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Toxicity of lanthanum oxide (La_2O_3) nanoparticles in aquatic environment

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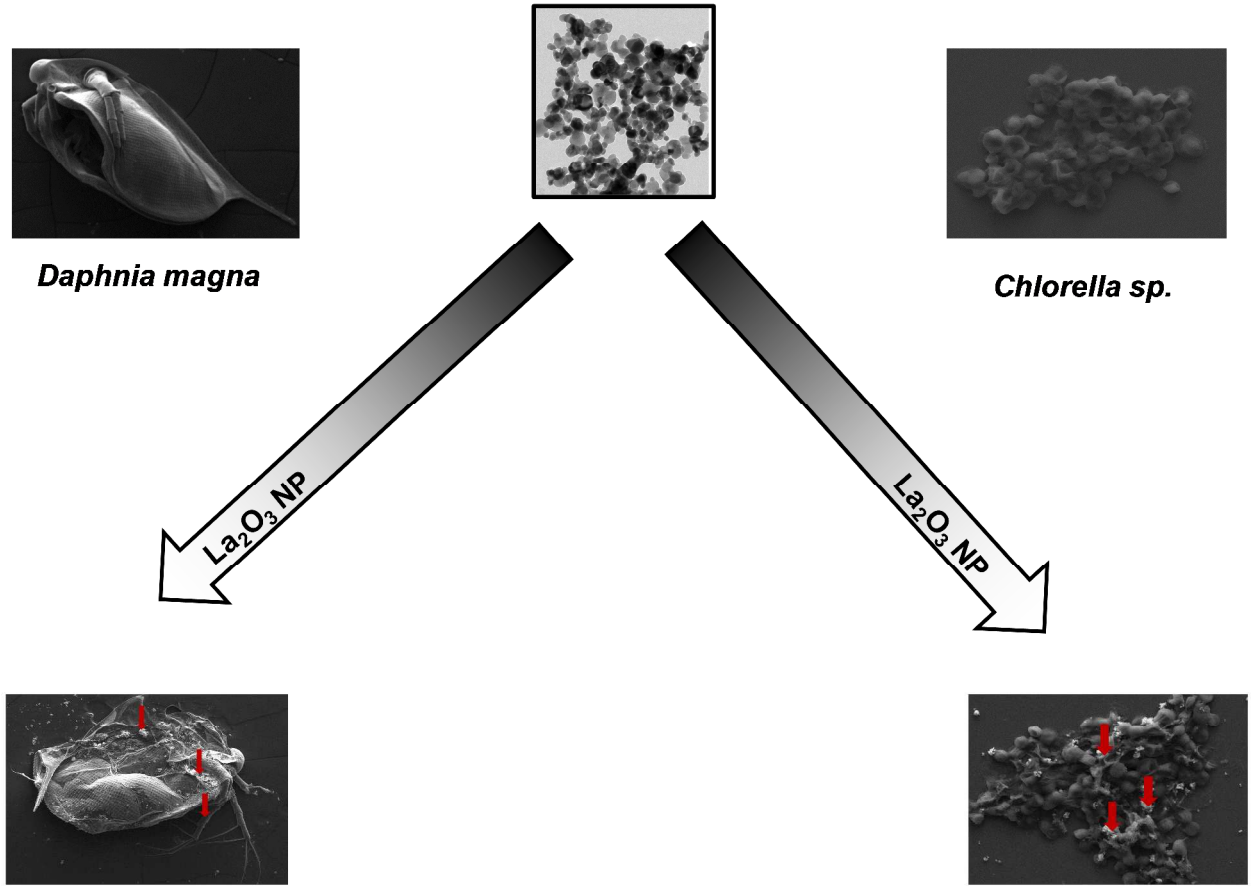
Graphical Abstract

The study demonstrated the ecotoxic potential of lanthanum oxide nanoparticles on fresh water aquatic microalgae *Chlorella sp.,* and crustacean *Daphnia magna*. The lanthanum oxide nanoparticles show nil toxic effect to algae and where as severe toxic effect observed towards crustacean. The results may play vital role in the risk assessment process for exposure of lanthanum oxide nanoparticles in aquatic environment.



