Influence of Different Concentrations of Nano-Magnesium Oxide on the Growth of *Coelastrella terrestris*

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Abstract: Magnesium ions regulate the electronic properties of the tetrapyrrole ring of chlorophyll. Without it, chlorophyll cannot capture sun energy needed for photosynthesis. This research dealt with the effect of three concentrations of MgO nanoparticles chosen (25, 12.5, 37.5 mg/l) according to the concentration of magnesium in Chu-10 medium represented by MgSO₄·7H₂O (25 mg/l) on the growth rate and Chlorophyll a, b and carotenoids content in the *Coelastrella terrestris*, which was classified in genotypic analysis and the partial 18S rRNA gene sequence submitted to the GenBank database and obtained the accession number MK336793. The results showed a decrease in all vital indicators compared to the control treatment which gave the best growth rate and Chlorophyll a, b, and carotenoids content of the alga. Despite the importance of magnesium, this study found that when it exists in nanoparticles form, it may behave differently as toxic materials leading to inhibit the growth of *Coelastrella terrestris*. These results confirmed by the results of the statistical analysis at P<0.05.

Key words: *Coelastrella terrestris*, Nanoparticles, Magnesium oxide, Chlorophyll, Carotenoids

1. Introduction

Nanotechnology has received attention and anticipation that was not received by any previous technology, it is considered the magic key to economic progress and development based on science and knowledge. It has become a major focus of world attention, namely materials technology, nanoparticles (NP), micro-technology, representing a new scientific revolution no less important than the industrial revolution, thus it is a technology that promises a huge leap in all branches of science[1].

One of the reasons for the intense interest in this science is that nanotechnology allows the preparation of materials in one dimension of the structure at least is less than 100 nm, so these substances show new properties and are different from their known characteristics and natural form[2]. The importance of nanoparticles is played a vital role in the various fields of life sciences such as medicine, pharmacy, and plants, the nanoparticles is a modern technology used in broad areas of life sciences. which has been used in many fields of industry or environmental sustainability[3].

Algae are the primary producers at the base of the aquatic food chain and are widely used in many industries. Algae are one of the typical model organisms used for the toxicity examination of NPs. Diverse physicochemical factors and morphology, including the size, shape, and aspect ratio of NPs, can influence the effects of NPs on algae[4]. Magnesium is one of the key elements required for chlorophyll synthesis and the co-factor for several important cellular processes[5]. Microalgae contribute not only to the environment through biodiesel
production and water purification system but also to economy and society., microalgae grown under metal nanoparticles that have recorded a significant increase in growth rate and biomass were selected for further cultivation in the presence of metal salts. Comparative experiments showed that metal nanoparticle exposed strain from Nano metal containing media has exhibited more growth rate, biomass, cellular pigments and lipid production than non-exposed microalgae thus indicating the positive effect of metal nanoparticles on microalgal growth[6].

He et al.[7] reported that High dose exposure to MgO nanoparticles limited increase in lipid productivity, possibly due to the repression on cell growth caused by nanoparticles-catalyzed reactive oxygen species generation, finally leading to reduction in biomass and lipid production in Scenedesmus obliquus. Other study evaluated the use of metal nanoparticles to induce metal resistance in Chlorella vulgaris thereby increasing the high-value products such as biomass, cellular pigments and lipid from microalgae. Initial experiments demonstrated the metal resistance development through metal nanoparticles and further experiments confirmed the positive influence of metal nanoparticles to improve microalgal growth, biomass and lipid production when grown in the presence of metal salts[6]. Aruoa et al.[8] explained that MgO was the only NP that was not toxic to any organism used in their study, in addition, the MgO NPS, showed no toxic effects below 100 mg/l.

The aim of this of this project to estimate the diverse effect of MgO nanoparticles on the growth rate, chlorophyll a, b and carotenoids of green algae Coelastrella terrestris.

