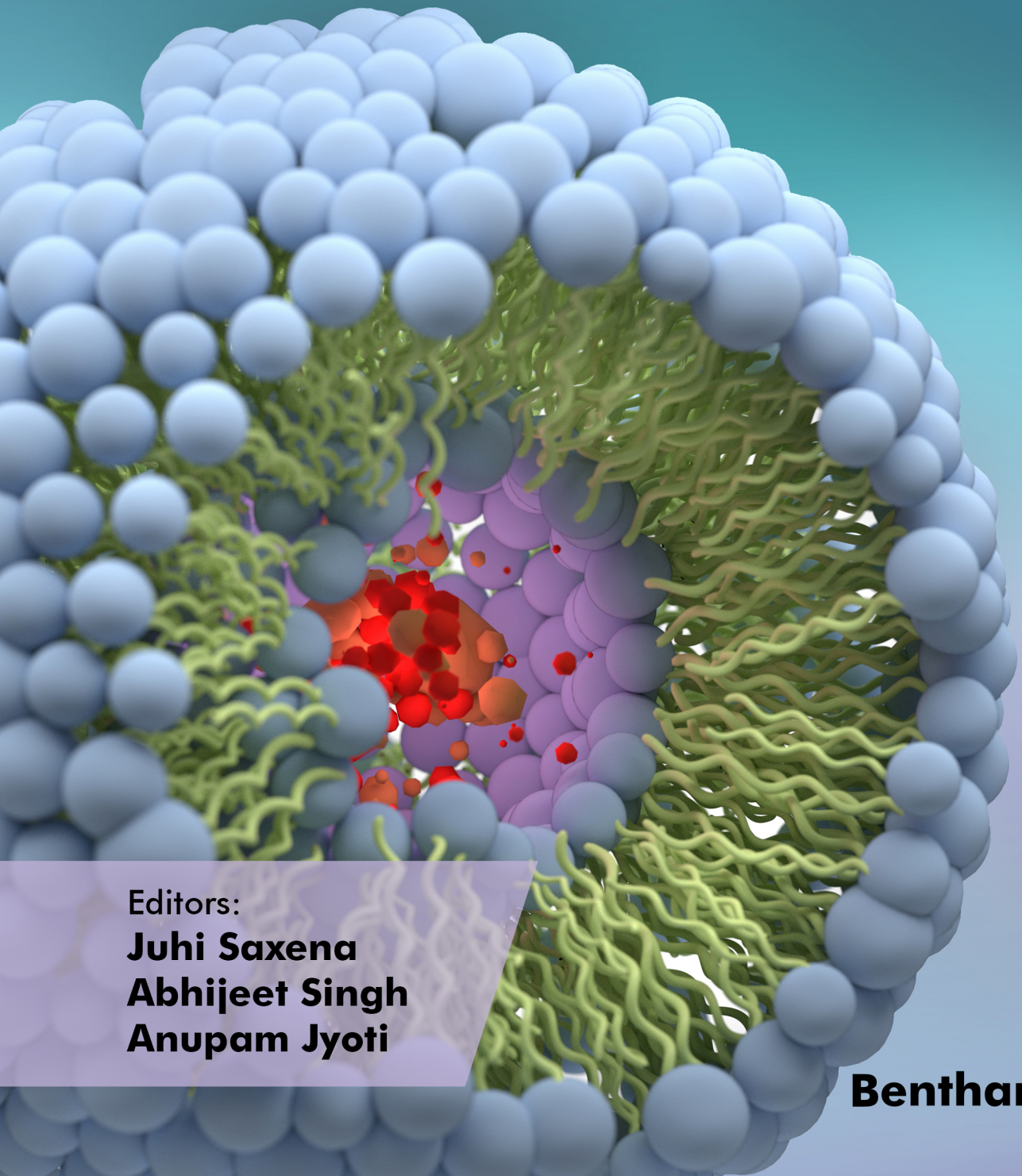
NANOBIOTECHNOLOGY

PRINCIPLES AND APPLICATIONS



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Nanobiotechnology: Principles and Applications

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FOREWORD

Research in nanobiotechnology is gaining prime attention as it is directly affecting many socio-economic sectors including medical, agriculture, food, textile, and other industries. Biological, chemical and physical sciences are the backbone of nanobiotechnology. In recent years, nanobiotechnology research has provided solutions for several problems including human health because of its integrated approach involving various disciplines. Lately, it has been integrated rapidly with new emerging branches like molecular biology, pharmaceutical chemistry, animal cell science and drug development and discovery for output-oriented research.

This eBook 'Nanobiotechnology: Principles and Applications' presents a broad overview of the principles and applications of nanotechnology in the diverse areas of biotechnology. The expert group of authors exhibit distinguished expertise and will belong to the academic world, creating a broad perspective. This volume covers the basics and applications of nanotechnology in drug delivery, combating pathogens, nanobiosensors, improving plant health by fertilizers, bioremediation, disease sensing, and diagnosis.

As a biotechnology scientist, I am happy to recommend this eBook to the students of universities as a text and reference book both. The theory, concepts and technique's part will be used as textbook and the application part as a standard reference. This eBook has been written in a way so that it is student-friendly with clean diagrams and protocols of specific techniques. I sincerely hope that the eBook has been prepared with scientific skills and will serve as a useful document for graduate and undergraduate students.

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PREFACE

This eBook titled 'Nanobiotechnology: Principles and Applications' will provide insight into the principles and practices of nanotechnology in biological fields. Nanobiotechnology, an amalgamation of nanotechnology and biotechnology has gained attention due to its diverse applications. It utilizes the power of nanotechnology to solve the problems of various aspects of biotechnology like agriculture, medicine, industry and many more. In view of this, there is an unmet need to compile different horizons of Nanobiotechnology. Additionally, the biological toxicity to nanomaterials needs attention.

We strongly believe this book is a reader's delight and will help in dealing with the fundamental principles, and applications of nanobiotechnology. This will help students to understand the importance of nano techniques in all domains of biotechnology which will set a benchmark for further research. This eBook will cover topics like nano drug delivery, nano fertilizers, nano bioremediation, nanotoxicology, and nano biosensors to be written by authors who have quality publications in their proposed chapter area. We sincerely hope our efforts will be embraced by students with appreciation and enthusiasm for learning.

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CHAPTER 1

The Roles of Nanoparticles in Ovarian Cancer Treatment and Diagnosis

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Abstract: Ovarian cancer, an aggressive epithelial cancer, remains a major cause of cancer mortality worldwide among women, but it can be diagnosed at an early stage also. Surgical removal of ovarian tumour is a good option for the initial treatment, but this is suitable only at the early stage of cancer. Surgery and other therapies like chemotherapy, hormone role therapy and immunotherapy alone are insufficient for the treatment of today's advanced ovarian cancer. The aim of this book chapter is to review the use of nano-particles in the treatment of ovarian cancer, along with surgery. It is believed that nano therapies have lots of advantages like they stabilize drugs in our body, deliver and penetrate the drugs to tumour-specific cells and can profile the toxicity of chemotherapy. This book chapter also covers the development of nanotherapies, types of nanocarriers and their role in ovarian cancer diagnosis and treatment.

Keywords: Apoptosis, Biomarker, Chemotherapy, Detoxification, Drug cargo, DNA repair, Drug resistance, Graft rejection, Gynaecological cancer, Heterogeneous nature, Hydrophilic corona, Intracellular delivery, M alignancy, Metastatic tumour, Nanocarriers, Nanomaterial, Nanotechnology, Nano transmitter, Photodynamic therapy, Prophylactic, Photo thermal therapy, Renal clearance, Silent Killer, Systematic toxicity.

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Juhi Saxena, Abhijeet Singh and Anupam Jyoti (Eds.)

INTRODUCTION

The most deadliest female reproductive cancer is ovarian cancer [1]. It is the sixth most common malignancy of females worldwide and the second most common malignancy of the female reproductive system. Ovarian cancer is responsible for 4% of all types of malignancies in women and 5% of cancer deaths [2]. Annual incidence rates vary from less than 5 per 1,00,000 in underdeveloped countries like Brazil, India, Thailand *etc.* to greater than 13 per 1,00,000 in developed countries like the United States, Germany, Denmark, Norway *etc.* It is the most common type of gynaecological cancer, ranking the third behind uterine and cervical cancers, and has the greatest incidence of mortality rates. Ovarian tumour pathology is one of the most challenging areas of gynaecology since the ovary produces a wider range and types of tumours than any other organ however; it is a high-grade serous subtype that is frequently misdiagnosed as a systemic disease. Because 75 per cent of Ovarian Cancer is found at an advanced stage, such as stage III or IV, it is also regarded as the "Silent Killer" [3]. The reason of high death rate is due to the fact that tumour grows secretly, and there is lack of appropriate examination to detect the certain stages. It is generally believed that the fatality rate from this type of cancer will surge very high in the following 20 years [4].

Because of the heterogeneous nature of ovarian cancer, prophylactic and early detection strategies have not yet shown effective result. Identifying risk factors and creating protective factors were the main prevention methods of ovarian cancer in the past [5]. But unfortunately these strategies did not greatly reduce the disease's occurrence. Although, surgery is the initial and effective treatment but most of the time, the disease re-occurs due to the aggressive nature of the tumours [6]. And most of the time it is seen that metastatic tumour of the ovary develops a very strong resistance to conventional systemic therapies (like Chemotherapy, targeted therapy and hormone therapy *etc.*). The resistance of cancer cells is caused by a variety of processes, including decreased absorption, increased excretion, drug inactivation and detoxification, and the loss of DNA repair power.

Currently, although many novel ways have been created to increase drug delivery to cancerous cells, nanotechnology has been identified as one of the best therapy methods for overcoming the barriers in advanced cancer treatment [7]. Nanoparticles have the ability to cope up very easily with molecular imaging, carrying drugs to the specific site, treatment, and tumour cell specific destruction. Conventional chemotherapies show very poor systematic toxicity and toxicological effects towards normal and tumour cells. However, nano therapy can be used to manage the cytotoxic effects of healthy cells while also lowering the toxicity of chemotherapeutics [8]. So, there is a hope for an effective treatment

of ovarian cancer with the efficient use of nanocarriers as a solution along with multiple chemotherapeutic drugs.

NANOTECHNOLOGY APPLICATION

Through the knowledge and control of matter at nanometre range, mostly 1 to 100 nm, novel functionalities and qualities of matter can be seen. Employed for a broad array of applications, nanotechnology creates Nano composites, sensors, and processes.

In biology, this technology is called nano biotechnology and in the medical field as nano medicine. The primary goal of nanotechnology in medicine is to improve the efficacy of cancer diagnosis and treatment procedures.

Nanocarriers

Nanocarriers are multifunctional nanomaterials and can be used for the treatment and diagnosis of cancer. Their surface can absorb different types of compounds, such as pharmaceuticals, are absorbed by physical absorption and antibodies by chemical conjugation interactions (Fig. **1**) [9]. Nanocarriers can be classified into several types like micelle, dendrimer, carbon nanotube, liposome, *etc.*

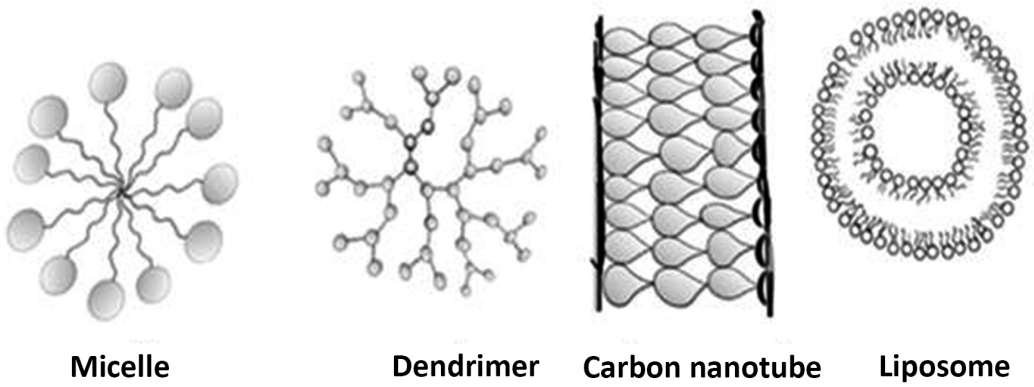

Fig. (1). Examples of some Nanocarriers.

As compared to conventional chemotherapies, Nanocarriers have lots of advantages like delivery of poorly soluble drugs, ability to reduce systematic side effects of chemical treatments, drug stability maintenance by extending their time in bloodstream, and reduced drug resistance by targeting cancer cells [10].

Nanocarriers have the ability to surround the poorly soluble drugs within the hydrophobic interface and can act as carriers for them in blood.

The mechanism behind the regulation of stability of drugs is by prolonging their presence in the bloodstream, protecting them against destabilization, and lowering renal clearance by the Nanocarriers [11].

Liposomes

Liposomes can be distinguished by the existence of two components: an inward hydrophilic component and also an outward hydrophobic component, as well as the presence of a lipid bilayer, which allows them to show multiple properties. Furthermore, they have the ability to change the polarity of these molecules.

Such a structure helps them to grab different types of hydrophobic and hydrophilic medications in liposomes and equip them with different pharmaceuticals.

Their main function is to deliver molecules that can tremendously increase the effectiveness of drugs, even though liposomes are molecules that try to conceal easily again from immune response and can stimulate the cell membrane. This increases the chances of retention of a drug concentration in its desired location for a prolonged period of time, allowing to solubilize poorly soluble therapeutics, and thus helping to mitigate risks of side complexity [12].

Dendrimers

Dendrimers are molecules that are radially symmetric. They have a very well-known morphology, which is a uniform and narrow size distribution structure in the form of tree arms or branches and looks like hyper branched macromolecules with carefully tailored architecture.

They are associated with a high number of functional groups and a molecular structure that is compact [13].

The end knob like structure of the dendrimers can be functionalized and ultimately can change their physicochemical and biological properties and that's why they have gained a vast range of applications in chemistry, particularly in host-guest reactions and self-assembly processes.

Micelles

This type of Nano composite is very important in diagnosis and treatment of tumours. Generally they are spherical in shape with a diameter of 10 and 100 nm. In an aqueous medium, self-assembled amphiphilic block co-polymers of micelles consist of a hydrophobic core and a hydrophilic corona.

Nowadays polymeric micelles are getting a lot of popularity due to their role in drug delivery system. They not only increase the solubility of a particular drug but also enhance the stability of the drug cargo [14].

Carbon Nanotube

Carbon nanotubes are considered a unique type of Nano transmitter because of their structure and property. In comparison to other Nanocarriers, they possess a huge surface area, a big aspect ratio, nonmetric size stability, and numerous chemical functionalities.

These are extensively used to deliver anti-cancer drugs, as well as proteins and DNA, among other things [15].

Carbon nanotubes can be employed as a carrier for both photodynamic therapy as well as photo thermal therapy to destroy cancer cells directly.

DIAGNOSIS AND IMAGING

In recent years, there have been several enhancements and major developments in diagnosis and imaging due to nanotechnology because of integration of technologies like biosensors and updated and improved imaging technologies as well as amalgamation of bioinformatics together with multiplexed assays.

Nowadays by applying diagnostic biomarker in nanoparticle platforms, we can get better contrasting images in devices like X-RAY, magnetic resonance imaging machine, position emission tomography machine, *etc* [16].

Targeted Imaging Agents

Non-invasive techniques cannot image molecules since they are too small. Therefore desired contrasting agents are applied in the desired type of tissue or cellular receptors for better imaging. A site-targeted agent has the ability to give direction to a particular biomarker so that they can differentiate the tissues.

The targeted desired contrasting agent must have the following properties like prolonged duration of their life in blood, highly site specific binding nature, acceptable toxicity profile, also promise for adjunctive therapeutic delivery, *etc* [17].

The core of vertebrate annexin is made up of four identical motifs containing roughly 70 amino acids, forming somewhat a curved circle around a central hydrophilic pore. The use of technetium-labelled annexin to membrane phosphatidyl serine epitopes revealed during apoptosis can be used to detect

cellular apoptosis. Liposomes are often used to identify sclerotic constituents as well as to visualise graft rejection; minute bubbles are used in magnetic resonance imaging and sonography.

Nano-Liposomal Imaging Agents

Liposome has the ability to encapsulate the biomolecules that are hydrophilic in nature, and can increase solubility through lipid bilayers of the cells.

Cholesterol can improve permanence by altering the permeability of the bilayer membrane, inhibiting phospholipid acyl chains from precipitation and causing steric barrier to their movement. Because of the high eliminating agents and poor systemic retention, as well as the rapid removal process from the body, it is important to add a molecule that boosts the imaging efficiency. And these problems can be solved by taking advantage of the EPR (Enhanced permeability and retention) phenomenon seen in tumours by encapsulating the imaging agent in a liposome [18].

FLUORESCENT IMAGES AND GUIDED SURGERY

There is indeed a need to have novel materials to improve the responsiveness, effectiveness, and durability of such imaging systems utilised during surgical treatment. That’s why, there were also numerous fluorescent nanoparticles created, analysed, and adapted for image-assisted surgical treatment. Two great examples are –

CF800 liposomes are commonly applied to encase the iohexol contrasting dye.

Magnetic iron oxide nanoparticles: These are targeting ligand nanoparticles that can be combined with optical magnetic resonance imaging.

NANOPARTICLE THERAPEUTICS (ANTI-CANCER)

Nanoparticles have a direct and target specific anticancer effect as compared to conventional treatments. They are more target specific and active intracellular delivery, but up to a certain extent, these two factors also depend upon the nanoparticles' structure and surface texture (Fig. **2**) [19].

Therapeutic lines like small-molecule drugs, proteins, peptides, nucleic acids and the chemicals that generate nanoparticle are the main components of nanoparticle therapy.

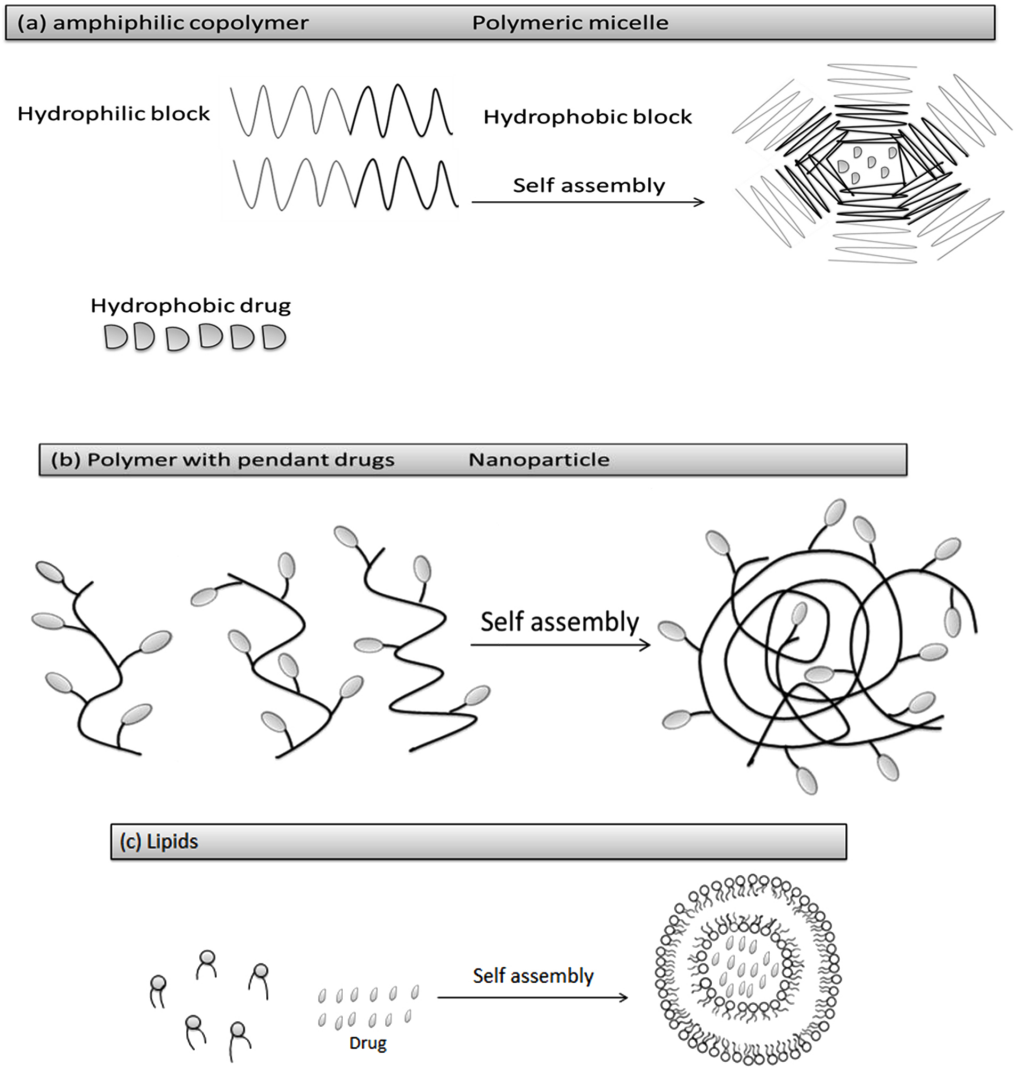

Fig. (2). Some of the most frequently applied nanoparticles in clinical testing **(a)** Nanoparticles containing medicinal ingredients. **(b)** Nanoparticles made of a polymer or a medicine. **(c)** Liposome-containing nanoparticle.

Size of the Nanoparticle

Anticancer nanoparticles are typically 10 to 100 nanometres in size. The glomerular sieving rates of the capillaries of the kidneys are used to calculate this dimension of the nanoparticles. For renal excretion, a threshold size of minimum 10 nm is utmost. On the other hand, vessels in tumour are subject to leak macromolecules, as a result, nanoparticles are unable to circulate in the blood for

long periods of durations and have a high possibility of reaching the blood through malignant tissue blood vessels. Here the size of nanoparticles is higher than six to twelve nanometres; which is also the diameter of the sieve in healthy tissues blood vessels and is blocked from entering and thus not being able to affect the normal tissues.

Cancer cells being specifically targeted by nanoparticles can be filtered through the kidney.

Nanoparticle Surface

Compared to the size of the nanoparticles, they have a very large surface area, and this design appropriately allows them to have easy contact with the molecule and its surroundings.

The nanoparticle's surface area as well as mixing components is exclusively responsible for deciding the nanoparticle's ultimate fate inside the body by regulating the degree of the nanoparticle's contact with its environment.

Surface properties of the nanoparticles also play a significant role. Nanoparticle’s surface having surface charges that are mildly negative or mildly positive, has much less self-self and self-non-self-interactions [20].

CONCLUSION

Nano therapies are far more effective than any other traditional chemotherapy in the detection and treatment of ovarian cancer. They are quite effective because of their potentiality to target a specific tissue and also to examine the living body of animals for adequate durations of time.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 2

Advances in Nano-remediation of Textile Dyes in Textile Industry Effluents: Current Developments and Future Prospects

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Abstract: Environmental clean-up for the removal of recalcitrant pollutants is a global concern, especially in the terms of industrial waste. Research over the years has led to the development of various conventional physicochemical and biological methods for the decontamination of numerous pollutants. These methods however are reported to be extremely expensive and with limited success. Nano-remediation has been reported as an effective alternative in this regard. The chapter outlines the use of various nanoparticles as an innovative and cutting-edge technology for the clean-up of environmental pollutants. It describes the use of fabricated nanoparticles to remove pollutants. The chapter offers an overview of current research developments in the emerging field of nano-remediation with special emphasis on textile dyes, elucidating the mechanisms involved.

Keywords: Adsorption, Environment, Nano-remediation, Textile dyes.

INTRODUCTION

Human activities have been constantly affecting the quality of air, water and soil. Constant inclusion of heavy metals, pesticides, particulate matter, oil spills, toxic gases, fertilizers, dyes and other organic compounds into the environment has become a major threat to the environment [1, 2] leading to the development of

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nanomaterial based remedial technologies for mitigation of toxic effects of these environmental pollutants through various clean-up mechanism [3 - 5].

Owing to the unique properties of the nano-sized materials, nanotechnologies have achieved immense attention during the last decades. Environmental remediation technologies have utilised the property of higher surface-to-volume ratio for nanomaterials in order to bring efficiency to the remediation processes [4, 6]. Apart from this, nanoremediation has also leveraged the surface chemistry of nanomaterials for trapping target-specific pollutant molecules [7]. Apart from surface chemistry, other tuneable physical parameters of nanomaterials such as size, porosity, morphology along with their unique chemical composition aid the process of remediation confirming additional advantages [8, 9]. The afore-mentioned advantages have therefore popularised the use of nanomaterials for the mitigation of environmental pollutants, especially from aqueous sources.

Furthermore, it is important to note that matrices utilised for the purpose of environmental remediation are not pollutants by themselves. In this connection, different biodegradable materials having desired properties along with nano-sized materials are considered more advantageous then using single nano platforms [10]. Such approaches of using nano-composites have been utilised for scaling up the nano-remediation technology by making it more acceptable amongst the consumers due to its greener and safer nature. Moreover, it also enhances the stability and specificity of the clean-up process by eliminating, off-targeting and promoting target-specific removal of contaminants from the wastewater [11]. Therefore, studies have focused on utilising the core principle of nanotechnology by combining physicochemical surface modifications for nano-composites or functional nano-materials for specific removal of a variety of pollutants from aqueous medium.

NANO REMEDIATION: DEFINITIONS AND AGENTS

Nano-remediation has been defined by various authors in different contexts. For instance, Ganie [9] defines Nanoremediationas “an innovative approach for safe and sustainable remediation of persistent organic compounds such as pesticides, chlorinated solvents, brominated or halogenated chemicals, perfluoroalkyl and polyfluoroalkyl substances (PFAS), and heavy metals”. Similarly, Grieger [12] defines it as “nano-remediation is the term used to describe various techniques and methods to clean up contaminated sites using engineered nano-materials”. Nanoremediation has also been defined as “Tiny Objects Solving Huge Environmental Problems” in simpler terms [13]. From the perspective of functionality, Zhang [14] simplies nanotechnology as “the use of small size, high specific surface area, reactivity and versatility of engineered nanomaterials to

potentiate them for the removal of recalcitrant contaminants and achieve selectivity of target contaminants in complex environmental media". Nano remediation has also been defined as "the practice of using various types of nanoparticles such as TiO_2 based NPs, dendrimers, Fe based NPs, Silica and carbon nanomaterials, Graphene based NPs, nanotubes, polymers, micelles, nanomembranes, *etc.* to diminish environmental hazards" [15]. Thus, leveraging the characteristics of nanoparticles such as high surface area to mass ratio, sensitivity, catalytic behaviour and electronic properties for removal/degradation of pollutants is termed nano-remediation.

Numerous nano-sized materials have been developed using different modes of synthesis for the purpose of environmental remediation. However, there seems no such classification of nanomaterial types utilised for nano-remediation. The chapter, therefore, classifies agents of nanoremediation into three major categories namely, polymer-based nanomaterials, inorganic nanomaterials and carbon-based nanomaterials on the basis of literature review.

Carbon-based Nanomaterial

Carbon-based nanomaterials are known for their unique physicochemical and electronic properties. The mutable hybridization property of carbonaceous materials helps to yield different structural configurations of these nanomaterials such as single-walled nanotubes (SWCNTs), multi-walled nanotubes (MWCNTs), *etc*. Earlier investigations have shown the utility of graphene and carbon nanotubes for environmental remediation applications. It has also been reported that surface treatment of these carbon materials helps in improving the efficacy of these materials as they are otherwise ineffective for remediation purposes. The literature demarcates the dominance of single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) owing to their absorption properties for the removal of a variety of pollutants from air as well as large-scale aqueous medium [16 - 19]. In order to enhance the adsorption properties further, researchers have been working on opening the close ends of pristine carbon nanotubes (CNTs) [16, 20]. It has been stated that the open-ended CNTs can typically absorb pollutants in four different available sites based on their adsorption energy. Apart from this, other modifications that have been proposed for improving absorption efficacy is by oxidation of CNT. For instance, post oxidation nitric acid treated CNTs proved to improve their heavy metal adsorption capabilities [21]. Furthermore, physical properties such as temperature, molecular weight, pH, and electric dipole moment also have a huge impact on the adsorption phenomenon by CNTs [20]. Thus, tuning physical parameters has also been employed as a strategy to activate carbonaceous nano-materials.

Apart from this, photocatalytic approaches have also been utilised to remediate contaminants using carbon-based nanoparticles [22]. In this context, graphene has been popularly utilised for fabrication of photolytic nano-composites [23 - 26]. These graphene composites have been amalgamated with TiO_2 for enhancing the photolytic activity of the composites by increasing the conductivity [23]. Table **1** demonstrates the use of carbon-based nano-material for the mitigation of various environmental pollutants.

Table 1. Remediation potential of graphene nanomaterials.

Graphene Nanomaterial	Remediation Potential	References
Graphene oxide nanoparticle	Effective adsorption of H_2S, SOx, NH_3, heavy metals, pharmaceuticals, volatile organic compounds and pesticides	[27, 28 - 31]
CdS-graphene/ZnO-graphene nanocomposites	Photo catalytic degradation of heavy metal like hexavalent chromium	[24]
Pristine graphene nanocomposites	Fluoride Adsorption	[32]
Multiwall carbon nanotube (MWCTs)	Adsorption of zinc	[33]
TiO_2-graphene nanocomposite	Photocatalytic degradation of benzene	[23, 26]
Carbon nanotubes/Al_2O_3 nanocomposite	Adsorption of Fluoride	[34]

Inorganic Nanomaterials

Inorganic nanomaterials used for environmental remediation can conveniently be classified into metal and metal oxide based nanomaterials and silica-based nanomaterials.

Metal and Metal Oxide Based Nanomaterial

Majority of studies indicate the use of metal and metal oxide based nanomaterials for the removal of various contaminants. These nanomaterials are extensively utilised for their adsorption capacity owing to their fast kinetics [35]. These materials are widely used for environmental applications because of their flexibility in both *ex-situ* and *in-situ* applications. Studies have focused on exploring efficient methods of synthesis for attaining shape-controlled, monodispersed, stable metal-based nano particles using physical, chemical and biological approaches [36, 37]. In this context, silver nanoparticles are extremely popular because of their water disinfectant properties owing to their antimicrobial nature [38, 39]. Moreover, silver nanoparticles conjugated with other scaffold materials have also been utilised for improving remediation efficacy [40]. Furthermore, titanium oxide nanoparticles are another popular remediation agent due to their non-toxic, energy converting, semi-conducting, cost-effective, photo-

catalytic properties [41, 42]. They are widely utilised for both air purification and water treatment. Apart from this, iron oxide nanoparticles have been extensively studied for the removal of various contaminants such as heavy metals, dyes, and chlorinated organic solvents [43 - 54]. Some major applications of metal-based nanoparticles in the environmental remediation of contaminants have been stated in Table **2** below.

Table 2. Remediation potential of metal and metal oxide based nanomaterials.

Metal and Metal Oxide Based Nanomaterial	Remediation Potential	References
Ag-doped TiO nanoparticles	Adsorption of 2,4,6-Trichlorophenol	[55]
Cu/Fe/Ag-doped TiO_2 nanoparticles	Photocatalytic reduction of nitrate	[56]
Titanate nanotubes	Catalytic reduction of nitric oxide	[57]
Iron/Iron-oxide nanoparticles	Adsorption of dyes, heavy metals, other organic pollutants	[58 - 64]
Nanosilver-decorated titanium dioxide nanofibers	Photo-degradation of Methylene blue dye	[65]
TiO_2 Nanoparticles	Adsorption of aromatic dyes, phenanthrene, hydrocarbons	[66 - 70]
Bimetallic Nanoparticles	Removal of brominated and chlorinated contaminants from soil and water	[71 - 80]

Silica Nanomaterial

The versatility and porosity of silica material have made it a highly explored option for adsorption applications. Silicanano-materials have been extensively reported for remediation of contaminants in the gaseous phase owing to their large pore volume, high surface area, adjustable surface modifications and pore size [11]. In this context, hydroxyl groups have been extensively explored for surface modification in the silica nano-materials to enhance gas absorption [4]. Apart from this, silica materials with amine groups on their surface have been reported to attribute to higher adsorption of pollutants [81 - 92]. For example, modified silicon nano-materials are popularly used for capturing carbon dioxide from impure air. Table **3** below demonstrates the application of silica nano-materials in the process of environmental remediation.

Table 3. Remediation potential of silica nano-materials.

Silica Nanomaterial	Remediation Potential	References
Amino-functionalized mesoporous silica nanoparticle	Adsorption of heavy metals.	[93 - 98]
Amine-modified xerogelsnanoparticles	Adsorption of gaseous pollutants like hydrogen sulphide and carbon dioxide.	[83]
Amino-functionalized mesoporous silica nanoparticles	Removal of heavy metals from wastewater through adsorption.	[93 - 98]
Carboxylic acid-functionalized mesoporous silica nanoparticle	Adsorptive removal of heavy metals and dyes.	[11, 99, 100]
Thiol-functionalized mesoporous silica nanoparticle	Adsorptive removal of heavy metals from wastewater.	[101 - 104]

Polymer-based Nano-materials

Aggregation of nano-materials has been reported as one of the major concerns for their application in environmental remediation. Aggregation consecutively leads to low stability and non-specificity which ultimately causes a lack of functionality. To address this issue, polymer-based nano-materials constituting a matrix or support holding the nano-materials have been utilised. The polymeric hosts which are often used for this purpose are emulsifiers, surface functionalised ligands, surfactants or stabilising agents [31, 105]. These hosts material not only enhance the stability but also enhance durability, mechanical strength and recyclability of the nano-material. Table **4** below shows various polymer-based nano-materials and their applications for environmental remediation purposes.

It is quite evident that nanoparticles are emerging as a potent tool for removal of a wide range of pollutants, textile dye being one of them. The following section thus focuses on the current developments in nano-remediation of textile dyes with special emphasis on the various studies on varying ranges of NPs and their remedial mechanisms.

Table 4. Remediation potential of polymer -based nano-materials.

Polymer-based Nanomaterial	Remediation Capacity	References
Amine-modified PDLLA-PEG nanoparticles	Target-specific adsorption of gaseous volatile organic contaminants (VOCs).	[4, 106]
Amphiphilic polyurethane nanoparticles	Adsorption of Polynuclear aromatic hydrocarbons from contaminated soil.	[107]
Polyamine-modified Cellulose nanoparticles	Target specific capture of gaseous VOCs	[10]

(Table 4) cont.....

Polymer-based Nanomaterial	Remediation Capacity	References
PAMAM dendrimer nanoparticles	Enhanced ultra-filtration of heavy metal ion of Cu(II) from wastewater.	[108]
Polymer nano-composites	Removal of metal ions like Cr^{6+},Cd^{2+}, Zn^{2+}, Pb^{2+}, $Cu^{2}$and adsorption of dyes.	[109 - 112]

NANO-REMEDIATION OF TEXTILE DYES: CURRENT DEVELOPMENTS

The simultaneous demand for clothing along with the growing population has imposed a huge pressure on the textile industries to produce more and more. This has led to the extensive application of textile dyes which in turn creates a huge demand for large-scale dye production. These dyes are mostly disposed of without treatment into the water bodies creating a serious threat to the environment. It is a well-known fact that, textile industry is a water extensive industry and the dyes used in the textile fibres do not get completely utilised and therefore most of the dyes are washed away during the washing process. This calls for efficient onsite treatment of textile effluents to remove dyes before their discharge into the environment. Many techniques have been explored for the treatment of textile wastewater such as membrane filtration, chemical treatments, *etc.* These methods however require a very high operation and maintenance cost leading to their failure at large scale. In this context, nano-materials are giving promising results both in terms of efficacy and cost-effectiveness. The unique properties of nanoparticles such as high aspect ratio, ordered structure, high surface area, thermal and electrical conductivity, high mechanical strength, and ultra-lightweight feature have led to the superiority of nano-remediation above the conventional physicochemical or biological treatment methods.

Agents and their Efficiency

Researchers have been actively exploring numerous nano-sized particles for their efficacy in the removal of a varying range of dyes. Some of these studies are enlisted in Table **5** below.

Table 5. Efficacy of fabricated nanoparticles and associated mechanisms.

S.No.	Nanoparticles	Name of Dye	Removal Efficacy	Mechanism	References
1	Carbon Nanotube (CNT)	Reactive Blue 4	309.2 mg/g	Adsorption	[113]
2	Carbon Nanotube (CNT)	Direct Blue 53	116.4 mg/g	Adsorption	[114]
3	Fe_3O_4 nanoprticles	Procion red	30.5 mg/g	Adsorption	[115]

(Table 5) cont.....

S.No.	Nanoparticles	Name of Dye	Removal Efficacy	Mechanism	References
4	Silver nanoparticles	Methylene blue	95%	Photocatalytic degradation	[116]
5	γ-FeOOH nanoparticles	Reactive orange 29	36.30%	Adsorption	[117]
6	TiO_2 nanoparticles	Malachite Green	65%	Photocatalytic degradation	[118]
7	Zinc oxide nanoparticles	Methyl orange dye	84%	Sonophotocatalytic degradation	[119]
8	Carbon Nanotube (CNT)	Methylene Blue	6.96 mg/g	Adsorption	[120]
9	MgO nanoparticles	Acid Red 73	100%	Photocatalytic degradation	[121]
10	Carbon Nanotube (CNT)	Blue 116	34.4 mg/g	Adsorption	[122]
11	Carbon Nanotube (CNT)	Yellow 81	35.1 mg/g	Adsorption	[122]
12	Graphene and Carbon nanotubes	Basic Red 46	60 mg/L	Adsorption	[123]
13	Amorphous iron nanoparticles	Congo Red	1735 mg g−1	Adsorption	[124]
14	Multi-Walled Carbon Nanotubes (MWCNT)	Alizarin yellow R	884.80 mg/g	Adsorption	[125]
15	Titanium dioxide nanoparticles	Malachite Green	0.6 g/L	Photocatalyticdegradation	[126]
16	Chitosan-Fe_3O_4 nanoparticle	Evans Blue	99%	Adsorption	[127]
17	Activated Carbon with loaded Ca-Fe_3O_4 nanoparticles	Methylene Blue	138 mg/g	Adsorption	[128]
18	Polyethyleneimine functionalized magnetic carbon nanotubes	Alizarin Red S	196.08 mg/g	Adsorption	[129]
19	Silver nanoparticles	tartrazine	28%	Reduction	[130]
		carmoisine	45%	Reduction	
		brilliant blue FCF	38%	Reduction	
20	Zero-valent iron nanoparticles	reactive black 5	99.60%	Reduction	[131]
21	Magnetic cobalt oxide nanoparticles	Malachite Green	238.10 mg/g	Adsoption	[132]
22	Multi-Walled Carbon Nanotubes (MWCNT)	Ismate Violet 2R	76.92 mg/g	Adsorption	[133]

(Table 5) cont.....

