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THE EFFECTS OF LOW-DENSITY POLYETHYLENE MICROPLASTICS ON BLUE GREEN ALGA *SPIRULINA PLATENSIS*

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Abstract: Microalgae play a critical role in the food web and biogeochemical cycling and produce compounds that are commercially exploited. However, their reactions and responses to microplastic contamination are not well understood. In this study, the widely distributed and commercially important cyanobacterium, Spirulina (Arthrospira platensis), was exposed to different concentrations (10, 20 and 30 mg/l) of low-density polyethylene microplastics over a 14-days period. Growth rate and photosynthetic activity decreased with increasing microplastic concentration, and an inhibition ratio was calculated from optical density measurements. In addition to, plastic concentrations caused oxidative stress and the intracellular production of SOD enzyme.

The microalga species Spirulina platensis Gomont was cultivated in Zarrouk media enriched with different low density polyethelene (LDPE) concentrations (10, 20, and 30mg/l) to study the effects of LDPE on growth rate, doubling time, chlorophyll, and SOD content.

The (maximum: minimum) range of the growth rate (0.31: 0.18cells/ml), and chlorophyll content (22.55: 19:88mg/l) were recorded in (control (0): 30mg/l) LDPE, respectively. While, the (maximum: minimum) range of the doubling time (18.35: 11.86 days) were recorded in (control(0): 30mg/l) LDPE, respectively. The maximum content of SOD was 15.25 unit/l in 25mg/l of LDPE and the minimum content of it was 14.84 unit/l in control (0mg/l) of LDPE.

Introduction

Due to further technical and medical advancements, plastics offer a variety of advantages for society in daily life. Nevertheless, plastic consumption contributes to environmental pollution given its poor biodegradability, improper applications, and ineffective disposal (Mariano *et a*l., 2021). The approaches of disposing of plastics results in the buildup of trash in landfills and natural habitats, thereby creating

physical problems for animals that eat or get tangled in plastic, leaching of chemicals from plastic products,

and the possibility of transferring chemicals to people and animals (Thompson *et al*., 2009). These events highlight the public health significance of proper disposal of plastic products. Guerrero *et al*., (2013) also identified insufficient waste management programs in most cities as the main cause of the massive amount of solid waste in the freshwater ecosystems.

Microplastics (MPs) is the general word for smaller plastic components, especially their microscopic versions that are less than 5 mm in size. Plastics that are already small, to begin with, are found in produced products such as cosmetics, detergents, drug vectors, and air-blasting media and are known as "microbeads" or primary MPs. These particles are broken down into plastic microparticles and nanoparticles by the Sun's ultraviolet (UV) radiation and by physical forces (Gewert *et al*., 2015). There are many kinds of polymers, such as polypropylene (PP), polyethylene (PE), polystyrene (PS), polycarbonate (PC), rubber, and polyvinyl chloride (PVC). Most of the microplastics result in relatively the same impact caused by exposure to a wide variety of novel chemical pollutants over the past decades. This is the result of high consumption, lack of regulation, and inadequate waste management of commercial items, and today they are viewed as ecological and health risks (de Souza Machado *et al*., 2018).

Primary producers, such as unicellular and multi-cellular algae, provide nutrients for a wide range ofbenthic species, besides serving as a food source forseveral species of aquatic and terrestrial herbivores(Carpenter, 1986; Wright *et al*., 2004). Microalgal biomass is considered a promising raw material for the production of clean and renewable energy (Perin *et al*., 2017). However, most studies have focused on the impacts of microplastics on consumers of aquatic foodchains and information about organisms at the base of the food chain is quite limited so far. Nevertheless,there are already some indications that microplastics can harm algae depending on the concentration, size,and type of polymer they are exposed to (Wagner and Lambert, 2017). Microalgae are differentiated organisms that are found in different shapes, with cell size between 0.5and 200 lm (Roy and Mohanty, 2019). The importantrole played by algae in different ecosystems, associated with the differences according to each species, justifies the great relevance of further studies on these organisms regarding their variation of responses when the culture is exposed with different pollutants presentin the aquatic environment (Almeida *et al*., 2019), including microplastics. Considering that any potential toxic effects on microalgae can cause damage to organisms of higher trophic levels, the influence ofmicroplastics in algae deserves further attention (Besseling *et al*., 2014; Wan *et al*., 2018).

Aim of The Study

1. Cultivation of cyanophyta species *S. platensis* in Zarrouk media in order to obtain a bulk of biomass.
2. Investigate the effects of different concentrations of microplastics (low density polyethelene) on *S. platensis* by the determination of growth rate, doubling time, chlorophyll content, and SOD activity.

Literature Review

Factors influencing MPs bioavailability:

Size

MPs are bioavailable due to their small size. Because of their small size, MPs can be mistaken by natural predators during regular feeding activities and consumed passively. Certain zooplankton species consume MPs ranging in size from 0.5 to 816 m (Cole and Galloway, 2015; Desforges *et al*., 2015). **1-2-2 Density**

The bioavailability of plastic debris in the water column would be assessed by its density. Filter feeders, even suspension eaters are likely to experience sustainable, lower-density plastics on their ocean face in planktivores' surface waters, such as polyethylene (PE). For example, PE 20×28 cm long food bags

showed a well-developed biofilm within one week, and due to the neutral elasticity, these PE bags started to drain after third week under the ocean's surface (Lobelle and Cunliffe, 2011).

