# Influence of polyethelene microplastics on the growth, photosynthetic efficiency and oxidative stress of marine microalga *Chaetoceros calcitrans*

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## Abstract

Low-density polyethylene (LDPE) is broadly utilized worldwide that increase more dramatically during the COVID-19 pandemic, and the majority ends up in the aquatic environment as microplastics. The influence of polyethylene microplastics (LDPE-MPs) on aquatic ecosystems still needs further investigation specially on microalgae as typical organisms represent in all aquatic systems and at the base of the trophic chain. Thereby, the biological and toxicity impacts of LDPE-MPs on *Chaetoceros calcitrans* were examined in this work. The results revealed that LDPE-MPs had a concentration-dependent adverse effect on the growth and performance of *C. calcitrans*. LDPE-MPs contributed the maximum inhibition rates of 85%, 51.3%, 21.49% and 16.13% on algal growth chlorophyll content, φPSII and $F_v/F_m$, respectively. The total protein content, superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities were significantly increased at 25 mg $L^{-1}$ LDPE-MPs by 1.37, 3.52, 2.75 and 1.84 folds higher than those of the controls to sustain the adverse effects of LDPE-MPs. Extracellular polymeric substance (EPS) and monosaccharides contents of *C. calcitrans* were improved under low concentration of LDPE-MPs, which could facilitate adsorption of MPs particles on the microalgae cell wall. This adsorption caused significant physical damage to the algal cell structure, as observed by SEM. These results suggest that the ecological footprint of MPs may require more attention, particularly due to the continuing breakdown of plastics in the ecosystem.

## Aim of the work

As a result, the current study aims to explore the potentially harmful consequences of LDPE-MPs on growth, chlorophyll, photosynthetic activities, total protein content, exopolysaccharides, and monosaccharides of *C. calcitrans*. Additionally, the antioxidant enzyme activities were evaluated, including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). Finally, the interactions between the microalgae cells and the LDPE-MPs were observed by estimating the zeta-potential and morphology of the algal cells using a scanning electron microscope (SEM).

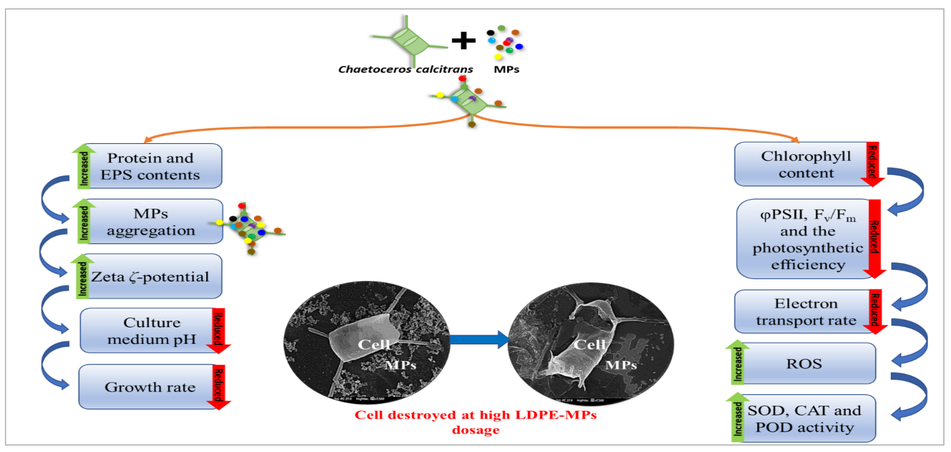
## Materials & Methods

***Strain and growth medium***

*Chaetoceros calcitrans* (Paulsen) Takano, was obtained as a sample from the Northeast Fisheries Science Center, Marine Service Center of the NOAA Fisheries, a United States Federal Agency (*The Aquaculture Sustainability Branch* –Stanford). *C. calcitrans* cultured on F/2 medium (Guillard, 1975). Cultures were preserved under continuous lights (60 mmol photons $m^{-2}$ $s^{-1}$). The experiments were performed at 25 ± 2 ˚C.