S.No.	Nanoparticles	Name of Dye	Removal Efficacy	Mechanism	References
23	Graphene oxide nanoparticle	Methylene blue	276.5 mg/g	Adsorption	[134]
		Methyl orange	423.15 mg/g	Adsorption	
24	Silver nano-composites	Organic dye	93.23%	Photocatalytic degradation	[135]
25	Cu-Ni Bimetallic Nanoparticles	Methylene Blue	0.086 mM	Reduction	[136]
26	CuO nanoparticle	Methylene Blue	100%	Reduction	[137]
27	CeO_2 nanoparticles	rose bengal dye	96%	Photocatalytic degradation	[138]
28	Magnetic Fe_3O_4 nanoparticles	Acid Green 25	0.5 mg/mL	adsorption & oxidative degradation	[139]
29	Graphene Oxide/Titanium Dioxide Nanoparticles	FD&C Red 40	55.23 mg/l	Adsorption	[140]
30	Zero-Valent Copper Nanoparticles	Mixture of Rhodamine B, Bromocresol Green, Methyl Orange, and Eriochrome Black T	90%	Adsorption	[141]

Mechanism

The core of nanotechnology application is based on downsizing the particles into nano-scale in order to enhance the surface-to-volume ratio which in turn improves magnetic, catalytic, optical electrical as well as chemical properties. The particle size is the major attribute for functionality of the nanoparticles [142]. Even in case of remediation, studies have shown that reduction of particle size to nano-scale influences characteristics like surface free energy, latent symmetry, stress and many other physiochemical parameters [143]. In this context, metal nanoparticles show unique absorptive capabilities due to their physic-chemical properties attributed by their edge surface size and highly dense corners. The small particle size leads to an increment in the number of surfaces which in turn causes interface atoms to create stress or strain. This leads to thermodynamic changes, which ultimately influence the structural attribute of nanoparticles. The diverse group of metallic oxide nanoparticles have also been popularly explored for nano-remediation purposes because of their highly tuneable surfaces that ultimately lead to higher absorption capacity. Apart from this, metal oxide nanoparticles are cost-effective as well as more efficient than core metallic nanoparticles in the adsorption of [151] pollutants. Many studies have reported the use of iron-oxide

nanomaterials for the absorption of dyes from aqueous phase [144 - 149]. Thus, metal and metal oxide nanoparticles act as effective nanosorbents for the removal of dyes. Similarly, carbon-based nanomaterials also are known to have adsorption sites on their surfaces. Major interactions of dyes with carbon-based nanomaterials are reported to be electrostatic interactions [150], van derwall forces [151], π -π staking interactions [152], hydrophobic interactions, *etc.*

Reduction has also been explored as a mechanism in the nano-remediation processes. Magnetic nanoparticles are preferred due to their low cost, magnetic susceptibility, low toxicity, high coerciveness and low curie temperature [153 - 155]. In this context, many methods for the fabrication of magnetic nano particles have been explored such as water in oil micro emulsion, co-precipitation, hydrothermal methods, *etc* [143]. It is worth noting that, different methods produce particles with different size distribution, shapes, dispersibility, and magnetic properties [24, 156]. These nanoparticles act as reducing agents to reduce recalcitrant dye structures. Gold nanoparticles are also effective reducing agents. However, the catalytic activity of gold nanoparticles depends largely on the charge, shape, particle size and distribution [157]. Many researchers have shown enhancement in the absorptive capacity of gold nanoparticles by using adjuvant like activated carbon as it acts as a redox mediator [158].

Furthermore, photo catalysis has also been explored to accelerate dye degradation on exposure to light. Metallic oxide nanoparticles such as zinc oxide and titanium oxide nanoparticles show such activities in the presence of light above its band gap which leads to the production of electron-hole pairs. They further react with an aqueous solution of dye to produce oxidising and reducing radicals such as superoxides. These radicals in turn accelerate some secondary reactions which ultimately disrupt the dyes. As these semiconductors are nano-structured, they have a high surface area which leads to high photocatalytic activity [159]. Many researchers have reported the photocatalytic activity of carbon nanotubes as a potential new edge technology for the degradation of recalcitrant azo dyes [160, 161]. Apart from this, zinc oxide and iron oxide nanoparticles have also been reported to be an effective photodegradation agent for textile dyes [162 - 166]. Other than this, copper-based nanoparticles have also been reported to drive thermodynamically favourable electron transfer reactions in the presence of light. Copper sulfide nanocomposites and zero-valent copper nanoparticles are effective photodegradation agents for a range of azo dyes [167 - 169]. Similarly, cadmium sulphide nanoparticle also acts as a photocatalytic agent for the treatment of dye-rich industrial wastewater when subjected to light above its bandgap [170 - 172].

SCALE UP OF NANOREMEDIATION: CHALLENGES & FUTURE PROSPECTS

Despite numerous advantages of the nanoremediation process, there have been ample gaps from synthesis to application of nanoparticles for environmental clean-up at a larger scale [35]. The chapter consolidates various gaps in the scale-up process and categorizes it into three major challenges.

Lack of Synchronisation between Stability, Activity and Selectivity

Most of the studies that have reported a higher absorptive or catalytic potential of nanoparticles have also simultaneously reported stability and selectivity issues. This is in line with the fact that, highly reactive nanoparticles have extremely vulnerable surface configuration and microstructure. This makes the nanoparticles become unstable when applied in harsh environmental conditions. The stability is thus an instrumental driver for the feasibility of nanoparticles for their use in practical situations [173, 174]. Many studies have thereby focused on integrating rational designs for optimization of nanoparticle synthesis in order to obtain extremely stabilised nanoparticles with a synchronisation between stability, activity and selectivity [149, 175 - 177]. This also calls for further integration of chemical engineering efforts in order to bring stringent control.

Toxicity of Nanoparticles

The toxicity of nanoparticles remains another challenge in the scaling up of nano-remediation technique. Nano-materials are often reported to interact with biological surfaces through bio interfaces leading to the production of various by-products which are more toxic in nature. The fate of nanoparticles in the environment is often described through various interactions such as dissolution, sulphidation, biosorption, sedimentation/deposition, and persistence [178 - 181]. Most of these interactions pose an inherent threat to nature by causing passive toxicity due to aggregation and agglomeration caused by electrostatic and steric stabilization [182]. For instance, sulfidation increases the toxicity of nanoparticles against microorganisms [176]. Similarly, biosorption also leads to the adsorption of nanoparticles and their aggregation on soil colloid which ultimately leads to its entry into plants [183]. Furthermore, absorption, sedimentation and aggregation of nanoparticles in the environment ultimately lead to the persistence of nanoparticles in the environment. This, therefore, calls for toxicity checks before using nanoparticles/composites for large-scale nano-remediation purposes.

Lack of Optimised Scale up Efforts

Despite lab scale remediation studies, very fewer efforts have been made to scale

up nano-remediation to field scale. Optimisation remains the priority in scale-up. Most of the studies depict the optimisation of process parameters only in the aqueous medium and not field samples. However, variation in effluent samples due to varying sub-surface conditions like pH, temperature or the hydrogeology of the water body highly impacts the remediation conditions. Also, the variation in the chemical constituents in the effluent due to the inclusion of other chemicals like fixatives and mordants also influence the efficiency of the nano-remediation agent in real field conditions. Thus, there is an urgent requirement to scale up lab proven nano-remediation agents to pilot scale applications for field scale success.

CONCLUSION

Nano-remediation is an emerging technology used for the removal of recalcitrant chemicals from the environment. Many nano-remediation agents have been fabricated using physical, chemical and green pathways to remediate numerous pollutants like heavy metals, dyes, hydrocarbons, and gaseous pollutants. These agents could be broadly bifurcated into polymer-based nanomaterials, inorganic nanomaterials and carbon-based nanomaterials. Remediation of dyes using these agents has been widely explored. Many azo and non-azo dyes have been successfully removed from aqueous medium using a varying range of nanoparticles and nano-composites. Moreover, engineered nanoparticles with surface modifications, size, shape and nano-structure variations have been explored for enhancing the dye removal efficacy of the nanoparticles. Adsorption, photocatalysis, and reduction are the dominant mechanisms behind nanoremediation which have been optimised for improving remedial abilities further.

At the same time, there are a number of issues prevailing with large-scale usage of nanomaterials for environmental remediation. It is quite evident from the literature that nano-remediation is scarcely tested at the field scale. This thereby points towards the utility of the method for its application in contaminated sites. Pilot scale studies are required. In-depth knowledge of the physicochemical properties of the nanomaterials such as size, shape, charge, bulk composition, surface chemistry and toxicological studies of the nanoparticles is also essential to understand and use nano-remediation as a scalable method for clean-up of environmental pollution of textile dyes in effluents.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 3

Interaction between Metal Oxide Nanoparticles and Terrestrial Plants: An Overview of the Mode of Action and Future Perspectives

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Abstract: Nanotechnological interventions have extensively been used as an efficient non-invasive approach in agriculture for disease protection, to improve yield and many more. The use of engineered nanomaterials (like metal-oxide nanoparticles) as fertilizers,pesticides, carriers for genetic material/RNA/protein, sensors for detection of contaminants and toxic compounds *etc.* have been extensively studied and reported. Interaction between plants and nanomaterials plays an important role in their applications for various purposes in agriculture and otherwise. In this chapter, mechanisms of uptake and mode of action of three commonly used metal oxide (TiO_2, CuO, ZnO) nanomaterials in plants have been reviewed. The chapter also summarises the various studies conducted on the effect of these nanomaterials on different agricultural food crops in the last 2 decades. The thorough review of existing literature on the aforementioned areas indicates that although the published data on terrestrial phytotoxicity of metal oxide NPs is increasing continuously but surprisingly the range of selected plants is still narrow (mostly agricultural crops and seed plants), thus random selection of plants (outside this narrow range) should be made to gain better insights into the various impacts of nanomaterials on plants.

Keywords: CuO, Mode of action, Phytotoxicity, TiO_2, Uptake mechanisms, ZnO.

INTRODUCTION

Nanotechnology has been identified as one of the most promising and revolutionary technologies, which is going to affect people's life. Now developing

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countries have started investing more in nanotechnology by considering the potential of NPs to overcome the challenges associated with development in key areas such as energy, water, agriculture, health and environment [1]. Therefore, the production of nanomaterials (NMs) has escalated in recent years due to their multifaceted utilities. The estimated global production of engineered nanomaterials (ENMs) in the year 2010 was 260,000 - 309,000 metric tons; out of which approx. 63-91%, 8 - 28%, 0.4 - 7%, and 0.1 - 1.5% were estimated to end up into landfills, soils, water bodies and atmosphere respectively [2]. The most common contaminating ENPs of the environment are carbonaceous nanoparticles (NPs), quantum dots, zero-valent metals, metal oxides and nanopolymers [3]. Certain exceptional properties of NPs such as high specific surface area, abundant surface reactive sites and mobility are greatly affecting the environment and health as well [4, 5]. Organisms especially algae, fungi and plants have direct interaction not only with NMs but also with their existing environment thus may be considered as the first target life forms to be exposed which are indirectly affecting higher species through the food chain [6]. Although, the evolution of plants took place in the presence of natural NPs, but because of expanded production of ENPs and their use in diverse processes and goods; the possibility of plant exposure has increased incalculably [7].

With these points of view, in this chapter, the interaction of NPs and plants has been discussed. As the most encountered group of NPs is metal oxides that are being produced largely for enormous applications, this chapter focuses on 3 leading candidates in line *viz*. TiO_2 CuO and ZnO for the study of interaction with plants.

Titanium Dioxide NPs (TiO_2 NPs)

Certain properties of TiO_2 NPs such as high stability, anticorrosion and photo-catalyst activity make them an excellent candidate to be used in cosmetic and skin care products, antibacterial and air cleaning articles; paints and pigments; and in organic matter decomposition in wastewater. In the year 2010, 64,000 - 81,000 metric tons of ENMs were used in coatings, paints, pigments and cosmetics, with approx. 34,000 and 10,000 tons/year for TiO_2 and SiO_2 NPs respectively. The above-mentioned applications contribute around 42% of the total global ENM flow, 82 - 87% of total ENM emissions to soil and 89 - 97% to water [2].

Copper Oxide NPs (CuO NPs)

Due to manifold uses of CuO NPs, they serve as potent NPs to enter the most important and sustaining environmental compartment *i.e.* soil [8, 9] and, therefore, catching the attention for numerous bio-toxicity studies [10 - 12]. These NPs are owing antimicrobial nature thus predominantly being used in

antimicrobial formulations [13]. Plentiful literature is available on the protection of wood products from fungi and insect-induced biodegradation using nano-CuO and nano-$CuCO_3$-based biocides [14]. 50% of the global wood preservation is occupied by the wood preservation market of North America with 79000 tons consumption of Cu salts annually [14, 15]. In the year 2010, the predicted world-wide production of Cu-based NPs was ~ 200 tons/year, and it is increasing continuously [2].

Zinc Oxide NPs (ZnO NPs)

In 2010, the estimated global annual production of ZnO NPs was 30,000 metric tons, which was used primarily in paints, medicine, cosmetics, optics, electronics, coatings and pigment products. Emission at the time of manufacturing was estimated to be around 32–680 tons/year, from which the highest amount is being contributed by emissions from the use of ZnO ENMs in cosmetics. Overall predicted emissions are 90–578 tons/year to atmosphere, 3,100–9,283 tons/year to soils and 170–2,985 tons/year to receiving water bodies [2].

Thus, the inevitable rapid use of metallic NPs in multiple areas have raised the demand for assessment of their impact on different biotic and abiotic components [16 - 18]. Scanty reports are available about the impact of NMs on food crops and the food chain [19, 20]. As plants are in direct contact with the environment and are first targets to face NPs, thus increasing the curiosity to know the way NPs affect plants, the method of their uptake and the way they act in plant systems (Fig. **1**). The focal point of this chapter is to discuss all these aspects of interactions of MONPs and plant systems.

UPTAKE AND TRANSLOCATION OF MO NPS IN PLANT SYSTEM

TiO_2 NPs

The worldwide production of titanium dioxide (TiO_2) NPs is up to 2 million tons/year [21], which eventually contaminate soils and plants on its release in the environment. In Arabidopsis thaliana, the uptake and translocation of nano-conjugate of an ultra-small TiO_2 (<5nm) complexed with Alizarin red were studied by Kurepa *et al.* (2010) [22]. They demonstrated that the inhibition or facilitation of entry of nano-conjugate depends on the pectin hydrogel capsule formed by the mucilage that was released from the surrounding roots. Numerous other studies show that depending on the plant species, toxic heavy metals in the rhizosphere could either be accumulated or inactivated by the polysaccharides present in the mucilage [23]. Asli and Neumann (2009) [24] investigated that in maize (Zea mays), TiO_2 NPs were not taken up by the root cells of excised roots with intact apices, the probable reason for this might be their large size compared to the size of pore diameter (6.6 nm). Servin *et al.* (2012) [25], evaluated the

uptake of TiO_2 in cucumber (*Cucumis sativus*) grown in hydroponic solution by using the micro X-ray fluorescence (micro-XRF) and micro X-ray absorption spectroscopy (micro- XANES) to track the presence and chemical speciation of Ti respectively, within the plant tissue. By micro-XRF, it was observed that Ti got transported from roots to the leaf trichomes, thereby, suggesting trichomes as the possible sink for Ti. Whereas the micro-XANES spectra showed the presence of TiO_2 within the cucumber tissues, hence, testifying that TiO_2 NPs were not biotransformed. Larue *et al.* (2012b) [21], treated hydroponically grown wheat and rapeseed plantlets to anatase TiO_2 -NP, either through root or leaf exposure. The quantification of absorbed Ti was done using Microparticle-induced x-ray emission (μPIXE) coupled with Rutherford backscattering spectroscopy (RBS) and accumulation upon leaf and root exposure was determined using Micro x-ray fluorescence (μXRF) based on synchrotron radiation. Larue *et al.* (2012a) [26], also investigated root-to-shoot translocation of anatase and rutile TiO_2-NPs along with root accumulation in wheat, where mapping, observation and quantification of Ti in plant tissues were done using synchrotron-radiation micro-X-ray fluorescence, transmission electron microscopy and micro-particle-induced X-ray emission respectively. Their results presented threshold diameters of 140 and 36 nm; above which in case of former diameter, NPs no longer accumulated in roots and in the latter NPs, accumulation took place in wheat root parenchyma but did not reach the stele and consequently did not translocate to shoot. Conclusively, the tested smallest TiO_2 NPs accumulate in roots in a limited amount and distribute to all plant tissues without dissolution or crystal phase modification. In another study, harvested fruits of cucumber cultivated in TiO_2 NPs treated sandy loam soil were evaluated using synchrotron μ-XRF, μ-XANES and ICP-OES to study macromolecule modification of cucumber fruit. This is the first report in which, μ-XRF and μ-XANES showed that TiO_2 got translocated without biotransformation from root-to-fruit in cucumber indicating that, TiO_2 could gain entry into the food chain with unrecognizable consequences. Jacob *et al.* (2013) [27], found that Ti originating from TiO_2 NPs is available for plant uptake. Soil grown tomato plants were investigated by Antisari *et al.* (2015) [28], and uptake and accumulation were studied by ICP-OES and the results showed NP component metal accumulation in roots. Electron microscopy (ESEM-EDS) detected NPs in tissues of tomato roots, suggesting the probability that translocation of NPs might be associated with the absorption patterns of water and nutrients within the root. Lack of reports on the uptake of TiO_2 NPs might be due to their high stability; treated plant samples are difficult to digest [29].

Conclusively, upon treatment with TiO_2 NPs, the uptaken entities, whether the metal component of NP or NPs itself, can accumulate and translocate in the whole plant body without biotransformation, as there are very few reports on biotransformation till now.

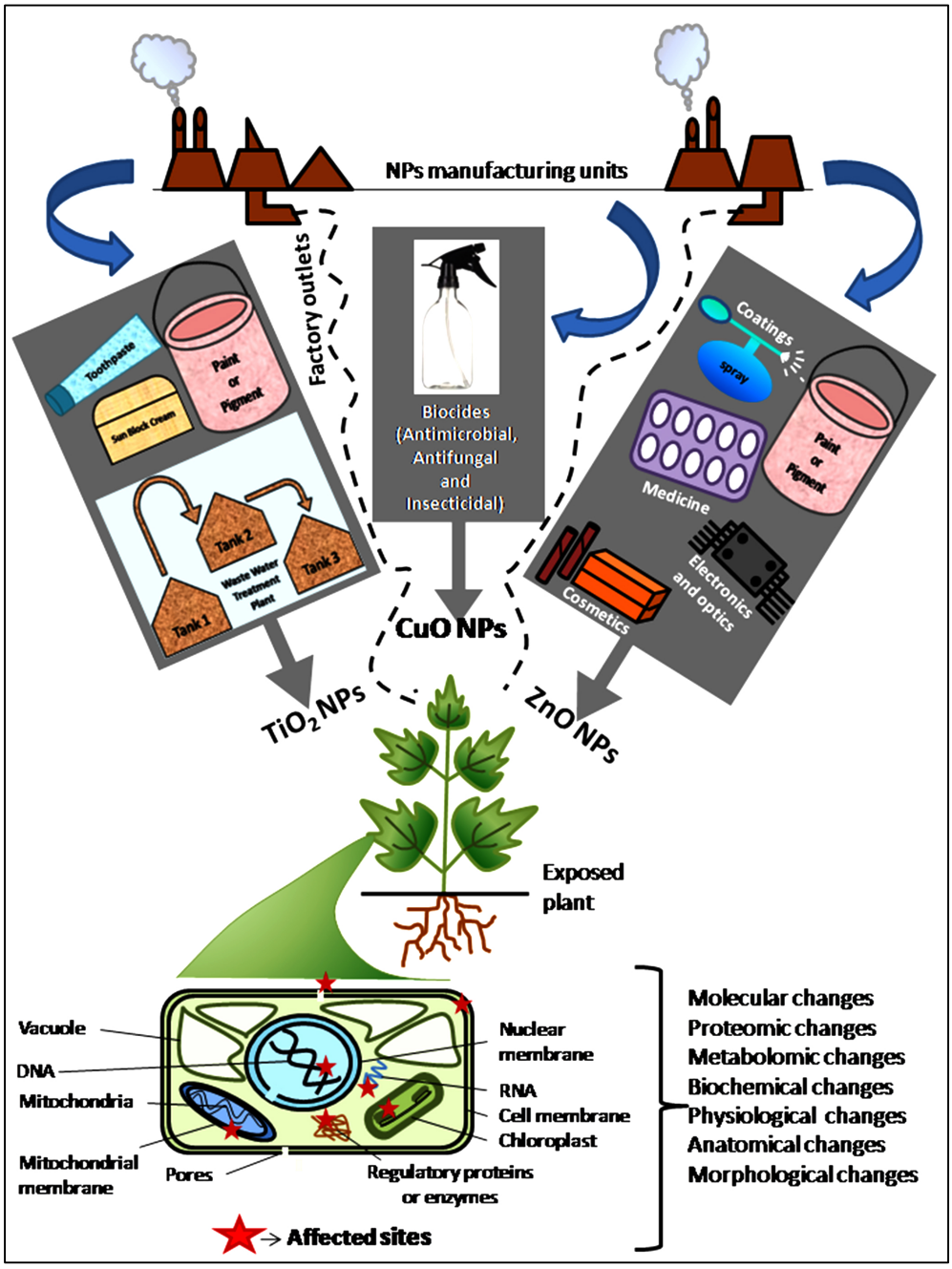

Fig. (1). Shows diverse sources of release of TiO_2, CuO and ZnO NPs in the environment and in turn their impact on various parameters of plant system.

CuO NPs

Before 2011, plants were not explored in terms of uptake, translocation and impact for CuO NPs. On wheat roots, methods were standardized by Zhou *et al.* (2011) [30] to differentiate the adsorption and uptake of CuO NPs by using various metal competing ions, surfactants, complexing agents such as NaOAc and Na_4EDTA and ultrasonic procedure. Upon treatment with CuO NPs, most of these were found adsorbed on root surface while some were mechanically adhered. It was observed that desorption of CuO NPs was not possible using competing ions but NaOAc and Na_4EDTA were quite effective in doing so. They observed that the uptake increased with increasing exposure concentrations, whereas absorption/adsorption ratio increased initially but then decreased. They reported that, at low concentrations of NPs, the adsorbed NPs at the plant root surface made their way into the cell easily but at the higher concentrations, only part of the absorbed NPs were transferred into cells. Toxicity, transport and redistribution of CuO NPs (20-40nm) on maize (*Zea mays* L.) were examined by Wang *et al.* (2012) [31], using TEM and EDS. They proved that the transportation of CuO NPs occurs from roots to shoots through xylem by locating them in xylem sap. They further authenticated the translocation of these NPs back from shoots to the roots through phloem by split-root experiments and high-resolution TEM. They affirmed the bioaccumulation and biotransformation of CuO NPs while translocation, as these could be reduced from Cu (II) to Cu (I). In a study conducted by Kim *et al.* (2012), TEM images confirmed the presence of CuO NPs in the endodermis [32]. It was observed that NPs form clusters either with themselves or with other cellular materials after penetrating the cell membrane. Also, the metal oxide NPs' deposition on the root surface hindering the uptake of available Cu. A probable explanation of CuO phytotoxicity is the accumulation of toxic Cu ions released from CuO NPs as suggested by Landa *et al.* (2016) [33]. These observations were also confirmed by Le Van *et al.* (2016), by studying the effects of CuO NPs on conventional and Bt-transgenic cotton [34]. They concluded that CuO NPs distinctly reduced the uptake of nutrients such as B, Mo, Mn, Zn, Mg and Fe. Also, at low concentrations of CuO NPs, growth and development of both transgenic and conventional cotton were hindered and there was an upregulation of Bt toxin protein of Bt-transgenic cotton. The presence of CuO NPs in TEM images was seen in conventional cotton to be aggregated on the epidermis while in transgenic cotton it was seen in the cells.

Conclusively as root is the first exposed organ if CuO NPs are provided through the soil, it can be worthwhile to target future studies on it.

ZnO NPs

Almost all studies on ZnO NPs have been carried out till the germination stage, which does not give an insight into uptake, translocation and accumulation of ZnO NPs due to lack of plant root and vasculature development [29]. In ryegrass, SEM and TEM images displayed that ZnO NPs were firmly bounded onto the root surface and some particles were seen in the apoplast and protoplast of the root endodermis and stele. Results also indicated little or no ZnO NPs were transported from the root to the shoot, as a translocation factor (TF < 0.02) was very low [35]. The probable explanation for this could be the agglomeration of NPs at high concentration which obstructed ZnO NPs uptake. The XAS spectra (x-ray absorption spectroscopy) of ZnO NPs treated roots showed that, in tissues, Zinc was present either in the form of Zn-nitrate or Zn-acetate *i.e.* in Zn+2 oxidation state instead of ZnO NPs. A study conducted by Hernandez-Viezcas *et al.* (2013), on Soybean seeds, germinated and grown in soil treated with ZnO NPs revealed that at the harvesting stage, ZnO NPs were not present as displayed by the X-ray absorption spectroscopy [36]. But the data of μ- XANES analysis surprisingly showed the presence of O-bound Zn resembling Zn citrate, which might be a component of Soyabean grain. In a recent investigation, on treating Maize plants with ZnO NPs, it was observed that NPs were not translocated to shoots; most of these were restricted to epidermis, a small fraction reached till the root tip cells and cortex, and some took the route to the vascular system *via* sites of the primary root–lateral root junction. Researchers found that zinc was agglomerated in the form of zinc phosphate out of which, the uptake of maximum amount of Zn was in the form of Zn^{2+} released from ZnO NPs [37].

As there are very few reports available, intending to explore the uptake, translocation and biotransformation of ZnO NPs in plants; those which are present are contradictory and not sufficient to make any conclusion. This situation creates a clear vision that more elaborative studies are required, to confirm the uptake, translocation and biotransformation of these metal oxide NPs for controlled application and treatment of damage done by them to the plants systems.

MODE OF ACTION OF MO NPS

Now, it is apparent that the physical and chemical characteristics of NPs (*i.e.* average size, element composition, surface area, dosage, porosity, surface charge, hydrodynamic diameter, aggregation, stability and coating with cellular or other constituents) and choice of crop have a direct relevance with toxicity in biological systems. In addition, life cycle stage, growth media and diluting agents may also influence toxicity in plants [38]. It is worth noting that due to the high surface area to volume ratio of metal NPs, they have more surface area to exchange their

valence electrons with biomolecules [39]. Due to which, metal NPs have the capability to take part in cellular redox reactions, leading to the altered antioxidant status of the treated plants [40]. The way in which plant growth is affected is still a topic of major concern for researchers and various efforts towards examining various crucial processes that work in plant systems are underway [41 - 43].

TiO_2 NPs

Initial investigations with TiO_2 NPs and plants were conducted by Hong *et al.* (2005) and Yang *et al.* (2007), concluding that upon treatment with Nano-anatase TiO_2, an improvement was observed in the absorption of light energy with its transformation into chemical and electrical energy, and induction of CO_2 assimilation and protection of chloroplast ageing during illumination for a long time [41, 43]. Moreover, it was also observed that the activity of non-cyclic photophosphorylation was much greater than that of cyclic photophosphorylation. It was envisaged that there is a possibility of entry of TiO_2 NPs in chloroplast and increase in the oxidation–reduction reactions of electron transport and oxygen evolution [41]. Whereas, Gao *et al.* (2006), concluded that there is an enhancement in the photosynthetic carbon assimilation when treated with Nano-anatase TiO_2 [42]. Thereby, activating Rubisco (complex of Rubisco and Rubisco activase) that might lead to carboxylation of Rubisco, and ultimately enhancing plant growth. These results were further confirmed using a molecular approach by Linglan *et al.* (2008), who suggested that anatase TiO_2 NP induces marker gene for Rubisco activase (rca) mRNA, resulting in an increased level of protein and activities of Rubisco activase [44]. According to Lei *et al.* (2007), anatase TiO_2 NPs promoted certain activities in chlorophyll such as photophosphorylation and evolution of oxygen under both UV and Visible light [45]. Anatase TiO_2 also enhanced the activity of the whole electron transport chain along with photo-reduction activity of photosystem II. Some additional supportive observations were also reported where TiO_2 NPs improve rate of transpiration, water conductance [46], plant growth and grain yield [47]. The mode of action of TiO_2 NPs, such as enhancing nitrogen assimilation, photo-reduction activities of photosystem II and electron transport chain and protecting the chloroplast membrane structure from reactive oxygen species, make these NPs promising as an efficient nutrient source for crops to improve biomass production [47]. But Servin *et al.* (2013), suggested that NPs have a promontory effect on nitrogen accumulation and thus protein formation [48]. It has been recognized that plants under optimum conditions tend to increase the duration of their development as much as possible, leading to improved leaf photosynthesis, light-use potential and higher yield [49]. Raliya *et al.* (2015), concluded that nano-TiO_2 also promotes microbial activities which can improve the utilization of native nutrients by plants [50]. On the contrary, some studies reported the occurrence of genotoxicity (DNA

damage) by TiO_2 NPs in plants [51, 52], whereas, Wang *et al.* (2011), found the microtubule disrupting nature of TiO_2 NPs while working with *A. thaliana* [53]. Among both the forms of TiO_2 NPs (anatase and rutile), it seems the rutile form has less toxicity than the anatase form, because of its lipophilicity, as rutile crystalline structure of NPs forms larger aggregates in the aqueous medium [54].

CuO NPs

The antibacterial activity of Cu based NPs is well documented, but the information underlying the mode of action of these NPs with reference to plants is comparatively limited. For instance, Adams *et al.* (2017) reported that Cu ions in the rhizosphere are released from the dissolution of the CuO NPs, due to the secretion of proteins [55] from root cells which chelated the released metal ions, which caused root shortening but promoted root hair proliferation *via* nitric oxide cell signaling and modified IAA distribution. Whereas, in another study, the reduction in hydraulic conductivity in roots was reported due to CuO NP treatment resulting in reduced water uptake, hence decreased root and shoot biomass [31]. DNA damage by CuO NPs in some agricultural and grassland plants was reported by Atha *et al.* (2012) and suggested the accumulation of oxidatively modified compounds which caused mutagenic DNA lesions, thereby, inhibiting plant growth [56]. As reported by many researchers, reactive oxygen species (ROS) stress is contemplated as the prime reason for obtained results.

ZnO NPs

Amongst all the three MO NPs focused in the present study, ZnO NPs are the most explored ones. As mentioned earlier, the well-identified reason for phytotoxicity is the ROS generation [57], which is perhaps generated by Zn ions formed by the dissolution of ZnO NPs. Not only plants, but enzyme activities of the contaminated soils may also be affected as heavy metals probably react with sulfhydryl groups, forming metal–sulfide equivalents, thereby inactivating or inhibiting enzyme activity [58]. It was suggested by Ghodake *et al.* (2011) that probably ZnO NPs penetrated radically into onion roots in turn damaging the whole cellular metabolism and stages of cell division, blocked the growth stages and showed severe hazardous effects at both cellular and chromosomal levels [59]. The accumulation and biotransformation of the MO NPs or the released metal from them are reported in various studies. Dimkpa *et al.* (2013) [10] stated that greater root toxicity may be correlated with a smaller particle size. The production of Phytochelatins, which are considered as the indicators of heavy metal toxicity in plants might occur in the presence of ZnO NPs or their dissociated Zn ions [36].

In spite of a number of reports for phytotoxicity, certain reports are available stating the positive mode of action of ZnO NPs. After treating with ZnO NPs, improvement in plant growth might be associated with the generation of fewer amounts of ROS causing less lipid peroxidation [60]. Since, Zn is a constituent of enzyme which influences the secretion of IAA, ZnO NPs showed a very positive response in seed germination, along with root growth, which may be accomplished due to oxygen vacancies in ZnO NPs, leading to increment in the level of IAA [61]. ZnO NPs also improved nitrogen assimilation soybean [62].

Initially, the modes of action of the MO NPs were not understood, but now, researchers are investigating un- or underexplored aspects very intensely, by using various high throughput machinery and protocols as evidenced by all the above-mentioned examples.

IMPACT OF MO NPS ON PLANT SYSTEM

Plant- NPs interaction is a nascent field that requires intensive research. Published data so far are focused on a particular group of plants, like in Angiosperms among terrestrial plants, whereas Gymnosperms are completely ignored worldwide. Probably the main reason of this ignorance is the complex life cycle of Gymnosperms and also the easy yardsticks of growth and development in Angiosperms. As the main concern of researchers is the accumulation of NPs in food web so all studies revolve around a very narrow range of crops (*e.g.* wheat, maize, rice, lettuce, cucumber, soybean etc.). The studies on the whole life cycle of plants are limited and recently this subject has caught attention. There is a complete lack of studies on ornamental and medicinal plants along with plants which are growing under severe environmental conditions like xerophytic plants. Fig. (**2**) represents the information about studies done so far with special context to terrestrial plants against discussed MO NPs (*i.e.* TiO_2, CuO, ZnO NPs).

Literature on the effects of major mineral elements on growth, development and biochemical aspects is well documented but nano-sized particles of these metals like (metal NPs and MO NPs) have just started making impact. Tables **1** - **3**, provide the summarized information about experimental design, results, possible hypothesis or expected mechanism of peer reviewed research articles. To emphasize on recent trends, only 2010 onward publications are included.

Table 1. Summary of the important studies (after year 2010) on terrestrial plant species exposed against TiO_2 NPs.

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studies endpoints/ parameters	Remarkable effects	Expected mechanism/possible hypothesis	References
1.	7-40 nm, Anatase	5 mg/L suspensions in DDW	NPs suspensions sprayed twice on 12^{th} and 16^{th} d foliarly	*Cicer arietinum*	Electrolyte leakage index (ELI), cDNA-AFLP analysis, Quantitative reverse-transcriptase polymerase chain reaction (qPCR) analysis, Gene ontology (GO) annotation, Enrichment analysis of GO terms, Functional annotation and network analysis	Number of generated transcript-derived fragments (TDFs) - 4200, Among them, 100 (~2.62%) expressed differentially, Differentially expressed TDFs of NPs-treated plants cloned – 60 (During cold stress), 10 of them formed readable sequences. These genes identified for- chromatin architecture, transcriptional regulation, metabolism pathways, cell connections and signaling and cellular defense, 2 out of 10 TDFs- unidentified genes, homology absent with known genes, Transcription level of these TDFs ↑	Probably TiO_2 NPs play a major role under cold stress conditions as an elevated level of transcription was observed in reported TDFs which are essential to build up cold tolerance by reducing ELI content in tolerant plants.	[63]
2.	20-160 nm	20 mg/kg soil	NPs in soil	*Lycopersion esculentum*	Morphological characters, Quantity of component metals taken up by treated plants from NPs, Nutrient contents in different organs	Root, stem elongation (-), Leaf dry matter ↓, Ca, K, Mg, Na, P, S in roots (-), Ca, Mg, P, S in stems ↑, P, S in leaves ↑	-	[28]

(Table 1) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studies endpoints/ parameters	Remarkable effects	Expected mechanism/possible hypothesis	References
3.	1 to 200 nm, Spherical, Types- Anatase (NAnT), Pristine rutile (NRuT), Rutile with hydrophilic surface (NLRuT), Rutile with hydrophobic surface (NBRuT)	0, 10, or 1000 mg/L of full strength medium	48 h germinated seeds exposed to 0.5 mmol/L $Ca(NO_3)_2$, During 5 weeks cultivation of seedlings, strength of nutrient solution increased gradually, After that seedlings transferred to full strength nutrient solution containing $Pb(NO_3)_2$ (0 or 1.0 mg/L Pb(II)) and 0, 10, or 1000 mg/L NAnT, NRuT, NLRuT, NBRuT for 7 d	*Oryza sativa*	Seedling biomass, Accumulation of NPs, Pb concentration in rice tissues, Iron plaque at the root surface	Seedling biomass (-), Only NAnT entered seedling roots through the apoplastic route, NAnT accumulates in rice roots, Translocation from roots to shoots absent, Iron plaque formation on root surfaces or their restriction effects on Pb uptake (by roots) not affected by the presence of NPs on root surfaces, Pb conc. in roots and shoots ↓, TiO_2-type dependent bioaccumulation of Pb, At high NPs exposure (1000 mg/L) bioaccumulation ↓	Probably casparian strip in root tissues hindered the translocation of NAnT from roots to shoots, NPs high sorption potential for Pb in nutrient solution might be the reason for reduced conc.of Pb in roots and shoots.	[64]
4.	< 100 nm, Mixture of anatase and rutile	0.2, 1.0, 2.0, 4.0% suspensions in DW	Seed soaked overnight in NPs suspension	*Vicia narbonensis, Zea mays*	Seed germination, Development and mitosis of root tip cells	Seed germination delayed, Mitotic index ↓, Aberration index ↑	NPs increased aberration index due to disturbances in the spindle apparatus.	[65]
5.	< 100 nm, Mixture of rutile and anatase	0.2,1.0, 2.0, 4.0% suspensions in DW	Seed soaked overnight in NPs suspension	*Vicia narbonensis*	Enzymatic and nonenzymatic antioxidant responses, Oxidative stress, Oxidative damage	Phytotoxic effects ↑, ROS production, DNA fragmentation	NPs generated stress condition may induce ROS production, Mechanisms of DNA repair not effective to eliminate early genotoxicity effects.	[66]

(Table 1) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studies endpoints/ parameters	Remarkable effects	Expected mechanism/possible hypothesis	References
6.	Sample S- <100 nm (tetragonal crystals), Sample P- <10 nm (spherical shape)	50 mg/L water	Seeds washed overnight in tap water than treated with NPs suspensions for 3 d	*Vicia faba*	Germination, Vigour index, Water contents, Root length, Relative water contents, H_2O_2 contents Activities of ascorbate peroxidase, glutathione peroxidase, catalase and Guaiacol peroxidase, Ascorbate and glutathione contents, proline contents, Thiobarbituric acid reactive substance contents	Germination, root length, water contents, thiobarbituric acid reactive substance contents and glutathione peroxidase activity (-), H_2O_2 and proline contents ↓, Activities of ascorbate peroxidase and catalase ↓, Vigour index- sample P ↑ and sample S (-), Relative water contents, total ascorbate and total glutathione- sample P ↓ and sample S (-), Guaiacol peroxidase activity- sample P ↓ and sample S ↑, Mitotic activity (MI) and occurrence of micronuclei in interphase (MNC) (-), Frequency of anomalies and aberrations (AI) in dividing cells- sample S ↑ and sample P (-)	Depending on their size and shape NPs may provoke major adverse effects in roots and exert specific actions at different levels of toxicity.	[67]
7.	15, 25 and 32 nm, Rutile and anatase	0.01-100 mg/L suspensions	Seed soaked in NPs suspension	*Linum usitatissimum*	Effect of size and crystal structure (anatase and rutile) of TiO_2NPs on their toxicity	NPs anatase crystal structure - toxic in whole set of tests	Lipophilic nature in rutile crystalline structure of NPs creates larger aggregates in an aqueous medium, which show less toxicity than anatase crystalline form	[54]

(Table 1) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studies endpoints/ parameters	Remarkable effects	Expected mechanism/possible hypothesis	References
8.	< 50 nm, Anatase	10, 20, 30, and 40 mg/ml of MS medium	Seeds cultivated in MS medium containing NPs	*Petroselinum crispum*	Germination %, Germination rate index, Vigor index, Fresh weight, Root and shoot length, Chl content	Germination, germination rate index, vigor index, fresh weight, root and shoot length and chl content of seedlings ↑, Best conc. of nano-anatase 30 mg/ml.	Nano-anatase can penetrate through seed and may stimulate the embryo. Improved seed germination might be observed due to reduced antioxidant stress, superoxide radicals, and MDA content carried out by increased conc. of nitrate reductase enzyme and activities of antioxidant enzymes. Nano-anatase assists the absorbance of minerals that promotes the formation of chl and activation of important enzymes for carbon fixation process.	[68]
9.	< 100 nm, Surface area >14.0 m^2/g	~ 91 mg/kg soil	NPs in soil	*Triticum aestivum*	Wheat growth, Soil enzyme activities (under field conditions)	Biomass ↓, NPs mainly adhered on roots surface	Cell membrane could be damaged due to extracellular ROS generated by NPs	[58]
10.	21 nm, Surface area 50 m^2/g	1, 2, 10, 100, 500 ppm suspensions in DW	Seeds germinated in petri dishes containing NPs suspensions	*Triticum. aestivum*	Shoot length, Seedling length, Root dry matters	Root length (-), At 2-10ppm conc. - shoot length and seedling length ↑ At higher conc. - shoot length and seedling length ↓	Appropriate conc. of NPs could promote seed germination and seedling growth.	[69]
11.	< 25 nm,	0, 0.1, 1, 2.5, 5% suspensions in growth media	NPs in growth media	*Nicotiana tabacum*	Germination rates, Biomass, Root lengths, Growth and development of seedlings, Expression profiles of microRNAs.	Germination rate ↓, Root length and Biomass ↓, ADH and APX upregulated, At low conc. - miRNA expression ↑	Water uptake inhibition might be due to the obstruction created by NPs clusters on root cell wall. Lipid peroxidation of cell membrane and oxidative DNA damage generated due to induced ROS.	[52]
12.	< 25 nm, Anatase	0, 6, 18 mM/L and 0, 12 mM/L suspensions in nutrient solution	Nutrient solutions with NPs	*Phaseolus vulgaris, Triticum aestivum, Rumex crispus, Elodea canadensis*	Biomass, Ti Associated with Roots, Uptake of Ti in roots, Translocation of Ti in shoots in rooted plants	For rooted plants – biomass production (-), Root ↑, In *R. crispus* – translocation of Ti into shoots	Roots increased because of Ti sorption and uptake.	[27]