Affluence (abundance)

MPs are typically more prevalent in marine environments. Certain types of MPs are more abundant in certain regions, whereas other types of MPs may be abundant in other areas. According to one study, expanded polystyrene was more abundant in Eastern and South-Eastern Asia, whereas polyethylene and polypropylene were found elsewhere (Shahul Hamid *et al*., 2018). Furthermore, seasonal variation was discovered to influence MP abundance (Kang *et al*., 2015). The greater the abundance of MPs in a given environment, the greater the chance of their consumption by organisms.

Color

The color of the MPs can significantly influence their consumption by aquatic organisms. The bioavailability of MPs may be enhanced by microplastic colours, and the similarity of MPs to prey particles may increase the likelihood of consumption (Wright *et al*., 2013). Only a few studies have looked at the effect of MP colour on zooplankton. Euphausiids and copepods are important MP grazers in the North-Eastern Pacific coastal waters, where they are mostly black, red, and blue in colour (Desforges *et al*., 2015). **1-2-5 Shape**

MPs can be introduced into the environment directly as cylindrical beads used in the treatment of sewage in treatment plants, in clothes-washed fibers and cosmetic products (Thompson, 2015; Napper and Thompson, 2016). MPs in the form of shaped components can be found improperly due to the weathering and deterioration of large plastic materials. A recent study has discovered that zooplankton *Calanus finmarchicus*easily consume microbeads, including microplastic fragments of size less than 30 mm (Vroom *et al*., 2017).

Sources of MPs

Several factors, including transport, dispersion, and deposition mechanisms, influenced the movement of pollutants in the atmosphere as shown in Figure (11). The movement of airborne MPs in the atmosphere is mainly influenced by these factor (Allen *et al*., 2019). Ambient wind flow and direction dominate the transport process. Deposition of the airborne MPs is mainly influenced by precipitation, scavenging, and sedimentation. While the local turbulence or disturbances are the main causes of dispersion (Kaya *et al*., 2018). The entire movement, including transport, dispersion, and deposition processes, is facilitated by the size, shape, and length of the MP particles (Zhou *et al*., 2017). The atmosphere is the main pathway for MPs transportation (Zhang *et al*., 2019). Airborne MPs must be transported by suspension due to their small particle sizes (Abbasi *et al*., 2019). Before being deposited on the ground, MP in the atmosphere can travel great distances (Dris *et al*., 2015). All environmental compartments, including freshwater, terrestrial, and the atmosphere, are affected by MP pollution (Bergmann *et al*., 2019). In addition to the shape and size of the MPs, meteorological conditions such as rain, snow, temperature, humidity, air pressure, and wind speed also impact the transport and deposition of MPs (Zhang et al., 2020; Hitchcock, 2020). According to Allen *et al*., (2019), the drivers of MP deposition in the remote regions of Pyrenees Mountains were suggested to be rain and snow. The deposition of MPs is significantly impacted by precipitation (Prata, 2018).

