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Effects of Fe_2O_3 and Co_2O_3 nanoparticles on Organisms in Freshwater

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Abstract: Nanoparticles (NPs) are causing threats to the environment. In this review, we examined how hematite (Fe_2O_3) and cobalt oxide (Co_2O_3) nanoparticles impact the species of freshwater green algae Chlorella vulgaris (C. vulgaris). We exposed laboratory cultures to five initial concentrations of nanoparticles and measured impacts on species in 24, 48, 72, 96, 120, and 144 hours in Karun River water at 20-25°C. Our results indicated that Fe_2O_3 and Co_2O_3 NPs significantly (dependent on concentration) reduced the chlorophyll a, b, and carotenoid contents of algae C. vulgaris compared to the control group ($P < 0.05$). Also, due to the combination of these two nanoparticles, Co_2O_3 (50 Fe_2O_3 +100 Co_2O_3) has a more negative effect on algae chlorophyll change. According to the data, the exposure concentration was also found to be a more effective factor in the Chlorophyll content in algae species as compared to the exposure time. Our study suggests this nanoparticle has potential to affect aquatic life and ecosystem properties of freshwater habitats.

Keywords: Algae; Chlorophyll; Nanoparticles; Toxicity.

Introduction

The use of manufactured nanoparticles (NPs) started recently and represents a human-made material which is being used increasingly .Currently available data on exposure to NPs and effects on organisms are currently insufficient to conclude on the risks involved (Warheit 2018).Iron oxide nanoparticles, with the chemical formula of Fe_2O_3, are a binary ionic compound of iron and nonmetallic oxygen atoms bonded by chemical bonds (Asadian et al. 2019; Jiang et al. 2018). These nanoparticles have drawn much attention in drug delivery, tissue engineering, heat treatment (Manickam et al. 2018), magnetic resonance imaging (MRI) (Zhu et al. 2017), and the removal of metal ions and pollutants from water Rahmatinia and Rahmatinia (2018), due to their unique characteristics such as magnetic properties (Adam et al . 2017; Omrani and Fataei, 2018) , and particle size. The iron in these nanoparticles is a transition metal (Zhu et al. 2017) that poses a serious threat to the exposed organisms with the rapid production of free radicals (Dhakshinamoorthy et al. 2017).Cobalt is an essential element for cells to synthesize cyanocobalamin (Kaweeteerawat et al. 2015). On the other hand, cobalt is known to be an oxidative damaging stimulator in mammalian cells (Zhang et al. 2012) and in bacteria (Kaweeteerawat et al. 2015). Co_2O_3 nanoparticles have caused the toxicity of single-celled sweet algae (Aruoja et al. 2015). It has also adverse effects on the ecosystem and the environment. (Chen et al. 2017; Adam et al. 2018).

Since surface waters are the final destination of many anthropogenic materials that can enter the body of aquatics, the relationship between nanomaterial and aquatic environments has become an important issue worldwide (Adam and Nowack 2017). At certain concentrations, nanomaterial may inhibit the growth and proliferation of plants and reduce the photosynthetic function of cells and alter the content of plant pigments. (Zhu et al. 2019). When the nanoparticles are introduced into algae cells, they damage the cell membrane and settle between the cell wall and the plasma membrane (i.e., the periplasmic space (Wang et al. 2016). The toxicity effect of nanoparticles on organisms can provide significant data on the widespread impact of nanoparticles in the aquatic environment. Considering this fact that Chlorella vulgaris algae have a key role in the aquatic food chain, and due to its simple cell structure, easy culture, short growth

period, and high resemblance to the cell structure of higher plants (Marutescu 2019). There have been no studies on the effects of Fe_2O_3 nanoparticles combined with Co_2O_3 nanoparticles on Chlorella algae. Therefore, in this study, the individual and combined concentrations of these two nanoparticles have been made available to Chlorella algae. This study was performed in Karun River water sample which is the innovation of this research.

Material and Methods

Concentrations of nanoparticles

Co_2O_3 nanoparticles in black, 99.7% purity, SSA [1] =75.8 m^2/g and APS [2] =50 nm and Fe_2O_3 nanoparticles in brown red, 99% purity, SSA=20 m^2/g and APS=20-40 nm for analysis and testing were made available. Different concentrations of nanoparticles (1, 5, 10, 20, 50, 100 mg/l of nano oxides) ;(10-10, 20-20,50-50, 20-50,50-20,50-100,100-50 mg/l Co_2O_3 + Fe_2O_3) were dissolved in water sample from in Karun River and dispersed for 40 minutes using an ultrasound machine (Elma E30H, 37 kHz Ultrasound frequency, Germany) with 400 rpm in 250 cc of water to homogenize. Scanning electron microscopes (SEM) (MIRA III model -TE- SCAN) and Transmission electron microscopes (TEM) (TE-SCAN) of nano oxides are presented in fig.1.

