanol fuel cell, and 3) direct ethanol fuel cell. The hydrogen fuel cell uses H_2 gas as a fuel and provides excellent fuel cell performance.

The half-cell reactions for the hydrogen PrEM fuel cell are as follows.

Anode: $H_2 \leftrightarrow 2H_+ + 2e^-$ Cathode: $\frac{1}{2}O_2 + 2H^+ + 2e^- \leftrightarrow H_2O$	$\Delta G^{o} = 0.00$ $\Delta G^{o} = -237.3 \text{ kJ/mole}$	(1) (2)

For the above cell, the both electrical and heat energies are produced by the cathode reaction. Theoretically, the Gibbs free energy of the reaction is available as electrical energy, but actually, a part of the Gibbs free energy will be converted to heat via the loss mechanisms.



Figure 6.1. Schematic diagram of PEMFCs.

$$\Delta G = \Delta H - T \Delta S \tag{4}$$

where ΔH is the change of enthalpy in reaction, T is the temperature, and ΔS is the entropy change of a reaction. Here, both the enthalpy and entropy of reaction are also dependent on the temperature at which the reaction taking place.

We know that the Gibbs free energy change of a chemical reaction is very well correlated with the cell voltage as.

$$\Delta G = -nFV_{o} \tag{5}$$

$$V_{o} = -(\Delta G / nF) \tag{6}$$

Where 'n' is the number of electrons involved in the reaction, 'F' is the Faraday constant (96485 C/mole), and 'V_o' is the cell voltage for thermodynamic equilibrium in the absence of a current flow i.e., open circuit conditions at equilibrium.

Substituting the standard conditions values for hydrogen and oxygen [2] into Equations (4) and (6), gives

$$V_{o} = -\frac{-237.3 \text{ kJ/mole}}{2 \times 96485 \text{ C/mole}} = 1.23 \text{ V}$$
(7)

The EMF of the equilibrium cell is the potential difference between anode and cathode at equilibrium conditions.



Figure 6.2. Photo of Fuel cell stack.

The schematic diagram of the fuel cell shown in figure 2, and the main components of PrEM fuel cell are:

- 1. Electrodes: Anode and Cathode
- 2. Polymer Electrolyte Membrane (PEM)
- 3. Gas diffusion layer (GDL)
- 4. Collector Graphite Plates

6.2.2.1. The electrodes

The electrodes are reaction centers of the fuel cells, where all the electrochemical reactions taking place. In order to attain suitable rates of electrode reaction, the operational area of active catalyst must be larger than the actual geometrical area of the electrode. Therefore, it is essential to made porous electrodes having a three-dimensional network, in which the three phase interfaces are located. The commercially available fuel cells always contain platinum or alloys of platinum or sometimes other noble metals like Pd as a catalyst. However, it is essential to maintain low temperature and low pH to get an optimum catalytic activity. Particularly, the cathodic oxygen reduction reaction (ORR) is very slow at elevated temperatures.

Nowadays, most researchers have been preferred the thin-film methodology, for their prototype instruments, in which the electrode materials are directly coated on the surface of the membrane. The thickness of a coated thin-film electrode is usually in the range of 5-15 μ m and the catalyst loading is between 0.1 to 0.3 mg per cm². The thin-film electrodes are more beneficial due to their low price, better catalyst utilization; additionally they show superior mass transport through the membrane [59].

6.2.2.2. Electrolyte membrane

The proton electrolyte membrane is a vital part in the membrane electrode assembly (MEA). It physically separates the electrodes by not conducting the electrons and reactant materials, but allows protons to pass through to the cathode side. The PrEM usually contains a sulfonate tailored PTFE based polymer backbone, which contain large number of freely migrate hydronium ions. The most common membrane material for commercial PEMFCs is Nafion®. In fact, to conduct protons, the PEMs must be in hydrated state, therefore the water management become a vital factor to achieve maximum performance PEMFCs due to the loss of water at the temperatures more than the boiling point of water. But, recently, water free PEMs were developed which can work at the temperatures more than 100 $^{\circ}$ C [60].

6.2.2.3. Gas diffusion layers (GDL)

The membrane assembly (MEA) contains a proton exchange membrane, catalyst layers/electrodes, and GDL. It is usually sandwiched by two mirrored flow field plates to make bipolar plate when

cells are stacked in series for greater voltages. Generally, GDL provides two major functions, one is making an electrical communication between the electrodes and bipolar plates and the other one is distribution of reactant to the electrodes, removal of heat and water from the electrodes. The GDLs fiber or non-fiber materials which are made by carbon cloth or carbon paper. They are porous materials with electrical conductivity and usually coated with a fluoropolymer at inner side and carbon black at outer side to improve the water management and electrical properties.

6.2.2.4. Bipolar plates

Bipolar plates are said to be the backbone of a PEMFC power stack by which we can isolate the individual cells. They act as a support structure in fuel cell stack and conduct current between the cells. Additionally, it facilitates water and thermal management through the cells by providing conduits to the electrodes through GDL for the transport of reactant gases as well as removal of reaction products.

Bipolar plate material must have high conductivity with least interfacial contact resistance, and as reactant gases and catalyst present, it should be impermeable to gases and high resistivity towards the corrosive environment, however, for commercial applicability, the material should be cheap, low weight, great mechanical strength, and suitable for high-volume manufacturing methods.

Most PEMFC bipolar plates are made of graphite/polymer composites, but also stainless steel has been used [61-63]. Solid graphite is highly conductive, high chemical stability in acidic conditions with pH < 4, but its manufacturing is expensive and their structures are brittle. Stainless steel is very affordable, have great mechanical properties but its machining process is expensive. However, the SS based materials must frequently be coated with resistive material to prevent corrosion and to reduce contact resistance. The best results have been achieved by using molded carbon-polymer composites [64-66].

6.2.3. Desirable properties of fuel cell membrane materials

Out of which the electrolyte membranes or polymer electrolyte membranes (PEMs) are core components of the PEMFCs and main intension of the book chapter is the PEMs.

To achieve high efficiency, the membrane must possess the following desirable properties.

- > High proton conductivity to support high current along with zero electrical conductivity,
- > Adequate mechanical strength and physical stability,
- > Chemical stability under operating conditions.
- > Extremely low fuel or oxygen transport to maximize columbic efficiency.
- > Productions costs compatible with intended application.

Even though, the polymer membrane synthesis is advanced in the recent decades, for the improvements in thermal and mechanical properties, a scientific approach based on the application of thermodynamics, mass transfer kinetics and structure-property relationship also considered in order to ensure satisfactory performance of the membrane. However, the suitability and membranes for fuel cell applications is depended on the level of hydration and thickness of the membrane.

6.2.4. Polymer electrolyte membrane fuel cells

Most of the PEMs investigated for DMFC and PEMFC applications are currently synthesized by using diverse approaches, like the production of new ionic and/or block copolymers, graft copolymerization of ionic monomers on hydrophobic polymers, blending of ionic and non-ionic polar polymers, synthesis of IPNs based on ionic/polar polymers and composite membranes using a large variety of fillers.

Generally, the polymer electrolyte fuel cells (PEMFCs) contain a thin solid polymer electrolyte called Polymer Electrolyte membrane (PEM). The benefits of PEM are high efficiency, high power density, reduced corrosion, high operational [67-69]. Nowadays, PEMFCs became one of the valuable chemical engineering technology due to their capability of quick start-up and low-temperature operation [70-72]. Finally, the PEMFCs are also environmental friendly as there is no emission of harmful gases like oxides of sulfur and nitrogen, which are environmentally benign [73-75].

In current world, Nafion is mostly using perfluorinated commercial polymer, even though several other perfluorinated polymers are available. Out of them XUS[®] by Dow chemicals, Aciplex-S[®] by

Asahi Kasei, Fumion[®] by Fumatech are very important and considering worldwide. Even though perfluorinated membranes granting excellent fuel cell properties, they also possess some shortcomings like high cost, high methanol crossover, and lowering of performance at elevated temperatures above 100 °C. Additionally, they lose fluorine in exhaust slowly by the attack of hydroxyl radicals, which also cause the loss of performance. The above limitations of perfluorinated membranes made researchers to investigate low-cost membranes with similar or high productivity even at elevated temperature (100-150°C).

The PEMs plays an important role in permitting ions to migrate from one electrode to another, where it undergoes electrochemical reaction to form water by reacting with electrons and oxygen at the same time it is barrier for fuel, oxidant and electrons (electronic insulator) between the two electrodes. Based on the PEM, the PrEMFCs can be classified as proton exchange membrane fuel cells (PrEMFCs), and alkaline anion exchange membrane fuel cells (AEMFCs). The Proton exchange membranes comprise replaceable protons, and hence they have been successfully integrated in various applications like fuel cells, batteries, and water desalination. There are two important factors to fabricate proton exchange membranes, one is well-defined conducting ionic channels of membranes and the other is good mechanical and chemical stability of membranes. The anion exchange membranes comprise replaceable hydroxyl ions, and hence they have been successfully incorporated in different technologies such as fuel cells, and water desalination. Compared with that of PrEMFCs, AEMFCs that operated under high temperature and pH conditions. Furthermore, the liquid hydroxide electrolyte present in the FCs reacts with carbon dioxide forming bicarbonates and subsequently carbonate salts. Moreover, the carbonate salts in supersaturated solutions block pores of the membrane, which leads to adverse effect on output power.

PEM plays a key role in fuel cells like DMFC, which transports protons from anode to cathode and it acts as a physical barrier to prevent methanol crossover from the anode to the cathode. However, the methanol crossover is the major drawback for the commercialization of DMFCs, which arises due to the diffusion of methanol from anode to cathode through polymer electrolyte [76-78]. Which may trigger the loss of fuel, causing cell polarization by mixed potential at the

cathode [79, 80]. Even commercially available nation also not an exception for this cell depolarization [79, 81].

To overcome the aforementioned issues, and to diminish the methanol crossover, several alternative PEMs have been developed and used for effective DMFC performance. The majority of these PEMs were made from cross-linked solid polymers due to their wide variability of barrier properties owing to their structures [6, 82-85]. In recent years, different natural and synthetic polymer composite membranes were fabricated by various scientists, which shows a significant impact on the polymer electrolytes for DMFCS [86-89]. Among these, natural polymers like chitosan (CS), sodiuim alginate (Sod Alg), pectin (Pn), and synthetic polymers like poly (vinyl alcohol) (PVA) have drawn considerable attention in the past decade [90-96].

Cs is a natural biopolymer, and is widely studied as an electrolyte in PEMFCs. Which having very attractive features for PrEM fabrication, even though poor proton conductivity due to rigid crystalline areas in membranes. In order to alleviate the above disadvantage many researchers utilised either chemical modification of Cs or chemical cross-linking [97, 98]. Nevertheless, the chemical reactions are often complicated and difficult to control accurately, furthermore it may lead to mechanical and stability problems. Therefore, making of organic-inorganic hybrid composites is a facile strategy for chitosan modification. Many researchers achieved the enhancement of proton conductivity by the incorporation of inorganic nanofillers such as TiO₂, graphene oxide, montmorillonite, and carbon nanotubes due to their long-range uninterrupted proton transport channels [99-101].

The introduction of other materials like GO, lignosulfonate into Cs membranes improves both the mechanical and FC characteristics like proton conductivity and selectivity [102]. High proton conductive Cs chemically modified Boehmite (Cs-CB) nanocomposite membranes were synthesized by Ahmed et al., which showed a proton conductivity almost equal to that of commercially available Nafion 117 (0.015 S cm⁻¹) [103]. Smitha et al., fabricated ionically crosslinked polyelectrolyte membranes of Cs and polyacrylic acid. Which were then tested for fuel cell applications. These membranes exhibited potential characteristics of FCs like high IEC, high

proton conductivity (0.038 S.cm⁻¹), and low methanol permeability ($3.9 \times 10^{-8} \text{ cm}^2.\text{s}^{-1}$) along with sufficient thermal and mechanical stability. [87].

Meenakshi et al., developed two different membrane systems (STA-CS-PVA hybrid membranes and CS-PVA-SPES mixed matrix membranes) for DMFC application based on natural polymers Cs, PVA [79, 89]. The STA-CS-PVA hybrid dual-network membranes containing Cs-PVA chains crosslinked with sulfosuccinic acid (SSA) and glutaraldehyde (GA) and modified with stabilized silicotungstic acid (STA). The optimized membrane is one with 10 wt.% STA showing lower methanol permeability compared to Nafion-117 membrane. The hybrid membrane STA-CS-PVA with10 wt.% stabilized STA delivers a peak power density of 156 mW cm⁻² at 400 mA cm⁻² of LCD. [79]. Further developed Mixed-Matrix Membranes with Cs, PVA, sulfonated polyethersulfone, and a cross-linker sulfosuccinic acid. These CS-PVA-SPES membranes evaluated for DMFC performance, which shows 33% lower methanol cross over about to with concerning Nafion-117 membrane. However, it delivers a PPD (peak power density) of 5.5 mW cm⁻² at an LCD (load current density) of 25 mA cm², at a 70 °C operational temperature. [89]. In conclusion, the low cost, easy processability, environmental benignity, and overall superior FC performance of CS-based membranes has been proved.

Shaari et al. developed sodium alginate based bio membranes by using different materials [93, 104]. They studied the FC performance and selectivity of alginate based membrane as a function of sulfonated GO and glycerol content. The optimized selectivity (12.956 x 10^4 S cm⁻³) was achieved by using the loadings of 2.78 wt% SGO and 3.11 ml of glycerol, in to the SA/SGO bio membrane. However, it shows max power density of 13.6 mWcm⁻² [104]. Further, they fabricated nanocomposite bio membranes for DMFCs, sodium alginate as the matrix phase and alumina as a nanofiller. The well-connected matrix through the filler leads an excellent performance by the SA/Alumina nanocomposite bio-polymer membrane. The proton conductivity of these membranes was 25.6 x 10^{-3} S cm⁻¹ at 60 °C temperature [93].

Mohanapriya et al. developed a series of mixed matrix membranes based on SA and PVA with different heteropolyacids, and GA as ae ex-situ cross linker. These fabricated membranes possess micro-domains in their networks, which restrict the methanol cross-over, and also, they exhibit a

maximum power-density of 68 mW.cm⁻² at 225 mA.cm⁻² of LCD, while operating at 70 °C [90]. SA based functionalized GO membranes were developed by Balappa e al. using polystyrene sulfonicacid-co-maleic acid as a macro cross-linker. the highest proton conductivity and peak power density of sodium dodecyl benzenesulfonate functionalized GO membrane is 0.145 S.cm⁻¹ and 0.42 W.cm⁻² respectively, at an operational temperature 80 °C. [105]

PVA is a synthetic semi-crystalline polymer, which is having its own signature properties like good film forming ability, favorable thermal and mechanical stability, and excellent cross-linking ability. PVA based PEMs are having widespread applications in fuel cells due to their desirable characteristics like required water uptake with low methanol permeability due to its dense structure formed by strong hydrogen bonding between the polymer chains [106]. However, the non-ionic nature of the PVA is major drawback for the fuel cell applications. Therefore, it is very essential to introduce proton sources like sulfonate groups, phosphate groups, and quaternary ammonium groups into the polymeric membranes. The incorporation of proton sources in to PVA membranes can be achieved by graft copolymerization, physical or chemical crosslinking, and blending with other polymers. Even though, the ionic conductivity and hydrophilicity of PVA membranes were improved by the introduction of proton sources due to ion-cluster effect, they cause some adverse effects like high methanol crossover, and loss of proton conductivity and slow cathode kinetics at elevated temperature. To maintain or improve fuel cell efficacy, we have to fabricate membranes with appropriate number of materials required.

S.D. Bhat et al., developed PVA-SSA-HPA mixed-matrix membranes and evaluated for DMFC applications. The optimized membranes of these system deliver effective DMFC performance with high proton conductivity and restricted methanol crossover [95]. Novel PrEM based on crosslinked PVA for DMFCs was developed by Pan Liu et al. These membranes possess high proton conductivity (in the order of 10^{-3} - 10^{-2} S cm⁻¹), with good water management. The methanol permeability of the membranes is in the order of 10^{-7} - 10^{-8} cm² s⁻¹, which is an average about 10 times smaller than that of Nafion-117. [107]. zeng et al. developed layered double hydroxide composite polymer membranes based on PVA, by using a solution cast method. The resulted membranes show a higher ionic conductivity, and also low permeable to ethanol. However, the DEFC based on this hybrid membrane yield a power density of 82 mW cm⁻² at 80 °C [108].

A series of PVA based organic-inorganic crosslinked polymer electrolyte membranes with PVA and poly (methacrylic acid-*co*-2-acrylamido-2-methyl-1-propanesulfonic acid-*co*vinyltriethoxysilicone) were prepared for DMFC applications. These membranes show better methanol barrier property (10 times) and two times better selectivity than the commercial membrane Nafion® 117 [109]. Jingmei Xu et al. synthesized a macromolecular crosslinked membranes based on PVA and sulfonated poly(arylene ether ketone sulfone) (SPAEKS). These membranes show low methanol permeability coefficients ranged from 4.01 x 10^{-7} cm².s⁻¹ - 8.71 10^{-7} cm².s⁻¹ and proton conductivities were greater than 10^{-2} S cm⁻¹ at 25 °C [110]. Rajaram et al, have developed PVA based sPMVEMA (sulfanilic acid (SA)-tethered poly(methyl vinyl ether-altmaleic anhydride)) polymer membranes with randomly orientated and distributed conducting ionic channels. [111].

PC is a least explored material in the class of polysaccharides with respect to DMFC application. as a biopolymer it is renewable and easily available in nature. Recently, it increased usage of PC in DMFCs due to advantages like low cost and environmental benignity. The PEMs of pectin in DMFCs, used either blending with other polymers or functionalized with proton conductive materials. Mishra et al. developed amidated pectin based PEMs for DMFC applications, which show highest proton conductivity of 1.098×10^{-3} S.cm⁻¹ [112]. Further, they tailored PEMs with highly methylated, and diethanolamine modified pectin, for DMFC applications [92]. Recently, pectin-PVA based green hybrid nanocomposite membranes with sulfonated titanium dioxide were synthesized by Mohanapriya et al. for DMFC application which having 27 mW.cm⁻² of peak power density at 70 °C [113].

Molavian et al. prepared semi- IPN membranes based on sulfonated poly(ether sulfone) and used as a PrEM for fuel cell application. The proton conductivity of these membranes is 16 mS.cm⁻¹ which consistent with the Nafion-117. Additionally, these membranes are having good mechanical strength and chemical stability. [114]. Zhu e al. developed Ionic-crosslinked IPN blend membranes based on polysiloxane tethered imidazole/triazole and PFSA ionomer were developed, which exhibited high proton conductivity (0.1 S. cm⁻¹) in presence of 30% phosphoric acid at 120 °C. [115]. Tang et al., prepared a robust proton exchange membrane based on acrylamide. These acids

retentive polyacrylamide/polyacrylamide interpenetrating polymer network (PAM/ PAM IPN) hydrogels were synthesized with low cost and easily processable. The highest proton conductivity of the IPN membrane ranges up to 83.3 mS cm⁻¹ at 183 °C in dry air and remains relatively stable over an 80 h period [116]. Annapurna et al., fabricated a series of blend membranes based on extracellular polysaccharides of microbia and sulfosuccinic acid. The FC electrode assembly equipped with the fabricated membrane showed a peak power density of 400 mW/cm² at a load current density of 1250 mA/cm² [117].

6.3. Polymeric materials for Drug delivery systems

The term 'drug delivery' includes the method (dosage form) and route by which a pharmaceutical compound is administered to achieve a therapeutic effect in humans or animals. The most common routes of drug administration are oral (through the mouth), parenteral (injection into systemic circulation), trans-mucosal (nasal, buccal, sublingual, ocular, rectal etc.), trans-dermal (skin) and inhalation [118, 119]. Usually, the drug delivery is carried out through a drug's chemical formulation called dosage form, and the general dosage forms are capsules, tablets, emulsions/liposomes, or gels (aerogels, nanogels) [120].

The systemic absorption of a drug molecules depends on the physicochemical properties of drugs, the nature of the dosage form on which it is incorporated and the anatomical and physiological properties of the sites where the drug is absorbed. However, the critical step in generating the therapeutic effect is the interaction of the drug with a receptor, which is located either on the cell membrane or inside the cell [121, 122], and the study of these interactions provides vital information for new drugs and drug delivery systems (DDS). Therefore, DDS systems are employed to reach sufficiently high amounts of API to its targets.

The drug delivery systems can be divided into two main types: 1. conventional drug delivery. 2. novel drug delivery. In the conventional DDS, the drug material is absorbed across a biological membrane, whereas the novel DDS releases the drug in a dosage form. The controlled drug delivery systems are having more advantages compared to conventional drug delivery systems, due to the limitations like requirement of higher and frequent dosages of drugs, lower effectiveness, toxicity and adverse side effects because of high drug dosage [123-125]. The novel

DDSs are three types: controlled drug delivery systems (CDDS), Sustained release drug delivery systems (SrDDS), and targeted drug delivery systems (TDDS).

6.3.1. Controlled drug delivery

Until the last century, the controlled and novel drug delivery was only a dream, but the extensive research from two decades make it reality at its best possibility. The controlled drug delivery systems based on polymers have attracted much attention, due to their flexibility in macromolecular synthesis [123, 126-129]. Furthermore, there are infinite methods for their synthesis because of enumerable polymers in terms of nature, properties, and ease of functionalization. Biopolymer based cross-linked hydrogels exhibit an exclusive property in terms of their, stimuli-responsive behavior and drug release characteristics along with favorable mechanical strength [124, 130-134]. A great deal of effort is now focused on the development of polymeric materials, and which makes them as efficient diagnostic and/or therapeutic tools against the diseases. Among them, the carbohydrate based polymer matrices have been attracted a sincere attention in this area, as they accompanying with several advantages as mentioned previous.

6.3.2. Sustained release Drug Delivery

The SrDDSs are intended to release a drug material at a predetermined rate by upholding a constant drug level for locally or systemically for a specific period of time. The main advantages of SrDDS is low amount of drug administered with less dosage frequency, which leads to enhanced drug efficacy with minimum side effects [126, 135-138]. Because of these merits patient acceptance & compliance were increased. However, the reservoir type dosage forms like Enteric coated tablets, capsules, and implantable tablets can only be useful for sustained release [139-144]. The sustained release systems are also stated as 'long acting' or 'delayed release' systems when compared with rapid 'conventional' release systems. The term 'sustained release' sometimes overlaps with 'controlled release', which infers more sophisticated control of drug release, but the prior one maintains drug release over a sustained period but not at a constant rate. The sustained release systems are not new but for better release properties several new modifications are being introduced.

6.3.3. Targeted Drug Delivery

Targeted drug delivery is another type of novel drug delivery system, which has shown lot of attention in pharmacotherapy. In this delivery system, the pharmacologically active agents or the drug molecules selectively targeted or delivered to the site of action [145-147]. The targeted delivery deduces the selective and effective localization of API by using specific properties of target cells, while restricting their access to non-target normal cells. It is a very effective drug delivery system by minimizing the toxic effects and promoting with maximum therapeutic index [145, 148]. In order to accomplish such a targeted drug delivery, the pharmaceutical agent can be encapsulated within the polymer matrices, lipids or surfactants to make nanoparticles, microspheres, and liposomes [149-155]. Hence the innovative polymer chemistry has been introduced a wide variety of polymers and polymeric matrices involved in drug therapeutic systems.

Broadly, there two strategies for targeting the drug to the desired organ/tissue: passive targeting drug delivery and active targeting drug delivery. The passive targeting is accomplished by the accumulation of drug at areas around the site of interest due to the enhanced permeability retention (EPR) effect. The EPR effect is governed by the non-specific physico-chemical properties such as size, surface charge etc. therefore, accumulation of drug is still found in healthy tissues. Whereas, the active drug targeting is attained through the ligand-receptor interactions between over-expressed receptors in target cells and the ligands present on the drug delivery vehicles [156-158]. This phenomenon is able to improve the therapeutic activity and reduce the adverse toxicity.

To achieve a successful system for targeted delivery, it should need four principal requirements, that are retain, evade, target and release. The properly loaded drug delivery vehicles and formulations must possess an ability to escape the body secretions that may degrade it [159]. Additionally, the delivery systems should not be too small to excrete by the kidney or not too large to recognize and trapped by the reticuloendothelial system (ESR). And hence they gain long residence time in blood circulation and thereby reach the site of interest and release the drug at the specific site. Different sites of interest within the body requires various drug delivery systems, depending upon the route of administration.

6.3.4. Wound Healing and Anti-microbial Activity

Antimicrobial dressings play a crucial role in wound care for the prevention and/or treatment of infections associated with a broad range of wound types, including traumatic skin tears and burns, chronic ulcers, surgical wounds dehiscence and wound dehiscence with implantable devices. The main goal in treating infections is to reduce the bacterial burden in the wound site to a level, where the regeneration process is stimulated to heal the wounds [160-163]. The wound healing process requires high local antibiotic concentration for an extended duration of time without exceeding systemic toxicity, and this can be achieved either by topical or a delivery device [164]. The antimicrobial delivery system should be made of biocompatible and biodegradable materials, and it should able to carry adequate drug concentration, and release the drug in a desirable rate for an optimal treatment of the infected tissue [159, 165]. In recent years, various platforms have been developed in order to carry different types of antimicrobial molecules and treat numerous organs and infections. Out of them, the polymer-based materials might play an important role as a DDS in case of sustained or targeted drug delivery at the site of infection in the body or on infected macrophages. These are based on polymer nanoparticles, polymer metal nanocomposites, fibers, dendrimers, liposomes, nanotubes, and films [166-176].

The polymer metal nanocomposites have gained tremendous interest in recent years, due to their unique physicochemical and biological properties such as high surface-to-volume ratio, wide range of optical, electrical properties, ease of preparation, facile surface chemistry and divergent functionalization. Hence, the silver nanoparticles (AgNPs) have been motivated towards intense research including molecular/disease diagnosis, bio-imaging and treatment of infections [177, 178]. In addition, AgNPs found interesting applications in various fields such as optics, sensors, biosensors, electronic devices, catalysis, cosmetics, composite fibers [179, 180]. In recent days the AgNPs are used as nano-medicine and wound dressing material due to their excellent antimicrobial activity against a variety of microbes [181].

However, the polymer immobilized and polymer functionalized AgNPs are seems to be more advantageous than pristine AgNPs and hence they are ideally suitable for biomedical applications such as clinical wound dressings, impregnated catheters, molecular diagnosis and so on [182, 183]. Remarkably, in the treatment of wounds, the several biomaterials are used either in combination

with drugs and /or metal NPs in order to achieve a synergistic effect, out of which AgNP impregnated biomaterials are predominately used as a wound dressing material.