2. Materials and methods

2.1 Isolation and identification

The samples collected from wastewater for isolating Coelastrella terrestris which is belonging to the class of Chlorophyta, for getting single alga adopted the streaking on agar [9]. Antibiotics used for obtaining an axenic culture of alga treated with as agreed [10]and then grown in the Chu 10 medium [11], after that alga isolate diagnosed according to some taxonomic sources [12]. The alga transferred to the sterile glass flask 250 ml containing 100 ml of Chu 10 sterilized medium and incubated in the growth chamber at 25 °C and 40 μmol m−2 s−1 Periodic illumination in 8:16 light: dark cycle.

2.2 Genomic DNA extraction

For 18S rRNA gene sequencing and analysis, genomic DNA of the microalgal strain was extracted using a DNA Mini Bacteria Kit (Geneaid, Korea), following manufacturer’s instructions with treated by liquid nitrogen and glass beads before using the Kit. Estimation of extracted DNA was checked by using Nanodrop (THERMO. USA) that measured DNA concentration (ng/µl) and checked the DNA purity by reading the absorbance at 260 /280 nm.

2.3 PCR Thermocycler Conditions and DNA Sequencing

the PCR master mix (Bioneer, Korea) contained 5 µl template DNA , 1.5 µl of reverse primer (10 pmol/µl), 1.5µl of forward primer (10 pmol/µl), 250µM of dNTP, 1.5 mM of MgCl2, 30 mM of KCl and 1 unit of Taq DNA polymerase with PCR water added to obtain 20 µl final volume in the PCR tube. Primers for green alga ChloroF (5′-TGGCCTATCTTGTGTCTGTGCT-3′) and ChloroR (5′-GAATCAACCTGGCAAGGCAAC-3′) [13], the PCR amplification was done by using conventional PCR Thermal Cycler Bio-Rad T100, USA, initial denaturation step (5 min at 95°C), Thirty-five incubation cycles followed, each consisting of 1 min at 95°C, 1 min at 60°C, and 1 min at 72°C. Final extension72 °C for 5min. The sequencing of the PCR products where the PCR products were purified from agarose gel by using (EZ EZ-10 Spin Column DNA Gel Extraction Kit, Biobasic, Canada). After that, the purified PCR products samples were sent to Macrogen Company in Korea for
performed the DNA sequencing by AB DNA sequencing system. The 18S rRNA gene sequence of the isolate was compared with the 18S rRNA gene sequences available in the NCBI site using the BLASTN search. Multiple sequence alignment and a phylogenetic tree were evaluated by using MEGA6 (Molecular Evolutionary Genetic Analysis).

2.4 Preparation of oxide nanoparticles
The nanoparticles were purchased as magnesium oxide from approved US company, Sky Spring Nanomaterial's Inc. With 99% purity and size 30-40 nm for ensuring they’re nanoparticles, it was examined by Atomic Force Microscopy (AFM) (figure 1). Stock Solution of MgO nanoparticles was prepared for use instead of MgSO4.7H2O magnesium salts exist in the chu-10 medium in three contractions 25, 12.5,37.5 mg/l which was dissolved in deionized water and store in the refrigerator under 4 °C until use. for treatment the alga with nanoparticles 10 ml of Coelastrella terrestris grown before, were added to four flasks, three of which were contained different concentrations of Nano-Magnesium oxide solution (25, 12.5,37.5) mg/l the fourth represented by proper chu medium without any addition of nanoparticles as a comparative.

![Atomic force microscopy (AFM) image of MgO nanoparticles](image)

**Figure 1.** Atomic force microscopy (AFM) image of MgO nanoparticles

2.5 Alga Biomass and Growth Rate Estimation
The Biomass of alga was estimated using the standard methods [14] based on the spectrophotometer measurement at a wavelength of 540nm. Whereas the specific growth rate (μ) of the microalgae was calculated according to the following formula [15].

\[ \mu = \ln \left( \frac{x_1}{x_0} \right) / (t_1 - t_0) \]

where X1 and X0 are the cell densities at times t1 and t0

2.6 Chlorophylls and Carotenoids Estimation
To estimate the chlorophyll a, b and carotenoids content of the alga about 10ml of the culture of Coelastrella terrestris was centrifuge at 5000 cycles/min for 5 minutes. The process was
repeated several times by using double distilled water. The supernatant was then removed and add 5 ml of acetone 90% to the precipitate and vortexed for 90 seconds then placed in a water bath at 25 °C for a one hour after that centrifuge at 6000 cycles/minute for 10 minutes and the supernatant measured at wavelengths (664, 647,630) nm using a spectrophotometer. Chlorophyll a and b contents of the microalga were estimated according to[16].