Toxicity of lanthanum oxide (La_2O_3) nanoparticles in aquatic environment

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Environmental Impact Statement

Nanomaterials attracted significant concern on their adverse effect on the aquatic organisms due to mass production and applications. Among Rare earth elements, lanthanum oxide nanoparticles are started to explore their use in various applications. The result presented here demonstrates the enhanced growth of *Chlorella sp*., with lanthanum oxide nanoparticles exposure. On the other hand, lanthanum oxide nanoparticles caused severe toxicity effects to *Daphnia magna* including mortality. These observations demonstrate the toxic effects of lanthanum oxide nanoparticles upon the release into aquatic environment.

Environmental Science: Processes & Impacts

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Various applications have evolved by the consumption of rare earth elements, instigating the need for a detailed study on them and their consequent effects on the environment. In particular, this study demonstrates the acute toxicity of lanthanum oxide nanoparticles (La_2O_3 NP) on two sentinel aquatic species, fresh water microalgae *Chlorella sp*., and crustacean *Daphnia magna*. The morphology, size and charge of the nanoparticles have been systematically studied. The algal growth inhibition assay, thus confirms nil toxic effect of La_2O_3 NP on *Chlorella sp*., even at higher concentrations of 1000 mg/L, following 72 h exposure. Similarly, no significant toxic effects were observed on *D. magna* until the test concentration 250 mg/L and considerable effects were noted in further concentrations (effective concentration (EC_{50}) 500 mg/L; lethal dose (LD_{50}) 1000 mg/L). In addition, attachment of La_2O_3 NP on aquatic species was demonstrated using microscopy analysis. This study proved to be beneficial in understanding the acute toxicity providing environmental protection as part of the risk assessment strategy.

1. Introduction

The past few decades have seen the extensive usage of nanoparticles (NP) in many industrial and house hold applications including sunscreens, cosmetics, paints and construction materials.[1-4] As a result, aquatic environments are considered as vulnerable source of different NP release, for which their subsequent impacts have not been clearly defined.[5] Consequently, the inevitable releases have gained significant attention for their adverse effect on the environment and human health.[6-8] However, owing to the differential nature of these NP compared to soluble chemicals, minimum information has been derived on their interaction with aquatic organisms. It has been found that transformation, agglomerations and surface properties play a vital role in determining their toxicological and bioavailability properties, once they are released into the aquatic environment.

Numerous studies on the ecotoxicity of NP have been displayed using a variety of algae, microorganisms, invertebrates and fishes as model aquatic organisms.[9-14] However, the underlying ecotoxicological effect on different aquatic organisms still remains unclear, creating a significant data gap. *Daphnia magna* and *Chlorella sp.,* are considered to be excellent biomonitoring aquatic species owing to their critical role in the aquatic food chain and their sensitive nature towards various pollutants.[15-16] The use of *D. magna* and microalgae for ecotoxicological studies have been highly recommended in various standard regulatory guidelines.[17-20] Until now, many studies were carried out on these species to evaluate the toxicity potential of NP.[21-29]

Due to the unique chemical nature and exceptional catalytic, magnetic and electronic properties, the rare earth elements (REE) have been prolifically used from various industries to biotechnology applications.[30,31] In addition, these elements are considered as industrial vitamins. Among the different REE, lanthanum oxide nanoparticles (La_2O_3 NP) have been exploited for their utilization in sensors, electronics, fuel cells, magnetic data storage, antimicrobial, catalysis, automobiles, water treatment, phosphate removal and biomedicine.[32-34] Because of their growing application, specific interest on studying their biological effects on the environment and human health has come to the limelight.

