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Environmental Toxicity of Nano Iron Oxides (Fe2O3 NPs) on Algal Growth *Klisinema persicum* and Cellular DNA Damage Using Comet Assay

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ABSTRACT

Nanometer oxides are widely used in medical, food and cosmetics. The widespread usage of nanoparticles may have hazardous consequences on numerous organisms. Thus, it is necessary to assess their fate in the environment. The environmental toxicity of nano iron oxides (Fe 2 O 3 NPs) in several concentrations (50, 100, 200, 400, 800, 1000, 1200, 1400, 1600) mg/L on algal growth *Klisinema persicum* was evaluated by assessing some photosynthetic pigments, pH, oxygen content and cellular DNA damage (genotoxicity), using Comet assay through some indicators. This includes eight parameters (olive tail moment, %DNA in tail, tail moment, tail length, head diameter, comet area, comet height, as well as comet length) that were assessed for each comet on each slide in which the average values were recorded. Upon rising the concentrations of Fe2O3 NPs, the treatment outcomes with Fe2O3 NPs revealed a definite influence on a photosynthetic pigment (carotenoids and chlorophyll). In addition, with increasing concentration of iron oxide NPs, the acidity increased, while the oxygen content of the culture medium decreased. Genotoxicity was also increased in all parameters of comet assay with increased concentrations of iron oxide NPS and a higher increase of genotoxicity with a significant effect (P<0.05) documented at 1600 mg/L of iron oxide NPs, compared to the molecular characteristics in the control group. This was evidence that the high values of Fe2O3 NPs have a destructive effect resulting in DNA degradation and inhibiting the growth of algae.

INTRODUCTION

In recent years, nanotechnology has gained a lot of attention due to the distinctive properties of nanoparticles. It has an enormous surface area per volume unit with respect to its interesting characteristics like physical, chemical, electrical, mechanical, magnetic, optical and thermal properties, which makes them attractive for research work **(Iriarte‐Mesa *et al*., 2020)**. Among those materials are iron nanoparticles, which have a broad application range, including applications in electronics, biomedicine, energy, agriculture, animal and biotechnology **(Ajinkya *et al*., 2020)**. In addition to its use as a catalyst, it has a catalytic property, which is widely used in laboratory tests **(Chen *et al*., 2018)**. Nanoparticles have gained a great interest owing to their ability to reduce a variety of environmental pollutants, as iron nanoparticles can break down organic pollutants and

kill pathogens **(Mosayebi *et al*., 2017)**. It is widely believed that greater usage of nanomaterials would result in the emission of more of these materials into the environment, increasing the possible dangers connected with them. Nanomaterials are projected to explore their way into atmospheric, terrestrial and aquatic systems. Nanoparticles have high movement in the water so that they can easily enter aquatic ecosystems. There are many concerns about whether nanomaterials pose a serious environmental threat **(Shirazi *et al*., 2015)**. Water is the most crucial natural resource connected to the different ecosystems and is needed by living organisms including algae, to support life in all its forms **(Commission, 2011)**. Algae are an important part of aquatic ecosystems, especially in the main production, where species with strong metabolic rates are important **(Magdaleno *et al*., 2014)**.

Given their capacity to show the number of contaminants in water and their strong bioaccumulation capacities, algae were employed as a bio-indicator for freshwater pollutants **(Barhoumi & Dewez, 2013)**. Furthermore, since algae's reactions to chemical pollutants may change depending on their species, it is critical to analyze diverse algal species to validate their reactions to specific contaminants **(Li *et al*., 2017)**.

Several studies dealt with the effect of iron nanoparticles on algae, including the study of **Demir *et al.* (2015)**, in which they explained the changes resulting from various Fe2O3 NPs concentrations on the growth and morphology of marine algae *Nannochloropsis* sp. and *Isochrysis* sp. They postulated that, the accumulation of iron nanoparticles on the surfaces of the algal cell walls in the form of clumps distorted its cells morphologically. Moreover, **Hazeem *et al.* (2015)** reported that, nano iron oxides (Fe3O4 NPs) influenced the photosynthetic pigments and growth in the alga *Picochlorum* sp. via accumulation, transport and the uptake of Fe3O4 NPs in cells which rely on the cellular structure including its permeability, these particles' size, as well as the algae's cell wall characteristics. Furthermore, **Toh *et al*. (2016)** assessed the Fe2O3 NPs' influence on total lipid, protein and carbohydrate content in *Chlorella* sp, which was exposed to gradient concentrations for seven days. The inhibitory effect of microalgae growth caused by nanoparticles became clear on the first day and increased with increasing concentrations, as the green cell turned brown in high concentration. Likewise, **Hurtado-Gallego *et al.* (2020)** found that, Fe2O3 NPs at certain concentrations can act as a potential environmental hazard, inhibiting the growth of *Chlamydomonas reinhardtii* and causing reactive oxygen species (ROS) production due to increased lipid peroxidation.

Due to the entry of nanoparticles into many practical applications that directly affect human life, it is necessary to find a mechanism to investigate the damage to cellular DNA, resulting from treatment with nanoparticles, given their high surface area and small size including other physicochemical attributes. These NPs may obstruct the cell division mechanisms, transcription and mechanisms of replication. Several studies have dealt with this aspect above, which determined the extent of cellular DNA damage in different organisms, including animal **(Wise Sr *et al*., 2010)**, human **(Foldbjerg *et al*., 2011)** and

plant **(Patlolla *et al*., 2012)**. Notably, algae were addressed in previous studies, such as that of **Barhoumi and Dewez (2013)** and the work of **Chen *et al.* (2018)**; those authors explained the oxidative damage caused by Fe2O3 NPs, with respect to *Chlorella vulgaris* in the aquatic environment. In addition, **Zhang *et al*. (2020)** demonstrated that, zinc oxide (ZnO) nanoparticles affect the gene expression of the alga *Chlorella sorokiniana*. In this context, **Schiavo *et al*. (2016)** mentioned that, ZnO nanoparticles could cause DNA damage which increased as a result of the rising concentration of ZnO NPs in the marine alga *Dunaliella tertiolecta* through estimating the cellular and genetic levels.

Many techniques are used in genotoxicity tests to determine DNA damage. The Comet assay technique is one of the best methods since it is fast, simple, acceptable and widely used. **Azqueta and Collins (2013)** reported that, this technique has many applications including testing the toxicity of some chemical compounds. Additionally, it is used in clinical tests to reveal the importance of therapeutic tests for some chemicals at the cellular level, repair and restore DNA in the damaged cell to reverse the type of damage in DNA and test the effect of NPs at the gene level **(Kumari *et al*., 2011)**. This includes applying Comet assay on phytoplankton to investigate the pollutants' genotoxic effects with respect to the population level and determine the trans-generational effects at the genetic level. Remarkably, it is an appropriate tool for assessing major DNA damage in microalgae **(Akcha *et al*., 2008)**.

Due to the importance of nanomaterials, which may cause increased health and environmental risks, a technique must be found to investigate the genetic damage resulting from these nanoparticles. Therefore, the current research intended to investigate the various iron nanoparticle concentrations' effect on some genetic traits of cyanobacterium *Klisinema persicum* using the Comet assay technique.

MATERIALS AND METHODS

Characteristics of iron nanoparticles employed in the research

Nano iron oxides (Fe_2O_3 NPs) were bought from the American company, the SkySpring Nanomaterials Inc, in the form of brown powder with a purity of 99% and a size of (20-40) nanometers (Fig.1).

Examination of iron oxide nanoparticles employing atomic force microscopy (AFM)

Iron nanoparticles were evaluated using AFM to identify and map surfaces with nanoscale dimensions**.**

Examination of iron oxide nanoparticles using X-ray diffraction analysis

X-ray diffraction (XRD) analysis of Fe_2O_3 was conducted employing a (Shimadzu X-ray Diffractometer XRD 6000) in the Central Service Laboratory of Ibn Al-Haytham College of Education for Pure Sciences / University of Baghdad to analyze the crystalline structure of the solids by projecting the X-ray spectrum of the material to be examined.

Fig.1 Iron oxide nanoparticles

Preparation of nano–solutions

Stock solution of Fe_2O_3 NPs was prepared by dissolving 2g of Fe_2O_3 NPs in a BG-11culture medium. Therefore, the solutions were sterilized using an autoclave and stored in the refrigerator at 4°C. Before its use, the stock solution of Fe_2O_3 NPs was suspended using an ultrasonic homogenizer for 15 minutes to prevent the agglomeration of these materials **(Haghi *et al*., 2012)**.

Algal cultivation and exposure to nanoparticles

Axenic culture of cyanobacterium *Klisinema persicum* isolated from soil was obtained from the environmental lab in the Biology department, College of Education, University of Al-Qadisiyah, guaranteeing their purity free of contamination and cultured in nutrient agar at 37°C **(Andersen, 2005)**. Then, *cyanobacterium* was preserved by growing in BG-11 medium, following the procedures of **Rippka *et al*. (1979)** at a growth chamber with photoperiod 16:8 light: dark unit, pH 7.2, temperature of 28°C and a light intensity of 40 µmol m^{-2} s^{-1}.

Toxic effects of Fe_2O_3 NPs on the activity of algal growth

10 ml of *K. persicum* was inoculated with different concentrations of Fe_2O_3 NPs (50, 100, 200, 400, 800, 1000, 1200, 1400, 1600) mg/L in addition to the control group with a BG-11 only free of **NPs (0 mg/L)**. To investigate the Fe_2O_3 NPs effect with respect to the cyanobacterium growth, samples were daily taken until the algae reach the death phase.