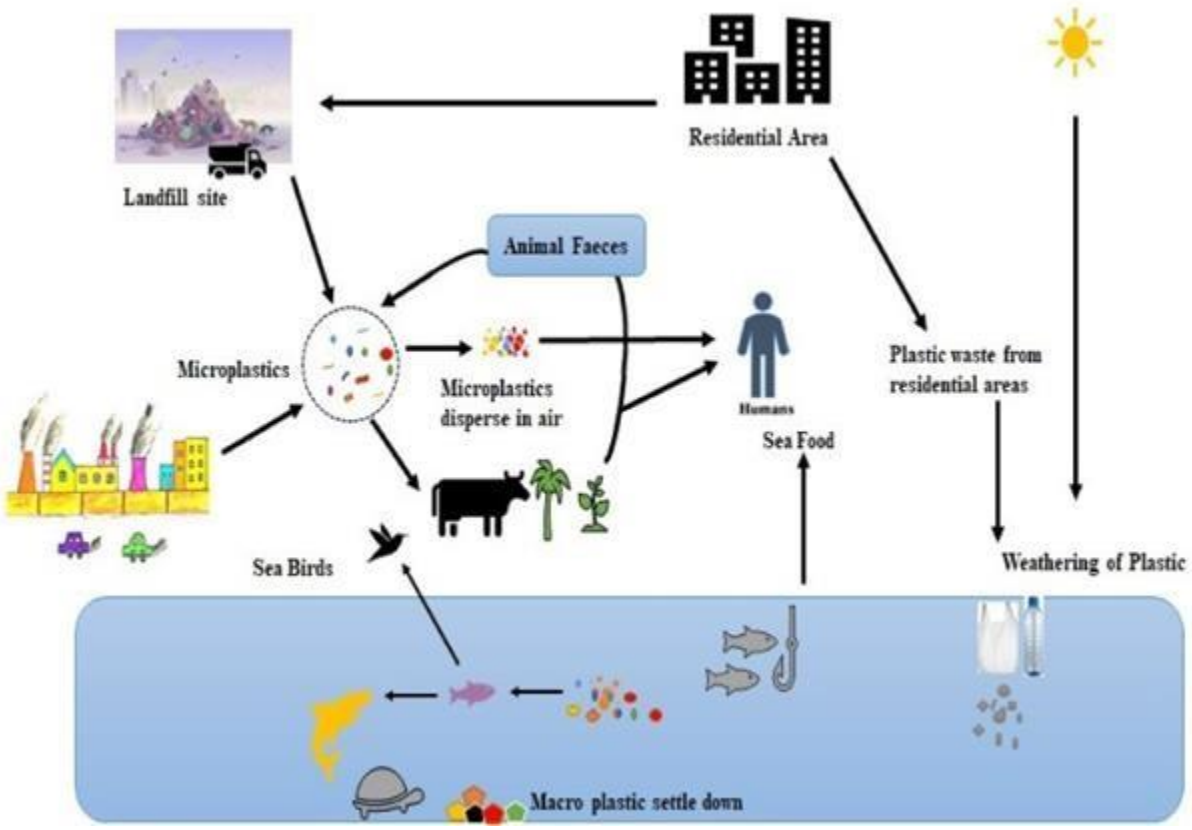


Figure (1): Sources, transport and disposition of MP (Ahmad *et al*., 2023)

Regarding origin, MPs fall within two categories: primary and secondary. Primary MPs are plastics that were industrially manufactured to be that size and they are found in textiles, sandblasting media, medicines, and such personal care products as facial and body scrubs (Cole *et a*l., 2011; Sundt *et al*., 2014; Browne, 2015). These particles enter the environment via 'leakage' during manufacture, transportation or use (Andrady, 2017). Secondary MPs, more abundant in the environment, mostly originate from the fragmentation of larger plastic liter (macro and meso plastics) but also from usual everyday processes such are laundering of fabrics and use of agricultural mulch plastics (Kyrikou and Briassoulis, 2007; Browne *et al*., 2011; Andrady, 2017). Plastics can be fragmented into MPs and subsequently NPs by abiotic and biotic processes. A solitary MPs will break down into billions of NPs particles suggesting that NPs pollution at one point become relevant across the globe (Yee *et al*., 2021). Generally, abiotic degradation precedes biodegradation and is initiated thermally, hydrolytically, or by UV light in the environment (Andrady, 2011; Yee *et al*., 2021). Environmental bacteria and other microorganisms can biodegrade MPs by the action of either intracellular or extracellular depolymerases (Liu *et al*., 2010).

Methods of identification of MPs

MPs can be identified using both physical and analytical/instrument-based methods. Instrument-based methods are more accurate and reliable. Table (1-1) depicts the methods of identification as well as their characteristics and disadvantages.

Table (1): Methods of identification of MPs

Method	Identification basis	Feature	Drawback
Visual identification	Characterizes the morphological and physical appearance of MPs	Usually carried to examine the big size range of MPs	It is a time-consuming technique as well as not accurate as compared to alternative approaches
Microscopy	Provides the data regarding surface quality, structural information, etc	Used for the identification method of MPs of the size ranges from 100 microns	Drawback of the microscopy includes poor separation of the light sediment particles in sediment sample
C:H:N ratio analysis	Based on the density and C:H:N ratio analysis, nature of the MPs can be detected	Recognize the type or origin of the plastic material	Takes more time for analysis, cannot analyse more samples, not applicable for the identification of the smaller particles
Thermal analysis	Based on the thermal stability of the material	Measurement of changes in the physical and chemical properties of the material in the thermal environment	The analysis of the certain minimum size of the particles, this results in the lower size limit of particles
Raman spectroscopy	Help in the analysis of microscopic plastic pieces by focusing a laser beam on a small spot to obtain Raman spectra.	Identifies plastic as well as provides a chemical composition of the polymer provides a contactless analysis of the sample	Sensitive to additive and pigment chemicals that interfere with MPs, which interfere with the identification of polymer types
Fouriertransform infrared spectroscopy (FTIR)	FTIR, in combination with the MP hunter software, proved to be a quick and accurate method of automatically identifying microplastics.	Provides information about the polymer	Time-consuming technique for the identification of the large samples

Toxicity of MPs

Microorganisms, birds, and animals in the aquatic environment uptake MPs particles due to their small size, lightweight, durability, and stability and ending up in the human body through the food items which increase the human health risk (Fu *et al*., 2020). MPs are found to have a large affinity to adsorb toxicants such as endocrine-disrupting compounds (EDCs), heavy metals, dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ethers (PBDEs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAH), and polychlorinated biphenyls (PCBs) due to large area/volume ratio and hydrophobic nature (Mammo *et al*., 2020). Moreover, a large number of studies reported the presence of MPs in more than 200 types of aquatic beings. Besides, the presence of MPs is reported in the digestive

tracts of invertebrates and vertebrates (Ma *et al*., 2020). Besides, different food items and bottles for sea salt, sugar, honey, milk, and drinking water were reported to contain MPs materials in significant amounts.

