Eco. Env. & Cons. 27 (2) : 2021; pp. (690-694)
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ISSN 0971–765X

Effect of different Magnesium Oxide Nanoparticles concentration on the growth of the *Lyngbya majuscula*

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(Received 5 September, 2020; accepted 15 October, 2020)

ABSTRACT

This study evaluated the effect of different concentrations of magnesium oxide nanoparticles (MgO NPs) was (37.5, 56.5, 75, 93.75, 112.5, 150) g/l in BG11 medium on blue green alga *Lyngbya majuscula* a species of filamentous cyanobacteria isolated from soil, on photosynthetic pigments and growth. The results showed that the increase of MgO NPs caused an increase in the growth and concentration of chlorophyll a, and carotenoids compared with the control group that gave higher concentrations of chlorophyll a, and carotenoid. Also, the genetic analysis showed matching the DNA sequences of cyanobacterium understudy with DNA sequences of species found in Gen Bank at a percentage of 99% which was *Lyngbya majuscula* CCAP 1446/ 4, the results of sequences deposited in Gen Bank of NCBI site with accession number MN567111.1.

Key words : *Nanoparticles, MgO NPs, Lyngbya majuscula, Chlorophyll a, Carotenoids*

Introduction

Nanotechnology is a branch of science that has received great attention from researchers due to the physicochemical properties of nanoparticles (Agrawal *et al*., 2014). Nanoparticles and nano material are present in the environment as a product of natural or industrial processes, or from the treatment and discharge of wastewater and the merging or integration of bio solids into the soil. Agriculture also contributes to adding many nanoparticles, which increases its access to the ecosystem (Morales-Díaz *et al.*, 2017).

The nanoparticles are less than 100 nanometers in one dimension at least (Kulacki *et al*., 2012) and are either metals or metal oxides. Nanoparticles oxides were used in water purification, medicine, cosmetics, engineering, and other fields (Yilmaz-Ozturk and Daglioglu, 2018). Increased use of nanomaterials means grow products of it very rapidly, and because the water ecosystem is the ultimate reservoir of rainwater, household waste, and materials from agriculture and industry, therefore the impact of nanomaterials on this system must be determined (Battin *et al.*, 2009).

Magnesium is one of the main major nutrients, in addition to its role in metabolic enzymes it plays an important role in regulating the energy balance in the cell and interacts with the pyrophosphate composition of nucleotide di and triphosphates (Zhao *et al.*, 2012). Blue-green algae spread in fresh surface water and are self-feeding bacteria negative to Gram stain (Chow *et al.*, 1998).

Regarding the effect of magnesium ion, the study of Ayed *et al.* (2015) aimed to know the effect of different concentrations of magnesium ranged between 8.9-465.0 mg/l that the magnesium ion is not toxic even in high concentrations on *Chlorella vul-*

garis, which showed high efficacy in removing this ion is 100% from the middle. The alga *Chlorella* sp. also showed a different response after its cultivation in an agricultural medium containing glucose and magnesium at concentrations of 0.2, 0.5, 1, 2.8 and 49 ppm. This is because magnesium is a necessary element in the processes of formation of chlorophyll as the increase in the addition of magnesium to the medium was accompanied by an increase in chlorophyll, which was reflected in the positive on the rate of growth (Finkle and Appleman, 1953).

Materials and Methods

The sample containing alga understudy was collected from freshwater, which is belonging to the class of Cyanobacteria, for obtaining unialgal filaments used the streaking on agar method adopted from Stein *et al.* (1973) and Andersen (2005). To obtain an axenic culture of this alga using Density Gradient Centrifugation which is described by Wiedeman, Walne and Trainor (1964) which was repeated twelve times until removed all contamination and to ensure that culture free off bacteria and fungi was cultivated in nutrient agar at 37 °C for 24h. The alga then transferred to the sterile flask 250 ml containing 100 ml of BG11 medium and incubated in the growth chamber at 25 °C and 40µmol m–2 s–1 until used (Rippka *et al*., 1981).

