

PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF *LEMNA MINUTA* KUNTH TO MULTI-WALLED CARBON NANOTUBES AND FULLERENE SOOT EXPOSURE

Ștefan Mihăiță OLARU^{1*}, Maria-Magdalena ZAMFIRACHE¹

¹Faculty of Biology, „Alexandru Ioan Cuza” University of Iași, Bd. Carol I, No. 20A, Iași, Romania

Abstract: Carbon-based nanomaterials (CNMs), such as multi-walled carbon nanotubes (MWCNTs) and fullerene soot, are potential contaminants in natural environments due to their increasing use in various industries and limited biodegradability. The present research investigates the physiological and biochemical responses of *Lemna minuta* Kunth specimens, an aquatic macrophyte with potential bioindicator properties, under experimental cultivation conditions (addition of MWCNTs, MWCNTs-COOH, and fullerene soot in the growth medium). The plants were exposed to two concentrations (50 and 200 mg/L) of each CNM under controlled cultivation conditions for 14 days. The evaluated physiological parameters included stomatal density and size, chlorophyll fluorescence, and photosynthetic pigment content (chlorophyll a, chlorophyll b, carotenoid pigments). Additionally, the amounts of total phenolic compounds, flavonoids, and the activity of antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (POD) were quantified. A significant dose-dependent toxicity of the experimentally applied nanomaterials was observed, including changes in stomatal number and surface area, degradation of photosynthetic pigments, induction of oxidative stress, and activation of enzymatic defence mechanisms in the test plants. These findings highlight the sensitivity of *Lemna minuta* Kunth to the presence of synthetic carbon-based nanomaterials and emphasise its relevance as a bioindicator in aquatic ecotoxicology.

Keywords: *Lemna minuta*, duckweed, MWCNTs, fullerene soot, abiotic stress, physiology, ROS, toxicity.

INTRODUCTION

Research conducted in the field of nanotechnology has led to the synthesis of innovative materials generically called carbon nanomaterials (CNMs), such as carbon nanotubes and fullerene soot, which foreshadow, through their properties, multiple applications in the electronics industry, agriculture, biomedicine, and environmental protection (wastewater treatment).

The realisation of these beneficial application prospects for CNMs has raised concerns about the risks of their accidental or non-accidental release into natural aquatic ecosystems and understanding their potential negative impact on the plants inhabiting them. CNMs, particularly multi-walled carbon nanotubes (MWCNTs) and fullerene soot, which are highly persistent in the environment and capable of accumulating in aquatic biota, possess unique physicochemical properties that enable interaction with them (Husen and Siddiqi, 2014; Narei et al., 2018). Recent studies suggest that these nanoparticles can stimulate plant growth by facilitating water and nutrient absorption, but may also induce toxic effects in plant organisms, manifested through oxidative stress due to generated reactive oxygen species, phenomena that disrupt their physiological balance (Tan et al., 2009; Jordan et al., 2020).

In this context, a detailed investigation of the effects of these nanomaterials on aquatic plant species is necessary, particularly on those frequently used as model organisms in ecotoxicology studies, such as species of the genus *Lemna* L.

While ecotoxicological studies frequently use specimens of *Lemna minor* L. as standard model organisms, *Lemna minuta* Kunth, a closely related

species with rapid growth and high environmental adaptability, is currently less studied (Ceschin et al., 2020). Research addressing its direct interaction with synthetic carbon-based nanomaterials is still in its early stages (Ceschin et al., 2016, 2018).

Preliminary studies on how *Lemna minuta* populations respond to the presence of synthetic nanoparticles as emerging pollutants in their habitats indicate significant morpho-anatomical and biochemical changes, including alterations in leaf surface and antioxidant activity. This suggests a potential negative impact of these nanomaterials on plant health (Chen et al., 2018). In this context, evaluating the physiological and biochemical responses of test plants exposed to CNMs provides essential information on the mechanisms inducing their sublethal toxicity in plant structures, particularly metabolic pathways generating oxidative stress.

Given this background, the research conducted in this study investigates how plants of the *L. minuta* species respond, in laboratory experimental cultures, to the presence of multi-walled carbon nanotubes (MWCNTs) and fullerene soot applied at different concentrations.