As there exists a void on the information about La_2O_3 NP and its impact on the aquatic biota, the aim of present study was to evaluate the toxicity of La_2O_3 NP towards aquatic organisms *Chlorella sp.,* and *D. magna*. Thus, investigations on the effect of La_2O_3 NP on behavioral change and ecotoxicity, continued by effective concentration (EC_{50}) and lethal dose (LD_{50}) values have been determined. Furthermore, the attachment and accumulation of La_2O_3 NP in the aquatic organisms have been investigated using optical microscopy (OM) and scanning electron microscopy (SEM).

2. Materials and Methods

2.1 Nanoparticle characterization

The La_2O_3 NP used in this study was gifted from CECRI,

Karaikudi, Tamilnadu, India. The Transmission electron microscopy (TEM, Tecnai G2 F30, FEI) was used to determine the morphology and chemical composition. The mean particle size and surface charge of La_2O_3 NP were studied by using Zeta sizer (Nano ZS, Malvern) in test medium (ISO test medium (pH 7.6) and BG 11 medium (pH 7.5)). X-Ray Diffraction (XRD) patterns of La_2O_3 NP were obtained using PANalytical X'Pert Multi Purpose X-ray Diffractometer with Cu Kα radiation. The surface composition of La_2O_3 NP was studied by X-ray photoelectron spectroscopy (XPS, Thermoscientific, K-alpha).

2.2. Algal growth inhibition assay

2.2.1 Test species and culture conditions

Firstly, isolation of the green algae *Chlorella sp*., from water supply was done at Sorgun, Yozgat, Turkey.[35] The medium BG 11 was used to conduct the algal growth inhibition assay based on OECD 201.[20,36] The microalgal cultures were inoculated at 0.1 g/L dry weight biomass and the flasks were illuminated by cool-white fluorescent lamps at 25 µmol/m^2s (1750 lx) light intensity at 25 ± 2 °C with 100 r/min.

2.2.2 Treatment and analytical methods

Exponentially growing algal cells were propagated in Erlenmeyer flasks containing La_2O_3 NP at 10, 50, 100, 250, 500 and 1000 mg/L of BG11 medium. In addition, control was prepared by having flasks without La_2O_3 NP. All experiments were carried out twice in triplicates. The flasks were maintained at 25 ± 2 °C under continuous illumination in a shaker (100 r/min). While exposed to various concentrations of La_2O_3 NP, the growth of *Chlorella sp*., was monitored by measuring the optic density, dry weight and specific growth rate parameters for the samples collected at 0, 24 and 72 h. At the end of the study, colony count was taken into account to elucidate the toxicities of different treatments involved.

The optical density was calculated with a Shimadzu UV 1800 model spectrophotometer at 600 nm. Microalgae was centrifuged at 3421 × g = 5000 rpm for 10 min (Hettich Universal 320 R model centrifuge), subsequent pellets were collected and dried at 80 °C overnight at MMM-MedCentre Ecocell model sterilizer, in order to preserve their dry weights. Maximum biomass productivity (Pmax) was calculated according to the equation,

$$Pmax = (X - X_0)/(t - t_0) \quad (1)$$

where X is the final and X_0 is the initial biomass concentrations (g/L), t is the final and t_0 is the initial time of the culture. Specific growth rate (µmax) was calculated according to the equation,

$$\mu max = (\ln X_2 - \ln X_1)/(t_2 - t_1) \quad (2)$$

X_2 and X_1 are the dry cell weight concentrations (g/L) at time t_2 and t_1, respectively.[37] The concentration for chlorophyll was obtained at 646.6 nm and 663.6 nm for Chlorphyll a and b, respectively.[38] The procedures of SEM (Quanta 200 FEG, FEI) and OM were used to observe and image the attachment of microalgae with La_2O_3 NP. Before SEM observation and Energy Dispersive Spectroscopy (EDS) mapping, a drop from the 1000 mg/L culture solution was air dried on a copper stage with subsequent coating with a layer of gold to confirm the attachment of La_2O_3 NP with the microalgae. Similarly, a drop of dried culture solution on a clean glass slide was used for OM observation.