Growth curve estimation

To estimate the growth curve of *K. persicum*, applying the technique of **Hotos *et al.* (2020)**, samples were daily collected to track the progress of the cultures, measuring the optical density at 750nm using (UV-Visible Apple, Japan) a spectrophotometer.

Effects of Fe_2O_3 NPs on the photosynthetic pigments

The photosynthetic pigments of algal cells (chlorophyll-a and carotenoids) were quantified according to the method of **Zavřel *et al.* (2015)** using the following equations:

$$Chl.-a\ [\mu g/L] = 12.9447\ (A665 - A720).$$

$$Carotenoids\ [\mu g/L] = [1{,}000\ (A470 - A720) - 2.86\ (Chla\ [\mu g/ml])]\ /\ 221.$$

Effects of Fe_2O_3 NPs on the pH and dissolved oxygen (O_2)

pH. pH value was calculated using a pH meter model (Milwaukee SM801) after calibrating it.

Dissolved oxygen. The dissolved oxygen value in the samples was calculated by the dissolved oxygen meter (YSI Incorporated model (55-25 FT), USA) after calibration, and the results were expressed in mg/L.

Investigating the genotoxic effects of Fe_2O_3 NPs on K. persicum

The comet assay technique was used to evaluate different concentrations of iron nanoparticles in influencing the genetic potential (molecular characteristics) of *K. persicum*.

Single-cell electrophoresis (Comet assay)

Comet assay for algae was conducted according to **Kumari *et al.* (2008)** using the following materials.

Lysing solution

It was prepared by dissolving (146.1, 37.2 and 0.6)g of NaCl, EDTA, and Tris, respectively, in a little distilled water, then completing the final volume to 700ml with distilled water. Then, 8g of NaOH was added and allowed to completely dissolve using a magnetic stirrer for 20 minutes, with an adjustment of pH to 13 by adding drops of a solution of NaOH or concentrated HCL. Next, the solution was supplemented to 890ml with distilled water. The final volume was then achieved by adding equal quantities of about 55ml of 1% Triton X and 10% DEMSO to complete the volume of the previous solution to 1000ml. The solution was kept in the refrigerator for half an hour before use.

Alkaline solution

The alkaline solution was prepared by dissolving 6g of NaOH as well as 1ml of 0.5% Na2EDTA and supplemented to 500ml of D.W**.**

Electrophoresis solution TBE (pH>13)

It was taken from the stock, the original bottle of 25ml, and was supplemented to 500ml with distilled water to obtain a concentration of 1X.

Low melting agarose

An amount of 0.6g of LMA agarose was dissolved in a glass beaker at high a temperature with 60ml of TBE (1X) solution.

The amount of DNA damage in algal cells was estimated by estimating some indicators related to the comet assay, which includes eight parameters (olive tail moment, % DNA in tail, tail moment, tail length, head diameter, comet area, comet height, as well as comet length). 50mg of dried algae was crushed with phosphate buffer solution on ice for 20 seconds, then 40µl of proteinase K was added to remove the proteins and then centrifuged at a speed of 13,000 cycles for 15 minutes at a temperature of 4°C. The precipitate was taken, and a small amount of phosphate buffer was added to it to suspend the DNA and maintain its vitality.

Afterwards, 50ml of the suspended cells were taken and carefully brushed on the slide, and 500ml of low melting agarose was added in a warm condition. Here, this process was done with the next steps with yellow light or faint light in order to prevent DNA damage by ultraviolet rays. Then, the slides were arranged in a horizontally rectangular package, and a lysing solution was poured on the tip of the package carefully and precisely to cover all the slides inside the package. With great caution not to move the slides, the package was carefully moved and kept in the refrigerator at 4°C for half an hour, after which the solution was carefully removed using the pipette to ensure that the slides were stable. An alkaline solution was added and left at room temperature for 20- 60min. Then, the solution was carefully withdrawn and transferred to the relay device, where the slides were horizontally arranged in the device. Electrophoresis solution TBE was poured into the device at a concentration of 1X. All slides were covered, and the electric relay device was turned on for 60 minutes. After the end of the migration period, the TBE solution was removed, and the slides were dyed with ethidium bromide dye solution and left for 24 hours. Then, the slides were monitored and the required dimensions were recorded using a fluorescence microscope, where the amount of DNA damage in the algal cells was estimated.

Statistical analysis

The statistically significant levels of difference between algae and control, treated with various Fe_2O_3 NPs concentrations as well as control group were assessed by employing SPSS v26 with the analysis of variance (ANOVA) one way and least signficat differences (LSD) test.

RESULTS AND DISCUSSION

Physicochemical characterization of iron oxide nanoparticles (Fe_2O_3)

Examination of nanoparticles using atomic force microscopy (AFM)

The size range and surface morphology of iron oxide nanoparticles (Fe_2O_3) were assessed by an atomic force microscopy (AFM). The two- and three-dimensional geography of Fe_2O_3 NPs was presented in Fig. (2). The type of each atom and its

location were identified on the 3-D diagram of the topography of the material's surface at the atomic level. A direct observation of the image exposed semi-spherical shapes of iron nanoparticles. Fig. (2A) (Topography image) shows the 2-D image of iron nanoparticles displaying molecular clusters, while Fig. (2B) shows the 3-D image of a section of iron nanoparticle surface, with a height of the molecular cluster of about 382nm. The average particle sizes were in the order of 24.2nm.

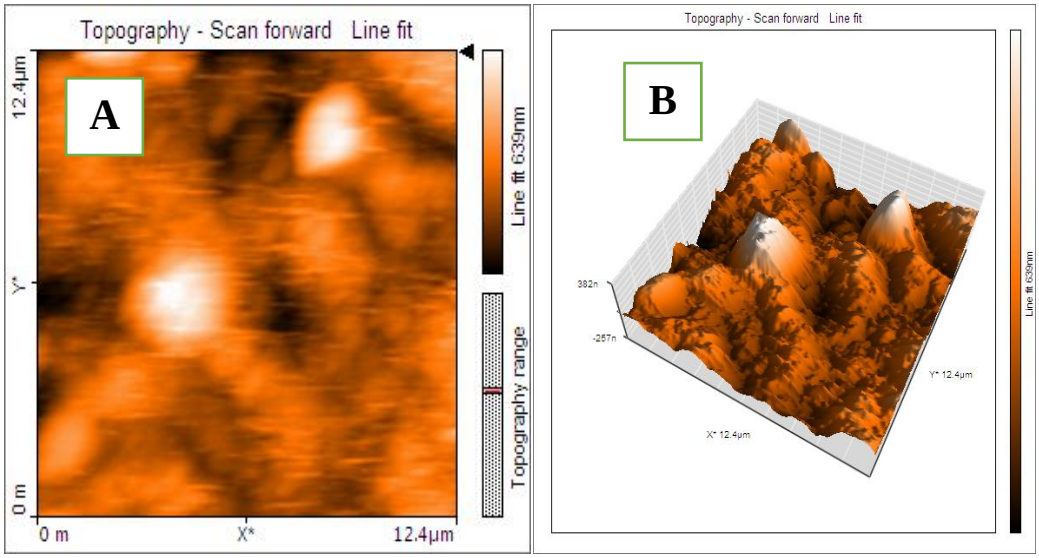

Fig. 2. Atomic force microscope (AFM) image of iron oxide (Fe_2O_3 NPs) nanoparticles showing: **A.** 2-D image (topography image) and **B.** 3-D image.

Examination of nanoparticles X- using ray diffraction (XRD) analysis

X-ray diffraction (XRD) analysis of Fe_2O_3 NPs was performed. XRD analysis is used to measure the crystallinity of particles, as sometimes, the crystallinity of these particles is not ideal. It is also used to reveal the nature of material particles **(Theivasanthi & Alagar, 2010)**. The findings revealed that the bio-crystallization on the Fe_2O_3 NPs' material surface was crystalline. Additionally, the examination results showed that, there are diffraction peaks indicating the extremely small size of the crystal, with the strongest peaks at 35, 38 and 52 **(Table 2 & Fig. 3)**.