Algae culture

Pure algae stocks Chlorella Vulgaris was cultured in vitro TMRL at 25°C, illumination of 2000 lux, and dark/illumination interval of 12/12h using an electric timer (TS-MD 20) and continuous aeration (Handy 2012). Further, 1 mg/L of algae with the density (OECD 2004) of $4.2x10^3$ was poured in 100ml Erlenmeyer flasks (Baumann et al. 2014) at 24, 48, 72, 96, 120, and 144 h, and were brought into contact with the Nano-oxides at 25°C (OECD 2004). Qualitative parameters of temperature, dissolved oxygen (DO) and pH of water was measured and controlled by a portable device (WTW-Multi 340 i) . The physicochemical characteristics of the test water are presented in table 1.

Chlorophyll measurement

For Chlorophyll measurement**,** 3ml of algae was centrifuged (Universal 320, Hettich,Germany) at 3000 rpm for 10 min. The supernatant was removed and acetone was added while stirring. After re-centrifugation of samples for 10 minutes, the supernatant was removed and chlorophyll a, b, and total carotenoid contents were reported by a spectrophotometer (T80 + UV/VIS)at 646, 663, and 480 nm in mg/ml. (Lichtenthaler and Weliburn 1983).

$$C_a=12.21A_{663}-2.81A_{666} \quad (1)$$

$$C_b=20.13A_{666}-5.03A_{663} \quad (2)$$

$$C_{x+c} = (1000A_{670}-3.27C_a-104C_b/198) \quad (3)$$

Where C_a is the a Chlorophyll concentration, C_b is the b Chlorophyll concentration and C_{x+c} is the total Carotenoids concentration.

The inhibition rate (IR) was calculated according to the following equation (Lu et al. 2008).

$$IR(\%) = \left(1 - \frac{T}{C}\right) \times 100 \quad (4)$$

Where, T is the biomass of the experimental groups and C represents the biomass of the control group.

Data analysis

Initially, the normality of the data was evaluated. Significant differences between different concentrations of treatments and duration of nanoparticle exposure were evaluated using two-way ANOVA, Turkey test, and Pearson correlation test. Statistical analysis was performed using $SPSS_{23}$ software.

Results

SEM and TEM of Fe_2O_3 nanoparticle & Co_2O_3 nanoparticle are presented in fig.1. The measured pH of the test dispersions remained within the range of 7.2 and 7.9 and did not vary by more than 1.0 unit in any given test. The water temperature ranged from 21.0ºC to 23.7ºC during all acute tests. The oxygen content of the test dispersions in all tests was between 6.3– 6.9 mgL^{-1} (Table 1). In river water samples, there was a significantly negative correlation between the concentrations of iron oxide as well as cobalt oxide combined nanoparticles and chlorophyll a and b and algae carotenoids ($P < 0.05$). (Figures 2 and 3). According to the data of chlorophyll content, with elevation of the concentration from 10 to 100 mg/L, the changing trends of chlorophyll content at different hours were insignificant and close to each other ($P < 0.05$). In the microscopic image of Fig. (4), the effect of toxicity of the nanoparticles to the algae is shown given its altered morphology and dimensions. Considering its morphology, the cells exposed to the nanoparticles were larger in size and more wrinkled than the control cells. The effect of the nanoparticles on the algae structure is shown at a concentration of 50 mg/L of the nanoparticles as the intermediate (Fig. 5). The results revealed that the concentration of cobalt oxide and iron oxide nanoparticles had a significant effect on the growth and inhibition rate of chlorella algae during 144 h of experiment ($p < 0.05$).

