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Review

Green Synthesis of Metallic Nanoparticles: Applications and Limitations

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Abstract: The past decade has witnessed a phenomenal rise in nanotechnology research due to its broad range of applications in diverse fields including food safety, transportation, sustainable energy, environmental science, catalysis, and medicine. The distinctive properties of nanomaterials (nano-sized particles in the range of 1 to 100 nm) make them uniquely suitable for such wide range of functions. The nanoparticles when manufactured using green synthesis methods are especially desirable being devoid of harsh operating conditions (high temperature and pressure), hazardous chemicals, or addition of external stabilizing or capping agents. Numerous plants and microorganisms are being experimented upon for an eco–friendly, cost–effective, and biologically safe process optimization. This review provides a comprehensive overview on the green synthesis of metallic NPs using plants and microorganisms, factors affecting the synthesis, and characterization of synthesized NPs. The potential applications of metal NPs in various sectors have also been highlighted along with the major challenges involved with respect to toxicity and translational research.

Keywords: green synthesis; metal nanoparticles; wastewater treatment; agriculture; food application

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Citation: Dikshit, P.K.; Kumar, J.; Das, A.K.; Sadhu, S.; Sharma, S.; Singh, S.; Gupta, P.K.; Kim, B.S. Green Synthesis of Metallic Nanoparticles: Applications and Limitations. *Catalysts* **2021**, *11*, 902. https://doi.org/10.3390/catal11080902

Academic Editor: Pierluigi Barbaro

Received: 20 June 2021
Accepted: 23 July 2021
Published: 26 July 2021

1. Introduction

During the last two decades, nanotechnology has taken massive leaps to become one of the most researched and booming fields due to its applications in various fields of human welfare. Nanoparticles (NPs) are naturally occurring or engineered extremely small sized particles in the range of 1 to 100 nm. They exhibit unique and valuable physical and chemical properties. At nanoscale, particles display better catalytic, magnetic, electrical, mechanical, optical, chemical, and biological properties. Due to high surface to volume ratio, NPs show higher reactivity, mobility, dissolution properties, and strength [1]. NPs are thought to have been present on earth since its origin in the form of soil, water, volcanic dust, and minerals. Besides their natural origin, humans have also started synthesizing NPs through various methods [2]. NPs and their derived nanomaterials are finding wide application in various sectors such as food, agriculture, cosmetics, medicines, etc. Application of NPs in food sector involves food processing and preservation (nanopreservatives, toxin detection, nanoencapsulated food additives, etc.) and food packaging (nanocoatings, nanosensors, nanocomposites, edible coating NPs, etc.) In agriculture, nanotechnology is being utilized for the production of nano-fertilizers, pesticides, herbicides, and sensors. In medicine, nanotechnology involves production of various antibacterial, antifungal, antiplasmodial, anti–inflammatory, anticancer, antiviral, antidiabetic, and antioxidant agents. Nanotechnology is also useful for the early detection of life-threatening diseases such as cancer. Besides, NPs have also been used for bioremediation due to their capacity to degrade various pollutants such as organic dyes and chemicals. Given the diverse scope of nanomaterials, different countries are investing in nanotechnology with USA and China emerging at the top. In 2019, the global market of different nano products was more than 8 billion US dollars, which is expected to show annual growth rate of around 13% by 2027.

Depending on their chemical composition, four major classes of NPs are described, such as carbon-based (nanotubes and nanofibers of carbon, etc.), metal and metal oxide based (Ag, Cu, etc.), bio-organic based (liposomes, micelles, etc.), and composite based [3]. NPs can also be classified as organic and inorganic in nature [4]. Organic NPs are biodegradable in nature and include polymeric NPs, lipid based nanocarriers, liposomes, carbon-based nanomaterials, and solid lipid NPs, while inorganic NPs are based on inorganic materials comprising of metals and metal oxides such as silver oxide, zinc oxide, etc. Among all the synthesized NPs, silver NPs (Ag NPs) are the most widely employed, showing their dominance in various consumer products (more than 25%) [5]. AgNPs are majorly used as antibacterial, antifungal, and antiviral agents. With each passing year, novel varieties of NPs are being developed using state-of-art technology having diverse applications in various sectors.

The synthesis of NPs can be carried out following two different approaches, viz., (i) top-down approach, and (ii) bottom-up approach [6,7]. Furthermore, three different strategies such as physical, chemical, and biological methods are adopted for the synthesis of NPs. A schematic representation of various methods adopted for NPs synthesis and its applications is depicted in Figure 1.

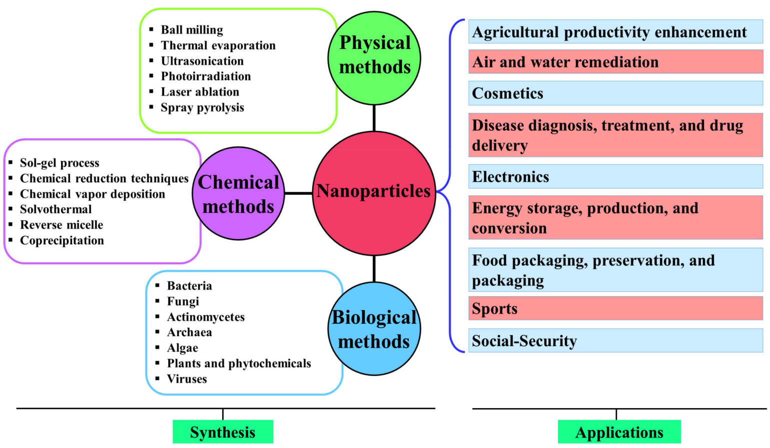


Figure 1. Schematic representation of various methods adopted for NP synthesis and its applications.

The physical methods belong to the category of top-down approach, while the chemical and biological methods follow the bottom-up approach for the synthesis NPs. Evaporation-condensation, electrolysis, diffusion, laser ablation, sputter deposition, pyrolysis, plasma arcing, and high energy ball milling are some of the most common physical methods used for the synthesis of NPs [8]. However, low production rate, expensive operations, and high energy consumption are the major limitations of these processes. Conversely, chemical synthesis methods that include chemical reduction, micro-emulsion/colloidal, electrochemical, and thermal decomposition are the conventional and most widely used methods for the synthesis of metallic NPs. The chemical reduction of NPs from their respective metal salt precursors by adding particular reducing agents is one of the most widely used methods for NPs chemical synthesis due to easy operational and equipment requirement. Several reducing agents, such as sodium borohydride ($NaBH_4$) [9], potassium bitartrate [10], formaldehyde [11], methoxypolyethylene glycol [12], hydrazine [13], etc., and stabilizing agents like dodecyl benzyl sulfate [14] and polyvinyl pyrrolidone [15] have been explored during synthesis. The chemical methods are economical for large-scale production; however, the use of toxic chemicals and production of harmful by-products cause environmental damage, thereby limiting its clinical and biomedical applications [16,17]. Hence, there is an increased demand for reliable, nontoxic, high-yielding, and eco-sustainable techniques for metallic NPs that can replace the conventional methods. The biological synthesis methods, therefore, provide an attractive alternative to the physicochemical synthesis methods.

The present article provides a critical overview on the synthesis of metallic NPs using biological methods and several factors affecting the preparation process. The applications of NPs in various sectors, such as medicine, wastewater treatment, agricultural sectors, etc., have been discussed in detail. Current challenges highlighting the toxic effects of NPs and future perspectives in each section gives us a comprehensive way forward in the near future.

2. Biological Synthesis of NPs

The biological synthesis of NPs can be carried out using a vast array of resources such as plants and plant products, algae, fungi, yeast, bacteria, and viruses. The synthesis of NPs is initiated by the mixing of noble metal salt precursors with biomaterials [18]. The presence of various compounds, such as proteins, alkaloids, flavonoids, reducing sugars, polyphenols, etc., in the biomaterials act as reducing and capping agents for the synthesis of NPs from its metal salt precursors [19]. The reduction of metal salt precursor to its successive NPs can be initially confirmed by visualizing the color change of the colloidal solution. Several studies reported the synthesis of Ag, Au, Cu, Pt, Cd, Pt, Pd, Ru, Rh, etc. using various biological agents in the recent past.

2.1. Plant-Mediated Synthesis of NPs

Figure 2 shows the Scopus search (with keywords “metal nanoparticles” and “plant extract”) results of the number of research published from last 10 years on biological synthesis of NPs. An increase in the number of research publications was observed with each year and approximately 468 publications reported in the year 2020. These data further corroborate that the research interest in the area of biological NPs using plant extract is increasing significantly every year.

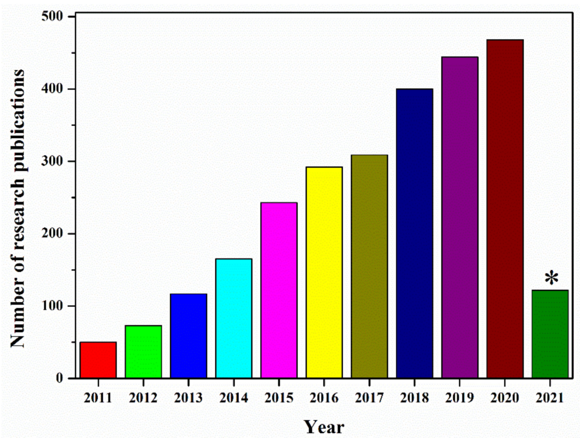


Figure 2. Number of research publications on biological synthesis of metallic NPs from last 10 years. Source: Scopus. * Number of publications reported as on 10 May 2021.

Synthesis of a wide range of metallic NPs has been reported using various plants [6,20,21]. Plant mediated synthesis of NPs can be achieved by three different methods, viz., (i) intracellularly (inside the plant), (ii) extracellularly (using plant extracts), and (iii) using individual phytochemicals. Several plants have the capability of metal accumulation and successive conversion of these accumulated metals to NPs intracellularly. The presence of several biomolecules such as amino acids, alkaloids, aldehydes, flavones, ketones, proteins, phenolics, polysaccharides, saponins, tannins, terpenoids, and vitamins in the plant plays a key role in the reduction of metals [22]. The variation in the size, shape, and properties of accumulated NPs are observed due to the variation in stabilizing and reducing potential of biomolecules present in the plant. The formation of gold NPs inside the living plant, alfalfa was reported by Gardea-Torresdey et al. [23] when the plants were grown in $AuCl_4$ rich environment. In a similar kind of study, Bali and Harris [24] observed the ability of *Medicago sativa* and *Brassica juncea* plants to accumulate Au NPs from aqueous solutions of $KAuCl_4$. The NPs were majorly located in the xylem parenchyma cells while some were also accumulated throughout the epidermis, cortex, and vascular tissue.

However, for the past several years, most of the works have been focused on using the inactive part of the plants either in powder form or as an extract for the synthesis of NPs [25]. Table 1 summarizes the green synthesis of various metal NPs using plants. Various parts of plants such as leaves, steam, flower, fruit, root, latex, seed, and seed coat are being exploited for the synthesis of metallic NPs.

Table 1. Summary on synthesis of metallic nanoparticles by various plant species.