The study examines growth metrics, changes in photosynthesising pigment content, and biochemical stress responses in the observed plants to validate their potential use in environmental quality monitoring. The study also provides a solid scientific basis for future applications of this species in monitoring and ecological remediation of waters contaminated with synthetic carbon-based nanomaterials, as a highly relevant applied research field.

*Correspondence: Olaru Ștefan Mihăiță, "Alexandru Ioan Cuza" University, Faculty of Biology, Bd. Carol I, No 20A, Iași, Romania, email: stefan.olaru@uaic.ro

MATERIALS AND METHODS

Cultivation and experimental design

Axenic cultures of *Lemna minuta* were maintained in SIS medium under standard growth chamber conditions (24 ± 2 °C, 16:8 h light/dark cycle, $115\text{--}118$ $\mu\text{mol/s/m}^2$). To minimise phenotypic variability, monoclonal cultures were established from a single individual. Plants used in exposure tests were selected from these cultures, with each cultivation vessel containing culture medium (10 mL volume) receiving a single individual with three fronds. To assess the effects of carbon-based nanomaterials (CNMs) on aquatic plants, they were exposed to two experimental concentrations - 50 mg/L and 200 mg/L for each type of CNM used. The total exposure period under controlled laboratory conditions lasted 14 days. Before use, aqueous CNM suspensions were subjected to 30-minute ultrasonic treatment to ensure uniform particle dispersion and prevent aggregation, which is essential for test reproducibility and biological relevance. Control samples were cultivated in parallel under the same experimental conditions but without nanomaterials, receiving only standard SIS nutrient medium as a control solution. All measurements were performed on the final day of the treatment period, specifically on day 14 of the experiment.

Nanomaterials

Multiwalled carbon nanotubes (>96% purity) and fullerene soot were purchased from certified suppliers: MWCNTs (multi-walled nanotubes) with outer diameter of 8 nm (product code NG01MW0101) and MWCNT-COOH (carboxyl-functionalized nanotubes) with outer diameter of 8-18 nm (product code NG01MW0303) were supplied by Nanografi (Ankara, Turkey), while fullerene soot, a mixture of C60, C70 and carbon black, usable in applications such as water purification, hydrogen storage, super-capacitors and nanoelectronics was supplied by Sigma-Aldrich (product code 572497-5G).

Physiological assessments

Stomatal density was evaluated by scanning electron microscopy (SEM) using intact leaves/fronds from treated plant variants and control plants. Plant material was harvested at the end of the experimental period, selecting only mature, well-developed specimens from ontogenetically and morphologically equivalent regions. These were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 hours at room temperature. After fixation, samples were rinsed three times in phosphate buffer and dehydrated through a graded acetone series (30%, 50%, 70%, 90% and 100%), with 10-15 minutes for each treatment step. Subsequently, samples underwent critical point drying with liquid carbon dioxide to prevent collapse of fine structures. After drying, leaf fragments were mounted on metal stubs with conductive double-sided adhesive tape and coated with a thin gold layer (~10 nm) applied by cathode sputtering to provide surface conductivity and reduce electrostatic charging artefacts during examination (Pathan et al., 2010).

Observations were made using a Tescan Vega II SBH scanning electron microscope at 30 kV accelerating voltage. For each experimental variant, at least three biological sample replicates were analysed, with a minimum of three viewing fields per sample. Stomatal density was expressed in stomata/ mm^2 , determined by counting visible stomata in SEM micrographs at known magnification and relating them to the analysed area (Xu and Zhou, 2008).

The influence of nanomaterials on the photosynthetic apparatus functioning of *Lemna minuta* plants was evaluated by determining the ΦPSII parameter (quantum efficiency of photosystem II), measured with the portable Hansatech FMS II fluorimeter. Measurements were performed by positioning the fluorimeter's optical fibre 1cm from the leaf/frond surface of duckweed plants, taking 5 measurements for each test plant container.

For quantification of photoassimilatory pigments and total polyphenols content, 5% g/v extracts in 96% ethanol were prepared from *Lemna* leaves/fronds, according to the method described by Wellburn (Wellburn, 1994; Zhao et al., 2017).