[1] *SSA: Specific Surface rea*

[2] *APS: Average Particle Size*

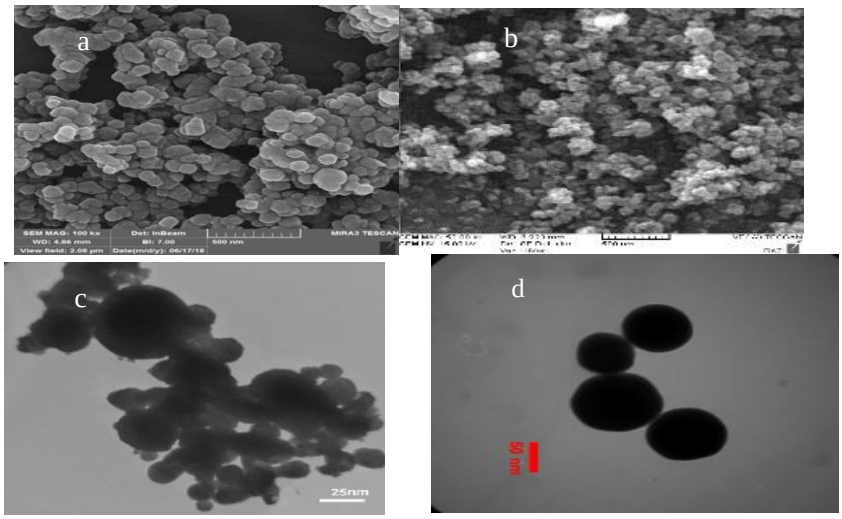

Fig. 1- SEM of Nano oxide. a) Co_2O_3, b) Fe_2O_3 TEM of Nano oxide. c) Co_2O_3, d) Fe_2O_3.

Table 1: Physico-chemical properties of the test.

Characteristics	Range	Mean ± SD
Room Temperature	27.2 – 29.5 (˚C)	27.8 ± 0.5
Water Temperature	21 – 23.7 (˚C)	21.4 ± 0.2
Dissolved Oxygen	6.3 – 6.9 (mg/l)	7.7 ± 0.12
pH	7.2 – 7.9	7.3 ± 0.1

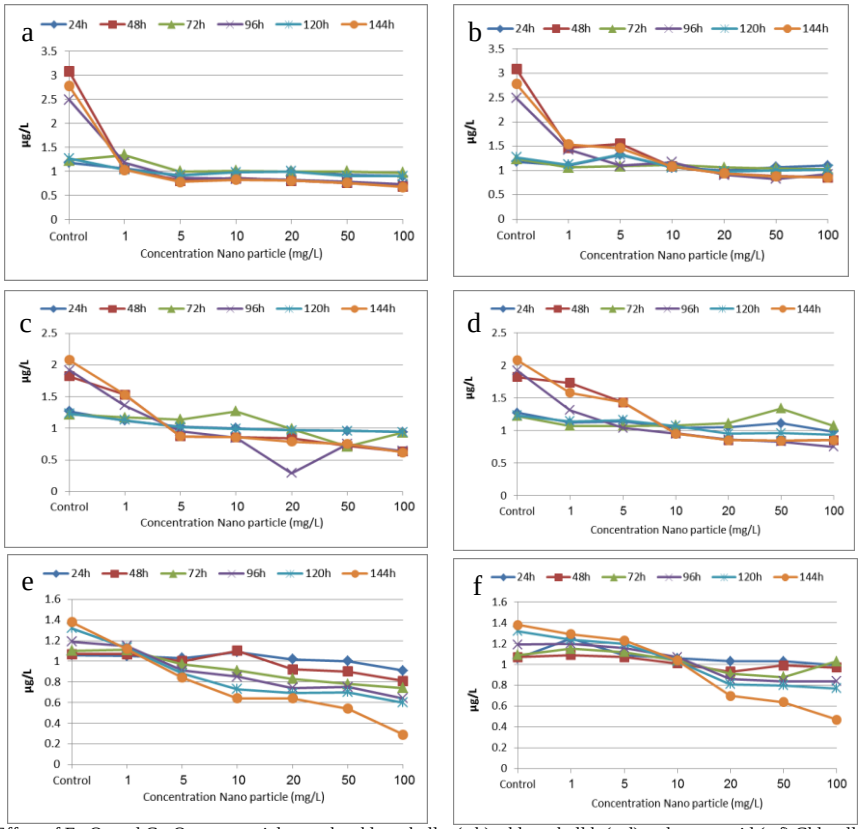

Fig. 2- Effect of Fe_2O_3 and Co_2O_3 nanoparticles on the chlorophyll a (a,b), chlorophyll b (c,d) and carotenoid (e,f) Chlorella vulgaris.