6.3.5. Polymeric Micro/Nano gels as Drug Delivery Systems

Hydrogels are three-dimensional networks derived from physical and chemical crosslinks, and this structure is responsible for their porous microstructure and which provide water absorption, permeability and filtration effect, and mechanical properties. The hydrogels can be found in various forms like thin films, brushes, membranes, colloidal particles, and foams etc. Broadly, the hydrogels can be divided into macrogels, microgels, and nanogels. Nanogel is a dispersion of discrete polymeric gel particles, which are in the range of tens to hundreds of nanometers (<200nm). In the same way, microgels can be described as gel macromolecules in the size range of 1 mm to 1 µm. The micro/nanogels are good candidates for drug delivery due to their advantageous properties such as tunable size ranging from micrometers to nanometers, large surface area available for multivalent bioconjugation. Again they have divergent physical and chemical structures to incorporate bioactive molecules. These particulate systems are stable even in biological fluids hence they prolong the circulation of drugs in blood stream. Consequently, the adverse side effects are minimized due to elimination of premature drug release. Additionally, the polymeric micro/nanogels can effectively protect the drug moieties from enzymatic degradation and hence the drugs are released only at diseased sites in high dosage while preventing adverse effects on healthy organs and tissues [184].

The polymer-drug conjugates, polymer-protein conjugates, micelles, vesicles and dendrimers are the good examples of micro/nanogels. The micro/nanogel based drug delivery systems deliver unique advantages over the other polymeric systems, due to their stability, ease of synthesis, wide possibility for functionalization and good control on the particle size. The interior network of these systems is also available for decoration with inorganic nanocrystals such as quantum dots, silver nanoparticles, carbon nanotubes and iron nanoparticles to attain responsive materials useful in pharmacotherapy, while the gold nanorods are used for photodynamic therapy [185]. The novel functionality prevailing on the surface of microgels and functionalized metal nanoparticles provides bio conjugation with site specific ligands for cancer cells such as folic acid derivatives, peptides, proteins and antibodies that can recognize the related receptors appeared on

diseased cancer cells [186]. The biodegradability is inserted into these systems by using biodegradable linkages such as peptides, oligo esters, disulfides, anhydrides, acetals and polyphosphoesters, which are cleaved in in external environmental conditions to give water soluble polymers, so as to release drug moieties at the site of action [187, 1877]. Therefore, the biodegradable cross linkers enable both the release of encapsulated cargos and the removal of the carriers [189].

6.3.6. Membranes, hydrogel membranes.

The membrane is nothing but a discrete, thin interface which controls the permeation of chemical species in contact with it. The membrane interface is either structurally and molecularly homogeneous having uniform composition or chemically and/or physically heterogeneous as they contain voids, holes or pores of finite dimensions. The membranes are very useful in various applications due to their ease of preparation, flexibility, high mechanical and chemical stability, biocompatibility and hemocompatibility.

Membranes are authoritative materials in chemical technology as they are widely used in biomedical applications, separation technology, fuel cell and battery applications. The key characteristic of the membrane for its utilization is the control of permeation rate of a chemical species which is permeating through the membrane. The membranes can be used for various biomedical applications like CDD, wound healing, selective transport/separation, bio sensing applications. In CDD applications, the major role of the membrane is the appropriation of drug permeation rate from reservoir to the body. Whereas, in wound healing applications, they involve in absorption of exudates and hemostasis along with the release of drugs. However, in separation applications, the main objective is the selective permeation of component or components through the membrane, while hindering other components. In case of fuel cells (DMFCs) it take part in transportation of H^+ ions but not methanol from anode to cathode.

In recent years, numerous studies have been conducted to develop specialized membranes for biomedical and other applications. Wei et al. developed amino alkylated bacterial cellulose membranes, which show a strong antibacterial property against Staphylococcus aureus and Escherichia coli [190]. Rao et al. developed organic-inorganic hybrid membranes based on CS and

PAPSQ by using a solution-casting method and further, they prepared silver nanocomposites using a sol-gel reaction between hybrid membranes and silver in presence of mild reducing agent. The CSSQ hybrid membranes having 7.5 v/v % of GA are showing controlled release of 5-FU for about 7 hrs at 7.4 pH [191]. Additionally, the silver nanocomposites of these CSSQ hybrids were exhibited excellent antibacterial activity towards E. coli and B. subtilis. Sadaf et al. synthesized PVA and starch-based membranes, which were then transformed as silver nanocomposites by reducing the silver using green methods, finally used for ciprofloxacin delivery in wound dressing application [192].

6.4. Conclusions

A wide verity of the polyelectrolyte membranes (PEMs) can be developed with multi-functionality and potential physico-chemical properties. The present book chapter provides the basic concepts as well as recent advancements in the fields such as fuel cells and drug delivery. In the case of fuel cells, PEM enhances the proton conductivity, reduce the methanol crossover and improves the overall efficiency of the fuel cell. As for as concern to the future prospective, PEMs needs to be developed with tunable swelling and long durability by increasing the length of side chains (with required functional groups) incorporation nano additives. As a drug delivery system, PEMs controls the drug release mechanism with stimuli-responsive behavior, which helps to improve the therapeutic efficacy of the drug.

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6.5. References

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Advancement in Hole Transport Materials and Device Architecture of Perovskite Solar Cells

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Abstract: Perovskite solar cells (PSCs) are of great interest and emerging technology belongs to third generation solar cells with low cost, easy fabrication and rapid progress in power conversion efficiency reaches to 25%. Interfaces in PSCs play an important role to enhance the efficiency and extend device stability. One such interface is formed by hole transport layer/materials (HTMs). HTMs play a dynamic role in PSC performance, charge dynamics, like interfacial charge separation and electron recombination. A wide range of HTMs explored for PSCs, which includes organic small molecule, organo metallic, polymer and inorganic p-type semiconductors.

Key words: Perovskite solar cells, Hole transport, p-type semiconductors, Organo metallics

7.1. Introduction

Ever growing of traditional fossil fuels depletion and energy consumption demands the research on sustainable and renewable energy resources like geothermal, wind, solar, tidal and biomass. Among these, solar energy is one of the abundant renewable sources. To meet increasing demand for energy conversion of sunlight to electricity is one of most promising studies. Inorganic photovoltaic cells have widely studied from past five decades and marketed for domestic and electric power generation. However, from inorganic silicon technology derived solar power price is pointedly higher than electrical grid, which restrict in large scale applications. Photovoltaic cells, directly transmute solar energy to electrical energy, epitomize renewable substitutes to fossil fuels. Clean and natural energy resources, can offer over 10⁴ times of power required to the entire world. [1-5] Next generation solar cell technologies fabricated through solution processable techniques like dye Sensitized Solar Cells (DSSC), quantum-dot solar cell (Q-DSC), organic photovoltaics (OPV), perovskite solar cells (PSC) appeared as cost effective substitutes to replace silicon based solar cells. In which, DSSC with attributes like lower production costs, appropriate fabrication procedure, improved performance under diffused light drive DSSCs to supersede solar cells based on silicon.

Further, in order to realize the wide scale applicability of DSSC technology in power production, fabrication of prototype device and subsequent modules has to be developed. Since 1991, efficiency levels up to 13 % by lab scale and 10% [6,7] for module under 1 sun illumination (100 mW/cm², AM 1.5G) condition have been attained by using much-researched Ru(II) polypyridyl complexes, with an iodide/triiodide redox couple based liquid electrolyte. Encapsulations become a major constraint of stability for a long-term of DSSCs, all these become hampers large scale applicability of DSSC. Bach et al reported the solid state DSSC (ss-DSSC) with efficiency of 0.74 % by replace of liquid electrolyte with spiro-OMeTAD organic hole transport material [8]. By modifying of Spiro-OMeTAD with cobalt (III) complex with organic dye sensitizer gave 7.2% efficiency [9]. In the year 2009, Miyasaka & co-workers attempted using of CH₃NH₃PbI₃ & CH₃NH₃PbBr₃ as light absorbers and demonstrated efficiencies of 3.8 & 3.1 % respectively [10]. In 2011, Nam Gyu-Park further improved the conversion efficiency of 6.1% for CH₃NH₃PbI₃ by modification of TiO₂ surface, exchange of the DMF solvent with g-butyrolactone (GBL) and method of deposition of perovsktie, and found unstable and degraded quickly due to altering of perovsktie with liquid electrolyte [11]. A great breakthrough was done by Snaith and Gratzel in 2012 using of CH₃NH₃PbI_{3-x}Cl_x and Susmitha Kalvapalli et al. 136

CH₃NH₃PbI₃ with spiro-OMeTAD and demonstrated efficiencies of 7.6% and 9.7% respectively [12,13]. Several groups put great efforts since from last decade for the optimization of perovskite materials, [14-24] design and deposition techniques, [25-30] device architecture, [31-34] p-type and n-type charge-transport materials [35]. A remarkable raise in power conversion efficiencies (PCEs) PSCs i.e, from 3.83% to 25.2% by the end of 2019 [36-38].

Earlier, the main disadvantage of PSC devices was unstable because of perovskite dissolution in liquid electrolyte, and replaced with hole transport materials [HTM]. Role of HTM was extract holes from perovskite, then transport to electrode. Conventional HTM in PSC was Spiro-OMeTAD, and the other HTMs in PSCs, like conducting polymers, organometallic complexes, organic small molecules and inorganic semiconductors. In this chapter, we discussed recent progress on HTMs.

7.2. Device architecture and operational mechanism

Earlier, perovskite solar cells are analogous sandwich architecture of solar cells sensitized by quantum dots/organic or inorganic dyes. Perovskite materials instability in liquid electrolyte driven use of solid-state electrolyte as hole transport materials. Later, n-type semiconductor such as TiO_2 / ZnO layer will be occupied with the inert mesoporous scaffold layer of Al₂O₃, which progressed into meso-superstructured PSCs with enhanced efficiencies. Recently, the TiO_2 with Al₂O₃ scaffold layer will be relieved leading to thin film p-i-n junction (heterojunction) based PSC which are analogous to the structure of planar heterojunction in polymer solar cell. Different architectures of PSCs are shown in Figure 7.1.

The mesoscopic architecture of PSC is a broad band gap mesoporous n-type semiconductor thin layers are employed to extract and passage electrons [39]. Then, the perovskites are permeated into mesoporous semiconductor layer with techniques of vapor deposition or solution [40], in some cases, they develop capping layer. Because perovskite able to transport holes, and is different compare with preceding architecture that perovskite can act as quantum dot sensitizer, easily dispersed on surface of the semiconductor. Layered structure is formed on transparent conducting tin oxide layer (TCO) casted a compact TiO₂ layer, and finally about 100–300 nm of inorganic or organic HTM in direct contact with sputtered Au/Ag as back contact electrode. This mesoscopic structure permits thin perovskite/TiO₂ film of 200 to 300

nm thick without losing the photocurrent [41]. Ambipolar nature of perovskite directed to a configuration of planar heterojunction, in which need of high-temperature processing of mesoporousTiO₂ layer was eliminated. Architecture of planar PSCs, was analogous to mesoscopic one except that scaffolding is fully detached and perovskite layer was sanded between the HTM and ETM (electron transport material). A further PSC architecture was inverted planar configuration, where perovskite film was located on electron blocking layer, the holes are collected by the lower FTO electrode.

7. 2.1. Working Mechanism

Solar photovoltaics perform two functions during their exertion: absorption of light to create electron-hole pairs, separation of charge to produce photovoltage and photocurrent. Light absorption by photosensitive materials, particularly in perovskite and charge separation by different electron-hole transport materials. In PSC, light is harvested by perovskite like CH₃NH₃PbI₃, bandgap is 1.55 eV, which is lower than inorganic (~3.2 eV) and polymers semiconductor (~2 eV), thus provides a comprehensive spectral sensitivity with 800 nm. After light absorption, photoinduced excitons have binding energy of 30-75 meV is enough for thermal dissociation in electrons & holes (free carriers). Later, the free charge carriers were transport through conductors of electrons and holes, respectively, finally reached to electrodes. As demonstrated in Figure.7.2, there is seven step process convoluted in PSC: photoexcitation (1), Transmission of electron to ETM (2), Transfer of hole to HTM (3), (4) to (7) denote undesirable recombination observe / practice with association of free charge carriers (4), transfer back from ETM & HTM to perovskite (5) (6), short circuit at absent of perovskite (7). In PSCs, one of their advantage is long carrier-diffusion length (102-103 nm) which contributes to low non-radiative recombination [42]. PSC will generate high open-circuit voltage (V_{OC}) under irradiation. Open-circuit voltage point to maximum energy obtainable from absorbed light is enclosed by band gap of perovskite. Typically, attained V_{OC} is lower than band gap potential (Eg/q). In PSCs, V_{OC} ranges from 0.9 to 1.19 V [43], attains loss-inpotential around 0.4–0.65 eV and rarely reported below 0.4 V [44-46].

HTMs play a dynamic role in PSC charge dynamics, like interfacial charge separation and electron recombination. Process of favorable charge transfer and unfavorable recombination influences the PSCs parameters, like short circuit current density (J_{SC}), open circuit voltage (V_{OC}) and power conversion efficiency (PCE). To achieve high J_{SC} , V_{OC} and efficiency in PSCs, typical HTMs have the following requirements.

(i) Hole transport materials should have companionable HOMO energy level (highest



Figure 7.1. Schamatic representation of (a) Mesoscopic PSC (b) planar PSC (c) Inverted planar strucure of PSC.

occupied molecular orbital) lower than valance band of the perovskite material [47].

- (ii) Stable thermal and photochemical stability
- (iii) Good hole mobility and
- (iv) Low Cost

7.3. Hole transport materials (HTMs)

From the last five years, number of HTMs are developed by the researchers and incorporated into the PSC devices. Again, they are classified to organic and inorganic HTMs in which they are divided into small molecules HTMs, organometallic complexes and conducting polymers, etc. are explored for PSC application.

7.3.1. Organic small molecule hole transport derivatives

Triphenylamine (TPA) oriented small molecule hole transport material exhibits excellent performance in PSCs, in which, spiro-OMeTAD has become most popular and showed efficiency of 4 % [48]. Snaith and Gratzel simultaneously demonstrated using of spiro-OMeTAD the efficiencies of PSCs reached to 7.6% and 9.7% respectively [12,13]. Due to *spiro*-bifluorene core is quite expensive, and involves enormous synthesis procedure, many scientist are focused on replacing the *spiro*-bifluorene core with pyrene center oriented N,N - di-*p*-methoxyphenylamine substituents. Jeon et al synthesized few spiro-OMeTAD derivatives studied their performance in PSC devices which exhibited comparable efficiencies (\approx 12.3%) to pure spiro-OMeTAD (12.7%). Nevertheless, observed performance is much lower for molecule-2 and is owing to inadequate driving force for hole injections associated to deeper HOMO (–5.41 eV) as related to energy level of valence band of CH₃NH₃PbI₃ (–5.43 eV) [49]. **Susmitha Kalvapalli et al. 139**



Figure 7.2. Schematic illustration of energy levels and electron transfer process in HTM/Perovskite /ETM.

The *spiro*-OMeTAD has low hole mobility $(10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1})$ few additives, complexes have been combined with spiro-OMeTAD solutions and succeeded to enhancement in the conductivity of holes and efficiencies of PSCs. The methoxy groups (OMe) are introduced in the ortho, meta positions of *spiro*-OMeTAD instead of its two para positions and its PSCs efficiency reached to 16.7%, that confirmed the OMe groups position is strongly dependent on

the performance [50]. The improved performance rises from high value of fill factor (FF) which is related with the low series resistance, high shunt resistance. A PSC of 12.8% efficiency is achieved based on star shaped hole transport molecule with fused quinolizino arcidine core by thiophene oriented side arms [51]. Later, Ko and Nazeeruddin group developed TPA-based hole transport materials by incorporation of a triphenylamine core, fused quinolizino arcidine core (molecule-16, 17), 1,3,5-triazine core (molecule-14, 15) and synthesized star shaped HTMs. The PSCs devices reveals reasonable effciencies of 10.79 to 13.63% are achieved for these star-shaped HTMs [52-54]. Gao et al synthesized p-doped single carbazole derivative HTMs and achieved a significant open circuit voltage is 1.5 V, owing to deeper HOMO of prepared HTM (-6.0 eV) (molecule-30) [55]. Two carbazole based complex (molecules-32) HTM synthesized by Sun & co-workers, exhibited high hole mobility and high conductivity compared with molecule-31, performed photo conversion efficiency (PEC) of 9.8%. HTMs with three carbazole based complexes based on phenylene diphenylene or a PTA core element performs 13% to 14.79% conversion efficiency [56].

Gratzel et al designed low band gap acceptor-donor-acceptor (A-D-A) oligothiophenes act as dual light harvesting structure with perovskite and also acts as hole transport materials (molecule-37, 38) [57] PCE of PSCs realized overall efficiencies of 9.5 to 10.5%, in which both HTMs absorption is 600-800 nm and these derivatives are complementary to the perovskite CH₃NH₃PbI₃. In low energy region of solar spectrum these two oligomers act not only as HTM, also act as light absorption region. Sun et al intended A-D-A phenoxazine based HTMs (molecule-39) showed comparatively higher hole mobility (2.71 x 10⁻⁴ cm² V⁻¹ S⁻¹), hole conductivity $(1.16 \times 10^{-3} \text{ S cm}^{-1})$ as compared by the spiro-OMeTAD $(1.32 \times 10.4 \text{ cm}^2 \text{ V}^{-1})$ ¹ S⁻¹ & 1.57 x 10-4 S cm⁻¹) which yield an efficiency of 13.2%. [58]. Dopant free tetrathiafulvalene derived HTM (molecule-40) based perovskite solar cell revealed 11.03% which comparable performance of spiro-OMeTAD with dopants (11.4%) [59]. Dopant-free hole-transport materials (DF-HTMs) 2,2'-bithiophene based act as main part (which is represented with BT-MTP & DFBT-MTP) were planned, synthesized by Y. T. Wang et.al. DFBT-MTP HTM in perovskite solar cells, shown an increased transportation of hole, effective passivation, efficiently extraction of charge revealed an efficiency of 20.2%, which is \sim 35% beyond that of the PSCs with the nonfluorinated HTM as BT-MTP [60]. Li et. al. developed a fused furan organic small molecule (C8-DPNDF) as dopant-free HTM introduced into inverted PSCs. Which showed 17.5% efficiency, compared with the conventional poly(bis(4phenyl)(2,4,6-trimethyl phenyl)amine) based device (PTAA) as HTM (17.1%), because of 141 Susmitha Kalvapalli et al.

their excellent properties like high mobility of holes, suitable energy level with perovskite, resistance to precursor solution of perovskite. Dopant-free HTMs based PSCs (C8-DPNDF) retained 92% of initial efficiency after 30 days in ambient air relative with humidity of $\approx 40\%$ [61]. DRSBDT-2OT was a mixed HTM, comoposition of spiro-OMeTAD and conjugate small molecule, exhibited high hole mobility and high molecular planarity. The PSC with mixed (HTM DRSBDT-2OT) shown an efficiency of 21.31% [62]. 2,3,5,6-Tetrafluoro-7,7,8,8 tetra cyano quino dimethane (F4-TCNO), was a high temperature impervious small molecule material, doped by sol-gel method into NiOx hole transport layer (HTL). The PSC with F4-TCNQ- NiOx as HTL revealed high recombination resistance (18804 Ω) compared with reference device (5349 Ω). Which results conversion efficiency of optimized device was 16.2% higher compared with undoped transport layer (15.7%) device [63]. Donor-acceptor small molecules (BTTI) with different alkyl chain length HTMs were developed for normal planar PSCs. BTTI-C6 HTMs was applied to PSC with configuration ITO/SnO₂/CsFAMA/HTMs/Au revealed an efficiency 19.69% which was higher than the PSCs with BTTI-C8 HTM (18.89%) and BTTI-C12 (17.49%). Due to shorter alkyl chain BTTI-C6 have better hole mobility good film-forming ability [64]. The effect of imparting polarity in inverted PSCs, dipoar molecules based on thieno-imidazole core based HTMs were developed [AI109 and AI112]. PSCs based on HTMs of AI109 & AI112 without any dopants and supplementary interlayers achieved an efficiencies of 13.32 & 13.42% these were higher than Spiro-OMeTAD based PSCs (11.62%), poly(3,4-ethylenedioxythiophene) polystyrenesulfonate [PEDOT:PSS, (12.01%)]. Higher efficiencies of AI-HTMs were account for the exceptional hole mobility and morphology of film [65].

7.3.2. Organometallic hole transport complexes

Kumar et al adopted HTM as copper phthalocyanine (CuPc) and the fabricated PSC displayed 5% efficiency with low fill factor (FF) (0.40) related with low shunt resistance, Voc is 0.75 V, Jsc is 16.3 mA/cm²[66]. The synthesis of Copper phothalocyanine (CuPc), is easy, and inexpensive, which has properties like low band gap, higher mobility of holes 10^{-3} to 10^{-2} cm² V⁻¹ S⁻¹ [67], good stability, longer excitation diffusion length (L_{ex}) from 8 to 68 nm range.[68,69]. Zhang et al fabricated low cost PSC with CuPc nanorods as the HTM and commercial carbon as counter electrode which delivered higher efficiency of 16.1%, J_{SC} is 20.8 mA/cm², V_{OC} of 1.05V and FF 0.74 and attained 91.5% of initial efficiency (16.1%) in 600 h durability test [70]. CuPC have outstanding properties like thermal stability and **Susmitha Kalvapalli et al.**

interfacial bonding. PSCs based on CuPC gives a high efficiency is $\sim 18\%$, this is maintained 97% of its initial efficiency at annealing of 1000 h more at 85 °C due to strong interfacial, conformal coating existing on perovskite surface, and is placed between the perovskite layer and CuPC. To develop highly efficient & thermally stable PSCs these results provide significant direction [71]. Ke et al demonstrated an efficient planar PSCs designed with fully-vacuumprocessed under room-temperature using tiny-molecule organic CuPc, accomplished an efficiency 15.42% with Voc 1.04 V, FF 77.47%, caliculated under reverse scanning voltage, and measured steady-state efficiency 14.5%. This cell presented an improvement in the stability related to reference cells with predictable organic hole selective layers. Here, our results recommend that fabrication of low-cost, flexible and large-area PSCs with long-term stability, CuPc which is evaporated thermally is an exceptional hole selective material. [72]. YT4 and YT5 is two novel HTMs with different dimensional structures developed by Wu et.al. A larger dimensional assembly YT5 has lower HOMO energy level, higher holetransport/extraction ability and also conductivity. An efficiency of PSCs configuration of FTO/SnO₂/Perovskite/HTM/Au based on YT5 exhibited 21.34%, higher than cell retaining YT4 (18.58%). Whereas, YT5 - inorganic PSC illustrated an outperforming PCE is 14.61%, and the Spiro-OMeTAD device (13.49%). Moreover, device with YT5- PSC showed long-Term stability as compared with YT4 due to greater hydrophobicity of YT5. The fallouts confirmed Spiro-OMeTAD was replaced with YT5 - HTM in PSCs [73]. Ding et.al. Designed new HTMs based on phenothiazine (PTZ) core building block (PTZ-Py and PTZ-Bz) by appropriate energy levels, electronic properties. The PSC based on HTM PTZ-Py reached the efficiency of 19.9%, sustained approximately 90% of initial efficiency subsequently aging of 1000 h under ambient conditions with RH 50-65%. By using PTZ-Py as layer of interface with Spiro-OMeTAD, enhanced efficiency is 22.1% has been achieved [74]. PbCdS nanocrystals prepared by a one step reaction (Cd_2^+ is doped into PbS) and was first applied in hole transport layer of inverted PSCs. PbCdS NCs as hole transport layer-based PSC achieved efficiency 17.1% with an average efficiency 16.5%. Which is due to corresponding HOMO energy levels of PbCdS NCs and CH₃NH₃PbI₃, improved charge extraction rate [75]. In PSCs Benzotrithiophenes and bithiophene (BTT -TT) used as hole transporting materials (HTMs). BTT-TT displayed superior hole mobility, good crystallinity and shaped plane uniform surfaces by covering active layer of perovskite, achieved power conversion efficiency of 18.58% [76].

7.3.3. Hole transport conducting polymer compounds

Other class of organic HTMs are conducting polymers, In which P3HT (polymer poly(3hexylthiophene-2,5-diyl), is extensively used, showed good results [59,77,78]. CH₃NH₃PbI₃-_xCl_x based PSC in combination with P3HT gave an overall efficiency of 10%. [77]. In addition, doped with LiTFSI and an additive based on pyridine- (2,6-di- tert -butylpyridine, D-TBP), performance of P3HT is to 12.4% [79]. Later, Qiu et al. studied the effect of PCBTDPP with MAPbBr₃ [80], gave Voc as 1.16 V compared with P3HT (0.50 V). The diketopyrrolopyrrole- polymer PDPPDBTE based on thiophene was also studied in mesoscopic MAPbI₃ [81] displays 9.2% efficiency which is more than spiro- OMeTAD (7.6%), attribute to deeper HOMO energy level (-5.4 eV), higher hole mobility ($10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ S}^{-1}$), associated with inferior series resistance, high FF. Seok et al. studied the PSC performance with PTAA, maximum efficiency 12% is reached [78]. PTAA based mesoscopic PSCs having MAPbI₃xBrx showed 16.2% [82]. Yang studied the influence of polyfluorene derivatives of fluorine and arylamine groups as HTMs with MAPbI₃ [83] revealed efficiencies (10.9–12.8%), are comparable to spiro-OMeTAD efficiencies (9.8-13.6%). Polyaniline (PANI) also tested as HTM in mesoscopic MAPbI₃ perovskite solar cells [84]. PANI nanoparticles based HTM reveals good J_{sc} is 18.0 mA/cm² and Voc 0.87 V with FF=0.40 results an efficiency of 6.3%.

