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High-Value Biochar from Sunflower Husk Pyrolysis Enhances Growth and Physiological Performance of *Spirodela polyrhiza*

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Abstract

Rising populations intensify food demand and place increasing pressure on already degraded soils, driving the need for sustainable, waste-derived fertilizers that can enhance plant growth. This research aimed to assess the potential use of biochar as a soil amendment to support plant growth. The biochar used in this study was produced from sunflower husk and examined in three variants obtained at pyrolysis temperatures of 300, 400, and 500 °C, applied at different dosage levels. *Spirodela polyrhiza* served as the model plant species. Assessment was based on changes in chlorophyll index, fluorescence, frond number, and overall plant condition. Temperature-dependent changes in biochar properties were further analysed using SEM, FTIR, BET, and GC-MS. The results indicate that the pyrolysis products used in this study did not exhibit toxic effects on *Spirodela polyrhiza* growth. Instead, all analysed variants showed a generally positive influence on plant development, reflected in a clear stimulatory response. The findings demonstrate that the impact of biochar on plant growth was strongly dependent on both the pyrolysis temperature and the applied dose, with the most favourable outcomes observed for biochar produced at 400–500 °C. Overall, the study identifies sunflower husk as a duckweed growth-stimulating material and supports its further consideration as an environmentally safe, waste-derived fertilizer component.

Keywords: biochar, pyrolysis, sunflower husk, *Spirodela polyrhiza*, stimulator, biochar-fertilizer

Highlights:

- Biochar produced at 400–500 °C most strongly stimulates frond production.
- Porosity was highest at 500 °C, resulting in the strongest growth response.
- Biochar from sunflower husks showed no toxicity; instead, it boosted growth.
- A 10 mg/10 ml dose of pyrolyzed sunflower husk increased fronds over 50% versus control.

1. Introduction

Global challenges such as climate change, rapid population growth, and continuous urbanization are putting pressure on agricultural systems, and recent projections suggest that total global food demand is likely to increase by approximately 50–60% by 2050 [1]. Fertilizers continue to play a central role in sustaining duckweed growth and crop productivity; however, ongoing limitations stemming from soil degradation and limited nutrient availability are prompting the search for complementary strategies to enhance their effectiveness [2,3]. Recently, interest has grown in incorporating amendments, such as biochar, alongside conventional fertilizers to further improve plant growth, while monitoring their effectiveness using bioindicators to ensure sustainable outcomes. Nowadays, chemical fertilizers are the most widely used type of fertilizers in agriculture. Supporters of these fertilizers claim they enhance crop yield and quality by providing nutrients in well-balanced, optimized proportions tailored to each crop's specific requirements. Nevertheless, chemical fertilizers also have disadvantages, the most important of which is their negative impact on the natural environment [4]. Their overuse poses a significant threat to both current and future generations. The production, use, and disposal of these substances pollute water and soil. It can also contribute to greenhouse gas emissions and land degradation [5].

Biochar can represent a promising option for enhancing plant growth [6]. It is a solid carbon product obtained by the thermal decomposition of biomass under conditions of limited oxygen access, a process called pyrolysis [7]. Pyrolysis is a thermal conversion method commonly used to transform organic-rich materials into more valuable products. This process initiates thermal decomposition of organic materials under oxygen-free or limited conditions. It can be conducted at various temperatures, ranging from 300 to 700 °C [8]. The most common feedstocks are agricultural and municipal waste, wood residues [9]. Thanks to its durable and highly porous structure, this material can contain extractable humic- and fulvic-like substances [10]. Furthermore, it has a high degree of microbial and chemical stability thanks to its molecular structure [11].

The properties of biochar are diverse and influenced by various factors. The type of feedstock plays a crucial role, as well as process conditions – including maximum temperature and holding time [12]. Also, the type of technological procedures used before and after the pyrolysis process can influence the biochar properties. Due to these factors, achieving optimal end-product characteristics requires the selective choice of both feedstock and

processing conditions [13]. For example, the lignocellulosic feedstock contributes to increased specific surface area in biochar. Plant-derived materials increase the cation exchange capacity, while animal waste feedstock enriches the product with biogenic elements [9].

Biochar (often applied as a modified or enriched fertilizer) can significantly improve aqua and soil quality, support plant growth, and increase yields [13]. Biochar can also contain a variety of harmful substances, including heavy metals, polycyclic aromatic hydrocarbons, polychlorinated dibenzodioxins, polychlorinated dibenzofurans, and volatile organic compounds. These toxicants can be present in the biomass used to produce biochar, or they can form during pyrolysis, although these were not analysed in the present study [14,15]. Due to the complex impact of biochar, which depends on its properties, as well as the diverse needs of soils in terms of nutrient availability, application methods, and crop types, it is crucial to continue research on different variants of this pyrolyzed biomass to identify the optimal conditions of biochar production [16]. In this study, the toxicity effects of biochar on *Spirodela polyrhiza* were analysed. The study was carried out using three fractions of biochar produced at 300 °C, 400 °C, and 500 °C from sunflower husks as the biomass feedstock.

The main objective was to determine whether sunflower husk biochar inhibits or promotes the growth and development of the macrophyte *Spirodela polyrhiza*. This study is highly novel, as it is the first to investigate the effects of this specific type of biochar on this species and, more broadly, among aquatic plants.

Our findings address a key research gap, demonstrating that even nitrogen-poor biochar can support plant growth through indirect mechanisms, such as modifying nutrient availability and environmental conditions. Previous research has primarily focused on *S. polyrhiza* in phytoremediation and nutrient recovery, with no reports on its response to biochar in aquatic systems. The *Lemnaceae* family comprises aquatic plants with simplified morphology, rapid vegetative reproduction (1–2 days), and high environmental sensitivity, which underpins their strong bioindicator potential [17]. In this study, *Spirodela polyrhiza* was selected due to its widespread use in toxicology, phytoremediation, and environmental monitoring [18].

Beyond the primary focus of this study on the effects of biochar on aquatic plant growth, it is important to note that biochar has considerable potential as an adsorbent for contaminants in wastewater treatment, effectively removing both organic and inorganic pollutants from effluent streams.

Moreover, surface modifications of biochar can significantly enhance its adsorption properties, making it a promising material in sustainable water purification technologies [19,20].

These results are particularly relevant in light of Commission Regulation (EU) 2025/153, which recognizes *Lemna minor* and *Lemna gibba* as novel foods, and may contribute to the development of sustainable biomass production strategies for related *Lemnaceae* species with food potential, including *Spirodela polyrhiza* [21].

2. Materials and Methodology

2.1. Plant *Spirodela polyrhiza*

Spirodela polyrhiza is characterized by a rapid development cycle and intensive vegetative reproduction, which enable rapid biomass accumulation and support its industrial use. Thanks to its high growth efficiency, this plant can be used for biomass and biofuel production, making it an attractive component of the circular economy. This species can remove and accumulate pollutants from aquatic environments. Additionally, it's highly effective in reducing the concentrations of heavy metals and nutrients [22]. It can adapt to a wide range of water types, further enhancing the ecological potential of this species [17]. *S. polyrhiza* has a high sensitivity to the presence of heavy metals such as silver, cadmium, copper, and chromium, responding with morphological changes, including reduced frond area and the length of regenerating roots [23]. The use of this aquatic plant enables the rapid acquisition of reliable results with minimal financial and technical effort, making this plant species particularly valuable as a bioindicator in toxicological studies and routine water quality monitoring [24]. *Spirodela polyrhiza* plants used in this study originated from a laboratory collection maintained at the University of Lodz, Poland, and were not collected from wild populations. The study complied with all applicable institutional regulations. No permits or licenses were required for the use of this plant material. *Spirodela polyrhiza* is not listed under CITES and is not considered a threatened species according to current conservation assessments.

2.2. Material: Sunflower husk

Sunflower husk is an eco-friendly biomass by-product of sunflower seed processing that can be used as a biofuel. In this study, biochar particles used were sieved to a nominal size of 8 mm. Due to its high calorific value of 21.57 MJ/kg and low moisture content, it is commonly used as a substitute for pellet or coal burners. Additionally, because it originates from agricultural waste,

it is a more cost-effective and sustainable alternative to wooden pellets. The sunflower husks used in this study were sourced from Ivano-Frankivsk, a region in Ukraine. The country accounts for approximately 25–30% of global sunflower production, with Ivano-Frankivsk contributing a smaller but still significant share of the national output [25].

2.3. Growth analysis

The *Spirodela polyrhiza* used in this study was collected from aquatic plants grown in vitro at the Department of Plant Ecophysiology, University of Lodz. The experiment was conducted in a plate system, where 10 fronds were initially introduced into each sample, each in 10 mL of tap water. The study used tap water with a stable and controlled physicochemical composition, in accordance with ISO 20079 [26]. This choice allows for a better representation of environmental conditions than synthetic media, which can introduce additional confounding factors (e.g., excess nutrients). This gives the obtained results greater ecological and interpretive significance. The pH measurements were conducted on day 0 and at the end of the experiment, showing a slight decrease from 7.4 at the beginning to 7.2 at the end, which is a natural phenomenon during plant cultivation. The composition of the tap water used in this study is presented in Table 1.

Table 1 Composition of growth media liquid

Mineral component	Value	Unit	Mineral component	Value	Unit
Calcium (Ca ²⁺)	72-76	mg/L	Aluminum (Al ³⁺)	<20	µg/L
Magnesium (Mg ²⁺)	1.70-10.40	mg/L	Zinc (Zn ²⁺)	0.003-0.009	mg/L
Sodium (Na ⁺)	5.08-6.40	mg/L	Copper (Cu ²⁺)	<0.004	mg/L
Potassium (K ⁺)	0.57-1.13	mg/L	Nickel (Ni ²⁺)	<4.0	µg/L
Chloride (Cl ⁻)	11.00-12.00	mg/L	Lead (Pb ²⁺)	<2.0	µg/L
Sulfate (SO ₄ ²⁻)	21-24	mg/L	Chromium (Cr ³⁺ /Cr ⁶⁺)	<2.0	µg/L
Nitrate (NO ₃ ⁻)	0.61-2.60	mg/L	Selenium (Se)	<1.0	µg/L
Nitrite (NO ₂ ⁻)	<0.04	mg/L	Boron (B)	<0.05	mg/L

Fluoride (F ⁻)	0.10-0.14	mg/L	Manganese (Mn ²⁺)	6-28	µg/L
Iron (Fe)	20-35	µg/L			

The growth environment was at room temperature (24 ± 1 °C) and under a 16/8-hour light/dark cycle with a light intensity of $85 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Plants were cultivated in sterile Tissue Culture Plates (6-well format, Falcon), with each well measuring 3.5 cm in diameter and serving as an independent microreactor. This configuration minimized environmental variability and ensured uniform conditions across experimental replicates. The system was not sealed; slight evaporation could occur during the experiment. To maintain a constant medium volume and stable concentrations of the tested factors (including biochar), water was replenished daily to the initial volume of 10 mL. This procedure minimized artifacts associated with concentration changes due to evaporation.

Three types of biochars obtained from sunflower husks were used as test agents. The pyrolysis temperatures used for this experiment were 300, 400, and 500 °C. Each biochar was added to the substrate at four doses: 10, 50, 100, and 250 mg. For comparison, a control sample without pyrolyzed products was also prepared. All experiments were conducted in triplicate to ensure the reliability of the results. Plant cultivation took place under natural climatic conditions, i.e., those occurring between July 5, 2025, and July 14, 2025. During this period, the temperature was 24-25 °C, the photoperiod was 15 hours of natural light and 9 hours of darkness. To create growth response curves and analyse the relationship between biochar doses and average macrophyte growth, all visible plant fronts were recorded daily. The experiment lasted ten days. All visible fronds in each sample were recorded daily, and their number was systematically documented. This activity provided the basis for determining the growth dynamics of the plant under test. Thanks to that, it was possible to analyze the effect of the applied biochars on the plants. It also helps to establish which doses may be toxic or have a stimulating effect. Deviations from the expected exponential growth were interpreted as a sign of either a negative or positive impact of the tested substances. A reduction in the number of fronds relative to the control was considered an indicator of an inhibitory effect. In contrast, an increase in the number of fronds was considered a possible stimulatory effect on macrophyte development. This was the primary indicator of this study. The resulting data served as the basis for subsequent statistical analyses and enabled the

determination of plant growth dynamics and the effects of different biochar doses on *Spirodela polyrhiza* development.