***MPs concentrations***

Low-density polyethylene (LDPE) chips (density 0.92 $g.cm^{-3}$) manufactured in ExxonMobil Chemical Company, Houston, Texas, USA, kindly provided by Dr. Mohamed Ghobashy, National Center For Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt. MPs were added to different glass bioreactors containing 2 L of *C. calcitrans* culture at exponential growth ($10^6$ cells $mL^{-1}$) to get the final concentrations of 0, 25, 50, 75, 100, 125, 150 and 175 mg $L^{-1}$.




***Growth parameters measurements:*** Every 2 days, the algal optical density (OD, 750nm) and cell numbers (Neubauer hemocytometer) were measured to determine the growth pattern of *C. calcitrans*. Then, the specific growth rates (μ) and growth inhibition ratio (IR) of *C. calcitrans* by LDPE-MPs were calculated at different concentrations (0-175 mg $L^{-1}$) and exposure times (0-8 d.) according to Eq. 1 and 2

$$\mu = \ln(N_e - N_i)/T_e - T_i \quad (1)$$

$$IR\ (\%) = 1 - N_e/C \times 100\% \quad (2)$$

Where, $N_e$ and $N_i$ were the cells count ($mL^{-1}$) at the end and initial exponential growth, $T_e$ and $T_i$ were times at the end and initial exponential growth, respectively. C was the cell count in control.

***Chlorophyll a, chlorophyll fluorescence and Quantum yield of photosystem-II (φPSII):*** Chlorophyll a (Chl a) content was estimated spectrophotometrically following equation (S. Wang et al., 2016)

$$Chl.a = \frac{(11.64 \times A663 - A750) - (2.16 \times A645 - A750) + 0.1 \times (A630 - A750) \times VA}{V}$$

Where: $A_{750}$, $A_{663}$, $A_{645}$, and $A_{630}$ are the absorbance of the extracted pigment at 750, 663, 645, and 630 nm, respectively; VA: volume of 90% acetone (mL), V: volume of microalgal sample (L).

The maximum fluorescence of dark-adapted cells, Fm, was determined, as well as the beginning fluorescence yield, $F_0$. The ratio of variable to maximal Chl fluorescence ($F_v/F_m$), obtained from (Fm-$F_0$)/ Fm, is used to indicate the maximum quantum yield of PSII in the dark-adapted condition. 10 s pulses of actinic light raised progressively from 0 to 1517 μmol photons/$m^2$/s were used to generate rapid light curves.

***Total proteins (TP) content and antioxidant enzymes***: The total soluble protein content of microalgae cells were estimated spectroscopically at 570 nm via the Lowry method (Lowry et al., 1951), using bovine serum albumin as standard.

***Superoxide dismutase (SOD):*** Estimated spectroscopically by Marklund and Marklund 1974) at absorbance 420 nm. The control tube was made by substituting distilled water for the sample. Up to 3 minutes, the change in absorbance was documented every 30 seconds.

$$Rate = \frac{OD_f - OD_i}{3}$$

$$\%\ inhibition = \frac{\Delta OD_C - \Delta OD_T}{\Delta OD_c} \times 100$$

$$SOD\ (Ug^{-1}) = \frac{\%\ inhibition}{50} \times \frac{VT(mL)}{VS\ (mL)} \times \frac{f}{fresh\ weight\ (g)}$$

where $OD_f$, $OD_i$ and $OD_c$ are the final, initial and control optical density, respectively; *VT* and *VS* are the total reaction and enzyme volumes, respectively; *f* is the dilution factor

***Catalase (CAT)***: Estimated according to Aebi (1974), CAT activity was monitored by the vanishing of $H_2O_2$ by decreasing absorbance at 240 nm up to 3 min.

$$CAT\ (Ug^{-1}) = \frac{2.303}{3} \times \frac{\log OD_i}{\log OD_f} \times \frac{f}{fresh\ weight\ (g)}$$

where $OD_f$ and $OD_i$ are the final and initial optical density, respectively; n is the dilution factor