(Table 1) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studies endpoints/ parameters	Remarkable effects	Expected mechanism/possible hypothesis	References
13.	21 nm	NPs- 0, 10, 100 and 1000 mg/L Cd- 0, 10, 20 mg/L	1 week grown seedlings transferred to ½ Kimura solution for 3 d, Then divided in 12 test groups and treated with combinations of Cd and NPs for 10 d	*Oryza sativa*	Biomass, Plant height, Root length, Presence of NPs in roots and shoots, Cd and Ti contents in root and shoot, Isothermal adsorption of Cd, Plant hormone conc. of abscisic acid, indole-3-acetic acid, trans-zeatin riboside, isopentenyl adenosine, gibberellic acid, brassinolide, methyl jasmonate, Antioxidant enzyme activities of catalase, super oxide dismutase, peroxidase, MDA contents, Net photosynthetic rate, Chl contents	Cd / NPs treatment-toxicity in plant (intensity Cd>NPs), MDA contents ↑ (at some conc.), On NPs treatment-NPs exist in plant Ti contents in root ↑, Root length and shoot height ↓, Fresh biomass ↑, On co-treatment (Cd + NPs)- Cd toxicity ↓, Ti and Cd contents in roots ↓, Net photosynthetic rate and chl-contents ↑, MDA contents ↓, Adsorption of Cd-in nutrient solution > in deionized water, Effects on hormones and antioxidant enzyme activities-treatment type and dose dependent	Cd treatment modifies/damages the structure and function of root's cell membrane which facilitates easy entry and accumulation of NPs in the roots. Lattice of TiO_2 NPs and Cd might be interacted in such a way that the adsorbed Cd becomes unavailable to plants eventually reduces Cd toxicity	[70]
14.	32-171 nm, Anatase, White powder form,	10, 100, and 1000 mg/L of Hoagland's solution	48 h germinated seeds exposed for 12 h light/12 dark photoperiod for 2 d, Then seedlings exposed to NPs amended Hoagland's solution for 14 d and various CO_2 conditions (Super-elevated- 5000 mg/L CO_2 and normal- 400 mg/L CO_2)	*Triticum aestivum*	Biomass, Lateral roots, root length and shoot height, Fresh weight of roots and shoots, Ti contents, Phytohormone determination-indole acetic acid (IAA), gibberellins (GA), abscisic acid (ABA), jasmonic acid (JA), brassinosteroid (BR), zeatin riboside (ZR), dihydrozeatin riboside (DHZR), indolepropioponic acid (IPA)	Under elevated CO_2 condition- Yellow/ light brown seedlings, Root biomass and lateral roots ↑, IPA and JA content ↓, JA content ↑ with increasing conc. of NPs, Ti accumulation and translocation in wheat treated with certain conc. NPs ↑, Under both CO_2 cultivation conditions- ABA content ↑, Ti accumulation in both shoots and roots ↑ in dose-response manner, Under normal CO_2 conditions- green and healthy seedlings	-	[71]

(Table 1) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studies endpoints/ parameters	Remarkable effects	Expected mechanism/possible hypothesis	References
15.	15 nm, Anatase	0, 0.1, 1, 10, 100, 1000 mg/mL suspensions in DW	Plant exposed to NPs suspension for 24 h or 72 h	*Allium cepa*	Macroscopic parameters-number and average length of roots and total length of the root system for each bulb, Microscopic parameters- mitotic index, portions of mitotic phases, chromosome aberrations and micro- nuclei	Duration and conc. dependent response of *Allium* roots, Mitotic activity of root meristem ↑	Biological reactivity of NPs may be interpreted with the help of some key parameters like exposure period.	[72]
16.	5-10 nm, Anatase, Crystalline, Tetragona system with dipyramidal habit, Few were rounded spherical grains	0–20% (w/w) NPs suspension of type I culture medium, 0, 100, 150, 200, 400, 600, and 1000 mg/L of type II culture medium	On type I medium- Initial 24 h, seeds on NPs amended medium kept in dark, then cultured for 5 d in a 16 h light/8 h dark cycle, On type II medium- 3 d germinated seeds exposed to NPs amended medium for 7 d with 16 h light/8 h dark cycle	*Hordeum vulgare*	Root and shoot length, Biomass, Chl-a and b contents, Ti contents	Plant absorbed NPs, Shoot growth ↓ (at 10 and 20% w/w in agar media), Shoot growth (-) (upto 1000 mg/L in hydroponic treatment), Root growth ↓, Chl- a and b contents (-), Biomass (-)	TiO_2 NPs exposure generates early root growth (indicator of potential effects of TiO_2 NPs), Reduced root length limits nutrient supply which inhibits shoot growth, Effective mechanical and physiological barriers present in roots may limit the transport of NPs into aerial parts.	[73]
17.	< 150 nm, Anatase:rutile:: 80:20	100 mg/L suspensions in media	NPs added to growth media	*Arabidopsis thaliana*	Gene expression in roots of the plant.	Weak impact	Nitrogen metabolism stimulated slightly showing no visible effects on plant growth.	[74]
18.	14- 655 nm, Anatase and rutile	100 mg/L in DW	Seeds germinated in sand, soaked with Hoagland solution then plantlets transferred to NP suspension	*Triticum aestivum*	Accumulation, Translocation, Impact of nanomaterials, Influence of diameter and crystal phase of NMs	Roots can accumulate – NPs < 140 nm, NPs translocated to leaves – size < 36 nm, Seed germination (-), Vegetative development (-), Photosynthesis (-), Redox balance (-)	Cell wall pores (40 nm) become enlarged due to Hypo-osmotic stress and radial diffusion of 36 nm NPs made possible up to Casparian band where further radial progression in roots was blocked and NPs accumulation occurs.	[26]
19.	14 - 25 nm	0, 10, 50, 100 mg/L suspensions in water	NPs treatment in hydroponics conditions either through root or leaf exposure	*Triticum aestivum, Brassica napus*	Plant development, Accumulation, Distribution in roots according to the size of NPs	Root elongation ↑, Germination (-), Evapotranspiration (-), Plant biomass (-), NPs internalized in roots and transferred to their leaves, NPs with smaller diameters- more accumulation.	Only the smallest NP modulated plant development and increased root length.	[21]

(Table 1) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studies endpoints/ parameters	Remarkable effects	Expected mechanism/possible hypothesis	References
20.	Pristine TiO_2 NPs (anatase 4 nm), TiO_2 MPs (rutile ≈ 150 nm), Aged paint (82% rutile and 18% anatase) leachate	Exposure of 1 µL per 25 mm^2 leaf area, Pristine TiO_2 NPs exposure – 0.125, 1.25 and 12.5 mmol/L ultrapure water deposited on leaves till final conc. of 12.5, 125 and 1250 nmol TiO_2 NPs/ gm fresh weight (FW), Paint leachate exposure- till final conc. of 35 nmol TiO_2/ gm FW	5-leaf staged plantlets exposed to NPs foliarly (on adaxial side of leaves) for 7 d	*Lactuca sativa*	Internalization and *in situ* speciation of Ti, Phytotoxicity biological markers- Fresh foliar biomass, Chl- a, Chl-b, carotenoid and pheophytin contents, Thiobarbituric acid reactive substance, GSH conc.	Internalization in leaves- TiO_2 MPs as well as NPs pristine and from aged paints (observed in all types of tissues), No change in speciation, No acute phytotoxicity, Variations observed in glutathione and phytochelatin levels (< typical values), Fresh foliar biomass, chl-a, chl-b, carotenoid and pheophytin contents, thiobarbituric acid reactive substance (TBARS) (-)	Involvement of both stomatal and cuticular pathways in the transfer of TiO_2 particles expected.	[75]
21.	30- 60 nm	750, 1000, 1250 mg/kg seeds	Seed treated with NPs	*Avena sativa, Trifolium alexandrinum*	Seed germination, Vigor and yield in fodder crops	Germination percentage ↑, Seedling vigour and yield ↑, Maximum seed yield at highest dose	Increment of Hill reaction and activity of chloroplast induced by NPs, accelerating Fe–Cy reduction and oxygen evolution	[40]
22.	24.5 nm, Anatase, Specific area- 55 m^2/g	0, 10, 30,60 µg/ml suspensions in growth medium	NPs in growth media	*Hordeum vulgare*	Callugenesis, Size of calli, Bactericidal activity	Callugenesis ↑, Size of calli in darkness ↑, Effective bactericidal activity	NPs stimulate plant cell division and cellular expansion but their mechanism in darkness is unknown.	[76]
23.	7- 40 nm	0, 2, 5, 10 ppm suspensions in DDW	Seeds exposed to NPs suspensions, Leaves sprayed with NPs suspensions at 12^{th} and 16^{th} d	*Cicer arietinum*	Plant response to cold stress	At 5 ppm conc. - electrolyte leakage and MDA content ↓	Plant-NPs interaction induces defense mechanisms in plant body which supported plants to cold stress	[77]
24.	P25 (representative of TiO_2 NPs), 29 ± 9 nm	10, 100, 1000 mg/kg of soil	5 d germinated seeds transferred to pots with substrate spiked with NPs	*Trifolium pretense,* (*Lolium perenne* used as reference of non-nitrogen fixing plant)	In *Trifolium pretense*- Biological nitrogen fixation by rhizobia, Root colonization of arbuscular mycorrhizal fungi (AMF), Number of flowers (flower heads), Dry weight of roots, shoots and total biomass	Plant growth, number of flowers, biological nitrogen fixation and AMF root colonization (-)	-	[78]

(Table 1) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studies endpoints/ parameters	Remarkable effects	Expected mechanism/possible hypothesis	References
25.	1-100 nm	0.01, 0.03% suspensions in DW	a. Spraying NPs at various vegetative and reproductive stages, b. Spraying NPs and bulk TiO_2 at different conc.	*Zea mays*	Chl contents (a and b), Total chl (a + b), Chl a/b, Carotenoids and anthocyanins contents	Amount of pigments- NPs treatment in reproductive stages > treatment of bulk and DW or NPs in vegetative stage	NPs elevated photosynthesis by activating photochemical reaction of crop chloroplasts	[47]
26.	30–50 nm	100, 250, 500, 2500, 5000 µg/mL in DW	Seeds germinated in petri dishes having NPs suspensions	*Cucumis sativus*	Seed germination rate, Germination index, Root elongation	Seed germination ↓, Germination index ↓, Root elongation ↓,	NPs generated stress in the seeds	[79]
27.	12–15 nm	10 mg/L suspension in DW	NPs suspension foliar sprayed on 14 d old seedlings	*Vigna radiata*	Phenological and physiological effects	Shoot length ↑, Root length ↑, Root area ↑, Root nodule ↑, Chl content ↑, Total soluble leaf protein ↑, Activites of dehydrogenase, phytase, acid phosphatase and alkaline phosphatase in the rhizosphere ↑	NPs stimulated plant metabolic activities along with rhizospheric enzymes activity and microbial population	[50]
28.	27 ± 4 nm, Surface area 51.5 m^2/g, Anatase 82%, Rutile 18%	0, 50, 250, 500, 1000, 2000, 4000 mg/L suspension in modified Hoagland nutrient solution	Plantlets transferred to Mason jars containing NPs suspensions	*Cucumis sativus*	Root length, Shoot length	Root length ↑, NPs absorbed by roots and transported to aboveground plant parts	NPs stimulated nitrogen accumulation and protein formation which promoted plant root growth eventually	[25]
29.	27 ± 4 nm, Surface area 51.5 m^2/g, Anatase 82%, Rutile 18%	0, 250, 500, 750 mg/kg soil	NPs in soil	*Cucumis sativus*	Macro and microelements accumulation in fruit, Chl Content, Catalase (CAT) and Ascorbate Peroxidase Activity	CAT activity in leaves ↑, P and K ↑, Total chlorophyll content in leaves ↑	NPs stimulated nitrogen accumulation and protein formation which promoted plant root growth eventually.	[48]
30.	< 100 nm,	100, 200, 300 mg/kg of soil	Seedlings grown in NPs containing soil contaminated with Cd (50-150 mg/kg)	*Glycine max*	Plant biomass, Relative water contents, Chl contents, Protein contents, Proline contents, MDA contents, Accumulation and distribution of Cd (translocation factor)	Plant growth due to Cd stress ↓, Highest accumulation of Cd in roots, On NPs treatment- Cd stress ↓, Photosynthesis rate and growth parameters ↑, Cd uptake ↑	NPs promoted plant growth rate and accumulation of Cd in plant tissues	[80]

(Table 1) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studies endpoints/ parameters	Remarkable effects	Expected mechanism/possible hypothesis	References
31.	Type 1- 27 nm, Aerosol, Anatase:rutile::80:20, Type 2- 10–20 nm, Specific surface area 55 m^2/g	Setup 1- 0, 100, 500, 1000, 2500, 5,000 mg/L suspensions in DW, Setup 2- 1,000, 2,500, and 5,000 mg/L suspensions in DW, Setup 3- 100, 1,000, and 5,000 mg/L suspensions in Hoagland solution	1. Seeds were soaked in NPs suspensions for 48 h for germination studies, 2. NPs suspensions added to seedling containing pot soil for pot experiments, 3.NPs suspensions for circular hydroponic system	*Brassica campestris, Lactuca sativa, Phaseolus vulgaris*	Germination, Root elongation, Uptake, Physiological responses	Seed germination (-), Enzyme activities (-), Chl content (-), Root elongation ↑	NPs penetrated the seed coat and accumulated inside seed tissues, it showed no toxic effects to plants	[81]
32.	>20 nm, Anatase, 2 fractions of NPs- 28.5 ± 0.5 nm (dominant) and 127 ± 7 nm	100, 250, 500 and 1000 µg/ml suspension in ultrapure sterile water	Seed treated with NPs suspensions for 48 h, then grown in soil for 5 weeks	*Arabidopsis thaliana*	Germination, Root length, Biomass, Tocochromanol and chl contents, Activities of antioxidant enzymes (SOD and CAT), Lipid peroxidation, Vitamin E (Vit-E) content, Real time analysis of expression of tocopherol biosynthetic genes (*vte1*-*vte5*) Element analysis	Ti contents ↑, At highest conc. of NPs- Elements conc. ↑ (copper- 2.5-folds, zinc- 2.5 folds, iron- 1.5 folds and manganese- 2 fold), Biomass and chl contents ↓, Root growth ↑, At higher NPs conc.- antioxidant level and lipid peroxidation ↑, Tocochromanol contents- ↓ for lower treatment conc. ↑ for highest conc., Vitamin E contents- ↓ for lower tested conc. ↑ for 1000 µg/ml treatment conc.	TiO_2 NPs alter gene expression of vit-E which could lead to antioxidant response in *Arabidopsis* plants, Generated local oxidative stress and enlarged cell wall pores and increased water flow and turgor in roots enhance root elongation eventually, NPs treatments inhibited photosynthesis and generated differences in mineral uptake which cause reduced biomass production.	[82]

(Table 1) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studies endpoints/ parameters	Remarkable effects	Expected mechanism/possible hypothesis	References
33.	25-70 nm, Elongated, High crystallinity with tetragonal rutile, Hydrodynamic diameters – 261-341 nm, Pristine sample-surface area- 20-40 m^2/g, zeta potential-- 14.5 ± 0.5 mV, Both hydrophobic and hydrophilic NPs-suface area- ~50 m^2/g, zeta potential-+ ~27.0 mV	125, 250, 500, and 750 mg/kg of soil	Cultivated for 65 d in soil amended with unmodified, hydrophobic (coated with aluminum oxide and dimethicone) and hydrophilic (coated with aluminum oxide and glycerol) NPs, Hydrophilic particles suspended in Millipore water before mixing with soil, Hydrophobic particles mixed directly with the soil	*Ocimum basilicum*	Ti and essential elements in tissues, Relative chl content, Carbohydrates, Antioxidant response, Biomass, Water contents	Hydrophobic and hydrophilic NPs-seed germination ↓, Unmodified and hydrophobic NPs-shoot biomass ↓, At 750 mg/kg, Ti contents in roots-(hydrophobic >hydrophilic >pristine) In shoots (-), All three types of particles affected homeostasis of essential elements, At 500 mg/kg, Unmodified particles-Cu and Fe ↑, Hydrophilic particles-Fe ↑, Hydrophobic particles-Mn ↑ but Ca, Cu and P ↓, Root elongation ↓ only by hydrophobic particles, Total sugar ↓ by all particles, Reducing sugar ↓ by unmodified particles, Starch ↓ by hydrophobic particles	Although all tested NPs affected plants but the highest impact on its nutritional quality was generated by coated NPs by altering more contents of starch, essential elements and reducing sugars	[83]
34.	2.8 ± 1.4 nm, Molarity 9.6 mM	2 µM suspension in phosphate buffer	Seedlings partially submerged to suspension of NPs	*Arabidopsis thaliana*	Microtubular (MT) network	26S proteasome dependent degradation of tubulin monomers ↑, Reorganization and elimination of MTs, Isotropic growth of root cells	NPs treatment causes MT disruption by generating ROS (indirectly) or by interacting with tubulin (directly)	[53]

(Table 1) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studies endpoints/ parameters	Remarkable effects	Expected mechanism/possible hypothesis	References
35.	20 nm, 293 ± 17 nm (in DI water),	0, 100, 250 or 500 mg/L of Hoagland nutrient solution	7 d seedlings exposed to NPs containing medium for 14 d	*Oryza sativa*	Biomass, Absorption and transportation of NPs in roots, stems and leaves, Antioxidant enzyme activities- catalase, peroxidase, superoxide dismutase, MDA contents, Metabolic profile	Biomass ↓, NPs gathered on root surface, subsequently absorbed and accumulated by root cells, Small amount of NPs transported through stems, Antioxidant defense system ↑, POD activity ↓, CAT (leaves) and SOD (roots and leaves) earlier ↑ later ↓, CAT (root) (-), Accumulation of Ti ↑ (root > leaves), 105 identified metabolites exhibited significant difference from control, Conc. of glucose--phosphate, glucose-1-phosphate, succinic and isocitric acid ↑ (most), Conc. of sucrose, isomaltulose, and glyoxylic acid ↓ (most), Tricarboxylic acid cycle and pentose phosphate pathway ↑, Starch and sucrose metabolism, and glyoxylate and dicarboxylate metabolism ↓, Biosynthesis of most of the identified fatty acids, amino acids and secondary metabolites ↑,	Epidermal cells absorbed NPs where they remain stuck to the root surface even after washing thus higher contents in roots over the leaves were observed, NPs treatment disturbed the metabolism of rice plants distinctly and showed mixed effects on their yield and quality.	[84]

(↑= increased, ↓=decreased, (-)= not significantly affected, DW= distilled water, DDW= double distilled water, DIW= deionized water, MS= Murashige and Skoog, Chl= chlorophyll, h= hours, D=days).

Table 2. Summary of the important studies (after year 2010) on terrestrial plant species exposed against CuO NPs.

S.N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanism/ possible hypothesis	References
1.	<50 nm, Round shaped, Smooth surface	3, 10, 30 and 300 mg Cu/kg soil	By mixing in Sand	*Triticum aestivum*	Root morphology	Root elongation ↓, Root hair proliferation, Shortening of zones of the division and elongation.	NPs released Cu ions in rhizosphere, which modified IAA distribution causing root shortening and by allowing Nitric oxide (NO) cell signaling Cu ions promoted root hair proliferation.	[55]
2.	47 nm, Crystalline nature,	50, 100, 200 and 400 mg/L of ½ strength Murashige and Skoog (MS) medium for germination experiments, 2.5, 5 and 10 mg/L of MS medium for callus induction	For seed germination experiments- Seeds exposed to ½ strength MS medium containg NPs for 15 d, For callus induction experiment- Seeds transferred on MS medium for 15 d and then stem and leaf explants inoculated on MS medium for 30 d	*Trigonella foenum-graecum*	Seed germination, Root and shoot length, Fresh and dry weight, Callus induction, Total Flavonoid content (TFC), Total Phenolic content (TPC), Total Antioxidant capacity (TAC), Total Reducing power (TRP), DPPH free Radical scavenging assay	Seed germination (-), Maximum root length, shoot length, fresh weight and dry weight at 50 mg/L then ↓, Fresh weight and dry weight of callus ↓, Presence of flavonoids and phenolics in the fresh weight extracts of treated plants ↑, Optimum conc. for growth considered- 50 mg/L (after this conc. toxicity occurs)	Protective nature and selective permeability of seed coat makes seed germination unaffected against treatment. Released Cu ions from NPs generated oxidative stress and interfered with normal growth of plants, which activated their defense system.	[85]
3.	<100 nm	10, 100, 500, and 1000 mg/L suspension in ultrapure water	Pre soaked seeds (in ultrapure water) exposed to 8 mL of NPs suspension in Petriplate	*Raphanus sativus, Lolium perenne, Lolium rigidum*	Germination, Root and shoot elongation, Uptake of Cu by plants, DNA damage studies	Plant growth (all species) ↓, Induction DNA damage by accumulation of oxidatively modified, mutagenic DNA lesions (7,8-dihydro-8-oxoguanine; 2,6-diamino-4-hydroxy-5-for-amidopyrimidine; 4,6-diamino-5-formamidopyrimidine)	Nature of CuO NPs is oxidative thus they withdraw electrons from various biomolecules within plant cells and transfer those electrons to other biomolecules (*i.e.* reducing agents), NPs promote damage to plant DNA due to direct redox interactions.	[56]
4.	50–60 nm	0.5–5.0 mg/ml suspension in water	Buffer of enzyme assay mixture replaced by equal volume of NPs suspension	*Hordeum vulgare*	*In vitro* effect on activity and stability of Barley Oxalate oxidase (OxO)	Adsorption of enzyme on surface of NPs, NPs-bound enzyme activity (-)	-	[86]

(Table 2) cont.....

S.N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanism/ possible hypothesis	References
5.	<50 nm	2.5, 10, 50, 100, and 1,000 mg/ L Suspension in Hoagland's solution	Pre-soaked (water) seeds treated with NPs suspension in petriplates for 6 d, Grown seedlings transferred to Hoagland's solution amended with NPs	*Oryza sativa*	Germination rate, Root length, Shoot length, Biomass, Photo-synthetic rate, Transpiration rate, Stomatal conductance, Maximal quantum yield of PSII photochemistry, Photosynthetic pigment, Oxidative and osmotic stress contents- MDA, Proline and ascorbate, Expression of antioxidant enzymes- APX and SOD	Germination rate, root and shoot length ↓, Biomass (at higher conc.) ↓, Uptake of Cu (in roots and shoots) with increasing conc. of NPs ↑, Accumulated in chloroplasts, No. of thylakoids per granum and size and no. of stomata ↓, Size and no. of trichomes; MDA, proline and ascorbate contents; and Expression of APX and SOD↑, At 1000 mg/L conc.- Photosynthetic rate, transpiration rate, stomatal conductance, the maximal quantum yield of PSII photochemistry and photosynthetic pigment contents ↓	Very small quantity of NPs transported from roots to shoots hence exerted more toxicity in roots. Released Cu accumulated in shoot and root tissues and reduced their length effectively. Damaged roots affect water uptake negatively thus reducing number of stomata and net photosynthesis. Reduced amount of PSII–LHCII complexes affects ultrastructural organization and light harvesting thylakoids.	[87]
6.	<50 nm	500 mg/Kg of soil	NPs added in soil	*Triticum aestivum*	Root length, Shoot length, Plant biomass, Bioaccumulation of metal, Lipid peroxidation, Chl contents, POD and CAT activities	Root and shoot length and Chl contents ↓, Number of roots, plant biomass, bioaccumulation, lipid peroxidation and activities of POD and CAT activities ↑, Glutathione oxidized	Dissolved Cu released from CuO NPs generated phytotoxicity and in turn altered behavior of plants.	[57]
7.	<50 nm, Round shaped, Smooth surface	500 mg/Kg of soil	NPs added in soil	*Triticum aestivum*	Root length	Root length ↓	Cu ions released from CuO NPs reduced root length	[3]
8.	<50 nm, Round shaped, Smooth surface	100, 250, 500 mg/kg of soil	NPs added in soil	*Phaseolus vulgaris*	Root growth, shoot growth, Conc. of other metals, Activities of ferric reductase and cupric reductase	At 250 and 500 mg/kg conc.- Root and shoot growth ↓, Mn, Zn and Ca in shoot ↓, Na in shoot ↑, Mg and K levels (-), Ferric reductase activity ↓, Cupric reductase activity ↑	Increased accumulation of Cu in shoots impaired the growth, NPs exposure altered activities of root metal reductases contributing to altered nutrient levels and growth inhibition	[88]
9.	10 -100 nm	0, 5, 10and 20 mg/L suspension in modified Hoagland's medium	Young plants grown for 10 d in modified Hoagland's medium, then transferred to modified Hoagland's medium amended with NPs	*Lactuca sativa, Medicago sativa*	Seed germination, Root length, Shoot length, CAT and APX activity	At 20 mg/L conc.- Shoot browning, Root length ↓, Iron content in roots and shoots ↓, Phosphorus content in roots of both plants ↓, Uptake and accumulation (with increasing concentrations) in Lettuce ↑, CAT activity- In lettuce (-), In alfalfa ↓, APX activity ↑	Different effects on plant growth due to differences in Cu accumulation and ROS generation. Uptake and translocation of Cu depend on copper compound and plant species (smaller size, greater uptake)	[89]
10.	40–100 nm, Irregular shape, Crystalline monoclinic cubic cuprous oxides	0.1, 1.0, 10, 100 and 1000 mg/L of MS medium	Seeds cultured on plain MS medium for 4 weeks, Then axillary shoot nodes excised and incubated in media with different conc. of NPs for 4 weeks	*Stevia rebaudiana*	Shoot length, Shoot organogenesis, Mean number of shoot per explants, Fresh weight, Analysis of steviol glycosides, Antioxidant assays- total phenolic content, total flavonoid content, total antioxidant capacity, total reducing power, DPPH free radical scavenging activity	Shoot length, mean no. of shoots per explant, fresh weight and production of steviol glycosides ↑ till 10 mg/L but after that conc. ↓, Total phenolic contents, total flavonoid content, total antioxidant capacity, total reducing power, % DPPH inhibition ↑ at 10 mg/L and least amount observed at 1000 mg/L	CuO NPs work as a stimulator in production of bioactive components and can be employed in *in vitro* batch cultures, Cu ions or free radicals released from CuO NPs into MS medium lead to oxidative stress hence increased antioxidant activities and secondary metabolites.	[90]

(Table 2) cont.....

S.N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanism/ possible hypothesis	References
11.	360 - 400 nm	125 and 625 µM in Hoagland's solution	After 4 weeks of culture, seedlings exposed NPs by adding them in Hoagland's nutrient solution	*Arabidopsis thaliana*	Fresh weight, Total chl content, Anthocyanin content, SOD and CAT activities, MDA contents, Gene expression study of APX gene	Fresh weight and chlorophyll content ↓, After 2 d- anthocyanin content and SOD and CAT activity ↑ then after 4 d ↓, MDA content ↑, During 2 d exposure- Up-regulation of APX1, APX3 and APX4 and no difference observed in APX2, APX5 or APX6 transcription, During 4 d exposure- down-regulation of APX1-6, After 4 d exposure- plant become bright yellow	Initial increase and then after 4 days exposure, a decrease in CAT activity might be due to initiation of adaptive response in plants to manage the overproduction of H_2O_2 at early stages of growth and then recover to normal conditions after stress.	[91]
12.	50 nm	0, 10, 50, 100, 500, and 1,000 mg/L suspension in ½ strength Hoagland's media	4 week old Soil grown seedlings transferred to ½ strength Hoagland's medium amended with NPs	*Cumumis sativus*	Biomass, Bioaccumulation, ROS enzymes activities-SOD, CAT and POD	At 1000 mg/L conc.- Biomass ↓, Bioaccumulation (dose dependent) ↑, SOD,CAT and POD activities ↑	With increasing conc. of NPs, more NPs crossed cell membrane and agglomerated, either with themselves or with other cellular materials within cells.	[32]
13.	65 ± 2.45 nm, Specific surface area- 14 m^2/g, Average hydrodynamic radius- 139 ± 16.2 nm, Zeta potential- 47 ± 0.1 mV	0.8 to 63.5 g/L of DW	Seeds treated with NPs. on 3rd d, seedlings added to 5 ml of suspensions of NPs	*Triticum vulgare*	Cell viability (WST and Evans Blue test), Cu Contents, Contents of individual reactive oxygen species, Degree of DNA fragmentation *in vitro* and *in vivo*	At higher conc.- small and significant effect on viability, Number of dead cells in seedlings with Evans Blue ↑, Total pool of ROS in roots ↑, 3.2 to 63.5 g/L conc. leads to DNA fragmentation and fragments less than 3000 bp ↑ (51.4–62.8%)	CuO NPs enter poorly into plant due to agglomeration in suspension medium or aggregation on rough surface of seeds and roots. NPs may accumulate in plant in ion form	[92]
14.	<50 nm, Surface area- 29 m^2/g	2, 4 and 10 mg/L of cultivation medium	Seedlings grown in 25% Hoaglands solution for 6 weeks, then cultivated in NPs containing medium	*Arabidopsis thaliana*	Weight of plant, Rosettes growth, Root transcriptome analysis	Rosette growth ↓, Upregulated genes- 111, Downregulated genes- 62	More solubility of ENPs than bulk particles, resulting in up-regulation of metallochaperone-like genes or down-regulation of aquaporins and metal Transmembrane transporters (also characteristic for ionic Cu^{2+} exposure)	[93]
15.	<50 nm, Surface area 29 m^2/g	10, 100 and 1000 mg/L Suspension in cultivation media	Seeds sown on filter papers in petriplates and cultivation media amended with NPs	*Sinapis alba*	Germination, Root length, Accumulation of NPs	Germination and root elongation (dose dependent) ↓, Accumulation of metal ions within seedlings	Toxicity created by ionic Cu released from CuO NPs	[33]
16.	30 ± 10 nm, Zeta potential 0.416 mV, Zeta average diameter 388.2 nm, Spherical shaped	0, 10, 200 and 1,000 mg/L suspension in DI water	Plants grown in nutrient solution for 4 d before exposure to different conc. of NPs,	*Gossypium hirsutum* (Used two types of cotton- Transgenic cotton-Bt29317, Conventional cotton- Jihe 321)	Biomass, Plant height, Root length, Nutrient contents, Hormone conc. (IAA, ABA, t-ZR, GA), Bt-toxin expression, Cu uptake and distribution	Growth, development, nutrient content, IAA and ABA conc. of transgenic and conventional cotton ↓, In conventional cotton leaves- NPs aggregated on epidermis, In transgenic cotton leaves- NPs reached into cells by endocytosis, Most NPs aggregates found on root outer epidermis, rest located in intercellular spaces of both conventional and Bt-transgenic cottons, Expression of exogenous gene encoding of Bt toxin protein in leaves and roots ↑	At certain treatment conc. increase in biomass suggests an optimal dose limit for the growth of both types of cottons, beyond which CuO NPs behaved toxic, Uptake of different nutrients in different type of cotton differently affected by CuO NP exposure on same treatments	[34]

(Table 2) cont.....

S.N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanism/ possible hypothesis	References
17.	<50 nm, Nearly spherical shape, Surface area 29 m^2/g	0, 50,500, 2,000 and 4,000 mg/ L suspension in ½ strength Hoagland's solutions	5 ml test solution added to filter paper in petriplates and seeds sown on it	*Fagopyrum esculentum*	Root growth, Root morphological features, Biomass, Localization of NPs, Genotoxic effects using RAPD	At 4000 mg/L conc.- Root length ↓, Biomass ↓, Root tip morphology changed, Number of hairs ↓, Genotoxic effects observed, Total number of bands↑	High conc. of NPs caused damaging effects on genomic DNA altering gene expression levels, Changed root tip morphology observed due to alterations in structure of cellular cytoskeleton	[94]
18.	-	0, 100,200, 400 and 600 mg/L suspension in DW	Seeds soaked in NPs suspension for 6h, then transferred to test solution containing filter paper in petriplates	*Cucumis sativus*	Seed germination, Root elongation, Discovering biomarker for phytotoxicity analysis (through SELDI-TOF MS analysis)	Germination↓, Root elongation ↓, 9 different proteins in CuO NPs treated seeds (in comparison to bulk and control), For figuring out CuO NPs generated phytotoxicity a 5977-m/z protein was the most apparent	Negative effect on seed development due to clogging of root opening and inhibition of hydraulic and nutrient uptake in roots	[95]
19.	<50 nm, Surface area 29 m^2/g	0, 0.5, 1, 2, 5, 10, 20, 50 and 100 mg/L suspension in ½ strength MS medium	Seeds sown on NP amended ½ strength MS medium	*Arabidopsis thaliana*	Germination, Root elongation, Plant biomass, Chl content, Anthocyanin content, Lipid peroxidation, Proline content, Lignin deposition, Antioxidant assay, Study of oxidative stress related genes	Germination (-), Root elongation, plant biomass, Chl contents ↓, Retarded primary root growth, Lateral root formation ↑, Loss of root gravitropism, Anthocyanin content, lipid peroxidation, proline content, lignin Deposition, SOD activity, H_2O_2 formation ↑, Induction of genes related to oxidative stress responses, sulfur assimilation, glutathione and proline biosynthesis	Due to the seed coat protection seed germination was unaffected, Hormonal imbalance changed root system architecture, Death of cells of lateral root apex, induction of P5CS genes and increased accumulation of proline observed due to excess ROS generation by NPs stress, NPs might be translocated through the vascular tissues and their dissolved Cu ions could have resulted in lignifications of vascular tissues.	[96]
20.	<50 nm, Surface area 29 m^2/g	0, 50, 100, 200, 400 and 500 mg/L suspension in ½ strength MS medium	Petriplate germinated seeds transferred to ½ strength MS medium amended with NPs	*Glycine max*	Shoot and root development, Total chl content, H_2O_2 generation, POD activity, Lignification of root cells, mRNA expression of genes - Lignin biosynthesis viz. phenyl alanine ammonialyase (PAL), Cinnamate 4-hydroxylase (C4H), Cinnamyl alcohol dehydrogenase (CAD), Peroxidase 2 (POD2), Peroxidase 4 (POD4), Peroxidase 7 (POD7)	Germination, shoot growth and weight, total chl content ↓, H_2O_2 level, POD activity, lignin contents of roots, ROS generation ↑, Upregulated genes – PAL,C4H and CAD (at 100, 200 and 400 mg/L), POD2 and POD4 (at 100, 200, 400, and 500 mg/L), POD7 (at 200, 400, and 500 mg/L)	Excess Cu in soybean roots leading to inhibited mineral nutrient uptake, deteriorating effects on plant growth and development and total chl content, Higher expression of genes PAL, C4H and CAD due to less toxicity at lower conc., Upregulated anionic POD genes produce POD enzymes, which might be involved in the final stages of lignification process in plants and degradation of auxins leading to retarded root growth.	[97]
21.	43 ±9 nm, Spherical, Hydrodynamic diameter- 240±23 nm, Specific surface area- 131 m^2/g	50, 100, 500, and 1000 mg/kg of soil (dry mass/air-dried soil mass)	2 weeks grown uniform seedlings transplanted in NPs amended soil	*Oryza sativa*	Root and Shoot Length, Fresh and dry weight, Water content, Number of filled and unfilled grains, Cu contents	Root length, shoot length and plant biomass ↓, Water content ↓ (in shoots at seedling stage and in roots at tillering stage), At higher conc.- grain yield and fresh weight ↓, At 500 mg/kg conc.- weights and numbers of filled grains < unfilled grains, Cu content ↑	NPs found to be translocated from soil to plant (especially to chaff) and promoted the Cu accumulation in aleurone layer of rice, but could not reach polished rice.	[98]

(Table 2) cont.....

S.N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanism/ possible hypothesis	References
22.	43 nm, Ellipsoidal or spherical, Surface area 131 m^2 /g	10 and 100 mg/L suspension in Milli-Q water	2 days Milli-Q water soaked germinated seeds sown onto filter papers moistened with 5mL NPs containing test solutions	*Oryza Sativa*	Root elongation, ROS detection, Lipid peroxidation, Mitochondrial membrane potential, Cell viability, Membrane integrity	Root elongation ↓, MDA content, lipid peroxidation ↑, Aberrations in root morphology and ultrastructure, Losses of cell viability and membrane integrity, Depolarization of mitochondrial membrane, Programmed cell death	Significantly higher production of ROS in the roots.	[99]
23.	<50 nm	0.5, 1.0 and 1.5 mM suspension in DDW	NPs suspensions added to cotton pads	*Oryza sativa*	Seedling growth, Modulation of Ascorbate-glutathione cycle, Membrane damage, Produced *In vivo* ROS, Foliar H_2O_2 and proline accumulation	Seed germination percentage and leaf carotenoids ↓, Loss of root cells viability, Foliar MDA level, proline contents, GR activity and GSH/GSSG ratio ↑, At 1.0 and 1.5 mM conc.- APX activity ↑	Severe oxidative burst under NPs treatment stress, Oxidative damage to lipid membranes	[100]
24.	<50 nm	0.5, 1 and 1.5mM suspension in ½ MS medium	Water soaked seeds were placed on cotton pads with NPs suspensions	*Hordeum vulgare*	*In vivo* ROS detection, Root cell viability, Chl fluorescence, Chl and epidermal flavonols contents, H_2O_2 measurement, MDA conc., Antioxidant enzymes assays-APX, SOD,GR, DHAR, MDAR, Foliar ascorbate and glutathione contents	Shoot and root growth (dose dependent), activities of DHAR and MDAR and GSH/GSSG ratio ↓, Maximal quantum yield of PS II photosynthetic apparatus (Fv/Fm) (-), Flavonol level, APX activity and H_2O_2 amount ↑, In 1.0 and 1.5 mM CuO NPs treated leaves-GR activity ↑	NPs induced oxidative burst, ROS/antioxidant imbalance and high membrane damages, Elevated APX activity and flavonol level not enough to decompose excess H_2O_2 produced under NP-stress, causing dark brown spots on leaves	[101]
25.	50 nm	10, 100 and 1000 mg/L suspension in DI water	Suspension of NPs provided *via* irrigation	*Spinacia oleracea*	Root and shoot length, Root and shoot weight, Chl and carotenoids contents	At 1000 mg/L conc.- Root length, shoot length, total weight and Chl and carotenoids contents ↓, At 10 mg/L conc.- nontoxic	Inhibition of growth at higher conc. attributed to aggregation properties of NPs, blocking pores present on root surface.	[102]
26.	20-30 nm	10 and 20 mg/L Suspension in Millipore water	Seedling grown in organic soil (initially) and Hoagland nutrient solution (1 week), lastly in NPs suspensions (15 d)	*Lactuca sativa*	Physiological parameters, Nutritional quality, Chl content, Activities of catalase and ascorbate peroxidase	Water content, root length, dry biomass and APX activity ↓, CAT activity ↑, Accumulation of cu in roots, In NPs-treated plants- Cu, Al and S ↑, Mn, P, Ca, and Mg↓	Plant exposure to Cu materials (bulk and oxide NPs) altered their capacity to absorb and transport some nutrients.	[103]

(Table 2) cont.....

