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ORIGINAL ARTICLE

Phytotoxic effects of seaweed mediated copper nanoparticles against the harmful alga: *Lyngbya majuscula*

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Abstract In this study, copper nanoparticles (Cu-NPs) were synthesized using *Corallina officinalis* Linnaeus and *Corallina mediterranea* Areschoug aqueous extracts. Transmission Electron microscope indicated that the biosynthesized Cu-NPs averaged 12.7 nm and 13.6 nm for *C. Officinalis* and *C. mediterranea*, respectively. As reported by the FT-IR analyses, the algal extracts contain phyto-chemicals such as proteins, carboxylic acids, complex carbohydrates; these compounds will act as encapsulating agents and be reduced from copper sulphate to Cu-NPs. Energy-dispersive analyses X-ray (EDX) confirmed the copper composition in the synthesized Cu-NPs. The biosynthesized Cu-NPs arrested the growth of *Lyngbya majuscula* and presented in time and concentration dependent trends. At a concentration of 2 μg/mL, Cu-NPs, synthesized by *C. officinalis* exerted 85 ± 4% reduction of the algae dry weight. Increasing Cu-NPs concentration led to excellent reduction, which is a very promising result. Cupper-NPs synthesized by *C. mediterranea* produced moderate effects on *L. majuscula*. The results also indicated that there were sharp decreases in chlorophyll a content in *L. majuscula* with the increase in Cu-NPs concentrations. Using 4 μg/mL of Cu-NPs derived from *C. officinalis*, chlorophyll a decreased by 48 ± 5%. On the other hand, lower reductions in chlorophyll a were recorded upon using Cu-NPs synthesized using *C. mediterranea* (36 ± 3% and 41 ± 5% reductions at concentrations of 2 μg/mL and 4 μg/mL, respectively). The results of this study suggested that the bioactive and allelopathic compounds derived from the two algal extracts coating the (Cu^{2+}) together with (Cu^{2+}) are responsible for the inhibitive impacts of Cu-NPs on *L. majuscula*.

1. Introduction

Algae represent the principal level of the trophic chain and assume an important role in the equilibrium of aquatic ecosystems. The impact of algae in the ecosystem incorporates

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both its positive and negative [26]. Cyanobacterial blooms can have detrimental impacts on aquatic ecosystems through altering trophic structure and functionality [20], water column deoxygenation leading to fish mortalities, and decreasing water quality [37]. Different bloom-forming species are known to produce strong toxins that can impact terrestrial and water-based organisms [16,47].

The absolute, most normal common toxin produces cyanobacteria, the N_2-fixing genera including *Lyngbya*; in tropical and subtropic marine ecosystem worldwide [7]. *Lyngbya* blooms often bring about oxygen depletion and have caused significant economic effects, diminishing recreational and commercial fisheries, and decreasing the recreational utilization of an affected region [35].

Distinctive species of *Lyngbya* are able to produce toxins, which may have damaging impacts on the marine ecosystems [30]. *Lyngbya majuscula* is considered as one of the most common species of *Lyngbya* worldwide [7]. Severe blooms of *L. majuscula* will obstruct ambient light reaching the sea grass and other primary producers, with their consequent suppress [46].

In marine ecosystems both nitrogen and phosphorus are usually limiting elements for biological productivity. Both of the two elements can improve the exponential growth of *L. majuscula* [3]. An increase in the nutrients levels occurs in the system, the alga only needs warm temperatures and high ambient light to stimulate growth [23]. Copper sulphate is usually used to control algae in water bodies at recommended concentrations of 1–2 ppm. According to Ansari and Amjad [6], maximum *Chlorella vulgaris* growth was observed at 5 ppm Cu (II) and decreased at higher copper concentrations [6]. The toxic component of copper sulphate is the cupric ion (Cu^{2+}) as earlier revealed by Palmer [32]. The toxic effect of Cu^{+2} on *Rhodella reticulata* was defined as algae static [43]. Copper toxicity; has been reported for *Chlorella* sp. with the intent to prevent algal blooms in aquaculture [18]. Furthermore, the toxic effects of Cu (II) on *C. vulgaris* and its chloroplast were investigated [15]. Moreover, growth inhibition assay of *Chlorella kessleri* due to copper toxicity was recorded at 72-h growth in the shake flasks [39].