Table 2. X-ray diffraction (XRD) analysis of iron oxide nanoparticles: the strongest 3 peaks

no.	Peak no.	2Theta (deg)	d (A)	I/I1	FWHM (deg)	Intensity (Counts)	Integrated Int (Counts)
1	35	33.1	2.7	100	0.21020	145	1742
2	38	35.6	5.5	72	0.18870	105	1028
3	52	53.9	1.6	49	0.25810	71	963

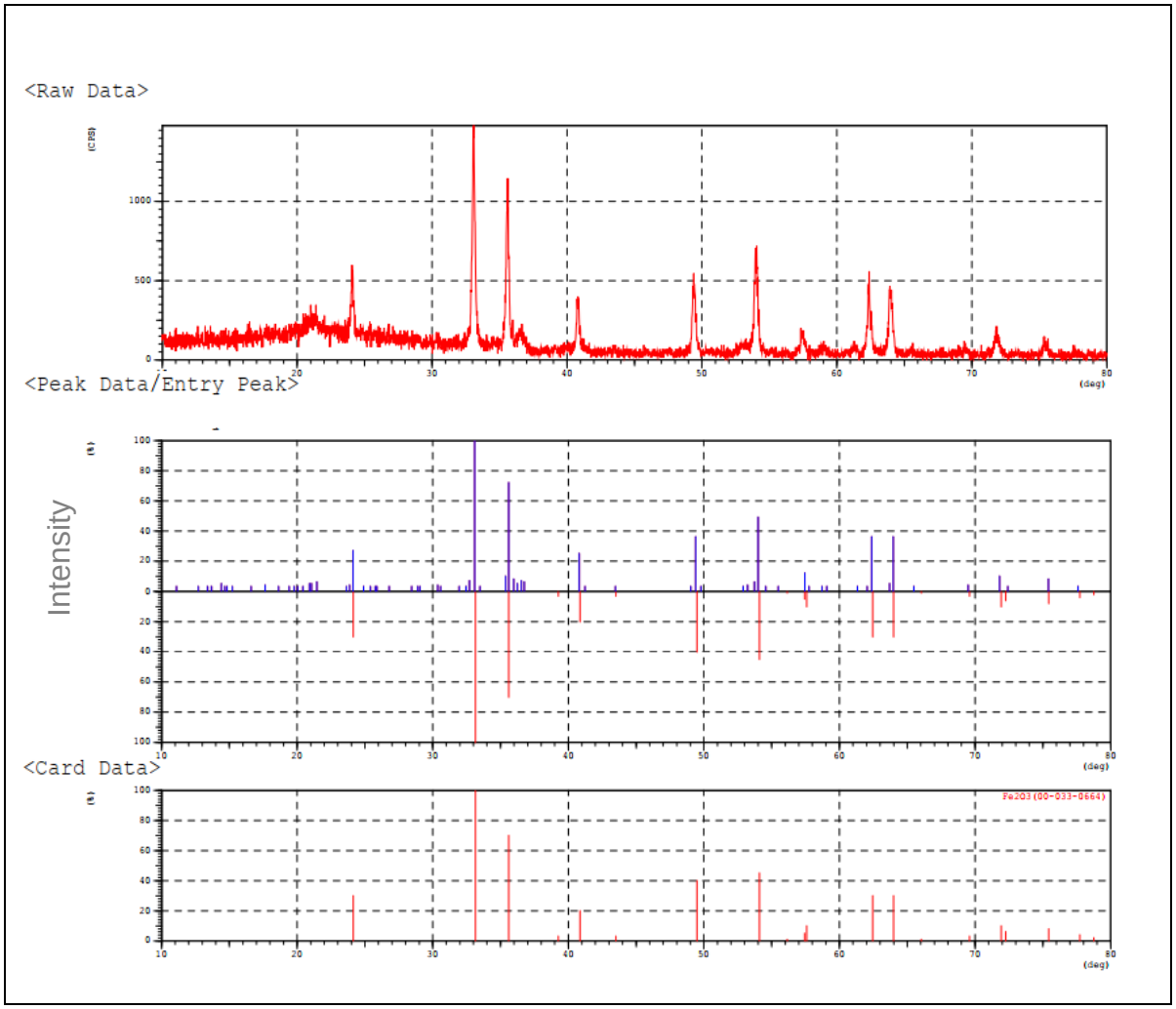

Fig. 3. X-ray diffraction (XRD) spectrum of Fe_2O_3 nanoparticles and their nanoscale

Microscopic examinations

The blue-green alga *K. persicum* was selected after sustaining its purity and was then grown on the culture medium. The algal light microscopic image without ZnO NPs treatment (control) revealed filamentous, membrane-intact cells (Fig. 4A). The phenotypic characteristics under the light microscope showed some diagnostic characteristics of the algae under study.

Klisinema persicum. Observations on the species under study revealed that, the algal trichomes are solitary, 3-5um broad, brilliant blue-green, generally straight, long, without sheat, extremely motile and not or slightly constricted. Cells are isodiametric or longer than 3-5um in length and conical; elongated terminal cell. This cyanobacterium exists in the soil **(Heidari *et al*., 2018; Guiry & Guiry, 2021)** (Fig. 4A).

Morphological examination of algal cells treated with Fe_2O_3 NPs

The effect of algae on nanoparticles is represented in several forms, including phenotypic, physiological or biochemical content. It was clear from the results of imaging by the microscope that iron nanoparticles influenced the blue-green alga *K. persicum* morphological characteristics when the concentration was enhanced. The treatment of NPs resulted in their adsorption and aggregation on algal cells (Fig. 4B). Algal cell aggregation, degradation and bleaching caused by chlorophyll loss via cell wall rupture, as well as the shrinking of algal filaments (Fig. 4C), resulted in a modification in the cells' shape due to cytoplasm contraction. As a result, the algae cells became deformed (Fig. 4D). This observations agrees with that of **Hazeem *et al.* (2015)** who reported the influence of the Fe_2O_3 NPs on the alga *Picochlorum* sp. They mentioned that iron nanoparticles collected on the surfaces of the algal cell walls in the form of clumps distorted its cells morphology. The results also align with those of **Djearamane *et al*. (2020)** who reported the effect of zinc nanoparticles on *Haematococcus Pluvialis*. The previous authors noticed the considerable cellular accumulation of ZnO NPs in algal cells as well as the comparable ZnO NPs' cytotoxic effects on *H. Pluvialis*.

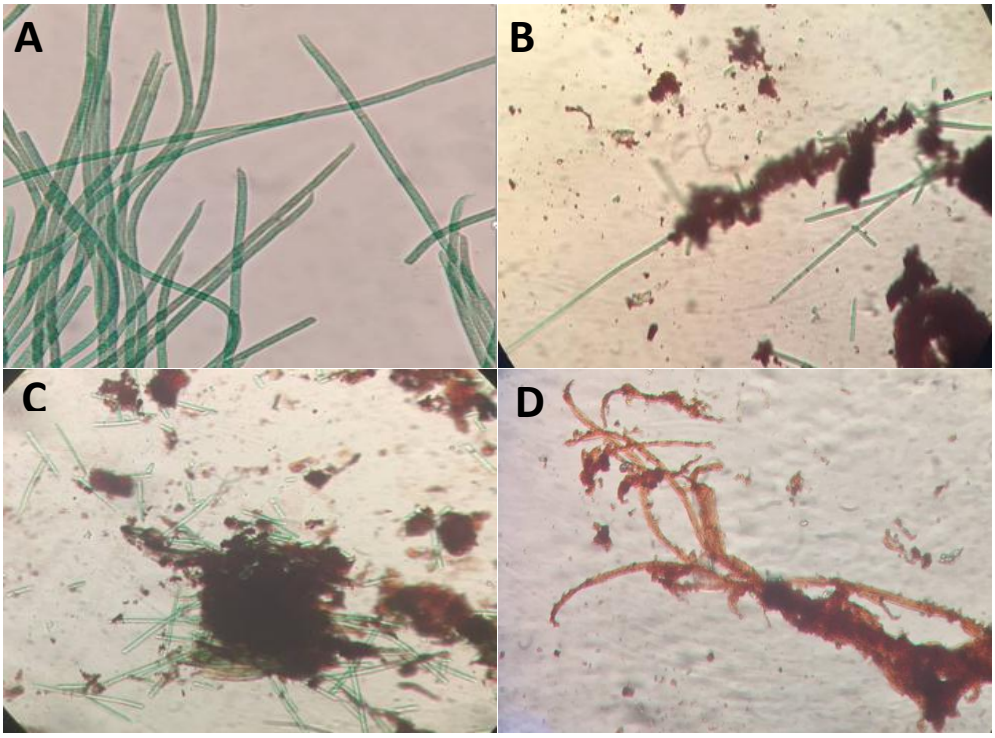

Fig. 4. Light microscopic images depicting the aggregation of NPs, **(A)** (control) filament of *K. persicum* without NPs; **(B)** filament *K. persicum* treated with Fe_2O_3 NPs 50 mg/ L; **(C)** 1600 mg/ L Fe_2O_3NPs, and **(D)** cell wall rupture.

Algal growth in culture media (BG-11) without nanoparticles (control group)

The growth curve of the blue-green alga *K. persicum* was studied in terms of absorbance values (Fig. 5). It was noted that, the exponential phase of the growth of the alga *K. persicum* started after four days of experiment and reached the stationary phase during the nine days of growth. Still, the death phase occurred in the 15 days and continued to decline until the death of the algae.