2.4. Physiological analysis methods

2.4.1. Determination of the chlorophyll index (a + b)

To accurately assess the potential toxicity of the biochars used, a key element of the analysis was to determine the chlorophyll content index- a physiological parameter reflecting the health of plants and their ability to conduct effective photosynthesis. The measurements were performed at three strategic time points: at the initial stage (before exposure), on the fourth day of the experiment, and at the end of the experiment. This three-phase measurement scheme enabled monitoring of chlorophyll content dynamics and assessing the impact of each biochar variant on photosynthetic processes. Chlorophyll content was measured using a handheld chlorophyll meter SPAD-502 (Konica Minolta, Japan). This device enables a rapid, non-invasive, and reproducible estimation of chlorophyll content based on leaf colour intensity [27]. This measurement enables accurate monitoring of plant physiological status, which is crucial for assessing the potential toxicity of the tested materials and their effects on basic metabolic processes in plants.

2.4.2. Chlorophyll fluorescence analysis

Chlorophyll fluorescence was measured using the Handy PEA (Hansatech Instruments Ltd., UK), an advanced fluorometer commonly used in plant physiology research. This instrument records fluorescence parameters with high temporal resolution, enabling accurate mapping of the dynamics of photochemical processes in chloroplasts. The Handy PEA can generate Rapid Light Curves (RLCs), which have been widely used in research on photosynthetic efficiency. For these measurements, individuals of *Spirodela polyrhiza* were collected from each experimental variant and grown under controlled conditions. But before the experiment began, each plant underwent a 20-minute dark adaptation. This allowed the complete relaxation of the photosystem II reaction centers and stabilization of their redox state. After that, the plant was exposed to a 670 nm light source, while chlorophyll fluorescence emission was recorded in the 680-760 nm range using a sensitive photodetector.

The basic parameter of this analysis was the maximum quantum efficiency of photosystem II (F_v/F_m), which can be calculated according to the equation:

$$F_v/F_m = (F_m - F_o)/F_m \quad (1)$$

Where:

F_m - maximum fluorescence obtained after the saturation pulse,

F_0 - initial fluorescence, recorded after dark adaptation.

To assess the changes in photosystem II efficiency during the exposure period, measurements were taken three times: on days zero, three, and six of the experiment.

The F_v/F_m typical reference value for plants in good health is approximately 0.83. A decrease in this parameter indicates environmental stress, PSII damage, or inhibition of photosynthetic processes [28,29].

2.5. SEM Analysis

Scanning electron microscopy was conducted on a Thermo Fisher APREO 2S instrument fitted with an EDAX energy-dispersive X-ray detector. Specimens were mounted on carbon-taped stubs and examined under high vacuum. Imaging employed a 1 keV accelerating voltage, beam current of 0.2 nA and the T1 segmented lower in-lens detector to resolve surface morphology.

Shimadzu EDX-8100P XRF spectrometer was used for bulk quantitative analysis, the samples were homogenized and then weighed at approximately 3 g. The sunflower husk samples — including raw biomass and pyrolyzed biochars were placed in a measuring cup lined with Mylar foil. Each sample was analyzed under a nitrogen atmosphere. A 10 mm collimator directed the X-ray beam, and the SSD detector, cooled by a Peltier module, captured fluorescent X-rays. Three energy channels (Al-U, C-Sc, S-K) covered elements from carbon ($Z = 6$) through uranium ($Z = 92$). Specialist filters and thin-window technology enable enhanced detection of low-Z elements down to the ppm level without requiring extensive sample preparation. Data were processed using Shimadzu's PCEDX-NAVI and PCEDX-PRO software to generate both qualitative spectra and quantitative concentration results.

2.6. Pyrolysis process

The biochar used in the study was obtained in a dedicated batch biomass pyrolysis installation. The biomass pyrolysis system consists of a batch reactor, a heating system with temperature control, and a condensation and combustion unit for the liquid pyrolysis products. The batch reactor used in the experiment is cylindrical, 350 mm in diameter, 500 mm high, and made of 3 mm steel, sealed with a DN350 flange and secured with 16 screws. It features thermocouple ports ($\varnothing 16$ mm) and a gas outlet ($\varnothing 63$ mm) for

monitoring. During the experiment, the reactor is placed axially within a ceramic heating element with Kanthal coils, which ensure even heat distribution, and is surrounded by insulation. Temperature is controlled by a type K thermocouple and a PID controller. Biomass is loaded and, upon heating, undergoes pyrolysis, producing gaseous and liquid products. The gaseous and liquid pyrolysis products (in vapor form) are rapidly removed through a DN50 pipe to prevent condensation and secondary reactions. The resulting products are directed to a disposal chamber with a gas burner, ensuring safe handling.

Additionally, a CHNS-O elemental analysis is being conducted to determine the biomass's elemental composition, a key factor influencing its thermochemical behaviour. This analysis also provides insight into the extent of pyrolysis, as the carbon content in the solid product typically increases with temperature, while the proportions of oxygen and hydrogen decrease. The elemental composition was measured using a CHNS-O Flash 2000 analyser (Thermo Scientific). The heating rate was approximately 5 °C/min. The exact heating rate may vary slightly across different grains (pellets) due to the large-scale reactor used for pyrolysis. In batch reactors, the heating rate cannot be estimated with the same accuracy as in, for example, TGA experiments. Therefore, the average heating rate for the entire reactor was determined by measuring the total process time and the temperature change within the biomass bed. The reactor was not purged with an inert gas due to the high bulk density of sunflower husk pellets and the resulting low air-to-biomass ratio. The reactor (50 dm³) contained approximately 32.5 kg of biomass and about 21 g of air, including ~4.8 g of oxygen. As the temperature increased, thermal expansion and evolving pyrolysis gases reduced the oxygen content to ~2.4 g, resulting in an oxygen-to-biomass ratio of approximately $1:1.3 \times 10^4$. Under these conditions, the effect of oxygen on the process is considered negligible, and the process can be regarded as true pyrolysis. When the temperature reached approximately 100 °C, residual moisture was released from the biomass, and the resulting water vapor further evacuated the remaining air. At around 200 °C, a temperature at which oxygen from air could potentially begin participating in surface reactions with the biomass, the oxygen concentration was already low enough that its effect would be negligible. It should also be noted that thermal decomposition of biomass begins around 200 °C. In the initial stage of decomposition, carbon monoxide is released, which is thermodynamically more prone to oxidation than the biomass itself. While inert gas purging is important in general, it is not critical for this type of batch reactor, especially

when processing biomass with high bulk density. The residence time was 1 hour, counted from the moment the target temperature was reached in the center of the biomass bed.

2.7. Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR analysis was performed using a Thermo Scientific Nicolet iS50 FT-IR spectrometer equipped with an iS50 ATR diamond crystal and an MCT-A detector. Spectra were recorded in the range of 400-4000 cm^{-1} at a resolution of 4 cm^{-1} , averaging 64 scans per sample, and processed using OMNIC™ Spectra software. Both raw sunflower husk and pyrolyzed samples (300, 400, and 500°C) were analysed to identify functional groups and monitor structural changes induced by thermal treatment.

2.8. Brunauer-Emmett-Teller (BET) Surface Area Analysis

The surface area, pore size distribution, and total pore volume of the raw sunflower husk and pyrolyzed samples (300, 400, and 500 °C) were determined by N₂ adsorption-desorption using a Micromeritics ASAP 2020 analyser equipped with NovaWin software. The procedure was adapted from methodologies reported by Onwudili and Scaldaferri (2023) [30]. Prior to analysis, all samples were degassed under vacuum at 120 °C for 12 h to remove residual moisture and volatiles. Nitrogen adsorption-desorption isotherms were collected at liquid nitrogen temperature (-196 °C). Specific surface areas were calculated using the BET method, while pore size distribution and total pore volume were obtained from the desorption branch of the isotherm using the Barrett-Joyner-Halenda model [31].

2.9. Gas Chromatograph

Gas chromatography-mass spectrometry was employed to identify organic compounds extractable from the produced biochars and to assess the presence of residual or newly formed organics following pyrolysis. Biochars obtained at 300, 400, and 500 °C were each subjected to solvent extraction. Approximately 1.0 g of biochar was transferred into a glass vial containing 2 mL of acetone, briefly vortex-mixed, and then sonicated for 10 min to maximise the desorption of loosely bound organic species. After extraction, the suspensions were passed through hydrophobic syringe filters (0.22 μm PTFE membrane) to remove particulate material prior to analysis.

Organic compounds in the filtrates were analysed following a previously established protocol by our research group [32]. The liquid sample was analysed using a Shimadzu GC-MS-QP2020 gas chromatograph equipped

with a capillary column SH-I-5MS (5%diphenyl - 95% dimethyl polysiloxane) (30 m long, 0.25 mm diameter, 0.25 μm film thickness). The helium (purity >99.9999) flow through the column was set to 1.0 mL/min. The temperatures of the ion source and the injector were set to 300 °C and 250 °C, respectively. For each analysis, 1 μL of sample was injected with a split ratio of 1:20. The oven temperature programme commenced at 40 °C, ramped at 10 °C min⁻¹ to 300 °C, and was held for 5 min.

3. Results

3.1. Pyrolysis results

In the conducted study, the pyrolysis temperatures were 300, 400, and 500 °C. After the pyrolysis product release has stopped, the reactor is switched off and subsequently cooled for approximately 10 hours. Afterwards, the reactor is unloaded, and the carbonized material is subjected to further analysis. The greatest amount of biochar was obtained at 300 °C, accounting for 67% of the solid mass. At 400 and 500 °C, the yields of carbonized solid mass were 36.3% and 31.7%, respectively. Thermogravimetric analysis (TGA) has been conducted on raw sunflower husk. The study, carried out using a NETZSCH TG 209F3 apparatus, enabled evaluation of the sample mass changes as a function of temperature over 35 to 800 °C, under a nitrogen atmosphere and at a heating rate of 10 K/min. The initial mass of the sample was 10.65 mg, and the measurement was performed using an open ceramic crucible (Al_2O_3 , 85 μl). The results are shown in Figure A1, provided in the Supplementary Materials. During the first stage (up to 130 °C), a slight mass loss of approximately 2% occurred. This corresponds to the release of moisture and volatile compounds from the material, as indicated by the first peak on the DTG curve at 73.5 °C, with a maximum mass-loss rate of $-0.70\%/ \text{min}$. The most significant mass changes occur between approximately 180 °C and 350 °C. Within this interval, a sharp decrease in the sample mass is observed - from about 95% to around 30%. The maximum mass loss rate is observed at 330.8 °C, at $-7.60\%/ \text{min}$. This temperature range is typical for the thermal decomposition of organic biomass components such as cellulose, hemicellulose, and lignin. The point corresponding to 50% of the initial mass is reached at 335.9 °C. Biomass weight loss is significantly reduced once it reaches 400 °C. Moreover, no further changes are observed at 500 °C. At the final temperature of 798 °C, the residual mass of the sample is 26.1%. This shows that a raw sunflower husk has a relatively high content of inorganic substances and bound carbon. Additionally, an elemental analysis was conducted, and the results are listed

in Table B1 provided in Supplementary Materials. In conclusion, the analysed biomass exhibits typical properties of lignocellulosic biomass: it contains a small amount of moisture, undergoes intensive decomposition in the range of 200–400 °C, and upon completion of the process, leaves a significant amount of inorganic residue.

3.2. XRF Analysis

The XRF results are presented in Table 2 below.

Table 2 Quantitative results for X-ray Fluorescence Spectrometer Analysis

Quantitative Result	Raw biomass	300 °C	400 °C	500 °C
K (Potassium)	1.494%	3.467%	4.099%	6.098%
Mg (Magnesium)	0.269%	0.551%	0.645%	2.475%
Ca (Calcium)	0.217%	0.849%	0.996%	1.503%
P (Phosphorus)	0.083%	0.429%	0.433%	0.744%
S (Sulfur)	0.150%	0.243%	0.226%	0.263%
Si (Silicon)	0.026%	0.179%	0.185%	0.108%
Cl (Chlorine)	0.084%	0.239%	0.278%	0.441%
Fe (Iron)	0.003%	0.222%	0.504%	0.344%
Mn (Manganese)	0.004%	0.004%	0.007%	0.008%
Zn (Zinc)	0.004%	0.005%	0.089%	0.138%
Cu (Copper)	0.001%	0.003%	0.003%	0.005%
Rb	0.001%	0.002%	0.003%	0.004%
Br	0.001%	0.001%	0.002%	0.003%
Sr	-	0.001%	0.002%	0.003%

As shown in Table 2, the analysis indicates that the potassium content in biochars made from sunflower husks increased with increasing pyrolysis temperature. In biochar obtained at 500°C, phosphorus content nearly doubled. The sulfur content remained unchanged during pyrolysis. As

pyrolysis progressed, the chlorine content increased significantly, while the contents of metals and compounds, such as Rb, Br, and Sr, did not change significantly.