PSC with conducting polymer PEDOT poly((3,4-ethylenedioxythiophene)) as HTM and carbon cathode, shows excellent performance of PCE 17.0% with V_{oc} 1.05V, J_{sc} 23.5 mA/cm² and FF 0.69, respectively [85]. Conjugated polyelectrolytes (CPEs) was one of the capable HTMs for PSCs. Liu et.al. synthesized CPEs anchored with-N(CH3)₃⁺, -SO₃⁻ and -NH₃⁺ ions, represented as BF-NMe₃, BF-SO₃ and BF-NH₃ HTMs. The three CPEs were substitute of the (PEDOT:PSS) and applied as HTMs in PSCs. EIS (electrochemical impedances pectroscopy) results revealed that the BF-NH₃ and BF-SO₃ devices have comparable charge recombination resistance, and was higher than BF-NMe₃ and PEDOT:PSS devices. BF-NH₃ HTM based PSC revealed a higher PCE of 17.7%. Thus, the cationic BF-NH₃ can act as an excellent HTM as the anionic BF-SO₃ [86]. Dopant-free polymeric HTMs constructed with 2,5-di-2-ethylhexyloxy-1,4-phenylene part and bithiophene part. polymer HTM based PSC shown a PCE of 19.68% (certified: 19.5%) and was comparable with spiro-OMeTAD- PSC (19.81%) [87]. PBT1-C was dopant free polymer HTM was produced from copolymerization between 1,3- bis(4- (2-ethylhexyl) thiophen-2-yl)-5, 7-bis(2-alkyl) benzo [1,2-*c*:4,5-*c*'] dithiophene-4,8-

dione and benzodithiophene parts. The PSC with PBT1-C shown a PCE 19.06% with high FF of 81.22%, is the uppermost efficiency reported for dopant-free polymeric HTMs and was due to excellent hole mobility and able to alter grain boundary and surface trap the perovskite films [88].

7.3. 4. Inorganic hole transport materials

Due to low production cost with ease synthesis and high hole mobility, the inorganic p-type semiconductors occupied in the place of organic hole transport materials. CuSCN was an excellent HTM for PSCs owing to its characteristics like good transparent in UV, visible & IR spectrum range, extensive band-gap of 3.6 eV, 0.01-0.1 cm²V⁻¹s⁻¹hole mobility, good chemical stability and comparable energy levels. Ito and Chavhan groups [89-91] extensively studied the copper thiocyanate (CuSCN) as HTM in PSC devices [Table 7.1]. Arora et al fabricated PSCs with configuration FTO/TiO₂/PVK CuSCN/rGO/Au. Hole transport layer CuSCN deposited on top layer of perovskite (CsFAMAPbI₃-xBr_x (MA=CH₃NH₃⁺, FA= $CH(NH_2)_2^+$)) using spin coating method. Device shows an efficiency is 20.8%, with J_{sc} 23.35 mA/cm², V_{oc} 1.13V and FF of 0.77, but it was unstable instigates from CuSCN/Au contact. So, they introduced spacer between CuSCN & Au layer, and achived a stabilized efficiency of 20.3%. Retained >85% of its initial efficiency for 1000 h under full-sun irradiation and >95% of their initial efficiency at 85 °C for 500 h [92]. Nazeeruddin et al reported the CuSCN based PSCs gave an efficiency of 12.4% by sequential deposition method of PSC fabrication and CuSCN caping layer thickness about 600 nm [67]. Planar heterojunction PSCs based on carbon electrodes (C-PSCs) were developed by Yang et.al., CuSCN used as HTL. Device with CuSCN revealed efficiency of 14.7% which is higher than the C-PSCs with spior-OMETAD (12.4%) [93]. Inverted planar PSCs based on the composite CuI/CuSCN hole transport layer gave an efficiency 18.76%. which was considerably higher than the pristine CuI HTL based PSC (14.53%) and PSC with CuSCN HTL (16.66%) due to high electrical conductivity and smooth and uniform film formation [94]. CuI as a p-type hole conductor, has inexpensive and stable material, band gap is 3.1 eV, good solubility and has excellent hole mobility. Kamat et al used CuI and dissolved in di-n-propyl sulfide and chlorobenzene and infiltrated into porous TiO₂ and about 2 microns CuI layer is coated on the top of TiO₂/ CH₃NH₃PbI₃, the PSCs yields 3.7 to 6% efficiency, and this low performance is attained to faster charge recombination rate. PSCs based CuI shows higher fill factor due to its higher electrical conductivity than the spiro-OMeTAD [95]. Shi et,al. developed hole transport films based on CuI with vacuum thermal 145 Susmitha Kalvapalli et al.

evaporation method for n-i-p PSCs. The film oriented of CuI of 200 nm thick showed best carrier extraction ability, optimized device with CuI films exhibited efficiency 8.07%. Reduced graphene oxides were introduced into the device, for further enhancement of device performance and the device revealed PCE of 8.69%, with J_{sc} 18.86 mA cm⁻², V_{oc} of 0.832 V and FF 0.550. Which avoid accelerated reduction caused by reaction of CuI with Au [96]. In Planar p-i-n PSC, CuI used as hole transport layer and deposited by spin coat method. The PSC with CuI HTM exhibited a PCE of 16.8% [97]. CuOx also used as HTM in palnner and mesoscopic PSCs. CuOx was introduced by superficial solution-processed method into inverted planar heterojunction PSCs. Optimized device, exhibited a champion efficiency of 17.1%, Voc of 0.99 V, J_{sc} of 23.2 mA cm⁻², FF of 74.4% [98]. Nickel oxide (NiO_x) was abundantly available on earth and low cost material and can easily synthesized. NiO_x was also used as HTM in planer and mesoporous PSC devices due to wide bandgap, appropriate work function with valence band 5.4 eVs, transparency and superior stability. Planar PSC with NiOx HTM shown PCE is highest 20.65% with fill factor 0.81 and offers J_{sc} (23.01mA/cm²) with V_{oc} (1.10 V) [99]. Recently, Carbon based materials occupied in the architecture of PSCs [100-104]. GO (Graphene oxide) is prearranged as hole conductor in PSCs, and device with CH₃NH₃PbI₃- $_{\rm x}$ Cl_x act like an absorber and achieve efficiency upto 12% [105]. Graphene oxide used as HTM and developed efficient planner heterojunction PSCs. Aqueous graphene oxide was deposited over perovskite layer, its device revealed 11.11% for 0.25 mg/ml graphene concentration-based device [106]. Feng Yan et al studied graphene effect with PMMA and PDMS as hole transport as well as transparent electrode for perovskite cell. Single layer graphene was grownup by CVD method on copper foil which is deposited with thin poly(methyl methacrylate) (PMMA), transported to flexible substrate. Poly (dimethylsiloxane) (PDMS) film was attached to the above substrate. The resultant film was laminated onto a solar cell. The film conductivity was improved with poly(3,4-ethylenedioxythiophene):poly(styrene sulfonic acid) (PEDOT:PSS) was deposited by using spin coat process on surface of graphene, this leads to Voc is 0.960 (0.945) V, J_{sc} is 19.17 (17.66) mA/cm², FF is 67.22 (71.72)%, efficiency is 12.37 (12.03)% under illumination of light from view of FTO side (graphene) [107]. Carbon nanotubes (CNTs), carbon nanomaterials, grapheme flakes were the another alternative HTMs and back contact electrode in PSCs owing to their chemical, physical and optical properties and stability contrary to moisture and heat. The carbon derived HTMs based PSCs were also shown good efficiency of above 15% [108]. Daniyal et.al fabricated inorganic p-n homojunction PSC with

HTM	Device configuration			FF	$(0/\mathbf{)}$	Ref
		(mA/cm²)	(v)		(%)	
DRSBDT- 20T	Planar	23.67	1.10	0.818	21.31	62
C8-DPNDF	Inverted PSC	20.48	1.065	0.803	17.51	61
BTTI-C6	Planar	24.00	1.10	0.746	19.69	64
CuPc nanorods	Planar	20.8	1.05	0.74	16.1	70
CuPc	FTO / <i>d</i> -TiO ₂ / <i>mp</i> -TiO ₂ / (FAPbI3)0.85(MAPbBr3)0.15 / HTM / Au	22.6	1.07	0.775	18.8	71
CuPc	Planar	18.91	1.04	0.774	15.42	72
BTT-TT	Mesoscopic	23.20	1.08	0.741	18.58	76
PANI	Mesoscopic	18.0	0.87	0.40	6.3	84
PEDOT	Mesoscopic	23.5	1.05	0.69	17.0	85
DTB	Meso	25.32	1.08	0.69	19.68	87
PBT1-C	Meso	22.37	1.05	0.812	19.06	88
CuSCN	Mesoscopic n-i-p	23.35	1.13	0.77	20.8	92
CuSCN	Planar	20.6	1.01	0.71	14.7	93
CuI/ CuSCN	Inverted Planar	22.33	1.11	0.76	18.76	94
CuI	Inverted Planar	22.6	0.99	0.71	16.8	97
CuI/rGo	Inverted Planar	18.86	0.832	0.55	8.69	96
CuO _x	Planar	23.2	0.99	0.744	17.1	98
NiO _x	Meso	23.01	1.10	0.81	20.65	99

non-toxic inorganic HTL. The device with inorganic HTM CuAlO₂ delivered a PCE of 16.48% and device without HTM shown an efficiency of 13% [109].

7.4. Conclusion

Perovskite solar cells are composed of multiple layers with the perovskite absorber film is inserted between the hole transport layer and electron transport layer. The role of perovskite absorber is cable of transport electrons and holes to their corresponding layers. Currently HTMs are under extensive investigation for high performance, cost-effective and large scale applications. There is a numerous HTMs are available, applied to PSCs including small molecule, orgono metallic, conducting polymer and inorganic semiconductors. This book chapter summarizes the different kinds HTMs for meso, planar and inverted PSCs. Widely used HTM in PSC is Spiro-OMeTAD (organic small molecule HTM) have the low mobility and high cost. Alternative to Spiro-OMeTAD with other HTMs are required with the high hole mobility, thermal stabilities, well-mached HOMO-LUMO levels with perovskite absorber layer, the organic small molecule HTMs based PSCs shown more than 20% efficiency. Conducting polymers are another class of HTMS shown excellent hole mobility. Due to low production cost with ease synthesis and high hole mobility, the inorganic p-type semiconductors occupied in the place of organic hole transport materials.

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Advanced Microbial Nanowires for Bioenergy Applications

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Abstract: The uncontrolled use of fossil fuels has created a global crisis, awakening interest in obtaining new sources of renewable energy with minimal impact on the environment. This led to thinking about the option of environmental microbiology, which in recent years has been aimed at optimizing hydrogen production, taking advantage of the methane generated in wastewater treatments, generating biofuels such as ethanol or biodiesel and renewable energy. Which suggests that the appearance of a new form of bioenergy, without interference in the food supply; whose exploitation will be a major biotechnological challenge in the coming years. This chapter shows a group of bacteria called Geobacter of the genus proteobacteria, these are anaerobic bacteria that have the ability to be useful for the production of electrical energy as a source of renewable energy in microbial fuel cells. This group of bacteria is capable of oxidizing many organic compounds using a variety of substances that function as electron receptors. This chapter focus on the main characteristics of bacteria, such as the mechanisms used to take advantage of the electricity it generates and an approximation of the system required for bacteria, a source of energy for the future to turn it into a competitive renewable energy source. The obtained literature revealed a comparative analysis of conventional and unconventional energy sources with respect to the Geobacter family of bacteria, being very useful in modern times. Due to the problems with current energy sources such as the depletion of fossil fuels, as seen in recent times, large companies have developed sustainable alternatives to obtain clean energy and thus try to mitigate in some way the environmental impact caused by obtaining energy, said fuels.

Key words: Geobacter, Microbial fuels, Proteobacteria, Renewable energy, Fossil fuels.

8.1. Introduction

The constant use of fuels destroys the landscape of many horizons, since it causes oil spills, pollutes our waters, causes acid rain and urban pollution; what ends up little by little with the forests, suffocates the cities and shortens the life of the human being that inhabits it. In the energy production and exploitation processes that are used to this day, cause a significant impact on the environment. Faced with such a problem, it is essential to obtain new sources of electrical energy that cause or mitigate to a certain degree of impact on the environment and do not depend on fossil fuels.

Many bacteria are associated with infection, disease, and decay. A wide diversity of microorganisms appears to produce protein nanowires [1-7]. However, due to internal respiration, a foundation of metabolic activity, there are bacteria such as the Geobacter species that humans have used as an ally in many investigations of multiple applications favorable to humans. Geobacter belongs to the Geobacteraceae family in which two species stand out, the *metallireducens* and the *sulfurreducens*, these two species have outstanding characteristics, which can contribute to humanity in multiple jobs.

The bacteria of the genus Geobacter has the ability to transfer electrons to molecules such as iron and Mn (IV) nitrogen oxides [8], substances that have a high molecular weight formed by the chemical and biological degradation of the remains of plants and animals. In addition, these allow the transfer of electrons directly to the electrodes, and thus generate small electrical currents, this characteristic allows the Geobacter species to play a very important role in the natural cycle of matter and metals in aquatic sediments and sub soils, in the bioremediation of organic compounds and polluted metal present in underground environments.

The ability of Geobacter to transfer electrons to and from the electrodes allows electricity to be obtained from organic matter; the application of electrodes as electron donors is also used to reduce contaminants such as; chlorinated solvents and nitrates. Furthermore, these species are capable of manufacturing Pili, which become very useful in the development of devices in the microelectronics industry.

Another characteristic of the genus Geobacter is it can very easily degrade oil and also its derivatives such as toluene, benzene, pesticides, ketones, and herbicides, among others. Heavy metals cannot be biodegradable, but these bacteria have the ability to concentrate them, *Jaffer Mohiddin et al.* 163

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therefore they are precipitated and eliminated more easily. This species has been used to promote in situ bioremediation of groundwater contaminated with uranium.

In the United States, these bacteria and some fungi have been used for the remediation of rivers, such as the River Mill located in Western Colorado, which in past years was analyzed and found high levels of contamination of radioactive material, such as minerals that are used to make nuclear weapons. In the bioremediation process, good results have been found when microorganisms were used and managed to reduce the polluting material existing in the river to a less toxic one when 5 years passed to recover 80% of the river Current studies suggest that Geobacter is capable of reducing radioactive uranium insoluble in neutral matter by 70%.

8.2. Bacterial Nanowire Diversity

Various types of exoelectrogens, which can produce nanowires, were identified so far (Table 8.1). Each and every species differ in its structure and composition of nanowires. Based on the available information, bacterial nanowires can be classified as follows.

8.2.1. Nanotechnology

Nanotechnology is a very extensive field and consists of the development and application of materials and many devices through the exploitation of phenomena that can be controlled on a small scale, such as the nanometer. The development of nanotechnology in the future may allow solving the problems of all kinds such as agricultural production; treatment and remediation of contaminated water, detection of pests, etc. To obtain these benefits, very small cables are needed, which are called nanowires that can be made of metallic materials; But the construction of nanowires is difficult and carries also highest costs. However, the genus *Geobacter sulfurreducense* and *synechocystis* (PiliA 1) within its cell produces some nanowires called Pili A138 that are easy to extract (Table 8.2).

These can be used as conductors in different devices; therefore, it is possible to grow bacteria in laboratories for the production of nanowires. They can be used in many ways another benefit is that the DNA of bacteria can be altered for the production of nanowires with different properties and functions, with the sole purpose of reducing costs for devices that are created based on nanotechnology [9]. Currently, according to UN (United Nations) report, [10], it is estimated that the world population reaches approximately 7.5 billion inhabitants and may exceed 9 billion by the year 2060 [11]. The fossil fuels produced daily (coal, oil and natural *Jaffer Mohiddin et al.*

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gas), which are limited and non-renewable resources, constitute 79.4% of the energy sector worldwide [12], being these as main sources of energy in the world, thus becoming the backbone of industrial growth and is the source of economic income for many countries.

However, the exploitation and extraction of these resources generates serious environmental problems of great importance on the planet, which include the formation of acid rain, an increase in greenhouse gases formed by the enormous amount of CO_2 that is emitted in the atmosphere, the emission of ash indirectly. Nuclear energy that generates large amounts of energy also has drawbacks, since it generates countless nuclear waste that is harmful to humans and the environment as such. Due to these situations, society faces the daily challenge of looking for alternatives that can mitigate pollution and thus satisfy many of the existing energy needs, and also be respectful with the environment [13].

Renewable energy is essential in mitigating environmental impact. However, the use of these new forms of energy also presents various drawbacks since, for example, for the production of biofuels large areas of land are required [14]. As is the case with solar and wind energy, these are dependent and have to occur in adequate meteorological conditions, they require large economic investments and constant planning [15].

Reimers et al., [16] showed that electrical energy could be obtained from the natural voltage gradient that is generated between the anaerobic zone. By studying in greater depth, the anode submerged in anoxic marine sediments, it was possible to show that the gradient was due to the presence of electroactive microorganisms such as the *Geobacteriaceae* family, which is capable of growing in the medium oxidizing a great variety of organic compounds that were present in marine sediments using an electrode as an electron acceptor, these triggered the beginning of the technological era of Microbial Fuel Cells (MFCs) [17].

As previous reports showed that it is possible to reduce an electrode in order to support bacterial growth. Where, many innovative systems have been developed that are related to MFCs, such as the case of microbial electrolysis cells (MECs) that are used for the electrochemical production of Hydrogen, microbial desalination cells (MDCs). It is used for the desalination of water in islands, microbial electroremediation cells (MERCs) that are used to restore polluted environments and finally, microbial electrosynthesis cells (MES). All the aforementioned technologies have the microbial electrochemical technologies (METs) platform integrated [17]. *Jaffer Mohiddin et al.* **165**

The MFCs can be used as a source of electricity to drive devices that require low power, such as telephones and computers [18], or to support constant monitoring devices in marine sediments, they can even be used in robotics since, it indirectly benefits from this technology [18].

Considering the limitations for the use of MFCs technology as an alternative source of energy, research has focused on the search for various applications for obtaining energy. However, it is not the fundamental objective, but a consequence of its operation [19]. Regarding the microorganisms that are responsible for catalyzing several electrochemical reactions that take place in MFCs, the ones that stand out most in this technology is the *Geobacteraceae* family, with *Geobacter sulfurreducens* being the microorganism that most closely matches the study model [17].

8.3. Bioremediation

Electrogens and their bacterial nanowires were used to treat the contaminated water and the biomass was successfully employed in MFC. As waste water is highly rich in sources of carbon and organic waste, it can act as an ideal substrate in MFC. Not only this organic waste and bacterial nanowires were also applied to treat the heavy metal contamination like uranium conatmination. Gene deletion mutation studies showed that the presence of bacterial nanowires in electrogens on Geobacter increase the efficiency of uranium contamination treatment [20]. Bacterial nanowires help in different ways to treat the heavy metal contaminations, such as it increases the surface area and thereby increases the bioavailability of uranium for absorption and it increases the cellular tolerance to heavy metal by preventing the cellular accumulation [20]. In addition to uranium, bacterial nanowires also help to precipitate arsenic, chromium like metal and there to facilitate the bioremediation process of heavy metals [3].

8.4. Conclusion

The genus Geobacter has a number of characteristics that can be easily controlled in microbial fuel cells that are beneficial for their growth, development and thus reach the production of electricity, therefore, while controlling the production of bacteria, it is also possible to control the energy source, which allows reducing and may avoiding the use of nuclear and fossil fuels

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Exoelectrogens	Protein component	Physiological	Conductivity	References
		Role		
I. Metal reducing				
G. sulfurreducens	Pilin subunit Pili A	Extracellular electron transfer	Along the length and width	
S. oneidensis MR1	Periplasmic extension with cytochromes	Unknown	Along the length and width	[22]
D. desulfuricans	Unknown	Unknown	Along the width	[23]
II. Photosynthetic				
Synechocystis sp. PCC 6803	Pilin subunit Pili A	Unknown	Along the width	[26]
Mi.aeruginosa	Unnamed protein (GenBank:CAO90693.1)	Unknown	Along the width	[26]
R. palustris RP2	Unknown	Unknown	Along the length and width	[24]
No. punctiforme	Unknown	Unknown	Along the width	[26]
III. Chemoautotrophs				
Aci. ferrooxidans	Unknown	Unknown	Along the width	[21]

Table 8.1. The major exoelectrogens which produce bacterial nanowires

Table 8.2. Structure and function of Pili in Geobacter sulfurreducense and Synechocystis

Exo electrogens	Subunit of Bacterial nanowire	Associated protein	Molecular mass of subunit	Dimensions	References
G. sulfurreducense	Pili A	Cytochromes	~10 kDa	Width/length: 3–5 nm/ 10– 20 μm	[25]
Synechocystis	Pili A1	Un known	~20 kDa	Width/length: 4.5–7 nm/2– 10 μm	[25]

that damage the environment and consequently global warming, greenhouse gases, and acid rain. It should be noted that the designs must be studied and optimized to offer competitive and positive results. The "Pili" nanowires that are found inside the cell and that are part of Geobacter can greatly revolutionize electronics and nanotechnology, thus opening a wide horizon to considerably reduce the environmental impact that is generated by industries and many of the technological fields. Infact the current production of nanowires can led to environmental repair, to help in this, devices such as the gasoline and diesel-based engine must *Jaffer Mohiddin et al.*

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also be replaced, therefore, the proposal to use Geobacter for energy and energy purposes takes force, this could be useful to reduce the harmful effects on the planet. Using the various microorganisms such as bacteria should be an option highly sought after by science in general and thus be able to generate technology that does not affect the environment and that reduces the current and future demand for electricity. The development of technologies based on bacteria that can generate electricity, reduce water pollution and produce hydrogen makes it quite attractive for in situ bioremediation treatments, generating electricity and environmentally friendly fuels such as biofuel.

Conflicts of interest

Authors declare there is no conflict of interest.

8.5. References

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Zeolite Remediate: A Multi Featured Green Technology

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Abstract: Zeolites in the present day found applications nearly in every modern scientific and engineering fields. The fundamental properties and applications of molecular sieve zeolites include inorganic chemistry, physical chemistry, biochemistry, geological sciences with emphasis on surface and colloid chemistry and catalysis. Geological and engineering applications include adsorption, separation, purification etc. The microporous property of zeolite has great variety of applications such as molecular sieve application used in recovering radioactive ions from waste solutions, separating hydrogen isotopes, ore beneficiation, agriculture industry, carbon rebreath, mitigation of air pollution and storage of high-volume gas in low pressure conditions. In the present study the multiple utilities of zeolites have been discussed. Zeolites are used in ore beneficiation of extraction uranium from uranium plant waste water and from the groundwater around uranium mining area. It is efficiently used in agriculture industry as zeofertilizer and zeo soil conditioner. The zeolites can be efficiently used in the mitigation of air pollution. The other very prominent use of zeolite is that it could be in the storage of high-volume gasses of LPG etc., at very low pressures even in the cylinders made of tough plastic.

Key words: Zeolite, Zeofertilizer, Agriculture, Renewable energy, Uranium.

9.1. Introduction

Microporous allumino silicate composition mineral has a general formula $M_{2/n}O.Al_2O_3.ySiO_2$. wH₂O is named as Zeolite, where M represents the group IA and group IIA elements like sodium, potassium, magnesium and calcium, *y* is 2-200, *n* is the cation valence and *w* represent the water contained in the voids of the zeolite. The tetrahedral made up of AlO₄ and SiO₄ linked by sharing oxygen ions. Zeolites can be tailored with the configured potential properties to get many impending applications. The molecular sieve action of zeolites over a larger surface at considerably low pressure is the primary motivation for engineering of membrane reactors. Their unique properties include ion-exchange, adsorption, sieving, and conductivity. Zeolite primary building units of AlO₄ and SiO₄ are assembled into secondary building units and form a polyhedral like cubes or hexagonal prisms or cubo-octahedra. These final framework structures exhibit voids of pore sizes 0.3 A⁰ to 20 A⁰ (1 A⁰=0.1nm). The selective adsorption and size discrimination of zeolite molecular sieve is controlled by Si/Al ratio. AlO₄ in the tetrahedron framework bear a negative charge. Addition of cation will counterbalance the negative charge and create a strong electrostatic force responsible for the adsorption or molecular sieve characteristics.

These cations are mobile and undergo ion exchange. By changing the type of cation, the pore size of the molecular sieve can be tuned. The sodium (Na) form of Zeolite A will have pore size of 4 A⁰ (4x10⁻¹⁰m) known as 4A molecular sieve, if Na ion is exchanged with potassium (K) ion, which is larger than Na ion, the pore size is reduced to 3 A⁰, known 3A molecular sieve. If the cation is exchanged with calcium (Ca) ion, as one Ca ion replaces two Na ions the pore size increase to 5 A⁰, known as 5A molecular sieve. This ability of zeolites to adjust the pore spaces to uniform desired sizes, enable them to adsorb molecules smaller than the pore size eliminating the larger molecules [1,2]. The zeolites have immense internal surface space of about 1000 m²/g [3]. They have capacity to adsorb high polar solutions or gases with maximum quantity even at low concentrations and low pressure [4].

Naturally occurring zeolites like chabazite, erionite, clinootiolite and mordenite are used as molecular sieves. Over 200 synthetic zeolites have been synthesized by slow crystallization of a silica-alumina gel in the presence of alkalis and organic catalysts [5]. Zeolites are formed from the volcanic rocks under the geological conditions of weathering, hydrothermal alteration

or metamorphism [6]. Silica rich volcanic rocks proceed to transformation from Clay \rightarrow modernite/heulandites (zeolites) \rightarrow epistilbite (zeolite) \rightarrow stilbite(zeolite) \rightarrow thomsonite/ mesolite/scolecite (zeolites) \rightarrow chabazite \rightarrow calcite(calcium mineral) while silica poor volcanic rocks proceed to transform as follows Clay \rightarrow cowlezite \rightarrow levyne/offertite(zeolites) \rightarrow analcime(zeolites) \rightarrow thomsonite/ mesolite/ scolecite (zeolites) \rightarrow chabazite(zeolite) \rightarrow calcite(calcium mineral)

The micro porous structures of zeolites have multi-faceted applications, which can be utilized in divergent fields maintaining friendly ecosystem. The fields of application discussed in the present chapter include i) Ore beneficiation from uranium mine waste and ground waters in Mining areas, ii) Agriculture, iii) Carbon Rebreath and iv) Mitigation of Air Pollution v) Storage of high-volume gas at low pressure [7-8].

9.2. Ore beneficiation from Uranium mine waste and groundwater in mining area

Uranium, by virtue of its ready reactive nature, it forms compounds with Cl⁻, Na⁺, SO₄²⁻ and CO_3^{2-} and at pH 7 it forms stable complexes with PO₄³⁻ and CO_3^{2-} . Groundwater in generally is in chemically reducing conditions due to low oxygen levels. Hence within the zone of uranium mineralization, the concentration of the uranium is high enough that it will get dissolved in the groundwater. Studies reveal that uranium leached out from the beneficiation plant blend with the groundwaters and contaminate [12].