Total phenolic content, flavonoids, and antioxidant activity

The total phenolic and flavonoid content was determined spectrophotometrically according to Ainsworth and Gillespie (2007), using alcoholic extracts (5% w/v in 96% ethanol). Results were expressed as gallic acid equivalent per g of fresh weight (mg GAE/g f.w.) for total phenolic content and quercetin equivalent per g of fresh weight (mg QE/g f.w.) for flavonoid content. The antioxidant activity of *Lemna* extracts was determined spectrophotometrically according to Sharma and Bhat (2009), by DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma, Schnellendorf, Germany) decolourisation. Results were expressed as percentage antioxidant activity (%).

Determination of superoxide dismutase (SOD), peroxidase (POD) activity, and protein content

Extracts were prepared with TRIS-HCl from *Lemna* leaves/fronds, following methods described by Winterbourn et al., adapted by Arteni et al. (Winterbourn et al., 1975; Arteni et al., 2008). These are based on Nitro Blue Tetrazolium reduction and spectrophotometric measurement of the colour intensity produced by ortho-dianisidine oxidation.

The data obtained through these determinations were statistically analysed using one-way ANOVA with Tukey's post hoc test ($p < 0.05$).

RESULTS

Stomatal density

Exposure of *Lemna minuta* plants to different types and concentrations of synthetic carbon-based nanomaterials significantly influenced stomatal morphology (Fig. 1). Stomatal density slightly increased in the treatment with multi-walled carbon nanotubes (MWCNTs) – 200 mg/L variant, though recorded values remained below control levels, suggesting a possible adaptation of the analysed plants

to experimentally induced abiotic stress with moderate values.

Functionalized nanotubes (MWCNTs-COOH) caused a pronounced stimulation of stomatogenesis, likely associated with intensified oxidative signalling in the test plants. Additionally, fullerene soot increased stomatal density, particularly at higher concentrations, possibly through a hormetic mechanism. Alongside the increase in stomatal number, a reduction in their size was observed across all treatment variants, a phenomenon more pronounced in the presence of

MWCNTs-COOH, indicating impaired stomatal development, likely caused by oxidative stress and the interaction of tested nanomaterials with the epidermal cells of *Lemna* leaves/fronds. The obtained results align with data from specialised literature (Begum et al., 2012; Tripathi et al., 2017; Calabrese and Agathokleous, 2021), highlighting an inverse proportional relationship between stomatal density and size as a complex adaptive strategy of test plants to abiotic stress induced by synthetic nanomaterials in their living environment.

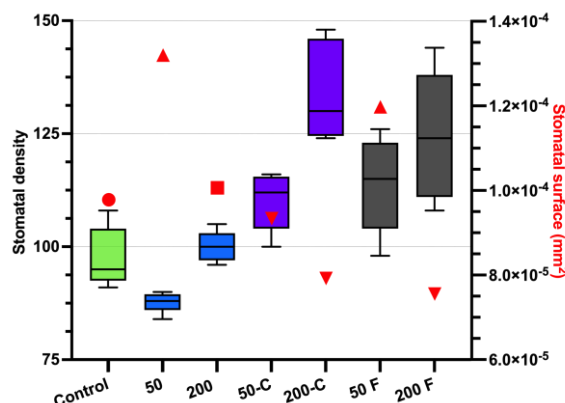


Fig. 1. Stomatal density of *Lemna minuta* Kunth plants grown under experimental conditions. Experimental variants: Control, 50 - 50 mg/l MWCNT, 200 - 200 mg/l MWCNT, 50-C - 50 mg/l MWCNT-COOH, 200-C - 200 mg/l MWCNT-COOH, 50 F - 50 mg/l Fullerene soot, 200 F - 200 mg/l Fullerene soot. Statistically significant differences are observed in the case of variants 50, 200-C, 50F, and 200F. On the secondary axis (in red) is marked the stomatal surface area (●- control, ■- non-significant difference, ▲- significant increase of stomatal surface area, ▼- significant decrease of stomatal surface area)

Chlorophyll fluorescence

Exposure of *Lemna minuta* plants to multi-walled carbon nanotubes (MWCNTs) resulted in a significant reduction of photosynthetic efficiency, expressed through the decrease of the Φ PSII parameter, depending on the applied treatment concentration (Fig. 2).