2.3 Acute immobilization test

2.3.1 Test species and culture conditions

We have used neonates of fresh water flea *D. magna* as test species in this study. The daphnids were maintained at a constant temperature of 20 ± 1°C and a photo-period of 16:8 h light:dark cycle. The daphnids were fed with suspensions of green algae (*Chlorella sp*.,).

2.3.2 Treatment

The acute immobilization test was conducted based on OECD 202 guideline.[19] Different concentrations of La_2O_3 NP (0, 25, 50, 100, 250, 500 and 1000 mg/L) were prepared in ISO test medium and allowed for a 48 h exposure to determine the sensitivity of *D. magna*. A total of 20 daphnids were divided in four replicates for each concentration tested. Following the 24 and 48 h exposures, daphnids were studied for their immobilization effects, with simultaneous comparison with the control. The experiment was repeated to ensure the consistency of the results. The pH of the culture medium was under check throughout the experiment. The change in morphology, La_2O_3 NP attachment in exterior surface and accumulation in intestinal tract of *D. magna* were examined using SEM and OM techniques.

3. Results and Discussion

3.1 Nanoparticle characterization

The TEM image shows that the particles are irregular spheres and less than 100 nm in size (Figure 1). Further, the EDS spectra confirm the presence of lanthanum and oxygen at 61.96% and 38.03%, respectively (Figure S1). The results of the Zeta sizer reveal the mean particle size of the La_2O_3 NP is 59 nm and 61 nm in ISO test medium and BG 11 medium, respectively (Figure S2). Similarly, the zeta potential value is 14.5 mV in ISO test medium and 14.9 mV in BG 11 medium. No significant differences in the diameter and surface charge were observed in test medium at different pH.

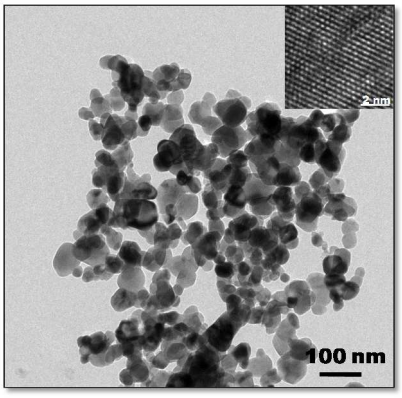

Figure 1: TEM image of La_2O_3 NP. The particles are irregular spheres in shape and less than 100 nm. Inset shows the lattice spacing (0.348 nm).

The XRD patterns of La_2O_3 NP are shown in Figure S3. The diffraction peaks are consistent with the values of standard card JCPDS file 65-3185.The surface composition of La_2O_3 was investigated by using XPS analysis. The survey spectrum confirms there are no other metal element impurities present in the surface of the sample except lanthanum (Figure S4a). The La 3d core level spectrum is shown in Figure S4b. The deconvoluted spectra show a two peaks separated by ~4eV. The La 3d core level spectrum is shown in Figure S4b. The deconvoluted spectra show a two peaks separated by ~4eV. The deconvoluted O1*s* spectrum having three peaks (526.6 eV, 529.3 eV and 531.6 eV) as shown in Figure S4c. These peaks are associated with chemical bonding state of O–La–O (O_L) and hydrated phases from air exposure (O_H).[39]

3.2 Algal growth inhibition assay

The effect of La_2O_3 NP on the growth parameters of *Chlorella sp*., were studied and analyzed under 24 and 72 h of incubation periods. Initially, during the 24 h observation, it has been found that with increasing nanoparticles concentrations, microalgal growth decreased. The highest growth was attained from the control culture at 0.133 g/L dry weight of microalgal biomass in 24 h. In addition, nanoparticle concentration at 10 mg/L showed nil toxic effect on *Chlorella sp.,* with the microalgae reaching 0.130 g/L biomass at this concentration. At 1000 mg/L nanoparticle concentration, lowest biomass has been obtained as 0.057 g/L (Table 1).

