A Review on Effects of Nanoparticles on Freshwater Single-Celled Microalga *Chlorella Spps.*

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ABSTRACT: With the increasing development in the field of nanotechnology and increased use of nanomaterials, there has been also increase in the release of Nanoparticles in the environment. These nanoparticles sometimes act as pollutants for living organisms. Due to release of these nanoparticles in the water bodies, they easily impact the aquatic organisms. Algae being the primary producers and acting as a food for higher aquatic animals, when encountered with nanoparticles, negatively affected by them or through biomagnifications, are directly or indirect become susceptible and also harmful for further food chain. Nanoparticles also hinder the growth of algae, thus providing less biomass. This review analyses the recent work done on the effect of different nanoparticles on the cellular structure of one of the most economically useful algae *Chlorella sp.*

Keywords: Algae, *Chlorella*, Nanoparticles, nanomaterials, pollutants.

1. Introduction:

Chlorella is a eukaryotic green microorganism which belongs to microalgae. It is economically very important organism in scientific as well as commercial field. *Chlorella* is an important source of proteins and carbohydrates. It is majorly used as food for humans and fodder for animals. *Chlorella* decrease CO2 level competently. It is also helpful in removing excess phosphorus and nitrogen nutrients preventing algal bloom and can also help in biomodulation of greenhouse gas and wastewater bioremediation. Nowadays *Chlorella* has been studied for its potential as an expression host for recombinant protein production that can alter the current used one. However there are many challenges remains to be addressed. *Chlorella* is also a good source for the production of biofuels, which could be a great step towards the eco-friendly environment [1].

Nanoparticles (NPs) are the substances that have any one dimension less than 100nm [2]. NPs can be divided into different categories which possess different properties such as physicochemical, chemical, optical and electrical properties. NPs are also used in different industrial purposes in wide areas [3] [4]. Nanoparticles can be classified into metal oxides such as Copper oxide (CuO NPs), titanium dioxide (nano-

TiO2), zinc oxide (ZnO NPs) are commonly used in nanomaterials. Considerable attention has been received by Gold nanoparticles (Au NPs), silver nanoparticles (Ag NPs) and zero-valent iron nanoparticles (nZVI) among noble metal materials [5]. These nanoparticles have very important role in nanotechnology due to their utilization in nano electronics, colorimetric techniques, development of biosensors, semiconductors, and DNA labeling [6][7][8][9] [10]. Graphene, fullerene, and carbon nanotubes (CNTs) and some other carbonaceous NPs are also increasingly produced and are useful in various industrial areas.

Metal oxide NPs are part of a family of nanomaterials frequently used in emerging technologies and have a wide range of applications, particularly in the manufacture of commercial products [11]. Metal oxide NPs were developed recently at the industrial level and have extensive applications in water treatment, medicine, cosmetics and engineering .For example Aluminum NPs are often used for mixing explosives [12]. ZnO-NPs have been used in the cosmetic and sunscreen industry on account of their capacity to absorb, reflect, and disperse UV radiation [13]. Once combined with materials such as surface coatings, paints, textiles and plastics, nanoscale ZnO materials are also potent antimicrobials [14]. CuO-NPs have the ability to substitute noble metal catalysts for oxidation of carbon monoxide [15] and are used in sensing materials, glass, ceramics and antimicrobials [16] . Fe_2O_3-NPs have broad applications in various products such as coatings, plastics, rubber, silicone, wear-resistant tools and sealing materials as an integral component [17][18]. Titanium dioxide NPs are primarily used as UV-absorbent photo catalysts in consumer goods such as sunscreen lotions and also serve as a catalyst in sterilization [19][20] [21] . Since the applications of engineered metal oxide NPs have increased in recent years, metal oxide NPs like titanium dioxide are expected to find their way into the aquatic environment, where their fate and behavior are largely unknown [22][23]. Titanium dioxide NPs photo catalytic activity was found to be dependent on concentration, crystal structure and light intensity [24]. The titania NPs have been shown to cause organism toxicity by developing reactive oxygen species (ROS) after contact with UV light; resulting in damage to the cell membrane [25].