The alga classified depend on morphological characterizations by using light microscope according to classification key (Desikachary, 1959). To confirm the phenotypic classification, a genetic analysis was used by extracting the DNA from the algae by taking 100 mg from alga and treated with glass beads to degrade the cell wall and then used genomic DNA extraction kit from Geneaid Company, Korea to the purification of extracted DNA. PCR techniques used for amplification the 16S rRNA gene found in cyanobacteria with forwarding primer CYA106F: CGGACGGGTGAGTAACGCG TGA and reverse primer CYA781R: GACTACTGG GGTATCTAATCCCATT, with product size 664bp the PCR condition performed under the following: 5 min initial denaturation at 95 °C; 35 cycles of denaturation 30 s at 95 °C, annealing1 min at 60 °C, and extension 2 min at 72 °C; a final extension at 72 °C for 10 min. (Nübel *et al*., 1997) for the PCR product was purified and subjected to AB DNA sequencing system to obtain the DNA sequences of the 16S rRNA gene, NCBI-Genbank-Primer-Blast and phylogenetic tree analysis used to the alignment DNA sequences of alga under study.

A stock solution of MgO NPS was prepared for use in preparing the culture medium instead of the Mg (MgSO4.7H2O) found in BG11 medium by dissolving (37.5, 56.5, 75, 93.75, 112.5, 150) g from MgO NPS in deionized water and store in the refrigerator under 4 °C until use. For treating the algae with nanoparticles, 10 ml of alga, which was grown before in BG11 medium, were added to 7 flasks with three duplicates, six of them containing different concentrations of Mg NPS and control group MgSO4.7H2O without addition of any nanoparticles.

The methods described by Tam and Wong, (1989) were used to estimate the growth based on optical density with absorbance at wave length 750 nm using the spectrophotometer.

To measure the chlorophyll a and carotenoid content of algae samples, the method presented by Zavøel, Sinetova, and Èervený (2015) has been adopted. To extract the chlorophyll-a and carotenoid, methanol 99.9% was added. The spectrophotometer measured the supernatant at 665, 720, and 470 nm wavelengths. The chlorophyll-a and carotenoid concentrations were determined on the basis of the following equations:

Chla [µg/L] = 12.9447 (A665 – A720)

Carotenoids [µg/L] = [1,000 (A470 – A720) – 2.86 (Chla [µg/mL])] / 221

Results and Discussion

The genetic analysis depends on 16S rRNA gene play vital role in classification of cyanobacteria, the results of DNA sequences of cyanobacterium under study showed matching with DNA sequences of species found in GenBank at a percentage of 99% which was *Lyngbya majuscula* CCAP 1446/4, the results of sequences deposited in GenBank of NCBI site with accession number MN567111.1., Also the phylogenetic tree analysis confirmed that matching (Figure 1)

Nanomaterials are an important emerging class of pollutants that have a wide environmental impact due to their small size, high effectiveness, and different toxic effects in different nanoparticles, and this characteristic is specific to materials in the Nano state. (Manzo *et al*., 2013). Estimating the effect of nanoparticles on phytoplankton is one of the necessary things to show their effect on food webs and

therefore on the entire ecosystem because they tend to congregate in aquatic environments (Handy *et al.*, 2008).