***Peroxidase (POD):*** POD activity was detected according to (Reddy et al., 1985); The increase in absorbance at 430 nm was recorded every 30 s up to 3 min.

$$POD\ (Ug^{-1}) = \frac{\Delta OD_T - \Delta OD_c}{12} \times \frac{VT(mL)}{VS\ (mL)} \times \frac{f}{fresh\ weight\ (g)}$$

where $OD_T$ and $OD_c$ are the treated and control optical density, respectively; 12 is the suppression coefficient of purpurogallin (1 mg $mL^{-1}$) at 420 nm; *VT* and *VS* are the total reaction and enzyme volumes, respectively; f is the dilution factor.

***Extracellular polysaccharide:*** The preparation of the extracellular polysaccharide and the estimation of the monosaccharide content of the carbohydrate fractions by reduction, acetylation and gas-liquid chromatography of the alditol acetates have been described by (Myklestad and Haug, 1972).

***Scanning Electron Microscope (SEM):*** The morphology and the interaction between 50% inhibitory LDPE-MPs concentration (75 mg $L^{-1}$). and *C. calcitrans* microalga was observed by SEM (JEOL-JSM5300), at the E-Microscope Unit; Faculty of Science, Alexandria University, Egypt.

***Zeta ζ-potential measurement:*** The control and the LDPE-MPs treated *C. calcitrans* culture samples were uniformly stirred using 50% inhibitory LDPE-MPs concentration (75 mg $L^{-1}$) for 1 min to permit polyethylene adsorbed to *C. calcitrans* cells. The sample was let to stand for 1 min, and the ζ-potential was then detected using a ζ-Potential and Submicron Particle Size Analyzer (Delsa™Nano HC, Beckman Coulter Inc., Japan) at 20±0.5°C in triplicates.

***Statistical analysis:*** Data were stated as the mean ± standard deviation (SD) of three independent trials. Data were analyzed by one-way analysis of variance (ANOVA) to estimate the differences between treated and control groups. Duncan's Multiple Range Test (DMRT) was performed if the results demonstrated a significant difference at $p < 0.05$ by IBM SPSS v27.0 (IBM, USA). Pearson correlation was used to determine the relationship between various variables using Paleontological Statistics (PAST3).

## Results

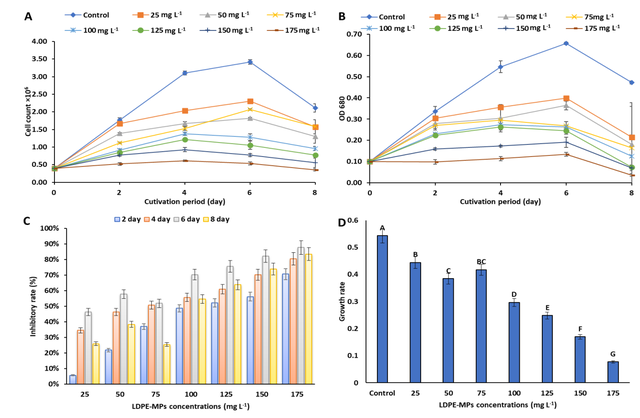



**Fig.1. Effects of different LDPE-MPs concentrations on growth of *C. calcitrans* with time A) Algal cell counts, B) Algal optical density, C) Inhibition rate (%) and D) Algal growth rate.**

The algal cell count and cell density reduced with increasing LDPE-MPs **(Fig. 1).** Moreover, the alga growth increased by incubation and reached the maximum on the 6th day for control and treated groups. Microalgae growth decreased significantly in the LDPE-MPs groups compared to the control during the cultivation period. This adverse effect of LDPE-MPs increased by LDPE-MPs concentrations, where 25 mg $L^{-1}$ concentration showed the lowest adverse effect on growth (32 % inhibition on the 6th day) and 175 mg $L^{-1}$ showed the most adverse effect (84 % inhibition on the 6th day) (Fig.1, C). While, 75 mg $L^{-1}$ showed 51.75 % inhibition which was selected as a 50 % inhibitory concentration ($IC_{50}$) for the SEM and Zeta ζ-potential.