Nanoparticles	Plant Species	Experimental Conditions	Shape and Size	References
Silver (Ag)	*Acalypha indica Linn*	Temperature: 27 °C; pH: 7.0; duration: 30 min	Spherical; 20–30 nm	[26]
	Chenopodium album leaf	Temperature: 20–100 °C; pH: 2.0–10.0; duration: 15 min	Spherical; 10–30 nm	[27]
	Hibiscus rosa sinensis leaf	pH: 7.2–8.5	Spherical; 13 nm	[28]
	Calendula officinalis seed	Temperature: 30 and 60 °C; pH: 3.0–9.0	Spherical; 7.5 nm	[29]
	Allophylus cobbe leaf	Temperature: 60 °C; pH: 8.0; duration: 6 h	Spherical; 2–10 nm	[30]
	Cissusquadrangularis leaf	Duration: 60 min	Spherical and cuboidal	[31]
	Piper nigrum, *Ziziphus Spina—Christi* and *Eucalyptus globulus* leaves	Temperature: ambient; duration: 1 h	Spherical; 8−35 nm	[32]
	Phyllanthus emblica fruit	Temperature: 65 °C; duration: 2 h	Spherical; 16.29 nm	[33]
	Blumea eriantha DC	Temperature: ambient; duration: 2–3 h	Spherical; 50 nm	[34]
	Brillantaisia patula, *Crossopteryx febrifuga* and *Senna siamea* leaf	Temperature: 70 °C; duration: 24 h	Spherical; 45–110 nm	[35]
	Ocimum tenuiflorum leaf	Temperature: ambient; duration: 10 min	Spherical and ovoid; 7–15 nm	[36]
	Annona squamosa leaf	Temperature: ambient	Spherical; 20–100 nm	[37]
	Aloe leaf	Temperature: ambient; duration: 20 min	Spherical; 20 nm	[38]
	Artocarpus heterophyllus Lam. Seed	Temperature: 121 °C; duration: 5 min	Irregular; 3–25 nm	[39]
	Trigonella foenum graecum seed	Duration: 5 min	Spherical; 17 nm	[40]
	Andrographis paniculata	Temperature: 30–95 °C	Spherical; 13–27 nm	[41]
	Podophyllum hexandrum leaf	Temperature: 20–60 °C; pH: 4.5–10.0; duration: 30–150 min	Spherical; 12–40 nm	[42]
	Syzygium cumini fruit	Temperature: ambient; pH: 7.0–9.0; duration: 2 h	Spherical; 5–20 nm	[43]
	Crassocephalum rubens leaf	Temperature: 50 °C; duration: 20 min	Spherical and hexagonal; 15–25 nm	[44]
Gold (Au)	*Cassia fistula* stem bark	Temperature: ambient	Rectangular and triangular; 55.2–98.4 nm	[45]
	Crassocephalum rubens leaf	Temperature: 50 °C; duration: 10 min	Spherical; 10–20 nm	[44]
	Simarouba glauca leaf	Duration: 15 min	Spherical and prism; <10 nm	[46]
	Hygrophila spinosa	Temperature: 30–100 °C; pH:2.0–12.0; duration: 15–60 min	Spherical, polygonal, rod and triangular; 68 nm	[47]
	Croton Caudatus Geisel leaf	Temperature: ambient	Spherical; 20–50 nm	[48]
	Moringa oleifera flower	Temperature: ambient; duration: 60 min	Triangular, hexagonal, and spherical; 5 nm	[49]
	Illicium verum	Temperature: 25–50 °C; pH: 2.0–10.00; duration: 15 min	Triangular and hexagonal; 20–50 nm	[50]
	Terminalia arjuna leaf	Temperature: ambient; duration: 15 min	Spherical; 20–50 nm	[51]
	Zingiber officinale	Temperature: 37 and 50 °C; pH: 7.4; duration: 20 min	Spherical; 5–10 nm	[52]
	Rosa hybrida petal	Temperature: ambient; duration: 5 min	Spherical, triangular, and hexagonal; 10 nm.	[53]
	Terminalia chebula seed	Temperature: ambient; duration: 20 s	Triangular, pentagonal, and spherical; 6–60 nm	[54]
	Eucommia ulmoides bark	Temperature: 30–60 °C; pH: 5.0– 13.0; duration: 30 min	Spherical	[55]
	Acorus calamus rhizome	Temperature: ambient; pH: 4.0–9.2	Spherical; 10 nm	[56]
	Curcuma pseudomontana root	Temperature: ambient; duration: 30 min	Spherical shape; 20 nm	[57]
	Citrus limon, *Citrus reticulata* and *Citrus sinensis*	Temperature: ambient; duration: 10 min	Spherical and triangular; 15–80 nm	[58]

Table 1. *Cont.*

Nanoparticles	Plant Species	Experimental Conditions	Shape and Size	References
Palladium (Pd)	*Hippophae rhamnoides* Linn leaf	Temperature: 80 °C; duration: 25 min	2.5–14 nm	[59]
	Cinnamom zeylanicum bark extract	Temperature: 30 °C; pH: 1.0–11.0; duration: 72 h	Spherical; 15–20 nm	[60]
	Banana peel extract	Temperature: 40–100 °C; pH: 2.0–5.0; duration: 3 min	50 nm	[61]
	Cinnamomum camphora leaf	Temperature: ambient; duration: 12 h	Quasi–spherical and irregular; 3.6–9.9 nm	[62]
	Catharanthus roseus leaf	Temperature: 60 °C; duration: 2 h	Spherical; 38 nm	[63]
	Terminalia chebula fruit	Temperature: ambient; duration: 40 min	-	[64]
	Rosmarinus officinalis	Temperature: ambient; duration: 24 h	Semi–spherical; 15–90 nm	[65]
	Anogeissus latifolia	Duration: 30 min	Spherical; 2.3–7.5 nm	[66]
	Daucus carota leaves	-	Rod; diameter—20 nm, length—38–48 nm	[67]
	Camellia sinensis leaves	Temperature: 100 °C; duration: 1 h	Spherical; 5–8 nm	[68]
Platinum (Pt)	*Anacardium occidentale* leaf	Temperature: ambient; pH: 6.0–8.0	Irregular rod shaped	[69]
	Cacumen platycladi	Temperature: 30–90 °C; duration: 25 h	Spherical; 2–2.9 nm	[70]
	Asparagus racemosus root	Duration: 5 min	1.0–6.0 nm	[71]
	Diopyros kaki leaf	Temperature: 25–95 °C	Spherical and plate; 2–20 nm	[72]
	Ocimum sanctum leaf	Temperature: 100 °C; duration: 1 h	Rectangular and triangular; 23 nm	[73]
Copper (Cu)	Mulberry fruit (*Morus alba* L.)	Temperature: ambient; duration: 5 h	Spherical and non–regular; 50–200 nm	[74]
	Crotalaria candicans leaf	-	Spherical; 30 nm	[75]
	Ziziphus spinachristi fruit	Temperature: 80 °C	Spherical; 5–20 nm	[76]
	Clove (*Syzygium aromaticum*) buds	Temperature: 30 °C; duration: 15 min	Spherical; 15–20 nm	[77]
Iron (Fe)	Tea leaves extract	Temperature: 80 °C; duration: 3 h	30–100 nm	[78]
	Moringa oleifera seeds	Temperature: ambient; duration: 30 min	Spherical; 2.6–6.2 nm	[79]
	Trigonella foenum–graecum seed	Temperature: 30 °C; duration: 5 min	7–14 nm	[80]
Selenium (Se)	*Ocimum tenuiflorum*	Temperature: ambient; duration: 75 h	Monodispersed and spherical; 15–20 nm	[81]
	Murraya koenigii	-	Spherical; 50–150 nm	[82]
	Zinziber officinale fruit	Temperature: ambient; pH: 9.0; duration: 75 h	Spherical; 100–150 nm	[83]
Nickel (Ni)	*Calotropis gigantea* leaves	Temperature: 80 °C; pH: 12.0; duration: 90 min	60 nm	[84]
	Desmodium gangeticum roots	Temperature: 80 °C; duration: 45 min	-	[85]

In general, the synthesis of NPs is carried out by mixing the plant biomass/extract with a metal salt solution at a desired temperature and pH. The primary confirmation of NPs synthesis can be checked by looking at the color change of the solution. The experimental procedure for the synthesis of NPs using plant biomass is depicted in Figure 3.

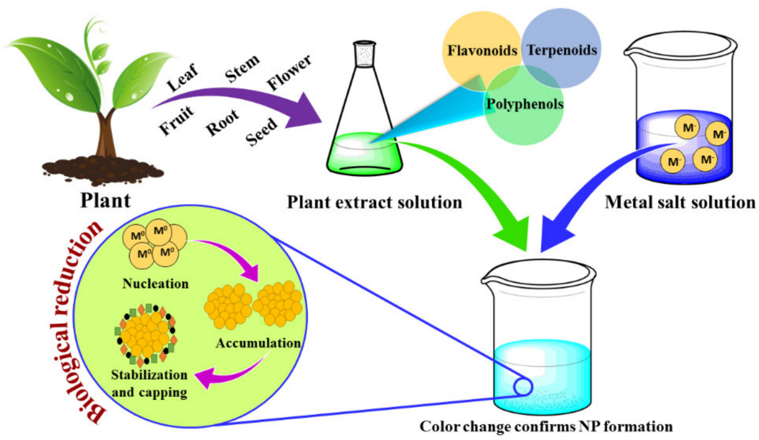


Figure 3. Schematic representation for plant-mediated biosynthesis of nanoparticles.

The plant extracts are prepared by using different methods such as hot extraction, cold extraction, and using Soxhlet apparatus, which were later used in NPs synthesis. This method of synthesis of NPs is more suitable in comparison to the intracellular method due to easy scale-up and downstream processing. Additionally, this method is renewable, non-toxic, biocompatible, and eco-friendly. Due to their biocompatible nature, these NPs are known to have various biological applications. The synthesis of metal NPs is initiated by adding the plant extract to the metal precursor solution containing the salts of respective metals. Metal precursor solutions such as $AgNO_3$, $HAuCl_4$, $PdCl_2$, H_2PtCl_6, $Cu(NO_3)_2 \cdot 3H_2O$, $FeCl_3 \cdot 6H_2O$, Na_2SeO_3, and $(NiNO_3)_2 \cdot 6H_2O$ are commonly used for the synthesis of Ag, Au, Pt, Cu, Fe, Se, and Ni NPs. The synthesis of metal NPs using plant extract mainly occurs in three stages. In the first stage, the reduction of metal ions (M^+ or M^{2+}) to metal atoms (M^0) and successive nucleation of the reduced metal atoms occurs. While in the second stage, the coalescence of small adjacent NPs into larger size particles occurs with simultaneous increase in thermodynamic stability. At the final stage, the termination of the process takes place while giving the final shape to the NPs [86,87]. The presence of various active biomolecules in the plant extract plays an important role in the reduction and stabilization of metal ions in the solution. However, due to the presence of a large number of phytochemicals in the plant extract, it is difficult to ascertain the exact reducing and stabilizing agents for NPs synthesis.

Salih et al. [32] used plant extract derived from leaves of three different plants, viz., *Piper nigrum*, *Ziziphus Spina—Christi* and *Eucalyptus globulus,* for the synthesis of AgNPs. The average particle size distribution was in the range of 8–35 nm and decreased with the increase in concentration of plant extract. In a similar kind of study, Dhar et al. [33] reported the synthesis of AgNPs using fruit extract of *Phyllanthus emblica*. The fabricated AgNPs were spherical with an average size of 60–80 nm. The extraction methods also play an important role which influences the antioxidant properties of Ag and Au NPs synthesized using leaves extract of *Crassocephalum rubens* [44]. Figure 4 depicts the size, shape, and morphological features of *C. rubens* synthesized AgNPs and AuNPs. The SEM and TEM images revealed spherical and hexagonal shapes of AgNPs with size 10–15 nm, whereas the size of AuNPs was in the range of 10–20 nm with spherical shape.