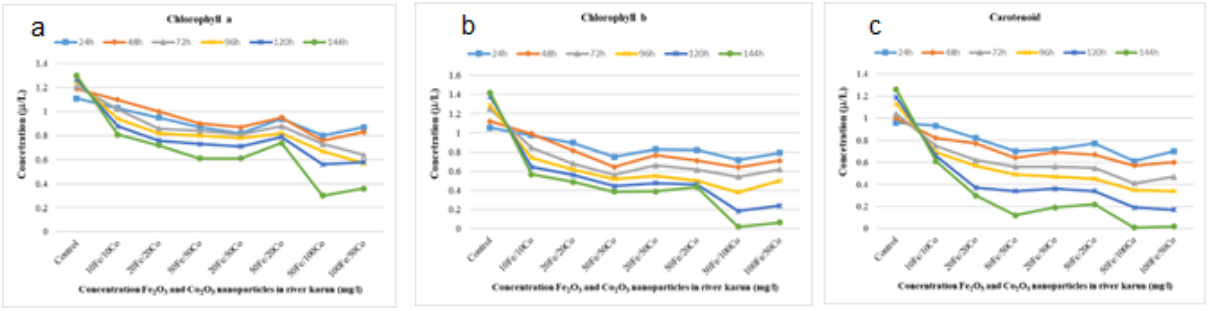

Fig. 3- Effect of Fe_2O_3 + Co_2O_3 nanoparticles on the chlorophyll a,b (a and b), carotenoid (c) of Chlorella.

Fig. 4- Chlorella vulgaris algae with an optical microscope. Treated sample (50 mg/L).

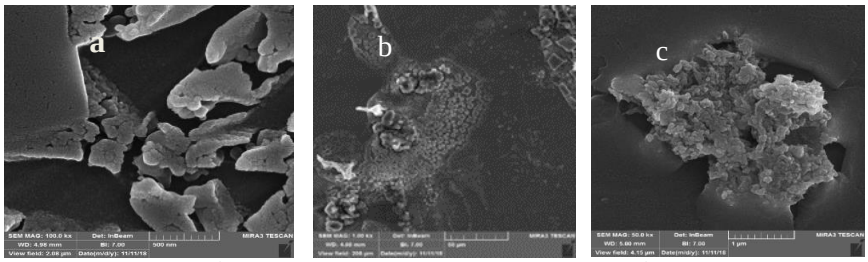

Fig. 5- SEM. Control Sample of Chlorella vulgaris algae (a) and the interaction of algae and nanoparticles with different magnifications. (b & c)

Discussions

The trend of using nanoparticles in the last decade has increased the probability that these substances are introduced into the aquatic environment affecting the primary producers in the food chain (Bundschuh et al. 2016).Different NPs may have different levels of toxicity and effects on various aquatic organisms (Wang et al .2019). Growth, cell division, and chlorophyll content are among the most important physiological parameters used to assess the risk of toxins in the environment (Wang et al. 2018). In the present study, the highest inhibition rate was measured at 144 h exposure and the concentration of 100 mg/L of nanoparticles, with 65.65% in iron nanoparticles and 90.04% in cobalt nanoparticles. In the study by Chen et al. 2018, the algal inhibition rate Skeletonema costatum was 23.9% at a concentration of 50 mg/L of cobalt nanoparticles at 24 h, reaching 73.6% at 96 h, which is consistent with the findings of the present study. Also, based on the inhibition rate, the intoxication rate of cobalt nanoparticles was also higher than that of iron nanoparticles. Various studies have shown that the toxicity of cobalt nanoparticles was higher than that of copper, gold, silver, and iron nanoparticles. (El-Sheekh and El-Kassas 2016). Note, however, that the toxicity of nanoparticles in different environments is also affected by the presence of other metals, e.g. it played an important role in the storage and toxicity of arsenic iron nanoparticles in Dubai. (Hu et al. 2012). Although the effect of the concentration of cobalt nanoparticles on the marine environment and most aquatic organisms has not been investigated so far, given the results as well as the higher inhibitory power of cobalt nanoparticles compared to iron nanoparticles, it is believed that this nanoparticle should be prevented from entering the environment considering its widespread use and its negative impact on the environment.

Scanning electron microscopy images (Figs. 5) also show that the nanoparticles have become strongly agglomerated and caked due to their low surface area /volume ratio. Thus, in the electron microscope images in the present study, the surface of chlorella algae was almost uniform in the control sample with only small agglomerations, while in the exposed sample, the surface was rough. Indeed, as the particle size shrinks, the surface area/volume ratio

