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CAT , SOD and MDA Variations in the *Spirulina platensis*in the Presence of MPs

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Abstract

the variation of Catalase (CAT), Superoxide dismutase (SOD), and Malondialdehyde (MDA) in Spirulina platensis microalgae due to the effect of four types of microplastics (Poly Ethylene, Poly Styrene and Poly Styrene Blue). CAT content values were found highest in the case of PVC (5.582 umol/L at 250 mg/L) and the lowest in the case of PE (3.92 umol/L at 250 mg/L) with a sequence of (PVC>PSB>PS>PE).On the other hand, SOD content values were found highest in the case of PVC (19.371 umol/L at 250 mg/L) and the lowest in the case of PE (17.85 umol/L at 250 mg/L) and the sequence of SOD content was found as follow (PVC>PSB>PS>PE).A deferent trend was obtained in the case of MDA, as the activity of MDA increased significantly continually until the last day of investigation with a small decrease at the last 5 days for the single treatment of (PE, PS, PSB, and PVC) as compared to the control treatment for all the range of concentration (12.5, 25, 50, 75, 100,125, and 250 mg/L) at a significance of ($p<0.05$). On the other hand, MDA content values were found highest in the case of PVC (18.64 umol/L at 250 mg/L) and the lowest in the case of PE (13.579 umol/L at 250 mg/L) and the sequence of MDA content was found as follow (PVC>PSB>PS>PE).The result of CAT content, SOD content, and MDA content recommended that the MPs presence can promote the microalgae cell to produce extreme ROS as it's confirmed by the increase of MDA content.

Keywords: *Spirulina platensis*, Microplastic, Toxicity, CAT,SOD, MDA.

1.Introduction

Approximately, 200 million metric tons of plastic materials are presented in the ocean in addition to 8 million tons are added each year (S. Sharma et al. 2020). The plastic materials are degraded into smaller pieces that float on the surface of the water and continue to degrade into more than 5 trillion fragments of a size less than 5 mm which is called micro-plastics (MPs) material (Romera-Castillo et al. 2018). Plastic materials are polymeric chemicals with additives used daily for different uses such as nylon, polyethylene (PE), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyester (PE), polyamide (PA), acrylic, etc (A. et al. 2020). Moreover, Plastics are used in our life due to their low cost, durability, resistance to temperature, light, and moisture in different shapes such as bags for shopping, different sizes of bottles and jars, as parts of cars, ships, airplanes, …etc. (Klemeš, Fan,

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and Jiang 2020). Human consumption of plastics material is increased dramatically due to the increase in the population worldwide.The toxicity mechanism of MPs can be explained briefly as the oxidative stress in the cell of the organism due to the exposure to toxic pollutant and causes eco-toxicity to the organism and the reactive oxidative species (ROS) is required for the organism at a moderate level (Du et al. 2018; Du et al. 2020). Prokić et al., (2018) found that the organism's antioxidant defense is induced by the extreme production of ROS as a result of the oxidative stress disturbance in the organism's capacity balance to deal with that. Bartoskova et al., (2013) found that the extreme production of ROS resulting damage of lipid, protein, DNA, tissue, the permeability of cell membrane, tissue, inflammatory response, cell death, and polyunsaturated fatty acid peroxidation. Moreover, free radical production and antioxidant defense system can adjust oxidative stress and overhaul cell damage (Sayre et al. 2005). Ribeiro et al., (2017) reported the oxidative damage, neurotoxicity, and DNA damage in Scrobicularia plana after exposure to 1 mg/L MPs for 14 days. Tang et al., (2018) concluded a decrease in alkaline phosphatase and GPx, and an increase in CAT, and SOD activities due to the exposure of scleractinian corals to MPs for a different time at (6,12,24 h). Lei et al., (2018) reported also excessive production of free radicals in Danio rerio and Caenorhabditis elegans after exposure to MPs and change cellular homeostasis. Qu et al., (2018) investigated the NPs effect on the gene expression of Caenorhabditis elegans required for control oxidative stress. Tang et al., (2018) explained the immune system and detoxification after P. damicornis exposure to MPs for 12 h through the ERK and JNK signaling pathways due to oxidative stress. Avio et al., (2015) reported the alteration of DNA by increasing the micronuclei frequency of marine mussels. Magni et al., (2018) studied the effect of Dreissena polymorpha by MPs after 6 days of exposure and found that GSH and CAT activities are affected significantly. Seoane et al., (2019) proposed another mechanism that depends on cytotoxicity and found that the exposure to MPs induces the reduction of photosynthesis and esterase activity which leads to reducing the growth rate of the plant. The researches that depend on cytotoxicity, not on oxidative stress are very rare in the aquatic system and there is a gap of knowledge used to be filled in this regard. The harmful effect of MPs on organisms in the aquatic ecosystem can be understood properly using the toxicity mechanism which is important for designing safe MPs-containing products suitable for humanity daily uses.The objective of the present work, is to investigate the variation of CAT, SOD, and MDA in *Spirulina platensis* microalgae due to the effect of four types of microplastics (Poly Ethylene, Poly Styrene and Poly Styrene Blue).

2.Materials and Measuring

2.1. MPs Characterization

Items of polyvinyl chloride (PVC), polystyrene (PS) (Blue and white),andPolyethylene (PE) mainly were collected from the water samples. the shape of the items was found different and the size ranged from 300-600 nm). Simulated solutions of different MPs concentrations were prepared by suspending a suitable weight of MPs in a distilled water and dispersed uniformly in water using ultra-sonic vibration for 45 min. Solutions of (0, 12.5, 25, 50, 75, 100,125, and 250 mg/L MPs per liter of Culture media were obtained and used in the present work for comparing the effect of MPs on the Algae CAT, SOD, and MDA contents. MPs

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characterization was done using a Bruker-Tensor II FTIR spectroscopy instrument, Germany witha resolution of 4 cm^{-1} in the mid-range of 4000-400 cm^{-1}. All chemicals used in the present study were analytical reagents.