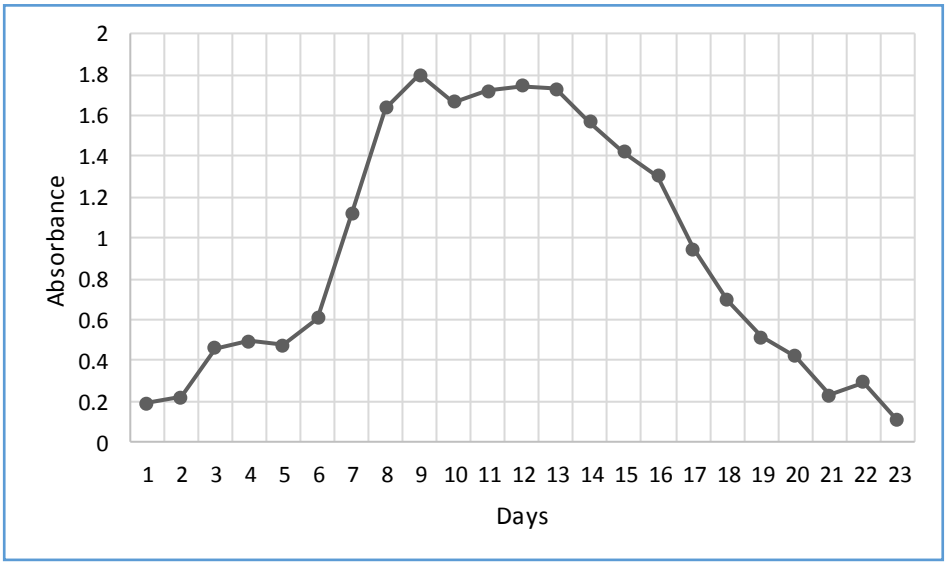

Fig. 5. The absorbance values of *K. persicum* under a (BG-11) mg/L medium

Effect of Fe2O3 NPs on the growth Of K. persicum

The findings revealed that Fe_2O_3 NPs inhibited the development of *K. persicum*, as evidenced by a decrease in algal biomass, photosynthetic pigments and cell viability. For all of the tested concentrations (100-1600 mg/L), Fe_2O_3 NPs induced a substantial decrease ($P<0.05$) in biomass and cell viability of *K. persicum*. The growth curve (Fig. 6) was estimated by the absorbance values of the effect of nine different concentrations of nano-iron (50, 100, 200, 400, 800, 1000, 1200, 1400 and 1600mg/ L) on the *K. persicum* growth. The findings indicate that the growth rate is affected by treatment with Fe2O3 NPs which begins with 50mg/ L and continued to decrease until it reached the 1600mg/ L Fe2O3 NPs, which registered a lower growth rate. On comparing the results of Fig. (5) of the control group to Fig. (6), ahigh growth inhibition occurred, and this inhibition increased with increasing the concentration of Fe_2O_3 NPs.

The use of limited concentrations of 50mg/ L of Fe2O3 NPs affected the rate of algal growth, as iron is an essential component of different effective compounds in the cell (enzymes, cytochromes, ferredoxin, etc.). Therefore, it plays a key role in cellular metabolism, and thus it is required for cellular number functions, including chlorophyll synthesis. Hence, it promotes the growth of algae, as iron enters the

photosynthesis process and carries the protein electron transport chain **(Kranzler *et al*., 2014)**. However, the drop in the algae *K. persicum* growth rate when treated with Fe2O3 NPs at greater concentrations reaching 1600mg/ L may be due to the toxicity of the nanoparticles through the shadowing effect **(Wu *et al*., 2018)**. The nanoparticles suspended in the cell culture diminish the light intensity, reducing the microalgae's growth rate. **Ko *et al*. (2018)** postulated that, iron nanoparticles affect the growth rate of alga *Chlorella vulgaris*, which decreases with an increased concentration of nanoparticles, as well as a study by (Hazeem et al., 2015) on the Fe_2O_3 NPs showing the effect on growth as well as photosynthetic pigments in the alga *Picochlorum* sp. It was shown that, particle concentration of 200mg/ L led to a substantial decline in the chlorophyll content and lived cells throughout the exponential growth phase, comparable to the current investigation's results.

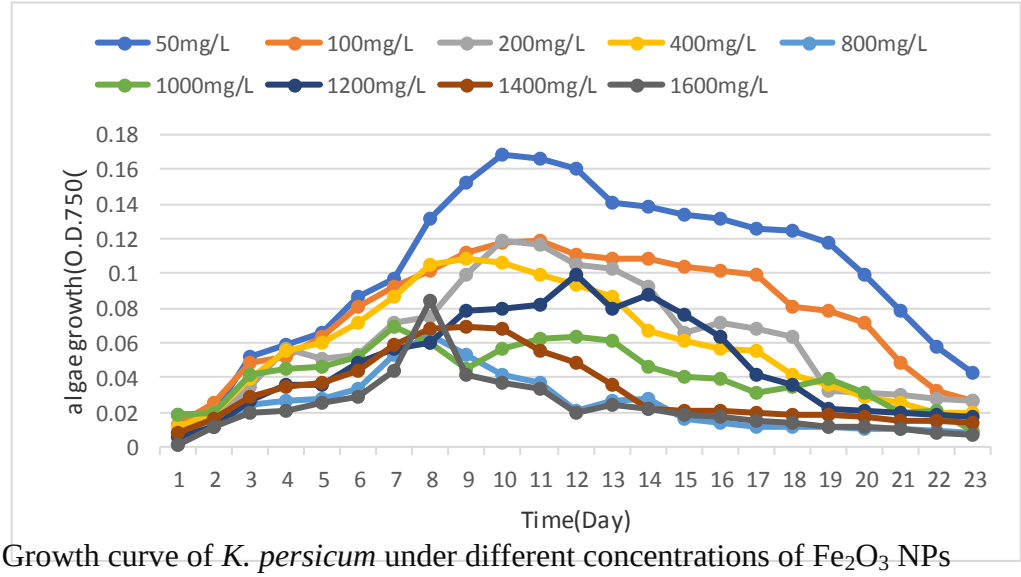

Fig. 6. Growth curve of *K. persicum* under different concentrations of Fe_2O_3 NPs

Effect of Fe2O3 NPs on the photosynthetic pigments of K. persicum

Figs. (7, 8) illustrate how various Fe_2O_3 NPs concentrations affect chlorophyll-a as well as carotenoid content in *K. persicum*. When contrasted to the control group, chlorophyll-a and carotenoid content dropped as Fe_2O_3 NPs concentration rose.

The results showed that the highest value of chlorophyll-a and carotenoid pigment for *K. persicum* reached 1.88 and 0.887µg/ l, respectively, when the algae were treated with Fe_2O_3 NPs of 50mg/ L. In contrast, the value of chlorophyll-a and carotenoid pigment decreased significantly at concentrations of nano-iron (100-1600) mg/L Fe_2O_3 NPs, compared to the control group, which added up to 2.3, 1.09µg/ l to the carotenoid and chlorophyll-a, as illustrated in the Figs. (7, 8). The findings of the statistical analysis revealed no substantial differences between the values of carotenoid pigment and chlorophyll-a at a substantial level of $P \leq 0.05$.

The high photosynthetic pigment values documented at 50mg/ L Fe_2O_3 NPs might be referred to the nanoparticles' role in enhancing biological activities **(Saxena, 2018)** and improving enzymatic activities, which stimulates chlorophyll divisions. In addition to stimulating photosynthesis enzymes, nanoparticles let the plastids live for a while. Hence, it increases the efficiency of photosynthesis **(Siddiqui & Al-Whaibi, 2014)**. The findings of the existing research agree with the research outcomes of **Karunakaran *et al.* (2017)** who explained that, various concentrations of FeO nanoparticles decreased the chlorophyll-a content of *Chlorella pyrenoidosa*. The low pigment concentrations occurred at high concentrations of Fe_2O_3 NPs may be justified by the NPs' features, which include the ability to wrap around algal cells, resulting in shade, or the ability to penetrate algal cells and bind to mitochondria, reducing cell photosynthesis (Aravantinou et al., 2017). Multiple investigations have discovered the outcome of shading caused by NPs, including the study (Schwab et al., 2011) of carbon nanotubes and their effect on *Chlorella vulgaris* and *Pseudokirchneriella subcapitata*, and the study of (Chen et al., 2018), which indicated the effect of CoNPs on *Skeletonema costatum*, *Chaetoceros curvisetus* as well as microalgae *Platymonas subcordiforus*.

Various investigations have shown that microalgal pigments are sensitive to metal toxicity and that treating Scenedesmus sp. and *Chlorella* sp. with Al2O3 NPs for 72 hours resulted in a time-dependent loss and dose of chlorophyll. Furthermore, when treated with AgNPs for 24 hours, two microalgae, *Chlorella vulgaris* and *Dunaliella tertiolecta*, showed a concentration-dependent drop in chlorophyll content (Oukarroum et al., 2012). Also, (Djearamane et al., 2020; Hurtado-Gallego et al., 2020; Sadiq et al., 2011) found that metallic oxide NPs had the most harmful effects on the electron transport chain of photosynthesis in *Chlamydomonas reinhardtii*, *Chlorella sp.*, as well as Scenedesmus sp., with a dose-dependent decrease in photosynthetic pigment. This research's findings are comparable to those of this research.