The negative effect was amplified when using carboxylated nanotubes (MWCNTs-COOH) - 200 mg/L variant, where Φ PSII reached minimum values, indicating their increased toxicity associated with their functionalization. This intensification of harmful effects may be attributed to better dispersion of these nanomaterials in the cultivation medium and their increased absorption at the leaf/frond level of *Lemna*. In contrast, fullerene soot treatments caused only a moderate reduction of Φ PSII, with values close to the control, indicating a lower photosynthetic impact on the test plants.

The results suggest that carbon nanomaterials, especially MWCNTs-COOH, negatively affect photosystem II of *Lemna minuta* plants, possibly by inducing oxidative stress and disrupting their energy balance, consistent with data presented in specialised literature (Ma et al., 2010; Wang et al., 2014; Meften et al., 2023).

Assimilatory pigments content

Treatments with multi-walled carbon nanotubes (MWCNTs) caused a slight, dose-dependent reduction in the content of photoassimilatory pigments (chlorophyll a, chlorophyll b, carotenoid pigments) in the leaves/fronds of *Lemna minuta* plants, suggesting possible inhibition of their biosynthesis or degradation of chloroplast structures caused by induced oxidative stress (Wellburn et al., 1972) (Fig. 3). In contrast, nanotubes functionalized with carboxyl groups (MWCNTs-COOH) induced an increase in the concentration of these pigments at the 200 mg/L treatment variant compared to the control and MWCNTs treatment variants, suggesting that functionalization may positively modulate the interaction between the plant and nanomaterial, indirectly stimulating the biosynthesis of photoassimilatory pigments and nutrient assimilation (Li et al., 2024). The application of fullerene soot similarly led to a significant increase in the content of photoassimilatory pigments, with peak values at the 50 mg/L concentration, indicating an adaptive response of test plants to moderate chemical stress in the cultivation environment, achieved through activation of intrinsic protective mechanisms for photoassimilatory structures, including regulation of photoassimilatory pigment biosynthesis pathways (Adil et al., 2022).

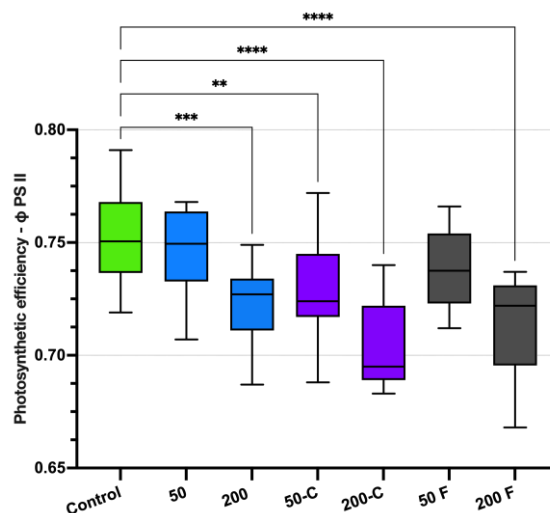


Fig. 2. Photosynthetic efficiency of *Lemna minuta* Kunth plants. Experimental variants: Control, 50 - 50 mg/l MWCNT, 200 - 200 mg/l MWCNT, 50-C - 50 mg/l MWCNT-COOH, 200-C - 200 mg/l MWCNT-COOH, 50 F - 50 mg/l Fullerene soot, 200 F - 200 mg/l Fullerene soot. Statistically significant differences are marked with *

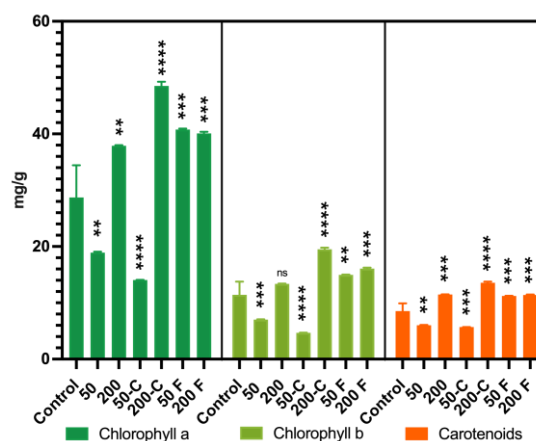


Fig. 3. Photosynthetic pigment content of *Lemna minuta* Kunth plants. Experimental variants: Control, 50 - 50 mg/l MWCNT, 200 - 200 mg/l MWCNT, 50-C - 50 mg/l MWCNT-COOH, 200-C - 200 mg/l MWCNT-COOH, 50 F - 50 mg/l Fullerene soot, 200 F - 200 mg/l Fullerene soot. Statistically significant differences are marked with *