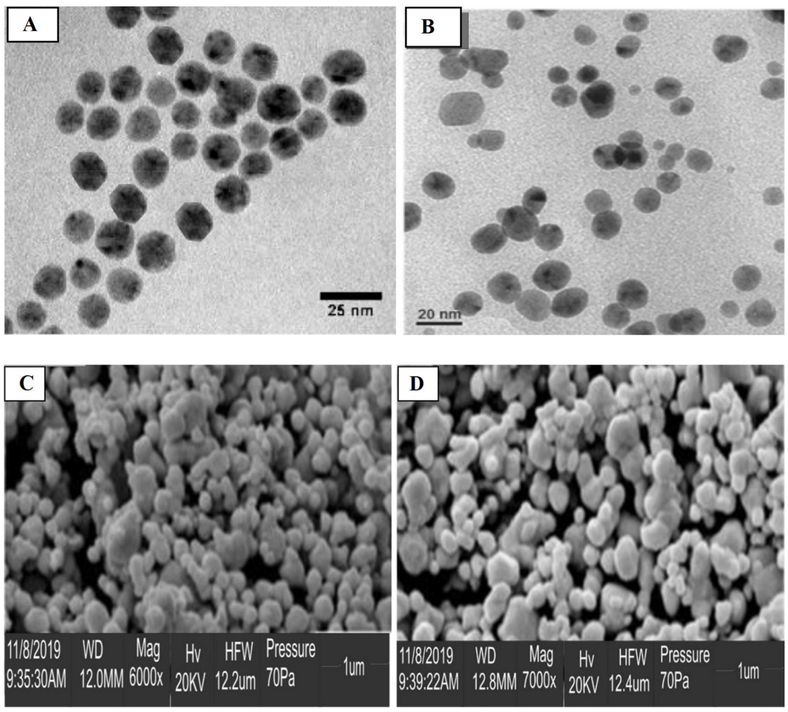


Figure 4. Characterization of AgNPs and AuNPs synthesized from *Crassocephalum rubens* leaf extract. TEM images of (**A**) AgNPs and (**B**) AuNPs; SEM images of (**C**) AgNPs and (**D**) AuNPs. Reprinted with permission from Reference [44].Copyright 2021 Elsevier.

The biosynthesized Au NPs using aqueous extract of *Hygrophila spinosa* exhibited enhanced cytotoxicity against various cancer cell lines compared to *H. spinosa* aqueous extract [47]. These green synthesized AuNPs with antioxidant and cytotoxic properties could provide a new direction for the development of nanomedicine. In addition to Au and Ag NPs, Pd NPs synthesized using *Rosmarinus officinalis* leaves extract demonstrated notable antimicrobial and antifungal activity against different bacteria and fungi [65]. Besides leaves, other plant parts such as root, fruit, flower, petal, seed, peel, bark etc. are used in the biosynthesis of different NPs [40,49,53,55,61,71,80]. Several previous studies tried to explain the mechanisms behind the antimicrobial effect of metallic NPs [88–90]. These metal ions could strongly interact with the negatively charged bacterial cell wall leading to its rupture. The other mechanism states distortion of the helical structure of bacterial DNA due to the interaction of NPs and interruption of internal and external cellular mechanisms.

2.2. Microbial Synthesis of NPs

In addition to the plant-mediated synthesis, several microorganisms such as bacteria, fungi, actinomycetes, and viruses are also reported to synthesize various metal NPs. The interaction between metals and microorganisms have been exploited in the past for various biological applications such as biomineralization, bioremediation, bioleaching, and biocorrosion [91]. However, recently microbial synthesis of NPs has emerged as a promising field of research due to certain advantages compared to other methods. The NPs are synthesized either intracellularly or extracellularly depending on the type of microorganisms [92]. Previous reports on the synthesis of metal NPs using various microorganisms have been summarized in Table 2.

Table 2. Summary on synthesis of metallic nanoparticles by microorganisms.

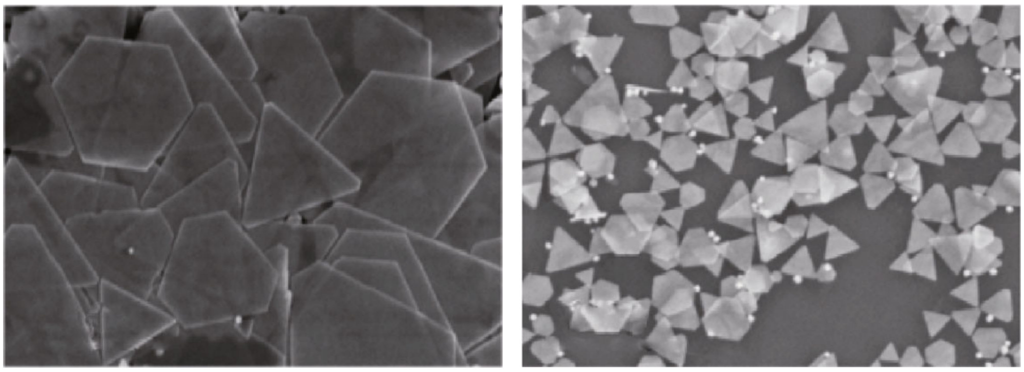
Microorganisms	Nanoparticles	Shape and Size	References
Bacteria			
Bacillus subtilis	Ag	Spherical; 3–20 nm	[93]
Pseudomonas stutzeri	Ag	Triangular; 200 nm	[94]
Bacillus licheniformis	Ag	40 nm	[95]
Ochrobactrum anhtropi	Ag	Spherical; 38–85 nm	[96]
Pantoea ananatis	Ag	Spherical; 8.06–91.31 nm	[97]
Actinobacter	Ag	Spherical; 13.2 nm	[98]
Pseudomonas aeruginosa	Au	15–30 nm	[99]
Rhodopseudomonas capsulata	Au	Spherical; 10–20 nm	[100]
Escherichia coli DH5α	Au	Spherical, triangles, and quasi–hexagons; 25 nm	[101]
Bacillus subtilis	Au	Spherical, 20–25 nm	[102]
Mycobacterium sp.	Au	Spherical; 5–55 nm	[103]
Shewanella loihica	Pt	1–10 nm	[104]
Shewanella oneidensis MR–1	Pt	2.83–61.03 nm	[105]
Jeotgalicoccus coquinae ZC15	Pt	Spherical; 5.74 nm	[106]
Shewanella loihica	Pd	1–12 nm	[104]
Shewanella oneidensis MR–1	Pd	10–100 nm	[107]
Lysinibacillus sp. ZYM-1	Se	Cubic; 100–200 nm	[108]
Bacillus subtilis	Se	Spherical; 50–400 nm	[109]
Lactobacillus acidophilus	Se	Spherical; 2–15 nm	[110]
Fungi			
Rhizopus stolonifer	Ag	Spherical; 2.86 nm	[111]
Candida glabrata	Ag	Spherical; 2–15 nm	[112]
Trametes trogii	Ag	Spherical and rod; 5–65 nm	[113]
Trichoderma longibrachiatum	Ag	Spherical; 10 nm	[114]
Fusarium oxysporum	Ag	Spherical; 21.3–37 nm	[115]
Aspergillus terreus	Ag	Spherical; 7–23 nm	[116]
Ganoderma sessiliforme	Ag	Spherical; 45 nm	[117]
Candida albicans ATCC 10231	Ag	Spherical; 10–20 nm	[118]
Cladosporium cladosporioides	Au	60 nm	[119]
Trichoderma harzianum	Au	Spherical; 26–34 nm	[120]
Pleurotus ostreatus	Au	Spherical; 10–30 nm	[121]
Aspergillus sp.	Au	Spherical; 4–29 nm	[122]
Rhizopus oryzae	Au	Spherical and flower like structure; 16–43 nm	[123]
Penicillium chrysogenum	Pt	Spherical; 5–40 nm	[124]
Fusarium oxysporum f. sp. *lycopersici*	Pt	Triangle, hexagons, square, and rectangles; 10–50 nm	[125]
Fusarium oxysporum	Si	Quasi–spherical; 5–15 nm	[126]
Fusarium oxysporum	Ti	Spherical; 6–13 nm	[126]
Yeast			
Rhodotorula sp. ATL72	Ag	Spherical and oval; 8–21 nm	[127]
Saccharomyces cerevisiae	Ag	Spherical; 2–20 nm	[128]
Cryptococcus laurentii	Ag	35–400 nm	[129]
Rhodotorula glutinis	Ag	15–220 nm	[129]
Rhodotorula glutinis	Ag	Spherical; 15.5 nm	[130]
Saccharomyces cerevisiae	Au	Triangle, truncated triangle, and hexagon	[131]
Magnusiomyces ingens LHF1	Au	Spherical and pseudo–spherical; 20–28 nm	[132]
Saccharomyces cerevisiae	Pd	Hexagonal; 32 nm	[133]
Magnusiomyces ingens LHF1	Se	Spherical and quasi–spherical; 70–90 nm	[134]

Compared to other microorganisms, bacteria are preferred for the synthesis of NPs due to their easy maintenance, high yield, and low purification cost. In recent years, cell-free extract of endophytic bacterium, *Pantoea ananatis*, was used for the synthesis of AgNPs [97]. These synthesized spherical shaped NPs with an average size ranging from 8.06–91.32 nm exhibited significant antimicrobial activity against various pathogenic microorganisms.

In a similar kind of study, Wypij et al. [98] reported maximum antimicrobial activity of AgNPs synthesized by using acidophilic actinobacterial strain against *E. coli*, followed by *B. subtilis* and *S. aureus*. Moreover, bacteria species belonging to *Pseudomonas stutzeri*, *Bacillus licheniformis*, *Ochrobactrum anhtropi*, *Bacillus subtilis*, and *Actinobacter* were used for the synthesis of AgNPs (Table 2). Gold NPs were synthesized by *Pseudomonas aeruginosa*, *Rhodopseudomonas capsulate*, *Bacillus subtilis*, *Escherichia coli DH5α*, *Mycobacterium* sp., etc. A larger amount of *Actinobacter* in the medium led to the formation of smaller size with uniformly distributed spherical AuNPs [135]. Intercellular and extracellular components of microorganisms play an important role in the synthesis of AuNPs [136]. Intercellular components such as reducing sugar, fatty acids, and enzymes and extracellular such as exopolysaccharides help in the reduction of AuNPs [136].

In addition to bacteria, the synthesis of NPs using fungi has received increasing attention due to various advantages such as easy scale-up and downstream processing, economic feasibility, and increased surface region due to the presence of mycelia. Different species of fungi such as *Rhizopus stolonifera*, *Candida glabrata*, *Trametes trogii*, *Trichoderma longibrachiatum*, *Aspergillus terreus*, *Fusarium oxysporum*, *Ganoderma sessiliforme*, *Candida albicans ATCC 10231*, *Cladosporium cladosporioides*, *Trichoderma harzianum*, *Pleurotus ostreatus*, *Aspergillus sp.*, *Rhizopus oryzae*, etc. are used in silver and gold NPs synthesis. Other species of fungi such as *Penicillium chrysogenum* and *Fusarium oxysporum* are used for the synthesis of Pt, Si, and Ti NPs.