Enhanced growth of *Chlorella sp.,* was observed with increasing nanoparticle concentrations at 72 h of incubation period. All of the treated culture showed higher microalgal growth than the control culture. The maximum growth achieved by the control culture reached only 0.237 g/L, whereas the culture containing 500 mg/L nanoparticle attained 1.5 times higher biomass rate than control culture (Figure 2). Thus, it was apparent that increasing nanoparticle concentrations did not exhibit any toxic effect on the growth of *Chlorella sp.*

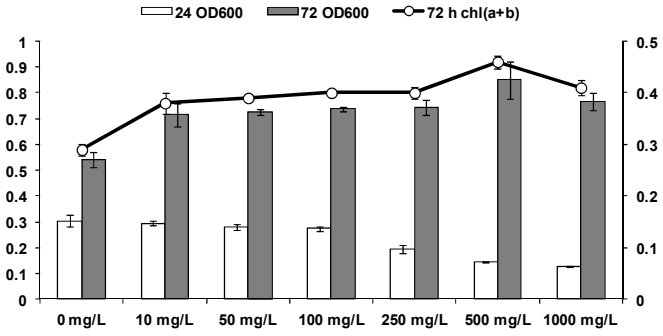

Figure 2: Interactive effect on optical density (OD_{600}) and chlorophyll content of *Chlorella sp.,* with La_2O_3 NP during the incubation period (24 and 72 h). The diagram represents the algal growth inhibition at 24 h exposure and growth enhancement observed at 72 h. The results are the mean value of triplicate cultures.

In addition, chlorophyll (a+b) concentrations of *Chlorella sp.,* were also evaluated for 24 and 72 h. Following 72 h exposure, maximum chlorophyll (a+b) concentration was found to be 0.46 µg/mL at 500 mg/L and this was found to be 13 times higher than the concentration of inoculated culture at 0 h (Figure 2). The visual observation of enhanced chlorophyll content production is presented in Figure S5.

The calculated µmax, Pmax and colony counts are presented in Table 1. As anticipated, 72 h values of maximum specific growth rate were lower than the 24 h values owing to the incubation time. Under the effect of La_2O_3 NP, maximum specific growth rate was obtained as 1.339 at 10 mg/L concentration in 24 h. The obtained value was found to be nearly close to the value of control culture (Table 1). Pmax was obtained at 0.116 at 500 mg/L in 72 h exposure. Similarly, the La_2O_3 NP exposure increased the viability of algal cells at the end of study. Since no significant toxicity was observed under illumination, shading effect of La_2O_3 NP on algal growth was considered unnecessary. Using OM and SEM, the attachment of microalgae with La_2O_3 NP were precisely demonstrated (Figure S6). Further, EDS mapping of the treated *Chlorella sp.,* confirmed the attachment of lanthanum on the surface of the microalgae without causing any morphological changes (Figure 3). The overall comparisons of chlorophyll and biomass production with La_2O_3 NP exposure over the control culture are presented in Figure S7.

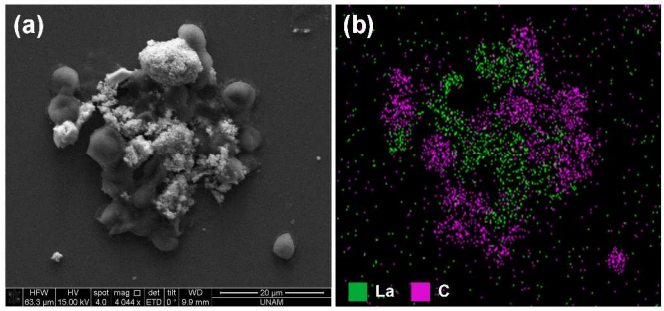

Figure 3: (a) SEM image of *Chlorella sp.,* following exposure to La_2O_3 NP (72 h; 1000 mg/L).(b) EDS dot map of corresponding SEM image. It shows the distribution of lanthanum and the attachment with *Chlorella sp.,* without any morphological changes.