Re-beneficiation of uranium beneficiation plant waste water and traces of uranium can be recovered from ground around uranium mining area by using zeolite molecular sieves. Normally radionuclides are immobile in nature, but when they mix with thermodynamic carriers like ferric iron oxide nanoparticles 15 nm size [13,14]. Regular procedure of beneficiation is followed. The samples of uranium plant waste water samples or groundwater in the vicinity of uranium mining are subjected to bar screens and gravitational settling, where by the sediments present in the samples will be settled in the settling tank. The solution after screening sill be sent through 15 nm EMT zeolite molecular sieve. In this process the ferric oxide molecules of 15nm size which are carriers of uranium will be separated. The separated liquid again passed through a 3.3 A^0 zeolite sieve. As the water molecular size is 3.2 A^0 , water is passed through the zeolite sieve leaving behind the uranium which will be collected, dried and sent for packing for different applications (Figure 9.1).



Figure 9.1. Uranium processing unit (Ragaranjan et al., 2013a)

9.3. Agriculture: Zeofertilizers and Zeo soil conditioners

The zeoite framework structures contain interconnected voids. They contain water molecules along with mobile cations which easily undergo ion exchange. The water molecules can be expelled by on heating the zeolites [1]. This unique property of zeolites is utilized in the agriculture field in the management of soil and irrigation, in the form of zeofertilizers, zeo soil conditioners for better agriculture yield. Biofertilizers are prepared by combining the biowaste with microorganisms such as anabaena, Nots, Rhizobium, Azitobacteria, Azospirilum and Ecotomycrorrhiza. The life of these microorganisms depends up on the moisture content in the surroundings. When Zeolites are mixed with biofertilizers at the ratio of 1 kg fertilizer with 200gm of zeolite powder known as zeofertilizer, will serve good moisture feed to the microorganisms for their survival. The water content within the voids of zeolites is naturally exerted out when temperature and pressure in the atmosphere is high in other words when environment becomes too dry. This reversibility water adsorption property of zeolites makes them perfect humidity stabilizers called zeo soil conditioners. These zeo soil conditioners adsorb and store water from the atmosphere when there is high humidity and return back to the soil when the atmosphere is too dry. Thus, the zeo soil conditioners supply water sufficiently for the plant growth even in the dry seasons [8].

9.3.1. Carbon rebreath

Tailored zeolites sieves are used for trapping of CO_2 for reuse. The outlet of refinery is connected to two vessels that are interconnected through solenoid valve and pressure switch.

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The vessels are filled with zeolite sieve of pore size less than that of CO_2 whose molecular diameter is 2.9 A⁰. The two vessels act in simultaneously and alternately i.e., when first vessel is in adsorption mode, second vessel will be in regeneration (removal of adsorbents) mode. The mixed gas is passed in to the first vessel with a flow rate of 4 m³/min. The first vessel allows the gasses with molecular diameter less than the pore sizes leaving behind the gases with molecular size more than the applied zeolite pore size, which will be removed. The gases from the first vessel move into second vessel where the remaining gases are captured and CO_2 is passed through the vessel, will be collected and send to reuse [8].

9.3.2. Mitigation of Air Pollution

Green House Gases can be captured by zeolite molecular sieves tailored in the form of membranes. The zeolite membranes can be prepared by applying aluminosilicate gel in a pore substrate and subjecting to hydrothermal reaction or through seed promoted hydrothermal crystallization of zeolite layers [15]. The zeolite membranes thus prepared are arranged one after the other with increased pore sizes. The setup is made in to a road side sign board and erected (Figure 9.2). In this sign board each membrane adsorbs the gasses of molecular size (Table 9.1) less that their specific pore spaces, which will be removed in the later phase by applying pressure on the membranes (Figure 9.3).

9.3.3. Storage of high-volume gas at low pressure

The CNG cylinders generally made up of steel, in which the cooking gas is stored at a pressure of 250 bar. The cylinders used to store gas naturally 10 times heavier than their gas holding capacity because, they are operated at high pressure. Zeolites property of adsorption can be used to store the high pressure gases in light weight cylinders made up of tough plastics [16]. The zeolite adsorbents can be made in different forms like moniths, granules, pellets, powders, tubes and membranes. The high pressure adsorbed and holds by the zeolite pore spaces by week wanderwals force and can be drive out very easily.

9.4. Conclusion

Zeolites are Microporous allumino silicate. Their unique properties include ion-exchange, adsorption, sieving, and conductivity. Among multifaceted applications of the zeolites, some selective utilities were discussed in the present study which includes Ore beneficiation from uranium mine waste and ground waters in Mining areas, Agriculture, Carbon Rebreath, Mitigation of Air Pollution and Storage of high-volume gas at low pressure. Uranium can be *K. Raghu Babu*

Name of the Pollutant	Molecular Diameter A ⁰	
Carbon dioxide	2.9	
Carbon monoxide	2.8	
Nitrogen	3.0	
Hydrogen sulfide	3.6	
Methane	4.0	
Chloro di fluorimethane (Freon22)	5.3	
Carbon tetrachloride	6.9	

Table 9.1. Molecular diameters of some pollutants (Raghu babu et al., 2020a)



Figure 9. 2. GHG capture by Zeolite membrane.



Figure 9.3. Removal of GHG by applying pressure.

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extracted from Uranium extraction plant waste waters by the molecular sieve property of zeolites. The property reverse storage of humidity within the voids of zeolites at high temperatures and release of at low temperatures can be used for agriculture as zeo-soil conditioners. Carbon rebreath is the utilization of carbon formed during the processing plants. Zeolites can be utilized for air pollution by the microporous molecular sieve property by application of different combinations of zeolite layers. Gases of high pressure can be stored in light weight cylinders filled with zeolites at very low-pressure conditions with the support of adsorption property of the zeolites.

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Recent Advancement in Hyper-Interconnected Porous Carbon Materials

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Abstract: Hyper crosslinked polymers are new class of porous materials which shows three different pores such as micro, meso and macro porous which is identified by BET and Electron microscopic methods. The main aim of this new porous polymers which shows high surface area, modification of various functional groups, carbonization and highly stable materials; owing to these the porous polymer materials used for the huge applications such as gas absorption, energy storage, conversion of epoxide to cyclic carbonates, sensor technology, and various catalysis studies such as esterification's, heterocyclic compounds synthesis and dyes degradations. Due to this importance the eminent researchers are focusing for the synthesis of more porous polymers with small organic monomers with template and without template methods.

Key words: Carbon materials, Hyper crosslinked polymers, Gas absorption, Energy storage.

10.1. Introduction

Hyper-interconnected porous carbon materials (HIPCs) are a subclass of porous many parts, which are primarily constructed by interlinking of monomers (Organic compounds) by Friedel–Crafts alkylation method with exterior inter linker. Example, the HIPCs are synthesized by the post-cross networking response of styrene-divinylbenzene compound or styrene in solution or a large puffy form of Davankov resin. Although, this procedure generated HIPCs with routine polymeric backgrounds of uniform or un-uniform pinholes, and wanted precious amalgamation routes. Very newly we have made-up highly uniform pinhole compounds constructed on a modest Friedel-Crafts reaction of aromatic hydrocarbons counting phenanthrene, naphthalene, anthracene, coronene and pyrene, displaying worthy performance as super capacitor constituents [1]. Since the consequential polymer materials are most stable either thermally and chemically owing to halogen functional groups on the surface; there are no possibilities to lose their surface morphology [Figure 10.1].

10.2. Importance of microporous organic polymers

The microporous organic polymers (MOPs) have potential applications in the areas of storage of gas, catalysis, conductivity, separation of molecules which has pores less than 2nm has substantial importance. MOPs are widely used in different synthetic approaches and extra chemical changes. Over the past few years, academics have established range of MOPs that are self-possessed of non-metallic elements such as C, H, O, N, and B, as well as polymers of intrinsic micro porosity, covalent organic bases, and conjugated microporous polymers. The monomers bring acetylenic group or stereo-controlled buildings from these resources, which need monotonous synthesis and refinement with costly catalysts.

Presently, in the area of catalysis, adsorption, sensor technology and chromatography methods derivatized polymer compounds with large surface space plays a vital role. Modifications of the polymer derivatives peripheral surface offers an effective limit contrary to acid erosion and oxidation. Numerous techniques have been established for functionalization as well as post-modification, copolymerization of skeleton molecules with functional groups, and self-polymerization of functional organic groups (such as –OH, NH₂, halogens---etc). These supports have benefits like large chemical and hydrothermal strengths and simple tailoring of rapid sites. For the functionalization on the surface of porous polymers Xiao et al.[2] employed sulfonic acid functionalized porous polymer for esterification's and Friedel–Crafts acylation reactions, Copper et al. [3] reported the post-modification of amine functionalized conjugated **Reddi Mohan Naidu Kalla**



Figure 10.1. Hyper crosslinked polymers formed with Phenanthrene and Pyrene.

microporus polymers for gas sorption, and Wang et al. studied organic polymers functionalized with *Tröger's* base and 4-(N,N-dimethylamino)pyridine as the heterogeneous organocatalyst, showing sensible catalytic activity in the addition reaction of diethyl zinc to 4-chlorobenzaldehyde and the acylation of phenols [Figure 10.2] and alcohols [4,5].

The porous organic polymers (POPs) have fascinated important potential applications in adsorption of gas, sensing, energy storage, and proton conduction. Therefore, investigators have endeavored to yield an innovative permeable resource like covalent and metal organic structure, conjugated microporous polymers (CMOPs), CTF, furthermore permeable resources like molecular sieves and active charcoal. Due to the exclusive characteristics like low skeletal density, large surface area, and high chemical stability MOPs and HCPs are considered over the porous materials. By the Friedel-Crafts reaction, Davankov resin is specific styrenic polymer that is hyper-crosslinked. Though, monotonous synthetic procedures are needed and *Reddi Mohan Naidu Kalla* 181



Figure 10.2. Hyper crosslinked polymers formed with Phenol and Bisphenol moiety.

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the resulting resin accept non-uniform pores. POPs are collected through the principle of reticular chemistry. POPs show excellent physical and chemical support and can have suitable extraordinary definite surface area and comparatively consistent pore form. For several bio-applications like delivery of drug and light therapy these physiognomies are very advantageous. Additionally, through hyper-cross-linked polymerization, POPs are functionalized with particular functional groups. To aim Bronsted acids like H3PO4, H2O3S and HCl are extremely used for conversion of biomass, and on the POPs, these are functionalized as surface acid sites with quite high density.

10.3. Methods for the development of HCPs

Even though outstanding physicochemical properties of these materials, the synthetic production of porous polymers usually needs extreme circumstances, like using complicated monomers, noble metal catalysts, and noble gases. HCPs, prepared by a one-pot Friedel-Crafts reaction and Scholl reactions, are superior to many POPs because of their efficient and stable synthesis, good heat resistant, excellent swelling capacity, and large absorbency. Among several structures, the hollow ones may grant attractive physical and chemical properties and extensive applications. Their exclusive basic physiognomies, like additional gap and porous structure offer simple availability to the acid locations for reactants and products. POPs with an excavated structure have described earlier as admirable carriers for dynamic resins and Nano sized partitions. During one-pot reactions, it is hard to synthesize hollow polymer nano spherical POPs using direct method, since exact backgrounds subsequent from the C-C coupling method. Freshly, a Lewis acid-base interaction-mediated self-assembly technique that can efficiently fabricate morphology-controlled HCPs was established in our group. This method does not need any templates as compared with the traditional template-related approaches, which removes problems like harmful etching solvents or refined precursor framework.

On the other hand, the denser walls of the hyper cross linked hollow globular polymers (HHGPs) formed through metal-catalysed Friedel–Crafts polymerisation sustain large diffusion limits, and subsequent HCPs inescapably produce metallic scums, later elongated Soxhlet extractor purification methods. In industrial uses like water treatment and catalysis it produces negative effect. Therefore, it waits to test another simplistic and green process for formulating HHGPs with tunable wall density with suitable template- and metal-free procedures. Additionally, abridge the groundwork of H₂O₃S functionalized hollow spherical **Reddi Mohan Naidu Kalla**

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polymers (SHSPs) and progress the catalytic efficiency for Methyl esters production, Il Kim et al., [6] synthesized and characterized 4 kind of H_2O_3S functionalized hollow spherical catalysts with tunable shell densities. Compared to metal-based catalytic technique, metal-free technique formed hollow spheres with diluent shell densities; thus, by regulated the catalysts varieties the shell densities of SHSPs can be simply adjusted. Without the template this may the primary case of changing the shell densities of SHSP, and to progress the catalyst base productivity, the metal-free technique is used. The surface area, pore size distribution, and thermal stability of these materials were orderly distinguished, and efficiency of the hollow spherical catalysts in Methyl ester structures were equated with other reported various methods.

HCPs can be synthesized by a simplistic and extensible Friedel-Crafts acylation, stand out from a sequence kind of NOPs, fugitive the usage of noble metal catalysts and noble gases. By changing the monomers, it is capable to regulate the pore assemblies of HCPs, and the dissimilar moieties on the polymer chain can be known to modify special features and functions. Furthermore, the methylidene connections give HCP-based NOPs with improved physicochemical stability, analyzing them appropriate for application in many fields like pollutant discharge, and manufacture of carbonic resources. Though, coursework added in the making of enhancement: (1) From the fast-cross-linking kinetics the dense microporous basis of HCPs is derived, usually results in ill-defined structures, strictly confining the morphology variety; (2) Through post-cross-linking dangerous byproducts are inevitable; (3) several catalyst structures, exactly metal-free ones, required to established structured well-controlled HCPs in mass production.

Over the past decade in research Lewis acid–base (LAB) collaboration are exploited. For an extensive range of conversions, this chemistry has been recognized as a potent procedure; example, Chen and coworkers established LAB-facilitate polymerization approaches for coupled polar alkenes. Qi and co-workers [7] freshly stated a strong LAB interface with perovskite that efficiently enhanced the productivity and stability of solar cells. Insight of this efficient and suitable method, imagine the presentation of the advantaged LAB method to polymerization-induced self-assembly, for particular HCPs which has been stated infrequently. In view of this, by combining the benefits of LAB collaboration for making a well-defined hierarchical NOPs and carbon nanomaterials we place a LAB-mediated self-assembly approach and the polymerization features of HCPs.

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Deprived of using any templates or surfactants by the one-pot Lewis acid-catalyzed Friedel-Crafts alkylation-induced crosslinking reactions of a sequence of functionalized aromatic mixtures, the hyper-cross-linked polymeric nanotubes, polymeric nanosheets, and hollow polymeric nanospheres, are obtained. By the N₂ sorption and molecular imitation the definite porous polymers possess tunable size of pores, and large surface areas. Throughout the one pot condensation polymerization, self- assembly is arising by the cross-linking study of reaction produced byproduct can compose the connections between the catalyst(acid) and monomer(base); in the meantime, also studied the effect of the categories of Lewis acid catalysts, concentration of monomer, and temperature reactions on LAB-mediated selfassembly. Remarkably, on the basis of examination the structural growth and developed mechanism of the HCPs, a nonmetallic technique as long as for the production of hyper-crosslinked hollow spherical polymers. These structured-engineered NOPs shows large physical and chemical stability and tremendous puffiness capacity, subsequently, under the treatment of harsh chemical environment they sustain their morphology and in common solvents they show studied amplification performance. The constant and swellable HHNPs naturally bear remaining functional groups on the skeletons used for additional chemical amendment, providing adaptability for presentation operation.

10.4. Conclusion

In summary, the hyper crosslinked porous polymer materials are highly stable and have various shapes based on the monomer and synthesis procedure. There are two methods are presents for the synthesis of porous polymers one is template and another on is template free methods. Template free method is cost effective method rather than two methods are involve same procedures and easy purification methods and vast applications. Due to this the preparation of porous polymers are still demand in the world wide.

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Biological Synthesis of Nanoparticles from Bacteria, Fungi, Algae and Plants

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Abstract: Nanoparticles (NPs) were synthesized through different approaches such as physical, chemical and biological approaches. Physical and chemical approaches are expensive and lethal to surroundings. Therefore, there is an urgent necessity to use non-toxic, environmental friendly and consistent approaches to produce nanoparticles to enlarge their advantages in the agricultural sector. The best way to attain this goal is to use biological creatures i.e., microbes and plant extracts to produce nanoparticles. The major aim of this chapter is to summarize the use of "eco-friendly nanofactories" (i.e., plant extracts and microorganisms) to synthesize nanoparticles. In the past ten years, metal nanoparticles (MtNPs) were gained great regard because of their unique physical and chemical properties along with anti-microbial, anti-cancer, catalytic, optical, electronic and magnetic properties. Initially, these MtNPs were produced by various physical and chemical approaches. Though, these conventional approaches have different drawbacks, like high energy consumption, high cost, and the participation of toxic chemicals. The microorganisms provide another platform for biosynthesis of MtNPs in an environmental friendly and commercial manner. In this chapter, we focused on different microbes used to synthesize different MtNPs. In addition, due to its many applications than chemically synthesized nanoparticles, microbial MtNP has unique dynamic characteristics and used in various fields such as farming, medicine, cosmetics and industries in the near future.

Key words: Nanoparticles, Bacteria, Fungi, Algae, Plant, Biological Synthesis

11.1. Introduction

Nanotechnology is an attractive research field in the areas of materials science, medicine, life science, physics and chemical science. Nowadays, metal nanoparticles have attracted the mindset of scientists because of their wide advantages in development of new approaches in various fields [1-5]. It is expected that the global biotechnology and pharmaceutical nanoparticle market will achieve approximately 79.8 billion U.S. dollars in 2019, with a compound annual growth rate (CAGR) of 22.0% from 2014 to 2019 [6]. Recently, physical approaches have attracted great attention in production of thin films and nanoparticles (NP) and their aggregates [7]. Environmental toxicity is the main alarm of physical and chemical synthesis approaches. Therefore, there is a growing demand for different sources of synthetic nanoparticles. In recent years, researchers noticed that different microbes were eco-friendly nanofactories [8-10]. Recently, copper nanoparticles (NPs) have been synthesized using pomegranate peel extract [11]. According to reports, a simple and effective biosynthetic approach can use yeast cells to prepare easy-to-harvest biocompatible cadmium telluride (CdTe) quantum dots (QD) with tunable fluorescence emission [12].

In the past few decades, because of the synthesis of metal nanoparticles (MtNP) and the wide application in various fields, viz., biology, food, agriculture, engineering, electronics, cosmetics, medicine, food and biomedical equipment, nanotechnology has achieved great development. MtNP is in an important position due to its specific physical and chemical properties and important biotechnology applications [13,14]. In the past decade, many scientists have produced MtNP through conventional physical and chemical approaches. The drawback of physical methods includes costly synthesis and low yields. Likewise, chemical approaches are also not safe due to the participation of harmful chemicals connected to the surface of MtNPs. These chemicals have harmful side effects in biomedical uses. Considering the above problems, research has turned to the synthesis of MtNPs using economical, biocompatible, non-toxic and environmental friendly biological components [15-18]. The biosynthesis of MtNPs is mainly performed by using various types of plants and microbes. The plant formation procedure of MtNP synthesis is economic and quite easy. However, due to its diverse photochemical effects, this process produces polydisperse nanoparticles [19,20]. In contrast, microbes are considered as potential bio-factories for green synthesis of MtNP. Because of their indispensable and important technical significance, they have

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recently attracted widespread attention. This is due to the wide variety of microorganisms that react with metal ions in the synthesis of MtNP. According to reports, due to the increased growth rate of various bacteria, fungi and yeasts, easy cultivation and ability to grow in certain temperature, pH and pressure conditions, microbial MtNPs of various sizes and shapes were noticed [21,22]. The biosynthesis of MtNPs and their alloys (gold, silver, gold-silver alloys, selenium, tellurium, platinum, palladium, silicon dioxide, titanium dioxide, zirconium oxide, quantum dots, magnetite and urea ore) was studied in number of microbes.

Various microbes have the capability to synthesize inorganic substances through endo and exocellular approaches. In endo-cellular method, microbial cell is composed of a remarkable ion transport system. Because of electrostatic interactions, negatively charged bacterial cell walls attract positively charged metal ions. In addition, bacterial cell walls contain enzymes that decrease metal ions into their respective nanoparticles. In exo-cellular approach, microbial cells secrete reductase for the biological reduction of metal ions into corresponding MtNPs [23]. The following sections of this chapter will give outline the use of prospective microbial flora for green synthesis of MtNPs, and also intracellular and extracellular mechanisms of microbes and their enzymes for MtNP synthesis will be described, as well as current challenges and future prospects.

11.2. Biosynthesis of Nanoparticles

Various physical, chemical, biological and hybrid approaches existing to synthesize various categories of nanoparticles [24-26]. Though physical and chemical approaches are commonly used to synthesize nanoparticles, the employ of toxic chemicals deeply limits their biomedical applications, especially in the clinical field [27]. Nature has devised different methods for biosynthesis of nanomaterials, which is an unexplored study field [28]. The synthesis and flock of nanoparticles through biological pathways may lead to development of clean, non-toxic and eco-friendly "green chemistry" process, which may involve biology from bacteria, fungi and plants too [29]. The three main steps for preparing nanoparticles should be evaluated from the perspective of green chemistry: selection of solvent media for synthesis, reducing agents that are not harmful to the environment, and non-toxic materials for stabilizing nanoparticles. So far, most synthetic methods mainly depends on organic solvents, which have negative effects on environment and are not safe for humans [30]. Therefore, investigation needs to turn to eco-friendly and

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biocompatible methods to synthesize nanoparticles because they are safe for agricultural uses. Therefore, microbes and plant extracts as a source of nanoparticle generation can be used as environmental friendly nanofactors.

Our own group and other scientists around the world have discussed in detail the use of plants for biosynthesis [31-34]. Both plant and microbial-derived synthesis of nanoparticles have their own benefits and drawbacks. Plant synthesis is time-saving and comparatively easy, but because it involves a variety of phytochemicals (such as phenols, flavonoids, terpenoids, etc.), plant synthesis usually results in polydisperse nanoparticles [20,35]. In addition, seasonal changes may change the phytochemical characteristics of extracts used for biosynthesis[36]. On the contrary, microbial synthesis does not have these shortcomings, but it needs to maintain a sterile environment and culture conditions, making it relatively complicated [37]. In this chapter, we discuss the potential of using microbial world as a nanofactory for biosynthesis of metal nanoparticles or metal matrix composites from the perspective of function and mechanism. Microorganisms have exciting potential for nanoparticle biosynthesis because this synthesis is environmental friendly and does not require use of harmful chemicals. Microbes are cost-effective and have low energy requirements. They can also accumulate and detoxify heavy metals by reductase, thereby reducing metal salts to consequent metal nanoparticles with smaller polydispersity and a narrow size range [38]. Schematic presentation of nanoparticles synthesis from microbes and plant in Figure 11.1.

11.2.1. Synthesis of nanoparticles by bacteria

It is reported that many organisms produce inorganic substances inside or outside the cell. Among microbes, bacteria have received the main interest in the area of metal nanoparticle synthesis. According to reports, bacteria such as *E. coli*, *Pseudomonas sternii*, *P. aeruginosa*, *P. tarsus*, *Salmonella typhi*, *Staphylococcus*, *Vibrio cholerae*, etc. form extracellular and intracellular metal nanoparticles [39,40]. *Bacillus licheniformis* bacteria synthesize silver particles with an normal particle size of about 50 nm, which are separated from sewage of municipal waste [41]. In recent past, a quick method for producing small (1-7 nm) monodisperse AgNPs by electrochemically active biofilm (EAB) by sodium acetate as an electron donor has been described [42]. Found the hypothetical mechanism of biosynthesis of nanoparticles: nitrate reductase. It is an enzyme in the nitrogen cycle is accountable for reducing metal ions in NP [43]. Previous research noticed that

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Figure 11.1: Schematic representation on synthesis of nanoparticles from bacteria, fungi, algae and plant.

bacteria that are key to the environment and ecology are also accountable for the biosynthesis of gold nanoparticles (GNP). *Rhodopseudomonas capsulata* [44], *Escherichia coli* [45], *Klebsiella pneumoniae* [46], *Arthrobacter nitrificans* [47] and *Brevibacterium* [48] have been studied for the reduction of Au3⁺ ions at room temperature through a one-step method. The precise mechanism of the synthesis of gold nanoparticles has not been elucidated, but some researchers have observed the involvement of the electron shuttle in enzymatic metal reduction procedure [44,49]. During

S.No	Name of the bacteria	Nanoparticles	Size	References
			(nm)	
1.	Pseudomonas sp.	Ag	20–70	[50]
2.	Bacillus mojavensis BTCB15	Ag	105	[51]
3.	Shewanella oneidensis MR1	Pd	10–100	[52]
4.	Shewanella loihica	Cu	10–16	[1]
5.	Shewanella loihica	Pt	1–10	[53]
6.	Mycobacterium sp.	Au	5–55	[54]
7.	Shewanella baltica	Те	8–75	[55]
8.	Bacillus endophyticus	Ag	5.1	[56]
9.	Bacillus brevis	Ag	41–68	[57]
10.	Streptomyces griseoplanus	Ag	19.5–20.9	[58]
11.	Nocardiopsis flavascens	Ag	5 and 50	[59]
12.	Caldicellulosiruptor changbaiensis	Au	<20	[60]
13.	Shewanella loihica	Pd	1–12	[53]
14.	Shewanella loihica	Au	2–15	[53]
15.	Micrococcus yunnanensis	Au	53.8	[61]
16.	Bacillus megaterium NCIM2326	ZnO	45–95	[57]
17.	Halomonas elongata IBRCM 10214	ZnO	18.11	[62]

 Table 11.1: Metal nanoparticles synthesized by bacteria.

biosynthesis of gold nanoparticles, bacteria reduce Au^{3+} to Au^{0} , indicating that NADH- and NADH are dependent on enzymes. Table 11.1 presents chosen instances of bacteria-mediated biosynthesis of metal nanoparticles. These nanoparticles have been widely used in various applications, but are mainly used for biomedical applications. Lately, silver nanoparticles (AgNPs) synthesized by *Bacillus brevis* have shown good antibacterial activity against multi-drug resistant strains of *S*.
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aureus and *S. typhi* [57]. *Pseudomonas stutzeri* has been noticed to accumulate AgNP using intracellular mechanisms [39].