Among the eukaryotic organism, yeast has been used for the synthesis of NPs like Ag, Au, Pd, Se, etc. Soliman et al. [127] synthesized AgNPs using pink yeast *Rhodotorula* sp., and the characterization revealed the NPs to be spherical and oval in shape with 8.8–21.4 nm size. These biosynthesized AgNPs exhibited significant antimicrobial activity with complete inhibition to wide range of bacteria (i.e., both Gram positive and Gram negative) as well as fungi. Similarly, the antifungal activity of biosynthesized AgNPs using two yeasts: *Rhodotorula glutinis* and *Cryptococcus laurentii* was evaluated against the phytopathogenic fungi [129]. The results of this study revealed that the antifungal activity of AgNPs from *R. glutinis* was higher than that from the ones prepared from *C. laurentii*. In another study, the morphology and size of AuNPs were controlled by varying the pH of the medium containing yeast [131]. In this method, various morphologies of gold nanoplates such as triangle, truncated triangle, and hexagonal nanoplates with uniform size were synthesized successfully (Figure 5).



(**A**) (**B**)

Figure 5. SEM images of gold nanoplates synthesized using yeast extract at different pH conditions. (**A**) Au nanoplates at low pH without NaOH, (**B**) small Au nanoplates synthesized at high pH. Reprinted with permission from Reference [131]. Copyright 2016 Springer Nature.

Similarly, the palladium (Pd) and selenium (Se) NPs are synthesized using aqueous extract of *Saccharomyces cerevisiae* and cell-free extracts of *Magnusiomyces ingens* yeast, respectively [133,134].

The NPs are formed during the microbial synthesis process is due to the oxidation/reduction of metallic ions by secreted biomolecules by microbial cells such as enzymes, sugars, carbohydrates, proteins, etc. [137]. However, a complete understanding of microbial NP synthesis is still unknown as the routes for NPs synthesis varies for each kind of microorganisms. The reduction of silver for the synthesis of extracellular and intracellular AgNPs by bacteria is mainly achieved by the action of deoxyribonucleic acid (DNA) or sulfur-containing proteins, whereas in the case of fungi, the process is carried out by nitrate-dependent reductase or carboxylic group [138]. The extracellular synthesis method is preferable due to easy and simpler purification steps. In contrast, the intracellular NPs synthesis method is challenging and expensive due to the involvement of additional separation and purification processes. Fungal-mediated synthesis of NPs holds additional advantages compared to algae or bacteria in terms of easier and simpler biomass handling and downstream processes along with the secretion of large amount protein that further increases the productivity by several folds. However, the microorganism mediated NPs synthesis process is extremely intricate and difficult due to the preparation of inoculum and growth media, isolation of strain, and maintenance of culture medium and operation conditions (pH, temperature, agitation). Conversely, the use of plant extracts or broths is simple and convenient, devoid of the complex methods of cell culture and maintenance. The time required to achieve a complete reduction of NPs using microorganisms is usually 24 to 120 h, while the reduction time is much less in using plant extract ranging from few hours to 48 h [138]. The reduction rate using the plant is much faster than microorganisms and in close agreement with the physical and chemical methods. The use of microorganisms for large-scale biosynthesis of NPs lacks feasibility compared with plants, which require less time for reaction completion. As reported in the earlier studies, the plant-mediated biosynthesis of AgNPs demonstrate better production rate, size, and morphological characteristics compared to other available biological techniques [138].

Hence, the plant-mediated synthesis of NPs proves to be a sustainable alternative not only to the other biological techniques but also to other synthesis methods such as physical and chemical. However, in-depth studies are required to understand the detailed mechanisms of action and to achieve better control over size, morphology, and production rate for making a plant-mediated synthesis method at par with chemical methods.

2.3. Factors Affecting NPs Synthesis

Adjustment of shape and size of metal NPs further enhances their functionality for various applications. The morphological parameters of NPs can be manipulated by changing various experimental parameters such as reaction time, reactant concentration, pH, temperature, aeration, salt concentration, etc. [139]. Precise control of these parameters can play a critical role during the optimization of metal NPs synthesis via the biological route. The size and shape of NPs can be controlled by varying the pH of the medium, while the acid pH leads to the formation of large-sized NPs [140,141]. During synthesis of Au NPs using oat (*Avena sativa*) biomass, Armendariz et al. [142] observed smaller sized gold NPs at pH 3.0 and 4.0 in comparison to the synthesized NPs at pH 2.0. This is due to the better accessibility of functional groups present in the extract for nucleation at higher pH compared to the presence of fewer groups at a lower pH range. In addition to pH, the concentration of biomolecules in the extract also affects the size and shape of synthesized NPs. Increase in the concentrations of *Aloe vera* leaf extract resulted in the synthesis of higher amount of spherical gold NPs instead of triangular which is due to the presence of carbonyl compounds in the extract [143]. In addition, the size of the NPs was modulated in the range of 50 to 350 nm by varying the extract concentration in the solution. The duration of reaction also plays a crucial role in the reduction of NPs and their size, which is primarily confirmed by rapid change in color of the reaction mixture. This duration can range from few minutes to few days.

A change in particle size of silver NPs was observed in the range of 10–35 nm by increasing the reaction time from 30 min to 4 h using *Azadirachta indica* leaf extract. Furthermore, the reaction temperature is one of the important parameters in the biological synthesis of NPs which also determines the shape, size, and yield of NPs. The average size of silver NPs decreased from 35 to 10 nm with the increase in reaction temperature from 25 to 60 °C using *Citrus sinensis* (sweet orange) peel extract [144].

2.4. Characterization of NPs

NPs have attracted significant attention of researchers due to their unique physical, chemical, and mechanical properties. Therefore, the physicochemical characterization of synthesized NPs is critically important before its application in various sectors. Analyzing various characteristics such as size, shape, surface morphology, surface area, structure, stability, elemental and mineral decomposition, homogeneity, intensity, etc. will provide important information about the NPs, which subsequently determined their end-use applications. Additionally, the electrical and thermal conductivity and purity of NPs can also be obtained by using these techniques. Size and shape of the synthesized NPs are mainly analyzed using X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM), Field Emission Scattering Electron Microscopy (FESEM), Transmission Electron Microscopy (TEM), High-Resolution Transmission Electron Microscopy (HRTEM), Atomic Force Microscopy (AFM), Dynamic Light Scattering (DLS), Condensation Particle Counter (CPC), Photon Correlation Spectroscopy (PCS), etc. Among these techniques, XRD, SEM, and TEM are most commonly used for this purpose. Further, SEM, TEM, AFM, etc. are used for studying the surface morphology. Superconducting Quantum Interference Device (SQUID), Vibrating Sample Magnetometer (VSM), Electron paramagnetic resonance (EPR), etc. are used for the determination of magnetic properties of NPs. More details about NPs characterization techniques are reviewed by previous authors [145,146].

3. Potential Applications of Metal NPs

These synthesized metallic NPs offer a diverse platform for various applications. Some of the most important applications of these metallic NPs are summarized in this section.

3.1. Agriculture

Nanotechnology has proven its potential to benefit the agriculture sector by finding solutions to agricultural and environmental problems in order to increase food production and security [147].

3.1.1. Effect of Nano Material on Plants

Seed germination and later growth phases benefit from nano-growth stimulants [148,149]. Owing to the small size and large surface area of nanoparticles, these particles are able to seep into the seed pores eventually activating the phytohormones required for seed growth and germination [150,151]. For example, the use nano-TiO_2 and nano-SiO_2 on soybean seedlings improved nitrate reductase activity thereby enhancing seed germination. However, combining both nanomaterials (NMs) was more advantageous [152]. Seed treatment with 0.25 percent TiO_2 improved nitrogen assimilation and photosynthesis rate in spinach (*Spinacia oleracea* L.), resulting in better growth [153,154]. Watermelon (*Citrullus lanatus*) seed soaking in Fe_2O_3 NPs improved germination and initiated plant development and fruiting behavior [155]. The use of low-concentration SiO_2 NPs on tomato seeds improved germination [156]. Nanomaterials can be used in a variety of ways in in-vitro cultivation. Zn as a ZnO nanomaterial resulted in an increased calli growth and physiological parameters in tobacco (*Nicotiana tabacum* L.). Although nanoparticles have been widely utilized for promoting plant growth [149,157], NM application may also be phytotoxic [158]. The positive and negative effects, however, are dependent on the dose, size, time, exposure, and make (synthetic/biological) of the NM [158,159]. Over the past years, application of chemically or physically synthesized NM have proven to be stimulant of plant growth; however, they pose a greater threat to ecology and environment (by seeping through the soil) [160]. Utility of green/biological NM in agriculture has therefore been advantageous due to their safety and feasibility [161]. An increased usage of biologically synthesized Au, Ag, Ti, Ca, N, Fe nanoparticles either in form of nanofertilizers or nanopesticides has been employed [161–165].

3.1.2. Application of Nanomaterials in the Field of Agriculture

Over the recent years, application of nanomaterials to boost agriculture has broadly been in two major forms, either as nanofertilizers, to enhance the agricultural productivity or in the form of nanopesticides, to eradicate the pest/pathogens/weeds hampering the growth of crop plants. In this section, we will be discussing the applicability of nanofertilizers and nanopesticides, in current agricultural practices.

Nanofertilizers

Huge increase in agricultural yields, particularly grain yields, has played an important role in providing the world's food demands over the last five decades. In this context, increased usage of chemical fertilizers acts as one of the key contributors to increased crop productivity. Although, use of chemical fertilizers has increased productivity of crops, their poor use efficiency due to volatilization and leaching has led to its excessive usage [147]. On the contrary, nanofertilizers are compounds that are applied in smaller amounts and can enhance the effect of fertilizers [166]. Enhancing the effect of fertilizers on plants is usually done by governing the fertilizers in nano form which results in controlled nutrient release, eventually minimizing the risk of environmental damage [167]. With the recent advancement in the field of nanobiotechnology, nanofertilizers can be utilized as intelligent fertilizers which are able to release desired amount of nutrients just when and where they are needed by plants, thereby limiting the conversion of excess fertilizers to gaseous forms or leaking downstream [168].

To date, various NPs have been employed in developing fertilizers, some of which include hydroxyapatite, polyacrylic acid, clay minerals, chitosan, zeolite, and many more. The small size and large surface area of these NPs give them an advantage over the conventional fertilizers. For example, strong interactions of hydroxyapatite with urea lead to release of nitrogen from urea until 60 days as compared to the ammonium nitrate fertilizer (normal form of urea) which releases nitrogen only until 30 days [169].