Chlorophyll a µg/ml=11.85 E664 - 1.54 E647 - 0.08 E630
Chlorophyll b µg/ml = - 5.43 E664 + 21.03 E647 - 2.66 E630
Carotenoids c1 + c2 µg/ml = - 1.67 E664 - 7.60 E647 + 24.52 E630

2.7 Statistical analysis
The statistical analysis was done using SPSS 24, the variation between three concentrations of MgO nanoparticles and proper magnesium exist in Chu10 medium among days was done by one-way analysis of variance (ANOVA) and least significant differences (LSD) at a level of p<0.05.

3. Results and discussion
In addition, to phenotypic classification under a microscope, the genotypic analysis showed that many sequences of strains in NCBI close related to the sequences green alga used in this study, in this case, the results of sequence alignment showed that the top one hit match much better to the sequence of green alga than the remaining BLAST hits (table 1, figure 2). at the same time, the partial18S rRNA gene sequence of the microalgal strain was submitted to the GenBank database and obtained the accession number MK336793, indicate that the isolate of this study was Coelastrella terrestris.

Table 1. NCBI homology sequencer identity between green alga isolate and NCBI Isolates

| NCBI Strains | Accession numbers | Max scores | NCBI sequencer identical |
|--------------|-------------------|------------|--------------------------|
| Coelastrella terrestris isolate CL2 small subunit ribosomal RNA gene, partial sequence | MK294227.1 | 846 | 100% |
| Tetradesmus sp. KG-2018 strain SAG 2564 small subunit ribosomal RNA gene, partial sequence | MH703775.1 | 846 | 100% |
| Tetradesmus sp. KG-2018 strain WD-1-6 small subunit ribosomal RNA gene, partial sequence | MH703774.1 | 846 | 100% |
| Tetradesmus sp. KG-2018 strain WD-7-1 small subunit ribosomal RNA gene, partial sequence | MH703773.1 | 846 | 100% |
| Tetradesmus sp. KG-2018 strain Hg-6-1 small subunit ribosomal RNA gene, partial sequence | MH703772.1 | 846 | 100% |
| Tetradesmus sp. KG-2018 strain Ru-6-2 small subunit ribosomal RNA gene, partial sequence | MH703744.1 | 846 | 100% |
| Tetradesmus obliquus isolate DOE-0152.Z small subunit ribosomal RNA gene, partial sequence | MH137236.1 | 846 | 100% |
| Scenedesmus sp. VP1 small subunit ribosomal RNA gene, partial sequence | MH277469.1 | 846 | 100% |
| Coelastrella saipanensis strain LY31-2 small subunit ribosomal RNA gene, partial sequence | MF407353.1 | 846 | 100% |
| Tetradesmus obliquus strain ABC-009 small subunit ribosomal RNA gene, partial sequence | MG971386.1 | 846 | 100% |
| Tetradesmus obliquus isolate CCAP 276/3C small subunit ribosomal RNA gene, partial sequence | MG022741.1 | 846 | 100% |
Graesiella emersonii isolate CCAP 211/8H small subunit ribosomal RNA gene, partial sequence
Coelastrella sp. DSA3 18S ribosomal RNA gene, partial sequence
Coelastrella sp. DSA2 18S ribosomal RNA gene, partial sequence
Scenedesmus sp. RT_F 18S ribosomal RNA gene, partial sequence
Scenedesmus bijugus 18S ribosomal RNA gene, partial sequence
Scenedesmus sp. FS 18S ribosomal RNA gene, partial sequence
Asterarcys quadricellulare isolate S2 18S ribosomal RNA gene, partial sequence
Coelastrella vacuolata strain CCNM 1028 small subunit ribosomal RNA gene, partial sequence
Acutodesmus sp. DPBB10 18S ribosomal RNA gene, partial sequence
Acutodesmus bajacalifornicus isolate BEA 0747B small subunit ribosomal RNA gene, partial sequence
Uncultured eukaryote gene for 18S rRNA, partial sequence