3.3 Acute immobilization test

It was found that immobilization of *D. magna* following 48 h exposure to various concentrations of La_2O_3 NP is concentration dependent. The results of immobilization upon exposure to La_2O_3 NP are presented in Figure 4. The No observed effect level (NOEL) and Low observed effect level (LOEL) was calculated to be 25 mg/L and 50 mg/L, respectively. The EC_{50} value of La_2O_3 NP against *D. magna* has been found to be 500 mg/L. Also, about 70 percentage of mortality occurred in the daphnids when treated at 1000 mg/L concentrations after 48 h exposure. This has given rise to the LC_{50} concentration as 1000 mg/L. The pH was in the range of 7 - 8 throughout the experiment.

At higher concentrations, ingestion of La_2O_3 NP was observed in the daphnids towards 48 h exposure. The OM images show no

Parameters	0 mg/L	10 mg/L	50 mg/L	100 mg/L	250 mg/L	500 mg/L	1000 mg/L
Dry weight (g/L)*	0.133 ± 0.01	0.130 ± 0.004	0.124 ± 0.005	0.120 ± 0.003	0.085 ± 0.007	0.064 ± 0.001	0.057 ± 0.001
Pmax*	0.108 ± 0.01	0.105 ± 0.004	0.099 ± 0.005	0.095 ± 0.003	0.060 ± 0.007	0.039 ±0 .001	0.032 ± 0.001
µmax*	1.340 ± 0.003	1.339 ± 0.001	1.338 ± 0.001	1.337 ± 0.001	1.328 ± 0.002	1.322 ± 0.001	1.320 ± 0.001
Chlorophyll(µg/mL)*	0.163 ± 0.012	0.159 ± 0.005	0.152 ± 0.062	0.147 ± 0.037	0.104 ± 0.087	0.078 ± 0.012	0.070 ± 0.012
Dry weight (g/L)**	0.237 ± 0.01	0.314 ± 0.016	0.317 ± 0.004	0.323 ± 0.003	0.326 ± 0.01	0.373 ± 0.052	0.335 ± 0.012
Pmax**	0.071 ± 0.01	0.096 ± 0.016	0.097 ± 0.004	0.099 ± 0.003	0.100 ± 0.010	0.116 ± 0.050	0.103 ± 0.012
µmax**	0.455 ± 0.001	0.462 ± 0.001	0.462 ± 0.000	0.463 ± 0.000	0.463 ± 0.001	0.467 ± 0.004	0.464 ± 0.001
CFU(10^7cells/mL)**	1.6 ± 0.040	2.2 ± 0.050	2.1 ± 0.070	2.6 ± 0.300	2.7 ± 0.090	3.3 ± 0.100	2.9 ± 0.500

Table 1: La_2O_3 NP effect on microalgae growth parameters during 24 h and 72 h exposure. * denotes the observation at 24 h whereas ** denotes the findings at 72 h (Values are expressed as mean ± standard deviation)

accumulation of particles in the intestinal tract of control *D. magna* whereas significant accumulation of La_2O_3 NP has been seen at 1000 mg/L (Figure S8). Further, the SEM images also affirm the no change in the morphology was observed at 0 mg/L and whereas severe damage of daphnid was observed at 1000 mg/L treatment (Figure 5). Interestingly, the images denote the attachment of La_2O_3 NP on the body surface of *D. magna* including their antenna, used for mobilization of the organism. In addition, the attachment of La_2O_3 NP further confirmed by EDS dot map which demonstrates the distribution of lanthanum and Oxygen (Figure S9).

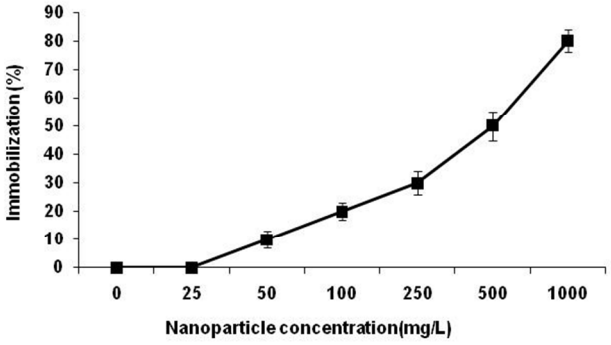

Figure 4: Effect of La_2O_3 NP on the mobilization nature of *D. magna* following 48 h exposure. The response curve shows the immobilization percentage is concentration dependent.