Nuclear metal oxides are the most used form of nanomaterials and are the most influencing the environment(Klaine *et al.*, 2008), as these oxides from industrial and domestic waste are discharged into natural waters (Aruoja *et al.*, 2009); (Ji *et al.*, 2011). Magnesium ion is an important factor in the growth of algae. It is a major component of chlorophyll, as it can be found in an equivalent rate of chlorophyll in the tissues of photosynthesis. (Ben Amor-Ben Ayed *et al.*, 2016). The effectiveness of nanoparticles may be affected by pH, nanoparticle concentration, exposure period, and ionic strength. (Sibi *et al.*, 2017); (Yilmaz-Ozturk and Daglioglu, 2018). (Manzo *et al.*, 2013). The effect of nanoparticles (NPs) depends on their size and the rate of absorption of the exposed cell through the cell membrane and transformed into ions or fully integrated or destroyed and carried out of the cell by the so-called "Trojan horse effect", or related to reactive oxygen species generation (ROS), which increases the oxidative stress, agglomeration, and shading effect (Aruoja *et al.*, 2009; Von Moos and Slaveykova, 2014)

It is clear from the results that the growth of algae under study was decreased with increase of MgO NPS concentration, and the inhibitory effect was observed even in high concentrations. (Figure 1). Although, inhibitory effect of MgO NPs treatment compared to the control group (free off nanoparticles) there was a growth of alga was seen and the higher concentration of 150 Mgo NPs gave a good growth approximated to the control group, this may be due to algae containing enzymatic and non-enzymatic antioxidant systems to prevent the oxidation effect and perpetuate the effective oxygen concentration to protect the algal cell from damage (Abd El-baky *et al.*, 2004). or the cell reduce the toxicity by exclusion, intercellular detoxification or by way of specific mineral binding (Rueter and Petersen, 1987). The results of the experiment also showed that, an increase in the concentration of Nano Mg caused an increase in growth, but it did not reach the level of control treatment. Sovová *et al.* (2009) showed that the toxic effect of Nano Mg decreases with increased concentration compared to magnesium if it is in a non-nanoparticle (Bulk), but the NPs has more toxicity.

The toxic effect of any substance was estimated on photosynthesis tests. It was found that the content of pigments (Chlorophyll a and Carotenoids) are the most stable in estimating toxicity and easy to measure in the Algae cells (Yilmaz-Ozturk and Daglioglu, 2018). Chlorophyll a is the most sensitive to measuring photodegradation, and this is due to its fundamental role in light reactions (PSII).

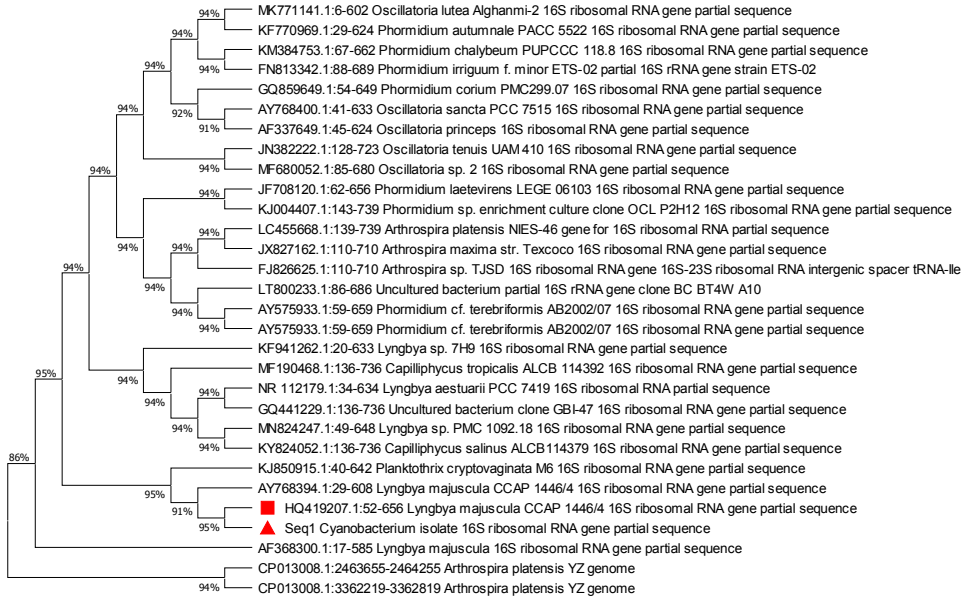

Fig. 1. Phylogenetic trees based on the cyanobacterium understudy 16S rRNA gene and other cyanobacteria organisms. Maximum likelihood algorithms were used, displaying only bootstrap values equal to 100%.