Apart from its interaction via entanglement and ingestion of MPs, plastics may contain stabilizers, plasticizers, and dyes that could leach, on weathering or aging of plastics, which could affect the biological activities of the zooplankton. Additionally, studies have shown that MPs in association with certain pollutants, owing to the interaction with inorganic and organic compounds, through adsorption is important determinant of the degradability, bioavailability, fate, toxicity, and dispersal, which could lead to adverse implications at various levels of biological organization. At the molecular level, reactive oxygen species (ROS) levels rise as a result of increased oxidative stress, and inflammation due to the ingestion of MPs inside the body. Studies have reported that excessive ROS production causes oxidative damage to biomolecules such as lipids, proteins, and DNA (Solomando *et al*., 2020; Tagorti and Kaya, 2022). Further, Imhof *et al*. (2017) reported that increased oxidative stress downregulates Hsp70 (Heat shock protein 70) gene expression, responsible for the enhanced transport of proteins in the nucleus, which, in turn impairs the DNA repair mechanism leading to DNA damage.

MPs affect the normal functioning of the organisms and may cause several organ-specific toxicities such as neuronal, digestive, reproductive, and developmental toxicity (Yin *et al*., 2021). Compromised sperm quality in men and infertility problems in women have been reported among plastic industry workers. (Hougaard *et al*., 2009). Indeed, micro- and nano-particles of plastics may pose more risk to the reproductive system. Various studies have been conducted on animals in order to understand the effect of MPs on male and female fertility (Wei *et al*., 2022). Moreover, MPs may also affect the growth of offspring when the mother is exposed for a longer duration, suggesting the detrimental effects of MPs on development and growth (Hu *et al*., 2021). Therefore, further research studies are required to understand the in-depth biological effects of MPs on the reproductive and development process, as they can affect future generations.

MPs Interaction with Microalgae

Microalgae, one of the most important primary producers in aquatic ecosystems, could suffer from microplastic contamination, leading to larger impacts on aquatic food webs. Nonetheless, little is known about the toxic effects of microplastics on microalgae populations. Thus, the objective of this review was to identify these effects and the impacts of microplastics on microalgae populations based on currently available literature, also identifying knowledge gaps. Even though microplastics seem to have limited effects on parameters such as growth, chlorophyll content, photosynthesis ac- tivity and reactive oxygen species (ROS), current environmental concentrations are not expected to induce tox- icity. Even so, microplastics could disrupt population regulation mechanisms, by reducing the availability or absorption of nutrients (bottom-up) or reducing the population of predator species (top-down). Microplastics' properties can also influence the effects on microalgae, with smaller sizes and positive surface charges having higher toxicity. Therefore, more research is needed to better understand the effects of microplastics on microalgae, such as adaptation strategies, effects on population dynamics and microplastics properties influenc- ing toxicity.

Algae are frequently used throughout tested microorganisms for investigating the harmful effects of microplastics. However, various algae, both photorespiration and heterotrophic, have been extensively researched for their key responsibilities in the microbial degradation of microplastics (Amobonye et al., 2021; Miloloža et al., 2022). They are capable of removing both inorganic and organic contaminants from a diverse range of environments by soaking up, removing impurities, or metabolizing them into healthy and safe levels (Hwang *et al*., 2020; Hoffmann *et al*., 2020). They colonize the outer layer of microplastics by secreting extracellular polymeric compounds, and this colonization could well result in effectual deterioration. The existence of polymeric materials, as well as plastic wastes, encourages the generation of

extracellular polymeric compounds (Song *et al*., 2020). Several algal species are effective at microbial degradation of microplastics. These include *Phormidium lucidum*, *Oscillatoria subbrevis*, *Scenedesmus dimorphus*, diatom *Navicula pupula*, *Chlorell*a, *Spirogyra*, *Nostoc*, *Spirulina sp*., *Anabaena spiroides*, and *Navicula pupula* (Kumar *et al*., 2017; Sarmah and Rout, 2018; Hadiyanto *et al*., 2021). Bioactive compounds produced by some algae have been found to biodegrade microplastics. *Phormidium lucidum* and *Oscillatoria subbrevis*, for example, can break down easily PE and LDPE (Chia *et al*., 2020). Discostella spp., Navicula spp., Amphora spp., and Fragilaria spp. algal biofilms have been discovered to deplete LDPE, PP, and PET in the marine ecosystem(Smith *et al*., 2021). After forming a biofilm on the plastic surface, algae use the carbon available on the plastic as a feed ingredient, softening and lessening the plastic. Furthermore, species can produce extracellular polymeric compounds and enzymes, such as PETase, which degrade PET (Ali *et al*., 2021). Plastic degradation by algae remains in its early stages and requires more research.



Figure (2): The modes of action and effects of microplastics (MPs) and nanoplastics (NPs) on microalgal cells and biomass production (Abomohra and Hanelt, 2022).

Chemical Materials and Equipments.

The Equipments and Apparatus:

The equipments and apparatus were used in this study are listed in Table (2-1).