| Gene Details | Accession | Length | Identity |
|--------------|-----------|--------|----------|
| MG022718.1   |           | 846    | 100%     |
| KX818836.1   |           | 846    | 100%     |
| KX818835.1   |           | 846    | 100%     |
| KY302863.1   |           | 846    | 100%     |
| MF069190.1   |           | 846    | 100%     |
| KY268297.1   |           | 846    | 100%     |
| MF039332.1   |           | 846    | 100%     |
| MF580078.1   |           | 846    | 100%     |
| KY315602.1   |           | 846    | 100%     |
| KY587455.1   |           | 846    | 100%     |
| LC109020.1   |           | 846    | 100%     |

Figure 2. Dendrogram phylogenetic tree of green alga Coelastrella terrestris evaluated by using MEGA6 program
To investigate the behavior of nanoparticles on *Coelastrella terrestris* growth, initially exposed to metal nanoparticles, namely, magnesium. The concentrations of MgO nanoparticles used in this study were determined according to the concentration of magnesium exist in the Chu-10m medium which was 25 mg/l same to the concentration of magnesium in the medium and 12.5, 37.5 mg/l less and higher than of it. The algae cultured in these three concentrations of MgO NPS in addition to the proper Chu-10 medium as a control. The results of this study shown in the table (2) and figure (3) that the growth rate of *Coelastrella terrestris* give a higher level in control treatment represented by MgSO4.7H2O 25 mg/l followed by MgO NPS 37.5mg/l, MgO NPS 25mg/l and MgO NPS 12.5mg/l. Many reports revealed the inhibitory effects of nanoparticles on microalgae growth and a positive effect at the same time. He et al. [17] demonstrated that 0.8 mg/l of MgO nanoparticles had inhibitory effect on *Scenedesmus obliquus*, Whereas [6] found that positive enhancement the growth of microalga *Chlorella vulgaris* when treated with metal nanoparticles.

**Table 2. Growth rate of *Coelastrella terrestris* under four different concentrations of magnesium**

| Days | MgSO4.7H2O 25mg/l control | MgO NPS 25mg/l | MgO NPS 12.5mg/l | MgO NPS 37.5mg/l | LSD |
|------|---------------------------|----------------|------------------|------------------|-----|
| Mean±SE | Mean±SE | Mean±SE | Mean±SE | Mean±SE | |
| 1 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000 |
| 2 | 0.347±0.002 b | 0.219±0.004 c | 0.024±0.002 d | 0.718±0.006 a | 0.0027 |
| 3 | 0.292±0.005 b | 0.157±0.003 c | 0.032±0.001 d | 0.501±0.003 a | 0.0092 |
| 4 | 0.507±0.003 a | 0.121±0.006 c | 0.056±0.003 d | 0.387±0.006 b | 0.0043 |
| 5 | 0.433±0.004 a | 0.094±0.003 c | 0.081±0.004 d | 0.305±0.004 b | 0.0024 |
| 6 | 0.380±0.004 a | 0.100±0.006 c | 0.103±0.003 c | 0.287±0.004 b | 0.0030 |
| 7 | 0.341±0.003 a | 0.109±0.003 d | 0.150±0.002 c | 0.263±0.004 b | 0.0036 |
| 8 | 0.305±0.001 a | 0.113±0.001 d | 0.160±0.003 c | 0.266±0.003 b | 0.0021 |
| 9 | 0.310±0.005 a | 0.111±0.007 d | 0.157±0.003 c | 0.242±0.002 b | 0.0026 |
| 10 | 0.303±0.002 a | 0.146±0.004 d | 0.170±0.004 c | 0.252±0.003 b | 0.0027 |
| 11 | 0.274±0.003 a | 0.134±0.003 d | 0.152±0.003 c | 0.299±0.005 b | 0.0026 |
| 12 | 0.253±0.004 a | 0.118±0.004 c | 0.141±0.004 b | 0.251±0.006 a | 0.0021 |
| 13 | 0.232±0.005 a | 0.094±0.002 c | 0.130±0.004 b | 0.230±0.003 a | 0.0029 |
| 14 | 0.207±0.003 b | 0.086±0.002 d | 0.117±0.003 c | 0.211±0.003 a | 0.0036 |
| 15 | 0.192±0.003 a | 0.064±0.001 d | 0.113±0.004 c | 0.186±0.004 b | 0.0042 |
| 16 | 0.175±0.003 a | 0.052±0.002 d | 0.095±0.005 c | 0.168±0.004 b | 0.0039 |
| 17 | 0.158±0.004 a | 0.048±0.004 d | 0.086±0.002 c | 0.149±0.002 b | 0.0029 |
| 18 | 0.140±0.004 a | 0.039±0.004 d | 0.065±0.003 c | 0.124±0.003 b | 0.0029 |