2.2. Microalgae Collection, Isolation and Purification

Spirulina platensis microalgae samples were collected from different aquatic environments in Al-Dywaniah governorate, Iraq using clean glass bottles and washed with tap water then with sterile distilled water and finally kept in a clean, sterile glass bottle for further investigation, isolation, and purification of microalgae species. To obtain a single algae culture, the method of agar streaking proposed by Stein (1973) (Andersen 2005) was used using a sterile pipette of 1 ml volume by taking 0.1 ml of the contaminated sample containing algae and placed at one of the edges of the Petri dish containing the nutrient medium solidified with the agar and prepared previously and then by inoculum Loop is sterilized with fire using a Burner lamp, cooled with the tip of the plate and then used to spread the algae sample in the form of lines on the dens in all directions, and then the dishes will be incubated upside down to prevent the occurrence of water droplets in the light incubator under appropriate conditions of light and temperature until the algal growth will happen. Moreover, the plate is wrapped with Parafilm tape to prevent the algae from drying out because the algal growth will take several days. After the growth appears, a sample is taken from the edge of the plate containing the target algae, and the streaking method is repeated more than once until a mono-alga culture is obtained.To obtain an axenic culture from the microalgae species, the density gradient centrifugation method was used, whereby 10 mL of liquid from the single algae culture that used to be purified was placed in a 15 mL thick-walled centrifuge tube and placed in the centrifuge machine at 3000 rpm for 5 min and the filtrate was removed, then washed with sterile distilled water and the process was repeated at least 12 times (Wiedeman et al., 1964). To ensure that the culture is free of bacteria and fungi, the final examination was conducted, which includes planting a sample of the algae culture on the special nutrient agar medium used for testing bacteria, and it was incubated at 37oC for 72 hours to ensure its purity (Andersen 2005).

2.3. Algae Cultivation

Spirulina p. (blue-green microalgae) are chosen for the study. Zarrouk culture media at pH of 10 are used for microalgae culturing at the conditions of (temperature: 29±1.5^{o}C, light intensity: 3000 Lux, optical density at initial: 0.05, the cycle of cultivation: 32 days and continuous aeration). A solution of 250 mL of microalgae was placed in an Erlenmeyer flask under light intensity (as explained in the culturing conditions) and shaken 4 times daily to prevent algae from settling.

2.4. Toxicity of MPs

The toxicity of MPs was estimated by measuring the activities of CAT, SOD enzymes, and MDA. 10 mL of algal culture sample was taken into centrifugation at 8000 rpm for 10 min to extract the algal cells. Then, algal cells were suspended in a phosphate buffer solution of pH=7 and placed in an ice bath for sonication stroke at each 5 s for 5 min to get the solution homogenized. Finally, the solution

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was placed in a centrifuge until getting the supernatant and use for measuring the activities of enzymes. CAT activity was tested through the change of absorbance as a result of decomposition in H2O2 using the spectroscopic method according to (Flerova, Bogdanova, and Flerova 2014). 0.1 mL of the supernatant was added to 2 ml of H2O2 solution (0.03%) as a sample for testing the CAT enzyme activity, while 0.1 mL of double distilled water was added to the blank sample and the reaction was stopped by adding 1 mL of ammonium molybdate (4%) after 10 min. Finally, the intensity of the mixture was tested at a wavelength of 410 nm against the control sample that containing water (2 mL) instead of H2O2. The Giannopolitis method is used for investigating the activity of the SOD enzyme (Giannopolitis and Ries 1977). Three identical tubes were taken two of them containing microalgae extract and reaction mixture of (0.9 mL phosphate buffer, 20 µL EDTA (24%), and 0.5 mL NBT (0.05%)) and one of them placed in the light, and the other in the darkness, while, the third one is control tube containing 100 µL of phosphate buffer and the reaction mixture. The reaction in the three tubes was initiated by adding 20 µL of riboflavin and continue for 15 min, while the optical density was tested at wavelength 410 nm using UV-spectrophotometer. The thiobarbituric acid (TBA) method was used for measuring the MDA content (Sabatini et al. 2009). In the case of MDA calculation, microalgae extract was suspended in a fresh reagent solution of TBA and without TBA, then the solutions were placed in a water bath at 95oC for 40 min. The absorbance at a wave length of 440, 532, and 600 nm was recorded for the supernatant after clearing. Finally, the amount of MDA was found as nano moles of MDA/106 cells using Hodges'equation (Hodges et al. 1999).

2.7. Statistical Analysis

Statistica 12 program is used for analyzing the control and experimental data in the present work. Based on the assumption of normality and homogeneity, the significant differences in the group tests were examined using the ANOVA analysis method with a standard level of confidence at 95%.

3. Results and discussion

3.1. CAT, SOD, and MDA Variation

Hydroxyl radical (HO•), hydrogen peroxide (H2O2), or superoxide (O2−) are reactive oxidative species that are produced in higher quantity and accumulated in the cell after exposing the algal cell to the MPs (Piddington et al. 2001). The peroxidation damage of lipid (LPD) has been detected due to the increasing ROS production which leads to an increase in the MDA byproduct of the LPO process. Moreover, lipid peroxidation (LPO) in the microalgae cell membrane is prompted and the MDA content is increased due to the high levels of the ROS. Also, CAT and SOD are produced as antioxidants enzymes and a part of the defense system in battle with ROS in the microalgae cell (Winston and Di Giulio 1991).