Table (2): - The equipments used in this study

The equipments & apparatus	**Company and origin**
FTIR spectroscopy	**Bruker, Tensor II, Germany**
Centrifuge	GEMMY-Taiwan
Thermometer	Germany
Vortex	Germany
Refrigerator	Gongord-Lebanon
Light meter	Gossen-England
Cooling centrifuge	Hettich-Japan
Oven	Memmert GmbH
Water bath	Memmert-Germany
Compound microscope	Olympus-Japan
Sensitive balance	Sartorius-Germany
UV-VIS spectrophotometer	UV-VIS-Germany
Magnetic stirrer	VELP-Scientieica
Autoclave	Webeco GmbH-Germany

Filter paper	Whatman
pH meter	WTW-Germany

Chemical Materials:

The chemical materials used to achieve this study listed in Table (2-2).

Table (2-2): - The chemical materials used in this study

Chemical materials	Origin
Acetone (C_3H_6O)	BDH-England
Boric acid (H_3BO_3)	FLUKA- Switzerland
Calcium chloride ($CaCl_2.2H_2O$)	BDH-England
Copper sulphate ($CuSO_4.5H_2O$)	BDH-England
Dipotassium hydrogen phosphate (K_2HPO_4)	FLUKA- Switzerland
Ferrous sulfate ($FeSO_4.7H_2O$)	BDH-England
Hydrochloric acid (HCl)	BDH-England
Manganese chloride ($MnCl_2.4H_2O$)	BDH-England
Na_2EDTA (disodium salt)	BDH-England
Phosphate buffer	BDH-England
Potassium sulfate (K_2SO_4)	BDH-England
Sodium bicarbonate ($NaHCO_3$)	BDH-England
Sodium chloride (NaCl)	BDH-England
Sodium hydroxide (NaOH)	BDH-England
Sodium molybdate ($NaMoO_4.2H_2O$)	BDH-England
Sodium nitrate ($NaNO_3$)	FLUKA- Switzerland
Zinc sulfate ($ZnSO_4.4H_2O$)	BDH-England

Materials and methods:

Algal Strain

Kits of blue-green alga, *Spirulina platensis*, were purchased from Algae Research and supply (UC San Diego, USA), SUNCOST MARINE AQUACULTURE (St. Petersburg, Florida) and HEALTHALGAE (Sweden)



(Photo 3): - Kits of blue-green alga, *Spirulina platensis*

Kit Contents:

1- Algae culture inoculum 2-Culture salts 3- Culture nutrients 4- Culture flask

Culture Kit Instructions:

1. Dissolve the salts: Pour a bag of salts into a half-liter bottle of distilled water. Shake until it dissolves.
2. Add the nutrients: Add the entire vial of the nutrients to the bottle of water.

This is now your culture media. Store it in a cool dark place.

3. Fill the culture flask with culture media: Fill to 2/3 full, ~50ml.
4. Add the inoculum: Cap the culture and shake.
5. Place in light: Dim light for 2 days. Avoid direct sunlight (culture can get too hot). A good place to start the culture the culture is next to a light source (a fluorescent bulb) with a timer (12- hour light cycle).
6. Shake as often as you can: Give the culture a little mixing at least once daily.
7. Refresh the culture: About two weeks after your culture has bloomed, discard half of the culture and refill with fresh culture media. This will keep the culture in log phase.

Microscopic Examination of *Spirulina platensis*

External morphology of the *Spirulina platensis* was observed by using a light microscope.