Flavonoid compound content

Exposure of *Lemna minuta* plants to multi-walled carbon nanotubes (MWCNTs) resulted in a modest, dose-dependent increase in flavonoid content, although recorded values remained below the control level (Fig. 4). This response suggests partial activation of antioxidant pathways in test plants due to oxidative stress generated by the presence of nanomaterials in the environment (Ma et al., 2010; Jiang et al., 2019). For treatments with functionalized nanotubes (MWCNTs-COOH), a more pronounced accumulation of flavonoid compounds was observed at the 200 mg/L treatment concentration, possibly as a test plant response to higher abiotic stress levels in the cultivation environment, increased nanotube dispersibility, and

their stronger interaction with plant surfaces (Lavagna et al., 2021). Fullerene soot treatments, in turn, led to increased flavonoid levels at both tested concentrations without a clear dose effect, suggesting a possible threshold effect in stimulating antioxidant metabolism (Gogos et al., 2012). The results support the idea that flavonoid biosynthesis is an essential component of *Lemna minuta*'s antioxidant response to abiotic stress induced by carbon nanomaterials, particularly functionalized structures or fullerene aggregates, which may amplify oxidative signalling and activate secondary metabolite synthesis in plants, playing a protective role for their vegetative structures (Tripathi et al., 2017).

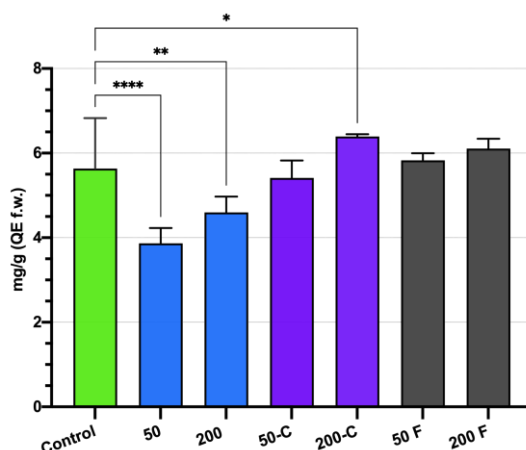


Fig. 4. The flavonoid compound content of *Lemna minuta* Kunth plants cultivated under experimental conditions. Experimental variants: Control, 50 - 50 mg/l MWCNT, 200 – 200 mg/l MWCNT, 50-C – 50 mg/l MWCNT-COOH, 200-C - 200 mg/l MWCNT-COOH, 50 F – 50 mg/l Fullerene soot, 200 F – 200 mg/l Fullerene soot. Statistically significant differences are marked with *

Polyphenols content

Treatment with carboxylated multi-walled carbon nanotubes (MWCNTs-COOH) induced a significant increase in total polyphenol content of *Lemna minuta* plants, indicating the activation of their antioxidant defence system (Fig. 5). This effect is associated with the superior dispersibility of functionalized nanotubes in the cultivation medium and their increased cellular absorption in plant tissues, phenomena that facilitate their interaction with test organisms (Aryal et al., 2019).

In contrast, exposure of *Lemna* plants to non-functionalized MWCNTs and fullerene soot led to a reduction in polyphenols levels; this response, independent of the applied concentration, suggests

either a lower metabolic impact of these compounds on test plants or their reduced capacity to induce oxidative signalling in plant organisms.

The differential response of *Lemna minuta* plants to exposure to various nanomaterials highlights the variability of their induced physiological effects, depending on their chemical nature, while also emphasising the role of polyphenols as sensitive biochemical markers in detecting sublethal stress manifested in aquatic ecosystems. Among all tested nanomaterial variants, MWCNTs-COOH showed the highest potential for activating phenolic biosynthesis pathways of *Lemna minuta* plants, confirming the increased bioactivity of functionalized nanomaterials with plant structures (Tripathi et al., 2017).

