

Understanding the Effect of Mixture of Metal Oxide Nanoparticles to Algae-Bacteria Consortia

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by

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Dedication

This thesis is dedicated to my beloved

Parents...Mumma and Papa

And

To my brother

for their endless love, support, and encouragement...

CERTIFICATE

This is to certify that the thesis entitled “**Understanding the Effect of Mixture of Metal Oxide Nanoparticles to Algae-Bacteria Consortia**” submitted by **Samridhi Rana** to the Indian Institute of Technology, Delhi for the award of the degree of **Doctor of Philosophy** is a bonafide record of research work carried out by her under my supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

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ABSTRACT

Due to the excessive use of nanoparticles in various fields since last decade, the concentration of nanoparticles has been increasing rapidly in the aquatic environment. In the aquatic ecosystem symbiotic relation of algae-bacteria consortia can be seen and they are responsible for maintaining the basic functioning of the aquatic ecosystem. Due to the presence of nanoparticles, therefore the possibility of interaction of algae-bacteria consortia with nanoparticles would be increased. Therefore, this research was carried out to understand the effect of metal oxide nanoparticles on algae/algae-bacteria consortia and the impact of nanoparticles on the functioning of algae-bacteria consortia.

The effect of single and mixture of nanoparticles on the algae-bacteria consortia was studied theoretically to understand the interaction mechanism. Algal Species, *Scenedesmus obliquus* and bacterial species *Escherichia coli* were used in the study as test organisms. The metal oxide nanoparticles, ZnO and CuO, were used in single and mixture form at various concentrations (0.1, 1, 10 and 100 mg/L) to understand the effect of the nanoparticles on the consortia. To understand the toxicity mechanism of nanoparticles, the long-term effect of mixture of nanoparticles on algae/algae-bacteria consortia was also evaluated in this study. The effect of time on the stability of media, nanoparticles, and their relation to toxicity to algae was also studied. It was observed that the inhibition time for biomass was observed between 14 days and 21 days at 10 mg/L NPs. At 1 mg/L, the order of toxicity of NPs to algae was found to be: CuO NPs (highest toxicity) > ZnO NPs > ZnO + CuO NPs (least toxicity). During exposure of algae cells to a mixture of NPs at 10 mg/L NP concentration, a smaller value of metal deposition was observed than that during exposure to individual NPs. Antagonistic toxic effects of two NPs on dry cell weight of algae were observed at both concentration levels. The usage of ZnO and CuO NPs is increasing tremendously, and 0.1 mg/L would be more realistic concentration of NPs in the realistic aquatic environment or wastewater. To understand the toxicity of mixture of nanoparticles on the algae in the realistic scenario, the wastewater and the pond water was collected and used in the present study. It was observed that at 0.1 mg/L concentration of the mixture of NPs, the reduction in the chlorophyll a content was $13.61 \pm 1.34\%$ (OECD media), $28.83 \pm 1.85\%$ (wastewater), and $31.81 \pm 2.23\%$ (pond water). Values of reduction in biomass were observed to be 42.13 ± 1.38 , 39.96 ± 1.03 , and $33.10 \pm 1.29\%$ for OECD media, wastewater, and pond water, respectively. The overall toxicity trend observed was Wastewater > OECD

media>Pond water in single and mixture of nanoparticles. For the consortia studies, 3 algae-bacteria ratios were considered (1:100, 100:1, and 1:1) and effect of observed in single as well as in mixture by analyzing various growth parameters such as Chl a, biomass, lipid, etc. and functioning was observed by observing the nutrient uptake efficiency. It was observed that at environmentally relevant NP concentration (0.1 mg/L), the order of toxicity of NPs to algae-bacteria consortia was found to be: CuO NPs (highest toxicity)>ZnO+CuO NPs>ZnO NPs (least toxicity).

Overall, this study helps in understanding the interaction between algae-bacteria consortia with single and mixture of nanoparticles. This study would help in understanding the effect of NPs on functioning and removal efficiency of algae-bacteria consortia for wastewater treatment and biomass production.

Keywords: *Mixture of nanoparticles; algae-bacteria consortia; long-term effect; toxicity*

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सार

पिछले दशक से विभिन्न क्षेत्रों में नैनोकणों के अत्यधिक उपयोग के कारण जलीय वातावरण में नैनोकणों की सांद्रता तेजी से बढ़ रही है। जलीय पारिस्थितिकी तंत्र में शैवाल-जीवाणु संघ का सहजीवी संबंध देखा जा सकता है और वे जलीय पारिस्थितिकी तंत्र की बुनियादी कार्यप्रणाली को बनाए रखने के लिए जिम्मेदार हैं। नैनोकणों की उपस्थिति के कारण, नैनोकणों के साथ शैवाल-बैक्टीरिया कंसोर्टिया की परस्पर क्रिया की संभावना बढ़ जाएगी। इसलिए, शैवाल/शैवाल-बैक्टीरिया कंसोर्टिया पर धातु ऑक्साइड नैनोकणों के प्रभाव और शैवाल-बैक्टीरिया कंसोर्टिया के कामकाज पर नैनोकणों के प्रभाव को समझने के लिए यह शोध किया गया था।