S.N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanism/ possible hypothesis	References
27.	20−40 nm	2,5, 10, 20, 30, 40, 50, 100 mg/ L suspension in nutrient solution	Seedlings grown by soaked (in NPs suspension) seeds in Petri- plates (having 5 ml NPs suspension), then transferred to nutrient solution without NPs, After emergence of third leaf shifted to NPs amended nutrient solution	*Zea mays*	Seed germination, Root elongation, Root morphology, Xylem and phloem based transport	At 100 mg/L conc.- Seed germination (-) Seedling growth, root elongation, root and shoot biomass ↓, NPs transported from roots to shoots *via* xylem, NPs could translocate from shoots to roots back *via* phloem	Phytotoxic effects on root elongation observed because after penetration of seed coats, emerging radicles rapidly absorb nutrients and water, thus maximizing NPs exposure. NPs treatment reduced water uptake and hydraulic conductivity in roots, hence decreasing plant biomass	[31]
28.	30-50 nm	13, 398and 228 mg/L suspension in DI water	Soaked seed (in NPs suspension for 2 h) grown in Petri plates having NPs suspension (5ml)	*Lactuca sativa, Raphanus sativus, Cucumis sativus*	Seed germination, Root elongation	Seed germination ↓, Germination of lettuce seeds more seriously inhibited than larger sized seeds (radish and cucumber seeds)	Phytotoxicity of MO NPs not only due to their dissolved metal ions, but also due to their interactions with the seed/root surface.	[104]
29.	40-60 nm, Specific area- 12.55 m^2/g	10 and 250 mg per plant (simulate aerial deposition and pollution of NPs)	Adaxial surface of leaves were treated using applicator brush	*Brassica oleracea* Used two varieties- Capitata L., Sativa L. cv. batavia blonde dorée	Accumulation of metal in tissues, Copper speciation in leaf, Fresh and Dry weight of shoots and roots, Water content, Net photosynthesis, Stomatal conductance	After 15 days of exposure- foliar Cu uptake ↑, At 250 mg- dry weight ↓, Water content and photosynthetic activity ↓, Formation of necrotic in Cu rich areas near deformed stomata	CuO-NPs may accumulate as CuO in the exposed leaves and partially transformed as Cu(II) – organic complexes in plant tissues. The presence of CuO-NPs generates an expression of two genes, involved in the regulation of root growth and ROS (oxidative stress)	[105]
30.	53 nm	500,1000, 1500 mg/L of agar media, For *in vitro* studies- 0, 1, 5, 10 and 20 mg/L of Basal MS media	Seeds germinated on NPs containing media, For *in vitro* studies- Seeds germinated on plain agar media and 14 d old seedlings used for explants (leaves and stem)	*Brassica nigra*	Seed germination, Seedling length, Fresh and dry weight, Total phenolic and flavonoid Contents, Total Antioxidant Capacity (TAC), Total Reducing Power (TRP), Free radical scavenging potential of seedlings and callus	Seed germination, plant length and fresh and dry weight ↓, Total Flavonoids and phenolics ↑ (till 500 mg/L) and then ↓ (at 1000 mg/L) and again ↑ (at 1500 mg/L), TAC (-), TRP (at 500 and 1000 mg/L) ↓ and then ↑ (at 1500 mg/L), DPPH Free Radical Scavenging Activity ↑, At 20 mg/L- direct root emergence from explants callus	Smaller NPs penetrate and destine at seed embryo that reduce seed germination efficiency, Variation in seed germination efficiency at the 3rd and the 5th day of inoculation depicts that the penetrated NPs did not damage the embryo but delayed the process of germination.	[106]
31.	25 to 80 nm, APS- 55 nm, Specific surface area- 23.9 m^2/g	5, 10, 50, 100 and 200 mg/L suspensions in agarose medium	NPs dispersed in agarose medium	*Triticum aestivum*	Optimizing the methods to distinguish and quantify the adsorption and uptake of NPs on wheat root	Most NPs adsorbed on root surface and some adhered mechanically, NPs uptake ↑ (dose dependent), Absorption/adsorption ratio ↑ then ↓	At low conc. of NPs- adsorbed NPs at root surface easily transported to cells At a higher conc. of NPs- NPs absorbed on root surface and only part of them further transferred into cell	[30]

(↑= increased, ↓=decreased, (-)= not significantly affected, DW= distilled water, DDW= double distilled water, DIW= deionized water, MS= Murashige and Skoog, Chl= chlorophyll, h= hours, D=days)

Table 3. Summary of the important studies (after year 2010) on terrestrial plant species exposed against ZnO NPs.

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanisms/ possible hypothesis	Referances
1.	< 100 nm	25, 50 mg Zn/g seeds	Coating of seeds with NPs, Stable suspensions of NPs in ethyl alcohol (having 2% crude pine oleoresin as binding agent)	*Zea mays, Glycine max, Cajanas cajan, Abelmoschus esculentus*	Chl estimation, Indole-3-acetic acid (IAA) auxin contents	Germination percentage, chl content, IAA contents and plant growth ↑	Total requirement of Zn of the crop can be fulfilled by coating seeds with NPs as plants take up Zn from ZnO NPs through seed coating, here NPs inhibited bacterial and fungal infections also of seeds	[107]
2.	< 50 nm, Surface area > 10.8 m2/g	50, 100, 200, 500, and 1000 µ g/ml suspension in DDW	2-3 cm long roots exposed to suspension/solutions of ZnO-NPs, ZnO-Bulk and Zn^{2+} ions for 12 h	*Allium cepa*	Root length, Analysis of mitosis, Cell viability and chromosomal aberrations in root cells, Lipid peroxidation, Antioxidant enzymes/ROS, Mitochondrial membrane potential	NPs present on outer and inner surfaces of cell and nuclear membrane and intracellular cell junctions, plasmodesmata, ROS production and genotoxicity or frequency of chromosomal aberrations (%) ↑, Mitotic Index (%) ↓, (Chromosomal aberrations observed- irregular prophase with vacuolated nucleus, during metaphase disorientation and stickiness, multipolar anaphase and drifted chromosomes, chromosome bridges with lag) Disintegrated root cells and ruptured root surface with spikes of root tissues	NPs penetrated into tissues and induced oxidative imbalance by producing ROS, leading to genotoxic and mito-depressive effects.	[108]
3.	< 30 nm	1.5 and 10 ppm aqueous suspensions	NPs suspensions sprayed foliarly	*Cicer arietinum*	Root/ shoot length, Biomass accumulation, Relative water contents, MDA assay, SOD and POD activity	At 1.5 ppm conc.- Shoot dry weight ↑, At 10 ppm conc.-Root growth ↓, Biomass accumulation ↑, MDA content ↓, SOD and POD activity ↓	Low level of ROS resulted in reduced lipid peroxidation.	[60]
4.	< 100 nm, Rhomboid initially (in water) to elongated rods (in aqueous phase of sand matrix)	500 mg NPs/ kg sand	NPs mixed with sand matrix	*Triticum aestivum*	Root/ shoot length, No of roots originated from shoot, MDA, POD, CAT, IAA and chl contents, Glutathione oxidation, Accumulation and speciation of Zn in plant body	Shoot length (-), Root growth and chl contents ↓, Zn as Zn-phosphate detected in shoots, Lipid peroxidation, oxidized glutathione, POD and CAT activities (in roots) ↑, IAA oxidase activity and MDA content ↑	Released Zn from NPs increased production of ROS in cells eventually inducing oxidative stress	57]

(Table 3) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanisms/ possible hypothesis	Referances
5.	< 100 nm	500 mg/ kg sand	NPs mixed with sand matrix	*Triticum aestivum*	Fate of ZnO NPs in plant environment, Root phytotoxicity,	Root length ↓, Root surfaces of plants whiter than control, Accumulations of Zn−phosphate species in shoots, Dissolution and aggregation of NPs altered by various factors from plant and soil	Smaller particle size of NPs generates more toxicity into exposed roots.	[10]
6.	< 100 nm, Slab like, Surface area 15–25 m2/g	5g NPs/110 kg soil	Seeds sown in spiked soil (after aging for 2 months; reflect field conditions), as 100 seeds per lysimeter	*Triticum aestivum*	Contents of Zn, Biomass, Soil enzyme activity	Biomass ↓, Dissolution of NPs in soil enhances uptake of Zn by plant having toxic effects, Soil protease, peroxidase and catalase activities ↓, Urease activity (-)	Heavy metals interact with sulfydryl groups, producing metal–sulfide equivalents and hence inhibit enzyme activities.	[58]
7.	20 nm	2 g/L	Foliar spray of NPs suspension on osmotically shocked plants	*Triticum aestivum* (var. Tajan and Kavir)	Plant height, leaf area, Chl contents, Shoot/root dry weight,	Plant height, leaf area, shoot dry weight and chl contents ↑, Root dry weight (-)	Positive effects of Zn on metabolism, photosynthesis and other biological activities promote vegetative growth of plants.	[109]
8.	<100 nm	3, 20 and 225 mg Zn/kg soils, Calcareous soil (pH 8.3), Acidic soil (pH 5.4)	Plants grown in soil having NPs for 90 d	*Phaseolus vulgaris, Solanum lycopersicon*	Zn accumulation, Photosynthetic pigments, Protein contents, APX activity, GPOD activity, CAT activity, ROS level, MDA contents	Accumulated Zn- Acidic soil > calcareous soil, ROS production ↑, Lethal effects- Acidic soil > calcareous soil, Calcareous soil- photosynthetic pigments ↑, Acidic soil- photosynthetic pigments for Bean ↓ and for Tomato (-), Protein level ↑ (calcareous > acidic soil)	Influence of NPs on biomarkers of oxidative stress greatly depends on exposure time, soil pH and plant species, Activity of free ions from ZnO NPs can induce toxicity, Increase in available ionic Zn (by definite addition) makes calcareous soil improved for plant growth by reducing its Zn- deficiency	[110]
9.	50 -100nm, Rod shaped, spherical, or hexagonal, Mostly clustered, Surface particulate and crystalline	5, 10, and 20 g/ml suspension in DI water	Hydroponic culture, Root exposure	*Allium cepa*	Root elongation, Root morphology, Cell morphology, Adsorption potential	Root elongation ↓, At 20 g/ml conc.- Almost no growth, Dehydrated root system, Damage normal morphology of root	NPs enter into onion roots radially and become accumulated in modules of chromosome and cell severely.	[59]

(Table 3) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanisms/ possible hypothesis	Referances
10.	10 nm	0, 500, 1000, 2000, and 4000 mg/L Suspensions prepared in Hoagland modified nutrient solution	Hydroponic culture in NPs containing Hoagland modified nutrient solution	*Prosopis juliflora*	Zn conc. in root, stem and leaves, Transformation of NPs, Distribution of Zn in tissues, Activity of APOX and CAT	Zn in vascular system ↑, Conc. of Zn in tissues- Root > Stem > leaf, At 2000 mg/L conc.- APOX (in stems and leaves) and CAT (in roots, stems, and leaves) ↑, NPs not observed in tissues, Zn present as Zn(II), similar to $Zn(NO_3)_2$, Wilting, stunting, chlorosis or necrosis not observed	This plant is tolerant against ZnO NPs and their released Zn ions at some extent.	[111]
11.	10 nm	500 mg/kg of soil	NPs mixed with soil matrix	*Glycine max*	Accumulation, Speciation of NPs	NPs not present and accumulated in tissues and grains, but Zn present in grain Plant translocate the form of O-bound Zn which resembles with Zn-citrate, Conc. of Zn in nodule epidermis and outer pod ↑, Most of Zn found in the phloem	Phytochelatins are the identified indicators of toxicity generated by heavy metasl in plants thus their biosynthesis can also be induced by NPs and released metal ions	[36]
12.	34 nm, Hexagonal wurtzite structure	0, 0.1, 1.0, 10, 100 or 1000 mg NPs/L of MS medium containing 3% (w/v) sucrose without any plant growth regulators	Shoot nodes (cut out from 4 weeks old plant) incubated in MS medium having NPs for 4 weeks	*Stevia rebaudiana*	Growth parameters, Shoot formation %, Shoot length, Fresh weight of produced shoots (*in vitro*), Number of leaves, Antioxidant activities, Production of Steviol glycosides (rebaudioside A and stevioside)	At 1 mg/L conc. of NPs- shoot formation % (89.6) highest, steviol glycosides ↑ (~ 2 × control), ROS production, DPPH scavenging activity, total reducing power, total antioxidant capacity, total phenolic and flavonoid contents ↑, Above 1 mg /L conc. of NPs- Physiological parameters, formation of secondary metabolites, antioxidant activities ↓, At 1000 mg/L conc. of NPs- Phytotoxicity maximum	Addition of NPs into MS medium released free radicals or ions of related metal resulting in oxidative stress.	[90]
13.	50 nm, Nearly spherical shape, Aggregated highly	2000 mg/kg of soil	NPs mixed with pot soils	*Cucumis sativus*	Root and shoot length, Biomass, Bioaccumulation, Soil dehydrogenase, Acid phosphatase, β-glucosidase	Root length, shoot length and biomass (-), Zn conc. in plants tissues and soil ↑, Activity of each tested enzymes ↓	Immobilisation and aggregation of NPs occur in the soil.	[112]

(Table 3) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanisms/ possible hypothesis	Referances
14.	<100 nm	25, 50, 75, and 100 g/ml in milli-Q water	Hydroponic culture, Root exposed for 4 h	*Allium cepa*	Cytogenetic and genotoxic effects, Mitotic index (MI), Lipid peroxidation (in cells of root), Chromosomal aberration index, Micronuclei index (MN index)	Internalization of ZnO NPs like particles, With increasing conc. of NPs- No. of pycnotic cells, indices of chromosomal aberrations and MN ↑, MI ↓	Internal physicochemical environment of cell could induce agglomeration of internalized NPs, These NPs can stimulate cytotoxic and genotoxic/clastogenic effects in exposed cells	[113]
15.	< 100 nm, Surface area 15–25 m2/g	100 mg/L in liquid MS media	Seedlings grown in MS medium for 3 weeks, then moved onto rafts in Magenta Boxes having 50 mL of liquid MS media with NPs, 7d exposure period	*Arabidopsis thaliana*	Gene expression in plant roots, Biomass	Plant biomass ↓, 660 up- and 826 down-regulated genes, Up-regulated genes- ontology groups related with stress responsive stimuli, Other up-regulated genes- genes related with functional categories (*e.g.* transport, signal transduction, developmental processes), Down-regulated genes- genes related with cell organization and biogenesis process like translation, microtubule and nucleosome assembly	Exposure of ZnO NPs significantly influenced genes related to response against environmental stimuli and stress.	[74]
16.	21.3 nm	Seed priming at 20, 40 and 60 mg/L NPs conc. for 12 hrs (marked as ZNPs1, ZNPs2 and ZNPs3 respectively)	Seeds (with or without NPs priming at various conc.) sown in plastic pots and exposed to 150 mM NaCl for 20 d	*Lupinus termis*	Root/shoot length, Fresh and dry weights of full-length plants, Photosynthetic pigment contents, Soluble sugar and protein, Proline contents, Total free amino acids, MDA contents, Antioxidant enzyme activities, Total phenols, Ascorbic acid contents, Na and Zn contents	Root length, shoot length, fresh weight and dry weight ↑- ZNPs3 > ZNPs2 > ZNPs1, Photosynthetic pigments, total free amino acids, soluble sugar and protein, total phenols, proline contents, antioxidant enzyme activities, ascorbic acid contents and Zn contents ↑, Na and MDA contents ↓	NPs- priming of seeds induces osmotic adjustments eventually improving salt tolerance of cells, These results were obtained because Zn plays important roles in various cell-mechanisms.	[114]
17.	30±5 nm, Surface area of 29 ± 1 m2/g, Crystalline phase Zincite, Nearly spherical shape, (+)ive surface charges at neutral pH, Aggregated	0, 2, 5, 10, 15, 20, 40, 60, 80 and 100 mg/L suspensions in 1% modified Hoagland solution	Hydroponic culture, Initially seedlings grown on 50% Haogland solution without NPs for 7d then transferred on NPs suspensions	*Zea mays,*	Uptake, Speciation, Accumulation of NPs	Zn metal accumulated in treated plants as Zn phosphate, NPs present in the epidermis, cortex, root tip cells, vascular system which probably entered from primary root–lateral root junctions, but not translocated to shoot	NPs dissolution in exposure medium enhanced by root metabolic activities, Biotransformation of ZnO NPs to Zn phosphate occurs in plant body.	[37]

(Table 3) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanisms/ possible hypothesis	Referances
18.	20 nm, Monodispersed with a narrow size distribution, Near spherical morphology, hexagonal, Crystalline nature	0, 10, 20, 50, 100, 500, 1000, and 2000 ppm for *Vigna radiata*, 0, 1, 2, 5, 10, 20, 50, 100, 500, 1000, and 2000 ppm for *Cicer arietinum* Suspensions prepared in DI water	Dual agar culture media (20mL, 2.5% agar + 10mL, 1% agar) + NPs suspensions, Exposure time - 60 h	*Vigna radiata, Cicer arietinum*	Root growth, Shoot growth	Adsorption of NPs on root surface, Maximum effects at 20 ppm conc.-for *Vigna radiate* and at 1 ppm conc.-for *Cicer arietinum,* At higher conc. - growth ↓	Uptake of NPs occurs by roots where it becomes accumulated.	[115]
19.	< 50 nm, Aggregation	5, 10, 25, 50, 75, 100, 125, 250, and 500 mg /L Suspensions in deionized water (DI)	Seeds soaked in suspensions for 2 h then transferred to Petri plates containing filter paper moisten with test media	*Brassica napus*	Germination, Root and shoot lengths, Dry weight of shoot (DWS) and root (DWR)	Germination percentage (-), Root and shoot length ↓, Root elongation affected more than shoot elongation, At some initialconc.- DWS ↑, DWR (-) but at higher conc.-DWR ↓	Germination remained unaffected because seed coat prohibited the entry of chemical substances, After radicles' emergence from seeds, phytotoxic effects were observed because of interaction of NPs with roots which induces dissolution of Zn from NPs	[116]
20.	20-45 nm, Uncoated, Rod shaped/ cuboidal/ spherical/ rectangular shaped	0, 20, 50, 100 and 200 mg/L in MS medium (half strength)	Seeds sown on NPs containing MS medium (half-strength)	*Arabidopsis thaliana*	Shoot and root Zn accumulation, Macro and micronutrients (P, K, S, Cu, Zn and Fe) contents, Regulation of transcription of genes related with auxin regulation and elemental homeostasis, Morphological studies	Zn mainly present in root tips as well as junctions of root-shoot and primary-lateral roots, but root to shoot translocation absent, At 20 mg/L conc.- lateral root formation ↑ (9%), At other conc.- fresh weight and length of primary root ↓, At > 200 mg/L conc.- highest reduction of P, K, S and Cu contents, At all higher conc.- leaf size reduced and chlorosis observed	NPs and their released metal ions follow different mechanism of toxicity like NPs may block nutrient uptake by roots and its growth as well whereas overindulgence of Zn can compete with other metal ions, in turn distinct patterns of root architecture can be formed due to deficiencies of P, K and S	[117]
21.	10 nm	50, 100 and 500 mg/kg of soil	NPs mixed with soil ~24 h before planting	*Glycine max*	Conc. of Zn in plant and soil, Bioaccumulation factors in pods, Micro and macro nutrient accumulation	Altered nutritional value of soybean, Zn accumulation in all analyzed organs ↑, At ≥ 100 mg/kg conc.- more Cu, Zn and Mn contents than control in pods, Significant correlations among P, S and Zn in pods with Zn in roots	Nitrogen assimilation could be improved in soybean plants by NPs as it dissolved Zn mostly in ionic form in soil solution	[62]

(Table 3) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanisms/ possible hypothesis	Referances
22.	10 nm, Procured as an aqueous suspension	0.01–1000 µg/mL suspensions in moderately hard water (MHW)	Seedling grown in Petri plates having filter paper (moisten with 3 ml nanopure water and 5 ml test solution)	*Zea mays, Brassica oleracea*	Germination rate, Root growth, Moisture content, Biouptake of metal, Primary root morphology and anatomical studies	In cabbage- germination ↓ (dose dependent), Water stress nil, In maize- Seed germination (-), Primary cells of elongation zone of roots modified in shape/size, 'Tunneling-like effect' in root apical meristem	Plant growth and development affected functionally due to metal biouptake	[118]
23.	25 nm, Crystalline, Slightly aggregated	400, 1000 and 2000 ppm (conc. referred to in terms of zinc content)	Seeds soaked in NPs suspension for 3 h and then devided into 2 sets: Set1- in Petriplate for germination and seedling vigor index Set2- in pot for rest studies Foliar application of NPs	*Arachis hypogaea*	Germination, Root growth, Seedling vigor, Chl content, Plant growth, Flowering, Pod yield,	At 1000 ppm conc.- Germination, stem/ root growth, seedling vigor index, chl- contents, pod yield per plant ↑, Establishment in soil and flowering- early, At 2000 ppm conc.- Inhibitory effects, For foliar application- dose of NPs = dose of Bulk/ 15 (recommended)	Micronutriuent, Zn delivered into seeds through NPs, Stomatal pathways are fundamentally different from cuticular pathways, giving different results when NPs applied by different ways	[119]
24.	1.2 and 6.8 nm, Monodispersed, Spherical and hexagonal, NPs samples having 98% atom of Zn element	10 ppm	Foliar sprayed on leaf of 14d pot cultured seedlings	*Cyamopsis tetragonoloba*	Root/ shoot lengths, Plant biomass, Root area, Chl-contents, Total soluble leaf proteins, Native phosphorous mobilizing enzymes, Gum production	Root/ shoot lengths, biomass, root area, Chl- content, total soluble leaf protein, seed gum contents, microbial population in rhizosphere and activities of rhizosphere enzymes (acid and alkaline phosphatases and Phytase) ↑	Leaf openings like stomata provide entry to the adsorbed NPs on leaves depending upon size and surface properties of NPs, Through cortex and central cylinder NPs enter into xylem may become accumulated in vacuole	[120]
25.	4 nm Spherical, Well dispersed	10, 20, 30, 40, 50 mg/L in DI water	Germinated garlic bulbs in DI water till 2.0 cm long redicals, Then directly placed on NPs suspensions for 24 h, Again transferred to DI water	*Allium sativum*	Root apical meristem mitosis, Root growth, Mitotic aberrations	Root length (dose dependant) ↓, At 50 mg/L conc.- Root growth blocked, Estimated IC_{50} - 15 mg/L, Mitosis index ↓, Total percentage of abnormal cells (conc. and time dependant) and mitotic aberrations (chromosome bridges, stickiness, laggings and breakages) ↑	NPs induced genotoxic effects in Garlic plants, It attributed to two different actions- (i) Release of (toxic) ions, (ii) Stress generation by NPs	[121]
26.	5 to 20 nm, Nearly spherical	100, 200µM in nutrient solution	8d grown seedlings transferred to 1/2-strength Hoagland's nutrient solution for 7d, Further shifted in NPs containing nutrient solution for 7d	*Triticum aestivum*	H_2O_2 contents, Lipid peroxidation, Photosynthetic pigments, Chl- fluorescence, Activities of enzymes of Ascorbate-Glutathione cycle (AsA-GSH) and contents of associated metabolites, Zn contents in phloem and xylem saps, Effect of NO on NP-phytotoxicity	Growth of seedlings, efficiency of photosynthesis, activities of AsA–GSH enzymes and Associated metabolites (ascorbate and glutathione) ↓, H_2O_2 level and lipid peroxidation ↑, NO reduces ZnONPs toxicity	Higher accumulation of Zn in phloem and xylem saps induces toxicity which can be reduced by NO treatment as it regulates Zn-accumulation and functioning of AsA–GSH cycle	[122]

(Table 3) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanisms/ possible hypothesis	Referances
27.	30 nm, Specific surface area $70m^2/g$	0.16, 0.8, 4, 20, and 100mg/L of cultivation media	Seeds cultivated in hydroponic conditions (25% Hoagland solution) for 4 weeks, Then exposed to NPs at various conc, for 2 weeks	*Arabidopsis thaliana*	Contents of auxins, cytokinins, abscisic acid, salicylic acid and jasmonic acid in roots, apical meristem and leaves	Cytokinins and auxins (growth promoting hormones) in shoot apical meristems ↓, Salicylic acid in leaves and roots↑, At 20 and 100mg/L ZnO NPs- cis-zeatin (cytokinin associated with stress response) in roots ↑, At higher conc. of NPs- abscisic acid (stress hormone) in apices and leaves↑, jasmonic acid (stress hormone) and its active metabolite jasmonate isoleucine ↓	NPs generate severe stress in plants where higher stress resistance in roots induced due to local accumulation of cis-zeatin	[123]
28.	Biosynthesized crystalline natures, Spherical and rod shaped, 2-54 nm	25, 50, 75, 100, and 200 mg/L Hoagland medium	Several days, soilrite grown seedlings transferred to hydroponic culture medium (Hoagland) for 1 week, Then different doses of NPs added to medium, Both treatments (NPs and control) amended with phosphorus (100 mM)	*Gossypium hirsutum*	Seedlings growth and biomass, MDA contents, Total soluble protein contents, Photosynthetic pigment contents, Antioxidant enzyme activities- SOD, POX, CAT, Isoenzymes expression pattern	Growth and total biomass, level of chl-a, b, carotenoids, total soluble protein contents, SOD and POX ↑, MDA contents in leaves and CAT ↓, NPs treatments changed expression patterns of isoenzymes.	Interaction of bioengineered NPs with meristematic cells triggers biochemical pathways which promoted growth whereas higher activities of SOD and POX enzymes help plant to fight against ROS production.	[124]
29.	Phycomolecules coated, Hexagonal, square and spherical shape, 2-64 nm, Crystalline nature	25 mg/L ZnSO4; 25 mg/L ZnONPs; 50 mg/L Cd; 50 mg/L Cd+25 mg/L ZnONPs; 100 mg/L Pb; 100 mg/L Pb+25 mg/L ZnONPs in Hoagland's medium	Seedlings transferred to Hoagland's medium in hydroponic system for 5 d, Then treated with optimum conc.of Cd and Pb ions (50 mg/L and 100 mg/L) and NPs in 7 types of combinations for 15 d	*Leucaena leucocephala*	Growth and plant biomass, Cd and Pb contents, Lipid peroxidation, Photosynthetic pigment contents, Antioxidant enzyme activity: SOD, CAT, POX, Detection of isoenzymes, Total soluble protein contents, Isolation of genomic DNA and RAPD-PCR	Root/ shoot length, biomass, growth tolerance index, photosynthetic pigment contents, Cd and Pb accumulation, total soluble protein contents, activities of SOD, CAT and POX ↑, MDA content in leaves ↓, Many bands of DNA disappeared and some new bands (amplicons) appeared in RAPD pattern	NPs induced genotoxicity explaining the absence of many normal bands of DNA in RAPD pattern whereas probably new bands were appeared due to mutation, NPs having coating of phycomolecules might activate genetic changes and many biochemical pathways in the desired way to diminish oxidative stress and cellular damages produced by heavy metals like Cd and Pb.	[125]

(Table 3) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanisms/ possible hypothesis	Referances
30.	Product reported particle size < 100 nm, < 35 nm (DLS), Estimated Particle size 67±2 nm	For solution culture: 25 mg Zn /L in nutrient solution, For soil culture: 500 mg Zn/kg soil	Solution or soil culture of plants with NPs amended growth matrix	*Vigna unguiculata*	Uptake of Zn and its transformation in tissues	Plants grown in soil (-), Toxicity in solution culture- Soluble Zn ($ZnCl_2$) > ZnO-NPs, NPs accumulated on root surfaces considerably, but not translocated from roots to shoots, On both soluble Zn and NPs treatments- Speciation of Zn in shoot tissues was the same.	After amendment in to soil, NPs rapidly show dissolution.	[126]
31.	< 100 nm, In water- rhomboid shaped, In aqueous phase of sand- elongated rods	125, 250 and 500 mg Zn/kg of soils	2 types of soils (acidic and calcareous alkaline) mixed with NPs	*Triticum aestivum*	Plant growth, Variation in phytotoxicity with soil properties, Solubility of Zn, Shoot uptake	Zn uptake and accumulation ↑, In acid soil - Root elongation ↓, Phytotoxicity ↑, In calcareous alkaline soil - Lateral root production ↑, Phytotoxicity ↓, Soluble Zn in soil- Acidic soil > alkaline soil, Soluble Zn in shoot- Acidic soil > alkaline soil	Soil properties influenced the phytotoxicity induced by ZnO NPs and soluble Zn in treated seedlings	[127]
32.	<100 nm,	500, 1000, and 1500 mg/L suspensions in DW used to prepare plain agar medium, For *in vitro* studies- 1, 5, 10 and 20 mg NPs /L of MS medium	Seeds inoculated on NPs amended plain agar medium for 14 d (germination and growth studies), Then stem pieces (explants) excised from seedlings and cultured on MS medium having NPs	*Brassica nigra*	Germination, Growth, Antioxidative potential, Total antioxidant and reducing power, Total flavonoid and phenolic contents	Germination, root growth, shoot fresh weight ↓, Shoot growth, antioxidative activities and contents of phenolics and flavonoids ↑, At lower conc. (1–20 mg/L)- thin roots (white) with thick root hairs	After entering into seeds NPs might be aggregated or show dissolution of Zn ions causing germination inhibition, At cellular level ROS and Ca^{+2} signaling might be induced by NPs eventually affecting organism physiologically, ZnO NPs have capability to induce roots from explants if culture conditions are appropriate	[128]
33.	380 nm (in DI water) to 1116 nm (in 100 mM NaCl), Zeta potential- +21 mV (in DI water) and negative (in NaCl)	100, 200, 400, and 800 mg NPs/kg of soil	Z n/ZnO NPs in sandy loam soil, Plants grown for 30d	*Zea mays*	Deposition of NPs and its release, Distribution of Zn in soil, Uptake mechanism, Distribution of NPs in tissues of root and leaf	Low mobility of NPs in soil column of different ionic strengths, Released Zn penetrated together with Fe and Al, Translocation and bioaccumulation Zn observed high, NPs aggregates were present in root epidermis, cortex and xylem vessels	The used experiment soil exhibits low environmental dispersion of ZnO NPs, but its colloids might help in the penetration of adsorbed NPs as Zn/ZnO NPs aggregates observed allied with minerals of soil, Aggregates passed epidermis by apoplastic and endodermis by symplastic pathway.	[129]

(Table 3) cont.....

S. N.	NP specifications	Range of conc.	Exposure method/ application method	Plant species	Studied endpoints/ parameters	Remarkable effects	Expected mechanisms/ possible hypothesis	Referances
34.	10±1nm	0, 400, and 800 mg/kg of soil	Organic rich soil amended with NPs, Plants were grown for 53d	*Cucumis sativus*	Chlorophyll contents, Gas exchange, Bioaccumulations	Plant growth, gas exchange, Chl content (-), At high conc.- Bioaccumulation of Zn in the fruit, Conc. of Zn in tissues- root > leaf > stem > fruit.	NPs are not generating stress	[130]
35.	10 nm	400 and 800 mg/kg of soil	Soil treated with NPs	*Cucumis sativus*	Total soluble and reducing sugars, starch, proteins, mineral nutrients, Total phenolics and flavonoids and antioxidants in the fruit	Starch and protein content ↑, Conc. of micronutrients Cu and Mo ↓	Probably NPs induced stress in the plants in turn the contents of starch and protein become increased.	[131]
36.	24 ± 3 nm	0, 400, and 800 mg/kg of soil	Soil amended with NPs	*Zea mays*	Bioaccumulation of Zn in tissues, Nutrient conc. and distribution in ears, Gas exchange in leaves, Relative chl contents, Shoot length, Dry weight of roots and leaves, No of leaves and ears, Area of leaf, Fresh and dry weight of ear	At 800 mg/kg conc.- Relative chl contents, net photosynthetic rate and stomatal conductance ↓, Yield ↓, Quality of corn altered	The expression of transcripts of plant growth regulators might be down regulated by NPs, Treatment of NPs has an influence on elemental translocation in reproductive structures as well as in silks ripening which directly affect yield.	[132]

(↑= increased, ↓=decreased, (-)= not significantly affected, DW= distilled water, DDW= double distilled water, DIW= deionized water, MS= Murashige and Skoog, Chl= chlorophyll, h= hours, D=days).

Table 4. The (Fig. 2) was made on the basis of this table. Table shows the complied data of explored plant species along with their taxonomic categorization and references. A research article may contain more than one plant or type of selected MONPs but we considered one plant or type of NPs as one case and given a separate row in the table. For the purpose, the total selected research articles were 108 (32, 42 and 48 articles for CuO, TiO_2 and ZnO NPs respectively including their common articles also) on random basis, having total 150 plant cases. All 150 cases belong to only 14 families and 50 terrestrial plant species, as explored repeatedly.

S.N.	Tested MO NPs	Plant	Angiosperm or Gymnosperm	Monocot or Dicot	Family	References
1.	CuO	*Spinacia oleracea*	Angiosperm	Dicot	Amaranthaceae	[102]
2.	CuO	*Lactuca sativa*	Angiosperm	Dicot	Asteraceae	[89]
3.	CuO	*Lactuca sativa*	Angiosperm	Dicot	Asteraceae	[103]
4.	CuO	*Lactuca sativa*	Angiosperm	Dicot	Asteraceae	[104]
5.	CuO	*Stevia rebaudiana*	-	Dicot	Asteraceae	[90]
6.	CuO	*Arabidopsis thaliana*	Angiosperm	Dicot	Brassicaceae	[91]
7.	CuO	*Arabidopsis thaliana*	Angiosperm	Dicot	Brassicaceae	[93]
8.	CuO	*Arabidopsis thaliana*	Angiosperm	Dicot	Brassicaceae	[96]
9.	CuO	*Brassica nigra*	Angiosperm	Dicot	Brassicaceae	[106]

(Table 4) cont.....

S.N.	Tested MO NPs	Plant	Angiosperm or Gymnosperm	Monocot or Dicot	Family	References
10.	CuO	*Brassica oleracea*	Angiosperm	Dicot	Brassicaceae	[105]
11.	CuO	*Raphanus sativus*	Angiosperm	Dicot	Brassicaceae	[56]
12.	CuO	*Raphanus sativus*	Angiosperm	Dicot	Brassicaceae	[104]
13.	CuO	*Sinapis alba*	Angiosperm	Dicot	Brassicaceae	[33]
14.	CuO	*Cucumis sativus*	Angiosperm	Dicot	Cucurbitaceae	[104]
15.	CuO	*Cucumis sativus*	Angiosperm	Dicot	Cucurbitaceae	[95]
16.	CuO	*Cumumis sativus*	Angiosperm	Dicot	Cucurbitaceae	[32]
17.	CuO	*Glycine max*	Angiosperm	Dicot	Fabaceae	97]
18.	CuO	*Medicago sativa*	Angiosperm	Dicot	Fabaceae	[89]
19.	CuO	*Phaseolus vulgaris*	Angiosperm	Dicot	Fabaceae	[88]
20.	CuO	*Trifolium alexandrinum*	Angiosperm	Dicot	Fabaceae	[40]
21.	CuO	*Trigonella foenum-graecum*	Angiosperm	Dicot	Fabaceae	[85]
22.	CuO	*Gossypium hirsutum*	Angiosperm	Dicot	Malvaceae	[34]
23.	CuO	*Fagopyrum esculentum*	Angiosperm	Dicot	Polygonaceae	[94]
24.	CuO	*Avena sativa*	Angiosperm	Monocot	Poaceae	[40]
25.	CuO	*Hordeum vulgare*	Angiosperm	Monocot	Poaceae	[86]
26.	CuO	*Hordeum vulgare*	Angiosperm	Monocot	Poaceae	101]
27.	CuO	*Lolium perenne*	Angiosperm	Monocot	Poaceae	[56]
28.	CuO	*Lolium rigidum*	Angiosperm	Monocot	Poaceae	[56]
29.	CuO	*Oryza sativa*	Angiosperm	Monocot	Poaceae	[87]
30.	CuO	*Oryza sativa*	Angiosperm	Monocot	Poaceae	99]
31.	CuO	*Oryza sativa*	Angiosperm	Monocot	Poaceae	[98]
32.	CuO	*Oryza sativa*	Angiosperm	Monocot	Poaceae	[100]
33.	CuO	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[55]
34.	CuO	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[57]
35.	CuO	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[10]
36.	CuO	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[30]
37.	CuO	*Triticum vulgare*	Angiosperm	Monocot	Poaceae	[92]
38.	CuO	*Zea mays*	Angiosperm	Monocot	Poaceae	[31]
39.	TiO_2	*Petroselinum crispum*	Angiosperm	Dicot	Apiaceae	[68]
40.	TiO_2	*Lactuca sativa*	Angiosperm	Dicot	Asteraceae	[75]
41.	TiO_2	*Lactuca sativa*	Angiosperm	Dicot	Asteraceae	[81]

(Table 4) cont.....

S.N.	Tested MO NPs	Plant	Angiosperm or Gymnosperm	Monocot or Dicot	Family	References
42.	TiO_2	*Lactuca sativa*	Angiosperm	Dicot	Asteraceae	[104]
43.	TiO_2	*Arabidopsis thaliana*	Angiosperm	Dicot	Brassicaceae	[22]
44.	TiO_2	*Arabidopsis thaliana*	Angiosperm	Dicot	Brassicaceae	[74]
45.	TiO_2	*Arabidopsis thaliana*	Angiosperm	Dicot	Brassicaceae	[82]
46.	TiO_2	*Arabidopsis thaliana*	Angiosperm	Dicot	Brassicaceae	[53]
47.	TiO_2	*Arabidopsis thaliana*	Angiosperm	Dicot	Brassicaceae	[134]
48.	TiO_2	*Brassica campestris*	Angiosperm	Dicot	Brassicaceae	[81]
49.	TiO_2	*Brassica napus*	Angiosperm	Dicot	Brassicaceae	[21]
50.	TiO_2	*Raphanus sativus*	Angiosperm	Dicot	Brassicaceae	[104]
51.	TiO_2	*Sinapis alba*	Angiosperm	Dicot	Brassicaceae	[33]
52.	TiO_2	*Cucumis sativus*	Angiosperm	Dicot	Cucurbitaceae	[79]
53.	TiO_2	*Cucumis sativus*	Angiosperm	Dicot	Cucurbitaceae	[25]
54.	TiO_2	*Cucumis sativus*	Angiosperm	Dicot	Cucurbitaceae	[48]
55.	TiO_2	*Cucumis sativus*	Angiosperm	Dicot	Cucurbitaceae	[104]
56.	TiO_2	*Cicer arietinum*	Angiosperm	Dicot	Fabaceae	[63]
57.	TiO_2	*Cicer arietinum*	Angiosperm	Dicot	Fabaceae	[77]
58.	TiO_2	*Glycine max*	Angiosperm	Dicot	Fabaceae	[80]
59.	TiO_2	*Phaseolus vulgaris*	Angiosperm	Dicot	Fabaceae	[27]
60.	TiO_2	*Phaseolus vulgaris*	Angiosperm	Dicot	Fabaceae	[81]
61.	TiO_2	*Trifolium alexandrinum*	Angiosperm	Dicot	Fabaceae	[40]
62.	TiO_2	*Trifolium pretense*	Angiosperm	Dicot	Fabaceae	[78]
63.	TiO_2	*Vicia faba*	Angiosperm	Dicot	Fabaceae	[67]
64.	TiO_2	*Vicia narbonensis*	Angiosperm	Dicot	Fabaceae	[65]
65.	TiO_2	*Vicia narbonensis*	Angiosperm	Dicot	Fabaceae	[66]
66.	TiO_2	*Vigna radiata*	Angiosperm	Dicot	Fabaceae	[50]
67.	TiO_2	*Ocimum basilicum*	Angiosperm	Dicot	Lamiaceae	[83]
68.	TiO_2	*Linum usitatissimum*	Angiosperm	Dicot	Linaceae	[54]
69.	TiO_2	*Rumex crispus*	Angiosperm	Dicot	Polygonaceae	[27]
70.	TiO_2	*Salix eriocephala*	Angiosperm	Dicot	Salicaceae	[135]
71.	TiO_2	*Lycopersion esculentum*	Angiosperm	Dicot	Solanaceae	[28]
72.	TiO_2	*Nicotiana tabacum*	Angiosperm	Dicot	Solanaceae	[52]
73.	TiO_2	*Nicotiana tabacum*	Angiosperm	Dicot	Solanaceae	[51]
74.	TiO_2	*Allium cepa*	Angiosperm	Monocot	Amaryllidaceae	[51]

(Table 4) cont.....

S.N.	Tested MO NPs	Plant	Angiosperm or Gymnosperm	Monocot or Dicot	Family	References
75.	TiO_2	*Allium cepa*	Angiosperm	Monocot	Amaryllidaceae	[72]
76.	TiO_2	*Avena sativa*	Angiosperm	Monocot	Poaceae	[40]
77.	TiO_2	*Hordeum vulgare*	Angiosperm	Monocot	Poaceae	[73]
78.	TiO_2	*Hordeum vulgare*	Angiosperm	Monocot	Poaceae	[76]
79.	TiO_2	*Oryza sativa*	Angiosperm	Monocot	Poaceae	[64]
80.	TiO_2	*Oryza sativa*	Angiosperm	Monocot	Poaceae	[70]
81.	TiO_2	*Oryza sativa*	Angiosperm	Monocot	Poaceae	[84]
82.	TiO_2	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[58]
83.	TiO_2	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[27]
84.	TiO_2	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[71]
85.	TiO_2	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[26]
86.	TiO_2	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[21]
87.	TiO_2	*Triticum. aestivum*	Angiosperm	Monocot	Poaceae	[69]
88.	TiO_2	*Zea mays*	Angiosperm	Monocot	Poaceae	[65]
89.	TiO_2	*Zea mays*	Angiosperm	Monocot	Poaceae	[47]
90.	ZnO	*Spinacia oleracea*	Angiosperm	Dicot	Amaranthaceae	102]
91.	ZnO	*Stevia rebaudiana*	Angiosperm	Dicot	Asteraceae	[133]
92.	ZnO	*Arabidopsis thaliana*	Angiosperm	Dicot	Brassicaceae	[74]
93.	ZnO	*Arabidopsis thaliana*	Angiosperm	Dicot	Brassicaceae	[117]
94.	ZnO	*Arabidopsis thaliana*	Angiosperm	Dicot	Brassicaceae	[123]
95.	ZnO	*Brassica napus*	Angiosperm	Dicot	Brassicaceae	[116]
96.	ZnO	*Brassica nigra*	Angiosperm	Dicot	Brassicaceae	[128]
97.	ZnO	*Brassica oleraceae*	Angiosperm	Dicot	Brassicaceae	[118]
98.	ZnO	*Sinapis alba*	Angiosperm	Dicot	Brassicaceae	[33]
99.	ZnO	*Cucumis sativus*	Angiosperm	Dicot	Cucurbitaceae	[112]
100.	ZnO	*Cucumis sativus*	Angiosperm	Dicot	Cucurbitaceae	[32]
101.	ZnO	*Cucumis sativus*	Angiosperm	Dicot	Cucurbitaceae	[130]
102.	ZnO	*Cucumis sativus*	Angiosperm	Dicot	Cucurbitaceae	[131]
103.	ZnO	*Cucurbita pepo*	Angiosperm	Dicot	Cucurbitaceae	[137]
104.	ZnO	*Arachis hypogaea*	Angiosperm	Dicot	Fabaceae	[119]
105.	ZnO	*Cajanus cajan*	Angiosperm	Dicot	Fabaceae	[107]
106.	ZnO	*Cicer arietinum*	Angiosperm	Dicot	Fabaceae	[60]
107.	ZnO	*Cicer arietinum*	Angiosperm	Dicot	Fabaceae	115]

(Table 4) cont.....