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3.2. CAT Variation

Figures 1-4 shows the variation of CAT activities with exposure time. In figures 1-4, it was found that the activity of CAT increased significantly until day 15, then start to decrease in the last 5 days for the single treatment of (PE, PS, PSB, and PVC) as compared to the control treatment for all the range of concentration (12.5, 25, 50, 75, 100,125, and 250 mg/L) at a significance of ($p<0.05$). Generally, CAT content values were found highest in the case of PVC (5.582 umol/L at 250 mg/L) and the lowest in the case of PE (3.92 umol/L at 250 mg/L) with a sequence of (PVC>PSB>PS>PE).

3.3. SOD Variation

Figures 5-8 show the variation of SOD content with the exposure time to MPs. On one hand, the same scenario was found for the SOD content as in the case of CAT content. The activity of SOD increased significantly until day 15, then start to decrease in the last 5 days for the single treatment of (PE, PS, PSB, and PVC) as compared to the control treatment for all the range of concentration (12.5, 25, 50, 75, 100,125, and 250 mg/L) at a significance of ($p<0.05$)). On the other hand, SOD content values were found highest in the case of PVC (19.371 umol/L at 250 mg/L) and the lowest in the case of PE (17.85 umol/L at 250 mg/L) and the sequence of SOD content was found as follow (PVC>PSB>PS>PE).

3.4. MDA Variation

Figures 9-12 show the change of MDA content in microalgae cells with exposure time to MPs. A deferent trend was obtained in the case of MDA, as the activity of MDA increased significantly continually until the last day of investigation with a small decrease at the last 5 days for the single treatment of (PE, PS, PSB, and PVC) as compared to the control treatment for all the range of concentration (12.5, 25, 50, 75, 100,125, and 250 mg/L) at a significance of ($p<0.05$). On the other hand, MDA content values were found in the case of PVC exposure (18.64 umol/L at 250 mg/L) and the lowest in the case of PE (13.579 umol/L at 250 mg/L) and the sequence of MDA content was found as follow (PVC>PSB>PS>PE). Alimi et al., (2018) showed that the smaller size of MPs is more damaging to the cell membrane receptors than the larger one by the oxidative effect. Moreover, a statistical linear relationship was concluded between the change in SOD and

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CAT content for all the periods of investigation at significance ($p<0.05$) to remove the extreme ROS. The result of CAT content, SOD content, and MDA content recommended that the MPs presence can promote the microalgae cell to produce extreme ROS as it's confirmed by the increase of MDA content. ROS production can cause damage to the receptors of the microalgae cell and decrease the activity of photosynthesis and microalgae cell growth (Prakash et al. 2020). Moreover, SOD and CAT content also was induced due to the ROS effect on the membrane of the microalgae cell. Xu et al., (2020) reported that the above effects depend on some factors such as microalgae species, MP's surface structure, functional group, chemical characteristics, size, and type. The results of the statistical analysis showed that there were significant differences between the MPs concentrations and the microalgae CAT, SOD, and MDA content, in addition to the existence of a correlation between the MPs concentrations for one day at the level of 0.05, meaning that ($P \leq 0.05$).

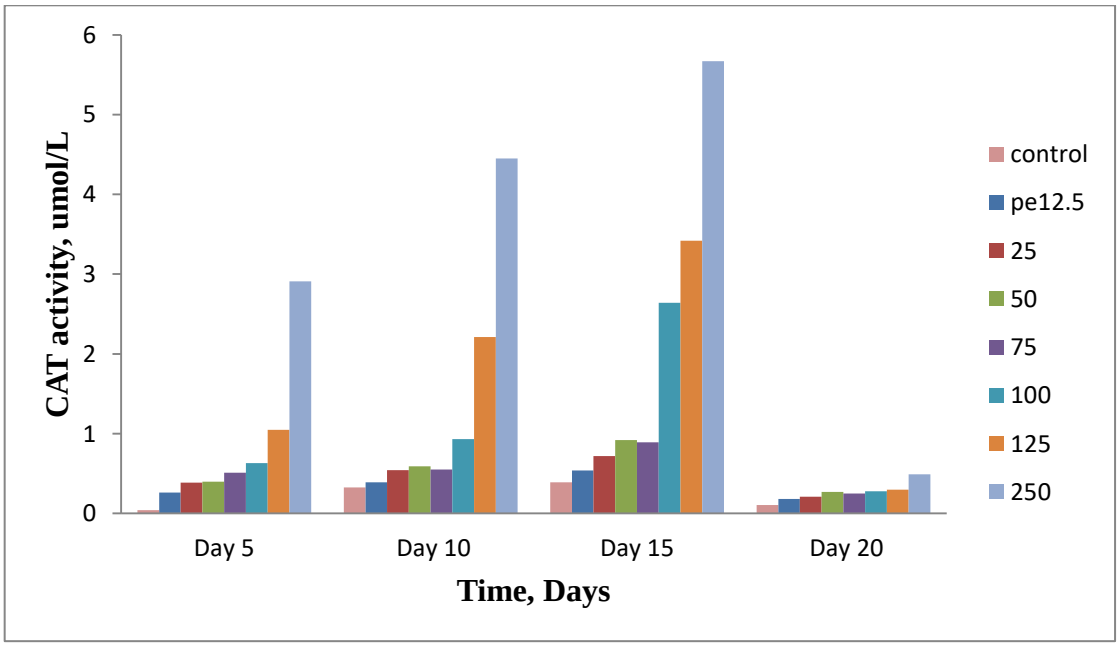

Figure 1Variations of the CAT content for *S. platensis* with culture time at different PE concentrations (12.5, 25, 50, 75, 100, 125, and 250 mg/L).