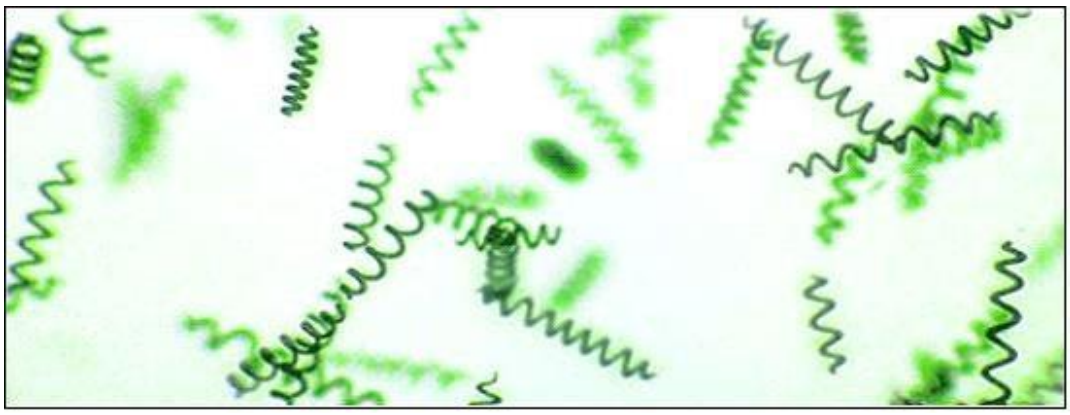


Photo (4): - Microscopic Examination of *Spirulina platensis*

Preparation of Media and Alga Cultivation for Biomass

It is essential to transfer algal strain into specific growth media to enhance and enrich its growth. For the cultivations, Zarrouk medium was used and its constituents are shown in Table (2-3) (Zarrouk, 1966). The solutions with the respective salts were sterilized separately by autoclaving at 121°C, for 15 minutes and mixed afterwards to achieve the final medium (Walter *et al*., 2011). The alga *S. platensis* cells were inoculated at a concentration of 10% ($V_{inoculation}/V_{media}$) in 500 ml Erlenmeyer flasks incubated in chemically defined Zarrouk Medium (photo 2-1). The experiment was carried out in triplicates at 32 ± 1°C, pH 9, under 135μEm^2 s^{-1} irradiance using cool white fluorescent lamps with a photoperiod cycle of 12:12 h light/dark and daily shaking by hand (Sarpal *et al*., 2016).



Photo (4): - Cultivation of algae for biomass

Table (3): - Compositions of Zarrouk media (Zarrouk, 1966)

Ingredients		Concentration (g/l)
NaCl		1.0
$CaCl_2.2H_2O$		0.04
$NaNO_3$		2.5
$FeSO_4.7H_2O$		0.01
EDTA (Na)		0.08
K_2SO_4		1.0
$NaHCO_3$		16.8
K_2HPO_4		0.5
$MgSO_4.7H_2O$		0.2
A5 micronutrient (H_3BO_3 (2.86g), $MnCl_2.4H_2O$ $ZnSO_4.4H_2O$ (0.222g), Na_2MoO_4 (0.390g), $CuSO_4.5H_2O$ (0.079g)	(1.810g),	1 ml

Preparation of Microplastics

Prepared solutions of low-density polyethylene (LDPE) at a concentration of 10, 20 and 30 mg/l of culture media was used in the present study and by using 0 mg/l as the control of the experiments.

Estimation of Growth rate

Spirulina platensis cell density was estimated with UV-Vis spectrophotometer by converting the OD at 560nm to the cell density (cells/ml) based on a linear relationship between these two parameters with the culture medium as blank (Saranraj *et al*., 2013). The specific growth rate, μ (day^{-1}), and doubling time were calculated during the exponential growth phase, according to the following equation (Fogg and Thake, 1987):

$K = 3.322 * (\log OD_t - \log OD_0) / t$

$G = 0.301 / K$

K: growth rate G: doubling time t: time

OD_0: optical density at the beginning of the experiment (zero time).

OD_t: optical density after (t) day.

Estimation of Chlorophyll

The estimation of chlorophyll was done by the method of Arnon, (1948). Algal cells were collected and resuspended in 1ml of 80% acetone. After centrifugation, the chlorophyll content of the supernatant was measured according to optical absorbance at 663nm and 645nm by using a UV-VIS spectrophotometer (Salman and Abdul-adel, 2016). The chlorophyll content was determined by the following Equation: Total chlorophyll (mg/l) = chlorophyll *a* + chlorophyll *b* = (20.2 × A645) + (8.02 × A663).

Measurement of Superoxide Dismutase (SOD)

S. platensis cells were collected by centrifugation at the rate of 4500 r/min for 20min, then the supernatant was decanted. The pellets were suspended in 0.9ml phosphate buffer (pH 7.4, 0.1mol/L) and then ground in an ice-bath for five min. Homogenized solution was centrifuged at 10000 r/min for 10min at 4C and the supernatant was used as the enzyme source for SOD spectrophotometric assay (Jia *et a*l., 2014). SOD activity assay was performed by pyrogallol autoxidation method as described by Marklund and Marklund, (1974).

Reagent:

1. Tris-buffer 50mM, pH 8.2: - this solution contains: -

Tris-base: - dissolve 0.285g of Tris-base in small amount of DW.

EDTA: - dissolve 0.111g of EDTA in small amount of DW.

After the adjustment of pH to 8.2, the volume was made up to 100ml by DW.

2. Pyrogallol: - This solution must prepared freshly. Pyrogallol solution was prepared as described below and the material should be added sequentially.

100ml of DW. , 60µl of HCl and 0.0252g of pyrogallol.

Procedure:

	Sample	Control
Enzyme source	50 µl	
Tris-buffer	1 ml	1 ml
Pyrogallol	1 ml	1 ml
D.W.		50 µl
After the addition of pyrogallol, immediately read the absorbance spectrophotometrically at 420nm against blank.		

Calculation:

$$\text{SOD activity} = \frac{\%\ \text{inhibition of pyrogallol}}{50\%\ \text{inhibition from standard}}$$

% inhibition of pyrogallol autoxidation = x 100% , where

ΔA of sample = Absorbance changes due to pyrogallol autoxidation in the sample reaction system

ΔAof control = Absorbance changes due to pyrogallol autoxidation in the control (without cell lysate)

Results and Discussion.

Effect of Low-Density Polyethylene on the Growth rate, and Doubling time of *S. platensis*

Cell growth of *S. platensis* is shown as a function of exposure time for the three different treatments. The highest growth rate was noted in the control treatment (0.31 cells/ml), followed by the 10 mg/l treatment (0.28 cells/ml), the 20 mg/l treatment (0.23 cells/ml), and the 30 mg/l treatment (0.18 cells/ml).