*a, b, c and d refer to significant differences between four treatments in one day*
Figure 3. growth rate of *Coelastrella terrestris* under three concentration of MgO NPs compared to control.

The concentrations of chl. a and b was registered high value in the control treatment comparative with MgO NPS as shown in table (3, 4) and figure (4, 5). The results thus obtained are in agreed with [5] who showed that 150 mg/l of nano MgO had produced highest biomass and chlorophyll content but is lesser than control. On the other hand, other study had reported that chlorophyll contents of cultures *Scenedesmus obliquus* were greatly reduced by 73.3%, 84.5%, 80.6% and 87.5% when exposed to nano MgO of 0.8, 8, 40 and 100 mg/l, respectively[6]. Although the concentrations of nano MgO that used in this study was less than of those concentrations in the study of [7] on *Scenedesmus obliquus*, but our results were agreed with them about that nano-MgO significantly inhibited the growth and the chlorophyll synthesis of *S. obliquus*, indicating that nano-MgO imposed a biological toxicity on *S. obliquus*. When treated with 100 mg/l Nano-MgO, the growth and increment of chlorophyll of the algal culture was completely inhibited, and the culture died after 4 d of incubation.

Table 3. Chlorophyll a µg/ml of *Coelastrella terrestris* under four different concentrations of magnesium

| Days | MgSO₄·7H₂O 25mg/l control | MgO NPS 25mg/l | MgO NPS 12.5mg/l | MgO NPS 37.5mg/l | LSD |
|------|---------------------------|----------------|------------------|------------------|-----|
| 1    | 0.569±0.009 a             | 0.536±0.008 b  | 0.462±0.010 c    | 0.550±0.008 a    | 0.021 |
| 2    | 1.138±0.011 a             | 1.072±0.011 c  | 0.923±0.011 d    | 1.100±0.012 b    | 0.027 |
| 3    | 1.165±0.011 a             | 1.048±0.011 c  | 0.965±0.008 d    | 1.120±0.014 b    | 0.027 |
| 4    | 1.267±0.014 a             | 1.052±0.012 c  | 0.863±0.010 d    | 1.189±0.012 b    | 0.030 |
| 5    | 1.293±0.010 a             | 1.187±0.020 c  | 0.983±0.005 d    | 1.169±0.010 b    | 0.031 |
| 6    | 1.309±0.006 a             | 1.082±0.010 c  | 1.058±0.011 d    | 1.226±0.010 b    | 0.022 |
| 7    | 1.635±0.009 a             | 1.205±0.012 c  | 1.084±0.009 d    | 1.359±0.011 b    | 0.024 |
| 8    | 2.128±0.012 a             | 1.392±0.006 b  | 1.013±0.014 c    | 1.385±0.010 b    | 0.024 |
| 9    | 2.227±0.008 a             | 1.533±0.006 c  | 0.965±0.010 d    | 2.103±0.010 b    | 0.026 |
| 10   | 2.233±0.009 a             | 1.564±0.014 c  | 0.833±0.010 d    | 2.191±0.011 b    | 0.026 |
Table 4. Chlorophyll b µg/ml of *Coelastrella terrestris* under four different concentrations of magnesium