In a study, two different isolates of *Pseudomonas aeruginosa* were used for synthesis of gold nanoparticles (AuNPs) to generate AuNPs of different sizes [63]. Spherical (10–50 nm) and triangular plates (50-400 nm) AuNPs are synthesized by *Rhodopseudomonas capsulate* [44]. *Serratia urate* mediates the synthesis of ZnO nanoflowers, which are used on cotton fabrics to provide antibacterial effect against *Staphylococcus aureus* and *Escherichia coli* [64]. It is also reported that *Lactobacillus plantarum* can be used for biosynthesis of ZnO nanoparticles [65]. *Aeromonas hydrophila* is a gram-negative bacterial strain used in synthesis and antibacterial applications of ZnO nanoparticles [66]. Recently, the slender *Haloonanas* was used to produce triangular CuO nanoparticles, and its antibacterial activity against *E. coli* and *S. aureus* was confirmed [67]. In another latest study, super paramagnetic iron oxide nanoparticles (29 nm) were produced by *Bacillus cereus*, and their anticancer effects on MCF-7 and 3T3 cell lines were reported in a dose dependent way [68].

11.2.2. Synthesis of nanoparticles by fungi

Compared with other microorganisms, fungi have many advantages in nanoparticle synthesis because they are relatively easy to separate. Compared with bacterial fermentation and culture, their downstream processing is very simple, and the culture secretes a large number of extracellular enzymes and has a wide range of diversity. Fungal synthesis of silver nanoparticles by 1 mM AgNO₃ solution as a precursor and culture supernatant of *Aspergillus terreus* [69], *Fusarium acuminatum* isolated from infected ginger (*Zingiber officinale*) [70] and *Fusarium pallidoroseum* [69]. It has been assessed that silver nanoparticles are formed within one minute of the silver ion contacting the battery. Due to their biocompatibility and stability, gold nanoparticles (AuNPs) were reported as a wide range of research tools in different areas of health centers, medicine and agriculture. The fungus-mediated synthesis of gold nanoparticles showed that the two main precursors, HAuCl₄ and AuCl, were dissociated into Au³ +ions and Au⁺, respectively [71]. The endophytic fungus *Aspergillus clavatus* was isolated from the surface sterile stem tissue of *Azadirachta indica*. When incubated in an aqueous solution of chloroauric acid ions, intracellular gold nanoparticles (Au NPs) will be produced, particularly a variety of mixtures of

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S.No	Name of the Fungi	Nanoparticles	Size (nm)	References
1.	Trametes trogii	Ag	5-65	[78]
2.	Macrophomina phaseolina	Ag/AgCl	5-30	[79]
3.	Fusarium keratoplasticum A13	ZnO	10-42	[80]
4.	Aspergillus niger G31	ZnO	8–38	[80]
5.	Aspergillus flavus	ZnS; ZnS-Gd	12-24 (ZnS); 10-18 (ZnS-Gd)	[81]
6.	Rhodotorula mucilaginosa	Ag	11	[82]
7.	Aspergillus niger	ZnO	53–69	[83]
8.	Trichoderma longibrachiatum	Ag	10	[84]
9.	Trichoderma harzianum	Au	32-44	[85]
10.	Fusarium oxysporum	Ag	21.3-37	[53]
11.	Pleurotus ostreatus	Au	10-30	[86]
12.	Aspergillus terreus	Ag	16-57	[87]
13.	Ganoderma sessiliforme	Ag	~45	[88]
14.	Phenerochaete chrysosporium	Ag	34-90	[57]
15.	Penicillium polonicum	Ag	10-15	[89]
16.	Candida glabrata	Ag	2–15	[90]
17.	Macrophomina phaseolina	Ag/AgCl	5-30	[79]
18.	Aspergillus nidulans	CoO	20.29	[91]
19.	Rhodotorula glutinis	Ag	15.45	[92]

Table 11.2: Metal nanoparticles synthesized by fungi.

nanotriangular (GNT) with a size of 20 to 35 nm [72]. Gold, silver [43] and bimetallic Au-Ag alloy nanoparticles [49], CdS nanoparticles [73] and zirconia nanoparticles [74] has reported *Fusarium oxysporum*. New fungi are also accountable for synthesis of metal nanoparticles, such as iron nanoparticles [75], bimetallic nanoparticles [76] and PbS nanocrystals [10]. In addition,

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nanoparticles with high mono dispersity and size can be gained from fungi [77].

Table 11.2 represents synthesis of various nanoparticles from different fungi. Fungal nanotechnology methods were successfully used to the synthesis of various metal nanoparticles. Similarly, in bacteria, biosynthesis may be intracellular or extracellular. In intracellular biosynthesis, metal salts are transformed into less toxic forms in mycelium, which may be used by fungi [93]. Because of the presence of many bioactive metabolites, high accumulation and increased yield, fungi are relatively richer in resources than bacteria in nanoparticle biosynthesis [38,76]. Recently, it was observed that different filamentous fungi are capable in synthesis of AuNPs. This study used various approaches for synthesis of AuNPs. The author proposes that fungal and media components may play a role in maintaining nanoparticles [93]. In another study, three various fungal species (i.e., Staphylococcus aureus, Fusarium oxysporum, and Fusarium spores) have been used to synthesize AuNP. The researchers pointed out that synthesis occurs in fungal vacuoles, and reducing sugars are involved in customization of spherical AuNPs. They also determined the role of specific fungal proteins in blocking of AuNPs [94]. A new investigation showed that rhizome extracts mediate the synthesis of monodisperse AgNPs (9.4 nm), while optimized conditions yielded 2.86 nm AgNPs [95]. Extracellular synthesis of AgNPs by Candida glabrata shows excellent antibacterial potential [90]. Aspergillus niger mediated ZnO nanoparticles have exceptional antibacterial potential and can also degrade up to 90% of Bismarck brown dye [83]. Recently, cobalt oxide nanoparticles were synthesized by A. nidulans [91].

11.2.3. Synthesis of nanoparticles by algae

In the synthesis of nanoparticles, the use of algae is becoming more and more common. *Sargassum muticum* is used in synthesis of ZnO nanoparticles and is reported to reduce the angiogenesis and apoptosis of HepG2 cells [96]. AgNPs biosynthesized by *amanita* show excellent antibacterial properties by forming a variety of biofilms against bacterial strains [35]. The macroalgae and seaweed *Sargassum crassifolium* were used in the synthesis of AuNPs. In addition, the researchers noticed a blue shift in UV absorption after enhancing the concentration of *S. crassifolium*, which was attributed to the increase in nucleation centers in the reducing agent resulting in a decrease in size [97]. *Cystoseira trinodis* is used in the synthesis of CuO nanoparticles (7 nm). It is recorded to have increased antibacterial activity and large potential as an antioxidant, degrading methylene

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blue [98]. Alumina nanoparticles (~20 nm) were synthesized by *Sargassum ilicifolium* [99]. For the synthesis of gold nanoparticles, algae strain, include *Sargassum tenerrimum* [100]. It has been used in the biosynthesis of AuNP. Table 11.3 shows synthesis of metal nanoprticles from different algae.

11.2.4. Synthesis of nanoparticles by plant extract

Plant mediated biosynthesis is a very easy and cost-effective approach for large scale production of nanoparticles with no pollution [11]. Due to contamination, there are problems in maintenance and preservation microbial cultures. Plants can be used to evade the prolonged steps of maintenance and preservation of cell cultures. Many plants for nanoparticle biosynthesis were reported [109]. Therefore, this is the first step for researchers to use plant leaves as nanofactories to synthesize silver nanoparticles. Several descriptions are existing for synthesis of silver nanoparticles with leaf extract as easy and simple experimental design like *phyllanthin* extract *Mentha piperita* [30], *Ocimum sanctum* [110]. Other parts of plants have also been studied as extracts of synthetic silver nanoparticles, such as ethanol extracts of marigold flowers [111] and kinnow extract [8].

S.No	Name of the Algae	Nanoparticles	Size (nm)	References
1.	Red algae Portieria	Ag	60-70	[101]
	hornemannii			
2.	Marine macroalgae Padina sp.	Ag	~ 25-60	[102]
3.	Chlorella vulgaris	Pd	70	[103]
4.	Chlorella vulgaris	CuFe ₂ O ₄ @Ag	20	[104]
5.	Anabaena flosaquae	Ag	5-25	[105]
6.	Microchaete NCCU342	Ag	60-80	[106]
7.	Macroalgae (Ulva lactuca L)	Ag	31 ± 8	[107]
8.	Marine algae <i>Gelidiella</i>	Au	5.8-117.6	[108]
	acerosa			
9.	Macroalgae (Ulva lactuca L)	Au	7.9	[107]

Table 11.3: Metal nanoparticle synthesized by algae.

Gold nanoparticles with an average particle size of 32.96±5.25 nm are produced from *Garcinia Cambogia* [112]. The synthesis of copper, gold-iron and silver-iron core-shell nanoparticles with pomegranate peel extract is also noticed [11, 113] and the characterization was done by UV-Visible spectroscopy, Fourier transform infrared spectrophotometer (FTIR) and TEM. The synthesis of nanoparticles was confirmed by U.V. spectrophotometer, X-ray diffraction (XRD) and scanning electron microscope (SEM). They observed the main morphology of the spherical shape with a particle size analyzer (PSA) and transmission electron microscope (TEM), with an average diameter of 5 nm [8]. List of different nanoparticles synthesized from various plant extracts presented in Table 11.4.

11.3. Nanoparticles as Antimicrobials

In the past decade, the appearance of nanotechnology has provided opportunities to explore bactericidal effects of metal nanoparticles. The bactericidal impact of metal nanoparticles is attributed to their small size and high surface to volume ratio, which enables them to interrelate directly with membranes, not just due to release of metal ions in solution [111]. Antibacterial activities of ZnO and gold nanoparticles against *E. coli* and *S. aureus* [48,111]. Various mechanisms with bactericidal effects were suggested, and these mechanisms have received more and more research due to their antibacterial properties and potential applications in food, environment, and medical care. Metal nanoparticles can be combined with polymers to form

S.No	Name of the Plant	Nanoparticles	Size (nm)	References
1.	Mulberry fruit (Morus alba L)	Ag	80-150	[114]
2.	Red cabbage extracts	Au	~ 25, 18-30,	[115]
			5-70, 27	
3.	Plant extract of	Se	15-20	[116]
	O. tenuiflorum			
4.	Mulberry fruit (Morus alba L)	Pd	50-100	[114]
5.	Calliandra haematocephala	ZnO	19-45	[117]
6.	Cymbopogon citratus	CuO	11.4-14.5	[118]
7.	Annona reticulata	Ag	7-8	[119]
8.	Camellia sinensis	Ag	2-4	[120]
9.	Tribulus terrestris	Au	~ 7	[121]
10.	Crotalaria candicans	Cu	30	[122]

Table 11.4: Metal nanoparticle synthesized by plants.

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composite materials to make better use of their antibacterial activity. Kaur, Thakur, and Chaudhury [123] observed the effects of chitosan silver nano-formulations on *Staphylococcus aureus*, *P. aeruginosa* and *S. enteritidis*, as well as plant pathogens, antibacterial and antifungal activity, isolated from seeds of chickpea, respectively. Although nanoparticles have many approaches attributable to antibacterial activity, DNA damage and cell wall destruction are the most common causes of cell death [48]. Nanoparticles adhere to cell wall and cell membrane due to electrostatic interactions, destroying cell wall, causing macromolecules to leak, or may pass through cell membrane and damage the cell DNA, leading to cell death.

11.4. Conclusions

For now, it can be concluded with certainty that, compared with metal chemical nanoparticles, biosynthesis of metal nanoparticles and their broad advantage in biomedical technology have broad prospects because of their low cost and ecological friendliness. If used appropriately, a large number of biological resources (microbes and microbial enzymes) can help biosynthetic NPs become prospective game changers in near future. Though, some challenges must be solved before industrial scale production and widespread use, which will take long time (5-10 years). However, microbial mediated metal nanoparticles have low toxicity, low cost, high degradability and can be used in many therapeutic applications, so they have great potential. It is also supporting that different groups are focusing on decoding whole mechanisms of microbial synthesis, which will finally lead to greater understanding and smarter applicability. Although mechanisms of absorption, diffusion, long term toxicity, and excretion of these nanoparticles are still vague, approaching biological uses make them a prospective candidate for a broad replacement of chemically synthesized NPs in future.

Conflict of Interest

The authors declare that they have no conflicts.

11.5. References

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Therapeutic Applications of *Nigella Sativa* and its Metal Nanoparticles

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Abstract: *Nigella sativa* is an annual flowering plant belongs to the family Ranunculaceae commonly known as Black seed, Black cumin or Kalonji. It is considered as one of the most miraculous healing medicines existed as a remedy for all diseases specified in varied traditional system of medicine and food. Phytochemically, it is enriched in fixed oil, terpenoids, essential oil, alkaloids etc and few key chemical constituents such as thymol, carvone, thymoquinone, nigellicimine, nigellicine, dithymoquinone and thymohydroquinone. Therapeutic properties of this plant are due to the presence of thymoquinone which is one of major active component and has different beneficial properties. Ample of phytochemical, pharmacological and clinical researches have been executed on this plant which may include antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, bronchodilator, hepato-protective, renal protective, gastro-protective, and antioxidant properties etc. This plant also been studied for nanoparticle synthesis via green route with varied biological applications. Considering above all these aspects, this plant catches the attention of research analyst to tactic the utility, proficiency and potency of *Nigella sativa* in diverse medicinal sectors.

Key words: Nigella sativa, green synthesis, thymoquinone, Kalonji seed.

12.1. Introduction

Herbal medicines are used for curing diseases as they are safe when compared to modern allopathic medicines from many centuries in different indigenous and folk systems of medicines. Many researchers are focusing on medicinal plants since only a few plant species have been thoroughly investigated for their medicinal properties, potential, mechanism of action, safety evaluation and toxicological studies. Nigella sativa commonly called black seed cultivated mainly in Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia [1] and the seed oil is widely used as significant drug in the traditional medicine like Unani and Ayurveda [2-3]. Nigella sativa is having wide range of biological activities and therapeutic potential and it possess wide spectrum of activities such as diuretic, antihypertensive, antidiabetic, anticancer and immunomodulatory, analgesic, antimicrobial, antihelminthic, analgesics and anti-inflammatory, spasmolytic, bronchodilator, gastroprotective, hepatoprotective, renal protective and antioxidant properties. The seeds of Nigella sativa are widely used in the treatment of various diseases like bronchitis, asthma, diarrhea, rheumatism and skin disorders. It is also used as liver tonic, digestive, anti-diarrheal, appetite stimulant, emmenagogue, to increase milk production in nursing mothers to fight parasitic infections, and to support immune system. Most of the therapeutic properties of this plant are due to the presence of thymoquinone which is a major active chemical component of the essential oil [4,3].

12.1.1. Description of plant

Nigella sativa commonly called as black seed is an annual flowering plant (**Figure 12.1A**) belongs to the family Ranunculaceae. Flowers are solitary pale blue and white, with five to ten petals. The fruit is a large and inflated capsule composed of three to seven united follicles, each containing numerous seeds which are used as spice. Macroscopically, seeds are small dicotyledonous, trigonus, angular, regulose-tubercular, black externally and white inside with bitter taste and odour slightly aromatic. *Nigella sativa* and its seeds (**Figure 12.1B**) popularly known as 'black caraway', 'black cumin', 'fennel flower', 'nigella', 'nutmeg flower', 'Roman coriander', and 'kalonji'.

12.1.1.1. Chemical Composition of Nigella sativa seeds:

Nigella sativa seed has many active important compounds that have been isolated, identified and reported so far like thymoquinone (30 % - 48 %), thymo-hydroquinone, di-thymoquinone, p-cymene (7 % - 15 %), carvacrol (6 % - 12 %), 4-terpineol (2 % - 7 %), t-anethol (1 % - 4 %),



Figure 12.1: Pictorial representation of *Nigella sativa* A) Flower B) Seeds (*Source: Adopted from internet*)

sesquiterpene longifolene (1 % - 8 %) α -pinene and thymol *etc*. Seeds contain two different types of alkaloids; *i.e.*, isoquinoline alkaloids *e.g.*, nigellicimine and nigellicimine-N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine [Figure 12.2] [5-6].

Seeds also contain other compounds *e.g.*, carvone, limonene, citronellol in trace amounts. The seeds of *Nigella sativa* contain protein (26.7 %), fat (28.5 %), carbohydrates (24.9 %), crude fibre (8.4 %) and total ash (4.8 %). The seeds are also containing good amount of various vitamin and minerals like Cu, P, Zn and Fe *etc*. The seeds contain carotene which is converted by the liver to vitamin A. Root and shoot are reported to contain vinic acid [5,7]. *Nigella sativa* seeds consist of quinine constituents most abundantly, thymoquinone attributes to the most of the therapeutic applications. The seeds reported to contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50-60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%). Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less. α -sitosterol is a major sterol, which accounts for 44% and 54% of the total sterols in Tunisian and Iranian varieties of black seed oils respectively, followed by stigma sterol (6.57-20.92% of total sterols) [8-9].



CHAPTER 12: Therapeutic Applications of Nigella Sativa and its Metal Nanoparticles

Figure 12.2: Pictorial representation of key chemical constituents of *Nigella sativa* (Source: Adopted from internet)

12.1.1.2. Traditional and Medicinal Uses

The seeds are acrid, bitter, aromatic, thermogenic, carminative, diuretic, emmenagogue, anodyne, antibacterial, anti-inflammatory, digestive, appetizer, antithelminthic, febrifuge, stimulant, galactagogue, expectorant and sudorific. The seeds are also used for cough, amenorrhea, flatulence, jaundice, dyspepsia, inflammation, paralysis, skin, eyes, respiratory, stomach and liver problems, diarrhoea, dysentery and dysmenorrhoeal.

12.2. Therapeutic Applications of Nigella Sativa Seeds

The extensive research has been done on black seeds using modern scientific techniques. This plant is believed to be a miraculous herb that has potential to cure multiple ailments and disorders and their probable potential therapeutic applications are displayed in **Figure 12.3** and few of them are discussed below.

12.2.1 Anti-microbial activity

Crude extracts of N. sativa exhibited good effect against some of the tested organism. Gram





Figure 12.3: Therapeutic applications of Nigella sativa

negative bacteria was affected more than the gram positive [10]. The antibacterial activity of black seeds extract on *S. aureus* was monitored and confirmed by using the positive control Azithromycin [11]. Antibacterial activity of *N. sativa* against and triple therapy in eradication of *Helicobacter pylori* in patients with non-ulcer dyspepsia was carried out. Clinically useful anti *Helicobacter pylori* activity was observed with *N. sativa* seeds comparable to triple therapy [12].

Promising antifungal effect was evaluated with methanol followed by chloroform extracts of black seeds in different *Candida albicans* strains [13]. Potential anti-dermatophyte activity of ether extract of *N. sativa* and thymoquinone was tested against 8 species: four species of *Trichophyton rubrum* and one each of *T. interdigitale*, *T. mentagrophytes*, *Epidermophyton floccosum*, and *Microsporum*. The results denote the potentiality and supports its use in folk

medicine for the treatment of fungal skin infections [14]. Kalonji seeds quinines, dithymoquinone, thymohydroquinone, and thymoquinone was examined *in-vitro*. Despite thymo hydroquinone and thymoquinone of Kalonji seeds exhibited significant anti-yeast activity [15].

12.2.2 Anti-schistosomiasis activity

Mahmoud et al [16] studied the effect of *Nigella* seed oil on liver damage caused by *Schistosoma mansoni* (S. mansoni) in mice. The result suggest that *Nigella* seed oil may play a role against the alterations caused by *S. mansoni*. The antioxidant and anti-schistosomal activities of the garlic extract (AGE) and *Nigella* seed oil (NSO) on normal and *Schistosoma mansoni*-infected mice was investigated. Result showed that, protection with AGE and NSO prevented most of the hematological and biochemical changes and markedly improved the antioxidant capacity of *schistosomiasis* mice compared to the infected-untreated ones. These results suggested that garlic extract and *Nigella* seed oil are the potential protecting agents for the treatment of *schistosomiasis* infection [17].

12.2.3 Anti-oxidant activity

The antioxidant, anti-inflammatory, anti-cancer and anti-bacterial activities of the shoots, roots and seeds methanol extracts from *N. sativa* were studied. The three organs exhibited strong antioxidant activity using the oxygen radical absorbance capacity method and a cell-based assay. Bourgou et al [9] suggest the suppression of oxidative stress was observed in Wistar rats by thymoquinone. It also suggests that dietary supplementation of black seeds powder inhibits the oxidative stress caused by oxidized corn oil in rats [18]. It was also reported that oral feeding on the diet containing black seed powder at 10 % level antagonized the oxidative stress effects induced by hepato-carcinogens like di-butylamine and sodium nitrate (NaNO₃) in Swiss albino rats by normalizing reduced glutathione (GSH) and NO levels [19]. The black seed oil and thymoquinone by intra-peritonial injection were found to shown protective effects on lipid peroxidation process during ischemia-reperfusion injury (IRI) in rat hippocampus [20].

Treating broiler chicks with black seed for six weeks prevented the liver from oxidative stress by increasing the activities of enzymes such as myeloperoxidase, glutathione-S-transferase, catalase, adenosine deaminase and by decreasing hepatic lipid peroxidation [21]. Significant *in-vitro* antioxidant activity of methanolic extract of *Nigella* was monitored by Mariod et al [22].

12.2.4. Anti-diabetic activity

Antidiabetic activity was monitored in diabetes induced rats with Kalonji seed extract and oil by Salama et al [23]. Treatment of rats with *N. sativa* extract and oil as well as thymoquinone significantly decreased the diabetes-induced increase in tissue MDA and serum glucose and significantly increased serum insulin and tissue SOD. The *N. sativa* oil restored normal insulin levels, but failed to decrease serum glucose concentrations to normal. In 2010, Abdel merguid et al [24] research on biochemical and ultra-structural findings suggests that *N. sativa* and thymoquinone are proved to be clinically useful in the diabetes treatment by pancreatic β cell integrity thereby decreasing oxidative stress.

The anti-hyperglycemic potential of thymoquinone on the activities of key enzymes of carbohydrate metabolism in streptozotocin (STZ)-nicotinamide (NA)- induced diabetes rats was evaluated. Oral administration of thymoquinone at 20, 40, 80 mg/Kg body weight for 45 days, dose dependently improved the glycemic status in STZ-NA induced diabetic rats. The levels of insulin, Hb increased with significant decrease in glucose and HbA (1C) levels [25]. The *N. sativa* showed the synergistic effect with human parathyroid hormone in improving bone mass, connectivity, biomechanical behavior and strength in insulin-dependent diabetic rats and found to be more effective as compared to the treatment with *N. sativa* or human parathyroid hormone alone [26]. *N. sativa* oil is effective in insulin resistance syndrome of diabetic and dyslipidemic patients [27].

12.2.5. Anti-inflammatory and analgesic activity

By applying the three different nociceptive experiments on the rats and mice i.e., hot plate method, tail-pinched method and acetic acid-induced writhing test it was concluded that the plant has ability to reduce the pain stimulus. The findings have confirmed that the plant black seeds fixed oil has potential anti-nociceptive actions that are may be due to an opioid part in the oil. This portion act as naloxoneis i.e., an inhibitory effect for the pain. Moreover, the plant oil has considerable CNS depressing activities [28].

12.2.6. Gastro-protective activity

Nigella seed oil and thymoquinone are proved to be protective against gastric lesions and alcohol induced lipid peroxidation [29]. Thymoquinone protects gastric mucosa against ulcers induced alcohol.

12.2.7. Hepato-protective activity

Administration of *Nigella sativa* protects liver tissue from toxic metals like lead and removes lipid peroxidation generated due to exposure to carbon tetrachloride [30]. Protection of rat liver against hepatic ischemia was determined by measuring biochemical parameters [31]. Fluconazole induced toxicity in wistar rats as reduced by the administration of *Nigella sativa* seeds. Black seeds restored the liver and kidney injury caused by fluconazole [32].

12.2.8. Nephro-protective activity

Nigella seed oil and thymoquinone reversed methotrexate-induced nephrotoxicity in albino rats by decreasing creatinine, preventing degenerative changes in kidney tissues [33-34]. Administration of NSO to Wistar rats exhibited protection against urolithiasis by decreasing urinary calcium, phosphate and acetate [35]. Hepato-nephroprotective activity of Nigella seed oil was demonstrated on paracetamol-induced rabbits. NSO maintained the shape of terminal hepatic venule (THV)/Central vein (CV) and sinusoids in the liver and normal shape of glomerulus and bowmans capsule in the kidney of Newzealand rabbit (*Oryctolagus cuniculus*) was observed [36].

12.2.9. Pulmonary protective activity and anti-asthmatic effects

The beneficial effects of NSO on rats with hyperoxia-induced lung injury were evaluated since oxygen-induced lung injury is believed to lead to the development of broncho-pulmonary dysplasia in premature infants. NSO significantly reduced the severity of lung damage due to hyperoxia [37]. The protective effect of Nigella sativa on tracheal responsiveness (TR) and lung inflammation of sulfur mustard GAS exposed guinea pigs was examined [38].

12.2.10. Testicular protective activity

Ethanolic extracts of Klonji seeds reduced the reproductive toxicity induced by fluoride. Aluminum in albino male mice. Fluoride and aluminum decreased the body weight, variation biochemical parameters like SOD, CAT, GSH levels in testis and epididymis, administration of kalonji seeds ameliorated toxicity [39]. Oxidative damage and tissue injury induced by formaldehyde in testicles may be prevented by using NSO which is a potential antioxidant [40]. Protective role of antioxidant property was reported against acetamiprid induced reproductive toxicity in male rats [41].

12.2.11. Neuro-pharmacological activity

Defatted infusions (aqueous and methanol) of Nigella Sativa seeds shows a commanding antidepressant and analgesic activity. Elongated management of Nigella Sativa boost learning and memory whereas its oil and thymoquinone are used in the treating anxiety [42-43]. In addition to these, NSO has its strong effect in tramadol tolerance and dependence. Nigella Sativa extracts such as Aqueous and hydroalcoholic have neuroprotective effects in cerebral ischemia and its supplements progresses Pb-induced neurotoxicity, antioxidant and thymoquinone helps against chemotherapy-induced neurotoxicity [44-46].

12.2.12. Anti-convulsant activity

Both *N. sativa* oil and Curcumin has potential effect on anti- convulsant and antioxidative property by dropping induction of seizures, excitability and oxidative stress in epileptic animals as well as removers from some of the contrary effects of antiepileptic drugs. NSO, seeds, aqueous infusion and Thymoquinone has antiepileptic effect when pass through maximal electroshock (MES)- induced convulsions and minimal neurological deficit (MND) test [44].