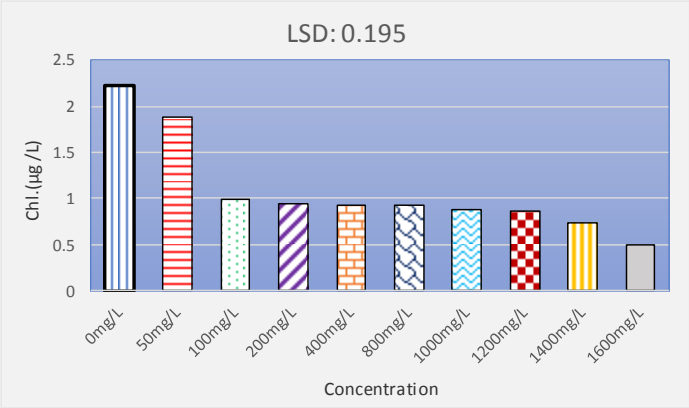

Figure 7. Effect of various **Fe_2O_3 NPs** concentrations on chlorophyll-a content of *K. persicum*

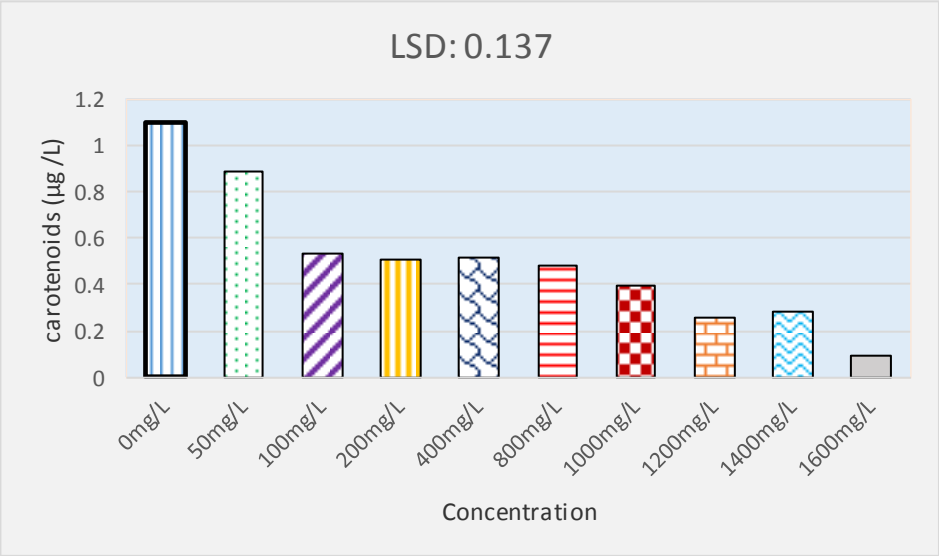

Figure 8. Effect of various **Fe_2O_3 NPs** concentrations on carotenoids content of *K. persicum*

Effects of Fe_2O_3 NPs on the pH and dissolved oxygen (O_2)

The pH values varied from 7.53 - 5.41 (Fig. 9). Variations in the pH were viewed in control and the rest of the concentrations of Fe_2O_3 NPs, as the pH values decreased with increasing concentration. Moreno-Garrido et al. (2015) indicated when studying the effect of nanoparticles on marine algae that changes in pH are expected due to a decrease in the metabolic activities of microalgae cells, the decrease in pH values which tend to acidity with high concentrations of nano-iron. The reduction in the growth of algae treated with high concentrations of nano-iron, which decreases the consumption of dissolved CO_2 along with its increase, results in a drop in pH values. This is compatible with the research (Aravantinou et al., 2017) on the nanoparticles' effect on algae, which discovered reduced pH values with rising nanoparticle concentration.

The findings of dissolved oxygen (O_2) showed that the highest value of dissolved O_2 during the growth period of *K. persicum* was 4.79 mg/L at a concentration of 50 mg/L Fe_2O_3 NPs, which is closest to the values documented in the control 5.03 mg/L, while the dissolved O_2 content decreased significantly at the concentration (100-1600) mg/L (Fig.10). The decreases in the dissolved O_2 content at high concentrations are due to the effect of Fe_2O_3 NPs in the activity of the photosynthesis process, which results in a drop in the dissolved O_2 release, as the dissolved O_2 in water is an important factor as it is dominant in the vital processes that take place in the aquatic system (Rasolofomanana, 2009).

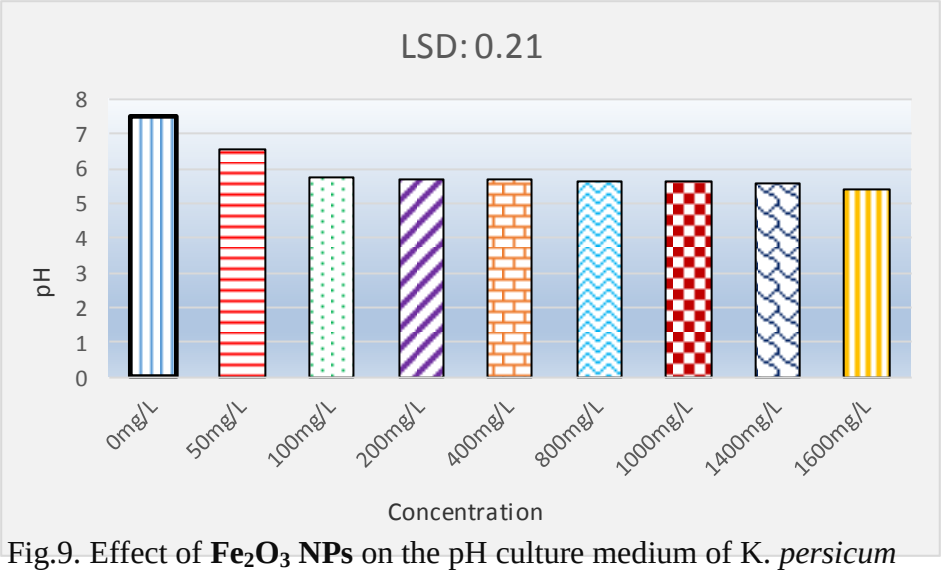

Fig.9. Effect of **Fe_2O_3 NPs** on the pH culture medium of K. *persicum*

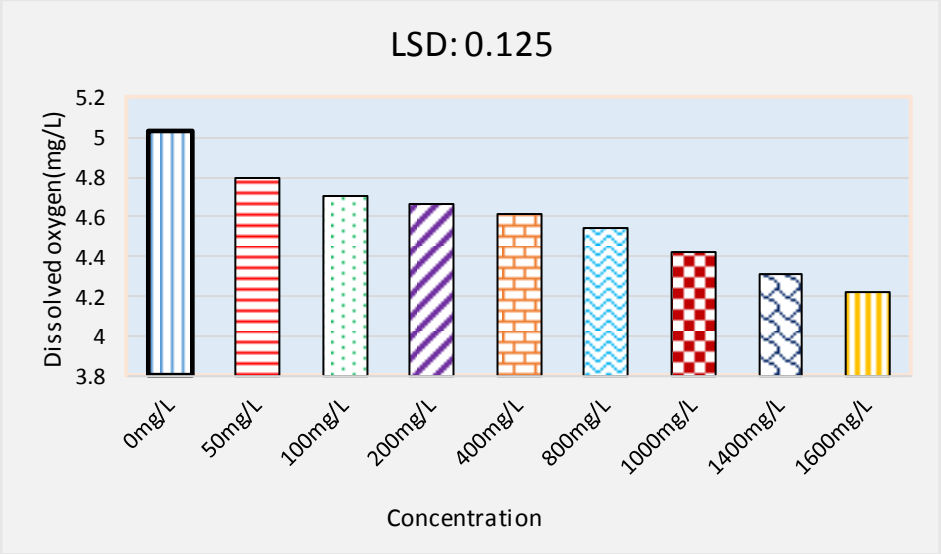

Fig.10. Effect of **Fe_2O_3 NPs** on the O_2 content of the culture medium of K. *persicum*

Effect of iron nanoparticles on molecular characteristics

Comet Assay (Single cell gel electrophoresis) method was applied to estimate the severity of damage in DNA molecules as a result of the treatment with iron nanoparticles. It was based on models taken from the organisms under study, represented by *K. persicum*. The amount of damage was determined in comparison with the control group, where the damage in the DNA molecule was classified into three categories according to the degree of damage, as shown in Fig.11.

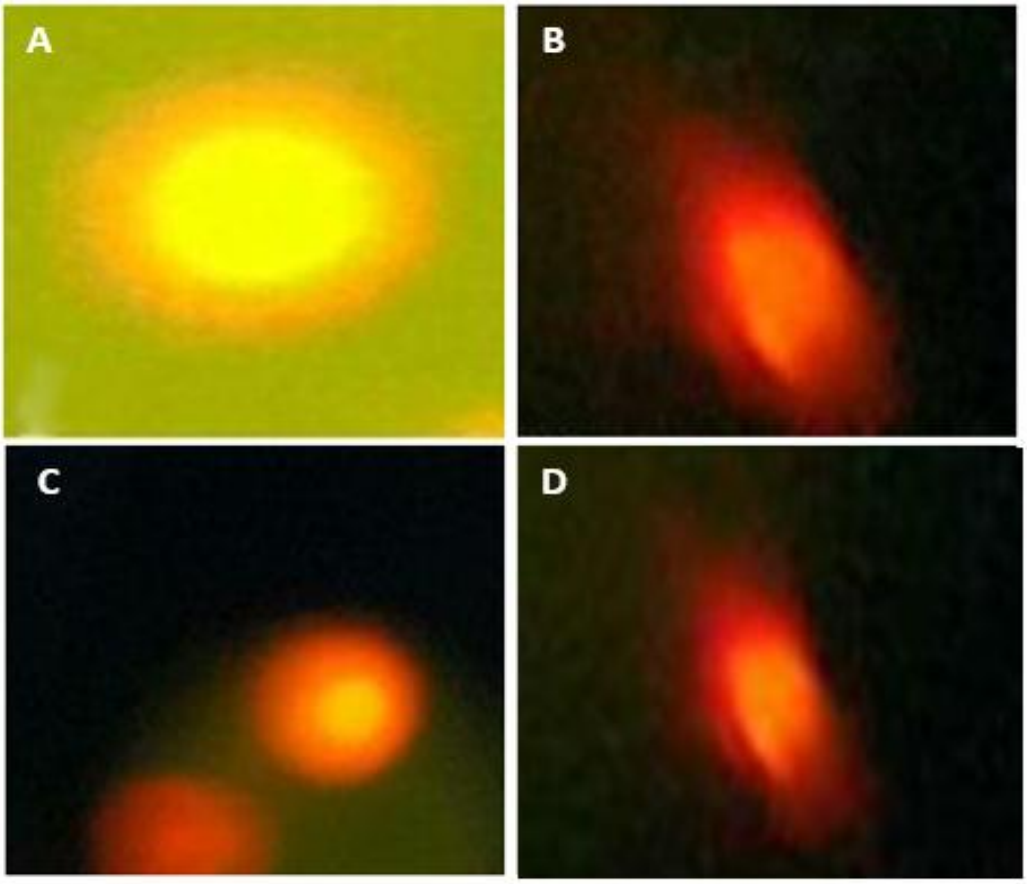

Fig. 11: Fluorescence microscopy images depicting DNA damage of *K. persicum* subjected to multiple concentrations of Fe_2O_3 as well as the control group as per the Comet assay (A: control – B: low damage- C: Medium damage; D: High damage)**.**

The genotoxic effect of iron nanoparticles in *K. persicum* was determined by some indicators related to the Comet Assay, which include eight parameters (olive tail moment, %DNA in tail, tail moment, tail length, head diameter, comet area, comet height, as well as comet length) were assessed for each comet on each slide, and the average of values was collected.