Algal toxicity tests are widely applied to determine the effects of dangerous substances in water since algae play an important role in the equilibrium of aquatic ecosystems, being the first level of the trophic chain to generate organics and oxygen. Various factors influence the toxicity of NPs on algae, in particular the characteristics and the aquatic properties of the algae, such as water chemistry, light and water temperature [26]. Hydro chemical conditions are important factors affecting the suspension of NPs including dissolved organic matter (NOM), ionic strength, and pH [27]. NOMs can be absorbed into NPs to modify the functional surface groups of NPs or to form thin films, and to strengthen their migration and delusions capabilities. By covering the surface of NPs due to electrostatic repulsion, NOM stabilized particle size [28]. NOM coating may limit the release of ions from NPs into water [29]. Prevent NP aggregation [30] and decrease the toxicity of NPs to algae [31]. Natural water bodies' ionic strength and pH can change the suspended state of NPs in water [32], which also affects the adsorption of NPs to NOM

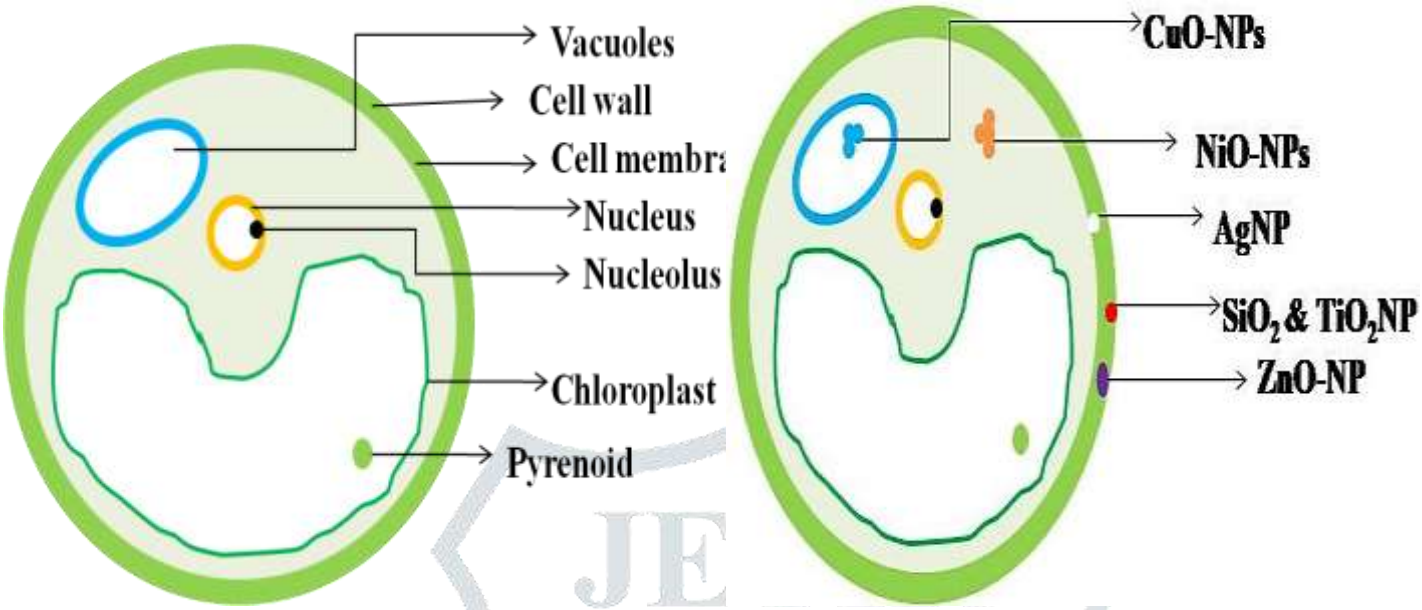
[33]. Another important mitigator of NP toxicity is water hardness, which encourages NP aggregation and decreases dissolution [34]. In addition , water temperature, light and emissions of contaminants will affect on the toxicity of NPs. Temperature is known to directly affect aquatic ecosystem communities [35] as it is considered an significant abiotic factor affecting primary producers such as algae growth and development. At an elevated temperature a higher dissolution rate of Ag NPs was obtained [36]. Therefore, due to changes in the physiological status of algal cells and the current state of NPs, the toxicity of NPs to algae may be expelled by temperature. Since some NPs are semiconductors with photo catalytic properties, UV-exposed NPs may cause a toxic effect on algae by generating highly reactive ROS [37][38] found NPs to be more harmful to UV-C irradiated algal cells (a high-energy radiation with wavelengths below 280 nm) than those treated under dark and visible conditions.