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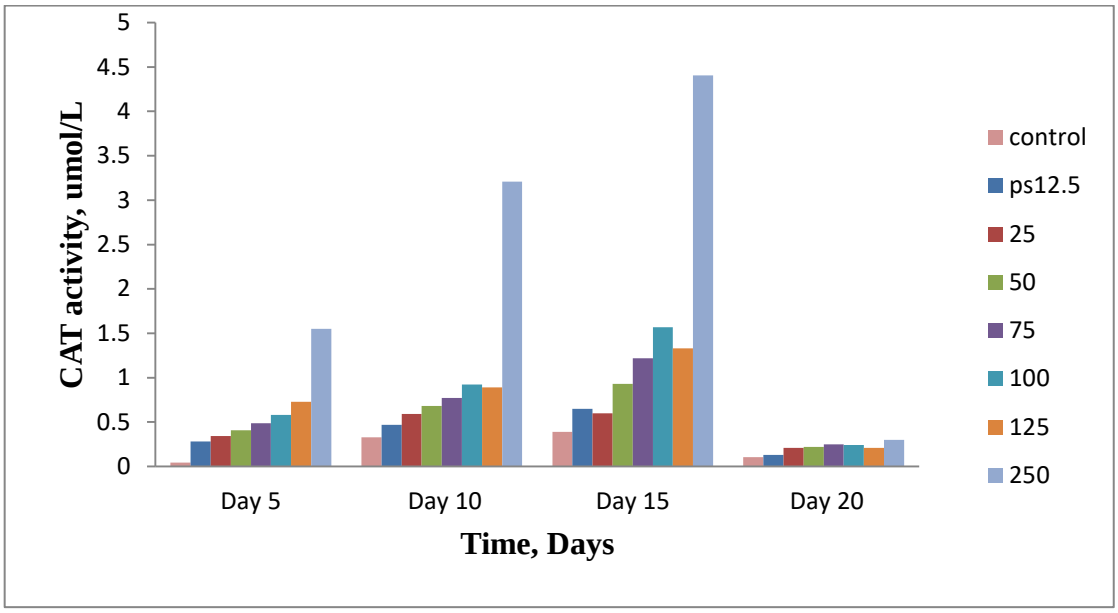

Figure 2 Variations of the CAT content for *S. platensis* with culture time at different PS concentrations (12.5, 25, 50, 75, 100, 125, and 250 mg/L).

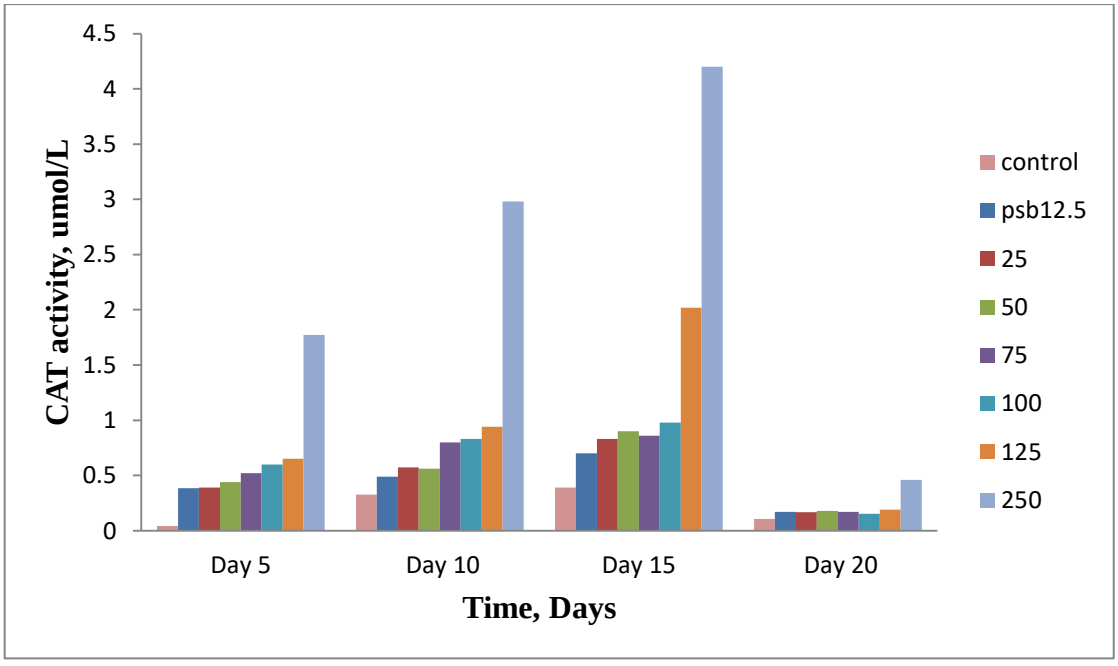

Figure 3 Variations of the CAT content for *S. platensis* with culture time at different PSB concentrations (12.5, 25, 50, 75, 100, 125, and 250 mg/L).

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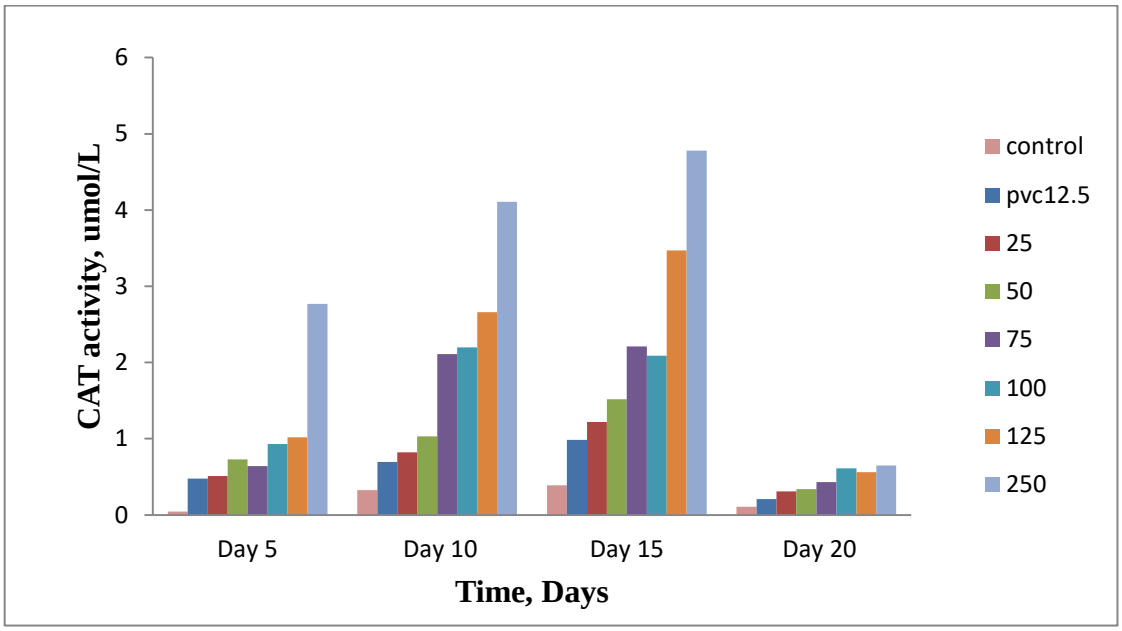