S.N.	Tested MO NPs	Plant	Angiosperm or Gymnosperm	Monocot or Dicot	Family	References
108.	ZnO	*Cicer arietinum*	Angiosperm	Dicot	Fabaceae	[61]
109.	ZnO	*Cyamopsis tetragonoloba*	Angiosperm	Dicot	Fabaceae	[120]
110.	ZnO	*Glycine max*	Angiosperm	Dicot	Fabaceae	[107]
111.	ZnO	*Glycine max*	Angiosperm	Dicot	Fabaceae	[36]
112.	ZnO	*Glycine max*	Angiosperm	Dicot	Fabaceae	[134]
113.	ZnO	*Glycine max*	Angiosperm	Dicot	Fabaceae	[62]
114.	ZnO	*Leucaena leucocephala*	Angiosperm	Dicot	Fabaceae	[125]
115.	ZnO	*Lupinus termis*	Angiosperm	Dicot	Fabaceae	[114]
116.	ZnO	*Phaseolus vulgaris*	Angiosperm	Dicot	Fabaceae	[88]
117.	ZnO	*Phaseolus vulgaris*	Angiosperm	Dicot	Fabaceae	[110]
118.	ZnO	*Prosopis juliflora*	Angiosperm	Dicot	Fabaceae	[111]
119.	ZnO	*Trifolium alexandrinum*	Angiosperm	Dicot	Fabaceae	[40]
120.	ZnO	*Vigna radiata*	Angiosperm	Dicot	Fabaceae	[115]
121.	ZnO	*Vigna unguiculata*	Angiosperm	Dicot	Fabaceae	[126]
122.	ZnO	*Abelmoschus esculentus*	Angiosperm	Dicot	Malvaceae	[107]
123.	ZnO	*Gossypium hirsutum*	Angiosperm	Dicot	Malvaceae	[124]
124.	ZnO	*Fagopyrum esculentum*	Angiosperm	Dicot	Polygonaceae	[94]
125.	ZnO	*Solanum lycopersicon*	Angiosperm	Dicot	Solanaceae	110]
126.	ZnO	*Allium cepa*	Angiosperm	Monocot	Amaryllidaceae	[108]
127.	ZnO	*Allium cepa*	Angiosperm	Monocot	Amaryllidaceae	[59]
128.	ZnO	*Allium cepa*	Angiosperm	Monocot	Amaryllidaceae	[113]
129.	ZnO	*Allium sativum*	Angiosperm	Monocot	Amaryllidaceae	[121]
130.	ZnO	*Avena sativa*	Angiosperm	Monocot	Poaceae	[40]
131.	ZnO	*Hordeum vulgare*	Angiosperm	Monocot	Poaceae	86]
132.	ZnO	*Lolium perenne*	Angiosperm	Monocot	Poaceae	[35]
133.	ZnO	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[57]
134.	ZnO	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[10]
135.	ZnO	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[58]
136.	ZnO	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[109]
137.	ZnO	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[122]
138.	ZnO	*Triticum aestivum*	Angiosperm	Monocot	Poaceae	[127]
139.	ZnO	*Zea mays*	Angiosperm	Monocot	Poaceae	[107]
140.	ZnO	*Zea mays*	Angiosperm	Monocot	Poaceae	[37]

(Table 4) cont.....

S.N.	Tested MO NPs	Plant	Angiosperm or Gymnosperm	Monocot or Dicot	Family	References
141.	ZnO	*Zea mays*	Angiosperm	Monocot	Poaceae	[118]
142.	ZnO	*Zea mays*	Angiosperm	Monocot	Poaceae	[129]
143.	ZnO	*Zea mays*	Angiosperm	Monocot	Poaceae	[132]

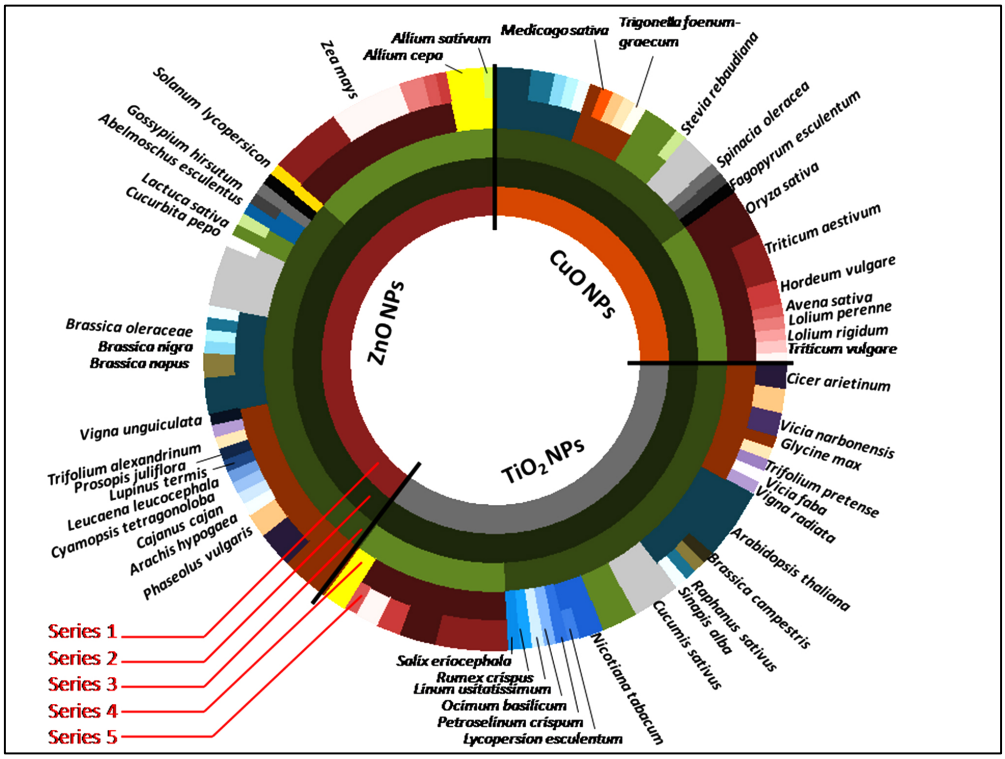

Fig. (2). Categorization of explored terrestrial plants for phytotoxicity studies under exposure of MO NPs (*i.e.* TiO_2, CuO, ZnO NPs). Here, series 1; 2; 3; 4 and 5 represent to the type of MO NP; category as angiosperm or gymnosperm; clade as monocot or dicot; taxonomic family and plant species respectively, with color codes. The same color is used for the same category in each series. In series 5, different shades of the same color indicate related species as having same taxonomic family (series 4). In series 3, dark shade was used for dicot and lighter one for monocot. In series 1, the length of bands is sign of the number of plant cases for each MO NP. To check their references one can go with the Table **4**.

FUTURE PROSPECTS

Undoubtedly, nanotechnology is rapidly encroaching all the areas with promising features of NMs but their underexplored impact on agriculture and plant system cannot be ignored. Here some problems and possibility of this field are being discussed.

1. However, numerous studies have been conducted on the phytotoxic effects of NPs, yet research intended towards the recognition of the beneficial effects of NPs on plants and agriculture remains inadequate.

2. Intrusion of nanotechnology in the field of tissue culture can generate some revolutionary results, but this field is almost untouched till now.

3. Moreover, nanotechnology may alter the secondary metabolite production and stress tolerance capacity in plants, thereby, increasing benefits. Though, some reports are available on these perspectives, but these are not enough and conclusive.

4. There are plenty of conventional and transgenic varieties of plants with so many qualities, demanding rigorous research and collaboration of nanotechnology for their betterment.

5. As metal oxide NMs are identified for their metal ion toxicity, it is recommended to compare closely related species with known differences in metal tolerance on these NPs treatments. This approach may influence the field of nano-fertilizers significantly.

6. Published data on terrestrial phytotoxicity by MO NPs is increasing continuously but surprisingly the range of selected plants is still narrow (mostly agricultural crops and seed plants). The same situation is expected for other NMs too but it should be discouraged as the earth is full of diverse terrestrial plants and all have the possibility of unintentional exposure by NMs, thus random selection of plants (outside this narrow range) should be appreciated.

7. It is often being argued about the standard NPs- phytotoxicity assessment tests, having universal consideration and acceptance.

8. Until now, the most commonly analyzed parameters were germination, root elongation, shoot length, plant biomass *etc*. but as opposed by some researchers, these parameters are not precise enough or appropriate for evaluation of NP toxicity in terrestrial plant species.

9. The studies on the interaction of NMs with plant growth matrixes (*e.g.* various types of soils, growth medium *etc*.) and their effect on life cycle of plants are very scarce, hence, demand attention.

10. Numerous appreciable efforts on uptake, biotransformation or accumulation of NPs in plant body cannot be overlooked, but the need of the hour is to understand the mechanism of NMs in affecting food chains and ultimately human health.

11. From the gathered information, it is apparent that most of the investigations are based on simple morphological and biochemical studies. Therefore, intense and in-depth work needs to be done in order to explore the physiological, molecular and biochemical mechanisms of plants in relation to NPs.

12. Due to dynamic climatic conditions and varied soil types, plants experience a number of abiotic stresses which lead to huge crop losses. Limited reports are available on the mitigation of abiotic stresses using NPs. Intense efforts should be done in this direction to minimize crop loss and sustain the population.

13. Most of the studies are restricted to controlled conditions, but to get a more realistic picture of the effects of MO NPs on different life cycle stages and in turn on yield and crop quality, field trials need to be done. These studies will also prove quite beneficial in studying the long-term multigenerational impact of MO NPs treated plants.

These types of approaches will turn the situation and it endow with better understanding, as well as provide good command over the behavior of NPs into living plant systems and the environment.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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Declared none.

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CHAPTER 4

Role of Nanofertilizers in Agriculture-Futuristic Approach

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Abstract: Chemical fertilizers are crucial in the production of cost-effective agricultural crops. However, long-term usage of chemical fertilizers will deteriorate the soil quality and it is hazardous to human health. Scientists and researchers across the globe are seeking the help of nanotechnology as a possible solution to combat the hazardous effect of chemical fertilizers. Nanotechnology is a branch of science and engineering concerned with the matter at the nanoscale or one billionth of a meter. Nanofertilizers are modified fertilizers that are synthesized using techniques of nanotechnology involving various physicochemical and biological methods. These methods aid in enhancing their attributes and composition, which leads to a positive effect on crop productivity. Nanofertilizers are far more beneficial when compared to chemical fertilizers as the former are cost-effective, less toxic and show controlled and regulated release of nutrients to plants. This chapter is primarily concerned with the various methods employed in nanofertilizer synthesis, the economic importance of nanofertilizers and their advantage over conventional chemical fertilizers.

Keywords: Chemical fertilizers, Cost-effective, Nanofertilizers, Nanotechnology.

INTRODUCTION

Soil is a storehouse of nutrients that serve as the medium in which plants grow. Nutrients are lost from the soil in several ways such as crop harvest, weeds,

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leaching, volatilization, and erosion thereby affecting the fertility status of the soil, thus affecting productivity. Therefore, these losses when combined altogether, a significant amount of nutrients are lost from the soil such that the crop requirement exceeds the soil supplying power thus nutrients are applied from external sources [1].

The bush-fallow strategy, which allows arable land to switch back to fallow after 3-4 years of intensive cropping, was once the conventional technique of sustaining soil fertility and production when the human population was low. With the increasing growth of the human population and other socioeconomic demands, an attempt was made to substitute the fallow system with the use of manures, mainly where significant numbers of animals were present. This highlights the agricultural benefits of organic manures such as farmyard manure, compost, green manure, poultry droppings, cow dung, and household refuse, among others. Nowadays, agriculture became more demanding with the usage of crops giving high yields. But such crops require more nutrition to grow than the natural nutrients present in the soil. It became evident that manures could not fulfill the nutrient requirements of these crops for increased productivity, and could not be procured in adequate quantities to meet farmers' needs. Even when manure is readily accessible, transportation and labor costs (inevitably) restrict its frequent use. In this case, mineral fertilizers were considered a viable alternative [2].

Fertilizers, also referred to as inorganic fertilizers are the mineral source of plant nutrients that are industrially manufactured and their nutritional content is higher than that of farmyard manures and are almost released instantly, thus meeting the nutrient demand of the crop. Fertilizers supply macronutrients such as Nitrogen (N), Phosphorus (P) and Potassium (K) which are necessary for plant growth and development. Fertilizers also supply micronutrients such as Zinc (Zn), Sulphur (S), and Iron (Fe) for plant uptake and utilization in various metabolic processes. Fertilizers can be straight fertilizers (such as Urea, SSP and MoP) containing only one type of primary macronutrients or complex containing two or more primary macronutrients that are chemically bound together [3].

Commercial chemical fertilizers are expensive and include substances that are harmful to the skin or respiratory system. Because of their huge particle size and low solubility, they are less bioavailable to plants. Furthermore, they cause toxicity and disrupt the soil's ecological equilibrium. Implementing nanoparticles in sustainable agricultural practice might be defined as using modern and advanced agro-nanofertilizers over conventional fertilizers in a sequence of environmentally and farmer-friendly inputs [4].

CONVENTIONAL FERTILIZERS

Today's agriculture is growing increasingly intensive, requiring higher dosages of chemicals such as fertilizers, herbicides, and pesticides to achieve maximum productivity per unit area to fulfill the demands of an ever-increasing human population. These chemicals have with no doubt increased crop productivity but simultaneously usage of these chemicals is more than optimum, severely affecting natural resources and ecosystem services.

Challenges of Fertilizers In Present-Day Agriculture Practices

Fertilizers play a significant role in obtaining higher crop yields as they contribute up to 40-60% of agricultural productivity [5]. However, applying higher doses of fertilizer than optimum does not guarantee an increase in crop productivity rather it results in several problems such as soil health degradation, environmental pollution, multi-nutrient deficiency (especially micro-nutrients), element toxicity, rise in the cost of production, among others [6]. The use of inorganic fertilizer helps in increasing the yield of the crop but it increases the cost of production (cost of fertilizer plus cost of transportation) and applying higher doses leads to environmental pollution. In addition, the application of higher rates of chemical fertilizer leads to significant land problems as a result of over-exploitation of land and land pollution [7]. Furthermore, applying higher doses of fertilizer more than crop requirement leads to losses of nutrients through various sources such as leaching (especially for nitrate), volatilization, immobilization, *etc.* The nutrients lost will not be utilized by the plant as such will increase the cost of crop production. Nutrients lost through leaching cause groundwater pollution while those lost through volatilization such as NO_2 (especially in rice field) is among the greenhouse gases that cause climate change. Pandey and Awasthi [8] concluded that using too many chemical fertilizers reduces soil health quality attributes (physical, chemical, and biological qualities) as well as crop productivity.

Solutions to the Use of Fertilizer

Despite all these challenges regarding the use of a fertilizer, it plays a significant role in obtaining higher productivity (contributes 40-60% of crop yield) as its nutrient concentration is high and is released immediately to the soil for plant uptake. Therefore, the use of fertilizer cannot be eliminated and this paved the way for several nutrient management practices to be employed in present-day agriculture to minimize many problems linked with the usage of fertilizer. The concept of integrated nutrition management is one of these nutrient management strategies. To improve crop and soil productivity as well as the sustainability of

production systems, integrated nutrient management entails a balanced use of mineral fertilizers in combination with organic and biological sources of plant nutrients. The combined use of organic manures and chemical fertilizers is effective in halting productivity declines by increasing fertilizer use efficiency (FUE) and correcting marginal deficiencies of secondary and micronutrients, as well as their beneficial impacts on the soil's physical and biological properties [3].

In addition, fertilizer nutrient use efficiency is another way of reducing the hazardous effect caused by the usage of fertilizer. Fertilizer nutrient use efficiency involves the efficient utilization of nutrients by applying the required amount of fertilizer to a crop at the right time at the right place using the right method. Adopting and using the techniques for increasing fertilizer use efficiency assist in limiting nutrient loss due to leaching, immobilization and volatilization, thus reducing environmental hazards and cost of production.

Moreover, the present-day agriculture being highly chemically intensive is also concerned with producing a higher yield of crops with little or no emphasis on the quality of crop production. However, in addition to quantity, there is also a need to produce a crop with a high nutritive value that is rich in protein, minerals, as well as other vital elements for human and animal intake. Therefore, to produce a higher yield of the crop with high quality while reducing the negative impacts of chemical fertilizers, the usage of nano-fertilizers is required. Nano-fertilizers are fertilizers developed using nano-technology. Nano-fertilizers are effective tools in agriculture for combating the negative impacts caused by the use of chemical fertilizers because of their small particle size, more penetration capacity, higher surface area and use efficiency. Nano-fertilizer particles are less than 100nm in size, allowing them to penetrate the plant more easily. The smaller particle size increases the surface area of the particles which boosts the rate of uptake and chemical reaction such as photosynthesis in the plant. Nano-fertilizers are characterized by the high use efficiency because they release nutrients slowly to the plant and are thus made available to the plant throughout the growth period hence no residue that may cause environmental hazards is left. Nano-fertilizer is thus served as an effective technology that has a great role to play in crop production as it is ecologically friendly and also ensures the sustainability of production systems and economic stability [6].

NANO FERTILIZERS (NF)

The word “Nano” is a Greek word that means “Dwarf” measured in one-billionth. The term “Nanotechnology” is defined as the understanding and control of matter typically in the size of 1-100 nm, unique properties of large surface area and extremely small size for nanomaterials such as biological, optical and physical

make the novel application possible [9]. The advances in science and technology such as Biology, Physics, Chemistry, Permaceuticals and engineering resulted in the emergence of the field of nanotechnology. They occurred naturally, but can also be engineered. Nanotechnology is now being explored in many fields like agriculture for new opportunities owing to its extremely small size [10]. Currently, the area of nanotechnology is currently receiving greater emphasis in the agricultural sector for the great potential of nanoparticles with characteristics such as; adherence effects, higher reactivity, surface effects and enhanced bioavailability [11].

The use of synthetic chemical fertilizers solely as a nutrient source remains a major constraint in agricultural production, it resulted in many environmental problems and poor nutrient use efficiency, which is a worse scenario that becomes a hindrance to agricultural sustainability [12]. Additionally, the profit margins for the framers reduce significantly due to the cost rises caused by the overuse of chemical fertilizers. The reasons for inadequate nutrient use efficiency are manifold; among which is usually the consequences of the high rate of conventional fertilizers release beyond the required nutrient requirement of the plants and/or inaccessible of nutrients/fertilizers to crops despite their transformation in the soil [13]. As a result, there has been a surge of interest in the direction of new innovative fertilizer sources that will enhance the efficiency of fertilizers used by crops [14]. Because of the regular consumption of fertilizers in the agricultural sector, the production of nanofertilizers is regarded as the most essential role of nanotechnology in the agricultural field, particularly in developing nations [15].

Effects of Nanofertilizers on Fertilizers

Food production in many countries has been achieved with the use of chemical fertilizers. In developing countries, the consumption of these fertilizers increases with the introduction of fertilizer-responsive crops and high-yielding varieties. Notwithstanding, the yield of many crops as a result of decreased soil organic matter and imbalanced fertilization has begun to decline [16]. Moreover, the major cause of groundwater pollution and eutrophication in aquatic ecosystems is considered to be the consequences of excessive applications of N and P types of fertilizers. The fact that the fertilizer use efficiency is 20% to 50% and 10% to 25% for nitrogen and phosphorus respectively, implies that efficiency in food production will have to be increased much more than previously [17].

To boost fertilizer use efficiency, a variety of solutions have been proposed, including split or targeted fertilizer application, precision fertilization, fertigation, and the use of nanofertilizers [18]. In the sustainable agriculture context, the

development of new fertilizers through the application of nanotechnology is viewed as one of the potentially likely options to attain sustainability, especially under the current scenario of climate change in addition to increased food production for the ever-increasing population food demands [19, 20]. Interestingly, nanofertilizers in a recommended dose can in a controlled manner feed the plants gradually without under or over application [21]. The side effects like environmental hazards, rate of leaching, and volatilization from the excessive use of chemical fertilizers can significantly be reduced while at the same time improving the fertilizer use efficiency [22]. In addition, enhancing the plants' ability for nutrient absorption [23]. Furthermore, the field of nanotechnology attracted more attention due to the increased efficacy and bioavailability of nanofertilizers applied to the soils and reduced risk of environmental pollution as a result of nutrient loss [24].

Advantages of Nanofertilizers

Mineral chemical fertilizers have been used and served the purpose of increasing crop yields in agriculture since the beginning of the dramatic increase in the human population. However, the overuse of these chemical fertilizers instead of increasing crop yield may contrarily reduce soil fertility and its productivity thereby causing crop loss in the near future. Nanofertilizers comprise the most important field of agriculture owing to their high capability to improve fertility, increase yield, mitigate pollution and make a favorable environment for soil microbes. The role of these smart fertilizers in plant and soil systems has been well-documented and can act efficiently for the enhancement of agricultural productivity [25].

Nanotechnology is crucial in the development and deployment of novel fertilizers because of its distinctive attributes, like high surface-to-volume ratio, controlled-release kinetics to defined locations, and sorption potential [20]. Nanofertilizers are nutrients that have been encapsulated or coated with nanomaterials to allow for the controlled and gradual release of one or more nutrients to meet plants' critical nutrient requirements [26]. These "smart fertilizers" are currently viewed as a potential option [27] and in some situations, are considered to be the preferred kind of fertilizer over conventional fertilizers [28, 29]. The nutrient delivery system of nanofertilizers has significant advantages over conventional chemical fertilizers [30]. They employ controlled and timely release strategies to regulate the nutrient availability in crops. Nanomaterials are coated with nutrients that have been connected to such slow nutrient delivery [22]. Delivery of nutrients is steady, gradual, and long-term which is beneficial for the producers to improve crop development. Nutrients, for example, can be supplied slowly over 40–50

days rather than the 4–10 days required by conventional fertilizers [31]. In traditional nutrient management methods, 50 percent of the fertilizer applied to the field is lost to run-off or becomes inaccessible to the crops due to high availability, obstructing root absorption and occasionally generating harmful effects. In addition, nanofertilizers cut down on transportation and application expenses [32]. Another benefit of utilizing in minimal amounts is that the soil does not become laden with salts, which can occur when using conventional fertilizers in either short- or long-term applications [33]. Another benefit of employing nanofertilizers is that they may be tailored to the nutrient requirements of the crops they are meant for [34]. In this case, biosensors could be coupled to a new novel fertilizer that regulates nutrient supply based on soil nutrient status, crop development period, or environmental variables[33]. Plants are susceptible to the availability of micronutrients throughout crop growth, and this has detrimental repercussions on nutritionally deficient fruits and vegetables [35, 36]. It is difficult to restrict the micronutrient distribution to a particular plant in a traditional nutrient management system, but nanofertilizers give the ability to distribute suitable levels of nutrients [37]. Since most horticultural growing areas across the world are deficient in key micronutrients, such as zinc and iron [36], nanofertilizers can be used as efficient and appropriate enrichment products for crops and perishable foods. Nanofertilizers promote nutrient absorption by having a large specific surface area, a small size, and high reactivity [30]. Nanofertilizers, on the other hand, help the plant to withstand diverse biotic and abiotic challenges by supplying balanced nourishment.

Limitations of Nanofertilizers

The widespread use of nanofertilizers in agriculture could have several significant drawbacks, such as new environmental and health-related issues that could restrict the technology's utility in horticulture crop productivity. Phytotoxicity from nanoparticles is also a concern in this area, as plants react differently to different nanomaterials at different doses [12]. These materials' reactivity and unpredictability are also a source of concern. This raises issues about the safety of farmers who may be exposed to xenobiotics as a result of their application [38]. This includes not just individuals who are involved in nanofertilizers' synthesis, but as well as those who have been involved in the application of nanofertilizers in the field. Given the expected benefits, it is necessary to investigate the practicality and applicability of these novel smart fertilizers. Indeed, transportation, toxicity, and bioavailability limitations, as well as unforeseen environmental consequences from contact with biological systems, restrict their applications in agriculture and horticulture [20]. Identifying and assessing the risks of nanomaterials, as well as nanomaterial or fertilizer life cycle evaluation

and toxicological research priorities, are crucial. This is especially true in light of nanoparticles' accumulation in plants and associated health risks. Food safety, along with human and food security, has been raised as a result of the use of nanofertilizers produced from nanomaterials [39, 40]. Nanoparticles' uptake, translocation, transformation, and accumulation in plants are influenced by species, dose, and application method, as well as NP composition, size, shape, and surface features [41]. It is critical to investigate the extent to which NP is harmful to specific crops. It is required for the examination and evaluation of nanofertilizer uptake and translocation. It also illustrates the various changes that occur in nanoparticles when they interact with soil compounds and phytochemicals. The accumulation of NPs in various plant locations can also be determined [42].

Comparative Analysis of Nanofertilizers over Conventional Chemical Fertilizers

Recent research has focused on a wide range of nanotechnological applications in the agricultural research area, with techniques being developed at the level of both academia and industry [43]. By inventing new techniques for plant disease treatment and pathogen identification, nanotechnology has the potential to improve every aspect of the current agriculture and food economy [44, 45], and improve the ability of plants to absorb nutrients [23, 46]. Furthermore, nanotechnology has begun to garner more interest in the agricultural field, particularly in the development of new nanofertilizers to increase the efficiency and bioavailability of these novel fertilizers while lowering the amount of material lost to the environment [24].

Many studies have shown that using nanofertilizers enhanced agromorphological parameters, photosynthesis, and crop yield. The result from the two years field experiment conducted in Egypt by Ahmed Shebl [47], revealed that; the spray of manganese oxide nanoparticles on the leaves of *Cucurbita pepo L.* delivers the best vegetative outcomes in terms of photosynthetic pigment, fruits, and yield. On the contrary, zinc oxide nanoparticles give the highest value of protein, energy, lipids, and organic matter content on the squash fruits. The application of ZnO-NPs on wheat plants and common beans improved both the vegetative and reproductive growth stages of the crops [24, 48], by the application as a foliar fertilizer. Furthermore, Khodakovskaya *et al.* [49] discovered that carbon nanoparticles improve tomato plant characteristics and yield. In a study conducted by Du *et al.* [50], ZnO-NPs were found to be more effective than $ZnSO_4$ in the germination and growth of wheat. The field experiment conducted by Nouraein [51] reported that the use of nanofertilizers (nanoboron, nanozinc and

nanocomplete) was observed to give the best result in protein percent, chlorophyll content and straw yield of maize crop compared to NPK mineral fertilizers and NP biofertilizers and control treatment respectively.

BIOSYNTHESIS OF NANO-FERTILIZERS: GENERAL APPROACH

The term "Nano fertilizers" can be defined as the nutrients which can be delivered to crops either by encapsulating them in nano-materials or by delivering them as particles or emulsions of nanoscale dimensions. This statement clarifies the definition of nano fertilizers, describing them as nanomaterials that are either nutrients (micro- or macro-nutrients) or operate as carriers/additives for nutrients (*e.g.*, by compositing with minerals) [34].

Nutrient encapsulation with nanoparticles can be accomplished in three different ways:

1. Plant nutrients are encapsulated in nanoparticles of variable origin and chemical composition.

2. Using a thin layer of nanoparticles, such as polymer film, to coat the nutritional particles.

3. Providing nutrients in the form of emulsions and particles with dimensions in the nanoparticle range.

The major processes of metal nanoparticle biogenic synthesis for nanoparticle formation include nucleation, nanoparticle growth, stabilization, and capping agents, which result in capped and persistent metal nanoparticles. The synthesis of nanoparticles (required for encapsulation) is carried out through a nucleation reaction between the metallic salts and the capping /reducing agent at a specific temperature. The actual entities involved could be electrons from the reducing/capping agent's functional group as well as electrons from metallic ions. These reactions are further assisted by a stabilizing agent which improves the procedure of nanoparticles' synthesis along with by-products. Engineered nanoparticles are made up of a variety of metals (Ag, Au, Zn, Pd, Pt), metal oxide nanoparticles, and even non-metal nanoparticles [52]. Various compounds including organic compounds such as sodium citrate, glycol ethylene, N, N-dimethylformamide, *etc.* with reducing properties are some of the common reducing agents utilized during the process. Various chemicals like polyethylene glycol (PEG), carboxymethyl cellulose (CMC), thiols, cellulose, *etc.* are used as stabilizers in nanoparticle formulation.

Approaches for the Synthesis of Nano Based Fertilizers

Nanotechnology is regarded as the future industrial revolution in the 21st century. The word nanomaterial refers to materials having a size between (1–100 nm) with distinctive structural, physical, and chemical properties [53]. Because the synthesis of nanoparticles by various chemical and physical methods has a harmful influence on the environment and living organisms, it is necessary to produce them utilizing bio-inspired agents. Currently, the green synthesis of NPs is a method that is regarded as environmental-friendly because no hazardous chemical is used in green approaches. By inventing new methods for plant disease cures, pathogen detection, and increasing plant physiological activities, nanotechnology can advance the overall existing agricultural and food sector [54]. Furthermore, nanotechnology has begun to bring further advances to the field of agriculture. New nano fertilizers are being developed to increase their efficacy and bioavailability while also reducing the loss of these components to the environment [24]. The advantages and disadvantages of top-down and bottom-up approaches in nanofabrication are described in Table **1**.

Synthesis and fabrication of nanomaterials can be done using two approaches as shown in Fig. (**1**);

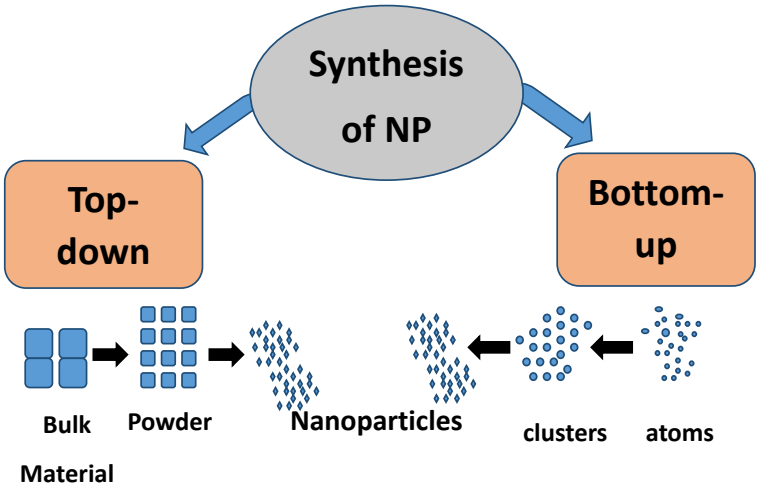

Fig. (1). Different approaches for synthesizing nanoparticles.

Table 1. Advantages and Disadvantages of approaches for the synthesis and fabrication of nanomaterials.

-	**Approaches**	
	Top-down	Bottom-up
Advantages	Chemical purification is not needed.	The parameters of the deposition can be controlled.
	It is useful for large-scale production.	Cheaper technique.
		Controlling ultra-fine nanoparticles, nanoshells, and nanotubes is possible.
Disadvantages	Controlling the deposition parameters is difficult.	Nanoparticles must be chemically purified.
	Expensive technique	Large-scale production is difficult.

1. Top-down approach

It is the process of slicing or cutting bulk material into nano-sized particles in a series of steps. In the top-down approach, the starting material is in a solid state. The method used in this approach involves mechanical methods such as cutting, etching, grinding, *etc*., and lithographic techniques such as photolithography and electron beam lithography.

2. Bottom-up approach

It refers to the atom-by-atom and molecule-by-molecule buildup of material from the bottom. Atom-by-atom deposition causes atoms/molecules and clusters to self-assemble, eventually forming self-assembled monolayers on the substrate's surface. A gaseous or liquid form of matter is used as the starting ingredient. This method employs several strategies;

• Physical techniques

I. The process of vapor phase species condensing is known as physical vapor deposition (PVD).

II. Evaporation (Thermal and e beam)

III. Sputtering

IV. Plasma arcing

V. Laser ablation

• Chemical techniques

I. The deposition of the vapor phase of reaction species is known as chemical vapor deposition (CVD).

II. Electrolytic deposition, sol-gel technique, microemulsion approach, and pyrolysis are all used to create self-assembled monolayers.

Methods of Biosynthesis of Nano-fertilizers

A variety of methods including physical, chemical, aerosol-based and biological have been developed which utilize either the bottom-up or top-down approach for the synthesis of nanoparticles. The advantages and disadvantages of methods of biosynthesis of nano-fertilizers are described in Table **2**. The methods are further discussed below:

Table 2. Advantage and Disadvantages of methods of biosynthesis of nano-fertilizers.

Methods	Size of Nanoparticles	Advantages	Disadvantages
Aerosol	100nm	Particle size and shape may be controlled to a high degree • Controlled nanocomposite synthesis. • Within a few percent, there is mono dispersion. • Surface passivation	Large aggregates are formed.
Physical	15-100nm	Rapid and scale-up synthesis.	Broad PSD and wide range of shapes.
Chemical	100nm	• Controlling the morphology of metal nanoparticles with precision.	Surface coating with harmful chemicals • Comparatively lesser biocompatibility
Biological	100nm	Environmentally benign • Natural macro and micro biomolecules are used to coat the surface. • Biocompatible	Particle synthesis occurs at a slow rate • Natural resources are required. • Broad PSD and wide range of shapes.

Physical Methods

The top-down method, which involves crushing the bulk material into fine particles, is often used in the physical method of nanoparticle production. External forces like crushing, impact, disruption, deterioration, cutting, cryo-grinding, grinding, processing, and homogenization are used to accomplish this. For the physical synthesis of metallic nanoparticles, a variety of techniques such as milling, attrition, sputtering, pyrolysis, and laser ablation can be used.

Micro-particle fracturing is a characteristic of the milling process, which can be processed using ball milling, high-energy ball milling (HEBM), grinding, cryo-grinding, refining, and homogenization, including medium-pressure homogenization (UHPH) and high process homogenization (HPH). A size-reducing process grinds macroscale or microscale particles in the same way that it grinds macroscale or microscale particles in the attrition process (*e.g.*, an ordinary or a planetary ball mill). The oxidized nanoparticles are then separated from the rest of the particles by air classification. The properties of the resultant nanoparticles are influenced by the milling material and time, as well as the atmospheric medium.

Sputtering is the process of ejecting atoms off the surface of a substance (the target) by bombarding it with strong particles. Sputtering is a momentum transfer phenomenon that occurs when bombardment ions push atoms away from a cathode/target. Sputtered atoms travel until they come into contact with a substrate, where they deposit to create the required layer. Pyrolysis is the process of forcing an organic precursor (either a liquid or a gas) through an opening under high pressure and burning it. To recover oxidized nanoparticles, the ash is air categorized. Laser ablation is the process of removing material from a solid (or often liquid) surface by irradiating it with a laser beam.

However, the production rate of these previously mentioned "physical" processes for attaining metallic nanoparticle synthesis is relatively poor, and the cost is also quite high. Even though these physical processes are adaptable strategies for producing larger nanoparticles in terms of size, diameter, and volume, they nevertheless produce surface flaws, mixed-phase crystals, and contamination, and are more expensive and time-consuming. The flaw regarding efficient and expensive maintenance was caused by biological entities traveling across space and nanoparticle production processes [55].

Chemical and biological processes are among the numerous options for making nanoparticles.

Chemical Methods

Wet-chemical processes are the most extensively utilized methods for the production of metallic nanoparticles. These chemical preparations use a bottom-up approach to generate nanoparticles in a liquid media containing a variety of reactants, including reducing agents and stabilizing agents. Various chemical preparation methods, such as co-precipitation, Hydrothermal synthesis, sol-gel process, and chemical vapor deposition (CVD) microemulsion process are available to produce nanoparticles.

In co-precipitation reactions, the processes of nucleation, growth, coarsening, and/or agglomeration all occur simultaneously. The metal nanoparticles are formed in an aqueous solution through reduction from non-aqueous solutions, electrochemical reduction, and decomposition of metallo-organic precursors. However, co-precipitation reactions are sometimes assisted with sonication or microwaves.

In hydrothermal synthesis, nanoparticles are produced by reacting chemical components in a sealed closed heated solution above ambient temperature and pressure. Mineral solubility in hot water at high pressure is required for nanoparticles' creation using this method. At the opposing ends of the growing chamber, a temperature gradient is maintained. The nutrients are dissolved at the hotter end, while the seeds are supported as they grow at the cooler end.

In another process termed CVD, a solid is deposited on a hot surface by a chemical reaction from the vapor or gas phase. To proceed with the CVC reaction, activation energy is required to break the chemical link between the reactant molecules. Several methods can be used to provide this energy: thermal, plasma, laser, and photo-laser.

The sol-gel method can be used for producing small nanoparticles using solid materials. The sol (or solution) progressively transforms into a gel-like diphasic structure throughout this chemical process. It comprises a liquid phase as well as a solid phase, with morphologies ranging from single particles to vast polymer networks. The microemulsion method is yet another technique used for the preparation of inorganic nanoparticles. Reactants are mixed in this technique, and exchange happens when water droplets collide in the microemulsion, resulting in a precipitation reaction in the nano-droplets. This is accompanied by primary particle nucleation and coagulation, forming final nanoparticles that are stabilized by surfactants and surrounded by water.

Ultrasonic irradiation can be used to induce ultrasonic cavitation in liquids. The production of nanoparticles with controllable morphologies is enabled by cavitation, which generates a unique condition for chemical interactions to occur under extreme circumstances.

Chemical processes are low-cost for large quantities, but they have downsides such as contamination from precursor chemicals, the use of hazardous solvents, and the formation of toxic by-products. As a result, there is a growing need to create high-yield, low-cost, non-toxic, and environment-friendly metallic nanoparticle production processes. As a result, the biological approach to nanoparticle manufacturing becomes critical [19].

Aerosol Route For Nanoparticle Synthesis

A gaseous suspension of solid/liquid particles is known as an aerosol. The Vapour phase method is considered the building block of nanotechnology for the synthesis of NPs [56]. Metal nanoparticles are prepared using a variety of inert gases through the evaporation method. Various processes such as atomization, chemical vapor deposition, flame, furnace, and electrospray are used to create nanoparticles utilizing aerosol. Nucleation and condensation of the initial gases and vapor molecules are the fundamental stages in all gas/vapor phase nanoparticle formation. Cluster formation and coagulation occur next, resulting in the production of primary particles, which can then be sintered, agglomerated, and aggregated to form aggregates [19].

The most common method for synthesizing metal nanoparticles is inert gas condensation (IGC). In an ultrahigh vacuum chamber containing helium (He) or argon (Ar) gas at a very high pressure, IGC vaporizes metals. Metal atoms lose kinetic energy when they evaporate and condense into small particles when they collide with the gas. Brownian coagulation and coalescence are then used to transform these particles into nanocrystals.

Biological Methods

Biological approaches involve the synthesis of nanoparticles from living things or substances. Plants and plant products, bacteria, fungi, viruses, algae, and yeast are just a few of the biological resources accessible in nature that could be used to make nanoparticles. Intracellular and extracellular inorganic minerals have been found in both unicellular and multicellular species. Metal nanoparticles may be made in the presence of metal salts from a variety of plant extracts that contain resins, latex, flavonoids, phenols, alcohols, and proteins. Because plants, fungus, bacteria, yeasts, and algae, are used as reducing and stabilizing agents, the biological approach is also known as "green synthesis." It is a more energy-efficient, safer, and waste-free procedure than the other techniques [57].