According to the previous results, the 6th day has been used to estimate the other tested parameters. The highest microplastics treatment of 175 mg $L^{-1}$ showed the lowest growth rate of only 0.07 $d^{-1}$ with 85 % inhibition and the highest growth rate among treated groups was recorded in 25 mg $L^{-1}$ treatment of 0.44 $d^{-1}$ with 18 % inhibition (Fig.1, D) with a highly significant difference.

**Table 1. Effects of LDPE-MPs different concentration on Chlorophyll a content, chlorophyll fluorescence**

| | LDPE-MPS (mg $L^{-1}$) | | | | | | | | F- value |
|---|---|---|---|---|---|---|---|---|---|
| | Control | 25 | 50 | 75 | 100 | 125 | 150 | 175 | |
| **Chl a content (μg $L^{-1}$)** | 0.17±0.008$^{a}$ | 0.17±0.005$^{a}$ | 0.14±0.011$^{b}$ | 0.13±0.014$^{bc}$ | 0.12±0.004$^{c}$ | 0.11±0.002$^{cd}$ | 0.10±0.011$^{de}$ | 0.08±0.014$^{e}$ | 28.27** |
| **% Inhibition** | - | 0% | 18% | 24% | 31% | 34% | 43% | 51% | |
| **$F_v/f_m$** | 0.81±0.03$^{a}$ | 0.74±0.02$^{b}$ | 0.72±0.01$^{bc}$ | 0.68±0.03$^{cd}$ | 0.67±0.04$^{cd}$ | 0.66±0.04$^{cd}$ | 0.66±0.03$^{d}$ | 0.63±0.02$^{d}$ | 11.53** |
| **% Inhibition** | - | 9% | 10% | 16% | 17% | 18% | 19% | 21% | |
| **φPSII** | 0.62±0.03$^{a}$ | 0.56±0.01$^{b}$ | 0.55±0.02$^{b}$ | 0.53±0.04$^{bc}$ | 0.52±0.02$^{bc}$ | 0.50±0.02$^{bc}$ | 0.47±0.05$^{c}$ | 0.47±0.03$^{c}$ | 6. 26* |
| **% Inhibition** | - | 10% | 12% | 15% | 17% | 19% | 24% | 24% | |

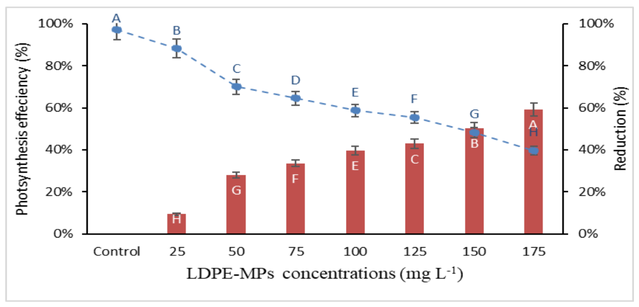



**Fig.2. Effects of different LDPE-MPs concentrations on the photosynthetic efficiency (%) of *C. calcitrans*.**

Chl. a content of the control and other treated groups was 0.17 μg $L^{-1}$ in control and under 25 mg $L^{-1}$ at 6th with no significant differences. Chl. a decreased 18 % under 50 mg $L^{-1}$ LDPE-MPs treatment and gradually decreased by increasing LDPE-MPs concentration and reached the maximum decrease at the highest MPs-PE level 175 mg $L^{-1}$ with 51% reduction compared to control **(Fig. 2)**

Regarding $F_v/F_m$ and φPSII, an identical trend was recorded in both parameters, with significant differences in the exposure concentration. The variation trend of three parameters of photosynthesis (chlorophyll, $F_v/F_m$ and φPSII) were diverse, so the photosynthesis efficiency was calculated depending on Chl. a content and φPSII to show the impact of LDPE-MPs on the whole algal photosynthesis process (Fig. 2).