12.2.13. Contraceptive and anti-fertility activity

The ethanolic extract of Nigella sativa seeds was found to possess an anti-fertility activity in male rats which might be due to inherent estrogenic potential. Oral administration of hexane infusion, their seeds prevent pregnancy in Sprague-Dawley rats as per reports of [44,47].

12.2.14. Anti-oxytocic activity

Nigella sativa seeds and its oil helps in inhibition of smooth muscle contraction of uterus induced by oxytocin stimulations and has anti- oxytocic effect in rats and guinea pig. [44].

12.2.15. Toxicological studies

Many toxicological studies related to Nigella Sativa seeds shows no toxicity whereas its oil has acute disorder. Moreover, the histo-pathological results also showed to be normal for the tissues of heart, liver, kidney, and pancreas [48]. Some studies pertinent to thymoquinone demonstrates the safety, particularly when given orally to experimental animals [44, 49].

12.2.16. Drugs-nigella interaction

Several studies demonstrated the potential of Nigella sativa interaction with co-administrated drugs and affect their intestinal availability and pharmacological effect. *In-vitro* studies reveals

that the extracts of Nigella sativa inhibit cDNA-expressed human cytochrome P-450, 3 A4, 2 C9, 3 A5 and 3 A7-mediated metabolism of marker substrates they may influence the affect and /or inhibit the metabolism of a wide range of drugs [50].

12.3. Green Synthesis of Nigella Sativa Nanoparticles and their Applications

Nanoparticles are small particles ranging between 1-100 nm in size and exhibit new properties based on their size, distribution and morphology. Research and development in nanotechnology have been growing rapidly around the globe. Nanoparticles are synthesized from metals by both chemical and biological methods. Despite to chemical synthesis, an alternative, eco-friendly and advantageous approach for synthesizing nanoparticles is the biological strategy which are synthesized from microbes (*Aspergillus flavus, Phomaexi gua pseudomonas* species), and plants such as *Chenopodium album, Acalypha indica, Diopyros kaki, Cynodon dacylon, Glycyrrhiza glabra, Nigella sativa etc.*

Silver nanoparticles (AgNPs) were synthesized from the kalonji seed extracts and they were characterized by using several techniques like UV Spectroscopy, FTIR, XRD, SEM-EDAX, TEM. The synthesized AgNPs were used for varied applications like antibacterial, anti-cancer, anti-inflammatory, and antidiabetic activity. Likewise, gold nanoparticles (AuNPs) were synthesized from the black seed extracts and their applications were studied such as antibacterial, anti-biofilm, and cytotoxicity and antioxidant activity. Nigella sativa oil was used for the synthesis of Fe₃O₄ NP and they were characterized by UV-Vis., FTIR, IRM and SEM. Iron oxide nanoparticles (Fe₃O₄ NPs) were used for the antimicrobial studies. MnFe₂O₄/BC NP of kalonji seeds were prepared by Siddiqui et al [51] and they characterized and evaluated the antibacterial activity. Zinc oxide nanoparticles (ZnO NPs) were synthesized from black cumin seeds and their antibacterial potential was evaluated. A list was prepared and summarized the green synthesis of *Nigella sativa* and their nanoparticles application in Table 12.1.

12.4. CONCLUSION

Herbal medicines can be used as drugs in traditional medicine and gain popularity globally. A lot of studies existed on *Nigella sativa* which validate its importance in folk medicine as antihypertensive, anti-inflammatory, immune stimulant, analgesic, anti-microbial, anti-fungal,

Sl.no	Plant	Type of metal	Characterization	Applications	Ref.
	part	Np	techniques		
1.	Seed	Ag NPs	UV-visible, XRD,	Anti-	[52]
			FTIR and TEM	inflammatory,	
				antioxidant	
				effects (Diabetic	
				neuropathy)	
2.	Seed	Ag NPs	UV-visible, XRD,	Antibacterial	[53]
			FTIR and TEM	activity	
3.	Seed	Ag		Hepatocellular	[54]
		nanocomposites		carcinoma	
4.	Seed	MnFe ₂ O ₄ /BC	FTIR, XRD, SEM,	Antibacterial	[51]
		NP	TEM, TGA and		
			DSC		
5.	Nigella	Fe ₃ O ₄ NP	TEM, FTIR, IRM,	Antimicrobial	[55]
	Sativa oil		SEM		
6.	Seed	ZnO NP	XRD, SEM,	-	[56]
			Ultrasonic		
			Irradiation		
7.	Seed	ZnO NP	UV-visible, XRD,	Food preservative	[57]
			FTIR and TEM	material inhibits	
				Quorum sensing	
8.	Seed	TiO ₂ @AG	TEM, SEM, DLS	Cytotoxicity	[58]
9.	Seed	ZnO NP	XRD, SEM, UV-	Photocatalytic,	[59]
			visible	antibacterial, and	
				antioxidant	
10.	Seed	CuO NP	PXRD, SEM, UV-	Antibacterial	[60]
			visible		
11.	Seed	AgNPs	FTIR, SEM, UV-	Antidiabetic	[61]
		(nanorods)	visible		
12.	Seed	Au NPs	FTIR, XRD, TEM,	Antibacterial,	[62]
			UV-visible	antibiofilm,	
				cytotoxicity	
13.	Leaf	AgNPs	SEM, FTIR	Phytotoxicity,	[63]
				cytotoxicity,	
				biofilm	
14.	Seed	Au NPs	XRD, EDAX, TEM,	Antioxidant	[64]
			UV-visible	activity	

 Table 12.1: Nano metals of Nigella sativa and their applications

anti-oxidant, and as an anti-cancer. Further work can be desired to discover the pharmacokinetics, biochemical and therapeutic activities of active constituents and their collaboration with modern drugs. Cellular and molecular target of various constituent of *N. sativa* seed may be explored to get more effective and safer drug. Detailed studies are required to ensure its safety and efficacy and possible use of phytochemicals as drug candidate. Further thorough clinical studies on N. sativa should be done so that humanity can be benefited.

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Conflict of Interest

The authors declare that they have no conflicts.

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Impact of Fungicides on Soil Enzyme Activities

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Abstract: The application of pesticides which include fungicides to the agricultural crops is the common practice in the modern agriculture to improve the crop productivity throughout the world. Continuous and indiscriminate use of these pesticides, which comprise fungicides, insecticides and herbicides, lead to the accumulation of parent compounds and their metabolites in the soil. These compounds mainly hinder the activity of various enzymes, which involved in the mineralization of many organic compounds in the soil. The enzymes are the indicators of the soil fertility; therefore, determination enzyme activity under the influence of pesticides is an important criterion to find out the soil health and quality. This chapter is mainly focused on the activity of enzymes under influence of fungicides.

Key words: Fungicides, amylase, invertase, cellulase, dehygrogenase, phosphatase, soils.

13.1. Introduction

Pesticides have become an important part of current agricultural systems since their application has benefited modern society by improving the quantity as well as quality of the world's production as keeping the cost of food supply reasonable. Owing to continuous pest problems, their usage probably cannot be terminated in the near future since the greatest contribution toward the control of these pests has come from the application of pesticides. Different pesticides used in the agriculture shows different time in the environment, with some persisting even for an indefinite period. For instance, certain lipophilic organo-compounds accumulate in the environment reaching very high proportions, which have been used extensively for control of a broad range of insect pests throughout the world [1]. The application of pesticides in the agriculture has been greatly increased in the last 40 years to increase the crop yield. On the other hand, nowadays most of the pesticides are contaminating water, soil, atmosphere as well as food. The pesticides, which comprise fungicides also impact soil enzymes, which are important catalysts ruling quality of the soil life. In particular, the activity of soil enzymes controls nutrient cycles, and, in turn, fertilization [2].

Few reports illustrate the existence of these toxic chemicals in aquatic system like drinking water [3] and terrestrial system composed of soil [4,5]. Between the two ecosystems, soil is an important source for the above pollutants and further it reduces potentially toxic dissolved concentrations of pollutants that might otherwise contaminate surface and ground water [6]. As inhabitants of soil environment, microorganisms encounter the insecticides released into the soil. These agricultural insecticides dumped in soil harm microbial function and in turn impair soil fertility. More knowledge regarding the function of the soil enzymes in the ecosystem may provide a distinct option for the united biological assessment of soils due to their decisive part in many biological activities of soils [7]. The soil enzyme activity could be influenced during the agronomic practices (fertilization) [8].

The soil enzymes are often associated with microbial activity as well as soil fertility. Pesticides have been using extensively to control plant pests as well as vectors of human and livestock diseases. The mode of application may vary and whether they are used in agriculture or in public health, pesticides never remain at the site of application and eventually reached to the soil. Though pesticides are intended to affect only particular target pests or processes, they may show toxic effects by interacting with biotic factors of the soil ecosystem. In general, the biochemical activity of soil results from a series of reactions catalyzed by the enzymes either

as intracellular components of the microbial community or as extracellular (cell-free) enzymes. Soil enzymes catalyze chemical, physico-chemical and biochemical reactions involved in nutrient cycling of soil [9]. The enzyme activity is considered as an index of microbial activity in the soil [10]. Measurement of enzyme activities are of great value in screening of the susceptibility of soil processes to agrochemical amendments [11-13]. Assay of soil enzymes like amylase, cellulase, dehydrogenase, invertase, phosphatase, protease and urease indicate the importance of their role in complement of chemical and microbiological analyses [9]. Besides the organo-chemicals like pesticides [14], enzyme activities are also influenced by heavy metals [15], soils type [16] and fertilizers [17]. The soil enzymes are good indicators of the soil fertility as well as soil quality. They are extremely sensitive to a variety of stress factors, comprising contaminants [18]. Floch et al. [19], reported the activities of enzymes are most credible and reliable indicators for evaluating the effect of pesticides on soils.

13.2. Amylase activity

The amylase enzyme, catalyses the hydrolytic depolymerisation of the polysaccharides in soil [20]. The starch hydrolysing enzymes are generally extracellular as well as inducible, however activity of the microorganisms to form amylolytic enzymes depending on type of the starch [21]. The stimulatory effect with insecticide treatment was observed by Tu [22]. He found stimulation with the addition of 17 pesticides, chlorfenvinphos, chlorpyrifos, diazinon, ethion, ethoprophos, fensulfothion, fonofos, leptofos, malathion, parathion, phorate, thionazin, triazophos, trichloronate, terbufos, permethrin and glyphosate, at 5 and 10 mg kg⁻¹ soil, on amylase activity, maximum stimulation was noticed with chloropyrifos and phorate. Further, similar results were obtained by Tu [23] in case of fensulphothion, diazinon, malathion, terbufos, carbofuran and permethrin in a sandy clay soil. Furthermore, Tu [24] stated that tefluthrin, trimethacarb and two experimental phosphorothioate insecticides promoted amylase enzyme in an organic soil. Significant stimulation at 2.5 kg ha⁻¹ of two organophosphates, monocrotophos and quinalphos, and two synthetic pyrethroids, cypermethrin and fenvalerate on amylase activity in four soils has been noticed by Rangaswamy and Venkateswarlu [25]. Experimental study made by Kumar and Prakash [26] revealed sharp increase in amylase activity. Same trend of stimulation was also demonstrated by Sabale and Misal [27] on soil application of lower doses of endosulfan.

Experimental facts indicate that the activity of amylase was not influenced by treatment of pesticides. Therefore, addition of 2,4-D had no effect on amylase activity. On the other hand,

initial non-toxic effect followed by the stimulation was observed on treatment with ethion, fonofos and parathion [28,29]. Further, similar trend was noticed with fenamiphos [30,31]. Srimathi and Karanth [32] also recorded same phenomenon in HCH treated soils. Cernakova [33] studied the effect of insecticide, nerametrine Ek-15 on cellulase activity at 0.3 mole/lit in four soil types and reveal that activity was stopped. Suyama et al. [34] worked on long term effects of chlorothlonil on cellulose decomposition in field experiments (2.25 and 11.25 m/plot) under upland conditions. The activity was suppressed in the high dosage plot that was attributed to difference in soil fungal flora. Reduction in enzyme activity was suggested by the experimental evidence of Tu [35]. Accordingly, imidacloprid caused inhibition in amylase activity for one week. However, significant recovery was made after 3 weeks. Mandic et al. [36] stated that dimethoate had a toxic effect on soil enzyme activity. Amylase catalyzes the hydrolysis of polysaccharides and release monosaccharides to provide labile carbon and energy sources for supporting living microbes in soil [37,38].

13.2.1. Invertase activity

The enzyme invertase catalyses hydrolysis of sucrose and results in the formation of D-glucose and D-fructose, and widely distributed in the microbes, animals as well as plants [13]. Tu [22] proposed that the use of ethion, phorate and trichloronate at 5 and 10 mg/kg improved the activity of invertase for 3 days in the organic soil. Identical results were noticed in sandy clay soil [23]. Srimathi and Karanth [32], reported that hexachlorocyclohexane as well as its isomers had steady raise on the activity of soil enzyme after 2 weeks at the level of 10 and 100 ppm. The rate of activity of invertase followed similar trend to the activity of amylase in the two soils as noticed by Rangaswamy and Venkateswarlu [25], which was further observed by Gianfreda et al. [39,40] with carbaryl at the level of 1 mM in soils. In contrast, Palaniappan and Balasubramanian [41] noticed inhibition at 2, 5 as well as 10 ppm carbofuran on the activity of invertase in laborotary condition. Decreased in invertase activity for 1 day has been noted by Tu [42] which was disappeared after 2 days in the sandy loam soil by captan as well as chlorothalonil. Likewise, El-Hamady and Sheloa [43] reported that the imidacloprid drastically decreased the activity of invertase in Egyptian soil. In contrast, Tu [35] stated that the initial inhibition followed by the recovery with imidacloprid in sandy soil.

13.2.2. Cellulase activity

Cellulase mainly acts on β -1,4-glucan bonds present in the cellulosic substance. Cellulosic material present in soil ecosystem was hydrolysed by cellulase enzyme [44]. Omar and Abd-

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Alla [45] stated the addition of 2 insecticide combinations promoted cellulase activity at 50 ppm in plant system. Further, Omar and Abdel-Sater [46] studied the effect of selecron at field rate and 5 times the field rate on cellulase activity up to 10 weeks. Activity was inhibited in soil incubation studies. The enzyme cellulase is involved in the decomposition of the organic matter in soil. The use of ridomil a gold fungicide led to an extremely significant stimulation of cellulase activity from day 7 to day 21 [47].

Conversely, Ross and Speir [31] proved that fenamiphos when applied at recommended doses were innocuous in the laboratory conditions. Similarly, Tu [24] demonstrated that malathion, an organophosphorus insecticide showed no drastic effect on enzyme activity. In contrast to this, benomyl and dithane M-45 reduced the activity of cellulase in culture filtrates [48]. Similarly, the pesticide fenamiphos at higher levels of 37 and 930 mg/kg had a deleterious effect on cellulase with 24 and 48% reduction even after 62 days under laboratory conditions [31]. In the same way, Satpathy and Behera [49] showed decrease in cellulase activity at very low concentration (10 ppm) in a loamy sand soil. Further, Jairaj and Rambadhram [50] revealed that production of the cellulase was significantly decreased by the addition of phorate at the level of 5, 10, 50 and 500 ppm. Experimental results of Ismail et al. [51] indicate similar type of results with metsulfuron-methyl in Malaysian soil. In a different study made by Gherbawy and Abdelzaher [52] variation in the activity of cellulase by metalaxyl was marked in the pure cultures of fungi. Similar observation was made by Arinze and Yubedee [53] that kelthane and fenvalerate caused inhibition to enzyme activity. Omar and Abd-Alla [45] also made a similar conclusion with fungicides, afugan and tilt. In addition, it was postulated that the enzyme activity was adversely affected by chlorothalonil [54]. The activities of invertase and cellulase were considerably enhanced tridemorph and captan at the levels of 2.5 and 5.0 kg/ha, respectively. But at higher concentrations (7.5 and 10 kg/ha) of tridemorph and captan were harmful to both cellulase as well as invertase activities [55].

13.3. Dehydrogenase activity

Of the many soil enzymes, dehydrogenase play a crucial role in the decomposition of organic matter and electron transfer reactions, and are widely studied using triphenyl tetrazolium chloride (TTC) reduction method. Dehydrogenases are enzymes involved in all living organisms. These enzymes, which have a remarkably conserved active site, take part in many reactions involving the transfer of pair of electrons and dehydrogenase activity of soil provides correlative information on the microbial population [56]. The activity of dehydrogenase had

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relationship with physico-chemical properties of soils [57] seasonal variations [58] and tillage [59]. The role of dehydrogenase activity (DHA) involves the biological oxidation of organic matter in soil through the transfer of hydrogen from an organic substrate to inorganic acceptors [60].

The spiroxamine, tebuconazole, and triadimenol S + Te + Tr mixture inhibited activities of dehydrogenases, urease, and acid phosphatase. The major changes were also noticed during growth of the spring wheat. The resistance index (RS) calculated on the basis of plant yield demonstrated that spring wheat shows extremely susceptible to the tested preparation administered to the soil in at the level of 13.80 and 27.60 mg kg⁻¹ [61].

Tu [24] noticed that 3 insecticides at 10 mg/kg induced stimulation in dehydrogenase activity of sandy loam at two weeks interval. In the same manner, dehydrogenase activity was promoted to a significant level at 2.5 kg ha⁻¹ of monocrotophos, quinalphos, cypermethrin and fenvalerate in groundnut soils [62]. As per the experimental data of Tu [35] and Tu et al. [63,64], formazen production was significantly activated with fenamiphos and other herbicides and five insecticides treatment for 1 week interval. Similarly, Nagaraja et al. [65] stated that aldrin at lower concentrations of 10 ppm promoted dehydrogenase activity in acid and neutral soils. In a related study of Megharaj et al. [66], increment in dehydrogenase activity at 1 to 10 kg ha⁻¹ of fenamiphos was proved in soils incubated for 20 days. Same trend of accentuation was further noticed by Andrea et al. [67] with endosulfan (2L), in two soils. The application of butachlor at 5.5 to 22 m μ g/g to dried paddy soil enhanced the activity of dehydrogenase with highest rate at 22 mg g⁻¹ on 16th day [68]. Soil dehydrogenase activity was stimulated (18-21%) with field application rate of benomyl (51 mg/kg), chlorothalonil (8-15%) at the rate of 37 mg/kg and also captan in wheat cultivated soils [69].

Reduction in enzymatic activity with insecticide dosage at varying concentrations was encoded in many previous reports. Palaniappan and Balasubramanian [41] examined the effect of carbofuran at 2, 5 and 10 ppm in lab conditions and found that activity was inhibited. In loam and silty clay soils, addition of dimethoate and phenmedipham affected microorganisms involved in dehydrogenase activity [70]. Further, Megharaj et al. [66] evaluated that DDT (10-100 mg/kg) and its metabolities affected dehydrogenase activity. This was further supported by the experimental evidence [71] on diazinon at 400 mg g⁻¹ of soils. In addition, Manisha and Rai [72] also confirmed inhibition of dehydrogenase activity on application of 750 g/ha of

endosulfan in soil samples. Whereas, Pozo et al. [73] demonstrated that activity was significantly decreased initially but recovered after 14 days. Chen et al. [69] also evaluated the effect of benomyl, captan and chlorothalonil on soil dehydrogenase activity and showed that activity was severely affected. Soil dehydrogenase activity was adversely affected in presence of chlorothalonil [54].

13.3.1. Phosphatase activity

The phosphatases play an important role in the process of biochemical mineralization of organic phosphorus and therefore, may act as a good indicator of the mineralization potential of organic phosphorus and the biological activity taking place in soil [74]. The phosphatase enzymes represent a broad range of intracellular and soil accumulated activities, which catalyse the hydrolysis of both the esters as well as anhydrides of phosphoric acid [75]. Phosphatases are hydrolytic enzymes that hydrolyze organic P to inorganic P and thereby make it bioavailable. It is produced by over 75% of soil microorganisms. It is also linked to metal ion concentration with Mg²⁺ increasing and Na⁺ decreasing activity [76]. The phosphatases speed up decomposition of soil organic phosphorus and improve the soil phosphorous concentration that is an important index to assess soil phosphorus bioavailability [77]. Addition of monocrotophos and malathion at 50 ppm increased the activity of alkaline phosphatase [78]. Rangaswamy and Venkateswarlu [79] also found stimulation in enzyme activity with monocrotophos, quinalphos, cypermethrin and fenvalerate in groundnut soils at 2.5 kg ha⁻¹ for 10 days. Organophosphate insecticides, malathion, pirimiphos methyl and dimethoate demonstrated higher levels of phosphatase activity in soils [80]. Yadav et al. [81] experimental results proved that carbendazim inhibited phosphatase activity in fungal cultures. The effect of four fungicides on alkaline phosphatase activity at one and ten times the recommended doses decreased enzyme activity in mycorhizal cultures [82]. Omar and Abd-Alla [45] studied the effect of two fungicides (propicanazole and afugan) and herbicides (butachlor and thiobencarb) which showed distinct stimulation in enzyme activity at 50 ppm in pure fungal cultures. Further, soil treatment with propicanazole and afugan on acid phosphatase activity was promotive at field application rates [46]. Contradictory to this, Tu [35] stated temporary suppression in enzyme activity with four insecticides in soils. Further, similar report was made by Megharaj et al. [66] with DDT in soils at 10-100 mg/kg for 20 days. Effect of fenamiphos and chlorpyrifos on soil phosphatase activity was insignificant [54]. On the other hand, enzyme activity was reduced with higher dose (100 ppm) of carbofuran in Karnataka soils [83]. Similar results were obtained by Tu et al. [63,64] with herbicides.

12.4. Conclusion

Soil enzymes play a tremendous role in cycling of nutrients and inturn involved in the fertility of soil. However, huge amount of fungicide application to the agricultural crops for controlling fungi pests, in order to increase the crop yield, led to the accumulation of pesticidal residues in the soil environment. These accumulated compounds dramatically affect the activities of soil enzymes. Whereas at lower concentration (< 25 ppm) of fungicides does not show any significant effect on enzyme activities, interestingly at field application rates (2.5 or 5.0 kg/ha), significantly enhances the soil enzyme activities such as amylase, cellulase, invertase, dehydrogenase and phosphatases. Hence, the application of higher concentrations of pesticides mainly affects the non-target native microbial enzymes, which play a key in the mineralization nutrients in the soil.

12.5. References

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Mechanisms Involved in Microbes and Heavy metal Interactions in Bioremediation

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Abstract: Anthropogenic activities raises pollutants in nature which is becoming a serious threat to all life forms. Elimination of pollutants at contaminated place is very important for safe ecosystem. Simultaneously removal of contaminants is attractive scheme for their remediation. Many conventional techniques are used as treatment solution, but they are not attractive due to different parameters. Now a days the novel, cost effective and ecofriendly technique is using the microorganisms. They take part a very important role in bio geochemical cycles and bio transformations, decomposition, mineral and metal transformations, bio weathering and sediment formation. Microorganisms have different mechanism which can effect changes in metal toxicity, mobility, speciation, mineral deterioration, dissolution and formation. These mechanisms play a crucial role in cycling of metals and elements which are associated with soil, rocks and minerals. Microbial mechanisms involve active efflux or sequestration with insoluble compounds or proteins, through which they may detoxify or metabolize the heavy meals like Zn, As, Cd Ni, Pd, Hg. This book chapter explains about the chemistry involved in different metal microbe interaction.

Key words: Microbes, Heavy metals, Environment, Bioremediation.

14.1. Introduction

Heavy metal pollution has become a very serious problem to all living organisms in an ecosystem [1-10]. Increased amounts of inorganic nutrients can inhibit the microbes [2]. Due to usage of fertilizers, and pesticides heavy metal contamination of fields increases which is reducing the crop yield, quality and plant metabolism. According to Environmental Protection Agency (EPA), transformation (degrade, transform or breakdown) of hazardous pollutants into less toxic forms by microbes spontaneously is called parameterization. This process eliminates pollutants from environment [11-15]. Chemical contaminants are used by microbes as their energy sources via metabolic process. Now a days innovative technologies are using different types of microorganisms for the removal of heavy metal pollutants from water bodies and soil [2,3]. Many studies reported some microbes can able to breakdown heavy metals into less toxic forms which are utilized for their growth [9].

14.1.1. Microbe/mineral interfaces

It is important to know about the nature of mineral/microbe interactions because how the bacteria controlling or influencing the fate and transporting of heavy metals polluted sites. Bacteria have exopolysaccharides which makes a distance of a few microns between the minerals and cells. Transmission and scanning electron microscopy have given the detailed pictures of bacteria and mineral surfaces. According to recent results steep oxygen gradients, nutrients and pH exist within the heavy network of bacterial biofilms. The biofilm structure responds to conditions such as hydrodynanic flow in such a way as to optimize the mass transfer of nutrients, substrates and electron donors to member of attached microbial community. By using Fluorescent Insitu Hybridization (FISH) with confocal laser microscopy we can define microbial structure on mineral asurfaces. Polysaccharide-specific fluorescent probes also provide the opportunity to image the extracellular matrix between cells and microbial community. These techniques provided morphology and structure of biofilms in correlation with metabolisms. Microbes are resistant to toxic metals the percentage ranges from few to hundred in highly contaminated environment [16-25]. The survival mechanisms are depending upon the metal speciation which leads to alteration in their mobility. Production of metal binding peptides and proteins, active transport, efflux, redox transformations and intracellular organizationa make metals significantly bind to cell walls and other structural components [17-19]. Other microbial properties make the metal solubilization from organic and inorganic metals which made a link between geochemical cycles and microbial responses for metals.

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14.1.2 Mechanisms in removal of Heavy metals by bacteria

In microbial transformations the mechanisms involved between insoluble and soluble metal species which depends on physical, chemical conditions, microbes, nature and relationship with Animals, plants and anthropogenic activities[9]. They are

Mobilization

- 1. Chemolithotrophic and chemo-organotrophic leaching
- 2. Autotrophic leaching
- 3. Methylation
- 4. Siderophores
- 5. Metal binding proteins

Immobilization:

- 1. Precipitation
- 2. Intracellular accumulation
- 3. Oxidation reactions
- 4. Redox reactions

14.2. Mobilization

14.2.1. Chemolithotrophic leaching

It is also called as heterotrophic leaching, in this mechanism According to Gadd [17] leaching is a process involve the complexing the metal anion with proton and efflux of the organic molecules in case of Al and Fe. Chemolithotrophic bacteria are hydrogen, sulphur iron oxidizers and nitrifying bacteria. These microbes accept electrons for the electron transport chain from the oxidation of inorganic molecules rather than NADH generated by oxidation of organic molecules. CO_2 is accumulated, flooded with proteins which creates acidic environment and changed the charge, it is followed by releasing of metal cations from complexes, this is because of competition between protein and metal. Table 14.1 showing some bacteria involving the chemolithotrophy of some element.