GREEN SYNTHESIS OF NANO-BASED FERTILIZERS

The use of environmentally friendly resources such as bacteria, fungus, and plants in the synthesis of NPs is known as green synthesis. It conserves energy and conducts the reaction at a very low temperature or pressure within the physiological regime. It is characterized as;

• A sustainable route

• Low toxic

• Environmentally benign

• Cost-effective, and

• More efficient modules.

There are three biological routes of nanomaterials synthesis, they are;

• Micro-organisms aided biogenesis

• Bio-template aided biogenesis

• Plant extract aided biogenesis

Micro-Organisms Assisted Biogenesis

Micro-organisms such as Prokaryotic, Bacteria, Fungi, Yeast, and Actinomycetes are used to synthesize nanomaterials, they are also called “Bioreactors”. Through enzymes produced by biological activity, these bacteria convert the metal ion into element metal. Therefore, the synthesis can be classified as;

I. Inter-cellular

II. Extra-cellular

Inter-cellular

Inter-cellular method occurs inside the cell (downstream processing) which involves enzymatic reactions at a very low temperature and pressure by transforming the metal into the microbial cell from nanomaterials.

Extra-cellular

The extracellular procedure occurs at the bacteria' surface, and it entails trapping the metal ion on the surface of cells and decreasing it in the presence of the enzyme.

Bio-Template Assisted Biogenesis

The following biomolecules are used as a template to design nanomaterials;

a) Nucleic acid

b) Membrane

c) Viruses

d) Diatoms (single-cell algae)

Among them, the nucleic acid is known as an excellent biomolecule template, especially for transition metal nanomaterial synthesis.

Plant Extract Assisted Biogenesis

This process is one of the most efficient, quick, clean, non-toxic, and environmentally friendly methods available [47]. It has mostly been used to make noble metal nanomaterials, metal oxides, and biometallicalloids. Because plant phytochemicals exhibit better decreases and stability, plant extracts are commonly employed [58].

Green synthesis of Iron (Fe) Nanoparticles

Green synthesis, which uses plant extract or biomass, is said to be more stable and has a faster rate of synthesis than traditional procedures because it is non-toxic, eco-friendly, cost-effective, simple, and easy to carry out [59].

The biosynthesis of Fe_2O_3NPs is mostly done with plant extracts, different proportions of plant extract and different iron precursor solutions are needed. When added together, the resulting mixture is sonicated at a certain temperature not higher than 40^{o}C and at a certain period of time no longer than 30 minutes. The appearance of black color in the solution signifies the presence of Fe_2O_3NPs [60].

Bibi *et al.* [59] employed pomegranate fruit extract to produce iron oxide nanoparticles and tested their photocatalytic activity for textile dye degradation in an experiment. It was observed that pomegranate seed extract may be utilized to synthesize Fe_2O_3 NPs in an environmentally friendly and cost-effective manner. Further, these synthesized NPs act as a photocatalyst to degrade dyes in wastewater.

In another experiment, Vitta *et al.* [60] also used an extract (aqueous) of *Eucalyptus robusta Sm* for the synthesis of Fe_2O_3NPs and to evaluate its antioxidant and antimicrobial activity on different pathogenic micro-organisms *viz. Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* On the various bacteria tested, the nanoparticles produced under various synthesis conditions demonstrated antibiotic action.

Green Synthesis of Silver (Ag) Nanoparticles

To make silver nitrate ($AgNO_3$), different concentrations of $AgNO_3$ and varied amounts of plant extract are required. The setup is incubated in a dark chamber to reduce photo-activation of the $AgNO_3$at room temperature. The appearance of a brown color solution from a colorless solution confirms the reduction of silver ions to the silver nanoparticle [47].

For the first time, a plant extract of *Salvia spinosa* cultivated *in vitro* was used to biosynthesize silver nanoparticles (Ag NPs). The study was carried out to identify the functional groups that existed in the plant extract responsible for the reduction of Ag ions to Ag NPs by using Fourier-transform infrared spectroscopy (FTIR) analysis. Both Gram-positive and Gram-negative bacteria were shown to be inhibited by the biosynthesized Ag NPs [61]. Also, bark extract of *Saracaasoca* indicated the presence of hydroxylamine and carboxyl groups responsible for the reduction of Ag ions to Ag NPs [62].

Green Synthesis of Zinc (Zn) Nanoparticles

As per the method of Elumalai and Velmurugan [63] for green synthesis of Zn NPs, the plant extract is heated on a magnetic stirrer, and a certain amount of Zinc precursor is added when the temperature reaches about 60^{o}C and is left for a while till white precipitate appeared. The mixture is then left in an oven at 60^{o}C for a period of time or till a creamy paste is formed. In the end, the paste is washed with a solution of distilled water: Ethanol (3:1) and heated in a furnace at 400 °C for 2 h until the resultant white powder is obtained. Chemical vapor deposition, microwave-assisted procedures, precipitation, Sol-gel, hydrothermal, spray pyrolysis, and ultrasonic techniques have all been established for the synthesis of Zinc NPs [64 - 70]. Jain *et al.* [71] reported the low-cost bacterium-based "eco-friendly" efficient synthesis of ZnO nanoparticles by using the zinc-tolerant bacteria *Serratia nematodiphila*.

Laurus nobilis L. leaves' aqueous extract is the potential for the synthesis of Zn NPs in a simple, fast and eco-friendly way [72]. Alamdari *et al.* [73] used *Sambucus ebulus* leaf extract to prepare and characterize Zn NPs, which showed strong antibacterial activity against a variety of species as well as the tolerable photocatalytic breakdown of methylene blue dye pollutants.

CONCLUSION

Nutrient shortage in soils has resulted in considerable agricultural productivity losses and significant economic losses in agriculture. Conventional fertilizers boost crop output, but their widespread use is unsustainable in the long run since they are inaccessible to plants. Furthermore, most of the macronutrients are less bioavailable with a low utilization rate as they change to an insoluble form in the soil. The delivery of macro-and micronutrients to plants is a crucial part of nanotechnology's application in agriculture. Nanostructured materials are used as carriers or vectors for the controlled release of fertilizers. Thus, these smart fertilizers can increase nutrient use efficiency while minimizing environmental pollution costs. In response to environmental changes and biological demands, a nanofertilizer precisely releases its active chemicals. For nano-fertilizer delivery to plants, both *in vitro* and *in vivo* approaches can be used. The uptake and transport of nanoparticles in plants are yet unknown, which has led to a slew of ethical and safety concerns about using nanofertilizers to boost crop plant output. Quantitative assessments are needed to better understand possible health consequences, environmental clearance, and safe and secure removal of nanomaterials, which will lead to better nanofertilizer design.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 5

Nanobiotics for the Treatment of MDR Infections

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Abstract: Nanoparticles are those agents that are made-up of single or a combination of single or multiple materials which are very small in size ranging from 1 to 100 nanometers. Several studies reveal that nanoparticles have features that interact effectively with microorganisms and can help in treating multidrug-resistant organisms. These have intrinsic antimicrobial activity and are of various types broadly divided into organic and inorganic nanoparticles. Nanoparticles can engage with bacteria and travel across the bacterial cells and host cell membranes, and help treat ESKAPE pathogens which are among the most notorious multidrug resistant superbugs. These pathogens have MDR features and have multiple types of MDR mechanisms including drug inactivation/alteration, modification of drug binding sites/targets, reduced intracellular drug accumulation and biofilm formation. For targeting different types of MDR, there are multiple types of nanoparticles such as metal nanoparticles, nanostructures, leukocyte membrane-coated nanoparticles, red blood cell membrane-coated nanoparticles, cancer cell membrane-coated nanoparticles, and platelet membrane-coated nanoparticles among others. Antimicrobial nanobiotics identified and synthesized to date harbor a vast diversity of intrinsic and modified physicochemical properties and have applications in diagnostics. No technology is without its challenges and the same is true for nanobiotics. The major challenges in this field of nanobiotic-based therapeutics are their allergic responses, assembly and pharmacokinetics. This chapter will elaborate on the mechanisms of action of various types of nanobiotics present as cost-effective solutions useful in a variety of applications in the treatment of MDR pathogens with a special focus on ESKAPE pathogens.

Keywords: Antimicrobial resistance, ESKAPE, Multi drug resistance, Nano biotics, Nanoparticles.

INTRODUCTION

The first antibiotic Penicillin was discovered and commercially produced in 1928. From the 1920s to the present, we have taken for granted that every infection can be cured completely by antibiotics. Because of the generous unchecked use of antibiotics in human therapy; it has resulted in the birth of pathogenic bacteria resistant to multiple drugs [1]. MDR is a serious threat to public health. Efforts for

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controlling MDR in "ESKAPE" pathogen (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter spp.)* have been hampered by their ability to escape drugs. They can cause life-threatening nosocomial infections. These pathogens also known as "Superbugs" [2] carry MDR genes on the bacterial chromosome, plasmid, or transposons. Drug resistance mechanisms fall into several categories, some bacteria produce enzymes that can irreversibly modify and inactivate the drug; these are *β*-lactamases, aminoglycoside-modifying enzymes, or chloramphenicol acetyltransferases. Some bacteria perform modifications of drug binding sites/targets to avoid recognition. The balance of antibiotic uptake and elimination determines the susceptibility of bacteria to a particular drug. Bacterial cells often reduce intracellular drug accumulation to develop antibiotic resistance. Biofilm formation [3] contributes to 65% to 80% of microbial infections and is advantageous in the survival of bacteria. Biofilm can be produced in recurrent tonsillitis, cystic fibrosis lung infection, urinary tract infections and chronic wounds. Clinical illnesses attributed to bacterial adhesion to implants and medical device-related infections are among the most challenging issues to be addressed in MDR infections [4]. Owing to the multidrug resistance nature of pathogens and the failure of the treatments, we must find a better option for treating these microorganisms. Nanobiotics, a revolutionary concept can be seen as a future of drugs for MDR [4].

Nanobiotics are small materials 1- 100 nanometers in size. Nanobiotics also known as Nano particles are categorized into numerous classes; these classes are based on their size, forms and qualities [5]. These categories include numerous subcategories which are elaborated further in the chapter. A brief glimpse into nanobiotics reveals that inorganic nanoparticles are introduced as nanobiotics and used for drug carriage [6]. Nanoparticles that are covered by metal oxide shell are known as metallic nanoparticles. After chemical modification, metal nanoparticles can be used in diagnostic imaging, and targeted drug delivery [7]. Organic nanoparticles focus on the utilization of nanoparticle-based materials having an organic structure [8]. Leukocyte membrane-coated nanoparticles, red blood cell membrane-coated nanoparticles [9], and cancer cell membrane-coated nanoparticles [10] are additional nanoparticles' categories that have been recently designed. In this chapter, we will be discussing about the roles and use of nanoparticles in MDR treatment and diagnostics.

Nanobiotics

Nanobiotics, also known as nanoparticles, are small materials that range in size from 1 to 100 nanometers. They are categorized into numerous classes based on

their qualities, forms, and sizes. Nanoparticles may consist of a single material or a combination of different materials. They are used in research and technology because of their small size and unique features. Nanoparticles show various physical as well as chemical properties that include the optical, mechanical, and magnetic properties [5]. Optical properties like absorption, transmission, reflection, and light emission of nanoparticles are dynamic. The optical property of nanoparticles is of great importance in several ways. It was discovered that their optical qualities are influenced by their internal electronic structure, providing a thorough understanding of the structure. They can use their electrical properties to develop quantum effects which may lead to variations in size, shape, and color they produce. The optical properties of nanoparticles can be recognized by using various spectroscopic techniques [11]. Nanoparticles' magnetic characteristics have a wide range of applications, including drug administration, therapeutic treatment, MRI imaging, and in-vitro diagnostics. According to one study, nanoparticles perform best when their size is less than the critical value, which is 10–20 nm. Nanoparticles' magnetic characteristics can dominate more effectively at this low scale, making them cost-effective and useful in a variety of applications. Nanoparticles have a magnetic property due to their unequal electrical distribution [12]. The unique mechanical properties of nanoparticles have numerous applications in the field of surface engineering, nanofabrication, and nanomanufacturing. Different mechanical parameters such as elastic modulus, hardness, stress and strain, adhesion, and friction can be examined to better understand the mechanical nature of nanoparticles. Surface coating, coagulation, and lubrication, in addition to these characteristics, play a role in the mechanical properties of nanoparticles. Controlling the mechanical properties of nanoparticles and their interaction with any type of surface, on the other hand, is critical for highlighting surface quality [13].

The Amalgamation of Nanoparticles with Antimicrobials

Antimicrobial resistance to hazardous bacteria is on the rise across the world, posing a serious threat to human health. This has led the researchers to look for new therapeutic options. One of the approaches that have been explored currently includes drug-associated nano systems. Several studies have revealed the intrinsic antimicrobial activity of various types of organic and inorganic nanoparticles. These nanoparticles have many unique properties, including small size and a high surface-area-to-volume ratio in comparison to bulk material, both of which are important for antimicrobial activity. Nanoparticles' special features allow them to engage with bacteria and rapidly traverse the bacterial and host cell membranes, obstructing the main microbial metabolic pathways and allowing the eradication of intracellular infections where antibiotics typically fail [5]. Nanoparticles can functionalize the surface, especially when it comes to linking chemical functional

groups for targeted medication delivery and antibiotic action enhancement. Nanoparticles, often known as "nano antibiotics," have antibacterial properties and can also operate as drug delivery vehicles for conventional antibiotics. As a result, nanoparticles have acquired favor in the scientific community as new generation antibiotics, allowing researchers to investigate many aspects of antibacterial action [14].

Multidrug Resistant ESKAPE Pathogens

'ESKAPE' pathogens consist of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* species that show potent virulence and multidrug resistance. These bacteria include both Gram-positive and Gram-negative species that can avoid or escape the bactericidal action of commonly used antibiotics and therapies because of their antimicrobial resistance property. In immunocompromised patients, ESKAPE pathogens are a common source of nosocomial infections. Antimicrobials are unable to combat these diseases due to a variety of resistance mechanisms, including changes in cell permeability, drug disintegration, modification of drug attachment sites/targets, and/or mutation [15]. They also form biofilms that prevent antibiotics as well as the host's immune-responsive cells to inhibit the pathogen. The continuous use of antibiotics gave rise to the development of extensively drug-resistant (XDR) and multidrug-resistant (MDR) bacteria, which renders even the most effective drugs, ineffective. Bacterial biofilms provide various survival advantages against antimicrobials. It has been found that biofilms contribute about 80% and 65% to chronic and microbial infections [3, 4]. Implants are used to replace and support body structures. Microorganisms clinging to the surface of the implant cause biofilm formation. Dental caries, chronic rhinosinusitis, recurrent tonsillitis, cystic fibrosis lung infection, urinary tract infections, chronic wounds, periodontitis, and device-related infections were among the clinical illnesses connected to bacterial adhesion to implants. ESKAPE pathogens are involved in the contamination of urinary catheters, central venous catheters, biofilm formation on mechanical heart valves among others. Because biofilms are resistant to antibiotics and the host's immune system, surface modification of implants is critical for enhancing their biocompatibility and anti-infection properties [16].

Nano Biotics-the Perfect Solution For MDR- broad-Spectrum Activity

The ability of microorganisms to withstand antibiotics has become one of the most pressing challenges in public health. Antimicrobial resistance has evolved through a variety of mechanisms, including enzyme inactivation, decreased cell

permeability, target protection, changed target site, and enhanced efflux by the overexpression of efflux pumps [17]. Infections produced by multidrug-resistant organisms (MDROs) and the lack of novel antimicrobials are major causes of morbidity and mortality worldwide due to this acquired antimicrobial resistance feature of bacteria. These clinical issues underline the urgent need for new antibacterial methods that are both effective and safe. Several strategies are being investigated to overcome this challenge, including the use of nanostructured materials. Nanoparticles represent a viable technique for controlling MDRO infections due to their unique features and uses. Nanoparticles (NPs) may easily penetrate pathogenic bacteria's cell membrane and disrupt major molecular pathways, allowing them to bypass bacteria's common resistance mechanism [18], [19]. Nanoparticles have antimicrobial efficacy through a variety of methods, including direct contact with the bacterial cell wall, biofilm inhibition, innate and adaptive host immunological responses, the formation of reactive oxygen species (ROS), and activation of intracellular effects. As a result, nanoparticles are an excellent way to tackle MDROs [17]. In bacteria, quorum sensing systems are one of the most significant pathogenic regulating mechanisms, specifically in biofilm formation. Biofilm generation, which permits bacteria to suppress antibiotic action, is one of the main causes of bacterial resistance to antibiotics. Biofilms are bacteria's most active component, consisting of cells connected on the surface within an extracellular polymeric substance (EPS) matrix. EPS acts as a barrier to antibiotic penetration and aids phagocytes in bypassing the innate immune system, creating antibiotic resistance, and posing a severe health danger to humans. Many studies have shown that nanoparticles can disrupt bacterial cell membranes and interfere with biofilm formation by interacting with EPS and quorum sensing, lowering the chances of bacterial survival [20]. The main target of most of the recent nanoparticles-based techniques to inhibit bacterial biofilm formation is to interfere with quorum sensing molecules. Quorum sensing system allows bacteria to communicate with one another *via* production and detection of signal molecules like auto-inducers which helps them to synchronize their gene expression, obtaining the advantage to react to environmental changes. In one of the investigations, it was shown that nanoparticles functionalized with -cyclodextrin (-CD) or N-acylated homoserine lactonase proteins (AiiA) can disrupt signaling molecules, preventing these molecules from attaching to their receptor and so turning off quorum sensing. In this way, nanoparticles provide a potential alternative strategy to disrupt bacterial biofilms and hence quorum sensing with the possibility to use antibiotic-free and antibiotic-coated techniques [17].

TYPES OF NANOPARTICLES

Inorganic Nanoparticles

As a result of recent breakthroughs in nanotechnology, various inorganic nanoparticles have been introduced, and several of these inorganic nanoparticles have been used as drug carriers. Preclinical research on inorganic nanoparticles as diagnostic and therapeutic systems in oncology for several applications, including tumor imaging and medication administration, has gotten a lot of attention. In comparison to organic materials, inorganic nanoparticles are biocompatible, non-toxic, hydrophilic, and highly stable [6].

Metal Nanoparticles

Metallic nanoparticles (MNPs) have an inorganic metal or metal oxide core that is surrounded by an organic or inorganic substance or metal oxide shell. Metallic nanoparticles are commonly employed in the fabrication of metal-based biopolymer composites due to features such as optical polarizability, biocompatibility, electrical conductivity, antibacterial activity, and chemical properties. MNPs can be modified with a variety of chemical functional groups to allow them to bind with ligands, antibodies, and medicines, allowing them to be used in biotechnology, diagnostic imaging, and targeted drug delivery [7]. Nanoparticles, such as gold, silver, and magnetic nanoparticles, have gained popularity in recent years. Despite the enormous advances of MNPs, they are highly toxic to living cells. Although there are some studies on MNPs toxicity suggesting that they might affect the biological systems at the cellular level, complete knowledge of MNPs toxicity is needed for large-scale production [21].

Gold Nanoparticles

Gold NPs are promising agents for cancer therapies and are important in imaging, drug carriers, and thermotherapy. Gold nanoparticles have unique physical and chemical features that improve medication efficacy, drug loading, and biocompatibility, allowing them to easily reach the target location with blood flow [22]. The various types of GNPs are gold nanoshells, gold nanorods, gold nanocages, and gold nanospheres. Gold nanoshells have recently been employed as imaging and therapeutic agents for cancer. The unusual core-shell nanostructure formed of a spherical dielectric core material such as silica or polystyrene, or sodium sulfide coated by a gold coating is the cause for their identification. Many in vitro experiments employing gold nanoshells targeting cancer cells have demonstrated that when exposed to near-infrared radiation, cancer cells are effectively destroyed [23].

Iron Oxides Nanoparticles

Iron oxide nanoparticles (NPs) are one of the most common types of inorganic materials. Iron oxide NPs are divided into two types based on their size: superparamagnetic and ferromagnetic. Superparamagnetic iron oxide NPs (SPIONs) are one of the most investigated inorganic materials for drug administration, hyperthermia therapy, and imaging. Superparamagnetic iron oxides have a significant magnetic response to external magnetic fields. They are non-toxic, biocompatible, and can be effectively removed from the human body using iron metabolism routes. Heike *et al*. proved that iron oxide NPs have an intrinsic therapeutic effect on tumors [24]. Iron oxide NPs treated tumor cells have a slower growth rate than that of control. Iron oxide NPs also exhibit an intrinsic enzyme activity. Due to their potential as MRI contrast agents combined with the ability for selective targeting, iron oxide nanoparticles play an essential role in MRI-based imaging and diagnostics. Other major applications of iron oxide nanoparticles are protein separation and purification, bio-sensing, and drug delivery [25].

Silver Nanoparticles

As a bactericide, silver nanoparticles (AgNPs) have been employed in a variety of products. Many human cancer cells, including breast cancer cells, have been tested with nano-sized silver particles and their anticancer properties. Silver nanoparticles can be produced by a variety of processes, including physical, chemical, and biological ones, and they can be utilized as biosensor materials. They have optical properties and can be used in the medical sector due to their antibacterial, antifungal, and anti-inflammatory effects [26, 27].

Nanostructures

Nanostructures are extremely essential in the field of nanoscience and nanotechnology. A nanostructure is a structure with at least one dimension of 100 nm or less. Nanostructures offer a variety of physical and chemical properties that can be exploited to create essential functional polymers, resins, and elastomers. Different types of nanostructures that are available are nanoparticles, nanotubes, nanorods, nanopores, nanowires, nanoribbons and nano scaffolds. The fact that these structures are size-dependent is their greatest advantage. Metallic nanoparticles, for example, produce tunable radiation and absorption wavelengths based on their aspect ratio and coating. These distinct properties are the characteristics of localized surface plasmon resonance (LPSR). Some nanostructured surfaces are appropriate for increased cell adhesion and

proliferation [28]. Carbon nanotubes possess extremely high current carrying capacity. Nanowires play a very important role in various sensing techniques like electrochemical, electrical, optical, and mass-based strategies. By taking advantage of nanoparticles' biodistribution, nanostructure-mediated medication delivery improves therapeutic impact and decreases side effects. Many scientists have created hybrid nanostructures that combine one or more nanoparticles. Hybrid nanostructures address fundamental issues in oncology, such as drug resistance and tumor heterogeneity, by combining many treatment modalities in a single nanocarrier and releasing it at the disease site in a regulated manner [29].

Organic Nanoparticles

Various studies are focusing on the utilization of nanoparticle-based materials having organic structure for bone, cartilage, skin, and dental tissue regeneration. Polymeric nanoparticles, carbon nanotubes, liposomes, and biomimetic nanoparticles are all examples of organic nanoparticles [8].

Polymeric Nanoparticles (NPs)

Polymeric nanoparticles (NPs) are colloidal particles that range in size from 10nm– 1μm. Polymeric nanoparticles (NPs) have several applications due to their properties arising from their small size. Polymeric NPs are known to clear heavy metals that are toxic to living cells. Nanoparticles affect their toxicity due to quantum size effects associated with genotoxicity, cytotoxicity, and oxidative stress. Polymer-NP composite materials have unique features like good electrical conductivity, high mechanical strength, optical and thermal properties. For oral drug delivery of quercetin. Kumar *et al*. produced a biodegradable polymeric NP. poly-ε-caprolactone (PCL) was the polymer used which is nontoxic, approved by FDA, biocompatible, permeable, and biodegradable. The authors concluded that the particles can be used in the pharmaceutical industry as they allow controlled release of the drug [30]. Nanosphere and nano capsules are the two forms of structures that can be formed by polymeric nanoparticles depending on the method of preparation. Polymeric nanoparticles can be produced from synthetic polymers. There are two types of synthetic polymers available - biodegradable and nonbiodegradable. For the treatment of cancer and advanced diagnosis, biodegradable polymeric nanoparticles show therapeutic potential for accurate drug delivery. Poly (d,l-lactic-co-glycolic acid) (PLGA) is a biodegradable polymer and has been used for the transdermal delivery of Spantide II, and ketoprofen. Polyacrylates are nonbiodegradable polymers. Polyacrylates are also used for dermal and transdermal drug delivery but to a lesser degree compared to biodegradable polymers. For the systematic transfer of chemotherapeutic drugs to tumor cells with the least damage to the healthy tissues, targeted PNPs are usually

used. PNPs can be viewed as ideal candidates for targeted antibiotics delivery, vaccine delivery, and cancer therapy [31]

Carbon Nanotubes

Carbon nanotubes are carbon allotropes. Carbon nanotubes (CNTs) are carbon-based tubes with diameters measured in nanometers. Their unique physical, chemical, and electronic properties offer great opportunities for nanometer-scale electronic applications. Carbon nanotubes can either be metallic or semiconducting based on their structure [32]. Macroscopic CNT-enabled materials are the most studied carbon nanotube composites. Single-walled carbon nanotubes (SWCNTs), double-walled carbon nanotubes (DWCNTs), and multi-walled carbon nanotubes are the three types of carbon nanotubes (MWCNTs). One or two graphene cylinders are found in SWCNTs and DWCNTs respectively, whereas MWCNTs contain many concentric graphene sheets. Because of their huge surface area, flexibility, remarkable strength, current capacity, low weight, and semiconducting properties, CNTs have been widely used in a variety of applications. Carbon nanotubes have sparked attention as a novel adsorbent due to their hollow and multilayer architectures, as well as their high chemical and thermal resilience [33].

Liposomes

Liposomes are phospholipid vesicles that are made up of one or more concentric lipid bilayers that surround discrete aqueous gaps that resemble the biological cell membrane. Because of their unique capacity to entrap lipophilic and hydrophilic molecules, these vesicles can encapsulate and transport a wide spectrum of medications. Hydrophilic molecules are integrated into the bilayer, while hydrophobic ones are confined in the aqueous center. Phospholipids such as phosphatidylcholines, phosphatidylethanolamines, phosphatidyl serines, and phosphatidylglycerol, as well as cholesterol, are typical liposome substituents. The most frequent nanocarriers for delivering targeted drugs are liposomes. They are known for developing more effective remedies for several biological problems. Due to its flexibility and versatility, a liposome is one of the most popular nanomedicines in cancer therapy and bioimaging, as well as a remarkable delivery mechanism. As a drug delivery method, liposomes have various advantages, including the ability to self-assemble, the ability to carry big pharmaceuticals, biocompatibility, and a wide range of physicochemical and biophysical properties. A lipid bilayer made up of cationic, anionic, or neutral lipids, as well as cholesterol, surrounds an aqueous center in traditional liposomes [34]. By altering pharmacokinetics and biodistribution to promote therapeutic

delivery to damaged tissue, traditional liposomal formulations reduce chemical toxicity *in vivo*. Ligand-targeted liposomes are used to deliver drugs to specific organs *in vivo* by selectively expressing certain ligands (receptors or cell adhesion molecules) at the ailment site. Commonly used ligands are antibodies, peptides/proteins, and carbohydrates. Polyethylene glycol (PEG), a hydrophilic polymer believed to be the best choice for manufacturing sterically stabilized liposomes, was discovered to improve liposomes' stability, and circulation time in the blood [35, 36].

Biomimetic Nanoparticles

Nanoparticles have one inherent property that they can be easily recognized as foreign substances by an immune system. Therefore, they have poor drug targeting effects. Biomimetic nanoparticles (NPs) are a new type of NP that has recently been identified as a unique drug delivery system that increases medication biocompatibility and specificity at the desired target region [37]. Nanoparticles (NPs) disguised in cell membranes are one of the most advanced biomimetic platforms for mimicking some of the functions and composition of cell membranes. This membrane-coating technology has lowered the constraints of nano-systems, such as quick elimination in circulation, allowing them to navigate more effectively throughout the body. Because of the various functional molecules present on the surface, cell membrane-based nanoparticles (CMBNPs) can interact with the complicated biological environment of tumor cells. According to the purpose and target disease, cell membrane coating can be produced from different cell lines such as platelets, RBCs, leukocytes, cancer, and stem cells, thus involving a wide variety of plasma membranes [9].

Leukocyte membrane-coated nanoparticles

WBCs, also called as leukocytes, are immune system cells that defend the body from pathogens and infectious diseases. WBCs can easily migrate to and from blood arteries to extravascular tissues due to amoeboid mobility. The progression of tumor cells is aided by a significant number of inflammatory cells, such as neutrophils, dendritic cells, macrophages, eosinophils, and mast cells, as well as lymphocytes [38]. Leukocytes are attracted by several chemokines and cytokines produced by tumor cells. Macrophages are considered one of the largest populations of cancer-related leukocytes. Nanoparticles coated with macrophage membranes have a prolonged blood circulation time, the ability to overcome vascular barriers and the ability to recognize tumor cells by molecular recognition. These NPs were also able to bind specifically to inflamed regions, allowing drug transport across the vasculature [39].

Red Blood Cell Membrane-coated Nanoparticles

RBCs, also referred to as erythrocytes, are common cells in human blood, with a total number of around 20-30 trillion. CD47 (transmembrane protein) is expressed on the cell membrane of red blood cells and is identified as a self-component that leads to long-term RBC circulation *in vivo*. CD47 binds to the inhibitory receptor signal regulatory protein alpha and releases the "do not eat me" signal, which prevents immune cells from phagocytosing RBCs. The CD47 signal indicated circulation time of RBCs. When injected into mice, different types of nanoparticles coated with RBC-membrane were found to have a longer circulation period and a regulated release of the encapsulated medicines, such as DOX (doxorubicin), with a higher LC50 [39].

Cancer Cell Membrane-coated Nanoparticles (CCNPs)

Cancer cells differ from blood cells in their limitless replicative potential, immune evasion, and homogenous targeting ability. Cancer cells can be easily separated *via in vitro* cell culture due to their proliferative potential. Homotypic cancer cell aggregation is critical for the formation of secondary lesions in various organs and tissues [37]. Because cancer cells have innate immune escape and homologous adhesion capabilities, many cancer-CMC nanoparticles are being designed for tumor targeting detection and therapy. A cancer cell membrane-cloaked-up conversion nanoprobe, was designed and it demonstrated low immunogenicity and homogeneous targeting effects [40].

Platelet Membrane-coated Nanoparticles

Platelet membranes have received a lot of attention because of their availability and distinct physiological significance. Because they have CD47 and P-selectin on their surfaces, they can address vascular damage and interact with circulating cancer cells, making them a unique platform for cancer targeting. Moreover, platelet-biomimetic NPs show higher blood circulation time. The presence of specific ligands like CD47 and CD55/59 on their surface platelet membranes provides potential advantages for nanoparticle coating like immune evasion and avoidance of complementing activation respectively. Hu *et al*. created platelet membrane-coated nanovesicles (PMNVs) with DOX and TRAIL ligands that can induce apoptosis in target cells in a recent study. Their ability to trigger apoptosis in MDA-MB-231 cells by delivering TRAIL to their membranes has been demonstrated. DOX-loaded platelet membrane-coated NPs containing RGD peptides were also demonstrated to evade immune-mediated purging and target cancer vasculature [38].

CHALLENGES IN NANOBIOTICS

Allergic Responses

Despite the potential advantages of employing nanoparticles in industry and health, there is growing concern regarding their biosafety. The interaction of man-made nanoparticles with the immune system has become vital and significant. These interactions between nanoparticles and the immune system can result in a variety of immunological reactions, which can alter the immune system and potentially cause immunotoxicity. For example, nanoparticles increase the level of reactive oxygen species in cells during a pro-inflammatory state, which can cause damage to protein, lipid, and membrane of human cells. Constant activation of the immune system can lead to the production of allergic and autoimmune diseases. Therefore, complete knowledge of the immunomodulatory effect of nanoparticles is very important for developing nanoparticles for biological purposes [18, 41]. Engineered nanomaterials have large biodistribution and tissue accumulation which can be serious from an allergic point of view. Boraschi *et al*. discovered that human primary monocyte-based *in vitro* tests may be used to evaluate the impact of manufactured nanoparticles on human innate immune responses. In one of the studies, it was shown that regardless of nanomaterial type i.e., single, or multi-walled, intranasal, or subcutaneous administration of carbon nanotubes (CNTs) increase the allergen potential of egg albumin. Another study demonstrated that the blood concentration of IgE was remarkably enhanced following iron oxide nanoparticles' single dose intratracheal administration. Furthermore, the mechanisms behind nanoparticle immunotoxicity are still not well understood and need more research to be determined effectively [42].

Assembly of NPs

Drug development areas have made extensive use of nanotechnology. Nanoparticle-based pharmaceuticals can efficiently deliver hydrophobic medications and biologics to target areas by crossing biological barriers. Despite these major advantages, only a small percentage of nanoparticle-based drugs have been licensed for clinical use, owing to a few roadblocks and barriers encountered throughout the research process. According to reports, the complexity of nanoparticles as a 3-Dconstruct necessitates careful design and engineering, detailed orthogonal analysis methods, and a repeatable production and scale-up process to achieve a consistent product with the required physicochemical properties, biological patterns, and pharmacological characteristics. Small variations in numerous parameters might alter the safety and efficacy of nanoparticle-based therapies, thus they must be thoroughly studied in preclinical and clinical research, particularly in terms of biodistribution and immunological

toxicities. The ability to construct simultaneous control structures on different length scales and their modification in time or on-demand is one of the most significant difficulties in nanoparticle self-assembly [43, 44].

Pharmacokinetics of NPs

Nanomedicines have physicochemical features, shape, particle size, and size distribution, and slight variations in the composition induced by manufacturing process deviations might influence the pharmacokinetics, biodistribution, and safety profiles of nanoparticle-based medicine. The achievement of a proper pharmacological and pharmacokinetic profile appropriate for the specified indication is critical for effective nanomedicine. The application of small-molecule pharmacokinetic criteria to the pharmacokinetics of nanomedicines poses several obstacles. Because only a small portion of the medication supplied reaches its intended location, the conventional criterion for assessing pharmacokinetic in the blood as the primary measure of nanoparticle *in vivo* behavior may be incorrect. With many nanomedicines available, it is doubtful that standard pharmacological approaches would be relevant to characterize their behavior. It was reported that there is no successful approach to engineer and design a nanoparticle-based medicine to attain an intended pharmacokinetic profile. The most popular method is to use nanomedicines with prolonged circulation times to take advantage of the EPR effect or target them. However, this technique may not always be appropriate for the intended reason, and it may compromise therapeutic efficacy and increase systemic exposure needlessly in some circumstances. Furthermore, rather than assessing plasma pharmacokinetic physicochemical properties, it may be more relevant to look at drug concentrations or accumulation at the intended sickness site to investigate the repeatability and activity of nanomedicines [43, 45].

Application of Nanobiotics in Treatment of MDR Infections

Antimicrobial nanobiotics identified and synthesized to date (i.e metal, metal oxide and organic and polymeric NPs) harbor a vast diversity of intrinsic and modified physicochemical properties. These properties are instrumental in their broad-spectrum effects which operate by numerous modes of action. This section explains how the various nanobiotics are used to treat bacterial MDR infections with a focus on their mechanism of action (MOA) and bioavailability.

Metallic and Inorganic Nanobiotics

Due to their ease of synthesis and tunable properties, metallic nanobiotics have been widely exploited for treating MDR bacteria in chronic wounds, infectious diseases, sepsis, and inflammatory syndromes. The intrinsic antibacterial effects

of metallic NPs are exerted through a range of broad-spectrum mechanisms. The small size enables high-level interaction with microbial membranes and protein transport activity. Metallic NPs engage in electrostatic interactions with sulfur proteins present abundantly in the bacterial cell envelope. This causes irreversible damage to cell wall structure resulting in disruption of cell membrane integrity and creating a leaky bacterial cell that exudes its cellular contents into the host environment; these can easily be detected and cleared by immune cells. Thus, metallic NPs offer an excellent alternative to traditional antibiotics for treating MDR bacteria. The Gram-negative ESKAPE pathogens have negatively charged lipopolysaccharides (LPS) on their surface which enables strong adhesion with metallic NPs which contain positive charges on their surface. This makes them even more susceptible to nanobiotic therapy. The free radicals are toxic to all the ESKAPE pathogens by directly damaging their DNA, proteins, and lipid biomolecules. In addition to the above, inorganic NPs modulate cell signaling and destabilize protein synthesis machinery.

Inorganic and metallic NPs have a large surface area to volume ratio and possess high cell permeability capabilities. Along with their intrinsic broad-spectrum bactericidal effects, these present themselves as viable candidates for carrying a variety of antibiotics. Gold nanoparticles combined with antibiotics generate nanobiotics with improved bactericidal action against a variety of Gram-positive and Gram-negative bacteria, according to efficacy tests. When compared to single antibiotics, these combined nanobiotics have a longer shelf life. Integrating inorganic NPs such as silica and graphene with metallic NPs enhances the bactericidal effects along with increased stability of drug release. One such example is the nano-assembly of ferric oxide (Fe_3O_4) core with a shell of mesoporous silica ($mSiO_2$) coated with graphene oxide and loaded with antibiotic cargo, which displays stable release of the drug. The high-affinity binding of metallic NP-antibiotic complex to the outer envelope of the bacterial cell enables an increase in local concentration and bioavailability of the drug and thus, antibacterial effects are seen at a much lower dose of the antibiotic. Integrating these particles into a polyethyleneimine surface resulted in an extension to control infections in biomedical applications. Several studies have shown that gentamicin and vancomycin can be successfully conjugated to gold NPs and these Au nanobiotics demonstrated increased antimicrobial activity against vancomycin-resistant enterococci (VRE) and *S. aureus* (VRSA). Importantly, the gold–conjugated antibiotics are quite stable even under harsh storage conditions as compared to the free antibiotic. Another group has shown increased absorption and activity of ciprofloxacin in the presence of ZnO nanoparticles. Interestingly, metallic/inorganic NPs can mimic the catalysis of natural enzymes, and these are termed as nanozymes. These nanozymes respond to changes in pH, GSH (glutathione) levels, and free radical contents of the human host, and accordingly

generate therapeutic agents through catalytic mimicry of enzymes. This effect has mainly been observed in antitumor nanobiotic therapy where inorganic nano-assemblies mimic peroxidases to generate toxic ROS to cause targeted tumor apoptosis. This has yet to be explored in detail for ESKAPE pathogens.

Liposomal Nanobiotics

Hydrophobic chemicals are loaded in the lipid bilayer with nano-sized liposomes, while hydrophilic substances are placed in the aqueous core. Antibiotics are better loaded into small unilamellar vesicles (SUVs) with a diameter of about 100 nm. These SUVs offer a flexible drug-carrier system that can be designed with the desired set of pharmacokinetic properties. Entrapment in liposomes also reduces the side effects of commercial antimicrobials.

Polyethylene glycol (PEG)-coated liposomal nanobiotic formulations improve the plasma circulations time of the antibiotic and consequently enhance clearance of infectious extracellular bacteria and their biofilms. The encapsulation in liposomes also protects the antibiotics from enzymatic hydrolysis which is a major resistance mechanism employed by ESKAPE pathogens. Previous studies have also shown that liposomal nanobiotics are highly effective and much less toxic, operating at lower doses for treating methicillin-resistant *S. aureus* (MRSA). In a remarkable experiment, the MDR variants of *Klebsiella pneumonia* regained susceptibility to levofloxacin *in vitro*, by administration of liposomal levofloxacin nanobiotic integrated with antimicrobial peptides. Liposomal nanobiotics are proficient in reducing drug toxicity and are mainly used to transform toxic drugs into safe therapeutic options.

Carbon based Nanobiotics

Carbon nanostructures were created as a revolutionary material with potential for several applications. One such recent application is the creation of nanobiotics. Functional carbon dots (CDs) have shown promise as bacterial inactivation and detection agents when conjugated with antibiotics. This field is in its nascent stages and a few initial studies have shown that CDs conjugated with ciprofloxacin provide controlled drug delivery under physiological conditions and display high antibacterial activity against *Pseudomonas aeruginosa*. These carbon nanostructures are also useful in increasing the stability and loading capacity of other drug carriers such as calcium alginate beads covered with CDs. Carbon nanodots hold the application in the detection of bacteria as well; this will be explored later in this chapter.