2. Uptake and Transportation Of Different Nanoparticles By Algal Cells:

Nanoparticles can penetrate different organisms and cells including mammalian cells, plant cells, bacteria, fungi, and viruses because of their size and surface properties [39][40][41][42][43]. This process can be energy-dependent intake or energy-independent entry (Navarro et al., 2008; [43] [44]). The former is often referred to as endocytosis, a mechanism through which cells consume molecules or particles through swallowing those up [45]**.** Nanoparticles join cells using various known endocytotic pathways such as clathrin-mediated endocytosis, caveolae-mediated endocytosis, phagocytosis, and macropinocytosis [44][46][47][48] [49]. Direct penetration occurs for 1D nanostructures or highly positively charged nanoparticles [43][50]. Nanoparticles may locate in endosomes, lysosomes, cytoplasm, mitochondria, endoplasmic reticulum or nucleus depending on the nature of the nanoparticles, after entering cells [44] [46] [47][48] [49] [51][52][53][54][55]. In addition to traditional TEM and confocal laser scanning microscopy (CLSM) studies, various imaging techniques have been developed for monitoring the cell uptake method [56][57]. For example, X-ray fluorescence microscopy is used to determine the distribution of chemical elements of nanoparticles in cells [58]. Magnetophotoacoustic imaging enables the separation of membrane-adhered nanoparticles or endocytosis [59]. Dynamic colocation microscopy enables the spatiotemporal characterisation of internalized nanoparticles [60]. In the absence of an external mark, Raman spectral imaging maps vibrational bands of nanoparticles in live cells [61]. Atomic force microscopy (AFM) tests the force between nanoparticles and the surface of the cell suggesting binding of the receptor and binding strength [62].

In an experiment studied a complex process of toxic effect of NiO-NPs on algal cells of *C. vulgaris*. The possibility of mechanism involved in toxicity is the uptake of soluble Ni2+ released from NiO-NPs by algal cells. The most common method of toxicity for several metallic NPs was considered the solubilization of NPs causing the release of toxic metal ions on aquatic microorganisms for several types of metallic NPs [63]. But in case of, it has been found that it is almost an insoluble material [64] having very

low solubility, thus the toxic effect in algal cellular component is not only due to release of free metal ions from NPs but also due to induction of strong oxidative stress.



(a) (b)

Figure 1. (a) showing cellular components of *Chlorella sp.* (b) transport of different nanoparticles to cellular components.

In recent studies, it has been observed that the penetration of NPs into the cells of algae could be the reason for toxicity [65][66] found that NiO-NPs were able to cross the cell membrane and to accumulate inside the cell. There could be a possibility of solubilization of bioaccumulated NiO-NPs inside the cell which offered more acidic pH environment than the culture media, and then the Ni^{2+} which was released in cellular system of algae contributed to the toxic effect. This hypothesis was also suggested for Ag-NPs toxicity process in algal cells [43].

3. Interaction between Nanoparicles And Algal Celllular Components

The interactions between ZnO NPs and green algae *Chlorella* sp. was studied by SEM and viability assay showed that cell damage was mainly induced by high concentrations of ZnO NPs which were associated with algal surfaces. Aggregation of the ZnO NPs and secretion of exudates from algae is the reason behind toxic effect induced by Zn ion released from ZnO NPs [67].