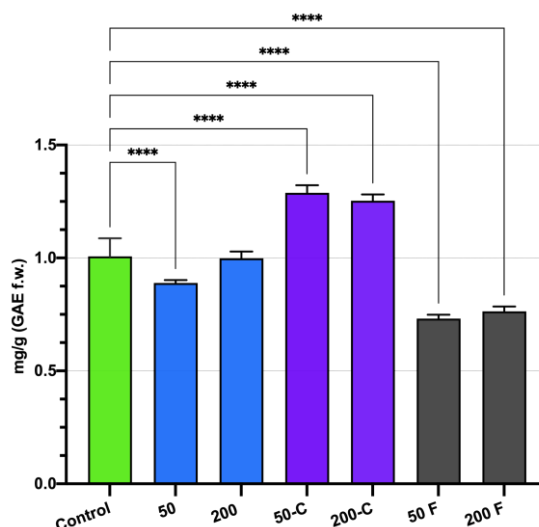


Fig. 5. Polyphenol content of *Lemna minuta* Kunth plants cultivated under experimental conditions. Experimental variants: Control, 50 - 50 mg/l MWCNT, 200 – 200 mg/l MWCNT, 50-C – 50 mg/l MWCNT-COOH, 200-C - 200 mg/l MWCNT-COOH, 50 F – 50 mg/l Fullerene soot, 200 F – 200 mg/l Fullerene soot. Statistically significant differences are marked with *

Peroxidase activity (POD)

Peroxidase activity (POD) showed a significant increase in *Lemna minuta* plants interacting with synthetic carbon-based nanomaterials at a concentration of 50 mg/L, indicating activation of their enzymatic antioxidant capacity in response to oxidative

stress induced by exposure to these compounds. However, at higher concentrations (200 mg/L), POD activity decreased considerably in test plants, reaching values lower than those determined in the control variant; this trend of reduced enzymatic activity with increasing concentration of disruptive chemical factors

in the environment suggests that high doses of nanomaterials may exceed the tolerance threshold of plants' antioxidant enzymatic system, leading to functional inhibition of peroxidase and, consequently, intensification of oxidative cellular damage processes in their structures (Ma et al., 2010) (Fig.6).

Superoxide dismutase activity (SOD)

The superoxide dismutase (SOD) activity was significantly and consistently suppressed, with concentrations of 200 mg/L inducing the most

pronounced disruptive effect on its activity in this context (Fig. 7). The resulting biochemical response indicates a dose-dependent inhibitory effect of the tested nanomaterials on this enzyme's activity, possibly associated with an initial phase of intense oxidative activation at the cellular level in test plants due to the presence of low nanomaterial concentrations, followed by severe oxidative tissue damage as environmental concentrations increased, leading to significant SOD inactivation (Ma et al., 2010).

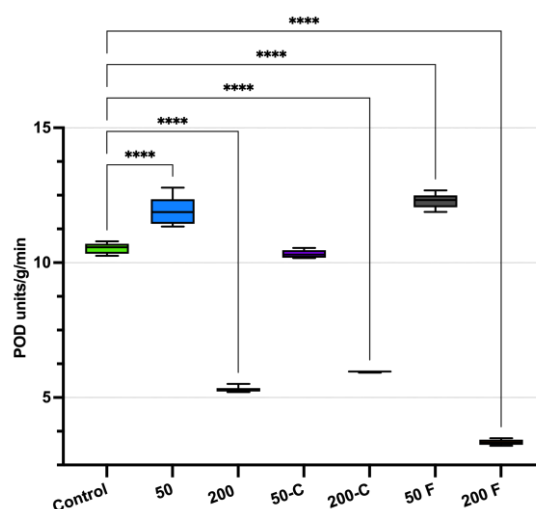


Fig. 6. Peroxidase activity of *Lemna minuta* Kunth plants cultivated under experimental conditions. Experimental variants: Control, 50 - 50 mg/l MWCNT, 200 – 200 mg/l MWCNT, 50-C – 50 mg/l MWCNT-COOH, 200-C - 200 mg/l MWCNT-COOH, 50 F – 50 mg/l Fullerene soot, 200 F – 200 mg/l Fullerene soot. Statistically significant differences are marked with *