Figure 4 Variations of the CAT content for *S. platensis* with culture time at different PVC concentrations (12.5, 25, 50, 75, 100, 125, and 250 mg/L).

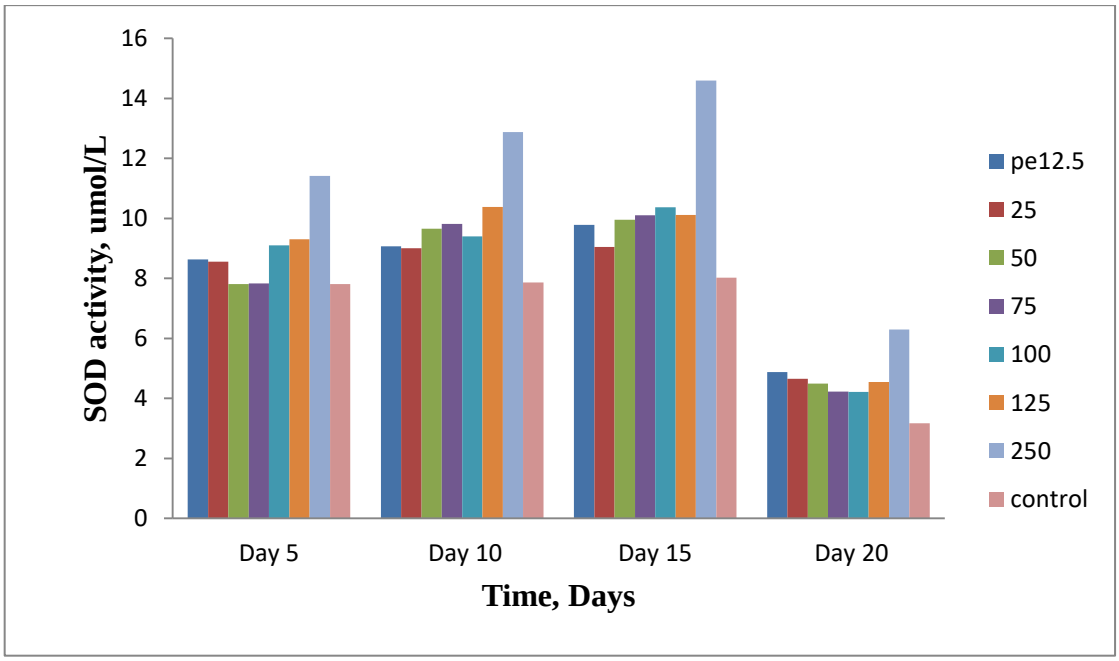

Figure 5 Variations of the SOD content for *S. platensis* with culture time at different

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PE concentrations (12.5, 25, 50, 75, 100, 125, and 250 mg/L).

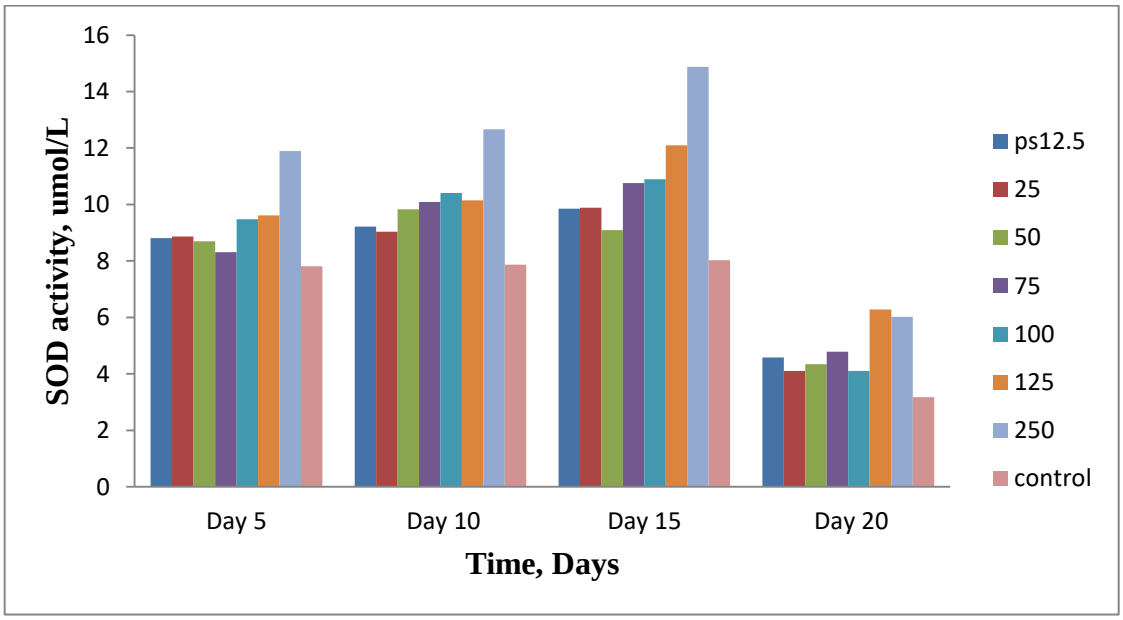

Figure 6 Variations of the SOD content for *S. platensis* with culture time at different PS concentrations (12.5, 25, 50, 75, 100, 125, and 250 mg/L).