14.2.2. Autotrophic leaching

In Autotrophic leaching CO_2 is fixed from the environment by the energy from oxidation or reduction of sulphide and ferrous ores. These are carried out by chemollithotrophic bacteria or acidophilic bacteria like Thiobacillus thiooxidans, Thiobacillus ferrooxidans. Oxidation of sulphur and Iron reduces the pH of that environment which lead the metal stabilization. This process involves two steps first step is oxidation of heavy metals which helps in leaching of metal then it is entrapped in the exopolymer Thiobacillus concretivorus and Thiobacillus

Chemolithotrophic Organism	Reactions
Alkaligenes, Hydrogenophaga and	$H_2+O_2 \longrightarrow H_2O_2$
Pseudomonas	
Nitrobacter	$NO_2^- + 1/2 O_2 \longrightarrow NO_3^-$
Nitrosomonas	$NH_3^+ + 1/2 O_2 \longrightarrow NO_2^-H_2OH + 2H^+$
Thiobacillus Denitrificans	$5S^{O}+6NO_{3}+2H_{2}O \longrightarrow 5SO_{4}+3N_{2}+4H$
Thiobacillus Ferro oxidans	$Fe^{+2} + 2H^+ + 1/2 O_2 \rightarrow 2Fe^{3+} + H_2O$

Table-14.1. Chemolithotrophic bacteria and reactions involved.

thiooxidans. Thiobacillus neapolitanus is the only bacterium in this group that oxidizes tetrathionate:

 $6 \operatorname{Na_2S_2O_3+5O_2} \rightarrow 4 \operatorname{Na_2SO_4+2} \operatorname{Na_2S_4O_62Na_2S_4O_6+6H_2O+7O_2} \rightarrow 2 \operatorname{Na_2SO_4+6H_2SO_4}$

Thiobacillus thioparus oxidizes thiosulfate first to sulfur, which in turn is oxidized to sulfate:

$$5Na_2S_2O_3+H_2O+4O_2 \rightarrow 5Na_2SO_4+H_2SO_44S_2S+3O_2+2H_2O \rightarrow 2H_2SO_4+H_2SO_4S_2S+3O_2+2H_2O \rightarrow 2H_2SO_4+N_2SO_4S_2S+3O_2+2H_2O \rightarrow 2H_2SO_4+N_2SO_4S_2S+3O_2+2H_2O \rightarrow 2H_2SO_4+N_2SO_4S_2S+3O_2+2H_2O \rightarrow 2H_2SO_4+N_2SO_4S_2S+3O_2+2H_2O \rightarrow 2H_2SO_4+N_2SO_4S_2S+3O_2+2H_2SO_4S_2S+3O_2+2H_2SO_4S_2S+3O_2+2H_2SO_4S_2S+3O_2+2H_2SO_4S_2S+3O_2+2H_2SO_4S_2S+3O_2+2H_2SO_4S_2S+3O_2+2H_2SO_4S_2S+3O_2+2H_2SO_4S_2S+3O_2+2H_2SO_4S_2S+3O_2+2H_2SO_4S_2S+3O_2+2H_2SO_4S_2S+3O_2+2H_2SO_4S_2S+3O_2+2H_2SO_4S_2S+3O_2+2H_2SO_4S_2S+3O_2+2O_2+2A_2S_2S-3O_2+2O_2+2A_2SO_4S_2S-3O_2+2A_2SO_4S_2S-3O_2+2A_2SO_4S_2S-3O_2+2A_2SO_2+$$

The facultative autotroph *Thiobacillus novellus* is able to oxidize thiosulfate directly to sulfate: Na₂S₂O₃+2O₂+H₂O \rightarrow Na₂SO₄+H₂SO₄

Some facultative autotrophs are able to perform this reaction, which is coupled with a rise in pH that is sometimes followed by a return to the original value:

 $2Na_2S_2O_3+H_2O+12O_2 {\longrightarrow} Na_2S_4O_6+2NaOH$

Although some of the *Thiobacilli* are able to form elemental sulfur, they never seem to store it. Instead, they oxidize it further or excrete it. Thiobacillus sp. Remediate about 95% of Cd from 270mg kg/g and 69%, of Pb from the 5g/kg of Pb

14.2.3. Methylation

Microbes play a significant role in biological methylation [16]. Several methylated derivatives are raised from biological methylation [Figure 14.1]. Under aerobic conditions some of the organisms like clostridia, sulphate reducing bacteria, methanogens and pseudomonas are involved in methylation. These can transform the methyl groups enzymatically to metals. The methylated metal compounds are differed in volatility, solubility and toxicity. Methylation of mercuric ion forms Metal mercury [(CH₃) Hg⁺] is toxic in nature. Other bacteria can methylate the methyl mercury to dimethyl mercury [5].

14.2.4. Siderophores

These are iron chelating compounds which can bind, shuttle or transport iron. These are low molecular mass coordination molecules secreted by many numbers of bacteria and fungi. Three types are present these are given in the Table 14.2 and the basic structure of siderophore is given in the Figure 14.2.

14.2.5. Metal binding proteins

Microbes synthesizes many peptides and proteins like phytochelations and metallothioneins which may bind to metals and regulate metal ion homeostasis in turn effect toxic responses [12,4, 20-30]. Phytochelations are glutathione oligomers. These act as chelators, for heavy metal detoxification. Metallothioneins have thiol groups, making them to bind a number of trace metals and can binds to several Zn ions. Zinc and Cadmium are coordinated to cysteine residues tetrahydrally, each metallothionein protein molecule may bind up to 7 atoms of Zn or Cd. Many minerals can improve important microbial processes like nutrient cell adhesion, formation of biofilm, energy generation [8]. Heavy metals can also be absorbed to mineral surfaces which is displayed by microbial activity. Minerals release some toxic metals have properties like surface composition, surface charge, microtopography and hydrophobicity which play a significant role in microbial attachment, thigmotropism and detachment which again showed their importance in ecology of microbial communities associated with mineral surfaces and biofilm formation [7].



Figure 14.1 Methylation reactions of metals.

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Types of siderophores	Organism producing	Туре
	siderophore	
Ferrichrome	Ustilago sohaerogena	Hydroxamate type
Desfemioxamine B	Streptomyces pilosis	
Desfemioxamine E	Streptomyces coelicolor	
Fusarinine C	Fusarium roseum	
Omibactin	Burkholderia cepacia	
Enterobactin	Escherichia coli	Catecholate type
Bacillibactin	Bacillus subtilis	
Vibriobactin	Vibriocholerae	
Azatobactin	Azatobacter vinelandii	Mixed ligand siderophores
Yersiniabactin	Yersinia pestis	
Pyoverdine	Pseudomonas aeruginosa	

Table 14.2. Types of siderophores.



Figure 14.2. Types of siderophores and their structure.

14.3. Immobilization

14.3.1. Precipitation

This process is involved in biomineralization. In this process microorganisms form minerals. Here organism can change some conditions which can precipitate minerals chemically [11]. In anaerobic environment bacteria such as *Desulfo vibrio* and *Desulfato maculum* produce hydrogen sulphide which then react with metals like lead and mercury and forms metal sulphides [10] [Figure 14.3].

Reactions of Heavy metals with H₂S



Figure 14.3. Reactions involved in production of H₂S anaerobically

14.3.2. Intracellular accumulation

This is a physical mechanism in which organic and inorganic metal species are absorbed in the contaminated site by microbes. The main chemical functional groups having strong electronegative charge such as phosphate, carboxyl, and nitrogen groups are removed from heavy metal compounds. In turn the possible groups present on microbial cell which can bind to metal are hydroxyl, carboxyl, amino, ester, sulfhydryl, carbonyl and polysaccharides, or internal such as in ketones and polysaccharides too and phosphate groups [26]. These groups have an important role in removal of toxic heavy metals by specific or nonspecific binding. Either living or non-living cells can participate in this process because it does not require any active cell metabolism.

The analysis of functional groups absorption can be done but using different analytical techniques such as X-ray spectroscopy (EDS), infrared spectroscopy (IR), X-ray photoelectron spectroscopy (XPS), energy-dispersive and X-ray absorption fine structure spectroscopy), raman spectroscopy (26). Hydroxyl, carboxyl, and amine groups of the *Aeromonas hydrophila* are involved in biosorption of lead (II) from water (26). According to new recent work by Liu et al., (28) these functional groups are involved in biosorption of uranium (VI) on to *Bacillus amyloliquefaciens*. Liu et al (28) observed by the XPS technique and suggested that there is a chemical bonding between oxygen containing functional group and uranium. According to their study, the main site of biosroption of uranium (VI) is functional groups which contains oxygen.

14.3.3. Oxidation and reduction

The microorganisms can transform toxic heavy metals into less toxic or non-toxic form with the help of oxidation and reduction reactions. These reactions may play a significant role in transformation of minerals and metals in their surroundings including iron and uranium. The degradation of metals by microbial activity is termed as biocorrosion. Iron oxidizing/reducing bacteria, Sulfate reducing bacteria, sulphur-oxidizing bacteria, manganese-oxidizing bacteria, bacteria secreting organic acids and slime are the main types which will form biofilm communities on surface of metals [23]. Here microbes use coupled reactions to transform the heavy toxic metals into soluble, nontoxic form. For example *Desulfovibrio vulgaris* reduces uranium ions (U16) into U14 ions by reductase enzyme while reduction of Cr16 to Cr13 was carried out by E.coli [24]. Oxidation and redox reactions of Heavy metals



14.3.4. Fungi in Bioremediation

Cell wall of fungi basically constituted with chitin, cellulose, mannan, glucan, some of proteins, lipids pigments and polyphosphates. Heavy metal ions can interact with extracellular enzymes

of fungi which make the uptake of metal by fungi, 20% is transported to vacuole, 30% will be present in cytoplasm and 50% of metal is bound to cell wall. In similarity with bacteria functional groups like hydroxyl phosphoryl and carboxyl involved in this mechanism known as biosorption. Heavy metals acts as enzyme inhibitors and protein denaturing agents. Hg bind to SH groups causes irreversible inhibition. Cd binds to aromatic amino acid residues by enzymatic reaction and can damage protein oxidatively. Immobilization is one of the techniques followed by fungi as resistant mechanism by means of intracellular and extracellular chelating compounds with small peptides like metallothioneins and phytochelations, melanin. In case of *Aspergillus niger* Cd uptake involves carrier mediated transport and rapid binding, fungi can also reduce the metal through extracellular precipitation and complexation strategies uptake.

14.3.5. Heavy Metal Removal by Cyanobacteria.

These are photosynthetic prokaryotes present in fresh, marine and terrestrial water bodies. The noval and cost-effective mechanism is removal of Heavy metals by cyanobacteria. It can take up the contaminants to enhance their metabolism as well as decrease or remove the pollutants. Wild type, genetically modified cyanobacteria are used for the bioremediation of heavy metals [1, 25]. Cynobacterial extrapolysaccharide has complex structure because of branched monomers and molecular weight is ranges from 10 kDa to 2 MDa. Different polysaccharides and proteins present on cyanobacterial surface help and enhances the binding with heavy metals [31-41]. Metallothionein cyanobacteria can produce metallothionins which are metal binding proteins having less molecular weight and rich in cysteine can bind to heavy metals (Zn,Cu, Cd, Hg, Pb) via cysteinyl thiolate bridges to cysteine ligands and their characteristic arrangement is -Cys-Cys-Cys-X-Cys-or -Cys-X-X-Cys (X: amino acids) [Figure 14.4]. Studies showed that MTs synthesis is elveveted in high Heavy Metal's concentration. Anacytis nidulans Showed resistance towards Cd (II), In Synechocystis sp PCC 6803 and Anabaena sp. PCC 7120 ZN-MT like sequence was present [6]. In presence of Cd (II), Cu (II) and Zn (II) up regulation of metal binding MT and heat-shock stress protein was occurred in Synechococcus sp. PCC 7942. Sequesterization of heavy metals like Cd and Zn by metal binding proteins was occurred in Nostoc linckia so it was tolerant to these metals [14]. In Anabaena doliolum metal binding protein gives resistnce against metal and environmental stress [29]. Due to the presence of sulfhydryl groups Apo-MT showed high affinity for Zn and because of reactive sulfhydryl groups Zn will be transferred from MT to another protein (exchange reaction) showed

CHAPTER 14: Mechanisms Involved in Microbes & Heavy metal Interactions in Bioremediation

resistance towards Cd (II). El-Enany and Issa [14] reported that *Nostoc linckia* was tolerant for Cd and Zn due to the sequestration of these metals by metal-binding proteins.

Pentose, ribose, xylose Hexoses, galactose, glucose, mannose, arabinose acidic hexoses, deoxy hexose, rhamnose, methyl rhamnose, galacturonic corrosive, are the primary structure blockers especially seen in cyanobacterial EPS [35]. Rossi and De Philippis [36] reported various sorts of building blockers, for example, N-acetyl glucosamine, 2, 4-O-methyl rhamnose, 2, 3-Omethyl rhamnose, 3-O-methyl rhamnose and 3-Omethylglucose, [15]. Oftnely glucose is present at elevated levels in cyanobacterial EPS. According to few reports sugars like xylose, arabinose, fructose and galactose present at higher amount than glucose [3]. Rossi and De Philippis reported that *Microcystis wesenbergii* has uronic acid as sole building blockers [35]. Thus, the cyanobacterial EPS with specific repeatable unit of building blocks is considered for specific species. These unique units are made up of 5-8 monosaccharides, this is why cyanobacterial EPS has complex behaviour. The presence of uronic acids, pyruvates and acyl bunches gives negative charge to EPS and bestows binding and adsorptive properties [22]. Gel like consistency of EPS is because of sulfates. The MT-like proteins in Anacytis nidulanshas beendescribed which has resistance towards Cd (II). Other examples are Synechocystis sp. PCC 6803 and Anabaena sp. PCC 7120 [6]. Cd showed a superior effect among all metals, and researchers stated that continued exposure of metals will upregulate the MTs and heat shock proteins. El-Enany and Issa [14] reported Nostoc linckia



Figure14.4. Domain nature of metallothionein.

was tolerant for Cd and Zn due to the sequestration of these metals by metal-binding proteins [14] *Anabaena dolium* a metal-binding protein showed resistance against metals and other environmental stresses [29].

14.4. Conclusion:

All Microbes play a significant geoactive role in the biosphere. Transformations of metals and minerals with Microbes has a significant part of natural environmental process and also have beneficial consequences for society. Understanding of chemistry between microbes and metals is the important area of microbiology and exploiting it in the applications such as bioremediation and other areas of biotechnology will clearly require a multidisciplinary approach.

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Biotechnology: A Field of Biological Advancement with Special Reference to Plant & Animal Agriculture and Medicine

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Abstract: Biotechnology a field of biological applications for the betterment human life. Advancement in biological sciences in combination with physical and chemical sciences to make products using microbes or living systems is called biotechnology. Various developments in proteomics, genomics, recombinant DNA technology, immunotherapies, pharmaceuticals, and disease diagnosis are included in human welfare. Role of biotechnology in varies fields of human interest is drastically improved in the early 21st century. This chapter deals about the advancements of biotechnology in the field of agriculture and medicine.

Key words: Biotechnology, r-DNA Technology, Transgenic Plants and Animals, Biological Products, Pharmaceuticals

15.1. Introduction

The term biotechnology introduced in 1919 by Karl Ereky. He defined it as using living cells (microbes) getting products from raw materials. Basically, biotechnology deals with product production by creating alterations in living systems or organisms. The world biotech industry plays an important role in producing various product of human interest and welfare in the recent years. Biotechnology is the combination of various biological science including microbiology, biochemistry, cell biology, genetics, embryology, molecular biology, immunology etc. Research methodology of these sciences are used in the strain improvement of life or microbes for crops, livestock, and drug production [1-2].

Ever growing human population on the earth, forecasting their needs to be fulfilled using various methods including biotechnology. Food the first among all, which is a basic need and must be produced using available agricultural land. The growth kinetics of human beings and the food produced conventionally are inversely related to each other. Using molecular biology through genetic engineering methods researchers are established procedure to increase agricultural yield. Biotechnology made drastic changes in crop improvement in agriculture and is not restrict to farm animals too. Secondly, the health of humans places an important role due to unexpected critical illnesses increase continuously. Biotechnology made a mark to produce pharmaceuticals for various ailments and chronic disorders. The products of human welfare in the row and are produced using tools of biotechnology for easy production and better usage. Besides this biotechnology has its own importance in the fields of immunology, gene therapy, drug production, horticulture, fisheries, etc [3-4].

Due to the role of microbes in the production of various products they are using industrially in the recombinant forms. Selection of right traits to isolate the gene function and introduce it into cells for industrial production is in practice through biotechnology. These cells are highly productive at bioreactors and a range of products releasing into market with affordable price. Biomass is another product of biotechnology producing mainly for feeding farm, poultry, and aquatic animals. Very few products prepared using biomass of microbes canned for human consumption with enriched vitamins, minerals, and protein [4-6].

Several biotech products and procedures are in use for the welfare of human beings. The present chapter educates the advancement of biotechnology tools in the major fields such as agriculture, medicine, industry, and health.

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15.2. Biotechnology in agriculture

Many factors limit the production of crop. Characterization of various genes of interest reduced the influence of various factors on crop yield through recombinant DNA technology. Biotechnology helped many crop plants for increased yield. Genomics and biotechnology combination elevated the food security through transgenic crops. Vegetable oil production has been improved in oelic acid producing crops. About 40% increase in transgenic oil consumption is expected in 2020 [7]. Various transgenic plants produced through biotechnology are discussed hereunder.

15.2.1. In vitro culture and Micropropagation

Tissue culture is an *in vitro* method of producing plants from cells in the right prescribed environment. A single cell from plants can become complete plant in this process. *In vitro* plant tissue culture initiated 30 years ago and is getting modified time to time with respect to new inventions in biotechnology. Several tissue culture methods viz anther, stem, meristem, embryo, leaf, root, seed ect., are developed since its discovery where it is demarcated with the plant part used as explant for *in vitro* [8-12].

An in vitro method of producing disease-free plants through placing high quality material planting for rapid production of productive plants of same type is referred as micropropagation. This method produces pathogen free like viral, bacterial, and fungal free cultivar plants. However, many cultivars are in use for agricultural production generated using micropropagation. Several pathogen free cultivars are in market belongs to banana, papaya, tobacco, tomato, potato, sweet potato, citrus, etc. are available [1, 9-15]. Especially meristem tip culture facilitates the production of virus free plants of various cultivars and are proved to yield high. Tissue culture and micropropagation not only restricted to crop plants these are extended to horticultural plants [1, 16-17].

15.2.2. Salinity resistant crops

Salinity one the factor that limits the yield of agricultural crop in many countries. Though salinity in soils restricts the growth of crop plants, a few of them effectively survive and yields productivity. The genetic analysis revealed that these plants are holding genes which are involved in the saline stress release through mediation of signaling pathways. A complex gene product responsible to show anti-salinity in plants and are reported [18]. However, knowing the complete mechanism involved in the salinity stress release is at primitive stage. It has been

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achieved by studying the genomics of various salinity resistant crop plants. Gene complexes responsible for salinity stress are isolated and characterized along with their signaling pathways which ultimately helps to improve the salinity stress responsiveness in crop plants [19]. Using r-DNA technology and in marker assisted breeding the genetic information is used to develop crops tolerant to salinity stress [20]. Chantre et al [19] discussed the recent progress in the genomic studies and its understanding in developing crop response against drought and salinity stress. Advancement in biotechnology moved forward to edit the genome with TALENs, Cas9/CRISPR, etc., for improved productivity and yield in the crop plants. These advancements made to produce wheat, rice, and some other crop plants against salinity stress. A total of 39000 potato genes were isolated from whole genome and the candidate genes of salinity stress also identified [21]. Van Os et al [22], Consortium et al [23] and Paz and Veilleux [24] sequenced potato genome with DM1-3518 R44 or DM and RH89-039-16 or RH. Salinity resistant genes of potato were identified from the genome analysis which helped to develop transgenic salinity resistant potato plants [25-26]. Moreover, Navarro et al [27] and Kloosterman et al [28] isolated genes responsible for maturity (StCDF1) and tuber initiation (StSP6A) in potato. The combination of these genes along with salinity resistance gene has been used for transgenic potato development.

15.2.3 Transgenic crops

One of the major areas of biotechnology much focused by the scientists is development of transgenic plants for various products synthesis and improving crop yield. Many studies in this direction were done and finding new varieties with varied additional qualities. Transgenics such as disease resistant, draught resistant, pesticide resistant, cold resistant, insect resistant, etc were already developed for certain crops. These plants are developed through genome modification using recombinant DNA-technology. Most of the cases desired DNA (genes) from other species (other species of plants, fungus, animal species, virus or bacteria) are introduced into crop plant cell genome through direct or *Agrobacterium* mediated gene transfer (Figure 15.1).

Cloning procedure are most prominent to produce transgenic crop plants [29]. According to Klumper and Qaim [30], in last two decades, the world's agricultural productivity has been increased drastically with the cultivation of transgenic crops. Which is reported as 22% productivity on an average of each transgenic crop along with 68% former profit. About 32 transgenic crops were approved by ISAAA with an event of 525 in which 238 highest events

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in maize, 61 in cotton, 49 in potato, 4 in argentine canola, 41 in soybean, 19 in carnation, ect [31]. The developmental history of transgenic crop plants was presented in Table 15.1. Various types of major transgenic crop plants produced are represented in Table 15.2. Transgenic crops with desired character were developed using genetic engineering tools. Linearized (restriction digested) Ti-plasmid of *Agrobacterium tumefaciens* or any other vector is used to introduce the desired DNA for making recombinant vector, which is transferred to the protoplasts of crop plant either by Agrobacterium mediated or direct gene transfer methods. The recombinant protoplasts can develop plantlets on tissue culture media after screening. The transgenic crop plantlets with specified character allowed to acclimatize and grow on the soil and finally in agricultural land.



Figure 15.1: Development of transgenic plant through r-DNA technology.

15.2.4 Production of plantibodies

Anti-bodies are produced generally using animal systems. The full-length recombinant immunoglobulins (Igs) are developed in pharmaceuticals through hybridoma and mammalian cell lines, various animal systems approved by the Food and Drug Administration and similar *Ramachandra Reddy et al.* **264**

organizations [32-33]. In general, eukaryotic protein synthesizing system highly conserved to express proteins in a systematic way which is commonly found both animal and plant systems. This is an indication that the plants were able to synthesize animal proteins with executing folding and assembly of full-size serum Igs [34]. Few changes in post-translational modification of plants to animals may not affect the function of plantibodies in mammalian systems. Hence, the plantibodies are producing in plants instead animal systems and especially expression of these antibodies in edible parts of the plant has great attention to have naturally through diet (Figure 15.2). Transgenic alfalfa with human IgG produced in 1999 by Khoudi et al [35] has shown IgG specific epitopes and are recognized in human systems.

Transgenic crops with desired character were developed using genetic engineering tools. Linearized (restriction digested) Ti-plasmid of *Agrobacterium tumefaciens* used to introduce the immunoglobulin DNA, which is transferred to the protoplasts of crop plant either by *Agrobacterium* mediated or direct gene transfer methods. Upon screening the recombinant protoplasts can allowed to develop plantlets *in vitro* and on to the land for acclimatization. Plantibodies are produced in the crop plants especially they targeted to express in the edible parts of the plant (tubers, roots, bulbs, leaves, fruits, flowers, stems ect.).



Figure 15.2. Development of anti-body producing transgenic crops through r-DNA technology.

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S.No.	Landmark discovery/activity	References
1	Discovery of natural ability of <i>Agrobacterium tumefaciens</i> for stable insertion of Ti plasmid DNA (T-DNA) into host plant cell genome and	[36]
	Ti plasmid was recommended as a vector to launch alien genes into plant cells.	
2	Using recombinant DNA and transformation technique specific gene sequence was first reported to be transferred to plant cell	[37-40]
3	Antibiotic-resistant tobacco and petunia the first transgenics were developed.	[39, 41]
4	Transfer of genes to taxonomically distinct angiosperm family is reported through expression of 'phaseolin' gene from bean in sunflower	[37]
5	Developed 'Flavr Savr' the transgenic tomato with longer shelf-life property (delayed ripening)	[42]
6	Approved commercialization of <i>Bt</i> Potato, canola with modified oil composition, <i>Bt</i> cotton, <i>Bt</i> maize, glyphosate-resistant soybeans, bromoxynil herbicide-resistant cotton, etc.	[43]
7	Genes and/or genetic elements of microbes into transgenic crops and its commercialization	[44]
8	Commercialization of 32 transgenic crops with a total of 525 modifications (latest)	[45]

Table 15.1: History of transgenic plant production

15.3. Biotechnology of Farm Animals

The traditional methods like genetics, breeding, feed supplementations used for improving farm animal productivity, but has limitations and still drawbacks are not addressed yet. Animal biotechnology has made tremendous changes in the livestock farming. Transgenic farm livestock produced has great potential to good health in humans along with increased nutrition, animal welfare, pollution free environment with lowered diseases. Gene modification is a tool of biotechnology used to develop transgenic animals with desired character. Livestock gene modification through biotechnology play crucial role in producing high quality food. Transgenes introduced by human intervention into livestock are tested for its heredity and found that they pass these genes from one generation to other (germ lines). Moreover, transgenic livestock can reveal the physiological processes and their genetic control, produce new products, improved animal traits and develop models for diseases [86].

During the process of introducing desired DNA fragment into livestock rapid gene introducing methods were used (gene cloning methods) (Figure 15.3). It has no relation with cross breeding, hybridization, and other traditional methods. A few methodologies have been developed to introduce transgenes into livestock [87-88]. However, there are several potential applications of transgenic livestock were discussed hereunder.