The findings of the statistical analysis showed a clear substantial difference between the control algae and the treated algae at $p \leq 0.05$, indicating that iron nanoparticles had a definite influence on the molecular properties of the algae under investigation. The values of the above-mentioned indicators increased in all algae after completing the treatment with iron nanoparticles except the 50 mg/L Fe_2O_3 NPs with low damage, and the damage was more evident at the higher concentrations. The findings indicate that when *K. persicum* was cultivated with Fe_2O_3 NPs concentrations of 100-1600 mg/L, the value of comet length rose dramatically, with the greatest value of 157.65px observed at 1600 mg/L. When the algae were treated with 50 mg/L, the lower value of comet length reduced substantially, reaching 33.24 px in comparison to 32.20 px in

the control group (Fig. 12). Comet height (px) with the greatest value of 106.5 px was obtained at 1600 mg/L test concentrations. In contrast, the smallest value of 36.06 px was reported when treated with a nano-iron concentration of 50 mg/L compared to the control group, which amounted to 33.36 px (Fig. 13).

Comet Area (px) reached the highest value of 8928.7px was registered at the test concentrations of 1600 mg/L, while the minimum value of 1468.9 px emerged at treated nano-iron concentrations of 50 mg/L in comparison to the control group, which is 1351.8 px (Fig. 14). When treated with nano-iron concentrations of 50 mg/L, the Head Diameter (px) attained the greatest value of 145.2 px. In contrast, the minimum value of 36.1 px was observed when contrasted with the control group, which was 33.4 px (Fig.15). Furthermore, the findings demonstrated that the greatest value of 18.66 px of tail length in *K. persicum* recorded when growing with nano-iron concentrations reached 1600 mg/L, while the tail length value decreased substantially when the alga was treated at a concentration of nano-iron 50 mg/L. The value was 2.08 px in comparison to the control group, which reached 1.84 px (Fig.16.) The greatest value of the tail moment in *K. persicum* was 5.66 px when cultivated with nano-iron concentrations of 1600 mg/L, while the minimum value of the tail moment was 0.12 px when cultivated with nano-iron concentrations of 50 mg/L, contrasted to 0.10 px in the control group (Fig. 17). When the algae were treated with a nano-iron concentration of 1600 mg/L, the % tail DNA reached 21.78%, whereas it reached 1.78% when treated with a nano-iron concentration of 50 mg/L, as opposed to 1.23% in the control group (Fig. 18). The olive tail moment was utilized to quantify the level of DNA damage determined by the Comet assay. In addition, olive tail moments reached the highest value of 10.23 was recorded at the test concentrations of 1600 mg/L,indicating damaged DNA structure, while the lowest value of olive tail moments reached 0.3 when treated with a nano-iron concentration of 50 mg/L contrasted to the control group, which amounted to 0.22 (Fig. 19).

The "tail" refers to the DNA streak that follows the nucleoid (Aslam et al., 2021). From the previous results, it is clear that the analysis of parameters for *K. persicum*, when grown with different nano iron oxides concentrations, showed an increase in tail length, tail area as well as percentage tail DNA in 1600 mg/L concentrations contrasted to the comets in the control group.

The results indicated increased DNA damage in the nanoparticles. The high rate of DNA damage and breakdown is associated with the oxidative effects that occur when organisms are exposed to nanoparticles (Zhang et al., 2020) which leads to ROS accumulation in living tissues which causes cell membrane oxidation and DNA damage (Ko & Kong, 2014). Given the purine nucleotides, oxidation is a major contributing factor in DNA damage induced by nanoparticles, in addition to chemical-mediated genotoxicity (Pakrashi et al., 2014). (Barhoumi & Dewez, 2013) pointed out the toxic effect of iron nanoparticles on *Chlorella vulgaris*, induced by the production

of free radicals ROS, an origin of DNA damage, caused by breaking strands, withdrawing nucleotides, or transforming the bases of nucleotides, which results in a deterioration in cell division, and that in turn influences the growth and morphology of the studied algae (Oukarroum et al., 2012), regardless of algae's capacity to repair DNA strand breaks (Desai et al., 2006). Nevertheless, genotoxic impacts on algae development and diversity can have long-term ramifications (Akcha et al., 2008). The high rate of damage and DNA damage as a result of treating algae with nanoparticles was recorded in previous studies, such as the study of (Zhang et al., 2020) on the green alga *Chlamydomonas reinhardtii* when treated with different concentrations of nanoparticles. They indicated the toxic effect of nanoparticles by inhibiting electron transfer and photosynthesis in chloroplasts, along with the suppression of photosynthesis at the gene expression level and the destruction of chloroplasts. The research of (Schiavo et al., 2016) showed adverse effects of nanoparticles on the DNA of the marine alga *Dunaliella tertiolecta* leading to DNA damage which increased with the increasing concentration of nanoparticles, similar to our findings. Finally, at nanoparticle concentrations where cytotoxicity was observed, the comet assay revealed damage at the level of DNA simple strain molecules suggesting that algae are models to include in ecological risk assessments for aquatic habitats (Martinez et al., 2015).

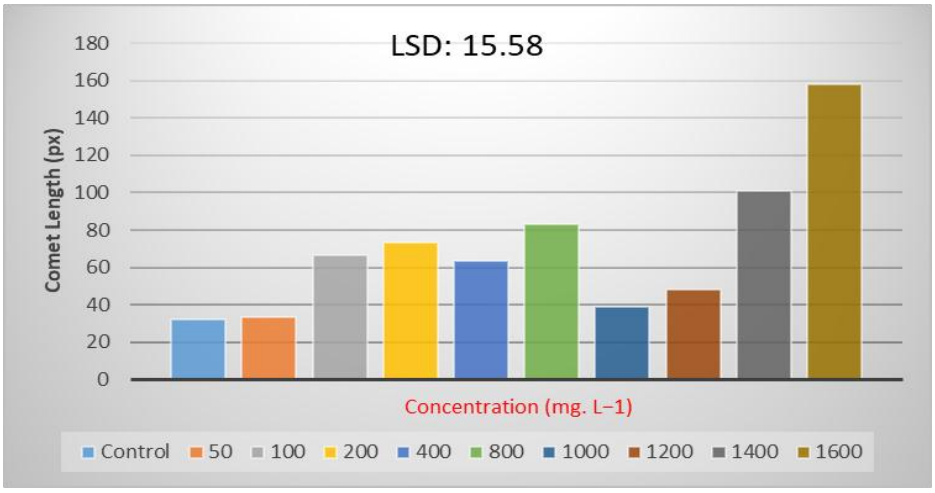

Fig. 12. Comparison of comet parameters comet length (px) of iron oxide nanoparticles NPs, and control of *K. persicum*.

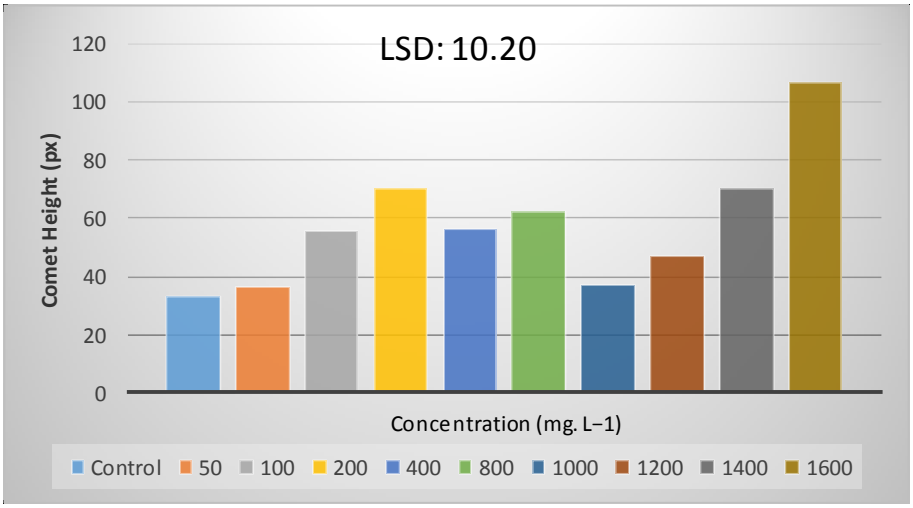

Fig. 13. Comparison of comet parameters comet height (px) of iron oxide nanoparticles NPs, and control of *K. persicum.*