अंतःक्रिया तंत्र को समझने के लिए शैवाल-बैक्टीरिया कंसोर्टिया पर एकल और नैनोकणों के मिश्रण के प्रभाव का सैद्धांतिक रूप से अध्ययन किया गया था। अध्ययन में परीक्षण जीवों के रूप में शैवाल प्रजाति, स्केनेडेसमस ओब्लिकस और जीवाणु प्रजाति एस्चेरिचिया कोली का उपयोग किया गया था। कंसोर्टिया पर नैनोकणों के प्रभाव को समझने के लिए धातु ऑक्साइड नैनोकणों, ZnO और CuO का उपयोग एकल और मिश्रण रूप में विभिन्न सांद्रता (0.1, 1, 10 और 100 mg/L) पर किया गया था। नैनोकणों की विषाक्तता तंत्र को समझने के लिए, इस अध्ययन में शैवाल/शैवाल-बैक्टीरिया कंसोर्टिया पर नैनोकणों के मिश्रण के दीर्घकालिक प्रभाव का भी मूल्यांकन किया गया था। मीडिया, नैनोकणों की स्थिरता पर समय के प्रभाव और शैवाल की विषाक्तता से उनके संबंध का भी अध्ययन किया गया। यह देखा गया कि बायोमास के लिए अवरोध का समय 10 मिलीग्राम/एल एनपी पर 14 दिनों से 21 दिनों के बीच देखा गया। 1 मिलीग्राम/एल पर, शैवाल के लिए एनपी की विषाक्तता का क्रम पाया गया: CuO NPs (उच्चतम विषाक्तता) > ZnO NPs > ZnO + CuO NPs (न्यूनतम विषाक्तता)। 10 मिलीग्राम/एल एनपी सांद्रता पर एनपी के मिश्रण में शैवाल कोशिकाओं के संपर्क के दौरान, व्यक्तिगत एनपी के संपर्क में आने की तुलना में धातु जमाव का कम मूल्य देखा गया। शैवाल के शुष्क कोशिका भार पर दो एनपी के विरोधी विषाक्त प्रभाव दोनों एकाग्रता स्तरों पर देखे गए।

यथार्थवादी परिदृश्य में शैवाल पर नैनोकणों के मिश्रण की विषाक्तता को समझने के लिए, अपशिष्ट जल और तालाब के पानी को एकत्र किया गया और वर्तमान अध्ययन में उपयोग किया गया। यह देखा गया कि एनपी के मिश्रण की 0.1 मिलीग्राम/एल सांद्रता पर, क्लोरोफिल सामग्री में कमी $13.61 \pm 1.34\%$ (ओईसीडी मीडिया), $28.83 \pm 1.85\%$ (अपशिष्ट जल), और $31.81 \pm 2.23\%$ (तालाब का पानी) थी। ओईसीडी मीडिया, अपशिष्ट जल और तालाब के पानी के लिए बायोमास में कमी का मान क्रमशः 42.13 ± 1.38 , 39.96 ± 1.03 और $33.10 \pm 1.29\%$ देखा गया। देखी गई समग्र विषाक्तता प्रवृत्ति अपशिष्ट जल > ओईसीडी मीडिया > एकल में तालाब का पानी और नैनोकणों का मिश्रण थी। कंसोर्टिया अध्ययन के लिए, 3 शैवाल-बैक्टीरिया अनुपातों पर विचार किया गया (1:100, 100:1, और 1:1) और सीएचएल, बायोमास, लिपिड जैसे विभिन्न विकास मापदंडों का विश्लेषण करके एकल और मिश्रण में प्रभाव देखा गया।, आदि और पोषक तत्व ग्रहण क्षमता को देखकर कार्यप्रणाली देखी गई। यह देखा गया कि पर्यावरणीय रूप से प्रासंगिक एनपी सांद्रता (0.1 मिलीग्राम/एल) पर, शैवाल-बैक्टीरिया कंसोर्टिया के लिए एनपी की विषाक्तता का क्रम पाया गया: CuO NPs (उच्चतम विषाक्तता) > ZnO + CuO NPs > ZnO NPs (न्यूनतम विषाक्तता)।

कुल मिलाकर, यह अध्ययन एकल और नैनोकणों के मिश्रण के साथ शैवाल-बैक्टीरिया कंसोर्टिया के बीच बातचीत को समझने में मदद करता है। यह अध्ययन अपशिष्ट जल उपचार और बायोमास उत्पादन के लिए शैवाल-बैक्टीरिया कंसोर्टिया के कामकाज और हटाने की दक्षता पर एनपी के प्रभाव को समझने में मदद करेगा।

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List of Abbreviations

NP	Nanoparticle
NPs	Nanoparticles
HDD	Hydrodynamic diameter(nm)
CuO NP	Copper Oxide Nanoparticles
ZnO NP	Zinc Oxide Nanoparticles
<i>S. obliquus</i>	<i>Scendesmus obliquus</i>
<i>E. coli</i>	<i>Escherichia coli</i>
OECD	Organization for Economic Cooperation and Development
BG-11	Blue Green-11
EPS	Extracellular polymeric substances
ROS	Reacting oxygen species
NT	Nanotubes
Chl a	Chlorophyll a
Chl b	Chlorophyll b
TEM	Transmission Electron Microscopy
EC50	Half Maximal Effective Concentration
COD	Chemical Oxygen Demand
BOD	Biological Oxygen Demand
TDS	Total Dissolved Solids
DOC	Dissolved Oxygen Carbon
CAT	Catalase
FTIR	Fourier Transform Infrared
%GR	Growth Reduction
DLS	Dynamic Light Scattering
EPA	Environmental Protection Agency
AWWA	American Water Work Association
APHA	American Public Health Association
POC	Aromatic Phosphates
NCIM	National Collection of Industrial Microorganisms
MTCC	Microbial Type Culture Collection