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3.3. SEM Analysis

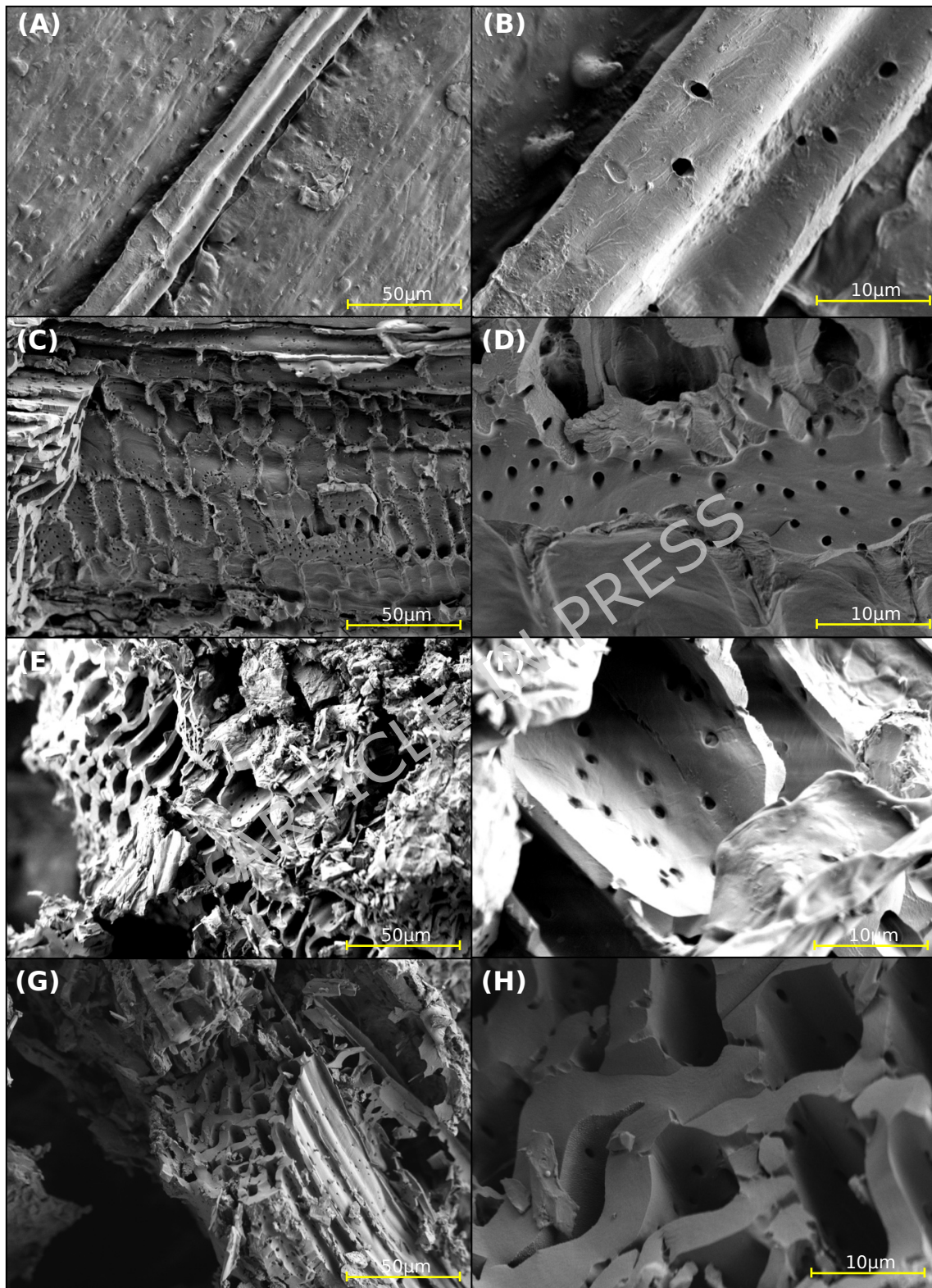


Figure 1 SEM views of (A, B) - raw material; (C, D) - 300 °C; (E, F) - 400 °C; (G, H) - 500 °C

SEM analysis of surface morphology changes showed that increasing the pyrolysis temperature leads to a more distinct surface voidage, as observed in Figure 1. Sunflower husk pyrolysed at 500 °C has a higher surface voidage than the raw biomass and samples pyrolysed at lower temperatures. In general, hemicelluloses are a group of polysaccharides located in the plant cell wall, consisting of branched amorphous structures that link cellulose and lignin [33]. In the raw sunflower husk, this branched structure is clearly visible; however, after pyrolysis, it gradually disappears, indicating that hemicellulose decomposes during the process. In the raw sunflower husk, the surface is relatively smooth, dense, and organized. The fibrous structures are intact, and the cell walls are mostly continuous. The thick nature of the structure indicates the presence of hemicellulose and lignin as binding materials between cellulose [34]. The visible vessels and long fibers are typical of the lignocellulosic material with high hemicellulose content [35]. No structural damage has been observed yet, confirming that the biomass is in a natural, undegraded condition. At a pyrolysis temperature of 300 °C, the surface begins to exhibit noticeable morphological change. It forms tiny voids, pores, and fissures, indicating the beginning of hemicellulose decomposition. The fibrous network remains recognizable, but it is coarser and less dense. Partial volatilization of amorphous material results in local shrinkage and mild fragmentation of cell walls. This temperature range corresponds to the main decomposition stage of hemicellulose and is responsible for the apparent loss of structural integrity [36]. At 400 °C, the degradation is significantly more severe. The cell wall structure collapses, leaving a surface with irregular, interconnected voids. The breakdown of hemicellulose and partial depolymerization of cellulose result in extensive loss of structural integrity. The surface is brittle and porous, with numerous microcracks and extensive devolatilization. At 500 °C, the sunflower husk exhibits a highly distorted, porous morphology. The fibrous structure of the raw sample is almost destroyed and replaced by a porous, carbon-rich network. The surface is characterized by numerous large voids, channels, and cracks, revealing serious thermal degradation and carbonization. The development of such pores increases the specific surface area, indicating that the majority of hemicellulose and cellulose have degraded, leaving behind a char-like substance with high aromatic carbon structures. The SEM analysis illustrates the clear correlation between pyrolysis temperature and structural degradation. As the temperature increases, the dense surface of the fresh sunflower husk transforms into a porous, fragmented carbonaceous

structure. The progressive formation of surface voidage and disintegration of the fibrous structure confirm the degradation of hemicellulose and cellulose, and the transformation of biomass into a thermally stable, carbon-rich product.

3.4. The effect of pyrolysis products on the growth of *Spirodela polyrhiza*

In the control sample, the average number of fronds was 17, with a standard deviation (SD) of 1.53. The result obtained provides a reference point for assessing the effects of biochar produced at different pyrolysis temperatures. Biochar was added directly in powder form after grinding and sieving. Figure 2 presents photos of the control sample and samples supplemented with pyrolyzed biomass at different pyrolysis temperatures and doses, taken on the final day of the experiment. The standard deviation for all variants ranged from 0 to 1.6 and did not differ significantly from that of the control group.

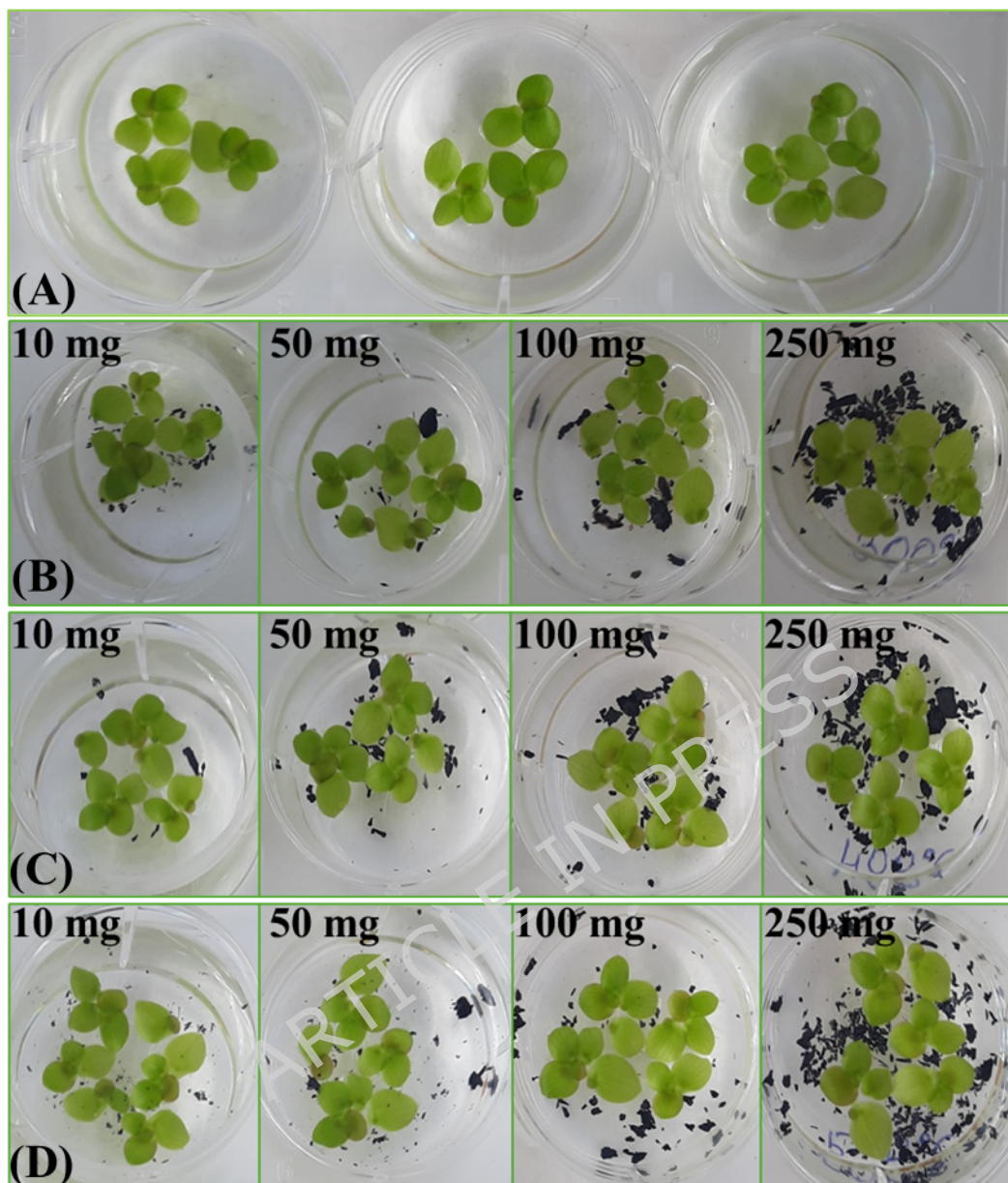


Figure 2

Growth of *Spirodela polyrhiza* plants. Variation in frond number among the samples on the final day of the experiment: (A) control sample, (B) biochar pyrolyzed at 300 °C, (C) biochar pyrolyzed at 400 °C, and (D) biochar pyrolyzed at 500 °C

Variants with biochar obtained at 300 °C showed a moderate, dose-dependent stimulatory effect, as shown in Figure 3. At the lowest dose (10 mg per 10 ml), the results were comparable to the control group, with an average of 16.3 fronds. However, with doses of 50 and 100 mg (per 10 ml), a significant increase in frond number was observed, reaching 19 and 20.5, respectively. The highest dose, 250 mg (per 10 ml), produced the greatest

increase in frond number, reaching 22. The results show that biochar pyrolysed at 300°C has a positive stimulatory effect on this plant's growth, particularly at higher doses.

The results were even more pronounced with biochar obtained at 400 °C. Plants treated with a dose of 10 mg/10 ml produced 21 fronds, while those with a dose of 50 mg/10 ml had 18 fronds. The highest stimulatory effect for this biochar was observed at a dose of 100 mg/10 ml, resulting in 24 fronds. The 250 mg/10 ml dose also produced a substantial effect, with a total of 22 fronds. This shows that biochar obtained at these temperatures has an even greater positive impact on frond number, with the optimal dose being 100 mg/10 ml. The greatest impact was observed with the variant containing biochar obtained at 500 °C. The highest number of fronds during the entire experiment was observed with the 10 mg/10 ml dose, reaching 26, an increase of over 50% compared to the control group. Other doses, including 50, 100, and 250 mg, also had a positive effect on growth, with counts of 19, 25, and 22 fronds, respectively.