Control of algal blooms through biological techniques such as alteration of normal physiology including the decrease in photosynthetic pigments may be promising ways of ecological recovery [49]. Copper nanoparticles are showing broad spectrum activities. The experiments suggest the possibility to use this material in various fields such as water purification, air antibacterial packaging, and so forth [41]. Copper-based nanoparticles (Cu-NPs) are used in water treatment [40], and as bactericidal in replacement of nano-silver. The chemicals used in the synthesis of copper nanoparticles are commonly available, cheap, and nontoxic, although, toxic impacts of Cu-NPs on different criteria cells have been well documented [2]. Up to current knowledge, not many studies have been accomplished on the impact and potential of Cu-NPs on cyanobacteria [1].

Seaweeds are an important component of our surroundings and ecosystem. Seaweeds have been one of the richest and most promising sources of bioactive primary and secondary metabolite [12,17]. Red algae are the most important source of biologically active metabolites in comparison to other algal [29].

Therefore the main objective of current study is to investigate the possibility of inhibiting cyanobacterium; *L. majuscula* growth using *Corallina mediterranea* Areschoug and *Corallina officinalis* Linnaeus cu-NPs. Furthermore, phytotoxic effects of the biosynthesized Cu-NPs, on the growth of the harmful cyanobacterium; *L. majuscula* will be evaluated.

2. Materials and methods

2.1. Samples collection, biosynthesis and characterization of copper nanoparticles

The red seaweeds *C. officinalis* Linnaeus and *C. mediterranea* Areschoug samples were collected from Abu Qir Bay, Mediterranean Sea, Alexandria, Egypt, on December 2015. The algae were brought to laboratory and cleaned thoroughly with fresh water. Seaweeds were spread on blotting paper to remove excess water and shade dried. The algae are classified as Phylum: Rhodophyta; Order: Corallinales; Family: Corallinaceae [4]. Algal aqueous extract was prepared following the method of Aboud et al. [1], and then were used for the synthesis of Cu-NPs.

The precursor (1.0 mM $CuSO_4$ solution) was prepared using (169 mg L^{-1} in deionized distilled water). For the synthesis of copper nanoparticles, equal volumes of heated $CuSO_4$ solution and heated algal extracts at 1:1 ratio was mixed and stirred for 10 min and then heated at 80 °C for 45 min. Cu-NPs synthesis is indicated by the turning of light brown colour to pale green. The change in the colour of the reactants indicates the formation of Cu-NPs that was confirmed also by UV–vis spectroscopy analyses. The Cu-NPs colloid was centrifuged at 5000 rpm 15 min. The residue was washed in deionized distilled water (DDW), three times. Then, Cu-NPs were collected and stored for further characterization. The biosynthesized Cu-NPs were characterized using TEM and EDX to get details on morphological and structural composition of the particles. FT-IR spectral analyses were carried out to recognize the biomolecules in the algal extracts accountable for synthesis and capping of Cu-NPs. The spectra were reported in the range of 500–4000 cm^{-1}. The UV–visible spectrum was recorded over the 300–700 nm range with a UV 1650 PC-Shimadzu B UV–visible spectrophotometer (Osaka, Japan).

2.2. Algaecide effect of the biosynthesized Cu-NPs against Lyngbya majuscula

The harmful Cyanobacterium species namely, *L. majuscula* was obtained from Phycology Laboratory, Faculty of Science, Tanta University, Egypt. The alga species was cultured in 1 litre conical flasks containing 400 mL in BG-11 medium for 15 days [5], and incubated in controlled conditions of continuous light (45 μmol/ms) at 25 ± 2 °C.

The stock solution of Cu-NPs (5 mg/mL) was prepared in sterilized deionized water, dispersed for 10 min using a sonicator (60 amplitude) to prevent aggregation and vortexed for 1 min. The stock solution of Cu-NPs was kept at 4 °C and used within one week for the experiments. Prior to each experiment, the stock solution was sonicated for 10 min, vortexed for 1 min and then immediately used in the working concentrations of 2,