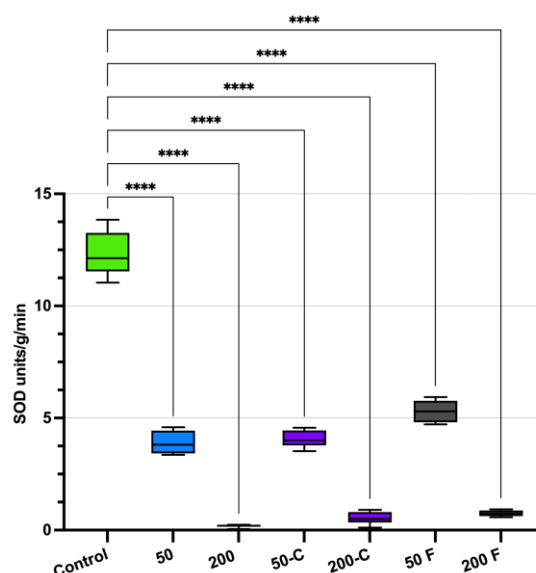


Fig. 7. Superoxide dismutase activity of *Lemna minuta* Kunth plants grown under experimental conditions. Experimental variants: Control, 50 - 50 mg/l MWCNT, 200 – 200 mg/l MWCNT, 50-C – 50 mg/l MWCNT-COOH, 200-C - 200 mg/l MWCNT-COOH, 50 F – 50 mg/l Fullerene soot, 200 F – 200 mg/l Fullerene soot. Statistically significant differences are marked with *

Soluble protein content

The soluble protein content increased in *Lemna minuta* plants interacting with synthetic carbon-based nanomaterials in most applied treatment variants compared to the control, as a result of activating metabolic processes associated with their defence

mechanisms and cellular adaptation to the induced abiotic stress. Among the tested variants, treatment with non-functionalized MWCNT at a concentration of 50 mg/L caused a severe reduction in soluble protein content, suggesting a possible metabolic inhibition of the biosynthesis of this category of cellular compounds

as a delayed physiological response of the test plants to the experimentally induced chemical stress. Meanwhile, treatments with functionalized nanotubes (MWCNTs-COOH) and fullerene soot significantly stimulated the cellular-level accumulation of soluble proteins, reflecting the activation of specific metabolic pathways for detoxification and cellular repair in the

test plants (Fig. 8). The results characterize total soluble protein content as a sensitive biochemical marker of metabolic changes and the response of aquatic plants exposed to oxidative stress caused by synthetic nanomaterials (Ma et al., 2010; Zhang et al., 2017).

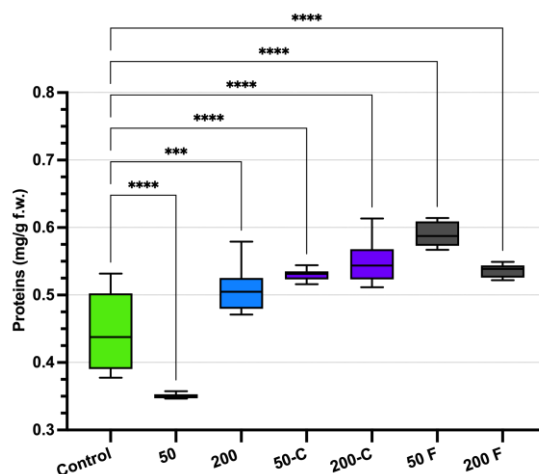


Fig. 8. Protein content of *Lemna minuta* Kunth plants cultivated under experimental conditions. Experimental variants: Control, 50 - 50 mg/l MWCNT, 200 - 200 mg/l MWCNT, 50-C - 50 mg/l MWCNT-COOH, 200-C - 200 mg/l MWCNT-COOH, 50 F - 50 mg/l Fullerene soot, 200 F - 200 mg/l Fullerene soot. Statistically significant differences are marked with *

Total antioxidant activity

The evaluation of the antioxidant response of *Lemna minuta* plants through the DPPH test revealed differentiated effects of synthetic carbon-based nanomaterials, depending on their chemical nature. Multi-walled carbon nanotubes (MWCNTs) and fullerene soot reduced the total antioxidant capacity of the tested plants, suggesting an impairment of endogenous defence systems, likely due to intense oxidative stress.