Nanofertilizers can be broadly classified into three categories: (1) nanoparticulate nano fertilizers, (2) micronutrient nanofertilizers, and (3) macronutrient nanofertilizers. Nanoparticulate nanofertilizers include NPs, such as CNTs, TiO_2, and SiO_2, responsible for plant growth. In soybean, an amalgamation of TiO_2 and SiO_2 results in overall increase in plant growth with increased nitrogen fixation and improved seed germination [152]. As utility of TiO_2 nanoparticle in plant growth has been well established, recently several works have been done for generation of non-toxic, cheap, and environmentally safe green synthesized TiO_2 from plant extracts of *Syzgium cumini*, *Moringa oleifera*, *Cucurbita pepo*, and *Trigonella foenum* [170–173]. Micronutrients such as molybdenum (Mo), copper (Cu), iron (Fe), nickel (Ni), manganese (Mn), and Zinc (Zn) packed in NPs serve as micronutrient nano fertilizers. A mixture of three micronutrients NPs (ZnO, CuO, and B2 O3) has been successfully established to ameliorate drought stress in soybean plants [174]. Recent studies on *Zea mays* have revealed utility of biologically synthesized micronutrient nanofertilizers (iron oxide nanorods) in better plant growth as compared to the chemically synthesized NPs [164]. Similar to micronutrients, macronutrient nanofertilizers are composed of a combination of macroelements (Mg, K, N, Ca, and P) [167]. Foliar application of Mg and Fe NPs on *Vigna unguiculata*, resulted in increased seed weight and photosynthesis ability thereby resulting in an overall improvement in yield [175]. When compared to crops administered with conventional fertilizer, phosphatic nanofertilizers have been attributed a 32 percent rise in growth rate and a 20 percent rise in seed production in soybean (*Glycine max* L.) [176]. A recent study on application of green synthesized multinutrient nanofertilizer (U-NPK) made from calcium phosphate NP doped with potassium and nitrogen resulted in reduction of 40% of nitrogen requirement of plants when compared to conventional approach due to slow and gradual release of major micronutrients [165]. Overall, use of nanofertilizer results in reduction in usage of fertilizer amount by allowing slow-release products.

Nanopesticide

Plants being sessile in nature are prone to various biotic and abiotic stresses such as pests, pathogens, heat, drought, pollution, etc. These stresses directly or indirectly affect the total yield of a plant. The ill effect of pathogens on an entire crop leading to famine like situations has been extensively studied by far, and in order to have a control over these forms of biotic stress, use of pesticides had been advocated. Utility of pesticides has been established in eradication of harmful pests and pathogens from crop field resulting in crop protection [177]. However, it has been found that use of pesticides leads to deleterious effects on environment and human health. As a result, numerous pesticides have been prohibited by state or international governments. Thus, development of effective yet safe pesticides is the need of the hour. Although biopesticides have emerged as a breakthrough, their use has been limited due to significantly higher cost of production. However, nanotechnology offers a new and better approach by introduction of nanopesticides [178].

One of the widely used examples of nanopesticide is nanostructured alumina (NSA). NSA acts as negatively charged insecticide which interacts with the positively charged bodies of the insects leading to dehydration. The dehydration of insect body results in detachment of insect's cuticle eventually leading to death [167]. Potent insecticidal activity of several other NPs has also been studied over past few years. In the year 2013, Paret et al. [179] studied the antimicrobial effect of TiO_2 and ZnO NPs against *X. perforans,* casual organism of tomato spot disease. Role of Imidacloprid (IMI) as an effective systemic insecticide against several sucking insects such as *Martianus dermestoides* has been established; in addition, use of the nano-IMI being more photodegradable increases its effectiveness and environmental safety over the conventional formulation [180]. Another study found that nanoformulation of permethrin had a higher absorption than the conventional form against *Aedes aegypti*. In a recent study, Zhao et al. investigated the insecticidal activity of Cu NPs which showed potential upregulation of exogenous microbial protein within plant tissue enhancing resistance against the bollworm, under the effect of at a low dose of Cu NPs [181]. These findings are encouraging for utility of nanopesticides against various crop pests which could be an important tool in future agricultural pest management practices [147].

3.2. Nanoparticles in Food Industry

NPs in combination with other technologies can bring impactful innovations in the production, storage, packaging and transportation of food products. Food processing transforms raw food ingredients into a palatable format with long shelf-life and in turn, ensure efficient marketing and distribution systems for the enterprise. Fresh foods, on the other hand, require robust logistics for their transportation from source to consumer. Nanotechnology based systems play an important role to maintain the functional properties by incorporating NP based colloids, emulsions, and biopolymers solutions. Nanotechnology has provided with a new dimension and ample opportunities to develop NPs for various applications with deeper knowledge of the material. Application of nanotechnology in food industry is based on nanostructures which target food ingredients as well as sensors. Nano-food ingredients cover a wide area of applications starting from processing of food to its packaging. Nanostructure based application in food processing comprises the use as antimicrobial agents, nanoadditives, nanocarriers, anticaking agents, and nanocomposites while in food packaging, they are applied as nano-sensors for monitoring the quality of food produced [182]. The nano materials can also serve as enzyme-supports because of their large surface-to-volume ratio with respect to their conventional macro-sized counterparts. Recent nano-carriers have the potential to function as selective and exclusive delivery systems in order to carry the food additives into the food ingredients without altering the basic physicochemical properties and morphologies. For delivery of the bioactives to the target sites, particle size is the most essential factor that affects the delivery rate as the micro particles cannot be assimilated in some cell lines. A number of researchers have developed various techniques of encapsulating these bioactives using nano-sized particles or nano-emulsions resulting in enhancement of their bioavailability due to the increased surface to volume ratio. Nanoencapsulation using nano-spray-drying is another promising technique for the development of nanoparticles which can serve the food industry for the production of bioactive ingredients.

However, challenges related to performance and toxicity of nanomaterials need to be addressed to induce active development and applications of NPs. Additionally, legislation for regulating the production, application, and disposal of nanomaterials for food industry is of utmost importance. Public awareness and acceptance of the novel nano-enabled food and agriculture products are also needed to be strengthened.

3.2.1. Application of NPs in Food Preservation and Packaging

The utility and global market of NPs have developed manifold in the recent years and is expected to reach USD 125.7 billion by 2024. In the domain of food packaging, the market is expected to reach a staggering USD 44.8 billion by 2030 [183]. Nanoceuticals and Nutrition-by-nanotech are the available commercial names for food nano-supplements. Nano-sized powders and nanocochleates are used for increasing absorption and delivery of nutrients without altering the taste, flavor, and color of the food products. For better absorption of micronutrients, vitamin spray-induced nanodroplets are used. The technique of nano-encapsulation is involved when probiotics and similar targets are required to deliver into the human system with the help of Fe and Zn nanostructured capsules. NP based food supplements are more effective than their common counterparts because they are able to react more efficiently with the human cells due to their nano-size.

Food preservation systems with antimicrobial packaging provide advanced barrier properties to the food [184]. NPs or nanocomposite materials such as starch and sorbic acid-based films are being utilized in various packaging applications for their microbial growth inhibiting properties. They are effective due to their high surface-to-volume ratio as well as enhanced surface reactivity of the nano-sized antimicrobial agents which assists in inactivating microorganisms more efficaciously in comparison to micro- or macroscale agents. Metallic and semiconducting NPs are the commonly used antimicrobial NPs. Metallic NPs such as Ag with Cu, Au, and Pt demonstrate different degree of efficacies. Among the semiconducting NPs, TiO_2, ZnO, WO_3, and MgO are proven antimicrobial agents. Other antimicrobial NPs consist of natural biopolymers like chitosan (CTS) and enzymes (peroxidase, lysozyme), organically modified nanoclay (e.g., quaternary ammonium-modified MMT, Ag-zeolite), natural antimicrobial agents (e.g., nisin, thymol, carvacrol, isothiocyanate, and antibiotics) along with synthetic antimicrobial agents (quaternary ammonium salts, ethylenediaminetetraacetic acid (EDTA), propionic, benzoic, and sorbic acids) [185]. Hybrid metal-polymer matrices is a new class of materials for diverse applications due to their distinct properties such as high surface areas, orderly crystalline structures, and pores with regular size and shape. Sensor composed of graphene oxide-nickel nanoparticle biopolymer films is capable of measuring glucose concentration in the body fluids. Nevertheless, it can also be employed in food application system because of the use of biocompatible materials low toxicity of Ni and cost-effective technology [186,187]. The antibiotic resistance mechanisms are irrelevant to the development of NPs since their mode of action is only to stay in direct contact of the microbial cell walls without penetrating it. The barriers that the natural NPs create can control microbial growth and consequently spoilage of pathogens. Ag-NPs are used in biotexiles, electrical appliances, refrigerators, and other kitchen-wares as they act in bulk form and their ions have the ability to inhibit a wide range of biological processes in bacteria [188]. The incorporation of AgNPs into the gelatin-based nanocomposite film promisingly enhanced its antimicrobial activity. Further, it was observed that nanocomposites, thus developed, showed potential antibacterial properties against both Gram-negative and Gram-positive food-borne pathogens [189]. ZnO NPs have antibacterial nature which increases with decreasing particle size that can further be stimulated using visible range light to incorporate in various polymers including polypropylene [190]. The contamination of *Escherichia coli* can be inhibited by using TiO_2 as a coating in packaging material, and in combination with Ag, it improves various disinfection processes.

Recent development of smart packaging, viz., oxygen scavengers, moisture absorbers, and barrier-packaging products, account for 80% of the market share. Bakery and meat industries significantly use nano-enabled packaging technologies. The food environment is so enabled that it can continuously sense oxygen content, temperature, and microbial load. Some examples include Ag-NP-incorporated enzymes for microbial detection and gas sensing and nanofibrils of perylene-based fluorophores for detecting gaseous amines from fish and meat spoilage. Additionally, ZnO and TiO_2 nanocomposites are used for detection of volatile organic compounds. Applications of NPs in the food industry are relatively recent and have demonstrated rapid developments in this area [191]. The major developments in this area include texture alteration, components and additives encapsulation, enhancing sensory acceptance, controlled release of flavor, and enhancing bioavailability of micronutrients [192]. NPs have also altered the novelty of packaging materials enhancing their mechanical barrier and antimicrobial efficacy. Hence, the recent advancements using NPs in food preservation and packaging may be used to overcome the disadvantages of the biopolymer-based packaging technologies. Nanocomposites exhibit enhanced barrier and mechanical and thermal properties compared to their polymers and conventional counterparts.

3.2.2. Applications of NPs in Food Supplements and Value Addition

The applications of nanotechnology and the use of NPs in food science and technology appear to have emerged from various sectors viz., pharmaceuticals, cosmetics, and nutraceuticals. The advent of nanomaterials, which can interact with biological entities at a near-molecular level makes it a common technology almost for various industries including the above. Current nanotechnology applications in food industry for developing nanotextured food constituents as well as the delivery systems for nutrients require techniques of nanoemulsions, surfactant micelles, emulsion bilayers, and reverse micelles. The nanotextured food ingredients claim to offer better texture, taste, and overall acceptability [193]. Low fat nanotextured spreads, mayonnaise, ice creams, and similar products claim to be as "creamy" as their full-fat alternatives, while offering a healthier alternative to the consumers.

Nanocochleates (50 nm in size), known to protect micronutrients and antioxidants from degradation during processing and storage, are based on a phosphatidylserine carrier derived from soybean and are generally regarded as safe. The Greek term "cochleate" means a 'snail with a spiral shell'. It can be derived by adding calcium ions to small phosphatidylserine vesicles in order to influence the formation of discs which are then fused to large sheets of lipid molecules and finally rolled up into nanocrystals.