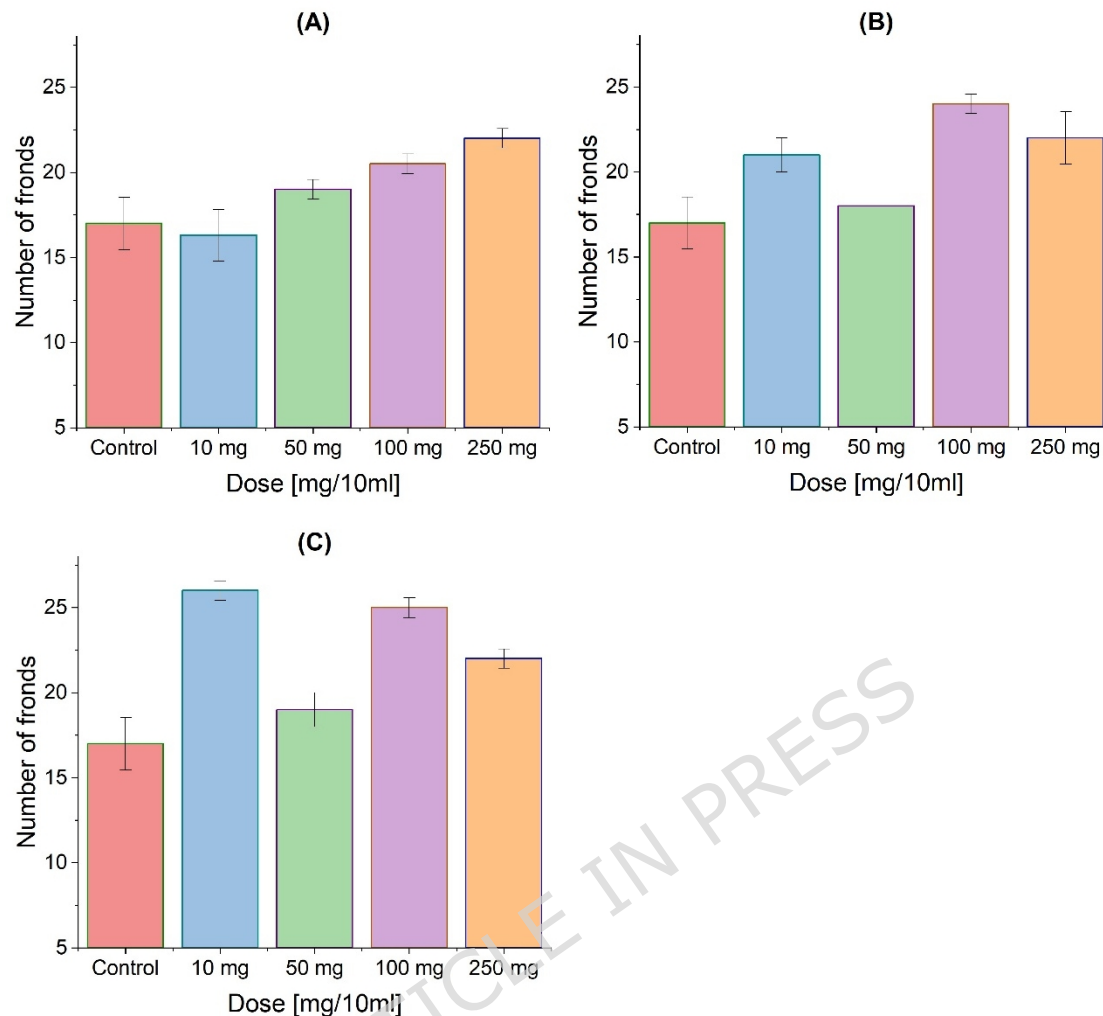


Figure 3 Effect of biochar produced at (A) 300 °C, (B) 400 °C, and (C) 500 °C on *Spirodela polyrhiza* growth in terms of the number of fronds

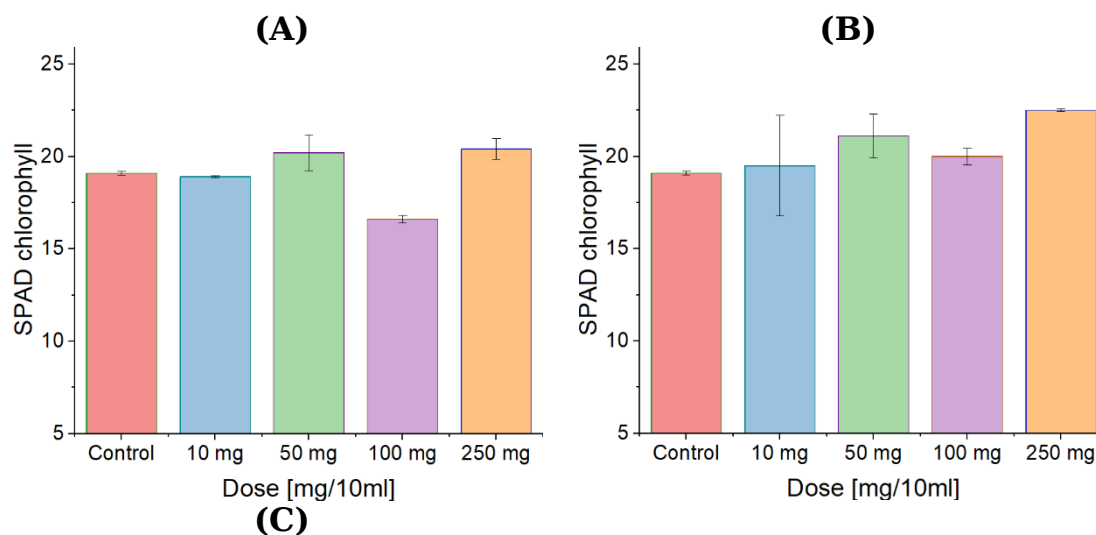
To complement the biological results with a rigorous assessment of statistical significance, an analysis of variance (ANOVA) was conducted using Statistica. The analysis evaluated the effect of biochar derived from sunflower husks on the growth of *Spirodela polyrhiza*. The results demonstrated that the model is statistically significant ($F = 17.29$; $p < 0.000001$), indicating significant differences among the experimental variants analyzed.

To identify differences between individual groups, Tukey's post hoc test (HSD) was applied. The analysis revealed numerous statistically significant differences ($p < 0.05$) among selected combinations of experimental factors. Significant differences were observed between the control sample and treatments involving elevated temperatures (300–500°C), as well as between different dose levels within these temperature ranges. At the same time, some comparisons, particularly those involving similar experimental conditions,

did not show statistically significant differences ($p \geq 0.05$), indicating a complex biological response. The obtained results clearly confirm that both the temperature of biochar production and its dose constitute significant factors determining the growth of *Spirodela polyrhiza*, with their effects depending on specific combinations of experimental parameters.

3.5. Chlorophyll index analysis

The chlorophyll index in *Spirodela polyrhiza* on a day t_0 was 17.46 SPAD units, with a standard deviation of 0.799. These results indicate that the plants subjected to the experiment are in excellent condition and provide a reference point for further research. In the control sample, which did not receive pyrolyzed products, the average SPAD value reached 19.1 at the end of the experiment. Variants with a 10 mg dose of biochar pyrolyzed at 300 °C had an average SPAD value of 18.9, similar to that in the control. The highest doses (50-250 mg/10 ml) showed slight fluctuations in the average values (20.2, 16.6, 20.4), with a moderate standard deviation. However, all of them remained within a range indicating good plant condition. The lowest SPAD value, 16.6 for the 100 mg dose, remained above the critical threshold associated with plant stress. All results are shown in Figure 4. The SPAD values obtained from biochar pyrolyzed at 400 °C varied with dose. The variant with a 10 mg biochar dose reached 19.5 SPAD units, while higher doses (50-250 mg/10 ml) produced average values of 20-22.5 SPAD units. At a pyrolysis temperature of 500 °C, the lowest dose of 10 mg resulted in a SPAD value of 18.7, while higher doses (50-250 mg/10 ml) produced average values ranging from 20.2 to 24.7 SPAD units. The highest SPAD value among all experimental variants was observed at this temperature with a biochar dose of 100 mg/10 ml.



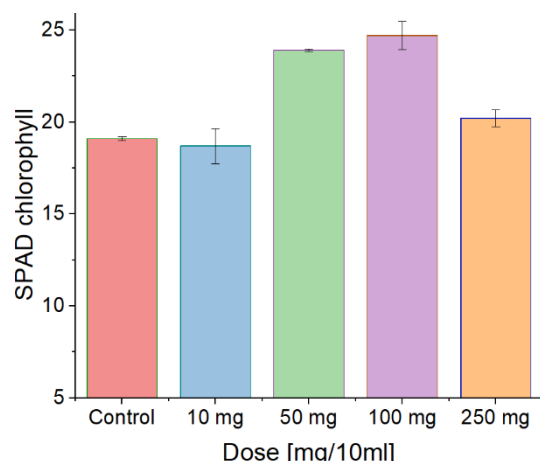


Figure 4 Effect of biochar produced at (A) 300 °C, (B) 400 °C, and (C) 500 °C on plant growth in relation to SPAD chlorophyll

3.6. Chlorophyll fluorescence analysis

Chlorophyll fluorescence in *Spirodela polyrhiza* L. tissues on day zero was 0.811, reflecting the excellent physiological condition of the plants before the experiment began. On the final day, the Fv/Fm parameter remained stable across all treatments, indicating that the plants maintained good condition throughout the experiment. The control sample achieved a value of 0.792, serving as a reference for the remaining measurements. Figure A2, which illustrates the effect of biochar on plant growth at each temperature relative to photosystem II, is available in the Supplementary Materials. For biochar obtained at 300 °C, fluorescence values ranged from 0.668 to 0.761. The highest result was recorded for the 10 mg/10 ml dose (0.761), while the lowest was observed for the 50 and 250 mg/10 ml doses (0.676 and 0.668, respectively). In variants with biochar obtained at 400 °C, fluorescence values were similar to or higher than the control, ranging from 0.695 to 0.795. The most pronounced effects were observed at doses of 100 and 250 mg/10 ml (0.795 each). The most stable values were achieved with biochar obtained at 500 °C. Regardless of the dose (10-250 mg/10 ml), chlorophyll fluorescence ranged from 0.759 to 0.799. The results for the 50 and 100 mg/10 ml doses (0.799 and 0.792, respectively) were almost identical to the control or slightly higher.

3.7. Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) of raw material (Sunflower husk) and biochar

The ATR-FTIR spectra (Fig. 5) of raw sunflower husk and biochar produced at 300–500 °C show systematic chemical transformation consistent with progressive decomposition of polysaccharides and formation of an

increasingly aromatic carbon matrix. The broad O-H stretching band (3400–3200 cm^{-1}), associated with hydrogen-bonded hydroxyl groups in cellulose/hemicellulose and adsorbed moisture, decreases in intensity and narrows as temperature increases, indicating dehydration and the loss of oxygenated functionalities. Aliphatic C-H stretching bands at 2920 and 2850 cm^{-1} ($-\text{CH}_2/-\text{CH}_3$ groups in biopolymers and lignin side chains) diminish markedly above 300 $^{\circ}\text{C}$, reflecting cleavage and volatilization of aliphatic structures. In addition, the carbonyl band in the 1730–1700 cm^{-1} region ($\text{C}=\text{O}$ in ester/carboxylic groups, commonly associated with hemicellulose components) weakens with increasing thermal severity, supporting deacetylation/decarboxylation reactions. In the fingerprint region (1200–900 cm^{-1}), the gradual loss of C-O-C/C-O stretching features characteristic of carbohydrates further confirms depolymerization of cellulose/hemicellulose. Conversely, bands assigned to aromatic structures (1600–1500 cm^{-1}) become relatively more pronounced at higher temperatures, indicating aromatization and development of a condensed carbon framework; lignin's higher thermal stability likely contributes to this aromatic backbone. Low-wavenumber features may also reflect increasing relative contribution of mineral/ash-related vibrations as organic matter is removed.