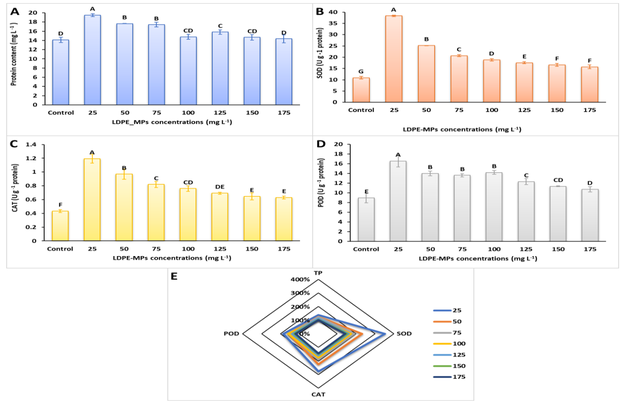



**Fig. 3. Effects of different LDPE-MPs concentrations on total protein (A), SOD activity (B), CAT activity (C), POD activity (D) and combined enzyme activities and TP content (E) of *C. calcitrans*.**

As shown in **Fig.3**, total soluble protein in *C. calcitrans* exposed to the low concentration of LDPE-MPs was greater than that of the control group, while there are no significant differences in the protein contents at 100, 150 and 175 mg $L^{-1}$. Total soluble protein content improved at 25 mg $L^{-1}$ LDPE-MPs with 1.37 times greater than control. The enzymatic activity of SOD, CAT and POD was significantly improved under LDPE-MPs as compared to the untreated control group, upon exposure to LDPE-MPs, for 6 days. SOD activity was superior, followed by CAT and POD, respectively. The SOD, CAT and POD activities peaked under 25 mg $L^{-1}$ LDPE-MPs, then reduced by increasing LDPE-MPs concentrations.

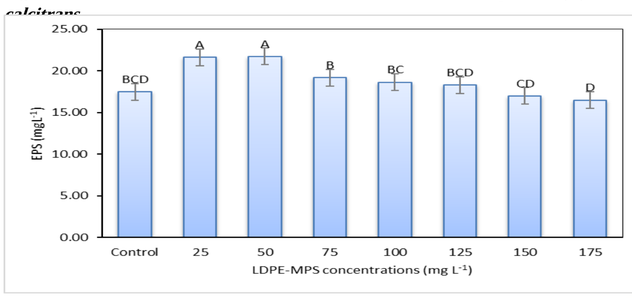



**Fig. 4. Effects of different LDPE-MPs concentrations on Extracellular polymeric substance (mg $L^{-1}$).**

It is obviously recorded that under LDPE-MPs treatments, EPS content was improved significantly by increasing LDPE-MPs concentrations at 25 and 50 mg $L^{-1}$ of 1.23 and 1.24-fold over control, respectively. Further LDPE-MPs increase, the EPS is increased insignificantly till 125 mg $L^{-1}$ then reduced to the minimum value (16.49 mg $L^{-1}$) at 175 mg $L^{-1}$ **(Fig. 4)**. Six monosaccharides have been identified among treatments, including rhamnose, fucose, xylose, mannose, galactose, and glucose. Rhamnose, fucose and galactose were the dominant monomer in all treatments as well as in control. Fucose showed the highest contents followed by rhamnose and galactose. The six monosaccharides have been detected in the MPs treatments of 50 and 75 mg $L^{-1}$ in addition to 125 and 175 mg $L^{-1}$.

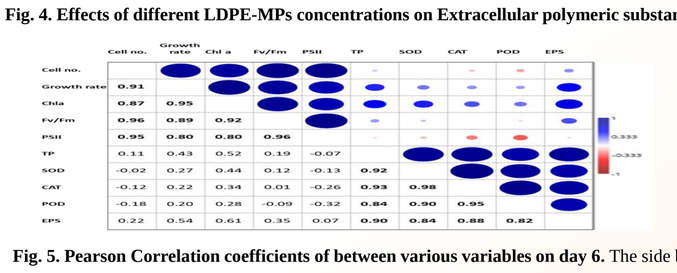



**Fig. 5. Pearson Correlation coefficients of between various variables on day 6.** The side bar indicates the probability. correlation value highlighted in bold refers significant at < 0.05 level.