REE including lanthanum have an extensive application as micro fertilizers in agricultural fields, due to their capability to enhance growth and productivity.[40-43] REE usage has significantly increased the chlorophyll content and production of the spinach plant.[44] The treatment of lanthanum at 12 mg/L significantly increased the germination rate, germination index and vigour index in sorghum.[45] Lanthanum supported the abscisic acid regulation and enhanced the root growth of *Arabidopsis*.[46] *Chlorella sp.,* belonging to the phylum Chlorophyta and considered as eukaryotic photosynthesizers, contains chloroplasts, growth regulators (auxins, cytokinins, gibberellins, abscisic acid and brassino steroids) similar to plants.[47] Regulation on these enzymes also promotes the growth of microalgae. Myers reported that trace metals at minimum concentrations can provide nutrients and in higher concentrations, they initiate interaction with proteins and affect enzymatic activities, leading to toxic effects.[48] Additionally, it is speculated that Lanthanide ions can also serve as an isomorphic replacement for Ca^{2+} in the

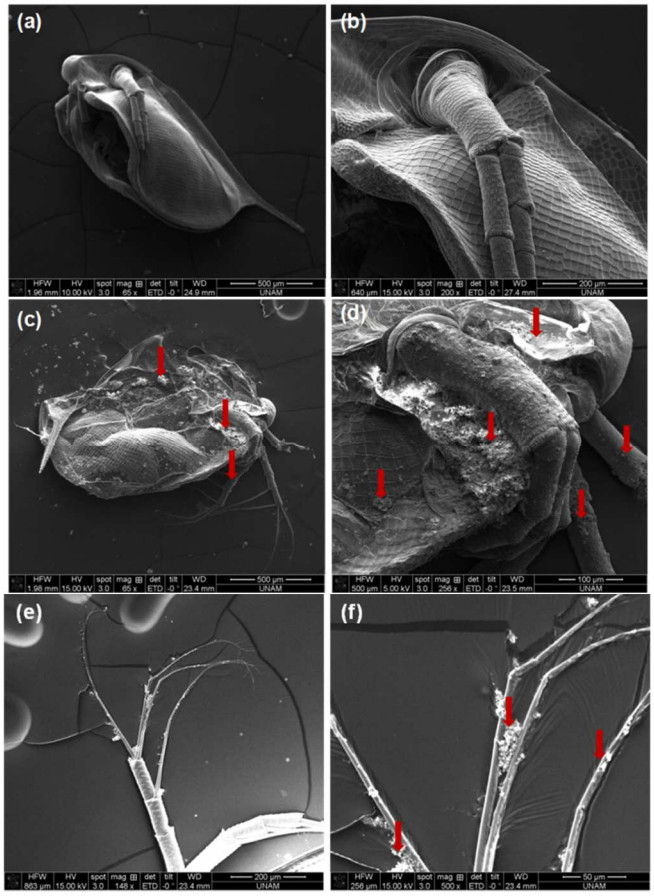

Figure 5: SEM image of *D. magna* (a&b) without La_2O_3 NP exposure, which shows no morphological changes. (c-f) treated with La_2O_3 NP (1000 mg/L) for 48 h. The images clearly illustrate the change in morphology, adhesion of particles on the body surface and antenna. The red arrows indicate attachment of La_2O_3 NP.

biochemical systems.[49] Thus, similar to trace elements, lanthanum also served as a nutrient to algae and enhanced their growth. The microalgae *chlorella sp.,* may be used for metabolic phenomenon to increase their productivity like any other plant.