Internalized MWCNTs can interact with organelles, leading to altered sub cellular structures within the algal cell. The control cell structure remains unchanged, with the plasma membrane adjacent to the cell wall. Exposure to o-MWCNT8 resulted in evident plasmolysis, starch grain shrinkage, larger pyrenoid, and lipid droplet formation. As the pyrenoid is the main site for the synthesis of starch synthesize. This organelle's enlargement may imply increased starch output. In the cells exposed to MWCNTs, however, shrinkage of starch grains was also seen, suggesting excessive starch intake. In Chlamydomonas sp. also a

related phenomenon was observed via cadmium [68]. Neutral lipids, which act as a large carbon and energy storage facility, are also contained in fat droplets. Earlier study indicated that synthesis of starch was a swift response to environmental stress, while lipids served a long-term function of storing energy [69]. Another important difference for the algae treated was the presence of dense electron granules within the cytoplasm vacuoles. In other microalgae species treated with toxicants such as cadmium and triphenyltin related structures have been found [68][70]. These electron-dense granules were known as polyphosphate bodies capable of providing a storage site for critical metals and toxicants, and thus serving as a detoxification route [71] o-MWCNT8 will adsorb nutrient cations from the OECD medium and release residue catalysts into the medium (mainly Ni). In polyphosphate bodies, the nutrient cations and the catalysts produced by MWCNTs could be sequestered. Consequently, the development of polyphosphate bodies and lipid droplets is most likely an adaptive response to stress / exposure from MWCNTs. Finally, the internalized CNTs may cause damage that is irreversible to the organelles and vacuolation of cells as well.

Table 1. Describing various effects of different Nanoparticles on *Chlorella spps.*

Nanoparticles	**Concentration & Duration**	**Algal Species**	**Effect Studied On Algae**	**Reference**
CuO-NP	EC_{50}- 45.7mgL^{-1} 72 h	*Chlorella pyrenoidosa*	Cellular membrane damage Increase in ROS level Mitochondrial depolarization	[72]
NiO-NP	0.1-100 mgL^{-1} 96 h	*Chlorella vulgaris*	Inhibition in chlorophyll systhesis Decreased viable cells Loss of cell membrane integrity Impairment of enzymatic and photosynthetic activity	[73]
ZnO-NP	48.6 mgL^{-1} 4-72 h	*Chlorella sp.*	Damaged and/ or deformed cell wall ZnO NP translocation Blocked nutrient uptake Delayed photosynthetic activity	[74]

TiO-NP	EC_{50}- 16.12 mgL^{-1} 72 h	*Chlorella sp.*	Disrupted cell membrane Decreased chlorophyll content Interrupted energy transduction	[75]
AgNP	0-10 mgL^{-1} 24 h	*Chlorella vulgaris*	Decreased chlorophyll content Decreased viable algal cells Increased ROS formation and lipid peroxidation	[76]
MWCNT	0-10 mgL^{-1} 1-8 h	*Chlorella pyrenoidosa*	Destabilized and disrupted membrane structure Altered enzymatic and photosynthetic activity	[76]

4. Biochemical and Physiological Changes In Clorella In Reponse To Nps:

The experimental studies showed that AgNPs induce a strong ROS formation chlorella. Cell structure damage and lipid peroxidation is caused by high levels of ROS induction [77]. High lipids peroxidation can lead to impaired cellular function and change in physico-chemical properties of cell membranes, which reduce viability of cells and disrupt vital functions [78]. The characteristics of medium also affect the toxicological effects of AgNPs. The contribution to algal toxicity is possibly the release of Ag. The most common mechanisms of toxicity for different types of NPs is solubilization of the NPs into free metal ions present in the media [79][80] in their study demonstrated that the inhibition in cellular division processes (relative cell size and granularity), the deterioration of photosynthetic apparatus (chlorophyll synthesis and photochemical reactions of photosynthesis), and the generation of ROS is the main cause for the loss in cellular viability of *C. vulgaris*. On exposing to NiO NPs suspensions, deterioration of photosynthetic and enzymatic systems necessary for was affected in algal cells of *C. vulgaris* due to strong oxidative stress effect. There were also some previous studies showed that cytological toxic effect of many NPs were due to formation of strong oxidative stress caused by generation of ROS [81] [82][80] [83].