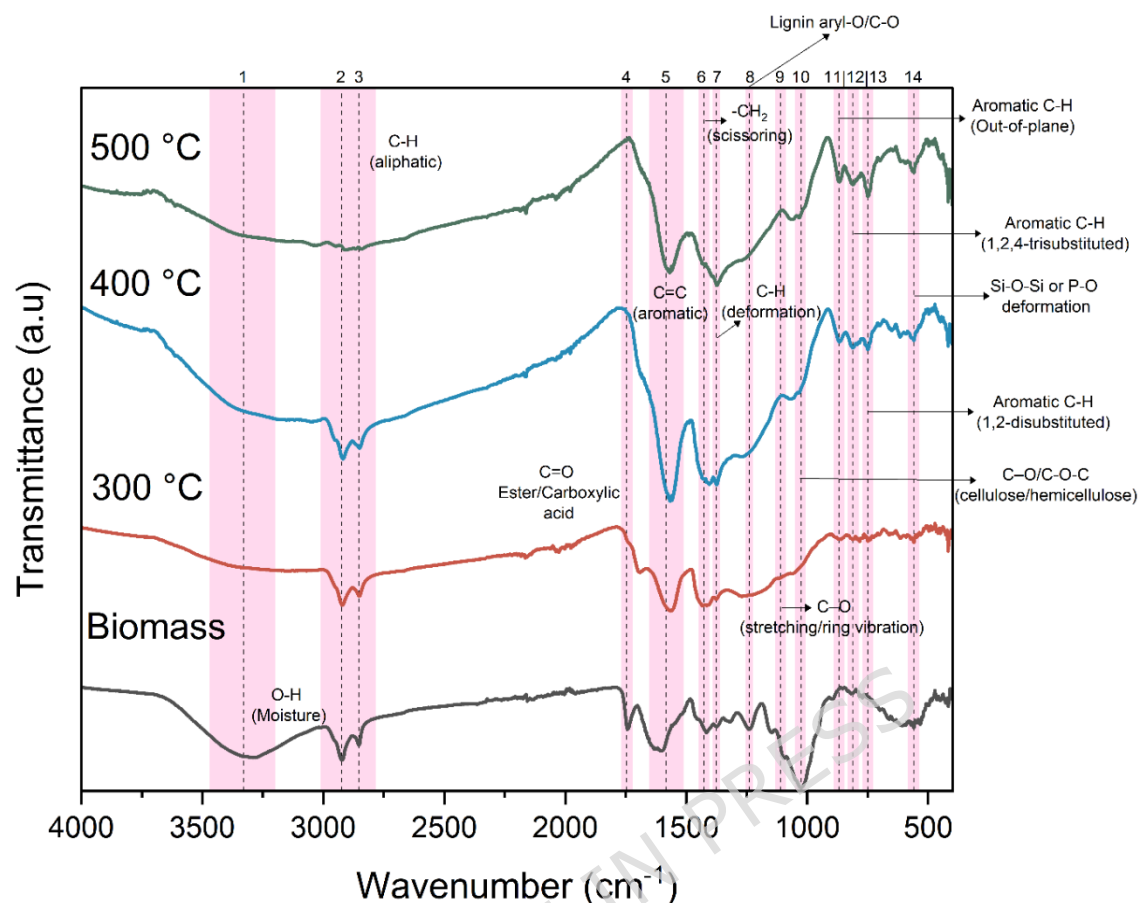


Figure 5 The ATR-FTIR spectra of the raw biomass and the pyrolyzed samples (300, 400, and 500 °C)

The ATR-FTIR results, showing the characteristic functional groups identified in the samples, are summarized in Table B2, provided in the supplementary materials.

3.8. BET Surface Area Analysis of raw material (Sunflower husk) and biochar

BET and BJH analyses reveal progressive textural changes in the biomass during pyrolysis. The very low BET surface area of raw sunflower husk reflects its dense structure and limited accessible pores, rather than a complete lack of surface area.

Pyrolysis at 300 °C slightly increases surface area and pore volume, indicating the formation of initial micropores. At 400 °C, a slight decrease in surface area is observed, likely due to pore collapse or densification. A significant change occurs at 500 °C, with pronounced increases in surface area, pore volume, and average pore diameter, suggesting the development

of mesoporous structures and enhanced porosity suitable for adsorption or catalytic applications.

The BET surface area results for the raw material and the resulting biochar are presented in Table 3.

Table 3 Brunauer-Emmett-Teller (BET) Surface Area Analysis results

Material	Surface area (m ² /g)	BJH Surface area (m ² /g)	Pore Volume (cm ³ /g)	Pore Diameter D _v (d) (nm)
Raw biomass	0	0.756	0.001	3.070
Biochar 300 °C	0.849	4.923	0.0188	3.088
Biochar 400 °C	0.577	3.914	0.013	3.049
Biochar 500 °C	1.124	19.603	0.118	24.114

3.9. Gas Chromatography-mass spectrometry (GC-MS) of raw material (Sunflower husk) and biochar

The GC-MS chromatogram identifies organic compounds in biochars pyrolysed at 300 and 400 °C, with significantly higher levels at 400 °C. For the biochar sample produced at 500 °C, none of the components were detected. The chromatogram and the sample images are included in Supplemental Material Figure A3. The GC-MS analysis of pyrolysis volatiles reveals a clear temperature-dependent evolution of organic compounds, as shown in Table 4. At 300 °C, the volatile fraction is dominated by a single compound, hexadecanoic acid methyl ester, indicating limited thermal decomposition and the release of fatty acid derivatives. At 400 °C, the volatile fraction becomes more complex, including methyl esters of hexadecanoic, octadecenoic, and docosanoic acids, as well as ketones (2-pentadecanone, 6,10,14-trimethyl-) and minor phenolic and alkyne compounds. However, at 500 °C, concentrations were below the detection limits, indicating that the majority of thermally unstable organic compounds had decomposed or condensed into the solid biochar. These results demonstrate the increasing thermal stability of the residual carbon matrix and the temperature-dependent release of low-molecular-weight organics during pyrolysis. These

results show that low-temperature pyrolysis primarily releases oil-like, lipid-derived compounds, whereas higher temperatures completely decompose these products into a stable, carbon-rich biochar.

Table 4 Identified organic compounds by GC-MS from biochar obtained at different temperatures

Retention time (min)	Compounds	Normalised Peak Areas (%)
300 °C		
35.8	Hexadecanoic acid, methyl ester	100
400 °C		
35.2	2-Pentadecanone, 6,10,14-trimethyl-	4.43
35.8	Hexadecanoic acid, methyl ester	13.30
38.9	1-Tetradecyne	7.39
39.0	11-Octadecenoic acid, methyl ester	29.56
39.1	11-Octadecenoic acid, methyl ester, (Z)-	25.62
39.3	Methyl stearate	9.36
39.6	o-Dodecylphenol	2.22
41.5	Docosane	1.48
42.3	Eicosanoic acid, methyl ester	2.96
42.5	Octadecane, 1-iodo-	1.72
43.8	Docosanoic acid, methyl ester	1.97
500 °C		
Below detection limit		

4. Discussion

Thermochemical valorization of biomass has become a widely studied and increasingly popular pathway for producing biochar. As a carbon-rich solid material, biochar offers remarkable versatility and can be used for a range of applications, including use as a renewable solid fuel and a plant growth stimulator. It was shown that applying biochar significantly improves organic duckweed growth. Abdul-Aziz et al. reported increased grain yield of 218.2% in 2022 and 106.3% in 2023 by adding 8 tons of biochar per hectare of soil [37]. Han et al. conducted even more advanced research by conducting a

meta-analysis to provide a comprehensive evaluation and overall impact analysis of results from various biochar studies. By reviewing 381 datasets from 63 studies worldwide, they found that adding biochar to soil increased crop yield, nitrogen use efficiency, and water use efficiency by 14.45%, 13.97%, and 14.28%, respectively [38]. However, in some cases, adding biochar results in neutral or even negative plant responses [39]. Therefore, to achieve optimal plant growth with biochar as a fertilizer additive, it is crucial to study various biochar feedstocks and production methods.

Sunflower husk biochar is characterized by limited nutrient availability, particularly nitrogen, which is significantly lost during pyrolysis. Unlike animal manure, which contains easily digestible forms of nitrogen, phosphorus, and other macronutrients, biochar primarily serves an indirect function—it does not directly provide large amounts of nutrients but influences their retention and availability in the environment. While sunflower husk biochar may contain moderate amounts of minerals (especially potassium and ash), it remains relatively low in nitrogen compared to other plant biochars. Its advantage, however, is its relatively high stability and ability to adsorb and gradually release nutrients, which can indirectly support plant growth.

This study primarily addressed the phytotoxicity of sunflower husk and its impact on the growth of the plant *Spirodela polyrrhiza*. The results of this study clearly indicate that biochar derived from sunflower husk, regardless of pyrolysis temperature, did not exhibit phytotoxic effects on *Spirodela polyrrhiza*. All variants exhibited either a stimulating or a neutral effect on plant growth and physiological properties. The produced biochar enhances the availability of mineral nutrients and modifies the environment's physicochemical properties, resulting in improved growth and development of the analysed plant. Those outcomes are consistent with findings reported by other researchers [6,40].

During the experiment, the effects of different biochars on plant responses and on the physicochemical processes underlying them were analysed. The results show that the biochar obtained at a temperature of 400-500°C has the strongest stimulating effect on the production of fronds. The increased production of fronds likely results from structural changes in the biomass during pyrolysis. As demonstrated by the SEM analysis, the material's porosity increased with higher pyrolysis temperatures, potentially enhancing water retention and gas exchange. These observations are consistent with BET and BJH measurements, confirming that the morphological evolution

observed under SEM is reflected in the overall textural development of the biomass. The measured specific surface area values (0.577 – 1.124 m²/g) are low, typical of sunflower husk, and consistent with previous studies (0.93, 1.3 m²/g) [41,42]. Although low, these values reflect the intrinsic nature of the biomass and do not compromise its potential functional properties. The combined evidence indicates that thermal treatment progressively transforms the material into a more porous, carbon-rich structure, linking physical changes to the chemical and structural modifications occurring during pyrolysis. Additionally, samples produced at elevated temperatures contained fewer volatile compounds, which often include phenolic substances and organic acids known to inhibit plant growth [14,15]. This relationship is further supported by the observation that the most efficient growth was observed with biochar produced at the highest pyrolysis temperature. The addition of 10 mg/10 ml of pyrolysed sunflower husk compared to the control sample enhances the number of fronds by over 50%. This proves that improvements in sorption properties, increased surface area, porosity, and pH stabilization in the aquatic environment stimulate plant growth. However, no linear relationship was observed between applied biochar doses and plant growth. It is possible that, with a higher addition of biochar to the environment, it may not be modified favourably. Increased alkalinity, micronutrient adsorption, or changes in dissolved substance concentrations do not support further intensification of macrophyte growth. The results obtained are consistent with those reported in the literature and highlight the importance of optimizing the dosage, especially in aquatic environments [9,43].

The chlorophyll index analysis also confirms a positive impact of biochar obtained at higher temperatures. The highest chlorophyll content compared to control samples was observed in plants grown with biochars produced at 400-500 °C. This indicates that those variants improve nitrogen metabolism and intensify photosynthetic processes. The results from X-ray fluorescence spectrometer analysis revealed that biochar obtained from sunflower husk contains significant amounts of K, Ca, and Si. Those elements can directly improve the stability of photosynthetic pigments and chloroplast function [44,45].

Fv/Fm values in the range of 0.759–0.799 may indicate the presence of mild stress; however, their relative stability suggests the absence of significant damage to the photosynthetic apparatus, particularly Photosystem II. In this context, the interpretation of results gains additional insight when considered alongside SPAD index values, which reflect the relative

chlorophyll content in plant tissues. Observed changes in SPAD values, combined with stable Fv/Fm parameters, may indicate the activation of adaptive mechanisms, such as regulation of chlorophyll synthesis or reorganization of the photosynthetic apparatus in response to changing environmental conditions. This response likely reflects physiological acclimation rather than direct damage to photosynthetic structures, highlighting the plants' ability to maintain photochemical efficiency even under stress [46]. The Fv/Fm ratio reflects stress differently in terrestrial and aquatic plants. In wheat, it remains stable throughout most of the drought period and only declines in the final phase, showing a strong correlation with yield loss, which makes it a good indicator of stress levels [47]. In barley, Fv/Fm decreases with increasing seeding rate, reflecting both environmental stress and competition among plants [48]. In contrast, in *Lemnaceae* (e.g., *Spirodela polyrhiza*), Fv/Fm remains at a high and stable level (approx. 0.75–0.80), and its decline occurs only under severe stress. This means that in aquatic plants, this index primarily reflects direct damage to the photosynthetic apparatus, whereas in terrestrial plants, it is more sensitive to various stress factors and competition.