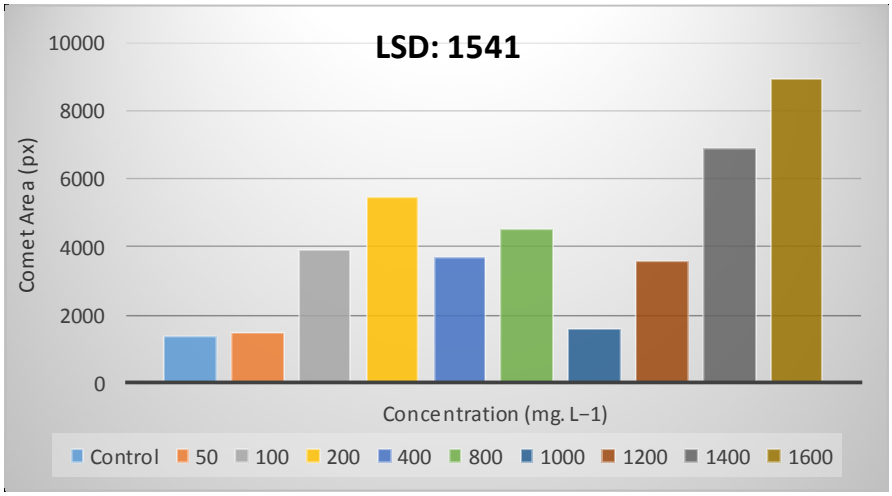

Fig. 14 Comparison of comet parameters comet area (px) of iron oxide nanoparticles NPs, and control of *K. persicum*.

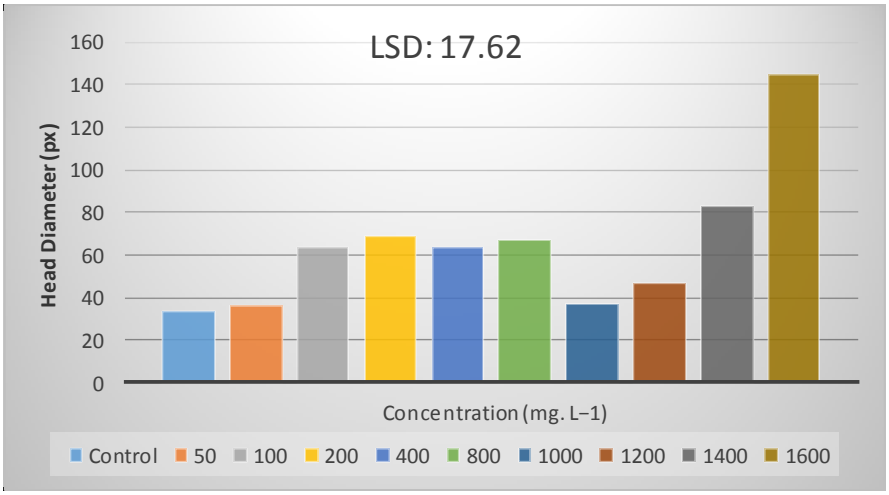

Fig. 15. Comparison of comet parameters Head Diameter (px) of iron oxide nanoparticles NPs, and control of *K. persicum.*

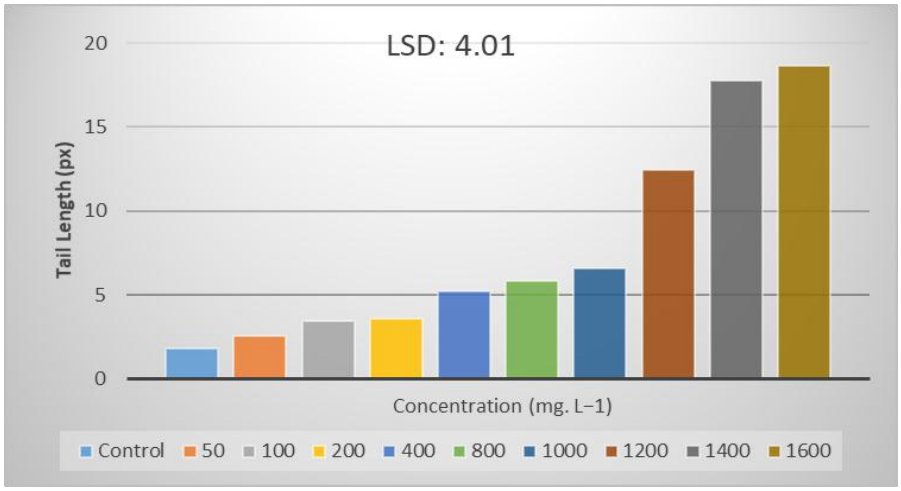

Fig. 16. Comparison of comet parameters Tail Length (px)) of iron oxide nanoparticles NPs, and control of *K. persicum.*

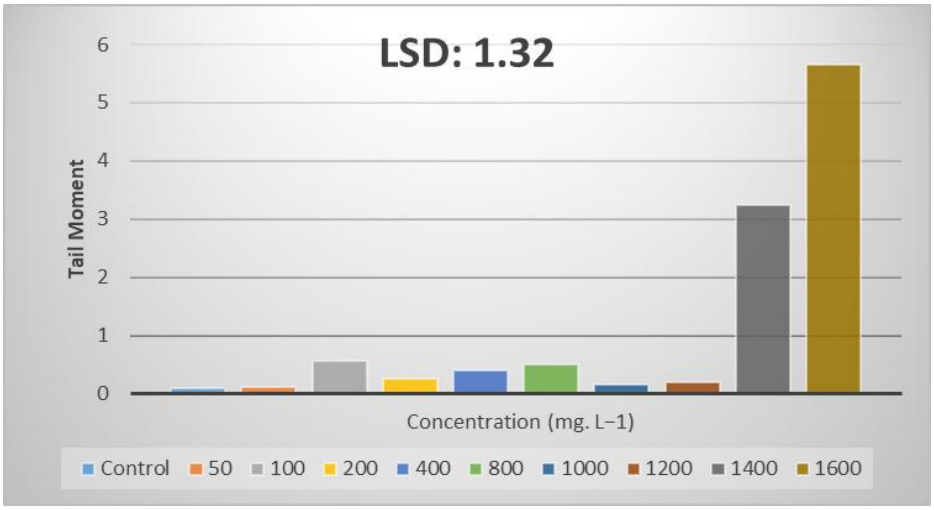

Fig. 17 Comparison of comet parameters (Tail Moment) of iron oxide nanoparticles NPs, and control of *K. persicum.*

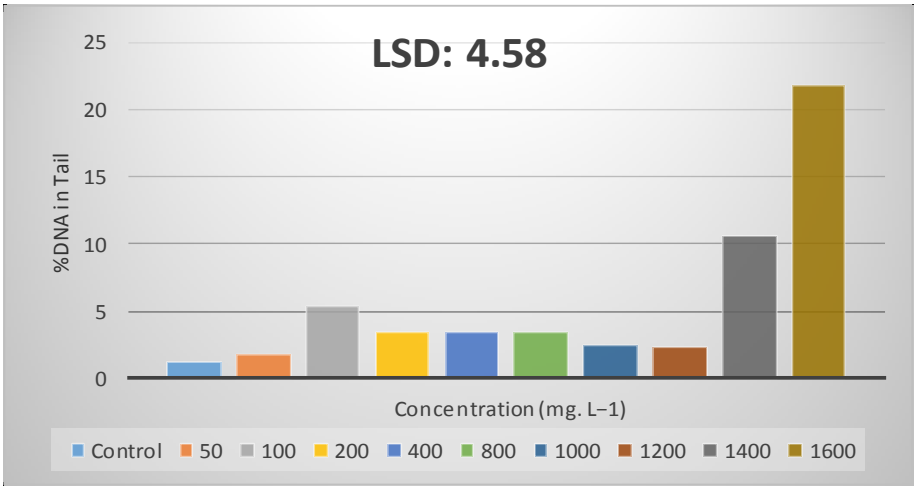

Fig. 18. Comparison of comet parameters (percentage DNA in tail) of iron oxide nanoparticles NPs, and control of *K. persicum.*

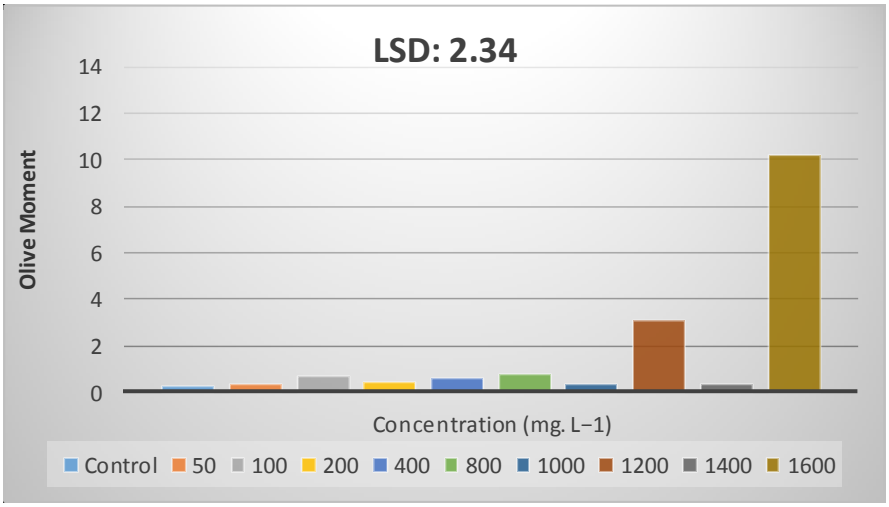

Fig. 19. Comparison of comet parameters (Olive tail moments) of iron oxide nanoparticles NPs, and control of *K. persicum*.