| Days | MgSO4.7H2O 25mg/l control Mean±SE | MgO NPS 25mg/l Mean±SE | MgO NPS 12.5mg/l Mean±SE | MgO NPS 37.5mg/l Mean±SE | LSD Mean±SE |
|------|----------------------------------|------------------------|--------------------------|--------------------------|-------------------------|
| 1    | 0.391±0.006 a                     | 0.319±0.011 c          | 0.252±0.006 d            | 0.365±0.008 b            | 0.021                   |
| 2    | 0.781±0.008 a                     | 0.638±0.005 c          | 0.504±0.004 d            | 0.730±0.008 b            | 0.016                   |
| 3    | 0.894±0.007 a                     | 0.825±0.005 c          | 0.594±0.004 d            | 0.769±0.005 b            | 0.014                   |
| 4    | 0.900±0.006 a                     | 0.752±0.007 c          | 0.642±0.006 d            | 0.827±0.005 b            | 0.015                   |
| 5    | 1.005±0.006 a                     | 0.684±0.005 d          | 0.771±0.007 b            | 0.729±0.007 c            | 0.018                   |
| 6    | 1.072±0.004 a                     | 0.843±0.006 b          | 0.846±0.008 b            | 0.749±0.008 c            | 0.016                   |
| 7    | 0.991±0.007 a                     | 0.902±0.009 b          | 0.840±0.010 c            | 0.802±0.005 d            | 0.019                   |
| 8    | 1.176±0.004 a                     | 0.699±0.005 c          | 0.876±0.005 b            | 0.859±0.004 b            | 0.036                   |
| 9    | 1.301±0.006 a                     | 0.466±0.006 d          | 0.813±0.009 c            | 0.922±0.008 b            | 0.018                   |
| 10   | 1.713±0.006 a                     | 0.546±0.006 d          | 0.753±0.009 c            | 1.284±0.006 b            | 0.017                   |
| 11   | 1.662±0.005 a                     | 0.592±0.008 c          | 0.487±0.011 d            | 1.278±0.004 b            | 0.019                   |
| 12   | 1.512±0.004 a                     | 0.573±0.011 d          | 0.595±0.007 c            | 0.992±0.007 b            | 0.019                   |
| 13   | 0.990±0.009 a                     | 0.505±0.007 d          | 0.688±0.005 c            | 0.794±0.005 b            | 0.020                   |
| 14   | 0.911±0.004 a                     | 0.212±0.010 d          | 0.677±0.010 c            | 0.800±0.007 b            | 0.020                   |
| 15   | 0.386±0.008 b                     | 0.229±0.003 d          | 0.579±0.007 a            | 0.544±0.004 c            | 0.015                   |
| 16   | 0.338±0.007 c                     | 0.133±0.007 d          | 0.452±0.004 b            | 0.476±0.007 a            | 0.015                   |
| 17   | 0.291±0.007 c                     | 0.200±0.007 d          | 0.458±0.005 b            | 0.575±0.006 a            | 0.015                   |
| 18   | 0.151±0.007 b                     | 0.127±0.005 c          | 0.119±0.005 c            | 0.366±0.006 a            | 0.015                   |

*a,b,c and d refer to significant differences between four treatments in one day*
Figure 4. Chlorophyll a concentration of *Coelastrella terrestris* under three concentration of MgO NPs

Figure 5. Chlorophyll b concentration of *Coelastrella terrestris* under three concentration of MgO NPs compared to control