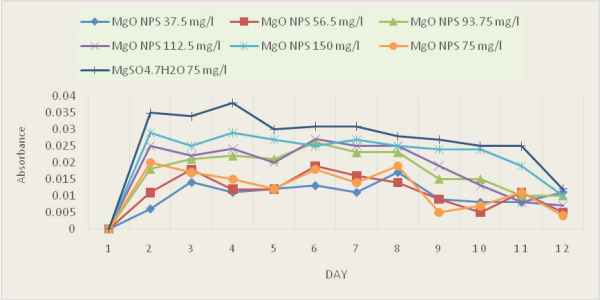

Fig. 2. Growth curve *of Lyngbya majuscula* under different concentrations of MgONPs compared to control.

(Rontani, 2001).

Results related to measuring the concentration of chlorophyll a showed an increase in the concentration of chlorophyll a with an increase in the concentration of Nano MgO, but the higher concentration of MgO NPs showed good chlorophyll a values, but not exceed the control treatment (Figure 3). While Anusha *et al.* (2017) confirmed that the increased concentration of Nano Cobalt caused a decrease in photosynthesis processes and this was due to the inhibition of photosynthesis pigments and cellular components. Nano cobalt may also inhibit the substitution process for magnesium in the chlorophyll molecule, causing the closure of chlorophyll manufacturing processes and the protoporphyrin accumulation, which results in the inhibition of the reducing step in biosynthetic pathways of this pigment.

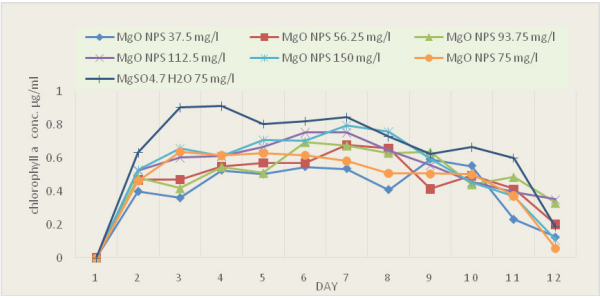

Fig. 3. Chlorophyll A concentration of *Lyngbya majuscula* under different concentrations of MgONPs compared to control group.

The difference in the response of living organisms to nanoparticles depends on the type of particles themselves, their size, the nature of the organism under test, and the method of testing (Ji *et al.*, 2011).

Carotenoid is a secondary pigment found in the plant and has an important vital role in increasing endurance for oxidative stress in vital metabolism reactions (Sanghera *et al.*, 2013).

The results indicated that carotenoids have increased in the control treatment compared to other Nano MgO concentration in which carotenoid concentrations have increased but less proportion than the control group (Fig. 4). That is consistent with the findings of each (Al-Khazali and Alghanmi, 2019; 2018) on green alga and did not agree with the results of a study Anusha *et al.* (2017) conducted on two blue-green algae.

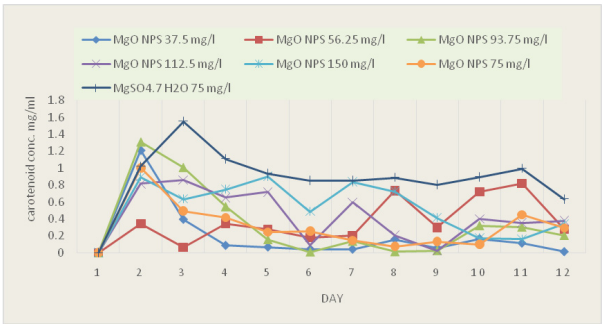

Fig. 4. Carotenoids content of *Lyngbya majuscula* under different concentrations of MgONPs compared to control group.

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