The initial inhibition observed during growth has been found to be based on the toxic effects produced by La_2O_3 NP. With prolonged exposure, microalgae have found to grow resistant,

utilizing La_2O_3 NP for enhanced growth. At higher concentrations, nanoparticles formed aggregates, wrapping the algal cells around, contributing to growth inhibition. It is also speculated that, La_2O_3 NP are well known for inhibiting broad range of microorganisms growth by competing available phosphate in the media. Hence, La_2O_3 NP restricted the availability of phosphates at the higher concentration and lead to microalgal growth inhibition.[50] These phenomena serve as the basis for the observed decrease in growth and biomass production at higher concentration (1000 mg/L). Hence, the lanthanide ions are considered responsible for enhanced growth with fresh water microalgae. Secondly, the regulation mechanism of lanthanum on the enzymes of *Chlorella sp*., has also arisen as the reason for growth enhancement. Further, the attachment of La_2O_3 NP on microalgal cells could be attributed to the electrostatic interaction between the positively charged nanoparticles and negatively charged cell wall of algae.[51,52] Electrostatic interaction of positively charged nanoparticles with different microorganisms and their effects are well reported.[53-56]

Remarkable feeding behavior of *D. magna* indicates the ingestion and potential toxicity of NP. Mendonca et al., demonstrated the effect of ingested NP on their gut cells.[57] In our study too, it is expected that the ingested La_2O_3 NP might get mixed with food and interfere in intestinal adsorption at higher concentrations. In cases of chronic exposures, accumulation has been noted at lower concentrations. Moreover, La_2O_3 NP are positively charged and known to adhere to the negatively charged biological molecules. Balusamy et al., has emphasized that the bacterial toxicity against interaction of *S. aureus* is based on the electrostatic interaction between the NP and the negatively charged cell wall content.[32] This statement also aligns in agreement with the OECD Draft Guidance Document, which states that hydrophobic substances are highly capable of getting attracted to the negatively charged biological materials.[58] In addition, it should be noted that La_2O_3 are well known for production of free radical among different rare earth elements and their effect on hepatic nuclei and mitochondria were reported.[59,60] Accordingly, we hypothesize the observed toxicity against *D. magna* has resulted from either mechanical disruption in feeding and carapace attachment of La_2O_3 NP, leading to eventual immobilization and mortality or due to production of reactive oxygen species (ROS), especially at higher concentrations. Again, this has also been in agreement with the findings of Asghari et al.,[61] Likewise, the experiments were conducted in the shaking platform, have been found to be highly relevant to environmental conditions, considering the natural water flows in the aquatic environments.

4. Conclusion

To conclude, our research highlighted the La_2O_3 NP treatment with *Chlorella sp*., emphasizing the absence of significant toxic effects, but enhanced growth rate and biomass production. On the contrary, the 48 h exposure acute toxicity test resulted in significant toxicity at concentrations 500 and 1000 mg/L on *D. magna*. The EC_{50} and LD_{50} values of La_2O_3 NP in acute immobilization test have been determined as 500 and 1000 mg/L, respectively. However, the observed toxicity effects of La_2O_3 NP concentrations have been found to be much higher than the regulatory recommendations. Therefore, the use of La_2O_3 NP in the consumer products can be considered safe. But, the release of La_2O_3 NP requires more attention at higher exposure levels since it has direct adverse effect on the environment. However, further research is needed to discover the appropriate biological phenomenon against toxicity and initiate the risk assessment process.

Acknowledgements

B.B thanks the Scientific & Technological Research Council of Turkey (TÜBITAK) (TÜBITAK-BIDEB 2216, Research Fellowship Programme for Foreign Citizens) for postdoctoral fellowship. B.B. also thanks Dr. L. John Berchmans, CECRI, India for providing La_2O_3 NP as gift. B.E.T and T.T. are grateful to the Scientific and Technological Research Council of Turkey (TÜBITAK) and ALGELA Biotechnology Ltd. Company for financial support.

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† Electronic Supplementary Information (ESI) available: Nanoparticle characterization, Optical microscopy of *Chlorella sp., D. Magna* and additional figures. See DOI: 10.1039/b000000x/

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