In another instance, self-assembled nanotubes were developed from a protein namely lactalbumin which is a natural alternative for nanoencapsulation of pharmaceuticals, nutrients, and supplements [194]. Nanotechnology comprises another major area, namely nanoencapsulation, which is effectively used for delivering susceptible food ingredients and additives. Microencapsulation can be employed to mask the taste and odor of tuna fish oil for enabling it to be used for supplementation for its rich omega-3 fatty acid content. Nanoencapsulated food ingredients and additives are used in a range of food products such as the delivery of live probiotic microbes for healthy metabolic function. Nanoemulsion is another use in food technology to improve the quality of sweeteners, processed foods, and beverages [195–197]. A summary on application of NPs in various aspects of food science and technology is given in Table 3.

Table 3. Applications of NPs/Nanotechnologies in various aspects of Food Science and Technology.

Application	NPs/Nanotechnology	Function	Reference
Food Production	TiO_2	Antimicrobial, coating in packaging material, detection of volatile compounds	[198]
	Nanoemulsion	Quality enhancement of beverages, sweeteners, and processed food	[195–197]
	Nanoencapsulation	Enhancement of taste, color, and odor of food materials	[194]
Food preservation and packaging	AgNPs, Ag–ZnO NPs	Packaging of meat, fruit, and dairy products by AgNPs—doped nondegradable and edible polymers and oils; antimicrobial property	[199]
	Low-density polyethylene film + Ag, ZnO NPs, TiO_2, kaolin	Orange juice, blueberry, strawberry	[200–202]
	Ethylene vinyl alcohol + AgNPs	Chicken, pork, cheese, lettuce, apples, peels, eggshells	[203]
	Polyvinylchloride + AgNPs	Minced beef	[204]
	Polyethylene + Ag, TiO_2 NPs	Fresh apples, white sliced bread, fresh carrots, soft cheese, atmosphere packaging milk powder, fresh orange juice	[205,206]
	Nanoclay-polymer nanocomposites	Meats, cheese, confectionery, cereals, boil-in-the-bag foods, extrusion-coating applications for fruit juices and dairy products, bottles for beer and carbonated drinks	[207]
	Ag-ZnO NPs	Nanostorage containers, bakeware, containers, cutting boards	[199]
	ZnNPs	Preservation and transport	[208]
Food supplement and value addition	Colloidal metal nanoparticles	Enhanced uptake	-
	Nanopowders	Increase absorption of nutrients	-
	Cellulose nanocrystal composites	Drug carrier	-
	Nanocochleates	Drug delivery, enhancement of taste and color of food materials	-

Various applications of NPs and nanotechnology provide numerous advantages for food quality and safety. From farm to fork, nanotechnology is proven impactful at every stage of food manufacturing, enhancing shelf-life, nutrition, quality control, and smart packaging. However, the unregulated applications of NPs can pose potential risks to human health and environment. Numerous studies have demonstrated the toxicological effects of NPs on biological systems. Since food contact materials are already available in the market in some countries, more data on the safety of such engineered NPs on human health are necessary to implement regulations for such products. More research on ecotoxicological effects of NPs will add on to the existing knowledge.

3.3. Drug and Medicine

Nanotechnology-based drugs have attracted a lot of attention in the last decade. The unique properties of NPs, viz., small size, ability to travel through fine blood capillaries, vessels, junctions, and barriers, have made them one of the most researched and studied domains [209]. They have great advantages in terms of improvement of bioavailability of drugs, solubility, toxicity safeguard, pharmacological activities, distribution, and prevention from chemical and physical degradation and increased stability of drugs inside the body [210]. Nanomedicines have shown higher capacity to bind with biomolecules as well as reduction of inflammation/oxidative stress in tissues. Thousands of different nanomedicines have been designed over the years; they have various applications in different types of diseases. Few are approved for clinical use, and many more are in the phase of clinical trials.

The use of nanomaterials as drugs and medicine implies nanotechnologies for medical application with highly advanced medical intervention at molecular levels to cure diseases.

It provides a platform for the discovery of therapeutic nanomaterials or nanomedicines. The growth in nanomedicines has introduced numerous possibilities in medical sciences, specifically in the drug delivery mechanisms. Their structural characteristics make them an excellent mode for targeting at specific sites and quick penetration inside the cell/diseased sites [211]. Depending on their application and origin, various types of NPs were discovered based on therapeutic need. Liposomal, polymeric protein, metal based, and iron oxide NPs have emerged as top-notcher. In this review, our discussion is mainly focused on the application of metal NPs.

3.3.1. Silver NPs (AgNPs)

Silver NPs are considered ideal because of their unique properties like catalytic activity and stability. They also contain anti-viral, anti-bacterial, and anti-fungal properties. One of the applications of AgNPs is used in the antibacterial nanodevices, because of its Ag^+ ion effect. They can be positively used as anti-cancer agents due to their anti-proliferative effect and ability to induce cell death [212]. AgNPs can be loaded/coated to reduce their toxicity and improve their biological retention time which allows specific targeting of cancerous cells. AgNPs from *Andrographis echioides* have been shown to inhibit the growth of MCF-2 cells and are widely used in human breast adenocarcinoma cell lines [213]. The viability of tumor cells declines with increase in AgNPs concentration. *Allium sativum* AgNPs have shown positive outcome in gastrointestinal carcinoma [214].

3.3.2. Gold NPs (AuNPs)

Gold NPs have anti-cancer properties and induce oxidative stress. They absorb photons and convert those incident photons to heat that destroys cancerous cells. Cationic gold NPs (2 nm diameter) are toxic at some dose [215]. Gold NPs exist in non-oxidized state. Smaller NPs had less protein-to-protein ratio as compared to larger ones. Reports show that gold NPs treated with Hela cervical carcinoma demonstrated increased reactive oxygen species (ROS), leading to oxidation of lipid, proteins, and other several molecules [216]. AuNPs of 10 nm size were widely distributed in organs whereas 50–250 nm (large) NPs were found to be distributed in liver, spleen, and blood when intravenously injected.

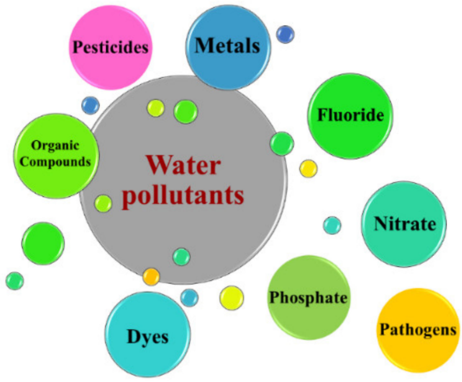
3.3.3. Iron Oxide NPs

Iron oxide NPs size lies between 1 and 100 nm in diameter. Their two main forms are magnetite (Fe_2O_3) and maghemite (γ-Fe_2O_3). Iron oxide NPs were synthesized by the process of precipitation in isobutanol (acting as surfactant) along with ammonium hydroxide and sodium hydroxide [217]. Iron oxide NPs are unstable in aqueous media without any surface coating; they aggregate and precipitate in vivo. The aggregates that are formed by unstable iron NPs inside the blood are sequestered by macrophages. Iron oxide NPs must be coated with different moieties to minimize the aggregation in certain conditions. Iron oxide magnetic NPs have many applications in anti-cancer strategy called hyperthermia; they destroy tissues nearby by generating heat. Iron oxide NPs have many properties like high solubility, stability, distribution, biocompatibility, and prolonged circulation time [218]. To increase the in vivo tumor imaging sensitivity, it is important to deliver large concentration of NPs in both tumor cells and in tumor mass. In a study, it was found that iron oxide NPs cause significant cellular morphological modifications, inducing apoptosis and necrosis in MCF-7 cell lines [219].

However, there is a need for restraint on the use and applications of these nanomaterials as drugs. A detailed understanding on possible hazards and toxicological impact of NPs on the environment as well as human health is needed prior to its application. Understanding the mechanisms of NPs access into the body, their function at cellular level, and their influences on public health is the call of the hour. Nanomaterial's characterization and understanding their surface functionality inside living systems are critical to understand their possible toxicological effects. All these parameters need more detailed studies before the approval of nanomaterial-based drugs for human usage.

3.4. Wastewater Treatment Process

The increase in population growth rate, industrialization, and excessive use of chemicals has contaminated the aquatic environment by releasing wastewater to the environment. The water from natural resources is not suitable for consumption due to the presence of organic (dyes, pesticides, surfactants, etc.), inorganic (fluoride, arsenic, copper, mercury, etc.), biological (algae, bacteria, viruses, etc.), and radiological contaminants (cesium, plutonium, uranium, etc.) [220,221]. Figure 6A depicts some of the common contaminants found in water. Several techniques such as physical, chemical, and biological have been adopted for the treatment of wastewater. However, search for new efficient technologies to improve water purification at low-cost is the current research focus. Currently, nanotechnology provides a new strategy for the removal of contaminants from wastewater with high efficiency. Several approaches have been developed in combination with various NPs for the successful removal of contaminants from wastewater as shown in Figure 6B.



(A)

(B)

Figure 6. Common pollutants found in water and the treatment processes. (**A**) Common pollutants present in water. (**B**) Treatment approaches used for wastewater using nanoparticles.

The adsorption process is defined as the attachment of gaseous or liquid molecules over the surface of the solid and forms a layer or film of molecules. This process is mainly limited to the surface of the adsorbent where the adsorbate accumulates. The adsorption process could be physisorption or chemisorption depending upon the nature of bonding between the adsorbate and adsorbent i.e., van der Waals forces, covalent bonding, or electrostatic attraction. Adsorption is the most commonly used technique for the removal of contaminants from water due to its low-cost, easy operation, and absence of secondary pollutants formation [146]. Due to the development of nanotechnology and its wide applications in the past few decades, several nanostructured materials have been explored as adsorbent for their potential application in the treatment of industrial effluents, surface water, groundwater, and drinking water [222,223]. Nanoadsorbents exhibit higher efficiency and faster adsorption rate compared to the conventional adsorbent due to their small size, high porosity, and large active surface area [224]. Additionally, these nanoadsorbents show high reactivity and catalytic efficiency. Nanomaterials such as carbon nanotubes (CNTs), ferric oxide (Fe_3O_4), graphene, titanium oxide (TiO_2), manganese oxide (MnO_2), zinc oxide (ZnO), and magnesium oxide (MgO) are successfully used as adsorbent for the removal of contaminants such as heavy metals, azo dyes, etc. from the water [223,225]. Several nanosized metal oxide adsorbents including ferric oxide, aluminum oxides, manganese oxides, titanium oxides, magnesium oxides, and cerium oxides are proved to be promising for the removal of pollutants from water [226,227]. Furthermore, different metal oxide NPs are superparamagnetic which allows the easy separation of these adsorbents from the reaction mixture with the application of an external magnetic field. Das et al. [228] reported the removal of methylene blue dye, Cu(II), and

Co(II) from aqueous solution using green synthesized magnetite NPs from crude latex of *Jatropha curcas* and leaf extract of *Cinnamomum tamala*. The removal of Cd(II) from contaminated solution was studied by using silver NPs prepared using leaf extract of Ficus tree (*Ficus Benjamina*) [229]. In a similar type of study, the removal of cadmium ions from contaminated solution was carried out using iron oxide NPs prepared by co-precipitation method with tangerine peel extract [230]. Maximum removal efficiency 90% achieved at pH 4.0 and adsorbent dose of 0.4 g/100 mL. Zinc oxide NPs synthesized from Aloe vera and Cassava starch used as copper ion adsorbent and higher removal efficiency was observed for Aloe vera synthesized NPs with the increase in adsorbate concentration [231]. These nanoadsorbents demonstrate remarkable efficiency in the removal of pollutants from wastewater; however, the toxicity of residual NPs in the wastewater and reduced potential activity due to the use of a huge number of NPs in the treatment process to minimize the process duration are the major shortcomings of this process [232].