Polymeric Nanobiotics

Antibiotics can be incorporated in polymer-based nanoparticles or conjugated to them. Polymeric NPs are nano-sized colloidal particles made up of biocompatible materials including poly (lactic acid), poly (lactic-co-glycolic acid), chitosan, and others. Polymeric NPs are efficient agents for stabilized drug release, drug solubilization, and targeted activity. These also have higher bioavailability and protect the antibiotic from rapid degradation in the alimentary canal by digestive enzymes and HCl. Polymeric NPs can be designed in the desired manner based on the targeted region. Their high permeability and stability allow drug administration through different routes apart from the oral route, such as nasopharyngeal and intravenous. The selection of polymer is based on the desired drug release profile, to allow effective and safe concentration of the antimicrobial at the site of infection. Biofilms are difficult to clear and are a major cause of chronic ESKAPE infections. A formulation of synthetic PLGA- nanobiotic dual conjugated with nitric oxide and gentamicin was able to effectively reduce 90% viability of *P. aeruginosa* biofilms. Other such PGLA- nanobiotics were able to penetrate through the thick mucus blocking airways of chronic pulmonary patients infected with *P. aeruginosa.*

Another polymeric nanostructure type is supramolecular gelatin nanoparticles (SGNPs). Substantial clearance of *S. aureus* infection was observed with SGNP formulation decorated with red blood cells and embedded with vancomycin. This hybrid nanobiotic was observed to be only effective against Gram-positive bacteria and showed no antibacterial against the Gram-negative ones. A great advantage of this hydrogel formulation is the capability of an extremely controlled on-demand antimicrobial release strategy. Chitosan, alginates and hyaluronic acid are natural polymeric NPs. Alginates and HA have been extensively used for drug delivery, whereas chitosan-based NPs are more useful for mucoadhesion and intracellular permeability. Antimicrobial and anticancer capabilities are found in AMPs, which are lengthy peptides containing 2-100 amino acids. Another component of nanomedicine that is being investigated to treat infectious diseases is the use of amplifiers and nanotechnology. An alginate-based spherical hydrogel containing AuNPs and nisin exhibiting AMPs on its surface could inhibit the growth of ESKAPE pathogens such as *S. aureus* and *E. faecalis*.

Biomimetic Nanobiotics

The amalgamation of biomimetic nanobiotics into the field of nanomedicine was done to explore the creation and application of therapeutic options adaptive to the complex functioning of the human body during the onslaught of infection. This technology has majorly been applied for vaccines and antitumor drugs but has

been showing promise for antibacterial therapy. In brief, biomimetic NPs are created by extracting cellular components for *in vitro* engineering of naked NPs to disguise parent cells. An example is the synthesis of leukocyte-mimicking liposomes (leukosomes) created by integrating extracted leukocyte membrane proteins into a liposomal NP. To further the adaptive properties of biomimetic NPs, the entire cell membrane is coated on the surface of inorganic or polymeric NPs. RBC membrane is the most common biomimetic membrane, mentioned in the previous section, coating synthetic NPs with RBC surface properties for avoiding macrophage capture, and also quenches the PAMPS such as a bacterial toxin, β-hemolysin/cytolysin of group *B Streptococcus*.

The Synergy Between Antibiotics and Nanoparticles

Antibiotics are one of the major medical inventions of the 20th century that have the capability to treat and cure bacterial diseases and harmful pathogens. But continuous inappropriate and overuse of antibiotics have resulted in the development of microbial drug resistance causing an adverse impact on mankind. Due to increasing bacterial resistance, antibiotics are becoming less powerful. New approaches for handling bacterial infections are instantly required and hence nanomaterials may be a useful approach. Nanobiotics as antibacterials complementary to antibiotics can perform the functions where antibiotics usually fail and can reduce the toxic effect of synthetic antibiotics and can reduce the problem of increasing bacterial multidrug resistance. Therefore, for antibiotics delivery, the use of nanoparticles has also been widely studied [40]. Most studies reported on nanoparticles as antibiotic carriers are confined only to some well-known antibiotic drugs like ciprofloxacin and penicillin. But recently, attempts have been made for the development of drug delivery approaches such as new lipophilic β-lactam antibiotics to solve bacterial drug resistance problems. For example, Poly (ethyl cyanoacrylate) (PECA) nanoparticles have been explored to entrap β-lactam antibiotics for using colloidal suspensions in aqueous media. In one of the studies, to produce amoxicillin-loaded poly(cyanoacrylate) nanoparticles, Fontana utilized pluronic (nonionic polyoxyethylene polyoxypropylene block copolymers) of different molecular weights. These PECA nanoparticles are specifically useful for delivering β-lactams to the stomach on a site-specific basis [41].

Application of Nanobiotics in Diagnostics

Treatment success is determined by the accuracy and speed with which a diagnosis is made, the better the prognosis, the faster and more accurate the diagnosis. Nanomaterials-based diagnostic platforms are much better equipped to display and amplify the signal of detection- associated molecules such as antigens

or antibodies owing to the high surface area-to-volume ratios and versatile surface chemistry. Metallic NPs have an added advantage of the nanoplasmonic effect which essentially means that light exposure (EM spectrum: visible to near-infrared) rapidly transforms the interactive events between detection-associated molecules and the NPs to generate outputs visualized optically and measured by photometrics.

Another sophisticated nanodiagnostic platform is biosensors. Biosensors can be of many types out of which a microfluidic chip based on biomimetic NPs is the most popular type. The microvesicles derived from nanoscale membrane fragments of leukocytes and neutrophils are embedded onto the chip and the diagnosis is done through fluorescence readings in a short response time of just 1.5 hours. Furthermore, nanobiotics can also be used as diagnostics through optical signal generation using an antibiotic conjugated with a fluorescence probe. Gao *et al*. created a biosensor with a vancomycin-fluorescent probe attached to a FePt magnetic NP. Van-FLA can recognize bacterial surface peptide D-Ala-D-

Ala through hydrogen bonds. This diagnostic chip can detect bacterial infection in under two hours with a low limit of detection of 10 CFU/ml and is more sensitive for Gram-positive bacteria like *S. aureus*. Gram-negative bacteria can be detected using a similar nano-biosensor with a polymixin B antibiotic that binds to the LPS. Another nanodiagnostic has been developed by Mou *et al*. that is based on a colorimetry strategy of redox chemical reactions. This quantitative diagnostic is made of azide and alkyne modified AuNPs that aggregate and changes color from red to blue *via* a chemical reaction catalyzed by copper ions (Cu+) generated by the redox enzyme system of bacteria that reduce exogenous Cu^{2+} to Cu+. This nanodiagnostic has been practically generated to identify and quantify *E. coli* in an experimental blood sample containing a mixed suspension of bacteria. The color signal in this nanodiagnostic platform is colorimetrically quantified through a smartphone at an LOD of 40 CFU/ml in a response time of under 1 hour. Carbon nanostructures can also act as theranostic (therapeutic and diagnostic) agents. One such notable example is if nano carbon dots (CDs) are conjugated with vancomycin for accurate detection of Gram-positive bacteria, including *S. aureus*, and *Listeria monocytogenes*. Thus, nanoplatforms present to us unique, rapid and sensitive diagnostic tools which might be the future of accurate detection and disease mitigation.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 6

Metallic Nanoparticles as Antibacterial Agents

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Abstract: Metallic nanoparticles against bacteria have increased recently due to their unique properties. Many metals like silver, gold, copper, aluminum, zinc and their oxides have been shown to have antibacterial properties. The activity of the nanoparticles is affected by their physico-chemical properties. Different types of mechanisms are proposed for the antibacterial actions against various types of bacteria. The metal-based nanoparticles are synthesized by the top-down methods and bottom-up methods. However, the latter methods are used effectively against many types of bacteria including antibiotic-resistant bacteria.

Keywords: Antibacterial activity, Antibiotic resistant, Metallic nanoparticle, Physico-chemical properties, Synthesis.

INTRODUCTION

Nanoparticles are exceptionally tiny particles that vary from 1-100 nanometer. These particles possess different chemical and physical properties in contrast to their larger counterparts. Since the particle size is extremely small, thus they follow the Brownian movement and do not sediment. Moreover, these are not seen by the naked eyes and with an ordinary microscope. Nanotechnology is one of the several techniques, which is employed in biology, chemistry, environment, food industry, agriculture, engineering, therapeutic application, sensor and medicines [1, 2]. The pharmaceutical field in medicine is mainly used for the

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improvement of drug solubility, bioavailability and delivery to various sites of action and real-time monitoring of drugs [3].

Nanoparticles are classified into different categories based on their physical and chemical properties. These may be metal nanoparticles, non-metal nanoparticles, ceramic nanoparticles, semiconductor nanoparticles, ceramic based nanoparticles, carbon-based nanoparticles, lipid-based nanoparticles, polymeric nanoparticles, organic based nanoparticles, *etc.* Among different types, metal-based particles are important in pharmacy. They are used in drug and gene delivery for their effectiveness against micro-organisms, in diagnostic assay, in thermal ablation and anticancer properties [4, 5].

Since ancient times, metals are used to cure several types of diseases and to combat infections against many micro-organisms like bacteria, fungi, viruses *etc.* Many types of metals are used among which silver, copper, gold, aluminum oxide, copper oxide, and titanium oxide have found their wide application against various diseases. In this chapter, we have discussed the properties, mode of action and the preparation of metallic nanoparticles.

PHYSICO-CHEMICAL PROPERTIES OF METAL NANOPARTICLES

Metal nanoparticles have quite distinctive features in comparison to their larger counterparts. Such properties provide them the mechanism of toxicity to different types of bacteria. Different types of physico-chemical properties affect their toxicity which are detailed as under:

Size of Nanoparticles

The size as well as the surface area of metal nanoparticles is crucial for the antimicrobial activity. The small size of nanoparticles has the larger surface area relative to volume that makes the nanoparticles more active by facilitating their entry in the bacterial cell membrane in comparison to larger nanoparticles [6]. The nano shape of the nanoparticles facilitates better contact with the plasma membrane of the bacteria mostly because of their larger surface area showing preferable interaction with the membrane than the larger nanoparticles [7]. The size of the nanoparticles thus greatly affects the various types of biological mechanisms [8, 9]. It clearly indicates that size and surface area of the particle govern the systems [10]. Due to the smaller size, nanoparticles are able to enter the biological system [11] due to which modification of various biomolecules takes place [12] which ultimately interferes the biological functioning of the cell. Although various mechanisms are attributed to the toxicity of nanoparticles, yet the production of ROS (reactive oxygen species) that is because of the creation of

free radicals since the liberated electrons try to make a stable bond is prime mechanism. The size of nanoparticles is essential in the generation of ROS which imparts hazardous effects on DNA in comparison to their larger counterparts [13, 14]. Besides, the size less than 100nm causes adverse respiratory effects in human beings in comparison to the larger nanoparticles. However, it is not necessary that the size of nanoparticles alone is responsible for antibacterial activity, other physico-chemcial properties of NPs should also be considered for antibacterial mechanism [15].

Shape of Nanoparticles

The shape of metal nanoparticles is important for their antibacterial activity. Shapes of nanoparticles interact with periplasmic enzymes of the bacterial cell thereby causing bacterial cell damage [16]. In nanoparticles, the most common shape is spherical however, other shapes like triangular, cubical, hexagonal, oval, helical, prism, tubes and rod-shaped are also found which impart toxicity and influence the wrapping process in the membrane during endocytosis and phagocytosis [17]. It has been shown that triangular and truncated (cut-off corners) sized nanoparticles show better inhibition [18] whereas nanotubes and rod shapes have been reported to be more effective due to their exposed planes. The exposed planes having higher density facets help in increasing reactivity because of the large surface area to volume proportion thus facilitating to increase the adsorption and binding of nanoparticles [8].

Charge of Nanoparticles

Charge on nanoparticles is a pivotal factor for the antibacterial property. Positively charged nanoparticles are attracted to the anionic cell wall of bacteria electrostatically thus alter the functioning of electron transport chain in bacteria which results in the creation of ROS [8]. Whereas, the negatively charged nanoparticles do not stick to the bacterial cell wall, however, the higher concentration of the negatively charged bacteria leads to interaction between bacteria and the nanoparticles due to molecular overcrowding [19]. The potential of nanoparticles increases vascular permeability [20].

Acidic Conditions

Acidic conditions favour bindings of the nanoparticles to the bacterial cell wall through electrostatic interaction [21]. Acidic conditions have been shown to increase the dissolution and release of Zn^{+} [22]. In acid medium, the silver

nanoparticles liberate the silver ions rapidly [23] whereas zero-valent copper nanoparticles possess the highest toxicity under acidic conditions [24].

Concentration of Nanoparticles

The concentration of nanoparticles has a direct effect on causing toxicity to bacteria. The higher concentration of nanoparticles releases more ions. Increased concentrations of silver ions produce oxidative stress in bacteria [25, 26]

MODE OF ACTION AGAINST BACTERIA

General Structure of Bacteria

Bacteria are the single-celled structure, which have no nucleus thus are called prokaryotic organisms. The DNA of bacteria is either present in the plastids or floats freely in nucleoids. Also present in the bacteria is a spherical structure which is known as a ribosome in which protein synthesis takes place by encoding the information from rRNA (Fig. **1**).

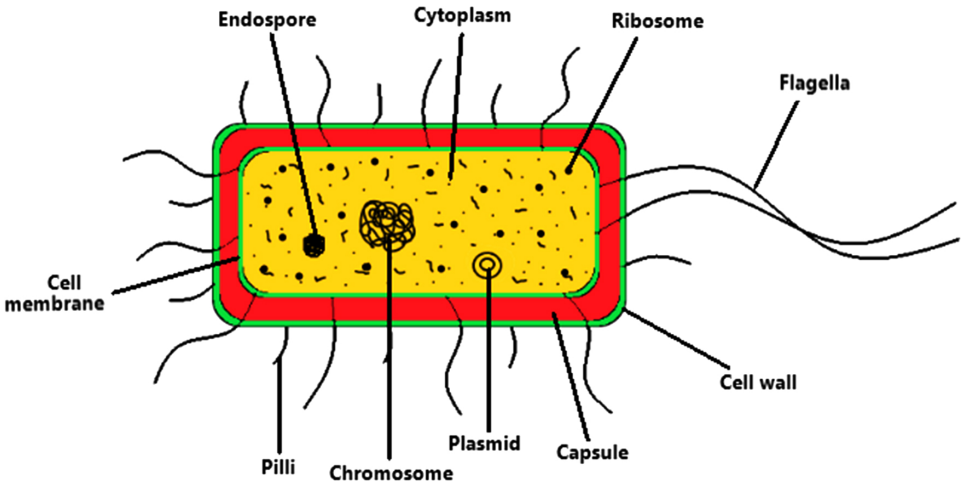

Fig. (1). General structure of bacteria

There are various structural differences in gram positive and gram negative bacteria. In the former, the outer wall has a thick laced layer of peptidoglycan protein and other polymers as teichoic acids whereas the latter is composed of multiple layers of peptidoglycan in which the outer membrane forms a barrier to the passage of many chemicals [27](Fig. **2**).

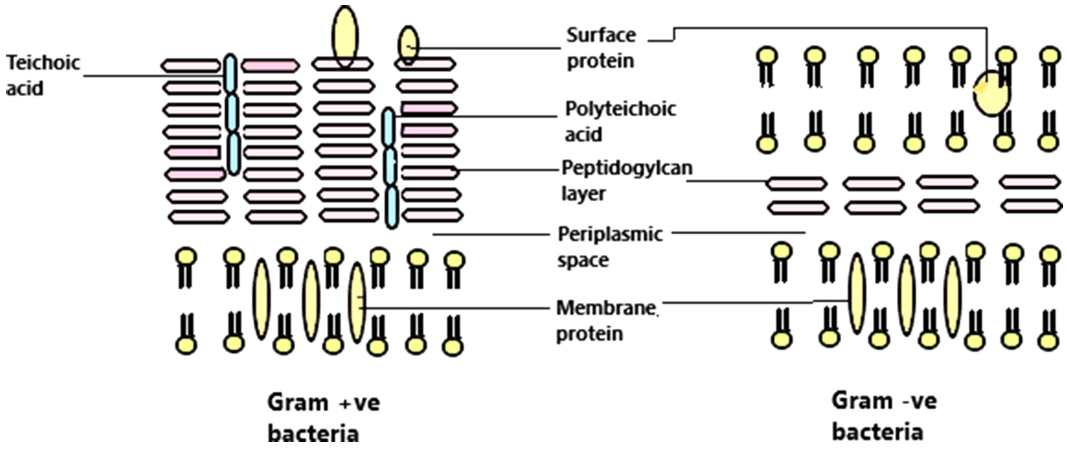

Fig. (2). Structural differences between Gram positive and gram negative bacteria.

Nanoparticles' Antibacterial Mechanism

Since nanoparticles are incredibly minute, they must be in close vicinity to the bacterial cell wall. The walls of bacteria are negatively charged, which is accomplished through a variety of mechanisms, involving electrostatic attraction, receptor ligand, van der Waals forces, and hydrophobic contact. In general, the nanoparticles are neutral so they cannot cross the cell membrane therefore the ions released by the nanoparticles cross the membrane which interferes the functioning of the cell membrane. Gathering of ions inside the cells leads to the disruption of functioning of cell organelles and enzymes. Oxidative stress, heterogeneous modifications, modifications in membrane permeability, and electrolyte imbalance are all induced by these processes. Furthermore, gene expression is influenced by enzyme inhibition and protein deactivation [28 - 30].

Various types of mechanisms are proposed for the antibacterial mechanism of nanoparticles (Fig. **3**). These are: Oxidative stress; Metal ion release and Non oxidative mechanisms.

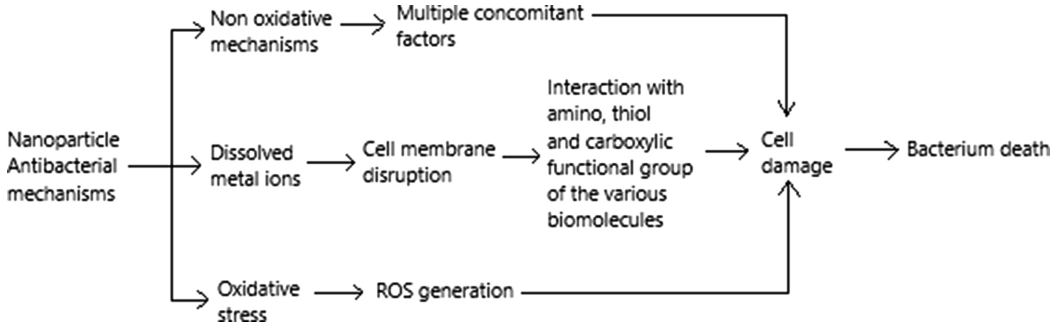

Fig. (3). Antibacterial mechanism of nanoparticles.

Oxidative Stress

This arises when the concentration of ROS is increased inside the cell. ROS are the highly reactive molecules that are formed due to the electron receptivity of oxygen which plays an important role in microbial metabolism. ROS related oxidative stress causes a change in the permeability of bacterial cell causing impairment [31]. The macromolecules (protein, lipid, carbohydrate and DNA) are also affected due to oxidative stress [32, 33]. ZnO has been found to possess antibacterial property and oxidative stress mechanism in a bacterial species, *E. coli* [34]. Intracellular oxidative stress has been found to lose the integrity of some bacteria when Al_2O_3 nanoparticles cross the bacterial cell membrane [35]. Nano silver ions check the multiplication of bacteria or kill them. These ions activate the oxygen in the air thus become reactive oxygen ions which are harmful to bacteria [28, 30]. ROS also been reported to play an active role by facilitating the relationship between DNA and bacterial cells [36].

Metal Ion Release

Metal ions are responsible for toxicity to bacteria. These ions are released slowly from their metal or their oxide nanoparticles. When these ions enter the bacteria by crossing the cell membrane, the cellular activity of the cell is disrupted which ultimately causes toxicity to bacteria [37, 38]. The toxicity to bacteria by these ions is directly proportional to their release. Besides, other mechanisms are also involved in causing toxicity to bacteria [38 - 43]. The release of ions depends on the metal from which these are formed. Copper nanoparticles release more nanoparticles than silver nanoparticles [44]. Silver and mercury that are heavy metals were found to have a detrimental effect on the physiology of bacterial cell [19] (Slavin *et al*, 2017).

Non-oxidative Mechanism

The non-oxidative energy system does not require oxygen to generate ATP. The antibacterial activities of MGO were studied by using nanomaterial-based techniques against a gram-negative bacterium (*E. coli)* and it was found that MgO in two was not able to form the ROS [45]. On the other hand, MgO in one could form only small ROS. The main mechanism of such a phenomenon was that lipopolysaccharides and phosphatidyl ethanolamine were not oxidised due to which the gene expressing ROS did not increase. This reveals that the toxicity was not only due to oxidative stress but it was due to the direct contact of MgO, the effect on pH and the slow release of Mg^{2+} ions [45 - 47].

SYNTHESIS OF NANOPARTICLES AND ANTIBACTERIAL ACTIVITY

There are two methods for the synthesis of nanoparticles (Fig. **4**); Top down method and Bottom up method or wet method.

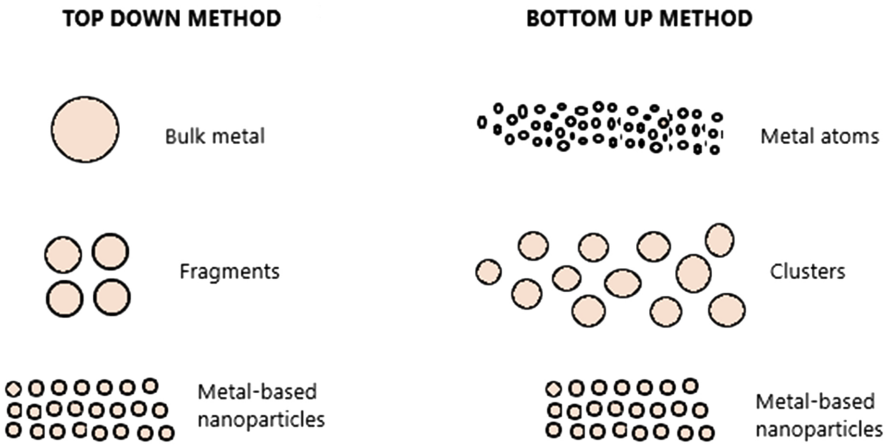

Fig. (4). Synthesis of metal based nanoparticles.

Top-down Methods

Also known as physical or mechanical methods, in which the body of a substance is mechanically reduced into tiny particles/molecules, resulting in the material's size being reduced to the nanoscale. Because of the particles' dispersion, these procedures are not suited for the synthesis of nanoparticles, despite the fact that the size of any nanoparticle has a significant impact on toxicity. The methods include Milling; Sputtering; Pulsed wire evaporation and Nanolithography.

Bottom-up Methods

In this method, the formation of nanoparticles takes place from nucleating atomic-sized materials by creating nanomaterials and objects within the same nanosphere. The methods are processed in such a way that allows the increase in the functioning of the structure of processed materials. Bottom-up approaches are categorized in many ways, the two approaches are more common: Chemical method and Biological methods.

Chemical Methods

In these methods, organic solvents are used. The synthesis encounters many types of problems associated with those which include the stability of products, aggregation of particles on long-term exposures, *etc.* Moreover, the toxic chemicals that are used for the synthesis limit their use for a long duration [48]. The metal nanoparticles synthesized in this way are detailed in Table **1**.

Table 1. Antibacterial activity of metal nanoparticles

NP	Size (nm)	Bacterial Strain	Antibacterial Activity	References
Silver	12.4	*E. coli*	Damage to cell occurs as a result of the development of pits in the bacterial cell wall and the deposition of nanoparticles in the bacterial membrane, which further causes the membrane's permeability to increase, leading to apoptosis.	[49]
Silver	1-10	*Salmonella typhimurium, Vibrio cholera*	Bacterial activity is size dependent with direct interaction with bacteria.	[41]
Silver	3-10	*E. coli*	Enhanced cell wall permeability leads to membrane damage and apoptosis.	[41, 49, 50, 51, 52]
Silver	1-10	*Pseudomonas aeruginosa, Vibrio cholera*	Interaction with the cell membrane, cellular membrane damage and DNA damage	[41]
Silver	3	*Bacillus subtilis*	Bactericide, damage of cell wall.	[52]
Silver	39	*E. coli*	Change in cell membrane and cell death.	[53]
Silver	13.5	*E. coli*	Free radical generation effect causes inhibition of bacteria.	[40]
Silver	92	*E. coli*	Antibacterial activity is due to smaller size.	[54]

(Table 1) cont.....

NP	Size (nm)	Bacterial Strain	Antibacterial Activity	References
Silver	22.5	*E. coli, S. aureus*	Enhanced the activity of some antibiotics.	[55]
Silver	3	*Staphylococcus aureus*	Disruption to the cell wall.	[52]
Silver	20-25	*A. baumanii, S. aureus, B. subtilis, P. aeruginosa, M. smegmatis, E. coli, M. bovis*	Cytotoxicity including DNA damage and cell viability.	[56]
Silver	10	*Gram+ and Bacillus*	Toxicity is determined by the shape, size, and capping agent.	[57]
Silver	-	*B.subtilis, B. barbaricus, Pseudomonas aeruginosa, Klebsiella pneumonia*	Cell viability is changed due to a change in zeta potential	[58]
Silver	1.5-10	*P. aeriginosa*	The size, form and composition of nanoparticles influence bacterial function.	[59]
Silver	9, 19, 43, 18, 23	*E. coli*	Size-dependent biological effect.	[39]
Silver	5-15	*L. monocytogenes*	Penetration of cell wall and plasma membrane of bacteria. Plasmolysis is triggered by the detachment of the plasma membrane from the cell wall.	[44]
Silver	95	*S. mutans*	The activity depends on size and concentration. Cytotoxic effect.	[60]
Silver	7.1	*E. coli, P. aeruginosa*	Production of ROS. Mechanical damage to membrane.	[61]
Gold	8.4	*A. baumannii, S. aureus, P. aeruginosa, E. cile*	Inhibition of cell growth.	[62]
Gold	55, 100	*S. oneidensis*	Surface attachment	[63]
Copper	10-40	*E. coli*	Antibacterial activity depends on type of copper nanoparticles.	[64]
Copper oxide	2 and 30	*E. coli and S. aureus*	Nanoparticles create ROS, which have antibacterial properties.	[65]
Copper	3	*S. aureus and E. coli*	-	[66]
Copper oxide	33	Gram negative and Gram positive bacteria	Both kinds of bacteria generate ROS.	[67]
Copper	4-18	*Pseudomonas aeruginosa and Staphylococcus aureus*	Inhibitory effect	[68]
Copper	63-160	*S. aureus, E. coli*	Sustained released of antimicrobial agent.	[69]
Copper	20-50	*E. coli and S. aureus*	Antimicrobial activity	[70]

(Table 1) cont.....

NP	Size (nm)	Bacterial Strain	Antibacterial Activity	References
Copper	10	*S. aureus*	ROS production	[71]
Copper	800	*S. aureus and Enterococcus*	Depolarization and damage of cell membrane of bacteria	[72]
Copper	191	*S. aureus*	Antimicrobial activity	[73]
Copper	10	*E. coli*	Antimicrobial activity	[74]
Copper	8	*S. aureus and E. coli*	The inhibition zone in bacteria is increased due to an increase in the amount of copper nanoparticles.	[75]
Copper	30-40	*E. coli, K. pneumonia, S. aureus*	Excellent antimicrobial activity.	[76]
Al_2O_3	11	*E. coli*	Toxicity is determined by the chemical makeup, size, surface charge, and form of the substance.	[77]
CeO_2	7	*E. coli*	Damage to cell membrane	[78]
CeO_2	6, 15, 22, 40	*B. subtilis, E. coli*	Growth inhibition of bacteria	[79]
CeO_2	2-4	*L. monocytogenes*	-	[44]
ZnO	12	*E. .coli*	Surface oxygen species of nanoparticles and abrasiveness.	[80]
ZnO	-	*E. coli, P. aeruginosa, Staphylococcus aureus, Enterococcus hirae, Bacteroides fragilis*	Loss of viability	[81]
ZnO	19	*E. coli*	Toxicity in aqueous media is caused by free zinc ions and its complexes.	[82]
Cu_2O	40	*E. coli*	Production of ROS	[83]
CuO	20-95	*E. coli, S. aureus, P. aeruginosa*	The emission of copper ions promotes antibacterial action.	[42]
CuO	30	*E. coli*	Production of ROS	[83]
MgO	4	*B. subtilis, B. megaterium,*	Bactericide. Disruption and damage to cell walls.	[84]
TiO_2	20	*Pseudomonas aeruginosa. Bacteroides fragilis, Enterococcus hirae*	Bactericide. Viability loss	[81]
TiO_2	12,17,21 25	*E. coli*	Damage to the cell membrane	[77]
TiO_2	23	*E. coli*	Massive cell leakage of K+ and depletion of intracellular ATP level	[85]

Biological Methods

These methods are based on the green-synthesis processes in which different types of microorganisms and plants are used. Various types of microorganisms are associated for the microbe-mediated synthesis of well-structured nanoparticles (details below) [86]. These are classified as Actinomycetes-based synthesis, algae-based synthesis, bacteria-based synthesis, fungi-based synthesis, yeast-based synthesis and plant extract-based synthesis.

The antibacterial activities of various biogenic metal nanoparticles are presented in Table **2** and the biogenic species included in this table are bacteria, fungus and plant species.

Table 2. Antibacterial activity of biogenic metal nanoparticles.

Biogenic Species	NanopArticle	Shape	Name of Bacteria	Antibacterial Activity	Reference
Deinococeus radiodurans	Gold	Spherical, triangular & irregular	*E. coli, S. aureus*	Cytoplasmic membrane of bacteria is damaged	[87]
Shewanella loihica	Copper	Spherical	*E. coli*	Cell damage due to ROS. Destruction of cell membrane and cytoplasmic components.	[88]
Enterococcus faecalis	Se	Spherical	*S. aureus*	Accumulation of nanostructure as extracellular deposits.	[89]
Bacillus mycoides	TiO_2	Spherical	*E. coli*	Antimicrobial activity	[90]
Aeromonas hydrophila	ZnO	Spherical	*Pseudomonas aeruginosa*	Inhibition of bacteria.	[91]
E. faecalis	Copper	Spherical	Both type of bacteria	Antibiofilm activity of nanoparticles upregulated	[92]
Aspergillus niger	Ag	Spherical	*Staphylococcus aureus, E. coli*	Antibacterial activity	[93]
Penicillium	Ag	Spherical	*E. coli, S. aureus, P. aeruginosa, B. cereus,*	Inhibition of bacteria	[94]

(Table 2) cont.....

Biogenic Species	NanopArticle	Shape	Name of Bacteria	Antibacterial Activity	Reference
Streptomyces sp	Gold	Spherical	*E. coli, K. pneuminaw, P. mirabilis, S. infantis, P. aeruginosa and B. subtilis*	Antibacterial activity	[95]
Sreptomyces viridogens	Gold	n.a.0F	*S. aureus and E. coli*	Antibacterial activity	[96]
Trichoderma hamatum	Gold	Spherical, pentagonal & hexagonal	*P. aeruginosa, Serratia sp., B. subtilis, S.aureus*	Antibacterial activity	[97]
Aspergillus flavus	TiO_2	Oval	*S. aureus, E. coli, P. aeruginosa and B. subtilis*	Bacterial inhibition	[98]
Aeromonas hydrophila	ZnO	Spherical	*P. aeruginosa*	Bacterial inhibition	[91]
Pichia kudriavzevii (yeast)	ZnO	Hexagonal	*E. coli, S. aureus, B. subtilis, S. epidermis, S. marcescens,*	Bacterial inhibition	[99]
Ananas comosus leaf extract	Silver	Spherical	*S. aureus, S. pneuminiae, P. mirabilis, E. coli*	Bacterial inhibition	[100]
Justica adhatoda leaf extract	Silver	Spherical	*P. aeruginosa*	Bacterial growth is inhibited.	[101]
Eriobotrya japonica leaf extract	Silver	Spherical	*E. coli and S aureus*	Antibacterial action	[102]
Melissa officinalis leaf extract	Silver	Spherical	*S. aureus, E. coli*	Inhibitory activity	[103]
Houttuyniacordata (PE)	Gold	Spherical	*A. subtillis, S. typhimurium, P. cereus, E. coli*	Antibacterial activity. Gram positive bacteria have more inhibition effect than Gram negative bacteria.	[104]

(Table 2) cont.....

Biogenic Species	NanopArticle	Shape	Name of Bacteria	Antibacterial Activity	Reference
Cresentia cujete	Gold	Spherical	*E. coli, O. aeruginosa, V. cholera, Salmonella typi, S. flexneri, B. subtilis*	Difference in cellular membrane thickness makes the Gram negative bacteria have a more inhibition effect than Gram positive bacteria	[105]
Disoscorea batata (rhizome)	Ag	Spherical	*C. albicans, S. cerevisiae*	Antimicrobial property	[106]
Citrus sinensis peel extract	Ag	Spherical	*E. coli, S. aureus, P. aeruginosa,*	Antimicrobial property	[107]
*Croton sparsiflorus morinaga leaves*leaves	AgNO3	Spherical	*S. aureus, E. coli, B. subtilis*	Antimicrobial property	[108]
Gum karaya(plant gum*)*	CuO	-	*E. coli, S. aureus*	Antimicrobial activity is due to the smaller size of nanoparticles.	[109]
Malva sylvestris leaf extract	CuO	Spherical	Both gram + and gram – bacteria	Significant effect against both types of bacteria	[110]
Phyllanthus amarus leaf extract	CuO	Spherical	*B. subtilis, E. coli, P. aeruginosa, S. aureus*	Inhibitory effect	[111]
Gloriosa superba leaf extract	Ruthenium	-	*Effective against gram + and gram – bacteria*	Antimicrobial property	[112]
Gloriosa superba leaves	CuO	Spherical	*S. aureus, Klebsiella aeroegenes, P. desmolyticum, E. coli*	Antimicrobial property	[113]
Cystoseira trinodis(Brown alga)	CuO	Spherical to irregular	*E. coli, Enterococcus faecalis, Salmonella typhimurium, Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis*	Inhibitory activity	[114]

(Table 2) cont.....

Biogenic Species	NanopArticle	Shape	Name of Bacteria	Antibacterial Activity	Reference
Costus pictus plant extract	ZnO	Hexagonal	*S. aureus, B. subtilis, E. coli, S. paratyphi*	Antimicrobial property	[115]
Camellia sinensis leaves	ZnO	-	*K. pneumonia, E. coli* and *P.aeruginosa*	Antimicrobial property	[116]
A. halicacabum	ZnO	Spherical & rod	*E. coli, S. aureus*	Gram-negative bacteria have more antibacterial properties than Gram-positive bacteria.	[117]

CONCLUSION

The studies on the antibacterial property of various metal nanoparticles clearly reveal their potential and further their exploitation in this area. Various types of bacterial species have been reported to be susceptible to metal nanoparticles, thus these can be considered effective agents for antibiotic drug-resistant bacteria. However, the exact mechanism for their mode of action against different types of bacteria needs to be investigated despite various types of proposed mechanisms.

FUTURE PERSPECTIVE

Since the widespread and uncontrolled use of antibiotics against bacteria has been exacerbated, the use of metal-based nanoparticles has increased and offered tremendous promise as antibacterial agents. However, detailed studies are required about their possible side effects before their introduction in a large scale to the market since some studies indicate their effect on human cells. Further, the investigation of the exact effective concentration, synthesis and formulations of metal-based nanoparticles may open new vistas in this field.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 7

Promises of Nanobiosensors in Pathogen Detection

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Abstract: Rapid and accurate identification of pathogens has always been challenging. There are a number of methods for the detection of pathogens, but still they face critical challenges. In general, rapidity, sensitivity, and accuracy are the important criteria that limit the applicability of classical methods. Nanomaterials-based biosensors have been proven to be effective for the early and accurate quantification of pathogens. Interactions between target pathogen and nanomaterials are very important, as they provide a measurable signal in biosensors. Nanobiosensors are effective in detecting pathogenic bacteria in various samples, including food, water, blood, and other matrices. In this chapter, we intend to discuss the existence and importance of electrochemical-based biosensors for quantification.

Keywords: Bacterial Sensing, Electrochemical, Nanobiosensors, Pathogenic bacteria.

INTRODUCTION

Bacteria, fungi, and protozoans are infectious agents that cause disease. Viruses and prions are molecular scale infectious agents, enter the body causing infection and lead to millions of deaths annually worldwide [1, 2]. The most prominent pathogens include bacteria such as *S. aureus* and *E. coli*, and viruses like influenza virus which bring exotoxins, mycotoxins and enterotoxins. They vary in several regards, like in contagiousness, virulence, transmittable dose and mode of spread. For example, the world is at present facing a global pandemic linked with the COVID-19 virus, for which infectious dose and virulence data are still promising.

Food products are the actual provision for a healthy life and are the strong transmitting media of more than 200 known diseases [3]. Drinking water is also a major source of contamination by microorganisms that have increased fast in

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recent years [4, 5]. Community concern has significantly increased regarding water and food safety in the past decades.

Therefore, it is of primary importance to observe these microorganisms for the avoidance of infections, the maintenance of human health at large and to comply with quality standards.

Different pathogen recognition techniques have been developed in recent times. Traditional methods containing immune-diffusion, latex agglutination, immune-precipitation, *etc.* were multi-step and time-consuming, taking several days to confirm the presumptive results [6, 7]. This has made them less appropriate for the fast and direct study of pathogens.

Some other techniques such as Enzyme-linked immunosorbent assays (ELISA), and Polymerase Chain Reaction (PCR) were frequently opted to detect pathogens but they could be time-consuming, sensitive and expensive to the given qualitative and quantitative information [8 - 10]. PCR technique not only requires expensive reagents for routine analysis but also bears the problem of false positives by amplifying nonviable cells [11]. Hence, it is required to develop suitable detection methods that authorize an accurate, rapid, and sensitive investigation to monitor pathogens.

Biosensors are integrated devices, developed to quantify and measure biomarkers particularly for infectious pathogens since they exhibit the advantages of selectivity, simplicity, rapidity, and high sensitivity [12, 13]. A biosensor has two elements, a transducer and a bioreceptor, which have great importance. A transducer converts the chemical recognition information into an assessable signal, and the bioreceptor can recognize and combine the target biomolecules. On the basis of signals, biosensors, and transducers are divided into colorimetric, fluorescent, electrochemical, SERS biosensors, and so on.

Electrochemical-based Biosensors

Electrochemical biosensors can perform chemical or biological analysis with simplified sample preparation and facilitate high sensitivity in lesser time. Electrochemical biosensors are made up of three elements, with signal transduction element, target recognition element, and electrochemical signal output elements. Combined with electrocatalytic activity, electronic properties and nanoparticles with a huge surface area [14], nanoparticles-based electrochemical biosensors have achieved significant attention for pathogen detection [15]. Nanoparticles offered an appropriate microenvironment for the immobilization of biomolecules to support the transfer of electrons between electrodes and immobilized biomolecules and enlarge the surface area of

electrodes for target identification. Therefore, electrochemical-based biosensors have a unique advantage of fast response, high sensitivity and ease of operation in thick media compared with conventional methods. Based on monitored electric parameters, electrochemical biosensors could be divided into voltammetric biosensors, amperometric biosensors, and impedimetric biosensors, and potentiometric biosensors.

Voltametric Biosensors

Voltammetric biosensors supervise the current changes caused by the reduction or oxidation reaction of the electrochemically energetic analytes, which can be studied by the unstable potential in the electrochemical system [16].

Shoaie&Omidfar (2018) prepared a voltammetric biosensor for rapid detection of *E. coli* based on AuNPs and a polyaniline customized screen-printed carbon electrode with a LOD down to 4 CFU/ml. The low LOD biosensors have great applications of AuNPs and polyaniline, which may significantly increase the surface area and conductivity for immobilized biomolecules [17].

Likewise, Zhu *et al.* (2014) developed a unique amperometric biosensor based on the rolling circle amplification (RCA) approach for *Salmonella* detection in milk samples. The *Salmonella* DNA was first arrested on the electrode surface by a DNA-AuNPs probe. After a chain of amplification processes, the DNA-AuNPs identified the RCA product and formed an enzymatic amperometric signal. The range for target DNA detection by proposed biosensors was from 10aM to 10pM and the LOD down to 6.76 aM [18].

Nze *et al.* (2019) also developed a technique for separating and electrochemically identifying *E. coli* in ground meat also developed by. In this technique, antibody-coated magnetic beads and hydrodynamic cavitation are used for the separation of immunomagnetic samples, which significantly amplified the detection potential of the biosensors [19].

In 2017, Chen *et al.* developed a voltammetric biosensor for the detection of *Mycobacterium tuberculosis* DNA. If nanoparticles are incorporated with DNA amplification approach, this can improve the detection limit of biosensors.