Chlorophyll fluorescence analysis indicates that plants are not under physiological stress. Fv/Fm values in the range of 0.759–0.799 are typical for healthy plants and indicate proper functioning of the PS II. The lower values obtained in samples pyrolyzed at 300 °C do not necessarily reflect metabolic disorders, as they may be associated with residual organic compounds characteristic of lower pyrolysis temperatures [46]. The findings of this study indicate that biochar obtained from sunflower husk and pyrolysed at ≥ 400 °C can be used not only as a fertilizer additive for aquatic plants but also as a tool to support phytoremediation processes and long-term carbon sequestration.

This study focused on physiological indicators, including chlorophyll content (SPAD) and photosystem II efficiency (Fv/Fm), which are widely recognized as sensitive and reliable measures of plant condition and performance in aquatic systems. The observed stability of Fv/Fm, together with maintained or increased SPAD values, indicates preserved photosynthetic functionality and adequate pigment levels, both closely associated with sustained plant growth potential. Such an approach, based on non-destructive physiological parameters, is commonly applied in studies of aquatic macrophytes as a robust tool for assessing plant performance and productivity.

Studies on *Vallisneria spiralis* (a submerged macrophyte) have demonstrated that biochar does not disrupt plant growth or interfere with biogeochemical processes, including sediment oxidation driven by oxygen released from the roots [49]. Its influence is particularly significant in the case of cadmium (Cd(II)), whose bioavailability is reduced thanks to the material's porous structure, high adsorption capacity, and alkaline pH. However, the literature also reports that biochar has limited short-term effectiveness for nutrient remediation, especially in organic-matter-rich sediments. This organic fraction occupies sorption sites (NH_4^+ , PO_4^{3-}) and reduces microbial activity within the first 30 days. The most significant potential of biochar lies in its pairing with biological agents. In combination with arbuscular mycorrhizal fungi (AMF) or plant growth-promoting bacteria (PGPB), its remediation efficiency increases, microbial stress is alleviated, and glomalin-like soil protein production is enhanced [50].

Biochar not only enhances plant growth but also helps immobilize heavy metals and reduce their bioavailability, thereby enabling phytoremediation plants to function more efficiently [6]. When combined with fast-growing macrophytes such as *Spirodela polyrhiza*, integrated systems emerge in which biochar acts as a sorbent, and plants remove contaminants via bioaccumulation, enabling practical applications in water and soil treatment. Additionally, biochar stabilizes organic carbon, sequestering CO_2 for centuries and enhancing plant photosynthesis, while macrophyte biomass can be reused for remediation, animal feed, energy, or further pyrolysis, creating a positive carbon balance [14,15]. Moreover, biochar can serve as a precursor for producing activated carbon used in the purification of flue gases and water [51].

GC-MS results indicate a predominance of lipid-derived compounds (fatty acid methyl esters) at lower pyrolysis temperatures, suggesting that the resulting biochar retains a notable fraction of thermally unstable organic matter. This observation is consistent with previous studies demonstrating that biochars produced under mild-to-moderate pyrolysis conditions can contain a variety of adsorbed organic contaminants, including water-soluble organic acids, phenols, and other low-molecular-weight organics [52]. Such residual organics may influence the chemical behaviour, stability, and environmental safety of biochar, for instance, by affecting aqua/soil microbial communities, biochemical oxygen demand, or the mobility of nutrients and pollutants when applied to soil [53].

The schematic in Figure 6 summarizes the multifunctional role of sunflower husk biochar, with an emphasis on its use as a slow-release aquatic fertilizer that supports duckweed growth by improving nutrient retention and gradual release in water. In parallel, biochar contributes to phytoremediation by sorbing contaminants and helping immobilize metals, while also enabling reduced reliance on chemical fertilizers and synthetic biostimulants through its nutrient-holding and conditioning effects. By converting biomass residues into a stable, carbon-rich material, the pathway supports circular-economy waste valorization and climate change mitigation through long-term carbon storage in soils.

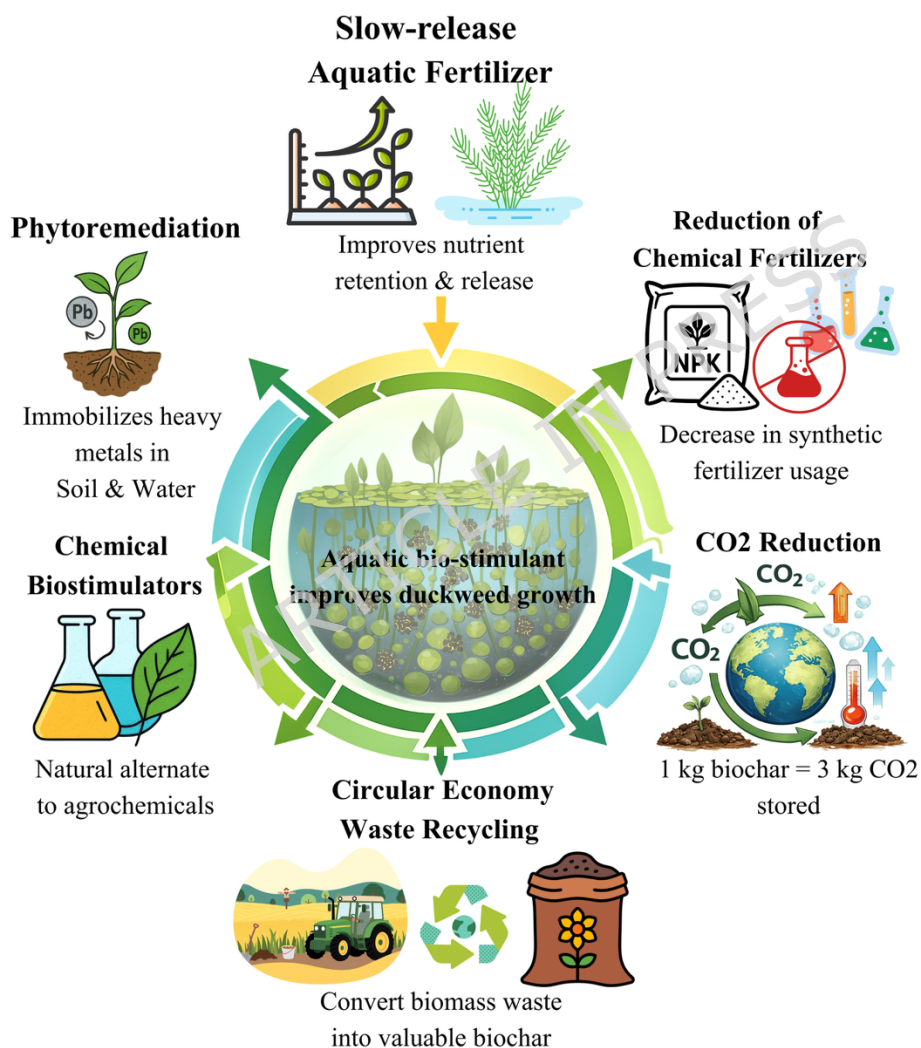


Figure 6. Schematic diagram for biochar-driven duckweed stimulation and co-benefits

Given these considerations, it appears advisable to explore post-treatment strategies (e.g., mild thermal treatment, controlled aeration, washing, or

aging) to reduce or remove residual lipid-derived or low-stability organic matter before the practical application of biochar, especially in environmental or agricultural contexts. Further studies are required to evaluate the effects of these post-treatment methods on plant growth and health, compared with untreated biochar, to confirm their practical benefits and safety. Many challenges remain despite its advantages, including the variability of biochar properties, which depend on pyrolysis conditions and feedstock; the potential reduction in the bioavailability of contaminants to microorganisms; and the short-lived effects reported in laboratory studies [54]. It is therefore essential that long-term investigation and optimisation of production parameters are conducted.

5. Conclusion

This study assessment indicates that biochar derived from sunflower husk, produced at pyrolysis temperatures between 300 °C and 500 °C, does not induce acute phytotoxic effects in *Spirodela polyrhiza* under the specific tap-water conditions tested. Within this experimental framework, the biochar variants analyzed demonstrated a stimulatory effect on plant development, characterized by a non-linear growth response. The most significant stimulation of frond production was observed with biochar produced at 500 °C applied at a 10 mg/10 mL dosage. These effects are attributed to enhanced porosity, increased surface area, and reduced volatile organic content at higher thermal treatment temperatures. While these results identify sunflower husk biochar as a potentially beneficial growth amendment for *S. polyrhiza* in a controlled aquatic environment, the findings are limited to the specific species. Consequently, long-term investigation and optimization of production parameters are essential to determine the sustained efficacy and environmental safety of this material across broader ecological and agricultural contexts.

6. Statements and Declarations

Ethical approval and consent to participate: Not applicable.

Data Availability: All data generated or analyzed during this study are included in this paper and its supplementary information files. The FTIR and EDX datasets generated during the study are available in the repository Lodz University of Technology RDBopen <https://doi.org/10.34658/RDB.A9AIU0>

Statement of informed consent, human/animal rights: No conflicts, informed consent, human or animal rights applicable.

Conflict of interest: The authors declare no competing interests.

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8. Author Contribution

Julia Osmanska: Conceptualization, Methodology, Software, Writing-Reviewing and Editing **Szymon Szufa:** Data curation, Writing- Original draft preparation, Funding acquisition **Romanowska-Duda Z.** Formal analysis, Methodology, Resources **Omar Mohammad** Formal analysis, Investigation, Methodology **Hilal Unyay** Data curation, Formal analysis, Project administration, Writing-reviewing and editing, Funding acquisition **Casey Wozniak** Resources, Methodology **Piotr Piersa** Supervision, Visualization **Justyna Czerwińska** Data curation, Resources, Software **Paweł Kazimierski** Data curation, Resources, Software **Panagiotis Regouzas** Formal analysis, Visualization, Writing - original draft **Alexandros Stefanakis** Formal analysis, Software, Writing - review and editing **Bartosz Panek** Data curation, Formal analysis, Methodology, Resources, Software **Jude A. Onwudili** Data curation, Formal analysis, Supervision, Writing - review and editing.

9. References

1. Falcon, W. P., Naylor, R. L. & Shankar, N. D. Rethinking Global Food Demand for 2050. *Popul. Dev. Rev.* **48**, 921-957 (2022).
2. Singh, H., Northup, B. K., Rice, C. W. & Prasad, P. V. V. Biochar applications influence soil physical and chemical properties, microbial diversity, and crop productivity: a meta-analysis. *Biochar* **4**, 8 (2022).
3. Pahalvi, H. N., Rafiya, L., Rashid, S., Nisar, B. & Kamili, A. N. Chemical Fertilizers and Their Impact on Soil Health. in *Microbiota and Biofertilizers, Vol 2* 1-20 (Springer International Publishing, Cham, 2021). doi:10.1007/978-3-030-61010-4_1.
4. Szufa, S. *et al.* Reduction of spruce phytotoxicity by superheated steam torrefaction and its use in stimulating the growth of ecological bio-products: *Lemna minor* L. *Biomass Convers. Biorefinery*

<https://doi.org/10.1007/s13399-025-06508-6> (2025)
doi:10.1007/s13399-025-06508-6.