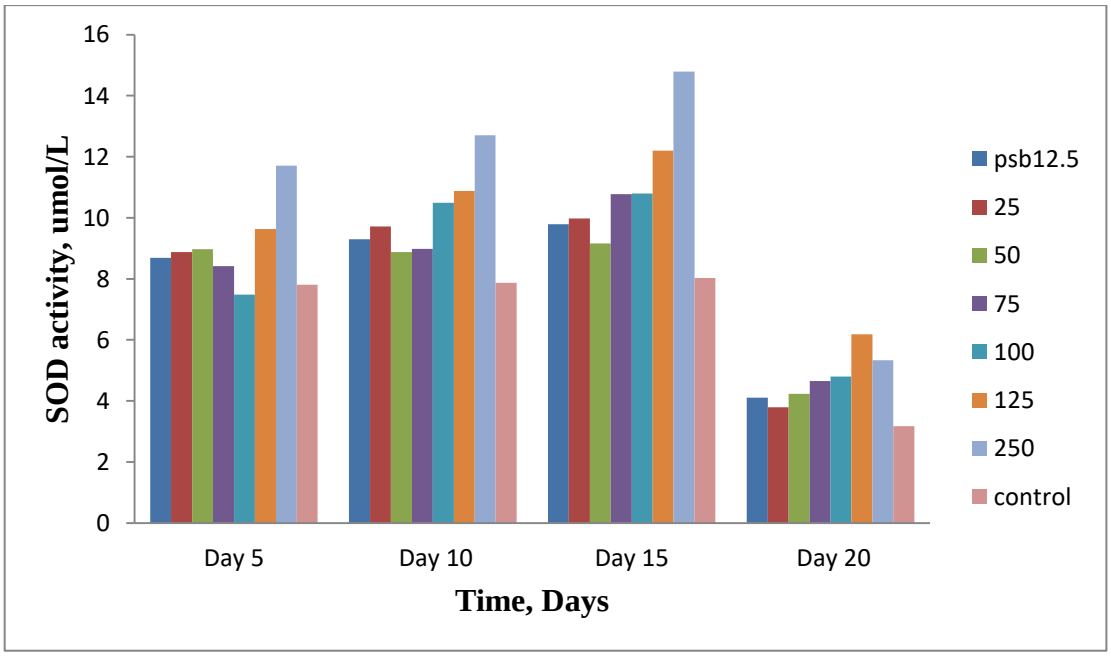

Figure 7 Variations of the SOD content for *S. platensis* with culture time at different PSB concentrations (12.5, 25, 50, 75, 100, 125, and 250 mg/L).

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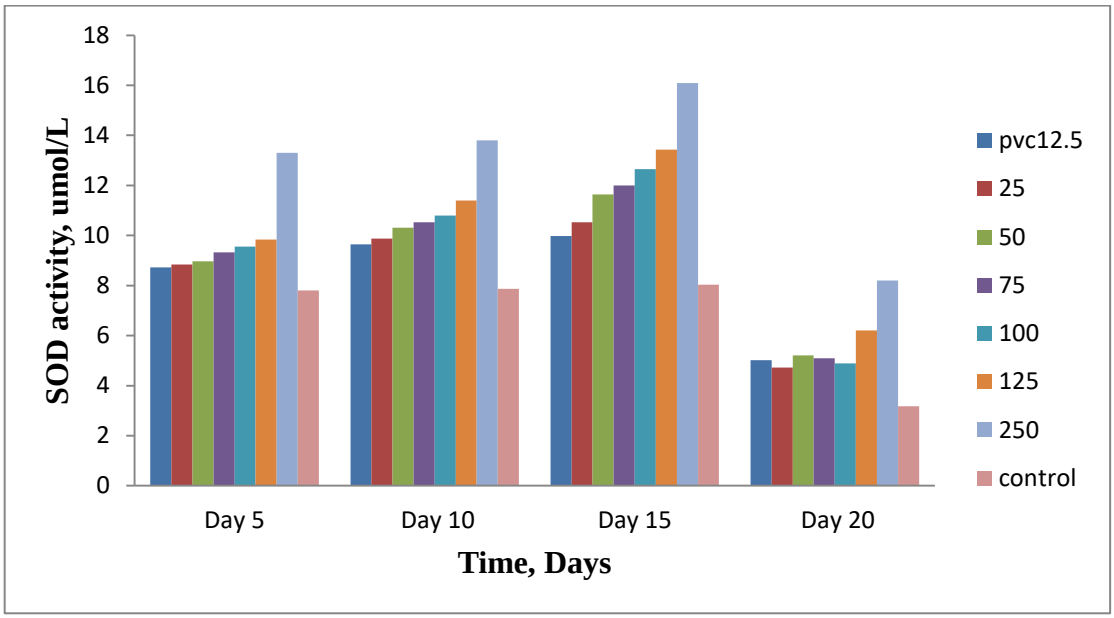

Figure 8 Variations of the SOD content for *S. platensis* with culture time at different PVC concentrations (12.5, 25, 50, 75, 100, 125, and 250 mg/L).