From the table 5. and figure (6) it can be seen that carotenoids content of *Coelastrella terrestris* used in this study was influence by Nano-MgO at three concentrations. While the higher content of carotenoids noticed at control treatment these results agreed with [6] whom found that the content of carotenoid was higher in wild strain of *Chlorella vulgaris* in control treatment with comparative to other concentration of MgO NPS (50 mg, 100 mg, 150 mg and 200 mg). another study showed adverse effect of metal Nanoparticles, [18]which Found there was a 69.3% and 73.2% decrease in carotenoid content of *Microcystis* and *Oscillatoria* respectively.
Table 5. Carotenoids µg/ml a of *Coelastrella terrestris* under four different concentrations of magnesium

| Days | MgSO4.7H2O 25mg/l control | MgO NPS 25mg/l | MgO NPS 12.5mg/l | MgO NPS 37.5mg/l | LSD |
|------|--------------------------|----------------|-----------------|-----------------|-----|
| Mean±SE   | Mean±SE          | Mean±SE     | Mean±SE          |
| 1   | 0.354±0.019 a | 0.338±0.020 a | 0.193±0.025 b | 0.380±0.039 a | 0.065 |
| 2   | 0.708±0.035 a | 0.676±0.033 a | 0.386±0.044 b | 0.760±0.015 a | 0.080 |
| 3   | 0.903±0.031 a | 0.664±0.015 c | 0.373±0.039 d | 0.743±0.020 b | 0.070 |
| 4   | 0.972±0.035 a | 0.693±0.028 c | 0.356±0.013 d | 0.804±0.025 b | 0.066 |
| 5   | 1.089±0.019 a | 0.710±0.019 c | 0.273±0.041 d | 0.909±0.033 b | 0.080 |
| 6   | 1.109±0.025 a | 0.712±0.026 c | 0.218±0.024 d | 0.936±0.022 b | 0.020 |
| 7   | 1.120±0.017 a | 0.578±0.043 c | 0.156±0.043 d | 1.024±0.023 b | 0.065 |
| 8   | 1.155±0.015 a | 0.691±0.020 c | 0.109±0.015 d | 0.900±0.023 b | 0.130 |
| 9   | 1.099±0.013 a | 0.593±0.033 c | 0.128±0.018 d | 0.969±0.015 b | 0.040 |
| 10  | 1.198±0.020 a | 0.477±0.007 c | 0.115±0.016 d | 0.826±0.021 b | 0.052 |
| 11  | 0.911±0.018 a | 0.332±0.014 c | 0.090±0.018 d | 0.615±0.015 b | 0.041 |
| 12  | 0.881±0.051 a | 0.327±0.048 c | 0.170±0.045 d | 0.561±0.045 b | 0.041 |
| 13  | 0.765±0.017 a | 0.253±0.005 c | 0.076±0.021 d | 0.511±0.022 b | 0.120 |
| 14  | 0.531±0.015 a | 0.070±0.012 b | 0.015±0.007 c | 0.491±0.019 a | 0.044 |
| 15  | 0.510±0.017 a | 0.107±0.014 c | 0.042±0.014 d | 0.183±0.018 b | 0.035 |
| 16  | 0.379±0.024 a | 0.075±0.021 c | 0.074±0.022 c | 0.198±0.025 b | 0.058 |
| 17  | 0.332±0.022 a | 0.099±0.016 b | 0.046±0.012 b | 0.348±0.017 a | 0.042 |
| 18  | 0.222±0.025 b | 0.129±0.016 c | 0.054±0.018 d | 0.288±0.023 a | 0.050 |

*a, b, c and d refer to significant differences between four treatments in one day*

Figure 6. Carotenoids content of *Coelastrella terrestris* under three concentrations of MgO NPs compared to control
4. Conclusions

Although magnesium is important to algae, but when its existence in nanoparticles form may behave differently as toxic materials lead to inhibit the growth of green alga *Coelastrella terrestris*. This study showed decrease in growth rate and chlorophyll a, b and carotenoids content of *Coelastrella terrestris* when exposed to the nanoparticle form of MgO, whereas the control treatment give a good consequence in growth rate and Chl. a, b and carotenoids.

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