Filtration of contaminated water or wastewater through membranes is another way to remove the pollutants from the water. Nanofiltration is efficient and effective for the removal of different types of contaminants (organic, heavy metals, pathogens, etc.) from wastewater, and the removal efficiency is mainly dependent upon the pore size and charge characteristics of the membrane [233]. Numerous studies have focused on the development and use of a composite membrane, prepared by the introduction of NPs into the polymeric or inorganic membranes for the treatment of water. The incorporation of metal oxide NPs like silica [234], alumina [235,236], zeolite [237], and TiO_2 [161,238] into polymeric membrane improved the membrane hydrophobicity and permeability. In addition to this, the incorporation of antimicrobial NPs like silver NPs into membrane matrix hinders bacterial attachment and biofilm formation [239,240].

Metal NPs are extensively used as nano-catalysts in water treatment due to their high surface-to-volume ratio and surface catalytic activity. These nano-catalysts improve the quality of water by degrading various contaminants, viz. dyes, pesticides, herbicides, polychlorinated biphenyls, nitro aromatics, etc. [241]. Various kinds of nano-catalysts such as electrocatalysts, photocatalysts, and Fenton-based catalysts are employed in the wastewater treatment process [220]. The mechanism behind photocatalysis is the photoexcitation of electron present in the catalysts. The light irradiation causes the generation of holes (h^+) and exited electrons (e^-). Further, the generated holes (h^+) are trapped by water molecules (H_2O) in aqueous media that subsequently form the hydroxyl radicals ($^\bullet OH$) [242]. These hydroxyl radicals are highly reactive and powerful oxidizing agents which oxidize the organic pollutants leading to the formation of water and gaseous degradation products [242]. Numerous studies reported photocatalytic activity of green synthesized Ag, Au, Pt, and Pd NPs in degradation of different dyes [63,243–247]. Additionally, various metal oxide NPs such as ZnO, CuO, FeO, SnO_2, TiO_2, NiO, CeO_2, etc. exhibited excellent photocatalytic activity for the degradation of different organic pollutants [248].

3.5. Antimicrobial Activity

In the past decade, application of nanomaterials to control microbial proliferation has garnered much interest from scientists worldwide [249,250]. The increase in resistance of microorganisms to antimicrobial agents, including antibiotics, has led to a spike in health-related complications. A vast body of work has revealed that by combining three forces of material science, nanotechnology, and the inherent antimicrobial activity possessed by certain metals, innovative applications for metal NPs can be identified [251]. Previous studies have reported that metal and their counterpart metal oxide nanoparticles have displayed toxicity towards numerous microorganisms [209,250]. These NPs may be used successfully to stop the growth of various bacterial species.

The surge in development of multi-drug resistant pathogens is presenting itself as a grave problem to public health, and thus, several studies have been conducted at improving the prevailing antimicrobial treatments [251]. It has been identified that approximately 70% of bacterial infections have developed resistance to one or more of the first- and second-line drugs that have been traditionally used to treat the infection [252]. The development of resistance in bacteria to commonly used chemical antibacterial agents may occur due to the lengthy production-consumption cycle, thus leading to reduction in efficacy. Moreover, the rampant use of poor quality or over-the-counter medicines in developing countries has led to a steep rise in antimicrobial resistance [253]. The need of the hour is to speed up the research and development and the synthesis of novel antimicrobial agents which are effective as well. NPs as antibacterial agents have turned out to be an emerging technology against this challenge, which have the ability to establish an effective nanostructure, which may be used to deliver the antibacterial agents, hence targeting the bacterial growth locally and more efficiently. In addition, nanoparticles have proved to have the potency that it leaves the pathogens with little device to develop resistance against them. Most of the available metal oxide NPs have zero toxicity for mammalian cells at the concentrations that have been used to kill bacterial cells, which in turn is an advantage for using them at a larger scale [254].

Metals like gold (Au), silver (Ag), titanium (Ti), copper (Cu), and zinc (Zn) are known to have their own properties and potency and display differential activity against microorganisms. This information has been understood and utilized across various cultures for centuries [255]. Numerous kinds of nanoparticles and their derivatives have been explored for their potential antimicrobial effects against several microorganisms. Metal nanoparticles such as gold (Au), silver (Ag), silicon (Si), silver oxide (Ag_2O), titanium dioxide (TiO_2), zinc oxide (ZnO), copper oxide (CuO), calcium oxide (CaO), and magnesium oxide (MgO) have been recognized to display antimicrobial activity. In vitro studies have suggested that metal nanoparticles have the potential to inhibit several microbial species, like *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa*, etc. [256–265].

The type of materials used in formulating the nanoparticles along with their particle size are the two most significant parameters, which can have an effect on the effectiveness of antimicrobial activity. It is well established that nanoparticles tend to possess different characteristics when compared to the same material having significantly greater dimensions. This is because the surface to volume ratio of the NPs considerably increases with a decrease in the particle size [266]. Certainly, in dimensions of nanoscale, the fraction of the molecule surface noticeably increases, which in turn can lead in improvement of some of the properties of the particles. For example, it may be mass transfer, heat treatment, catalytic activity, or the dissolution rate [267]. Additionally, the morphology and physicochemical properties of NPs have also been demonstrated to wield an effect on their level of germicidal activities. Literature survey has pointed that the particle size plays a role as vital parameter that can determine the effectiveness of antimicrobial activity of the metal nanoparticles [268,269]. The use of combination therapy with metal nanoparticles has the potential to be a strategy that can help tide over the emergence of bacterial resistance to multiple antibacterial agents [270,271]. More studies need to be developed to understand if green synthesized nanoparticles have better efficacy over traditionally synthesized nanoparticles. Current studies have displayed the same level of antimicrobial effects [272,273].

The shape of nanoparticles also has major influence on their antimicrobial effects [268]. Several research studies have investigated these shape-dependent characteristics of nanoparticles. A study described antibacterial activity of Ag NPs in three dissimilar shapes, namely, spherical, rod-shaped, and truncated triangular. It came to the conclusion that the truncated triangular NPs were more inclined to be reactive owing to their high atom density surfaces and consequently displayed greater antimicrobial activity [274]. In another study, the size and shape-dependent antimicrobial activity of fluorescent Ag nanoparticles (1–5 nm) was studied against some selected Gram-positive and Gram-negative bacteria [275]. They highlighted that the size and shape of the particles generated an effect on its activity. These investigations reported that the smaller the particles size, the easier they breach the cell wall exhibiting heightened antimicrobial activity. Furthermore, the authors proposed that these AgNPs could be used for multiple diverse procedures such as wound dressing, biofilms, bio-adhesives, and coating of certain biomedical materials. It was also found that antimicrobial property of TiO_2 is related to the size, shape, and structure of its crystal [252]. It is proposed in this particular study that the generation of ROS, ultimately leading to development of oxidative stress in the cells, may be a significant mechanism for TiO_2 nanoparticles to show its germicidal activity. It is well known then that ROS has the capability to cause site specific DNA damage, ultimately leading to the death of the cell.

The exact mechanisms in which nanometals present the antibacterial effect are still an area of active investigation. However, two common options have been proposed in this aspect. Firstly, toxicity associated with free metal ions can arise due to the dissolution of the metals from the surface of NPs. Secondly, oxidative stress could be triggered through the production of reactive oxygen species (ROS) on the surface of the NPs. Based on literature review, there are some intrinsic factors that can have an influence on the ability of nanomaterials in reducing the number of cells or completely eliminating the cells [255].

NPs are a promising technology, and owing to its vast application, understanding nanotoxicity and its consequences is of utmost importance. For decades, the pharmaceutical industry has used NPs as a tool to reduce toxicity and side effects of drugs [276]; nonetheless, one needs to be careful when using NPs, as certain safety concerns still exist. Several reports have identified damage to neurological and respiratory organs issues in the circulatory system. In addition, other yet unknown toxic effects of NPs are few of the foremost apprehensions in using NPs as part of a systemic therapy. Undeniably, numerous NPs seem non-toxic, and a few of them are reduced to having non-toxic properties, which ultimately has beneficial effects on health [209,277–279]. Nevertheless, further studies need to be executed focusing on minimizing the toxicity of metal and metal oxide NPs, that will eventually be applied in therapy as a proper substitute to disinfectants and antibiotics especially in biomedical applications. Moreover, current research should make the application of antimicrobial activity of NPs in eradication of microbial infections as one of their priorities.

4. Toxicity of Metal Nanoparticles

NPs have wide applicability in different sectors such as electronics, agriculture, chemicals, pharmaceutical, food, etc. due to their unique physicochemical properties [280]. The most commonly used NPs by various sectors include metal oxide NPs such as silicon oxide (SiO_2), titanium dioxide (TiO_2), zinc oxide (ZnO), aluminum hydroxide [$Al(OH)_3$], cerium oxide (CeO), copper oxide (CuO), silver (Ag), nanoclays, carbon nanotubes, nanocellulose, etc. [281,282]. However, massive release of NPs into the environment (air, water, and soil) by various industries is resulting in production of nanowaste and proving to be dangerous for the living organisms and causing threat to ecosystem balance. Various characteristics of NPs affecting their toxicity are size, nature, reactivity, mobility, stability, surface chemistry, aggregation, storage time, etc. NPs cause adverse consequences on human health and animals. Use of NPs has intensified the risk of various diseases in humans such as diabetes, cancer, bronchial asthma, allergies, inflammation, etc. [3]. The animal reproductive system has also been shown to be affected due to toxicity of various NPs such as Au, TiO_2, etc. [283,284]. NPs enter the animal body through ingestion and inhalation and get absorbed by the cells through the processes of phagocytosis and endocytosis and induce the generation of reactive oxygen species (ROS), ultimately resulting into lipid peroxidation, mitochondrial damage, etc. Different NPs such as Ag, Cu, ZnO, Ni, etc. have also reduced the enzymatic activity of various microorganisms. In addition, excessive production of NPs is also affecting the food web of the ecosystem [285]. Toxicity effects of NPs over plants, animals, and microorganisms are shown in Figure 7.

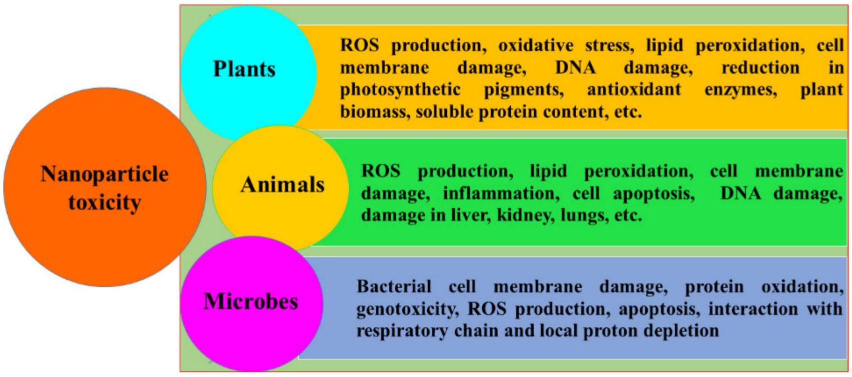


Figure 7. Toxicity effects of NPs on microbes, plants, and animals.