There were strong positive correlation between the growth parameters where the algal growth (Cell no.) had a strong positive correlation with growth rate, Chl a, $F_v/F_m$, and PSII (0.91, 0.87, 0.96 and 0.95, respectively, $P < 0.05$), while showed negative correlation with antioxidant enzyme activities. On the other hand, total protein content showed a strong positive correlation with SOD, CAT, POD, and EPS (0.92, 0.93, 0.80.98, 0.9, and 0.84, respectively, $P < 0.05$) **(Fig.5)**.

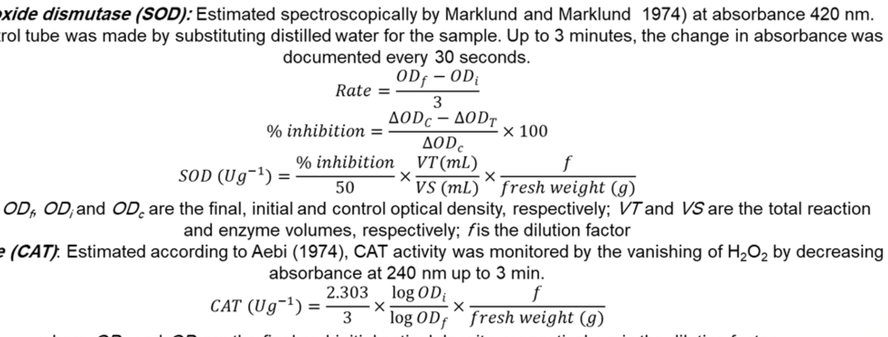



**Fig. 6. Photos of the interaction between LDPE-MPs and alga. A and B, *C. calcitrans* as control; C and D, PE MPs adsorbed on *C. calcitrans*; E and F, physical damage on *C. calcitrans*.**

The change of morphological feature and the interaction between *C. calcitrans* and LDPE-MPs was detected by SEM, which showed that LDPE MPs are absorbed on the surface of algal cells. MPs are wrapped up in surface microalgae cell walls. Additionally, LDPE-MPs were embedded in the microalgal cells and exerted physical damage and roughness on the cells surface, causing a negative effect on the growth of *C. calcitrans* (Fig. 6).

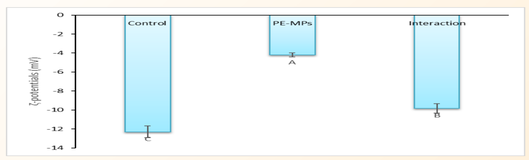



**Fig.7. Effects of exposure to LDPE-MPS on Zeta potential of *C. calcitrans*.**

LDPE-MPs particles have the potential to bind to *C. calcitrans* cells, according to our findings. Zeta-potentials of *C. calcitrans* cells and LDPE-MPs were evaluated to assess the adhesion abilities of LDPE-MPs to *C. calcitrans* cells, as shown in Fig. 7. Zeta potentials of LDPE-MPs and *C. calcitrans* cells in the medium were −4.2 mV and −12.2 mV, respectively. They had a higher zeta potential (−9.83 mV) after interacting with LDPE-MPs concentration.

## Conclusion

LDPE-MPS showed an inhibitory effect on the growth rate of *C. calcitrans* cells, and chlorophyll content and consequently retard the photosynthesis process that inhibits 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. Variations in SOD, CAT and POD activities could be regarded as a defense mechanism for scavenging the excess ROS in algal cells and sustaining the balance inside the cell. On the other hand, soluble proteins are increased under LDPE-MPs treatments, which could improve the water holding capacity of cells to protect biofilms and important algal compounds. SEM confirmed the LDPE-MPs aggregation causes physical damages to the algal cell . These findings are respected for improving awareness of the hazards of LDPE-MPs on *C. calcitrans* and for evaluating the environmental pollution of other MPs. This work might give experimental evidence and a theoretical foundation for elucidating the toxicity mechanism of MPs to marine diatoms and appropriately estimating their environmental concerns in the marine environment.