CONCLUSION

The iron nanoparticles showed higher toxicity to cyanobacterium *Klisinema persicum* through apparent impact on the growth rate, carotenoids contents and chlorophyll-a, in addition to the genetic toxicity which estimation by Comet assay, which is a good indication of the influence of the DNA molecule by Fe2O3 nanoparticles, especially with concentrations (100-1600) mg/L. Also, we recommend using the Comet assay test for monitoring environmental pollution levels because it is an undoubtedly valuable test for determining the genetic toxicity of chemicals.

REFERENCES

Akcha, F.; Arzul, G.; Rousseau, S. and Bardouil, M. (2008). Comet assay in phytoplankton as biomarker of genotoxic effects of environmental pollution. *Marine environmental research*, *66*(1): 59-61.

Andersen, R. A. (2005). *Algal culturing techniques*. Elsevier.

Aravantinou, A. F.; Andreou, F. and Manariotis, I. D. (2017). Long-term toxicity of ZnO nanoparticles to Scenedesmus rubescens cultivated in different media. *Scientific reports*, *7*(1): 1-11.

Aslam, M. S.; Zaidi, S. Z. J.; Toor, R. H.;Gull, I.; Iqbal, M. M.; Abbas, Z.; Tipu, I.; Ahmed, A.; Athar, M. A. and Harito, C. (2021). Interferon α2–Thymosin α1 Fusion Protein (IFNα2–Tα1): A Genetically Engineered Fusion Protein with Enhanced Anticancer and Antiviral Effect. *Materials*, *14*(12): 3318.

Barhoumi, L. and Dewez, D. (2013). Toxicity of superparamagnetic iron oxide nanoparticles on green alga Chlorella vulgaris. *BioMed Research International*, *2013*.

Chen, X.; Zhang, C.; Tan, L. and Wang, J. (2018). Toxicity of Co nanoparticles on three species of marine microalgae. *Environmental pollution*, *236*: 454-461.

Desai, S. R.;Verlecar, X. N. and Goswami, U. (2006). Genotoxicity of cadmium in marine diatom Chaetoceros tenuissimus using the alkaline Comet assay. *Ecotoxicology*, *15*(4): 359-363.

Djearamane, S.;Wong, L. S.;Lim, Y. M. and Lee, P. F. (2020). Oxidative stress effects of zinc oxide nanoparticles on fresh water microalga Haematococcus pluvialis. *Ecology, Environment and Conservation*, *26*(2): 663-668.

Guiry, M.; andGuiry, G. (2021). Algae Base. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 27 October 2021.

Haghi, M.; Hekmatafshar, M.; Janipour, M. B.; Gholizadeh, S. S.; Faraz, M.; Sayyadifar, F. and Ghaedi, M. (2012). Antibacterial effect of TiO2 nanoparticles on pathogenic strain of E. coli. *International Journal of Advanced Biotechnology and Research*, *3*(3): 621-624.

Hazeem, L. J.; Waheed, F. A.; Rashdan, S.; , M.; Brunet, L.; Slomianny, C.;Boukherroub, R. and Elmeselmani, W. A. (2015). Effect of magnetic iron oxide (Fe3O4) nanoparticles on the growth and photosynthetic pigment content of Picochlorum sp. *Environmental Science and Pollution Research*, *22*(15): 11728-11739.

Heidari, F.;Hauer, T.;Zima, J. R. H.; and Riahi, H. (2018). New simple trichal cyanobacterial taxa isolated from radioactive thermal springs. *Fottea*, *18*(2): 137-149.

Hotos, G. N.;Avramidou, D.; and Bekiari, V. (2020). Calibration Curves of Culture Density Assessed by Spectrophotometer for Three Microalgae (Nephroselmis sp., Amphidinium carterae and Phormidium sp.). *European Journal of Biology and Biotechnology*, *1*(6).

Hurtado-Gallego, J.;Pulido-Reyes, G.;González-Pleiter, M.;Salas, G.;Leganés, F.;Rosal, R.; and Fernández-Piñas, F. (2020). Toxicity of superparamagnetic iron oxide nanoparticles to the microalga Chlamydomonas reinhardtii. *Chemosphere*, *238*: 124562.

Karunakaran, G.;Jagathambal, M.;Gusev, A.;Kolesnikov, E.; and Kuznetsov, D. (2017). Assessment of FeO and MnO nanoparticles toxicity on Chlorella pyrenoidosa. *Journal of Nanoscience and Nanotechnology*, *17*(3): 1712-1720.

Ko, K.-S.;Koh, D.-C.; and Kong, I. C. (2018). Toxicity evaluation of individual and mixtures of nanoparticles based on algal chlorophyll content and cell count. *Materials*, *11*(1): 121.

Ko, K.-S.; andKong, I. C. (2014). Toxic effects of nanoparticles on bioluminescence activity, seed germination, and gene mutation. *Applied microbiology and biotechnology*, *98*(7): 3295-3303.

Kranzler, C.; Lis, H.; Finkel, O. M.; Schmetterer, G.; Shaked, Y. and Keren, N. (2014). Coordinated transporter activity shapes high-affinity iron acquisition in cyanobacteria. *The ISME journal*, *8*(2): 409-417.

Kumari, S.;Rastogi, R. P.;Singh, K. L.;Singh, S. P.; and Sinha, R. P. (2008). DNA damage: detection strategies. *EXCLI J*, *7*: 44-62.

Martinez, R. S.;Di Marzio, W. D.; and Sáenz, M. E. (2015). Genotoxic effects of commercial formulations of Chlorpyrifos and Tebuconazole on green algae. *Ecotoxicology*, *24*(1): 45-54.

Moreno-Garrido, I.;Pérez, S.; and Blasco, J. (2015). Toxicity of silver and gold nanoparticles on marine microalgae. *Marine environmental research*, *111*: 60-73.

Oukarroum, A.; Polchtchikov, S.; Perreault, F.; and Popovic, R. (2012). Temperature influence on silver nanoparticles inhibitory effect on photosystem II photochemistry in two green algae, Chlorella vulgaris and Dunaliella tertiolecta. *Environmental Science and Pollution Research*, *19*(5): 1755-1762.

Pakrashi, S.;Jain, N.;Dalai, S.;Jayakumar, J.;Chandrasekaran, P. T.;Raichur, A. M.;Chandrasekaran, N.; and Mukherjee, A. (2014). In vivo genotoxicity assessment of titanium dioxide nanoparticles by Allium cepa root tip assay at high exposure concentrations. *PloS one*, *9*(2): e87789.

Rasolofomanana, L. V. (2009). *Characterization of ranomafana lake water quality-antsirabe madagascar* University of Stavanger, Norway].

Rippka, R.;Deruelles, J.;Waterbury, J. B.;Herdman, M.; and Stanier, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Microbiology*, *111*(1): 1-61.

Sadiq, I. M.;Pakrashi, S.;Chandrasekaran, N.; and Mukherjee, A. (2011). Studies on toxicity of aluminum oxide (Al2O3) nanoparticles to microalgae species: Scenedesmus sp. and Chlorella sp. *Journal of Nanoparticle Research*, *13*(8): 3287-3299.

Saxena, P. (2018). Nanoecotoxicological reports of engineered metal oxide nanoparticles on algae. *Current Pollution Reports*, *4*(2): 128-142.

Schiavo, S.; Oliviero, M.;Miglietta, M.; Rametta, G. and Manzo, S. (2016). Genotoxic and cytotoxic effects of ZnO nanoparticles for Dunaliella tertiolecta and comparison with SiO2 and TiO2 effects at population growth inhibition levels. *Science of the total environment*, *550*: 619-627.

Schwab, F.;Bucheli, T. D.;Lukhele, L. P.;Magrez, A.;Nowack, B.;Sigg, L. and Knauer, K. (2011). Are carbon nanotube effects on green algae caused by shading and agglomeration? *Environmental science & technology*, *45*(14): 6136-6144.

Siddiqui, M. H. and Al-Whaibi, M. H. (2014). Role of nano-SiO2 in germination of tomato (Lycopersicum esculentum seeds Mill.). *Saudi journal of biological sciences*, *21*(1): 13-17.

Theivasanthi, T. and Alagar, M. (2010). X-ray diffraction studies of copper nanopowder. *arXiv preprint arXiv:1003.6068*.

Wu, Y.;Wang, Y.-j.;Li, Y.-w.; Du, J.-g.;Wang, Z.-h. and Deng, S.-h. (2018). Effects of single-walled carbon nanotubes on growth and physiological characteristics of Microcystis aeruginosa. *Journal of Central South University*, *25*(7): 1628-1641.

Zavřel, T.;Sinetova, M. A. and Červený, J. (2015). Measurement of chlorophyll a and carotenoids concentration in cyanobacteria. *Bio-protocol*, *5*(9): e1467-e1467.

Zhang, H.; Chen, Z. and Huang, Q. (2020). Study of the toxicity of ZnO nanoparticles to Chlorella sorokiniana under the influence of phosphate: spectroscopic quantification, photosynthetic efficiency and gene expression analysis. *Environmental Science: Nano*, *7*(5): 1431-1443.