4.1. Impact of Nanoparticle Toxicity on Plants

Plants are of high fundamental significance as they perform photosynthesis and release oxygen in the atmosphere. As all the plant parts (roots, shoots, and leaves) are in direct contact with environmental matrices (air, water, and soil), they may get affected more by the NPs contamination as compared to the other living beings. NPs present in the atmosphere can enter into the plant body through the stomatal openings on leaves [286], while those present in soil and water can be selectively up taken by the plant roots [287].

NPs proved to be toxic to plants and hamper their growth and development. The toxicity in plants is mainly due to ROS production, causing lipid peroxidation and ultimately leading to DNA damage, reduction in photosynthetic pigments, plant biomass, soluble protein content reduction, etc. [288]. However, plants have a defensive system against oxidative stress in the form of enzymatic and non-enzymatic antioxidants, which may become inefficient under higher oxygen concentrations [289].

Assessment of NPs Phytotoxic and Genotoxic Effects on the Plants

While phytotoxic effects of NPs on the plant can be assessed by analyzing morphological and physiological changes, genotoxicity can be observed by observing DNA damage in the plant cell. By evaluating various physiological parameters such as germination, biomass production, leaf number, photosynthetic ability, root and shoot length, etc., phytotoxicity levels of NPs on the plants can be assessed. To observe genotoxic effects on the plant, assessment of cytology of plant roots for the determination of mitotic index, chromosomal abnormalities, etc. is the simplest approach [290].

SiO_2 NPs are among the top metal oxide NPs produced by various industries such as cosmetics, pharmaceuticals, food, etc. due to their worldwide demand. Positive effects of SiO_2 NPs on various plants such as *Oryza sativa*, *V. faba*, *S. lycopersicum*, *Medicago sativa*, etc. have been shown in different studies [291–294]. Toxic effects of SiO_2 NPs on plants have been investigated by Slomberg and Schoenfisch [295] in *Arabidopsis thaliana*, Karimi and Mohsenzadeh [296] in *Triticum aestivum*, and Silva and Monteiro [297] in *A. cepa*, etc. Regarding TiO_2 NPs, negative effects have been shown in *Z. mays* and *Vicia narbonensis* [298], *V. narbonensis* [299], *V. faba* [300,301], etc. ZnO NPs are majorly used in agriculture sector (as pesticides, fertilizers, etc.) [302].

In green algae, *Chlorella vulgaris* and *Dunaliella tertiolecta*, AgNPs proved to be inhibitory for their growth and displayed higher ROS production and lipid peroxidation [303]. AgNPs also proved to be toxic for the growth and development of green algae, *Pithophora oedogonia* and *Chara vulgaris* [304].

4.2. Toxicity of Nanoparticle-Based Drugs

The commercial applications of NPs as therapeutics for treatment of diseases is a double-edged sword. Even though many studies are being done worldwide to analyze the toxic effects of NM exposure, the possible mechanism of NMs interactions with biological systems and their consequences are still unknown. Research has shown that NPs can travel through the bloodstream and easily cross membrane barriers. This in turn can adversely affect tissues and organs at molecular and cellular levels [305]. NPs have demonstrated the capacity to cross the blood–brain barrier (BBB) and gain access to the brain [306]. Small size, large surface area to mass ratio (SA/MR), and surface characteristics determine nanoparticle's interaction with biological milieu and the resultant toxic effects that ensue. The unique nature of the NMs allow them to easily pass-through cell and tissue membranes and cellular compartments to cause cellular damage. The large SA/MR of NPs also remains open for active chemical interactions with cellular macromolecules. Increase in surface area of the identical chemical further enhances adsorption properties, surface reactivity, and potential toxicity [305].

NPs have the tendency to translocate across cell barriers from the entry point i.e., the respiratory tract to secondary organs, reach the cells by various mechanisms and start interacting with subcellular structures. These properties make NPs uniquely suitable for therapeutic and diagnostic uses. NPs are transported neuronally, involving retrograde and anterograde movement in axons and dendrites as well as perineural translocations. The target organs such as the central nervous system (CNS), however, bear the brunt of potential adverse effects (e.g., oxidative stress) [306]. The size of NPs has an important role in renal clearance and in avoiding immune activation, enhancing the efficacy and circulation time of the drug inside systemic circulation [209]. In case of nickel NPs, small particle sizes (less than 200 nm) are preferable for entering into epithelial cells, whereas larger NPs are phagocytosed by macrophages present. Another issue is surface charge which restraints the fate of NPs. Positively charged surface NPs with amorphous nature do not enter inside the cells, whereas, negatively charged crystalline nickel sulphide and sub-sulphide particles can enter cells by phagocytosis. Inhalation of MnO_2 NPs leads to the formation of ROS causing oxidative stress in brain [210].

Silver NPs of different sizes, i.e., 20 or 40 nm (Ag20Pep and Ag40Pep) were analyzed in THP-1-derived human macrophages through their cellular uptake. Results demonstrated a majority of the AgNPs spread throughout the cells. Formation of protein carbonyls or induction of heme oxygenase I are some of the associated responses due to oxidative stress are also observed. The charged Au NPs sized 15 nm cause cell death by apoptosis, whereas neutral Au NPs cause necrosis in HaCaT (human epidermal keratinocyte) cell lines [307]. Several NPs can penetrate inside the nuclear envelop, and they play an important role in inducing genotoxicity. Silver NPs have been found to be more toxic than gold NPs. TiO_2 NPs are considered as biological inert material in vitro and in vivo, while TiO_2 NPs larger than 15 μm are highly toxic, generating ROS. TiO_2 is toxic to PC12 cells [308]. It was reported that toxicity of NPs increases with increasing surface charges, i.e., lower positive charge NPs have less electrostatic interaction with the cells. Positive ZnO NPs have more cytotoxic effect in A549 cells as compared to same sized and shaped negatively charged ZnO [309]. Rod shaped Fe_2O_3 NPs produce high cytotoxic responses compared to spherical Fe_2O_3 NPs in RAW 264.7 (murine macrophage) cell lines [310]. Rod-shaped CeO_2 NPs cause more toxic effects and produce LDH and necrosis factor-α in RAW 264.7 cells [209]. NPs cytotoxicity depends on assay, cell line, and physical and chemical properties. Copper oxide NPs have been found to produce toxic side effects in liver and kidneys when examined on lab animals; after oral administration and interaction with gastric juice, they form reactive ionic copper. It was reported that silver NPs and iron oxide NPs can penetrate and cross the blood–brain barrier [306]. Iron oxide has the capacity to accumulate inside liver, spleen, lungs, and brain and has the capacity to cross the BBB after inhalation [305]. Iron oxide shows less cytotoxic effects at high concentration (300–500 kg/mL, 6 h) than in low concentration (25–200 μg/mL). At low concentration, it generates Reactive oxygen species (ROS), DNA damage, and causes lipid peroxidation. Silica based NPs of size 70 nm at 30 mg/kg concentration have been found to alter biochemical parameters [210]. Hence, a number of studies show evidence of NPs causing DNA and membrane damage, protein misfolding, and mitochondrial damage.

In a clinical scenario, several factors need to be addressed, viz., bioavailability, adverse reactions, cellular interactions, biodistribution, biodegradation, etc. The successful clinical translation of nanomedicines is therefore a long and onerous process of weighing the benefits against risks of toxicity involved. Scientists must proceed with caution and refrain from premature launch of nanomedicines without assessing the adverse effects involved.

Compared to physio-chemical methods, biologically synthesized NPs have been proved to be non-toxic/less toxic due to non-use of external stabilizing agents and hazardous chemicals/solvents during the synthesis process. Hasan et al. [164] compared the morphological, physiological, and biochemical responses of biologically and chemically synthesized iron oxide NPs in *Zea mays*. The biological synthesized FeO NPs promoted better plant growth as compared to the chemically derived NPs. The chemically synthesized NPs proved toxic and hampered the plant growth even at lower concentrations. In a similar kind of study, Anna et al. [311] observed better growth of green algae *P. kessleri* while using biologically synthesized NPs compared to chemical synthesized NPs.

5. Major Challenges and Future Perspective

In the recent past, research on NPs and their potential applications have progressed by leaps and bounds. Numerous studies have reported the green synthesis of metallic NPs using various biological sources such as plants, bacteria, fungi, and yeast. However, several challenges persist, which limit its large-scale production and consequent applications. Some of the major challenges observed during the synthesis are summarized below:

- Detailed optimization studies on reactants (plant extract, microorganism inoculum, fermentation medium composition, etc.) and process parameters (temperature, pH, rotational speed, etc.) are required to control the size and shape of the NPs.
- Studies also need to be focused on enhancing various physicochemical characteristics of NPs for specific applications.
- The involvement of each metabolite of plant extract and cellular components of microorganism in the synthesis of NPs should be completely analyzed.
- Scale-up of NPs production for commercial purposes using green synthesis methods needs to be prioritized.
- Improvement of NPs yield and stability with reduced reaction time is needed by optimizing various reaction parameters.

Addressing these challenges could make the green synthesis methods cost-effective and comparable to the conventional methods for the large-scale production of NPs. Additionally, the separation and purification of NPs from the reaction mixture is another important aspect that need to be explored. A detailed toxicological study of the NPs on plants and animals is necessary for expanding its application in diverse fields. In addition to wild type strains, genetically modified microorganisms with the ability to produce greater quantity of enzymes, proteins, and biomolecules could further enhance the biosynthesis as well as the stabilization of NPs. Further, enhancement of metal accumulation capacity and tolerance of genetically modified microorganisms could provide a futuristic approach for the production and application of metal NPs using the green synthesis method.

6. Conclusions

The present review focuses on the green synthesis of metal NPs derived from plants and microorganism and their applications. Green synthesis methods provide a clean, non-toxic, and eco-friendly approach for the synthesis of metal NPs compared to other conventional techniques like physical and chemical methods. A wide range of plant materials including leave extract, fruit extract, seed, fruit, bark, etc. and microorganism such as bacteria, fungi, actinomycetes, etc. have shown potential for synthesis of various metal and metal oxide NPs (viz., Au, Ag, Pt, Pd, Ni, Se, Cu, CuO, and TiO_2). The size and shape of NPs and the reaction rate strongly depend on various experimental parameters such as reaction time, reactant concentration, pH, temperature, aeration, salt concentration, etc. Different characterization techniques such as UV-VIS spectroscopy, FTIR, XRD, SEM, TEM, EDX, and AFM have been used to determine the shape, size, and morphology of biosynthesized NPs. However, in terms of translational research, several factors, viz., bioavailability, adverse reactions, cellular interactions, biodistribution, and biodegradation, need to be addressed. The accumulation of these NPs in the environment and their uptake by biological systems can lead to disastrous consequences as a number of studies show evidence of NPs causing DNA and membrane damage, protein misfolding, and mitochondrial damage. Although numerous studies reported the biological synthesis of metal NPs, a thorough investigation is the need of the hour for widening their applications and successful commercialization.

Author Contributions: Conceptualization, P.K.D.; writing—original draft preparation, P.K.D., J.K., A.K.D., S.S. (Soumi Sadhu), S.S. (Sunita Sharma), S.S. (Swati Singh); writing—review and editing, P.K.D., J.K., A.K.D., S.S. (Soumi Sadhu), S.S. (Sunita Sharma), S.S. (Swati Singh), P.K.G., B.S.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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