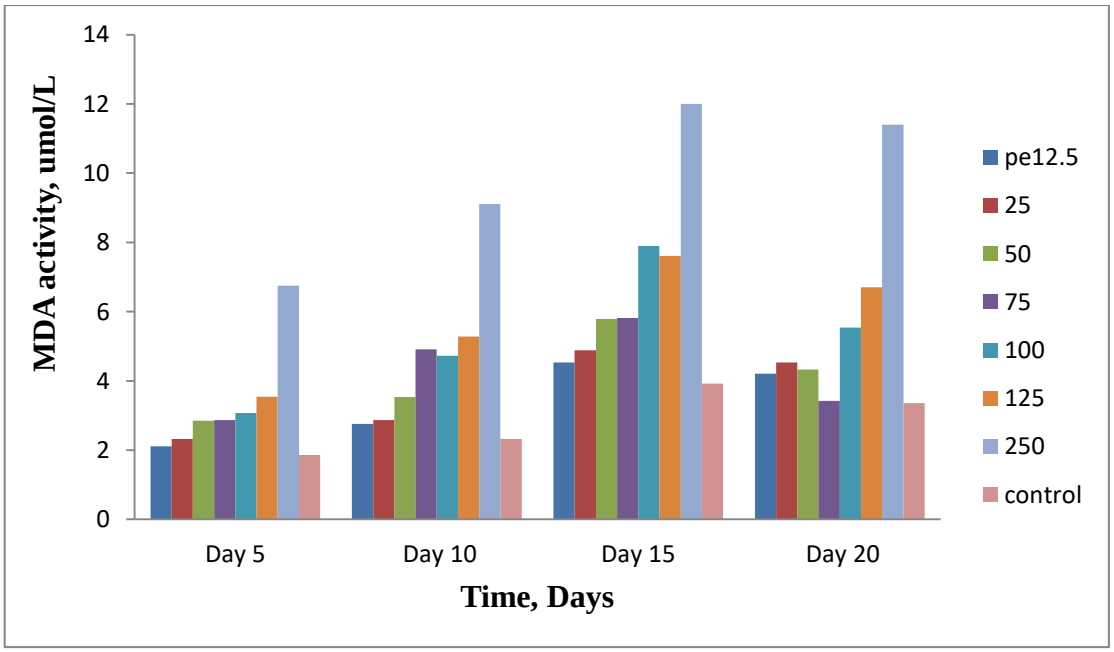

Figure 9 Variations of the MDA content for *S. platensis* with culture time at different PE concentrations (12.5, 25, 50, 75, 100, 125, and 250 mg/L).

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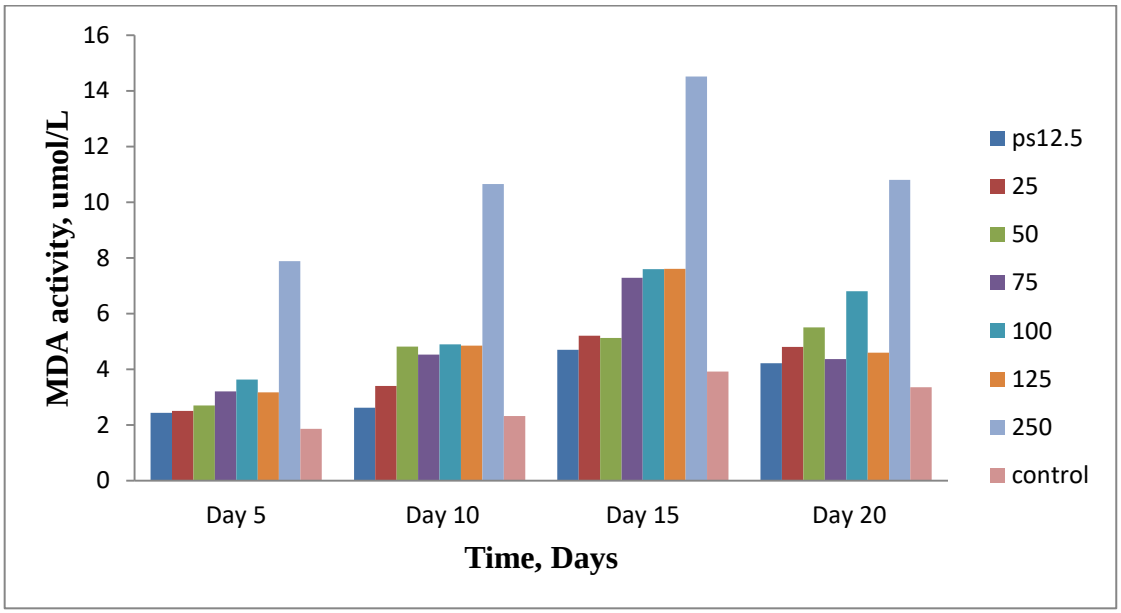

Figure 10 Variations of the MDA content for *S. platensis* with culture time at different PS concentrations (12.5, 25, 50, 75, 100, 125, and 250 mg/L).