Metal-based nanoparticles, such as MWCNTs and GO carbon-based NPs, have also been commonly applied in electrochemical biosensors because of their excellent electron transfer properties and high surface area.

Amperometric-based Biosensors

Amperometric biosensors are working on the principle that the number of

transferred electrons is linear with respect to the concentration of the analyte [20]. A sensitive and simple amperometric immunosensor was proposed by Zhu *et al.* (2013), for simultaneously identifying three analytes, with carcinoembryonic, alpha-fetoprotein and *Streptococcus suis* Serotype 2 [21].

The biosensor uses a graphene sheet, AuNPs, *etc.* functionalized as a tracer for the second antibody. On the surface of the electrode, the antibodies were first immobilized to prepare a sandwich structure with the tracer in the occurrence of the target. The LODs were reduced to 4.2pg/ml with a linear range from 0.012 to 50 ng/ml for streptococcus suis Serotype 2.

Villalonga *et al.*, (2019) designed a core-shell MNP-based amperometric biosensor for fast recognition of *Brettanomyces bruxellensis* in wine. Fe_3O and SiO_2 nanoparticles were customized with antibodies for arresting *Brettanomyces bruxellensis.* The amperometric indicator rises with the increased concentration of *B. bruxellensis* and the series of the amperometric biosensor started from 10 to 10^6CFU/ml with 5 CFU/ml LOD down [22].

CONCLUSION

Nano therapies are far more effective than any other traditional chemotherapy in the detection and treatment of ovarian cancer. They are quite effective because of their potential to target a specific tissue and also to examine the living body of animals for adequate durations of time.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 8

Breaking the Barriers of Nanotoxicological Assessments: The Importance of Available Models and Future Perspectives

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Abstract: Nanoparticles (NPs) and nanotechnology have penetrated every walk of life. The nanotechnology-based products include pharmaceuticals, cosmetics, electronic goods, food, food packaging, and household products of daily use. The unique physicochemical properties of nanoparticles also make them a potent toxicant. The evidence suggests that nanoparticles are used in humans' neurological disorders, pulmonary disorders, and other ailments. The situation is alarming as NPs may make their way to the human fetus. The regulations for checking the use of NPs are still in their early stages. The NP toxicity has not only affected the human race but the entire Biosphere. The chapter discusses the different assays and models to study nanotoxicity. The models used in deciphering the molecular mechanism are primarily *in vitro* models, particularly 2D and 3D cell cultures of primary, cancerous and normal cell lines. 2D cultures are monolayers, while 3D cultures can be spheroids and organoids derived from stem cells. Cell culture models serve to be a good assessment model but due to lack of systemic complexity, results may not be explicitly extrapolated to humans. In order to fill the gap, *in vivo* models are available. *In vivo* models are helpful in assessing the systemic toxicity in organisms. The *in vivo* models are further categorized as models to study human nanotoxicity and the models to study nanoecotoxicity. Out of the plethora of models, certain specific models are briefly discussed here. The ethical regulations for the usage of animal models are stringent which sometimes make it challenging to acquire animal models. Such challenges can be overcome by developing futuristic models like a lab or animal on a chip, and other computation models which may make nanotoxicological assessments easy and accurate, thereby helping in making efficient regulatory policies for NPs usage in various consumer products safeguarding the mankind and the biosphere.

Keywords: Cell viability, Cytotoxicity, Ecotoxicity model, *In vitro* models, *In vivo* models, Nanoparticles (NPs), Nanotoxicity.

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INTRODUCTION

The advances in nanotechnology have been unprecedented in the last two decades. The technology is penetrating every aspect of human life in the form of nanomedicines, disease diagnostics, food preservatives, cosmetics (sunscreen), detergents, personal wears, vehicles, paints, surface coatings and electronic goods. Nanoparticles (NP) are everywhere. "Nanotechnology product Database," a website that maintains the data of nanotechnology based products worldwide, shows currently there are 9422 products that are being manufactured by 2797 companies set in 64 countries across the globe [1]. The number of such products has increased more than seven times in the last ten years as it was just 1317 in 2011.

The database shows that the electronics, cosmetics, medicine and textile industries are amongst the top industries having maximum numbers of nanotechnology enabled-products [1]. This ever increasing trend shows how fast we are dumping our environment with such nanotech enabled products containing various NPs whose fate and effects on the human and the biosphere is drastically unknown. NPs are more reactive as compared to their larger counterparts with similar particle mass due to their smaller size, higher surface area, and high tensile strength. The characteristics that make them such an advanced technology, also make them a threat to us and our Biosphere. The term 'nanoparticle' was coined in 1970s but its potential to be a toxicant was acknowledged in the year 2004 when the term 'Nanotoxicity was used for the first time [2]. The immense increase in the uses of NPs in consumer products has enhanced the chances of human exposure due to which the instances of toxic outcomes have also increased. There are studies which highlight the neurological, pulmonary, vascular, and genetic toxicities in humans caused by NPs' exposure. For example, in a clinical study performed over 22 human subjects, chronic exposure to Fe_3O_4 NP of size less than 20nm is reported to be neurotoxic. Age-associated bio-mineralization of Fe_3O_4 in the brain is manifested as Alzheimer's disease [3]. In another study, 37 human subjects showed symptoms of neurodegenerative disorders due to an enhancement in ROS levels upon exposure to Fe_3O_4 with a size ranging up to 150 nm [4]. To investigate the pulmonary toxicity of nanomaterials, Khatri *et al*. studied the effects of acute exposure to NPs (30-40 nm) from photocopiers in 9 healthy volunteers. These nanoparticles can trigger immune responses in the upper airways, resulting in systemic oxidative stress with the generation of pro-inflammatory cytokines [5]. In a study dealing with vascular dysfunction, an incidental exposure of diesel fumes consisting of NP (<100 nm) showed increased systolic blood pressure in 16 human subjects, which might be due to vasodilation induced by oxidative stress [6]. Genotoxicity associated with exposure to silver NPs is also reported in mononuclear leukocytes

in 76 subjects employed in the silver jewellery industry. The genotoxicity was attributed to oxidative stress induced by silver NPs [7].

The situation is alarming as NPs are not only affecting human health, but also being accumulated in our ecosystem. The release of NPs in water bodies and landfills is 69000 and 189200 metric tons per year [8], respectively. The biological magnification of NPs is still in its early stages of research. In the early 1960s, pesticides and chemical fertilizers came up with a lot of hope and promise in solving hunger issues by increasing plant yield. However, after decades of prolonged usage, we now know that these toxic chemicals enroute to our biological system and cause various diseases ranging from mild allergies and hormonal disorders to severe genotoxicity and cancers [9 - 11].

The human race would certainly not want to be caught off guard in the case of NPs, and that is why it is important to study the behaviour of NPs in terms of toxicity for which we need to device a vast setup of model systems along with the robust test batteries. The fate of NPs in the environment depends upon the aggregation, disaggregation, chemical interaction, and change in their surface properties. There is very little research available regarding these aspects of various NPs in both biological and ecological systems. Due to a lack of robust knowledge about the prospective harms of using these NPs in various consumer products like cosmetics and other routinely used stuffs, developing countries like India fail to make stringent policies for regulating the usage of NPs in consumer products. Thus, the area needs appropriate model systems for nanotoxicity evaluation in order to decipher the potential threats as well as to form stringent regulations.

Here in this chapter, we would discuss the present advances in nanotoxicology research in terms of various assays and various available models for the assessment of toxicity of such NPs along with the future perspectives just for the ease of the young researchers because very few such articles are available that illustrate all these assays and models in one write-up.

NANOTOXICITY CAUSES AND MECHANISM

Nanoparticles enter the human body *via* oral ingestion, inhalation, occular exposure, deposition on the skin, and intravenous administration. NPs then translocate *via* the bloodstream to distant organs and tissues. While translocating NPs may interact with serum proteins, thus resulting in the structural changes of interacting proteins, causing them to accumulate around the NP (protein corona formation) and may change the protein's functionality. Subsequently, NP may also trigger certain pathways that lead to immunotoxicity, loss-of-function in proteins, new antigenic site formation, and may hinder gene expression [12, 13]. Some of

the main mechanisms include NPs attachment to the cell membrane, dissolution to toxic ions, and oxidative stress (Fig. **1**).

Membrane Damage: The cell membrane is the first line of protective barrier against the potential toxic effect of nanoparticles. NPs may directly interact with the cell membrane *via* electrostatic attraction, and this interaction can lead to NPs uptake by endocytosis without being involved in any specific receptor-mediated interaction [14]. It can physically damage the cellular boundary by removing the lipid membrane. In addition, it can initiate or disrupt internal signalling pathways [13, 15].

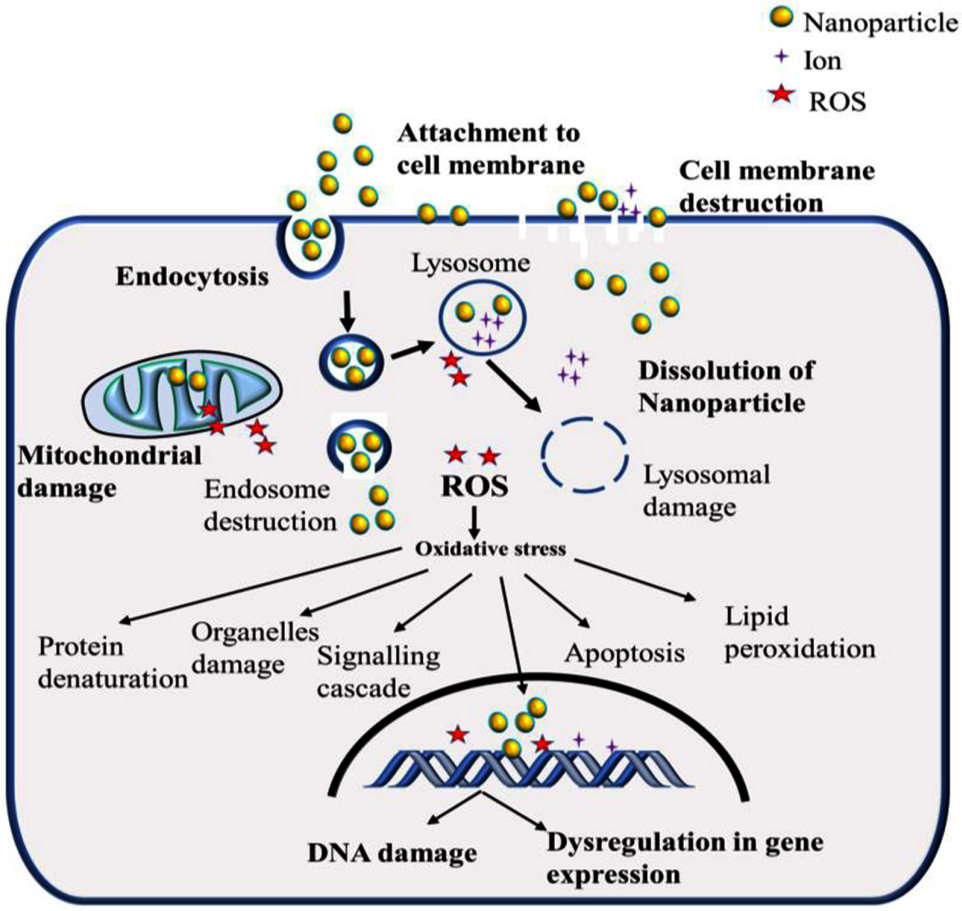

Fig. (1). Schematic representation of NPs toxicity inside the cell.

Dissolution to Toxic Ions: The dissolution of toxic elements from NPs is an essential mechanism whether that dissolution occurs after binding to an organism

or in the surrounding environment. There are different ways through which released ions can cause toxicity. Several ions can bind to proteins and enzymes, thus critically impairing their functions and inhibiting cellular metabolism [16]. Metal ions may directly interact with and damage the phospholipid membrane and/or genetic material and alter the gene expression of the organism [17 - 19].

Oxidative Stress: After internalizing the membrane, nanoparticles or their ions can generate excessive amounts of reactive oxygen species (ROS). The amount of ROS generation depends on the shape, size, and charge on the surface of the NPs. Because of the strong oxidation potential of NPs, the excess ROS induced by them can cause damage to biomolecules and organelle structures, and lead to protein oxidative carbonylation, lipid peroxidation, DNA/RNA breakage, and membrane structure destruction, which further cause necrosis, apoptosis, or even mutagenesis [20].

IN VITRO SCREENING METHODS AND ASSAYS FOR NANOPARTICLE CYTOTOXICITY

As new nanomaterials are developing, rapid screening of their biological and health impacts is required to assess their possible risk to provide insight into proper handling and care. Presently a wide variety of cytotoxicity and cell viability assays are in use in the field of nanotoxicology and pharmacology. An ideal assay for *in vitro* viability and/or cytotoxicity determination should be rapid, safe, reliable, efficient, and time and cost-effective. Also, it should not interact with the test compound. The choice of assay method is also crucial in the assessment of the interaction type. Therefore, the assay method should be chosen with caution, considering the mechanism of action of the test compound. The tissue or cell type used in the study also influences the results of cytotoxicity and/or cell viability assays. Therefore, different methods should be tried and compared to determine cytotoxicity and/or cell viability in *in vitro* studies to increase the reliability of the obtained results. Most commonly used assays for determining cytotoxicity are cell viability assays, oxidative stress or ROS assay and apoptosis assays.

***In Vitro* Cell Viability Assays:** Nanomaterials can affect cell health and metabolism *via* various mechanisms for example, destruction of cell membranes, prevention of protein synthesis, permanent binding to receptors, oxidative stress, and enzymatic reactions, leading to cell death. Therefore, there is a need for cost-effective, reliable and reproducible short-term cytotoxicity and cell viability assays to decipher the potential as well as the mechanism of toxicity [21]. *In vitro* cell viability and cytotoxicity assays with cultured cells are extensively used for toxicity testing of NPs. These assays can determine number of viable and dead

cells at the end of the experiment. Various cytotoxicity and cell viability assays are used in the field of nanotoxicology based on their detection principle. For example i) Lightmicroscopy based dye uptake or exclusion assays using various dyes/stains such as trypan blue, hematoxyline, eosin, congo red, erythrosine b *etc*. ii) Colorimetric assays such as MTT assay, XTT assay, MTS assay, WST-1 assay, WST-8 assay, LDH assay, SRB assay, NRU assay and crystal violet assay. iii) Fluorometric assays like Alamar Blue assay (resazurin) and CFDA-AM assay and iv) Luminometric assays such as ATP assay and real-time viability assays.

ROS Assays Oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and a cell's antioxidant defences, is associated with human diseases as well as aging. Oxidative stress in the cell can be determined by quantifying the glutathione, changes in ROS, and the ratio of reduced to oxidized glutathione as indicators of cell health. ROS is most common marker for the determination of oxidative stress. Generation of ROS within the cytoplasm beyond natural levels is measurable *via* introduction of ROS sensitive dyes such as the nonionic, nonpolar, membrane-permeable fluorophore 2′,7′-dichlorofluorescein diacetate (DCFH-DA). After internalization in the cell, it converts into the nonfluorescent polar analog dichlorofluorescein (DCFH) by the cellular esterase enzyme. Hydroxyl radicals and cellular ROS oxidize DCFH into highly fluorescent dichlorofluorescein (DCF), which is monitored using fluorescence microscopy or flow cytometry, or fluorescence based multiwell plate reader [22].

Apoptosis Assays Usually, the toxicants cause toxic outcomes in the form of apoptosis, which has a vital role in aging, development, and diseases. Apoptosis is originated by a tightly regulated signalling cascade that involves various apoptosis related proteins (*e.g*. Bax, Bad, Bcl2, Caspases *etc*) which play their specific roles in the execution of apoptosis under the influence of certain toxicants. Key features of apoptosis include cell shrinkage, membrane blebbing, chromosome condensation, nuclear fragmentation, DNA laddering, and the eventual engulfment of the cell by phagosomes. An apoptosis assay depends on detecting and quantifying such cellular events like caspase activation, mitochondrial damage, cell surface exposure of phosphatidylserine (PS), and DNA fragmentation and these events can be detected by Caspase-Glo, JC-1, Annexin V, and TUNEL method respectively [22]. These methods are mainly based on Colorimetric or Fluorometric principles of detection.

MODELS

Despite increasing exposure to NPs and their potential toxicity, there is no sufficient data to predict its hazardous impact on humans as well as on biosphere.

To formulate any regulation for the potential future threat, there must be a robust database on nanoparticle toxicity, and to achieve it, a series of suitable models are needed. Currently, the toxicity assessments depend upon cellular models i.e. *in vitro*, as well as the animal models *i.e., in vivo.*

Cellular or *in vitro* Models: NP has potential to induce toxicity at molecular, systemic, and ecosystem levels. According to regulatory bodies, NPs undergo toxicity screening before it reaches for human usage. The first phase of the screening is performed in *in vitro* models. *In vitro* models are comparatively easier in terms of ethical and regulatory guide lines, cost and maintenance, as compared to *in vivo* models. Based on the exposure paradigm, relevant cell types can be selected and grown in both 2D and 3D culture setups.

2D Cell Culture This is the commonly used culture setup. Most of the cell types are grown as a monolayer in 2D space. Cells are grown in artificial nutrient media supplemented with growth factors (generally animal serum). Different cell types are grown in different media, *e.g*., MEM, DMEM, RPMI *etc*.

Cancerous and Normal Cell Lines The cell line could be cancerous or normal based on the origin. Cancerous cell line derived from cancerous tissues and has potential to divide infinite times. Normal cell lines are primary cells transformed into immortal cells by inducing certain mutations *via* radiation, chemicals, or viruses. Cancerous cells have a different microenvironment, acidic pH, activated replication pathway, overburdened translational machinery, and a changed phenotype, in such an environment, NPs react differently. In the acidic environment of cancerous cells, ZnO NP releases Zn ions that kill cancerous cells but not normal cells [23, 24]. Similarly, Ag NPs coated with polyvinylpyrrolidone (PVP) can induce the apoptotic pathway in cancerous cells (HepG2: human hepatoma cells)) but not in normal cells (L-O2 cells (normal hepatic cells) [25]. Also, it has been reported that SiO_2, Fe_3O_4, and TiO_2 NPs can kill 16HBE (immortalized human bronchial epithelial cells) but not A549 cells (human non-small-cell lung carcinoma cells) [26].

Primary Cell Culture System: The primary culture cells are isolated from the desired organ or part of the organ by harvesting the animal. Such cells can also be isolated from human biopsy tissues or placenta. Such isolated primary cells are then cultured in artificial media having various growth factors. The cells in primary culture are isometric with actual organs and serve as a good model system but, the method is a bit lengthy, technically challenging, and prone to contamination. Also, the whole method depends upon the availability of the desired tissue or model. Apart from this, primary cells have a limited lifespan due to the inability to proliferate indefinitely.

Stem Cell Culture System Stem cells are undifferentiated cells that can form any type of cells on proper activation *via* suitable signals. There are embryonic stem cells (ES), adult stem cells, and induced pluripotent stem cells (iPS). Embryonic stem cells (ES) are derived from the innermost cell of an embryo (blastocyst), and they can develop into any cell form indefinitely. Adult stem cells are derived from the tissues and have the limited self-renewable ability. iPS cells are somatic cells induced to pluripotent stem cells by activating a cascade of genes. The Stem cell has an advantage over primary cells as it can be passaged like immortal cells.

Limitation of 2D Cell Culture: Cells in 2D culture setup lie in dishes in the form of monolayers, whereas, in the actual living body, it remains in 3D space interacting with neighbouring cells, extracellular matrix (ECM), and a number of physiological factors. These interactions shape the cells' functionality and help to maintain homeostasis of the *in vivo* system, which remains missing to a certain extent in the 2D cell cultures. 2D culture also lacks a complex ECM, which is sometimes very important for the transportation of the toxicants/NPs across the cells. So, it can be understood that though the 2D cell culture model provides an effective yet simple cause-and- effect environment, it still fails to mimic the actual environment of the *in vivo* systems. Probably due to these differences, there sometimes occurs a sharp contrast in toxic outcomes assessed in *in vitro v/s in vivo* systems. For example, the toxicity studies of quantum dots [27], magnetic NPs [28], carbon nanotubes [29], and fullerenes [30] performed using *in vitro* 2D cell culture setup showed low viability of cells, but when similar experiments were conducted in the *in vivo* models, there were no adverse effects [31 - 34]. So, to overcome this limitation of the 2D culture setup, 3D culture setups can be used as alternative or midway options between 2D culture setup and *in vivo* models.

3D Cell Culture System: In this cell culture system, a permissive environment is provided for cells to interact among themselves and the surrounding extracellular matrix in three dimensions. 3D cell culture used to screen for small molecule drugs or genetically manipulated to understand disease pathways. Compared to 2D cultures, 3D cell cultures more precisely predict the effectiveness or toxicity of drug treatment. The 3D cell culture can be of two types: Spheroids and Organoids.

Spheroids Culture: They are generated by spontaneous aggregation of cells *via* non-covalent interaction between surface integrins and ECM. The NP toxicity varies a lot in 3D culture as compared to 2D. NP has to pass through layers of cells in 3D culture, which is absent in 2D culture. One of such experiments showed that gold NP stabilized by cetyltrimethylammonium bromide (CTAB) can induce significant morphological change in 2D HepG2 culture but did not cause any effect in 3D culture [35]. Spheroids culture lacks vasculature and different

types of cells and organ level complexities.

Organoids Culture: They are derived from primary tissue or stem cells (embryonic, induced pluripotent stem cell (iPSC) and adult stem cell (ASC)). The organoids ensembles various types of cells, thus exhibiting organ functionalities. Organoid culture involves usage of various growth factors and is a time-consuming process that takes 2-3 months, depending on the type of tissue to generate [36, 37].

Readouts for the Toxicity: The morphological observation for alive cells is complicated in 3D culture as compared to 2D. In 3D culture, the dead individual is represented as small blobs. But the biochemical analysis of the viability of the cells is identical for both 2D and 3D cell cultures, and the assays like LDH, MTT *etc*. are used for assessing the cell viability.

Limitations of 3D Culture Models: Though 3D culture systems are a bit closer to the physiological systems as compared to 2D culture setups, but still the 3D organoids lack the systemic complexity of the animal model and hence, NP toxicity may not be predicted accurately. These limitations can be overcome in complex organism level *in vivo* model systems.

Animal or *in vivo* Models: As the scientific community has started acknowledging the threat of NPs, it becomes necessary to assess the ill effects of such NPs on mankind, but studying such effects directly on human being is not ethically viable; that is why we need appropriate and easy to use model systems. We have also discussed that the *in vitro* cellular models are suitable for assessing the toxicity of NPs but in a controlled environment, whereas, it is crucial to assess the toxicity of such NPs in complex biological systems, for which it is necessary to use suitable animal models from where the results can be extrapolated for establishing the safety guidelines for human usage of NPs. Here in this section, we will discuss about such commonly used *in vivo* models of toxicity. There are several well-established *in vivo* models ranging from simpler organisms like C. *elegans* to the complex organisms like mice, rabbits and monkeys. The selection criteria of an organism as a model are the resemblance at the systemic level with humans, ease in maintenance and handling, ease of availability, and the extent of limitations put on by ethical and regulatory bodies. The simple multicellular models are modified according to particular human organs or related pathologies studies.

Zebrafish: The Zebrafish (*Danio rerio*) is a freshwater fish of the family Cyprinidae, order Cypriniformes. Due to simple maintenance and ease in genetic manipulation, George Streisinger (University of Oregon,1972) used Zebrafish for the first time as a model for developmental toxicity studies since all vertebrates

have highly conserved embryos. The Zebrafish embryo (ZFE) is considered a common, well-established *in vivo* aquatic model for NP toxicity [38 - 40]. The model has several advantages like ease of culturing and maintenance. The embryo is transparent, which is advantageous to monitor the fluorescently tagged proteins or dye under a microscope. The adult to offspring ratio is significant, and hence larger litter is available for the study. The PubMed search "Zebrafish as nanoparticle toxicity model" results in 272 papers in the last 11years (2010-2021). Several Nanoparticle toxicological studies show systemic toxicity, cytotoxicity, genotoxicity, immunotoxicity, and neurotoxicity response in Zebrafish [41, 42]. The exposure of silver NPs has been reported to damage the integrity of gills, developed goblet cell hyperplasia, vacuolization and partial loss of microvilli, and inhibition of Na^+/K^+ ATPase pump in the intestine [41 - 44]. Cadmium and TiO_2 NPs has also been found to cause DNA damage in Zebrafish *via* ROS generation [45, 46].

***Caenorhabditis Elegans*:** *Caenorhabditis elegans* (*C. elegans*) is a soil-dwelling nematoid that is wildly used as a model for molecular genetics, developmental, and neurobiology. In 1963, Sydney Brenner was the first researcher to use *C. elegans* as model animals to study developmental biology. In 1998, *C.elegans* became the first multicellular organism to have its whole genome sequenced. It is an instrumental tool to study the toxicant's effect on fundamental biological phenomena. The model has several advantages: it is economical to culture, has a small body, and is transparent (easy to study gene expression and localization of NP) [47]. The number of offspring per adult is very high (300:1) and the life cycle is approximately three weeks. They are quickly adaptable to the lab condition and which helps in exposure study. They are non-infectious to humans. There are several nanotoxicological studies performed on the model. The PubMed search "*Caenorhabditis elegans* as nanoparticle toxicity model" results in 80 papers in the last 11years (2010-2021). Few of the studies are briefly discussed here. On exposure to Graphene Oxide (GO), there is upregulation of genes related to antimicrobial peptides [48]. NP like Graphene oxide and Cadmium induced abnormal immune response by causing immune cell death [48, 49]. Further Exposure to Silica NP has been reported to impair reproduction, development, and movement. It also affected serotonergic neurotransmission resulting in neurodegeneration in the nematodes [50].

***Drosophila Melanogaster*:** *Drosophila melanogaster* (*D. melanogaster*) is also known as the fruit fly. The first reported document of D.*melanogaster* use in the laboratory was William Castle's group at Harvard in 1901. Thomas Hunt Morgan did his pioneer work on heredity using *D. melanogaster*. *D. melanogaster* has developed into a prominent model to understand how the cascade of gene expression orchestrates single-cell embryo development into a multicellular

organism. Though the model is old, its utilization for nanotoxicity is recent. The PubMed search "Drosophila melanogaster as nanoparticle toxicity model" results in 50 papers in the last 11years (2010-2021). *D. melanogaster* is very easy and economical to culture, and it can grow at 18-25 ºC. The model culturing requires a cornmeal-molasses-yeast-agar medium, a stereo dissecting microscope with a light source, and a CO_2 anesthetizing station with a block and blowgun. The model has a short life cycle of 10 days. Also, it has simple genetic architecture (~15,000 genes harboured on four chromosomes), mutants are easy to make, can easily transport, and are freely available [51]. The adult *D. melanogaster* has organs equivalent to mammals' hearts, lungs, kidneys, gut, and reproductive tract [52, 53]. The models can be used to determine and characterize molecular mechanisms and cellular pathways of NP toxicity. In this regard, E. Demir has reviewed the various studies in which *D. melanogaster* was used to investigate the genotoxicity and cytotoxicity of potential nanopesticides with metallic nanoparticles such as Ag, Si, Co, Au, and TiO_2 NPs [54]. The mentioned NPs can cause somatic mutation, gene mutation, impaired fertility, and longevity. The fly is also used as a model to study nanoparticle immunotoxicity [55].

Rat (*Rattus*): Rat has been an important animal model for ages. The Brown Rat, Rattus norvegicus, was first used for fasting experiments two centuries ago. The first scientific documentation was made in 1856 by J. M. Philipeaux for adrenalectomy in albino rats. Rat is physiologically and genetically similar to humans and can mimic human diseases as well. The familiar strains are Brown-Norway (BN), Sprague-Dawley (SD), and Wistar rat. The most significant advantage of using rats as the model is its legacy. The plethora of research on rats make it the most accepted model for nanotoxicity studies. The PubMed search "Rat as nanoparticle toxicity model" results in 996 papers in the last 11years (2010-2021). The rat model is used for research on various aspects of nanotoxicology. The nanotoxicity studies conducted on rat models show that the exposure of various NPs like ZnO, TiO_2, and Ag NPs may cause complications in the gastrointestinal tract [56 - 58]. The smaller nanoparticles can trigger more intense reactions [59]. Silica NPs are reported to cause pulmonary lesions, lung inflammation, and damages to alveoli and lung tissue in Wistar rats [60]. Few neurotoxicity studies reported that Ferric oxide and Copper oxide NPs could cause a decrease in the nerve cell body accompanied with damages to the dopaminergic terminal and neuronal vasculature [61, 62].

Mice (*Mus* spp): Mice is a standard animal model and is practiced for a long time. Mice were first used for biomedical research in 1678 by William Harvey for reproduction and blood circulation studies. There are hundreds of strains available for research. The most common of them are C57BL/6 and BALB/c mice—the whole-genome sequencing of the mouse (C57BL/6 strain) completed in 2002. The

mouse has 85% gene similar to humans. Like a rat, mice also have many research documents available covering all human disease models and verified protocols. Mouse and rat usage depends upon the study's requirement. The mouse has a slight edge over the rat in terms of shorter life span, smaller size, abundant availability of genetically modified strains (CD-1, SCID, A/J, ICR, NOD, C3H, and many more). The mouse model has been in use for nanotoxicology studies. The PubMed search "mice as nanoparticle toxicity model" results in 3003 papers in the last 11years (2010-2021). The tons of data available on the toxicity of NPs on various human aspects. The toxicity of NP can be observed at a systemic level. The role of ZnO and TiO_2 NPs has been studied on insulin resistance and plasma glucose levels in mice [63, 64]. The single-walled and multiwalled carbon nanotubes, when administered intravenously, resulted in embryolethal and teratogenic in mice [65]. Nickel NPs cause acute lung inflammation and mechanical injury on prolonged exposure [66]. On exposure to carbon nanotubes (CNT), there was a decrease in sperm viability, and count, accompanied by damaged testis in mice [65]. The prime reason for the cytotoxicity of NPs was oxidative stress [67].

Models for Ecotoxicity: The NPs may enter the ecosystem either directly through nanopesticides or indirectly through NPs containing consumer products. It is essential to understand the routes of entry and the fate of various nanomaterials in the environment, and it is also imperative to understand its harmful effect on organisms, from cells to complex communities. The nanoecotoxicity studies are challenging as it is sometimes difficult to differentiate whether the nanoparticle is engineered or has a natural origin (volcanoes, leaching by water bodies). The behavior of NPs varies with the kind of media it is interacting and the bioavailability or bioaccumulation at each trophic level. The complexicity of ecotoxicity caused by nanoparticles' demands appropriate models for its study. There are many models out of which, few are discussed here briefly.

Artemia salina: *Artemia salina*, commonly known as Brine shrimp, is a microcrustacean that grows in hypersaline environments. The organism is widely used in ecotoxicology experiments. The PubMed search "*Artemia salina* as nanoparticle toxicity model" results in 14 papers in the last 11 years (2010-2021). It is an entry-level trophic model for aquatic systems. Its cultivation, and the cyst production are easy in controlled lab conditions. Artemia-based assays and protocols are also readily available [68]. Studies show that Ag NPs are toxic at nanomolar concentration for artemia. The toxicity manifest *via* DNA damage, apoptosis, and aggregation of nauplii (first larval stage) at the gut region. LC_{50} for the Ag NP is 10 nm. The exposure of Ag NP can cause mobilization defects, oxidative stress with increased ROS production and decreased Superoxide dismutase expression [69].

Daphnia: *Daphnia magna* and *Daphnia galeata* are common water flea used in ecotoxicity studies. *Daphnia* is a small planktonic crustacean having a size of 0.2-0.6 mm. It is a member of order Anomopoda. It is a well-established model for nanoparticle toxicity for the ecosystem. The PubMed search "*Daphnia* as nanoparticle toxicity search" results in 90 papers in the last 11years (2010-2021). The short life span and ease of culture at lab conditions make it an excellent study model. It forms an essential connection in the food web, which is highly significant in higher trophic levels. This could be a good source of measuring ecotoxicity as wild *Daphnia* from boreal lakes has been reported to shows toxicity against Ag NPs. In lake conditions, LD_{50} was ranged between 34-292 μgL^{-1} [70]. However, in the lab conditions of 48 hrs window, the LD_{50} range is 27 and 247 μgL^{-1} [71]. Another group showed that nanoplastic could cause reproductive disorder in *Daphnia* species [72].

Plants: Plants represent one of the significant trophic levels in the food chain in any ecosystem. With the increase in usage of nanomaterials in nanopesticide and other nanotechnology enabled stuffs, bioaccumulation and biomagnification of NPs have increased many folds [73, 74]. The nanoparticles come in direct contact with the plant and lead to absorption [75, 76]. The studies related to plant interaction with nanoparticles are few. Here, the authors will discuss a few plants used for NP's morphological, physiological, and genotypic toxicological impact. The plants used for studies vary upon the viability in the geographic location. Several studies on *Cucurbita pepo* (genus Cucurbita) were done as it is easy to cultivate and maintain. The NPs like multiwalled carbon nanotubes, Ag, Cu, ZnO, and Si were studied [77]. Except Cu NPs, no other NPs have been found to show germination defects. The plant biomass reduces in the presence of Ag NP. The plant's root length is also a good readout for nanotoxicity. The rare earth oxide NPs of Cerium (Ce), Lanthanum (La), Ytterbium (Yb), Gadolinium (Gd) can cause a reduction of root length in the Lettuce plant [78]. *Arabidopsis thaliana*, a weed, is used mainly as a plant model for toxicity studies. A study, assessing the effects of Ag NPs in *A. thaliana,* has reported the upregulation of 286 genes and downregulation of 81 genes. The genes upregulated were generally related to metals and oxidative stress genes. The down-regulated genes belonged to pathogens and hormonal stimuli [79]. Germination of seeds has also been found affected by Ag NPs in *A.thalliana*. The size and mode of Ag NP availability affected germination. The 75 μgml^{-1} Ag NP dose is toxic for seed germination in soil but not in hydroponics culture [77]. *A.cepa* has also been studied for the toxicity of TiO_2 NPs. The exposure of 6 mM and 8 mM TiO_2 NPs caused a significant reduction in root elongation [80]. There are several other plant species which have been studied for NP toxicity. *Triticum aestivum*, *Solanum lycopersicum*, *Nigella sativa*, *Salvia mirzayanii*, *Alyssyum homolocarpum*, *Sinapis alba*, and many other plant species have been used for NP toxicity studies [81].

FUTURE PERSPECTIVES

The currently used models of nanotoxicity assessments are primarily old models which were developed even before the term nanotoxicity was coined. However, they are good enough to serve the purpose of establishing the toxicity paradigm of currently available NPs, yet their limitations cannot be ruled out as the toxicity assessments of the NPs have their own challenges due to the distinct behaviours of various NPs. The models, especially the *in vivo* ones, are challenging to work upon, as they differ in setup, regulations, and ethical clearance, which is a long and time-consuming process. The maintenance can also be an issue as it is quite costly. The reproducibility of experiments and the data is also a significant concern because it is challenging to have the same set of model animals which behave identically. *In vitro* cellular models do have the answer for such issues as they are mostly identical, and the culturing and maintenance are relatively cheaper and easy to handle, but they are far from the actual complex environment of the animal system, and cannot beat the accuracy and extrapolatability of the toxicity data obtained from animal experiments. So here it can be inferred that both *in vitro* and *in vivo* models do have their pros and cons, and to overcome their respective limitations, there is a need to look for new scientifically validated model systems which possess the ease of handling as well as the accuracy for extrapolation. The quest for such futuristic model systems has resulted in few perspectives like lab or animal on chip or the use of artificial intelligence in computational models.

Lab on a Chip/Organ on Chip: Lab-on-chip has been introduced as a means to mimic laboratory experiments in miniaturized conditions. This concept gained much attention from researchers, which soon moved up to applications such as organs-on-chip. The organ-chips are developed to mimic the physiological conditions and mechanical forces that cells experience in the human body. The chips are lined with living human cells and their tiny microfluidic channels reproduce blood and/or air flow just as in the human body. Microfluidic devices consist of chambers with inlets and outlet for seeding, culturing, sampling, and assaying the cells. The chip's transparency allows the analyst to see the organ's functionality, behaviour and response at the cellular and molecular level. Chips are consistently being developed that cover almost all organs present in the human body. Microfluidic devices that are commercially available are made up of poly(dimethylsiloxane) (PDMS). PDMS is considered biocompatible as it does not have deleterious effects on cells and tissues. It is transparent, permeable to gases, exhibits low autofluorescence, and is cheaper [82].

Organism on a Chip: Organisms on chips are microfabricated devices capable of

precisely manipulating single simple organism and their environment. It is expected that these devices will play a major role in the investigation of nanoparticle toxicity involving small-scale model organisms. Particularly, *C. elegans* is the first animal that has been successfully grown in microfluidic chamber due to its small size (35–40 mm in diameter, 1 mm in length). This device offers several advantages over conventional approaches as they can: maintain well controllable micro-environments, create reproducible experimental conditions, automate tedious experimental protocols and enable high-throughput studies [83, 84].

Computational Models: Previously mentioned models for toxicity assessments are both time-consuming and costly. Computational modelling or computational toxicology is an emerging alternative to the other biological models, which applies advanced mathematical and statistical approaches for analysing the available scientific data to get an insight about the mechanisms by which any toxicant or NM causes the damages, and eventually, provides the capability of predicting the adverse outcomes of the NMs/toxicants in the human and the environment [85].

In the last few decades, scientists and researchers have determined the effects of various chemicals and toxicants including several NMs as well, using various biological models. Recently the majority of such data are made accessible to the public through various data sharing projects like Toxicity Reference Database (ToxRefDB) [85, 86]. This vast scientific knowledge is used by the computational models which use computer programs to simulate and study complex systems using an algorithmic approach. Simulation is done by adjusting the variables alone or in combination with observing the results. The computational model allows researchers to conduct thousands of simulated experiments by computer [86]. Applications of these models to predictive toxicology will be important in prioritizing NMs for further testing and uncovering mechanistic information that is valuable in customizing testing programs for each NM in an informed way and supporting the risk assessments. Computational methods also seem likely to be effective in other areas of the risk evaluation process, especially in estimating the degree of variability in response to the human population, supporting more sophisticated aggregate exposure assessment, and providing a pragmatic approach to evaluating the risks posed by cumulative exposure to mixtures of compounds [85].

CONCLUSION

The inception of nanotechnology has instigated a huge spike in the manufacturing of nanomaterials-based consumer products. Engineered nanoparticles have unique

properties like small size, large surface to volume ratio, high tensile strength, *etc*, which are advantageous to ease our lives, but they could be disadvantageous to humankind too. There are chances for nanomaterials to pose another threatening experience as happened with the wide usage of pesticides which created a serious environmental nuisance. It is important to study nanomaterials' mechanism of toxicity and predict the fate of those materials on physiological systems. The appropriate toxicological models are needed for studying the effects of nanomaterials. *In-vitro* cellular models are the primarily used models for the assessment of nanotoxicity at the molecular level. The traditional 2D cultures comprise cancerous cell lines, derived continuous cell lines, stem cells, and primary cell lines. The recently developed 3D *cultures* like spheroid and organoid cultures help in better mimicking the actual tissue-like environment, but these *in vitro* models lack the systemic complexity of humans, and hence the experimental results cannot be extrapolated to complete accuracy. To negate this problem, *in vivo* models are available, comprising invertebrate and vertebrate organisms that can mimic (wholly or partially) the human systemic complexity. These are established biological models, and nanotoxicologists use them for routine nanotoxicity investigations. The ethical clearance for the use of animal models is a big challenge for researchers. The usage of animals for toxicity studies needs a vast number of regulatory clearances, thus posing an obstruction for efficient nanotoxicological studies. A few futuristic models like a lab on a chip (microfluidics-based) and animal on-chip are the need of the hour to overcome these challenges.

Apart from human toxicity, the nanoparticles can be ecotoxic at various trophic levels. The nanoecotoxicity investigation is very complex and challenging. The toxicity at different ecosystem levels needs to be investigated thoroughly with the help of available model organisms like Daphnia and Artemia, and with other plant models. Contrary to these, prudently designed machine learning-based computational or *in-silico* models might help understand the complex interplay of nanoparticles and their interaction, bioaccumulation, and the resultant toxicity.

Ultimately the nanotoxicology data obtained by using these model systems will help to formulate stringent rules to curb the indiscriminate usage of the toxic NMs, thereby minimizing the toxic effects of such NPs on humans and the overall Biosphere.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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