5. Chandini & Kumar, Randeep & Kumar, Ravendra & Prakash, O. *Research Trends in Environmental Sciences*. (2019).
6. Ding, Y. *et al.* Biochar to improve soil fertility. A review. *Agron. Sustain. Dev.* **36**, 36 (2016).
7. Wang, J. & Wang, S. Preparation, modification and environmental application of biochar: A review. *J. Clean. Prod.* **227**, 1002–1022 (2019).
8. Muzyka, R., Misztal, E., Hrabak, J., Banks, S. W. & Sajdak, M. Various biomass pyrolysis conditions influence the porosity and pore size distribution of biochar. *Energy* **263**, 126128 (2023).
9. Joseph, S. *et al.* How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. *GCB Bioenergy* **13**, 1731–1764 (2021).
10. Lin, Y., Munroe, P., Joseph, S., Henderson, R. & Ziolkowski, A. Water extractable organic carbon in untreated and chemical treated biochars. *Chemosphere* **87**, 151–157 (2012).
11. Cheng, C.-H., Lehmann, J. & Engelhard, M. H. Natural oxidation of black carbon in soils: Changes in molecular form and surface charge along a climosequence. *Geochim. Cosmochim. Acta* **72**, 1598–1610 (2008).
12. Unyay, H. *et al.* Dry vs wet torrefaction of oxytree biomass: A comparative study on fuel properties and energy yield. *Energy Convers. Manag.* **350**, 120974 (2026).
13. Oni, B. A., Oziegbe, O. & Olawole, O. O. Significance of biochar application to the environment and economy. *Ann. Agric. Sci.* **64**, 222–236 (2019).
14. Kabir, E., Kim, K.-H. H. & Kwon, E. E. Biochar as a tool for the improvement of soil and environment. *Front. Environ. Sci.* **11**, 1324533 (2023).
15. Domene, X., Enders, A., Hanley, K. & Lehmann, J. Ecotoxicological characterization of biochars: Role of feedstock and pyrolysis temperature. *Sci. Total Environ.* **512–513**, 552–561 (2015).
16. Mohammad, O. *et al.* Assessments of sustainable chemicals and bioenergy potentials of selected lignocellulosic biomass feedstocks in Poland via physicochemical characterisation and pyrolysis. *Biomass and Bioenergy* **203**, 108302 (2025).

17. Baek, G., Saeed, M. & Choi, H.-K. Duckweeds: their utilization, metabolites and cultivation. *Appl. Biol. Chem.* **64**, 73 (2021).
18. Acosta, K. *et al.* Return of the Lemnaceae: duckweed as a model plant system in the genomics and postgenomics era. *Plant Cell* **33**, 3207–3234 (2021).
19. Ali Alshehri, M. & Pugazhendhi, A. Biochar for wastewater treatment: Addressing contaminants and enhancing sustainability: Challenges and solutions. *J. Hazard. Mater. Adv.* **16**, 100504 (2024).
20. Samuel Olugbenga, O., Goodness Adeleye, P., Blessing Oladipupo, S., Timothy Adeleye, A. & Igenepo John, K. Biomass-derived biochar in wastewater treatment- a circular economy approach. *Waste Manag. Bull.* **1**, 1–14 (2024).
21. European Union. *COMMISSION IMPLEMENTING REGULATION (EU) 2025/153 of 29 January 2025 Authorising the Placing on the Market of Lemna Minor and Lemna Gibba Plants as Novel Food and Amending Implementing Regulation (EU) 2017/2470.* (2025).
22. Ahammed, T., ISLAM, S., AMIN, M. R., KAMAL, M. M. & FARUQUE, M. O. EFFECT OF SPIRODELA POLYRHIZA ON PHYSIO-CHEMICAL CHANGES IN INDUSTRIAL WASTEWATER. *Pollut. Res.* **42**, 413–423 (2023).
23. Lee, H. *et al.* Giant Duckweed (*Spirodela polyrhiza*) Root Growth as a Simple and Sensitive Indicator of Copper and Chromium Contamination. *Toxics* **11**, 788 (2023).
24. Kaur, L. & Kanwar, N. Duckweed: a model for phytoremediation technology. *Holist. approach to Environ.* **12**, 39–58 (2021).
25. ReportLinker. Global Sunflower Seed Production Share by Country. (2023).
26. International Organization for Standardization. *ISO 20079:2005 Water Quality — Determination of the Toxic Effect of Water Constituents and Waste Water on Duckweed (Lemna Minor) — Duckweed Growth Inhibition Test.* (2005).
27. Ling, Q., Huang, W. & Jarvis, P. Use of a SPAD-502 meter to measure leaf chlorophyll concentration in *Arabidopsis thaliana*. *Photosynth. Res.* **107**, 209–214 (2011).
28. Ritchie, R. J. Consistent Sets of Spectrophotometric Chlorophyll Equations for Acetone, Methanol and Ethanol Solvents. *Photosynth. Res.* **89**, 27–41 (2006).
29. Kalayi H, L. T. *Chlorophyll Fluorescence in Plant Biology Research.* (SGGW Publishing House, Warsaw, 2010).

30. Onwudili, J. A. & Scaldaferri, C. A. Catalytic upgrading of intermediate pyrolysis bio-oil to hydrocarbon-rich liquid biofuel via a novel two-stage solvent-assisted process. *Fuel* **352**, 129015 (2023).
31. Razaq, I., Simons, K. E. & Onwudili, J. A. Parametric study of pt/c-catalysed hydrothermal decarboxylation of butyric acid as a potential route for biopropane production. *Energies* **14**, 3316 (2021).
32. Mohammad, O., Onwudili, J. A., Yuan, Q. & Evans, R. Advancing CO₂ utilisation via suspension-based carboxylation of single and mixed biomass-derived phenolics to produce high-value hydroxybenzoic acids. *Chem. Eng. J.* **515**, 163498 (2025).
33. Yoshimi, Y., Tryfona, T. & Dupree, P. Structure, Modification Pattern, and Conformation of Hemicellulose in Plant Biomass. *J. Appl. Glycosci.* **72**, 7201301 (2024).
34. Szufa, S. *et al.* Numerical modeling of the co-firing process of an in situ steam-torrefied biomass with coal in a 230 MW industrial-scale boiler. *Energy* **263**, 125918 (2023).
35. De'Nobili, M. D., Bernhardt, D. C., Basanta, M. F. & Rojas, A. M. Sunflower (*Helianthus annuus* L.) Seed Hull Waste: Composition, Antioxidant Activity, and Filler Performance in Pectin-Based Film Composites. *Front. Nutr.* **8**, (2021).
36. Sikora, A., Hájková, K. & Jurczyková, T. Degradation of Chemical Components of Thermally Modified *Robinia pseudoacacia* L. Wood and Its Effect on the Change in Mechanical Properties. *Int. J. Mol. Sci.* **23**, 15652 (2022).
37. Abdul-Aziz, A.-L. *et al.* Biochar effects on soil properties and yield of maize in Northern region, Ghana. *Discov. Agric.* **3**, 103 (2025).
38. Han, M., Zhang, J., Zhang, L. & Wang, Z. Effect of biochar addition on crop yield, water and nitrogen use efficiency: A meta-analysis. *J. Clean. Prod.* **420**, 138425 (2023).
39. Gale, N. V., Sackett, T. E. & Thomas, S. C. Thermal treatment and leaching of biochar alleviates plant growth inhibition from mobile organic compounds. *PeerJ* **4**, e2385 (2016).
40. Ghorbani, M. *et al.* The Impact of Pyrolysis Temperature on Biochar Properties and Its Effects on Soil Hydrological Properties. *Sustainability* **14**, 14722 (2022).
41. Wystalska, K., Kwarciak-Kozłowska, A. & Włodarczyk, R. Influence of Technical Parameters of the Pyrolysis Process on the Surface Area, Porosity, and Hydrophobicity of Biochar from Sunflower Husk Pellet. *Sustainability* **15**, 394 (2022).

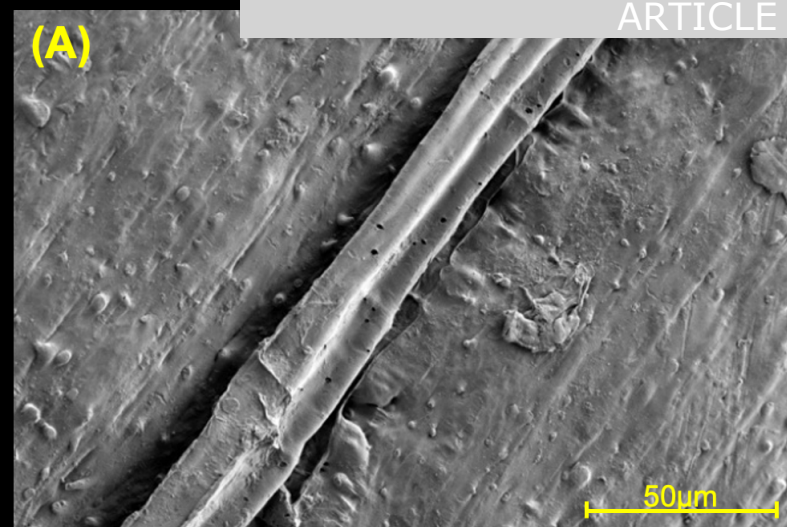
42. Nguyen, T.-B. *et al.* Hydrothermal and pyrolytic conversion of sunflower seed husk into novel porous biochar for efficient adsorption of tetracycline. *Bioresour. Technol.* **373**, 128711 (2023).
43. Kozioł, A., Paliwoda, D., Mikiciuk, G. & Benhadji, N. Biochar as a Multi-Action Substance Used to Improve Soil Properties in Horticultural and Agricultural Crops—A Review. *Agriculture* **14**, 2165 (2024).
44. Weng, X. *et al.* Calcium Regulates Growth and Nutrient Absorption in Poplar Seedlings. *Front. Plant Sci.* **13**, (2022).
45. Song, A., Li, P., Fan, F., Li, Z. & Liang, Y. The effect of Silicon on photosynthesis and expression of its relevant genes in rice (*Oryza sativa* L.) under high-zinc stress. *PLoS One* **9**, e113782 (2014).
46. Kalina, M. *et al.* The Effect of Pyrolysis Temperature and the Source Biomass on the Properties of Biochar Produced for the Agronomical Applications as the Soil Conditioner. *Materials (Basel)*. **15**, 8855 (2022).
47. Sommer, S. G., Han, E., Li, X., Rosenqvist, E. & Liu, F. The Chlorophyll Fluorescence Parameter Fv/Fm Correlates with Loss of Grain Yield after Severe Drought in Three Wheat Genotypes Grown at Two CO₂ Concentrations. *Plants* **12**, 436 (2023).
48. Nowak, R., Szczepanek, M., Błaszczuk, K. & Hassanpouraghdam, M. B. Response of photosynthetic efficiency parameters and leaf area index of alternative barley genotypes to increasing sowing density. *Sci. Rep.* **14**, 29779 (2024).
49. Morini, L. *et al.* Phytoremediation by rooted macrophyte *Vallisneria spiralis*, not biochar amendment, reduces eutrophication from nutrient release in organic-rich sediment of a polluted wetland. *Sci. Total Environ.* **1001**, 180470 (2025).
50. Liu, K. *et al.* Arbuscular mycorrhizal fungi and biochar synergistically enhance Cd(II) phytoremediation in *Koeleria bipinnata*. *Ecotoxicol. Environ. Saf.* **303**, 118882 (2025).
51. Marczak-Grzesik, M. *et al.* Modified Fly Ash-Based Adsorbents (MFA) for Mercury and Carbon Dioxide Removal from Coal-Fired Flue Gases. *Energies* **14**, 7101 (2021).
52. Cantou, G., Wang, J. J., Zhou, B., Lee, J.-M. & Park, J.-H. Toxicological fate of biochar according to feedstock and pyrolysis temperature. *Ind. Crops Prod.* **236**, 122094 (2025).
53. Azeem, M. *et al.* Biochar-derived dissolved organic matter induced changes in the bacterial communities structure and metabolic functions in As and Cd contaminated soil. *Environ. Res.* **286**, 122749

(2025).

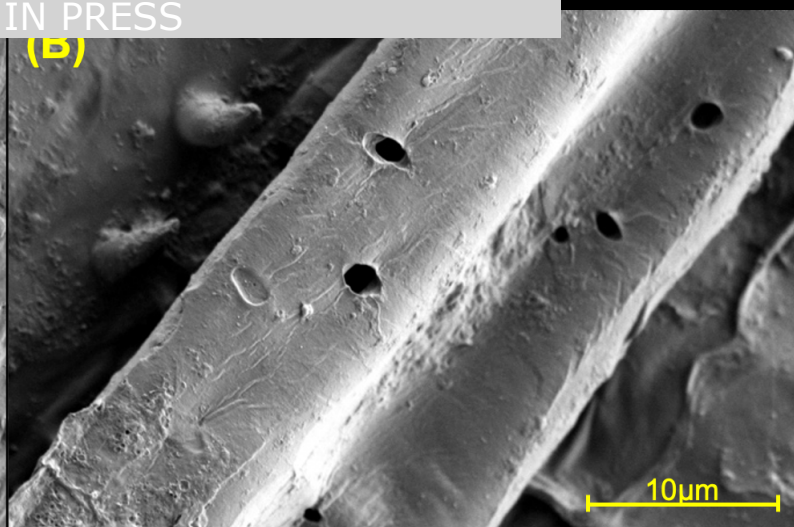
54. Kamyab, H. *et al.* Biochar as a carrier for plant growth-promoting bacteria in phytoremediation of pesticides. *J. Hazard. Mater. Adv.* **18**, 100673 (2025).