The shortest doubling time (G) was 11.86 days at the 10mg/l, while the longest was 18.45 days at 30mg/l treatment.

Table (4): - Effect of different NaCl concentrations on the average of growth rate (K) and doubling time (G) of *S. platensis*

LDPE Concentrations (mg\l)	Growth rate (K)	Growth rate inhibition %	Doubling time (G)
30	0.18	41.93	18.45
20	0.23	25.80	14.44
10	0.28	9.67	11.86
0	0.31		9.49

This study is in line with the findings of other studies. Ye *et al*., (2023) studied the interaction of microplastic and 12 species of microalgae and revealed growth inhibition by microplastics. He *et al*., (2022) also reported the increasing trend of growth inhibition (7−17%) in *Chlorella pyrenoidosa* during the cultivation days. Moreover, similar results were stated by Abbasi *et al*., (2023) who exposed the widely distributed and commercially important cyanobacterium, *Spirulina* (*Arthrospira platensis*), to different concentrations (1–100 mg L−1) of low-density polyethylene microplastics (<5 μm) over a 20-d period. Various end-points were combined with different microscopic techniques in order to examine physiological and biochemical effects and interactions between the plastic and microalga. Growth rate and photosynthetic activity decreased with increasing microplastic concentration, and a maximum inhibition ratio of about 9% was calculated from optical density measurements. Plastic concentrations above 10 mg L−1 resulted in oxidative stress and the intracellular production of proline.

Effect of Low Density Polyethelene on the Chlorophyll Content of *S. platensis*

Comparisons with a control, which supported 2.255mg/l of chlorophyll content showed that increasing LDPE concentration to 10, 20, and 30 mg/l affected *Spirulina platensis* by the inhibition of chlorophyll. Maximum reduction of chlorophyll was 1.988mg/l in the presence of 30mg/l LDPE.

Table (5): - Effect of different LDPE concentrations on the chlorophyll content of *S. platensis*

LDPE Concentrations (g\l)	0	10	20	30
Chlorophyll (mg/l)	2.255	2.141	2.133	1.988

Besides the effects on microalgae growth, studies have found that microplastics seem to affect algal photosynthesis, as both chlorophyll content (Prata *et al*., 2018; Zhang *et al*., 2017) and photosynthetic efficiency (Mao *et al*., 2018; Zhang et al., 2017) decreased under microplastic exposure. This possibly related to a decrease in the expression of photosynthesis genes (Lagarde *et al*., 2016), interference in substance exchange and increase in energy demand for motility due to surface adsorption of microplastics (Bhattacharya *et al*., 2010). Furthermore, microplastics may hinder photosynthesis by affecting the electron donor site, the reaction center of photosystem II (responsible for energy conversion) and the electron transport chains, also leading to electron accumulation and the production of reactive oxygen species (ROS) responsible for oxidative stress (Bhattacharya *et al*., 2010; Mao *et al*., 2018).

In agreement with that Senousy *et al*., (2023) demonstrated that LDPE-MPS showed an inhibitory effect on the growth rate of *C. calcitrans*cells and chlorophyll content, consequently, retard the photosynthesis process. Resulting in inhibition of the energy conversion and electron transport chains at PSII, causing reactive oxygen species (ROS) accumulation that are responsible for oxidative stress to the photosynthetic process and the whole algal cell.

Effect of Low-Density Polyethylene on the superoxide dismutase

Content of *S. platensis*

SOD activity increased to 14.91 and 15.25 and 14.97 unit/ml at 10, 20, and 30mg/l of LDPE as compared with control that is contain 14.84 unit/ml of SOD content (table 3-3, figure 3-3).

Table (6): - Effect of different LDPE concentrations on the average of SOD content of *S. platensis*

LDPE Concentrations (mg\l)	**0**	**10**	**20**	**30**
SOD (unit/ml)	14.84	14.91	15.25	14.97

Superoxide dismutase (SOD) is a protective enzyme used in the antioxidant enzyme system to regulate intracellular reactive oxygen radicals (ROS). SOD is widely distributed in various organisms, and its activity level can reflect the degree of oxidative stress on cells (Huang *et al*., 2020). In the absence of external influences, the antioxidant system of algal cells maintains a dynamic equilibrium. When faced with external stress, SOD is rapidly activated to mitigate the ROS as ROS accumulation. Low levels of ROS can regulate multiple physiological and biochemical reactions in cells; however, ROS is evidently toxic to organisms at high concentrations (Huang *et al*., 2020; Li ., 2022). The finding of this study agreed with Wang *et al*., (2023) who found that the SOD activity of microplastic-treated algal cells exhibited a pattern of initial increase followed by a subsequent decrease. Moreover, there was a dose–effect relationship between the concentration of microplastics and SOD activity during the later stage of the experiment, indicating a significant inhibitory effect. This implies that highly sensitive algal cells in the logarithmic phase of growth rapidly activated SOD activity within a short period of exposure, in response to the explosion of ROS also as a way to maintain a normal state (Wan, 2021). However, as time progresses, and the degree of external stress gradually increases, ROS accumulation reaches the threshold of clearing by the algal cells themselves. At this juncture, the intracellular antioxidant system was imbalanced, the antioxidant system of the organism was damaged, and the SOD enzyme activity was significantly inhibited, indicating that the cells were seriously damaged (Cao., 2022). Moreover, Senousy *et al*., (2023) found that superoxide dismutase (SOD) activity were significantly increased at 25 mg L^{-1} LDPE-MPs by 3.52 folds higher than those of the controls to sustain the adverse effects of LDPEMPs.