In contrast, treatments with carboxyl-functionalized nanotubes (MWCNTs-COOH) led to a significant increase in antioxidant activity, indicating a possible

activation of reactive oxygen species neutralization pathways of the tested plants, favoured by improved absorption of this type of nanomaterials at the plant tissue level, with an effect of intensifying intracellular antioxidant protection reactions (Fig. 9). These results highlight the importance of nanomaterial functionalization and concentration in modulating physiological responses of aquatic plants and validate the use of the DPPH test as a sensitive indicator of their metabolic adaptation capacity or suppression of antioxidant mechanisms. (Ma et al., 2010; Begum and Fugetsu, 2012; Tripathi et al., 2017).

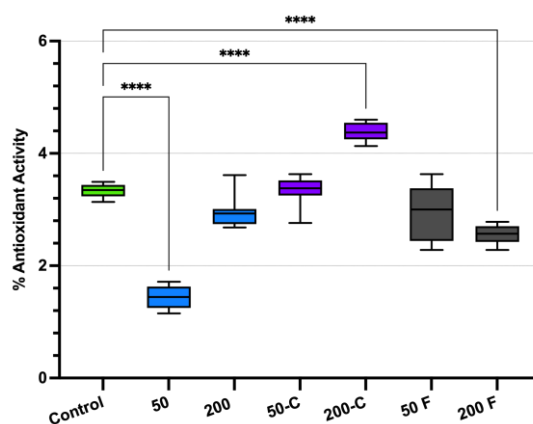


Fig. 9. Total antioxidant activity of *Lemna minuta* Kunth plants grown under experimental conditions. Experimental variants: Control, 50 - 50 mg/l MWCNT, 200 - 200 mg/l MWCNT, 50-C - 50 mg/l MWCNT-COOH, 200-C - 200 mg/l MWCNT-COOH, 50 F - 50 mg/l Fullerene soot, 200 F - 200 mg/l Fullerene soot. Statistically significant differences are marked with *

CONCLUSIONS

The results obtained during the conducted research highlight the high sensitivity of the species *Lemna minuta* to moderately prolonged exposure (14 days) to synthetic carbon-based nanomaterials, with differentiated responses depending on the chemical nature and concentration of the tested compounds. This underscores the species' role as a bioindicator in the ecotoxicological assessment of contaminants in freshwater aquatic ecosystems.

Among the analysed nanomaterials, multi-walled carbon nanotubes functionalized with carboxyl groups (MWCNTs-COOH) induced the most pronounced phytotoxic effects on the test plants, reflected through morphophysiological and biochemical alterations: changes in stomatal density and dimensions, reduced efficiency of photosystem II, and disruption of photosynthetic pigment balance. Notably, while low concentrations of functionalized nanotubes led to a decrease in photosynthetic pigment content, a concentration of 200 mg/l of this compound category and fullerene soot induced an increase, suggesting differentiated response mechanisms of *Lemna minuta* plants to chemical stress factors present in the cultivation medium.

At the biochemical level, MWCNTs-COOH stimulated the accumulation of phenolic compounds and increased the total antioxidant capacity of the test plants, indicating a compensatory adaptive response. Antioxidant enzymatic activity was variably influenced by the applied treatments, with marked and consistent inhibition of superoxide dismutase (SOD), possibly associated with severe oxidative stress or advanced cellular damage. In contrast, peroxidase (POD) exhibited sustained activation, supporting its use as a sensitive biomarker of sublethal stress in aquatic plants exposed to nanomaterials.

The increase in total soluble protein content, observed in most treatment variants, reflects the activation of plant defence metabolic pathways through the synthesis of protective and detoxifying proteins. This global response confirms that the effects of synthetic nanomaterials on aquatic plants are strongly influenced by their chemical nature and concentration.

The obtained results allow us to conclude that *Lemna minuta* Kunth proves to be a suitable biological model for testing acute nanotoxicity induced by synthetic carbon-based nanomaterials in its habitats. We advocate for its integration into biomonitoring schemes and the development of standardised protocols for assessing ecotoxicological risks associated with the accidental introduction of these compounds as potential pollutants in freshwater ecosystems.

AUTHORS CONTRIBUTIONS

Conceptualisation: Ș.M.O. and M-M. Z.; methodology, Ș.M.O.; data collection, Ș.M.O.; data validation, Ș.M.O. and M-M. Z.; data processing Ș.M.O.; writing—original draft preparation, Ș.M.O.; writing—review and editing, Ș.M.O. and M-M. Z.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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