Figure 2. Showing events of processes taking place after interaction with NiO-NP

Membrane damage is an important consequence of CuO NPs exposure which is mainly caused by oxidative stress [84] or physical interaction/penetration [85]. The main effect of CuO NPs on the algal membrane is due to direct physical contact/penetration is likely the reason of membrane damage rather than ROS formation due to oxidative stress. Exposure of CuO NPs increased the mitochondrial depolarization. The dysfunction and mitochondrial depolarization is due to direct contact of NPs, which physically damaged structure of mitochondria [86] whereas oxidative mitochondrial damage and/or disrupted electron transfer within the mitochondrial inner membrane occurred due to amassing of excess ROS [87][88]. ROS accumulation could be a major cause for the observed mitochondria depolarization Nps may not directly affect the mitochondrial structure because many factors are not in favour as layered structure of mitochondria, CuO NPs size, less appearance of CuO NPs in the cytoplasm.

However, membrane damage was mainly caused by the direct interaction of CuO NPs with membrane whereas the released $Cu^{2}+$ was the main cause of ROS accumulation and mitochondrial depolarization.

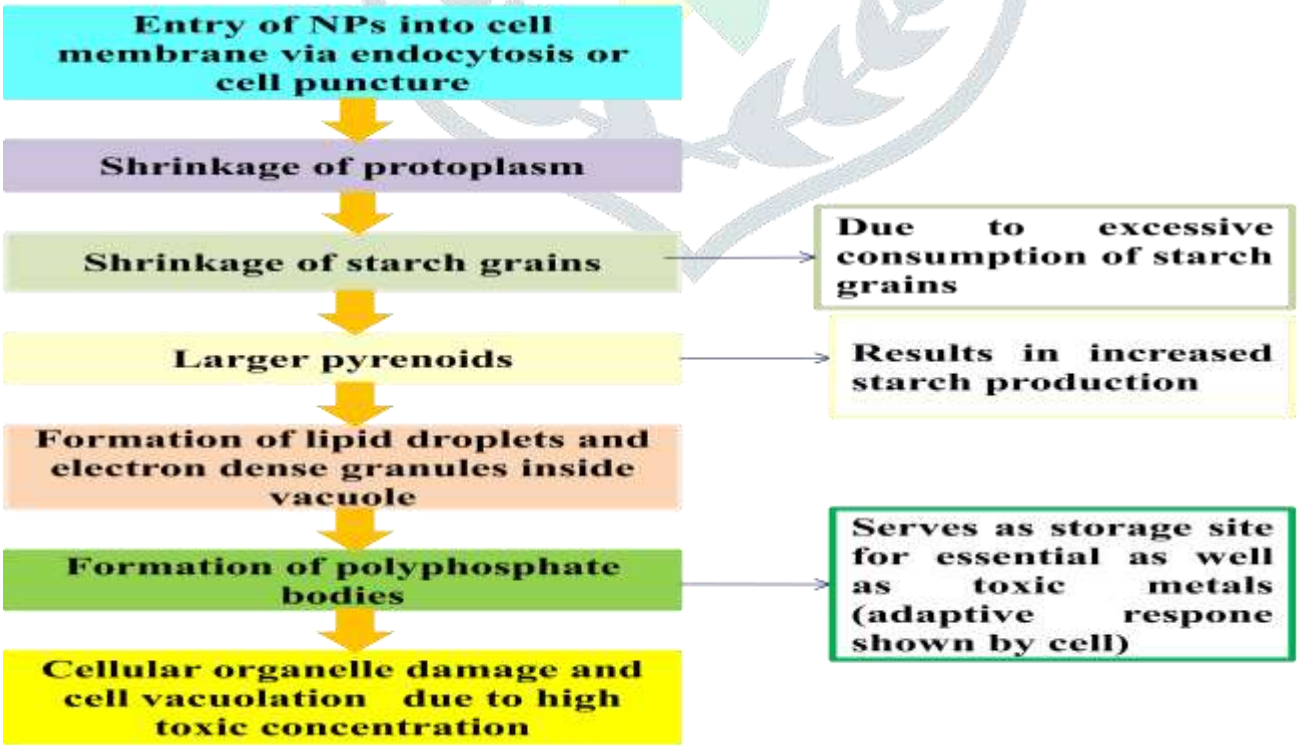


Figure 3. General response mechanism shown by algal cells encountered with NPs.