increases, causing enhanced gravitational force between the particles, resulting in severe agglomeration. All these eventually culminate in larger and swollen algal cells compared to the control sample. Irreversible changes in the microalgae exposed to nanoparticles such as nickel (Manzo et al. 2013)has also been reported (Oukarrom et al. 2012) that silver nanoparticles directly affect the surface of the algae, making them appear larger and denser. Indeed, 50 nm silver nanoparticles, such as iron oxide and cobalt oxide nanoparticles about 40 nm in size cannot enter the cell but can serve as a bridge to another cell, which eventually accelerates their aggregation in cells (Chen et al. 2018) reported that the adsorption of nanoparticles by algae Chlorella & Scenedesmus decreased and the rate of light absorption due to the decrease in chlorophyll content as a major factor in photosynthesis, and it indicated a reduction in the algal growth in the present study (Wang et al. 2013). In the presence of cobalt nanoparticles, these nanoparticles disperse the protein and chlorophyll-forming amino acids and, by affecting the protein-pigment interaction of the thylakoid wall, result in damage to the wall. In previous studies, the main cause of toxicity of silver, cerium, zinc, and other nanoparticles with metal ions was the release of metal ions. According to Fig. 2, chlorophyll a and b levels were significantly reduced in the treatments with different concentrations of nanoparticles compared to the control treatment. In the case of carotenoid pigment, there was also a significant decline with increasing concentration and storage time (144 h), which confirms the effect of nanoparticles on the algal inhibition rate and survival. Other nanoparticles like Ag NPs significantly reduced chlorophyll content and inhibited the growth of the green algae *Chlorella vulgaris* (Zheng et al.2019).The results showed that chlorophyll a, b and Carotenoid had the highest levels in the Control medium at 144 h and the lowest at $50Fe_2O_3/100Co_2O_3$ and $100Fe_2O_3/50Co_2O_3$ concentrations. In fact, the two - time parameters and the nanoparticle concentration have been effective on chlorophyll a, b and Carotenoid levels. In fact, the two- time parameters and the concentration of nanoparticles have been effective on chlorophyll levels a, b and Carotenoid. Due to the stronger effect of MgO nanoparticles, the combination of this substance with Fe_2O_3 nanoparticles and its proximity has caused synergistic behavior against E coli (Torabi et al .2017) .The results also showed that the simultaneous presence of nanoparticles Ti_2O and ZnO reduces chlorophyll a in algae ($P < 0.05$) Hazeem et al .2016).These results are consistent with those of Wang et al. 2013; Chen et al. 2018), in which copper oxide and cobalt nanoparticles inhibited photosynthesis in C. algae and Skeletonema costatum, Platymonas subcordiforus, Chaetoceros curvisetus and algae by reducing chlorophyll. Nanoparticle shading due to the binding of these particles to each other (Chen et al. 2018) degradation of chloroplast and photosynthetic apparatus (Rao and Shekhawat, 2014), are the main causes of reduction in chlorophyll content and increase in the inhibition rate. Thus, density and adsorption of the nanoparticles by algae were the main reasons for the toxicity of cobalt oxide and iron oxide nanoparticles on C. algae.

Another factor in the reduction of photosynthetic potency in algae Chlorella vulgaris and increasing inhibition is related to the effect that nanoparticles have on photosynthetic organisms as a defense mechanism against oxidative stress. Carotenoids are one of the non-enzymatic antioxidant compounds that eliminate free radicals, inhibit lipid peroxidation, and protect photosynthesis Rao and Shekhawat (2014). Meanwhile, in a number of studies, such as Wong et al. 2016), carotenoids increased with the presence of nanoparticles, which could be attributed to differences in the nanoparticle type and storage conditions.

Conclusions

The uptake and toxicity of NPs depend on the inherent properties of NPs and also the chemistry of the surrounding environment (Park et al. 2015) .With the concentration rise of iron oxide and cobalt oxide nanoparticles, chlorophyll b and a decreased significantly at different times compared to river water, which can be caused by an increase in the algal structure degradation and also to the surface of the algae. The combined presence of nanoparticles has different effects. In this study, Co_2O_3 had a stronger effect than iron Fe_2O_3 in reducing the amount of algae chlorophylls. Algae chlorophylls have reached their peak over time. On the other hand, the more nanoparticles available at the time and concentration of chlorella vulgaris algae, the more negatively they will affect the concentration of chlorophyll a, b and carotenoid. There was a significant difference between the simultaneous presence of Fe_2O_3 and Co_2O_3 nanoparticles. Although this was an in vitro study, it is expected that the introduction of high levels of Nano-oxides into the aquatic environment has adverse effects on aquatic health and ecosystems. Furthermore, marine algae are potential organisms for usage in Nano pollution bioremediation in aquatic system because of their ability to adapt to the long exposure to NPs. The results of this study emphasize the importance of studying the possible interactions of different NPs in assessing their potential environmental risks .Thus, taking measures to prevent threatening amounts of nanoparticles from entering the aquatic environment and avoiding excessive use of

nanoparticles in various marine and aquaculture industries seems to be essential. Further, the amount of various metallic pollutants and their environmental effects on the tissues of various aquatics should be monitored regularly.

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