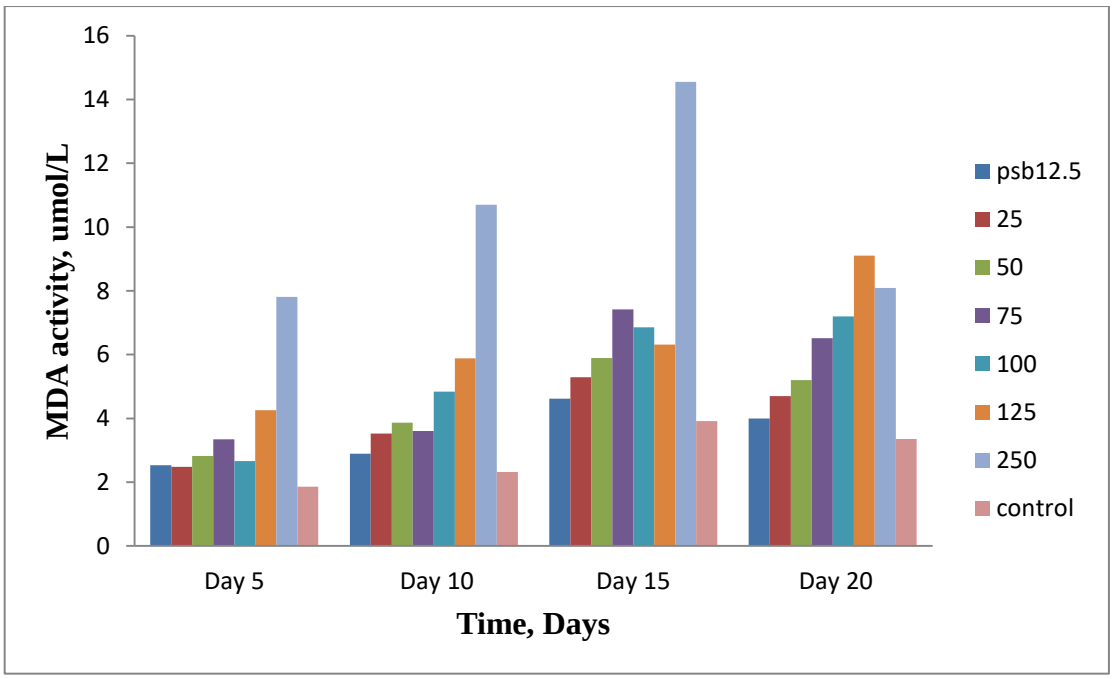

Figure 11 Variations of the MDA content for *S. platensis* with culture time at different PSB concentrations (12.5, 25, 50, 75, 100, 125, and 250 mg/L).

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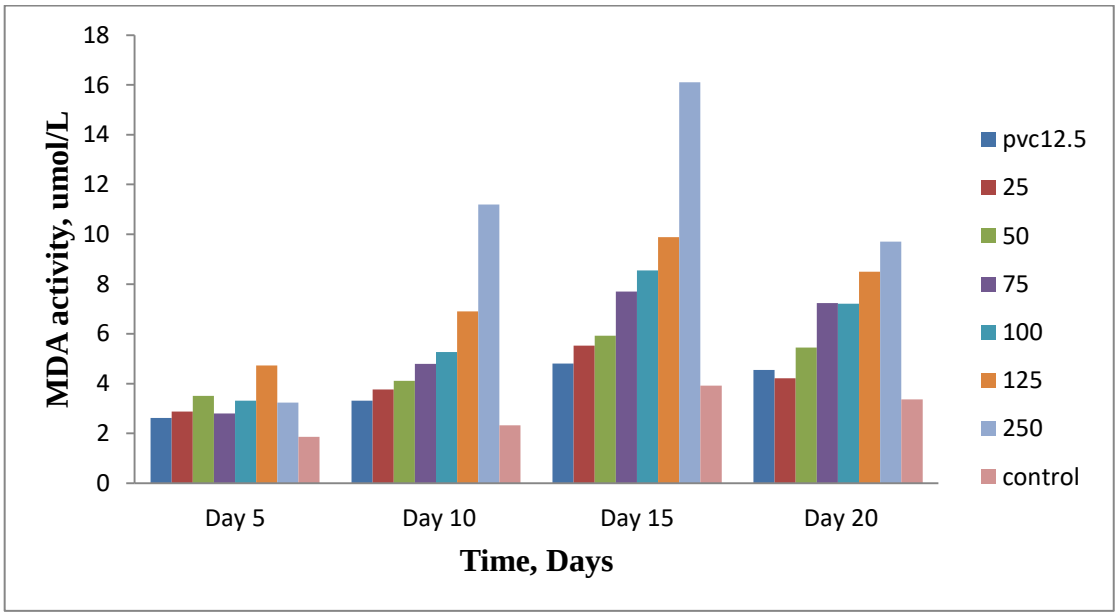

Figure 12 Variations of the MDA content for *S. platensis* with culture time at different PVC concentrations (12.5, 25, 50, 75, 100, 125, and 250 mg/L).

4. Conclusion

The following points were concluded in the present work:

1. it was observed that the presence of MPs harms the microalgae *S. platensis*,CAT content, SOD content, and MDA content.
2. The accumulation of MPs in Shatt Al-Furat in Al-Dywaniah in November 2020 showed an increase by two times compared to the recorded concentrations in November 2019 and a real monitoring system should be used on the disposal of plastics materials in the Shatt Al-Furat in Al-Dywaniah.
3. PVC showed a higher effect on the CAT content, SOD content, and MDA contentin *S. platensis* compared to the other MPs types.
1. Generally, CAT content values were found highest in the case of PVC (5.582 umol/L at 250 mg/L) and the lowest in the case of PE (3.92 umol/L at 250 mg/L) with a sequence of (PVC>PSB>PS>PE).
4. It was observed that the presence of MPs in the Shatt Al-Furat in Al-Dywaniah harms the two types of microalgae growth and chlorophyll content.
5. SOD content values were found highest in the case of PVC (19.371 umol/L at 250 mg/L) and the lowest in the case of PE (17.85 umol/L at 250 mg/L) for *S. platensis* and the sequence of SOD content was found as follow (PVC>PSB>PS>PE).

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6. MDA content values were found highest in the case of PVC (18.64 umol/L at 250 mg/L) and the lowest in the case of PE (13.579umol/L at 250 mg/L) for *S. platensis* and the sequence of MDA content was found as follow (PVC>PSB>PS>PE).
7. SOD and CAT content also was induced due to the ROS effect on the membrane of the microalgae cell.

5. Recommendations

The following recommendations can be used for further future work:

1. The result of CAT content, SOD content, and MDA content can be studied in case of the combined toxicity of MPs and some other organic materials present in the aquatic environment to understand the mixed toxicity issue.
2. The size of MPs found of great effect on the CAT content, SOD content, and MDA content, and a detailed study especially in case of combined toxicity are recommended to be done

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