In various stresses, membrane fluidity changes as an adaptive response of cells., enabling reduction of cellular functions by altering fatty acid composition [89]. The increase in membrane fluidity is supposed related to cell plasmolysis [90]. Membrane structure was destabilized and disrupted resulting into acute toxicity. Methyl palmitoleate, monoolein, and monopalmitin levels were increased on MWCNTs exposure,which indicates the formation of lipid droplets as these are related to the formation of lipid droplets. MWCNTs are also capable to alter DNA/RNA biosynthesis and the gene expression which can be suggested due to induction in the production of nucleosides and nucleotides, including guanosine, inosine, and adenylic acid AMP.

There was a change in the contents of some metabolites which was dependent on concentration indicating that the algal cells responded to o-MWCNT according to concentration. MWCNT decreased citric and malic acid content which are intermediates of the tricarboxylic acid (TCA) cycle associated with glycolysis, suggesting less production of adenosine triphosphate (ATP) due to slow processing of TCA cycle [91]. Whereas the accumulation of glucose, lactose, and galactose indicates the inhibition of glycolysis [92]. When algal cells were exposed to MWCNTs, increase in ribulose 5-phosphate was observed, which indicates enhancement of pentose phosphate pathway by which nicotinamide adenine dinucleotide phosphate (NADPH) was generated. Excess production of NADPH has been observed as a defense mechanism against oxidative stress [93]. Different contents involved in osmotic regulation of algal cells such as mannitol and D-arabitol was also decreased whereas glycerol level was increased. However, cell wall damage due to exposure of MWCNT was indicated by the reduction of mannose.

Different amino acids levels as well as polyamines which were related to stress such as putrescine were also increased. Putrescence inceases the activity of antioxidant enzymes such as SOD which results in the increased oxidative stress in their experiment also found the increased SOD activity on MWCNTs exposure, which further increased on increasing concentration.It has been found in different studies that increased accumulation of putrescine and other amino acids is defense response to different stress [94].

The expression mRNA level of 13 genes involved in different functions such as cell division, photosynthesis, lipid synthesis and mitochondrial function were evaluated in control and treatment groups to study the response of cells *C. pyrenoidosa* at the genetic level. It was observed that there was an increase in the expression of the gene *fts*H, which encodes protein FtsH responsible for cell division which indicates that MWCNTs may increase cell division of algae.

Exposure of o-MWCNT increased the regulation of genes that encode acetyl-CoA carboxylase (*acc*A and *acc*D) and diacylglycerol acyltransferase (*dgat*7494 and *dgat*2354) and accumulation of triacylglycerols (TAGs) was observed by the overexpression of these genes as these two enzymes catalyze the first and the final steps in TAG biosynthesis, respectively. Formation of lipid droplets was also observed having TAGs as major components. Gene *ME* that encodes malic enzyme was also increased

which suggests that MWCNTs hastened the change between malic acid and pyruvate, thereby decreasing the content of malic acid. The depletion of malic acid content was supplemented with increased activity of phosphoenolpyruvate carboxylase (PEPC).There was very little increase in the expression of gene *HLA*3 that encodes an inorganic carbon transporter protein which is involved in photosynthesis.

MWCNTs mainly had a negative effect on photosynthesis of algal cells. Photosystem II P680 chlorophyll A apoprotein, encoded by gene *psb*B was decreased. The genes *rbc*L and *CAH*2, which are mainly involved in carbon fixation for photosynthesis and chloroplast gene *atp*B is involved in synthesis of ATP were also very much decreased. Expression of gene *cox*2 encoding cytochrome *c* (Cyt *c*) oxidase subunit II was lowered which indicated the impairment of mitochondrial respiration. It has been suggested that CNTs altered electron transfer of Cyt *c* which derogated mitochondrial respiration [95].

5. Conclusion:

As now-a-days, nanomaterials have been used widely in different fields, their existence is a threat to the living organisms residing in water bodies. NPs effect algal bodies in different manner and are considerably toxic to them. Different studies have been held to study the transportation and uptake mechanism of NPs by algae and the phenomenon involved in the toxicity of NPs to algae.

However, there is not enough clarification about the toxicity mechanism from the solubilization of bioaccumulated metallic NPs which need to be performed with new advanced analytical technologies. There is also need of understanding of transportation of NPs into cytoplasm and other cell organelle.

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