S.No.	Type of Transgenics	Characteristics induced	References
1	Insect-resistant	Major crops have been transformed with insecticidal genes (most commonly different variants of <i>cry</i> gene, and in a few events <i>vip</i> gene) which control the harmful insects attacking crops	[46]
		Insect-resistant transgenic crops growing 23.3 million hectares and occupies second largest area cultivated for the year in 2017.	[47]
		Genes represent <i>Cry</i> proteins from <i>Bacillus thuringiensis</i> used for insect (Coleopterans, Dipterans and Lepidopterans) resistant crop varieties.	[48]
		Cry genes were inserted first commercial lepidopteron resistant cotton.	[49]
		<i>cry</i> genes introduced in many crop plants including tomato, soyabean, rice, potato, chickpea, maize, canola, alfalfa etc.	[50-53]
		Genes (<i>vip</i>) from <i>Bacillus thuringiensis</i> and <i>Bacillus cereus</i> code for vegetative insecticidal proteins are developed in commercial crops.	[54]
		Vip genes (vip $3A(a)$ and vip $3Aa20$) genes were introduced and expressed heterologous sites in maize and cotton.	[45]
		Fungal, bacterial or plant protease inhibitors are introduced into crop plants to control insects by stopping the development and growth of larvae through reduction of gut amino acid assimilation.	[55]
		Insect control in crops by engineering the trypsin (<i>Cpt I</i>) inhibitor and potato protease (<i>pin II</i>) inhibitor for inhibiting digestive enzymes in insects.	[56]
		Rice, tobacco and cotton transgenic crops were made for wide range of insect resistant by introducing <i>cpt II</i> and <i>pin II</i> genes.	[45]
2	Herbicide-resistant		
a	Glyphosate-resistant	Genes epsps (Agrobacterium tumefaciens) and grg 23 (Arthrobacter globiformis) which are responsible for glyphosate resistance has been introduced in to crop plants.	[57]
		Commercialized crops resistant to glyphosate	[58]
b	Glufosinate-resistant	Insertion of glufosinate-resistant genes <i>pat</i> and <i>bar</i> isolated from <i>Streptomyces spp</i> . into crop plants can induce the release of glufosinate detoxifying enzyme phosphinothricin acetyl transferase.	[59]

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Table 15 2. Details of differen	nt transgenic cro	n nlante prov	duced through	genetic engineering
Table 13.2. Details of unities	in transgeme cro	p plants plot	Juceu unough	genetic engineering.

c	2,4-D, mesotrione, dicamba, sulfonylurea, isoxaflutole, oxynil resistant	A total of 351 herbicide resistant transgenic cultivars have produced through biotechnology and are approved for cropping.	[45]	
3 Drought-resistant		Transgenic maize with CspB protein genes raised against Monsanto's drought and it is commercially released with the trade name Genuity [®] drought guard TM .		
		Insertion of drought-resistant gene <i>Hahb 4 of helianthus annuus</i> into crop plants helps to induce dehydration in plants.	[62]	
4	Osmatic-resistant	Genes related to osmoprotectants like sorbitol, trehalose, fructan, mannitol, proline, glycinebetaine help transgenic crop plants to survive under osmotic stress	[63]	
		Water stress in crop plants is overcome by choline dehydrogenase encoding gene <i>bet A</i> , whose product (glycinebetaine) acts as osmoptotactant.	[63-65]	
		Sugarcane with drought-resistant transgenes tolerate water stress up to 36 days with high sugar (10 to 30%) content than non-transgenics.	[61, 66]	
		Dehydration stress is rectified in transgenic crops with RNA and protein chaperones.	[67]	
5	Cold-resistant	Introduced cold shock proteins of bacteria into crop plants like rice and maize to grow them at cold places.	[67]	
		RAN-binding protein and GRP 2 from Arabidopsis thaliana reported to have both salt and cold resistant functions in transgenic crops.	[68]	
		Cold-resistant protein (<i>Csp</i> 3) and its role in drought and salt resistance was tested in crop plants.	[69-70]	
6	Disease-resistant	About 19 potato diseases-resistant genes (<i>cry 3A, pvy, cp, plrv-orf 1, plrv</i> etc.) were introduced and was commercialized.	[71]	
		Transgenic papaya with replicase gene (PRSV) developed for viral-resistance and is commercialized in the name of Huanong No.1.	[72]	
		Transgenic Phaseolus vulgaris against bean golden mosaic virus was developed with the introduction of sense and anti-sense RNAs of viral replication proteins and commercialized this crop.	[73]	
		Transgenic crop of holding <i>rpi_vnt 1</i> (Solanum venturi) gene shows resistance to foliar late blight disease.	[74]	
7	Nutritionally fortified transger	nics		

a	Biofortified	rice	with	Golden rice with yellow colour provitamin A produced in transgenic rice by introducing	[75]
	provitamin A			β -carotene synthesis genes expressed in endosperm of rice.	
				Transgenic American golden rice 1 introduced with <i>psy</i> and <i>crt 1</i> an endosperm expressive gene for producing carotenoids up to 6 µg per gram rice.	[76]
				Transgenic American golden rice 2 introduced with <i>psy</i> (maize) and <i>crt 1</i> (bacterial) an endosperm expressive gene for producing carotenoids upto 37 μ g per gram rice which is 23 times more compared to original one and have highest amount of β -carotene (84%).	[77]
)	Fortified fatty a	cids or o	oil	Transgenic crops holding medium chain triglycerides have resulted in lowered metabolic rate and less storage of adipose tissue when it is included in the diet of humans.	[78]
				Transgenic safflower, soyabean, Argentine canola were produced with low lipid content with a total 18 events all together.	[45]
				Transgenic <i>Camelina sativa</i> with genes producing elevated levels of ε-3 long chain plyunsaturated fatty acids (docosahhexaenoic acid and eicosapentaenoic acid) of marine microbes has been introduced.	[79-80]
;	Essential An fortification	mino	acids	Lysine rich protein from pea is introduced into transgenic wheat and rice and has expressed in the endosperm.	[81]
				AH protein transgenic maize showed high amounts of lysine, isoleucine and tryptophane the essential amino acids along with 32% increase in protein content in the seeds.	[82]
				Genes responsible for lysine rich protein (α -lactalbumin) form animals introduced into maize and is produced 47% of higher lysine content.	[83]
				Introduction of dihyrodipcolinate synthase gene (<i>cordap A</i>) into transgenic crops elevates the lysine levels by beyond the lysine feedback inhibition.	[84]
				Lysine feedback insensitive bacterial DHDPS gene was introduced into transgenic crops which shown 50-fold more lysine production.	[85]



Figure 15.3: Methods used for producing transgenic livestock.

15.3.1 Growth enhancement

The growth control of transgenic animals has been revealed through biotechnology methods. Tested the possibilities of altering various factors of growth including receptors, modulators, manipulators. One of such biological molecules play a crucial role in animal growth is 'growth hormone', a potential manipulator identified to induce growth in livestock. Manipulated transgenic livestock with growth hormone shown tremendous productivity in pigs, fish, poultry etc. [89-91]. In addition to growth hormone omega-3 fatty acid production in fish to other animals and altered biosynthetic pathway or uptake of fatty acids including cholesterol in cheese, meat and eggs from livestock also modified [92]. In this process, the most targeted genes that lowers the cholesterol in transgenic animal products are hormones such as leptin and receptors of low-density lipoproteins (LDL).

15.3.2 Milk production

Biotechnology advancement increased the opportunity increase the volume or to change the composition of milk from transgenic livestock. Due to new methodologies of biotechnology, the milk industry grown drastically in the past two decades with transgenic animals of producing high quantity of milk with greater content of nutrients and nutraceutical proteins. Several modifications from the traditional milk producers have presented in the Table 15.3.

S.No.	Transgenic	Expressed protein	Promoter used	Reference
	animal			
1	Cattle	Lactoferrin	Bovine αs1-Casein	[93]
2	Rabbit	Insulin-like Growth	Bovine αs1-Casein	[94]
		Factor-1		
3	Pig	α-Lactalbumin	Lactalbumin	[95]
4	Pig	Insulin-like Growth	Bovine α-Lactalbumin	[96]
		Factor-1		
5	Cattle	Bovine β , κ -Casein	Bovine β-Casein	[97]
6	Cattle	Lysostaphin	Bovine β -Lactoglobulin	[98]
7	Goat	Lysozyme	Bovine αs1-Casein	[99]
8	Goat	Lactoferrin	Goat β-Casein	[100]
9	Goat and Cow	G-CSF ^a	Cattle and Goat β -Casein	[101]
10	Goat	Human antithrombin	Goat β-Casein	[102]
11	Goat	Vitamin-A	Goat β-Casein	[103]
12	Goat	human α-lactalbumin	Cytomegalovirus	[104]
		gene integrated with β -		
		lactoglobulin gene		

Table 15.3: Transgenic livestock producing various molecules of our interest in milk.

15.3.3 Reproduction and fecundity

Genes are responsible for reproductive performance and proliferation in any organism. In livestock, diversified transgenic pig breeds were used to produce high litter number by introducing estrogen receptor gene or mutation in this gene. Similarly, Piper et al [105] in Merino sheep identified Boroola autosomal fecundity gene for elevated ovulation with a rate of 1.5 ova per gene. Since the regulation of reproduction is a complexed process, not so easy to manipulate it for enhanced reproduction and fecundity in the livestock. However, the scientist groups are working in this direction to improve the livestock in a reasonable way.

15.3.4 Nutrition enhancement

Animal products with improved nutrients is an aspect to affect the health of humans directly by the transgenic livestock. It is a known fact that gene recombination alters the nutritious value of livestock. Based on this, enhanced nutrition of livestock has been increased by gene alterations. The best example for this is explained in sub-section 3.1 [92].

15.3.5 Environment protection

One of the major problems flinging human life into risk is environmental unevenness due to anthropogenic activities. Over exploitation of natural resources, deforestation, conversion of agricultural land into real estate are some of the major causes of destroying natural environment. A major risk is at near future in the form of known or unknown natural disaster due to increased imbalance in nature. In one-way transgenic livestock helps to protect environment by producing high quantity quality products in a small farm area with less utilization of natural resources to meet the demands of ever-growing human population. Especially transgenic livestock can protect the soil and ground water resources. Golovan et al [106] demonstrated that transgenic pig with salivary phytase expression at the age from day 7, increased 75% digestibility of dietary phytate phosphorus which lowers a major pollutant produced in pig farming. This transgenic swine is named as Enviro-PigTM indicating environment protection with high amount of commercial pork production. Similar to swine other products like meat, eggs and milk from transgenic livestock facilitating the low utility of natural resources including land and water with high productivity.

15.3.6 Disease resistant livestock

An important aspect in animal husbandry is maintaining disease free environment. Since ancient days, the livestock has maintained in a separate place of with hygiene to prevent disease of contagious, airborne and soilborne. No methodology has known to ancestors for identifying and curing livestock genetic disorders. Disease-free environment and healthy condition in the livestock can enhances the productivity. Development of biotechnological methodologies show a way for maintaining the disease-free or superior disease resistance livestock in the recent past. For this Donovan et al [107] introduced a gene producing lysostaphin into milk of dairy cows for showing resistance to mastitis majorly caused by *Staphylococcus aureus* and protects the mammary glands. Similar way, Golding et al [108] and Richt et al [109] the causatives of mad cow disease or bovine spongiform encephalopathy in chattels and Creutzfeldt-Jacob disease in humans are curbed prion and prion-free respectively in livestock through r-DNA technology.

15.3.7 Enhancement of fiber and hair

Hair, fiber, wool, and yarn are the byproducts of cattle farm industry. Quality improvement of these by products can increase the economic status of the farmers. Most importantly gene manipulations are essential to increase hair and wool length, fitness, strength, crimp, and quality. Hollis et al [110] and Powell et al [111] tested all these parameters in transgenic goat *Ramachandra Reddy et al.* **272**

and sheep. Another aspect in this area is quality improvement on the surface of wool and fiber for minimizing the shrinkage.

15.4. Biotechnology of Medicine

Human health is one of the important aspects to be taken care throughout the world. Change in the lifestyle, food habits and anthropogenic activities causing damage to the natural environment and has showing adverse effects to health of all organisms including humans. Biotechnology the way of living can facilitate the supplementation of medicine for curing various diseases. Health can be maintained through biotechnology products such as medicines which include antibodies, drugs, various pharmaceuticals, hormones, etc.

15.4.1 Plantibodies and other immunological molecules

Plantibodies are in trend to avoid complications of various diseases by supplementing them as food supplements. The Table 15.4 has presented the plantibodies of various types in different plant systems. It is notable that major studies of plantibodies and other immunological molecules production through r-DNA technology has been done in *Nicotiana* (tobacco) *spp*. system.

15.4.2 Humilin and other biopharmaceuticals

Human insulin produced through genetic engineering methods are commercially called humilin. Insulin is two polypeptide chains A and B joined by cysteine mediated disulphide bonds. It is notable that type-2 diabetes a major metabolic disorder identified in the populations of developed and developing countries due to various reasons including obesity in all age groups. Insulin response to elevated blood glucose levels is dropped in this condition and causes hyperglycemia. Human insulin gene (chain A and B) introduced into the bacterial or yeast or any other mammalian cell lines/systems using cloning strategies can yield humilin in a large quantity for medicating the population who are suffering with diabetes.

A number of biopharmaceuticals have been developed in the recent days by different pharmaceutical companies. Through biotechnology many molecules like protein-based molecules, small drugs, human growth hormone, β -interferon, interleukins, hepatitis B surface antigen vaccine, γ -interferons, α -interferons, granulocyte colony stimulating factor, insulin and other molecules produced using recombinant bacterial or yeast system and few of them through mammalian cell cultures (Table 15.5).

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S.No.	Plant system	Details	Antigen used	Plantibody	References
1	Arabidopsis	Accumulates in	h-Creatin Kinase	Ig G1, Fab	[112]
	and Nicotiana	apoplast or			
		nucleolus. A 13%			
		TSP reported.			
2	Nicotiana		Colon cancer	Ig G	[113]
3	Nicotiana,	Yields 100 µg per	HIV (2G12)	Ig G	[114]
	maize	g seeds	gp120		
4	Soyabean	Reported in	Herpes simplex	Ig G1	[115]
		apoplast	virus2		
5	Alfalfa	Reported in	h-Ig G	Ig G1	[35]
		apoplast			
6	Rice	Reported in	Embryonic	scFv	[116]
		apoplast or ER	carcinoma antigen		
7	Pea	Reported in ER	Embryonic	scFv	[117]
			carcinoma antigen		
8	Wheat	Reported in	Embryonic	scFv	[116]
		apoplast or ER	carcinoma antigen		
9	<i>N</i> .	Toxin	Bacillus anthracis	Ig G	[118]
	benthamiana	neutralization	antigen		
		both in vitro and			
		in vivo			
10	Barly grains,	Yields 150 mg	HIV blood	scFv fussion	[119]
	tobacco	per g	antibodies		
	leaves, tubers				
	of potato				
11	Tobacco roots		Interleukin-6	scFv	[120]
12	Tobacco	Purified 41.7 µg	Salmonella	scFv	[121]
		per g leaves	enterica		
			lipopolysaccharide		
13	Tobacco		Ebola virus	ZMapp,s	[122]
				protein	
				Mabs	

Table 15.4: Plantibodies produced in various plant systems.

S.No.	Company Produced	Product	Use of product
1	Reddy Labs, India	Humilin	Anti-diabetic
2	Amgen, Johnson &	Erythropoietin	Against anemia
	Johnson		
3	Amgen, Sankyo	GCSF	Against WBC
4	Schering, Roche	α-Interferon	Anti-hepatitis
5	Chiron, Biogen	β-Interferon	Against multiple sclerosis
6	GeneTech,	h-Growth Hormone	Deficiency of growth
		Activator of tissue	Blood clot thinner
		plasminogen	
		Tumor antibodies	Against cancers
7	Genzyme	Glucocerebroidase	Treating Gaucher's disease
8	Bayer	Factor VIII	Against hemophilia
9	Amgen and Glaxo	Tumor antibodies	Against cancers

Table 15.5: Various biopharmaceuticals produced and their use in treating diseases.

15.4.3 Antibiotics

Microbial products which inhibit the growth of other microbes are antibiotics. These are commonly used for the prevention and treatment of bacterial, protozoan, viral and fungal infections in animals and humans. Antibiotics of varied chemical nature are identified, isolated, and characterized from various microorganisms. Though the wide range of antibiotics are identified and are using for treating against diseases, their production in the microbes is low and is not sufficient to meet the demand. An alternative found for improved production industrially is by following the methods of genetic engineering to generate recombinant antibiotic producing microbes (Figure 15.4).

4.4 Vitamins and other compounds

Vitamins play a crucial role in the physiology of an organism, especially in vertebrates. Human's mental strength and good health is maintained by vitamins and its related compounds (provitamins, antioxidants and bio-pigments) obtained through balanced and proper diet. Modernization took us to have imbalanced diet with a lot of change in the dietary components leading to notorious avitaminoses. The vitamins/provitamins also play a crucial role in many formulations of chemicals, processed foods, cosmetics, animal feeds, pharmaceuticals, multi or single vitamin preparations, etc. Commonly produced industrial vitamins are ubiquinone, B₂, B₁₂, C, D₂, K₂, pyrrolquinoline quinine, coenzyme Q₁₀ through fermentation from *Saccharomyces cervisiae/Candida utilis* [6, 123]. *Corynebacterium* fermentation converts



Figure 15.4: Industrial production of antibiotic through genetic engineering using recombinant *E. coli*.

glutamate into γ -aminobutyric acid [5]. Many microbial producers modified through r-DNA technology are in use to produce several vitamins, provitamins, antioxidants, bio-pigments and other health related compounds [124].

4.5 Gene therapy

Therapy of hereditary disease through gene replacement known as gene therapy. In 1990, first clinical gene therapy for adenosine deaminase (immune modulator) deficiency was done in a 4-year girl child. It is understood that gene therapy is a process of improving genes by correction of mutated or altered sites in target tissue or cells. Many gene therapy strategies are in use often for therapeutic purpose [125]. However, these studies are restricted to laboratory and waiting for reality in near future [3, 126].

15.5.Conclusion and Future Outlook

DNA, the material of cells programmes the functional activity of each organism by producing proteins thereby controls all cellular metabolic reactions producing various molecules of

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different nature shows cellular activity. Biotechnology a world for making impossible events of organisms into possible. Through understanding molecular biology of cell and applying it using genetic engineering tools many products are producing in large quantities including medicines, transgenic crops, transgenic animals, pharmaceuticals, industrial products etc. Gene therapy a modern method of eliminating human diseases have great importance at present situations. Vaccines, health beneficiaries, food, cosmetics, drugs are some other products produced as a development of biotechnology. Future expects more biotechnology products for human welfare.

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Conflict of Interest

Authors have no difference of opinion on this chapter and declare no conflict of interest.

15.6. References

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Proteomics Studies in Plants under Abiotic Stress Tolerance

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Abstract: In the recent years proteomics is a necessary tool to understand the plant abiotic stress responses. Plants under changing environmental conditions like drought, soil salinity, light, temperature, nutrient deficiency, excess metals in the soil have negative impact on the plant growth. All these alterations in the climate results in economic loss in agriculture due to decrease in crop yield. Plant stress related alterations affect plant growth, development and productivity, which ultimately limits crop yield. The main aim of the present chapter is to discuss the abiotic stress related plant proteomes. Plant abiotic stress responses include differential expression of genes; stress responsive protein expressions leading to cellular metabolic alterations, changes in defense response proteins, over expression of compatible solutes like proline, glycine betaine, sugars, polyamines, amino acids, organic acids and increased synthesis of stress specific proteins. The chapter describes the several proteomic studies on stress treated plants, stress sensitive and tolerant plants.

Key words: Proteomics, Plants, Abiotic Stress, Tolerance.

16.1. Introduction

Plants are exposed to various environmental factors which adversely affects the plant growth and development leading to reduction in crop yield. Major environmental stress factors include drought, soil salinity, light, temperature, nutrient deficiency, excess metals, these abiotic stresses have negative impact on the plant growth (Figure 16.1). Plants have developed various adaptive mechanisms to overcome stress. Adaptive mechanisms include alterations at physiological, biochemical and molecular levels leading to changes in protein pool. Therefore, proteins play major role in stress response which helps the plants to cope up with changing environment. The term proteome was introduced by Marc Wilkins in 1994 [1], it represents whole proteins in an organism at a given time period. Now a day's proteomic analysis is a significant molecular tool for protein profiling and identification of abiotic stress response thereby to understand the mechanisms involved in stress tolerant response.

16.2. Different abiotic stresses

Plants are sessile organisms and challenged by different environmental conditions; to cope up with the conditions several genes are upregulated or down regulated leading to synthesis or degradation of different proteins (Figure 16.1). Proteomics studies of abiotic stresses like drought [5], extreme temperatures [6]. Plant proteome responses to salinity [3], stress responses of major crops [7] including rice [8], maize [9], wheat and barley [10,11], soybean [12,13], common bean [14], Solanaceae species [15], stress proteomics of crops grown in temperate climate [16], and others.

16.3. Stress related responses

Salt stress decreases growth of a plant by affects many physiological and metabolic processes. According to the Food and Agriculture Organization (http://www.fao.org/nr/land/information-resources/terrastat/en/) of the United Nations saline soils include more than 800 million ha, which represents more than 6% of the global land area. Plants adopt two strategies to acclimatize, either avoidance or tolerance to stresses. These strategies at the metabolic and cellular level help the plants to withstand under adverse conditions. Acclimatization involves alterations in the plant proteome and these alterations are directly associated with the plant stress related responses (Figure 16.1). Proteomic analysis may be useful to screen and identify these proteins and their

implication in plant stress response to different abiotic stresses. In this chapter stress related responses to various types of abiotic stresses like drought, salinity, temperature, excess metals, nutrients deficiency and light are discussed. Various physiological changes occur, especially respiration and photosynthesis processes leading to the increased production of reactive oxygen species. Changes at molecular level involve osmotic adjustment by enhanced synthesis of osmolytes like proline, glycine betaine etc. and other stress specific proteins. Proteomic analysis revealed the differential expression of proteins like 30 % of stress defense proteins, 21 % energy and carbohydrate metabolism, 17 % photosynthesis proteins, 13 % protein synthesis and degradation in Banana, explains the mechanism of salt stress (Figure 16.2). The differentially upregulated and downregulated proteins of different proteomics studies under different environmental factors in various plants/crops are listed in Table 16.1.



Figure 16.1. A schematic representation of plant abiotic stress and stress responses

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Drought stress causes decreased relative water content due less availability of water in the soil, reduced soil water potential decreases the root water uptake. Accumulation of compatible solutes and LEA proteins are the changes at the molecular level. Stomata closes during drought to conserve water leading to reduction in CO_2 uptake resulting in imbalancing the photosynthesis process. Alterations in Rubisco large subunit. Total protein content was extremely reduced in drought susceptible wheat cultivar with worsening effect on photosynthetic proteins, sugar and nitrogen metabolism enzymes. Contrasting results were observed with minor changes of protein levels in drought resistant wheat [17]. Phenylpropanoid metabolism proteins are also associated with the drought response was identified by Chmielewska et al. 2016 [18].





Elevated temperatures increase the risk of protein misfolding, limiting the plant growth and reproduction. Heat stress induces synthesis of several heat shock proteins. Heat stress inhibits photosynthesis and various primary and secondary metabolic pathways are involved in heat

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tolerance mechanism. Many heat responsive proteins and phosphoproteins related to calcium signaling, reactive oxygen species (ROS) homeostasis and membrane transport are reported in Spinach [20]. Heat causes water deficit due to evaporation from soil surface and leaf transpiration resulting in oxidative stress and dehydration stress [16].

Salt stress increases the ion concentration in the soil, increment in sodium ion levels leads to osmotic stress, oxidative stress. Plants have developed several adaptive mechanisms at morphological, physiological, biochemical and molecular levels to overcome salinity [2, 21]. Plants detoxify the ions by active ion exclusion or by intracellular compartmentation of sodium ions. To understand the salt tolerant mechanism, iTRAC (isobaric tags for relative and absolute quantification) based protein profiling is done in several crops to analyze the differentially expressed proteins, like rice, banana, cotton, alfalfa in response to salt stress (Table 16.1). Signal transduction is also affected, up regulation of ATP dependent ion transporters like Na⁺/H⁺-ATPases, V-ATPases, and inorganic pyrophosphatases (PPi ases). Osmotic stress is common in all dehydration conditions while ionic toxicity is time-progressive and specific to salt stress [22] Drought stress is frequently associated with salt stress.

Plants growing at tropical and subtropical regions are susceptible to low temperatures. Low temperatures (cold and frost) forms ice crystals in soil resulting water deficit. Freezing temperatures cause freezing injury leading to decreased growth; roots are chilled causing plants to wilt. Respiration rates are decreased, protein synthesis is inhibited, degradation of proteins takes place during cold stress. Synthesis of special plant proteins called as antifreeze proteins occurs. Membranes lose their properties due to cold conditions as a result photosynthesis is inhibited leading to decreased carbohydrate translocation [16]. Low temperature induced proteomic changes were reviewed by Janmohammadi et al. 2015 [23].

16.4. Conclusion

Plant is affected by several environmental factors; proteins play critical role in plant stress response. Plant stress response depends on the type, duration, intensity, severity of stress, proteins play critical role to overcome the stress leading to stress adapted or damaged phenotype. Numerous abiotic factors cause oxidative stress, several stresses lead to cellular dehydration. Plant stress response is a dynamic process with specific proteome composition. Differentially expressed proteins include defense, photosynthesis, energy and carbohydrate metabolism, proteins synthesis and degradation, lipid metabolism. In tolerant plants enhanced levels of detoxification and stress related proteins leads to weaker disturbances in the cellular homeostasis during stress conditions.

S. No	Types of	Crop / plants	Differentially	References
	stress		expressed proteins	_
			Upregulated / Down	
			regulated proteins	
1.	Salt	Alfalfa	254	Long et al. 2018
2.	Salt	Cotton	54 %	Gang et al. 2017
3.	Salt	Rice	28 (LS), 368 (MS), 491 (HS)	Hussain et al, 2019
4.	Salt	Halogeton	97 / 192	Wang et al. 2016
		glomeratus		
		(Halophyte)		
5.	Salt	Banana	77	Ji et al. 2018
6.	Salt	Wheat	243	Singh et al.2017
7.	Salt	Leymus chinensis	274	Li et al. 2017
8.	Salt	Spica prunellae		Liu et al 2019
9.	Drought	Maize	Tolerant 572/671	Zeng et al. 2019
			Sensitive 172/247	
10	Drought	Sugarcane	586/618	Salvato et al. 2019
11.	Drought	wheat	65/111	Wang et al. 2019
12.	Drought	Brassica rapa	440	Kwon et al. 2016
13.	Temperature	Brassica	1022	Yuan et al. 2019
		campestris	172/324	
14.	Heavy metal	Maize	252	Li et al. 2016
	stress (Lead Pb)			
15.	Lead	Gracilaria	14	Du et al. 2018
		lemaneifemin	2/11	

Table 16.1. List of proteomic studies in different crops/plants under different stress conditions

LS: Low stress, MS: Moderate stress, HS: High stress

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16.5. References

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