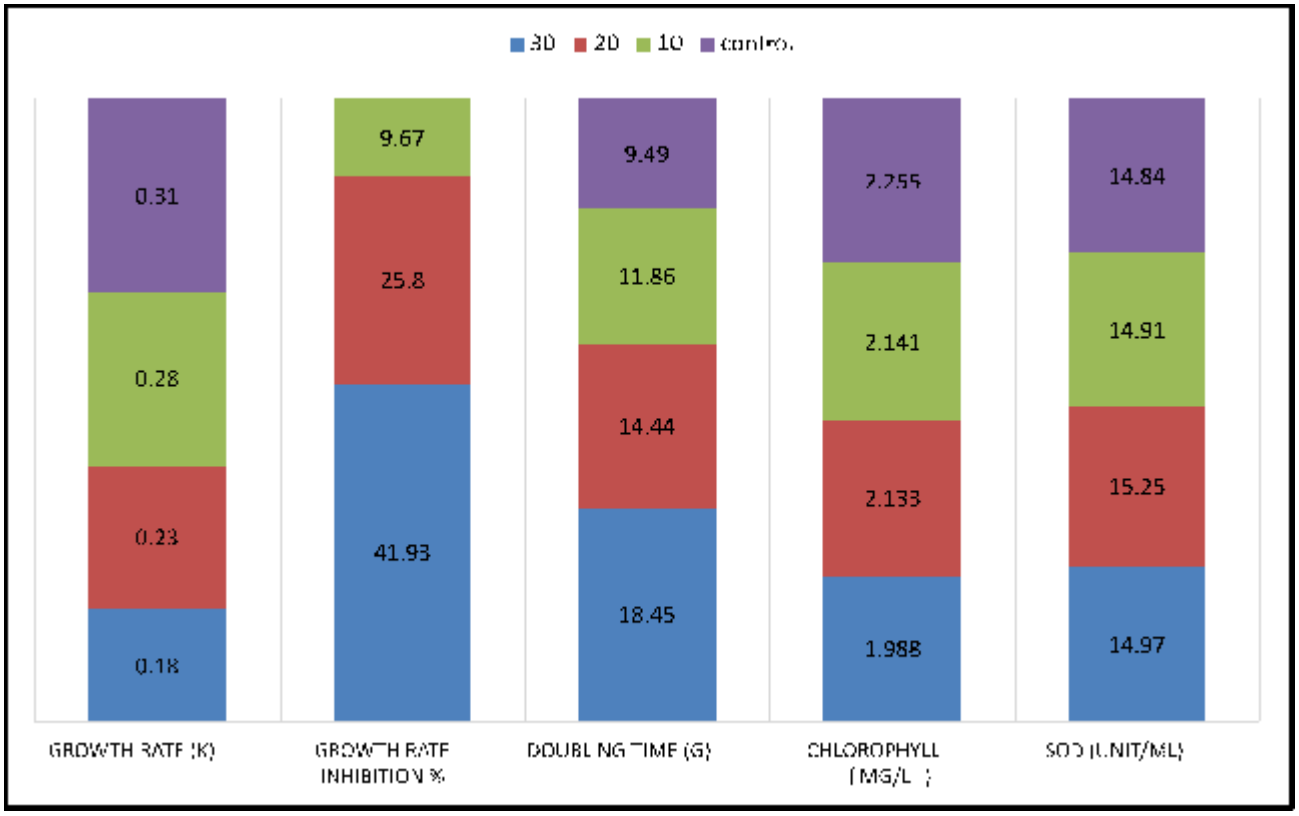


Figure (5): Growth rate, Growth rate inhibition, Doubling time, chlorophyll, and SOD content of *S. platensis* at different LDPE concentrations

Conclusion

Microplastics at concentrations in the low ppm range have negative effects on microalgae by inhibiting growth, reducing chlorophyll and photosynthesis and inducing oxidative stress.

Recommendations:

1. Examine in the combined toxicity of MPs and some other organic materials present in the aquatic environment to understand the mixed toxicity issue.
2. Study the effects of other microplastic types like polypropylene (PP), polyethylene (PE), polystyrene (PS), polycarbonate (PC), rubber, and polyvinyl chloride (PVC) on *S. platensis* or other microalgae species.
3. Using of *S. platensis* or other types of microalgae for bioplastics production.
4. Study the combined effects of other nutritional and physical stress factor on the *S. platensis* like change in culture media composition, pH, temperature and light intensity with the addition of LDPE.

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