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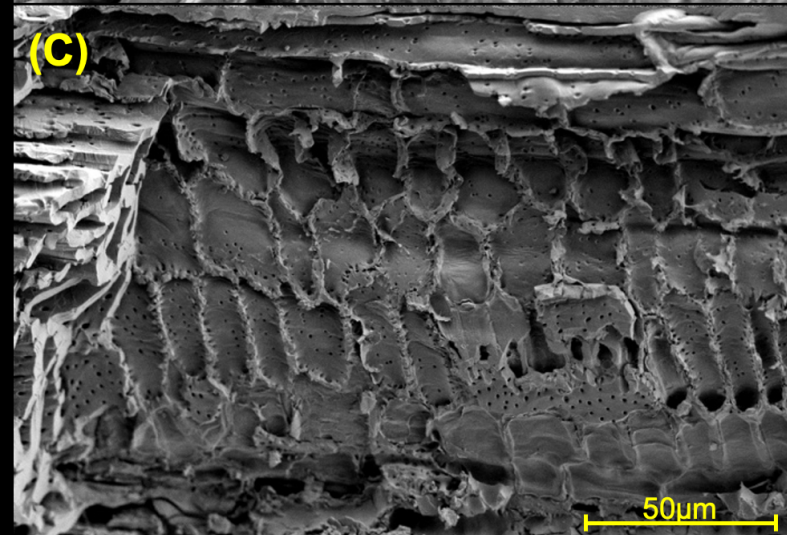
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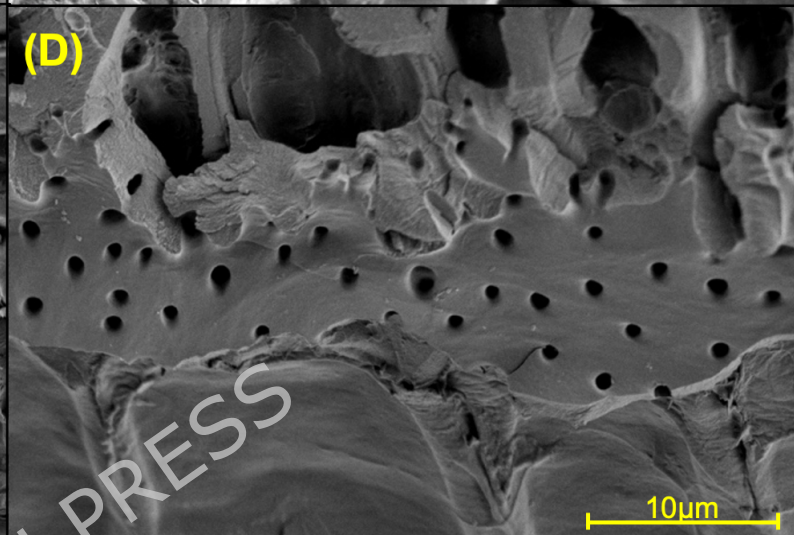
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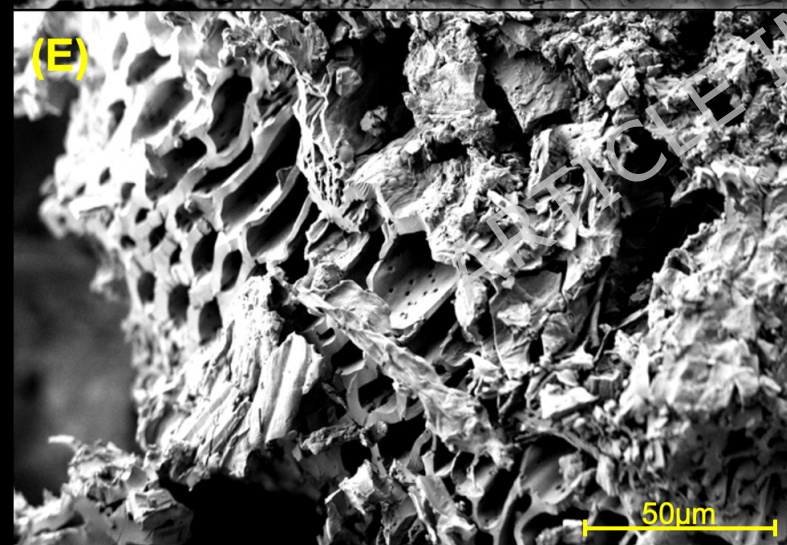
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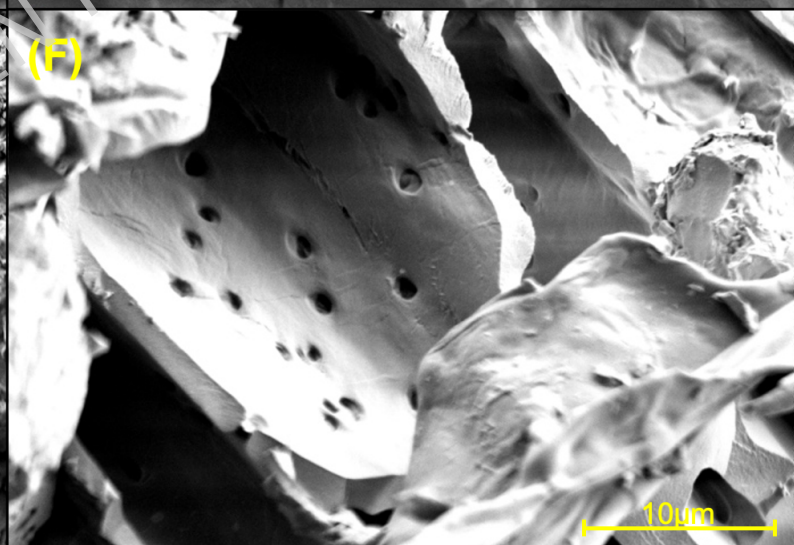
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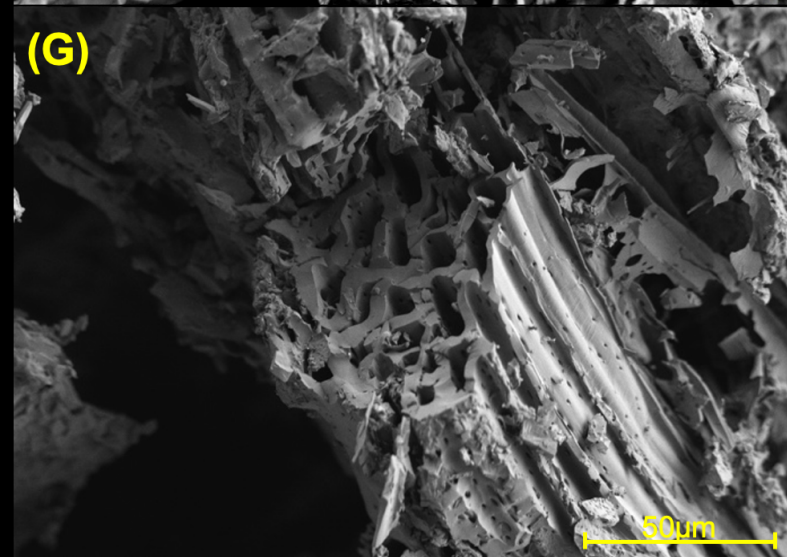
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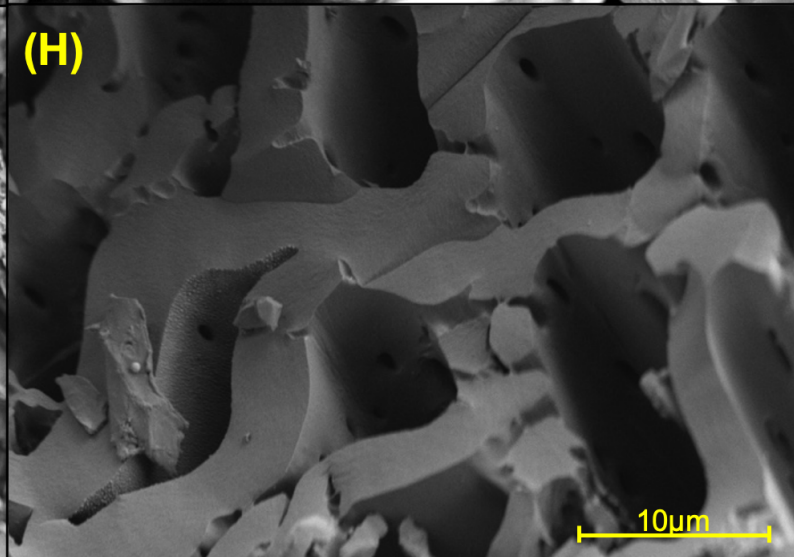
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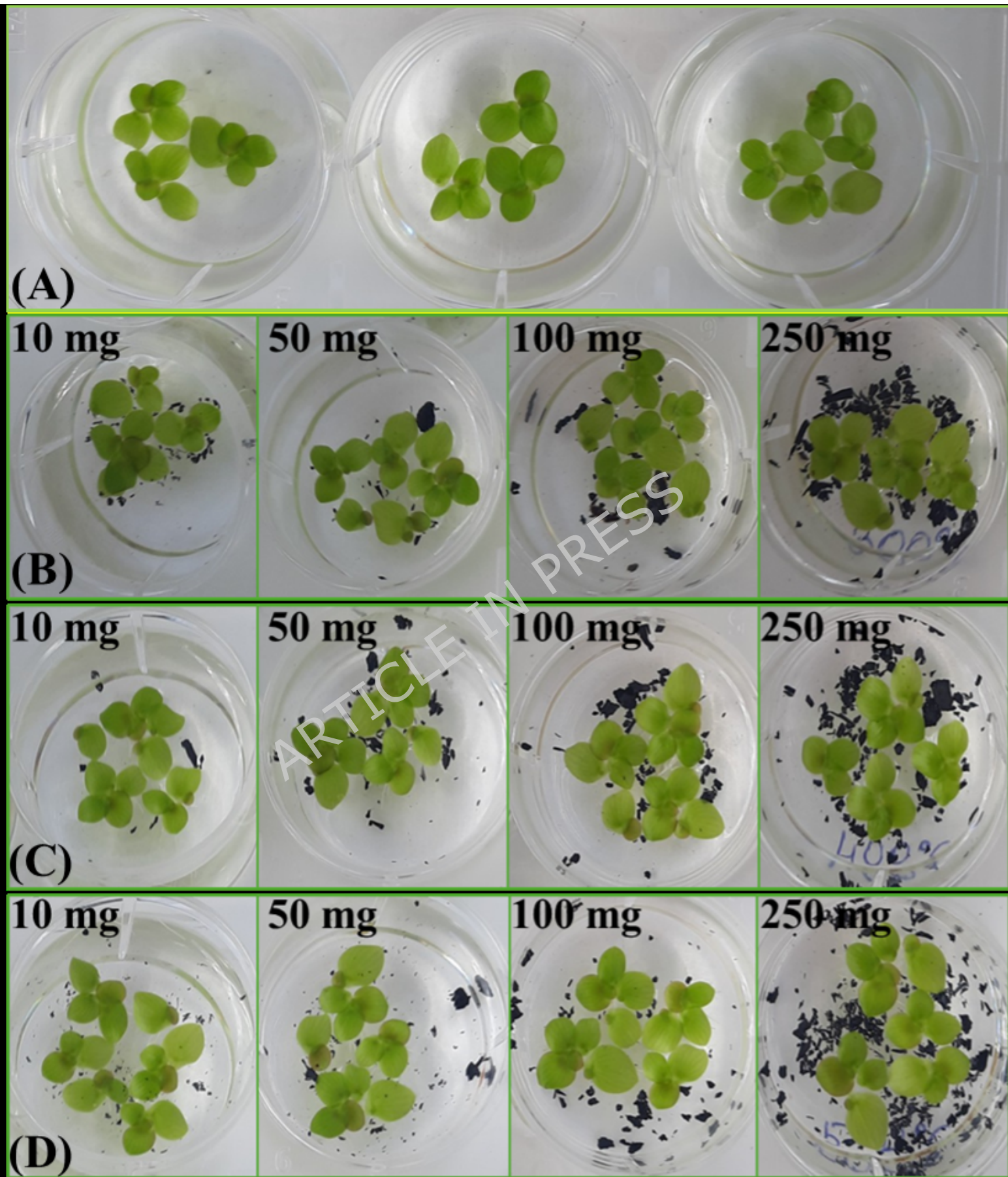


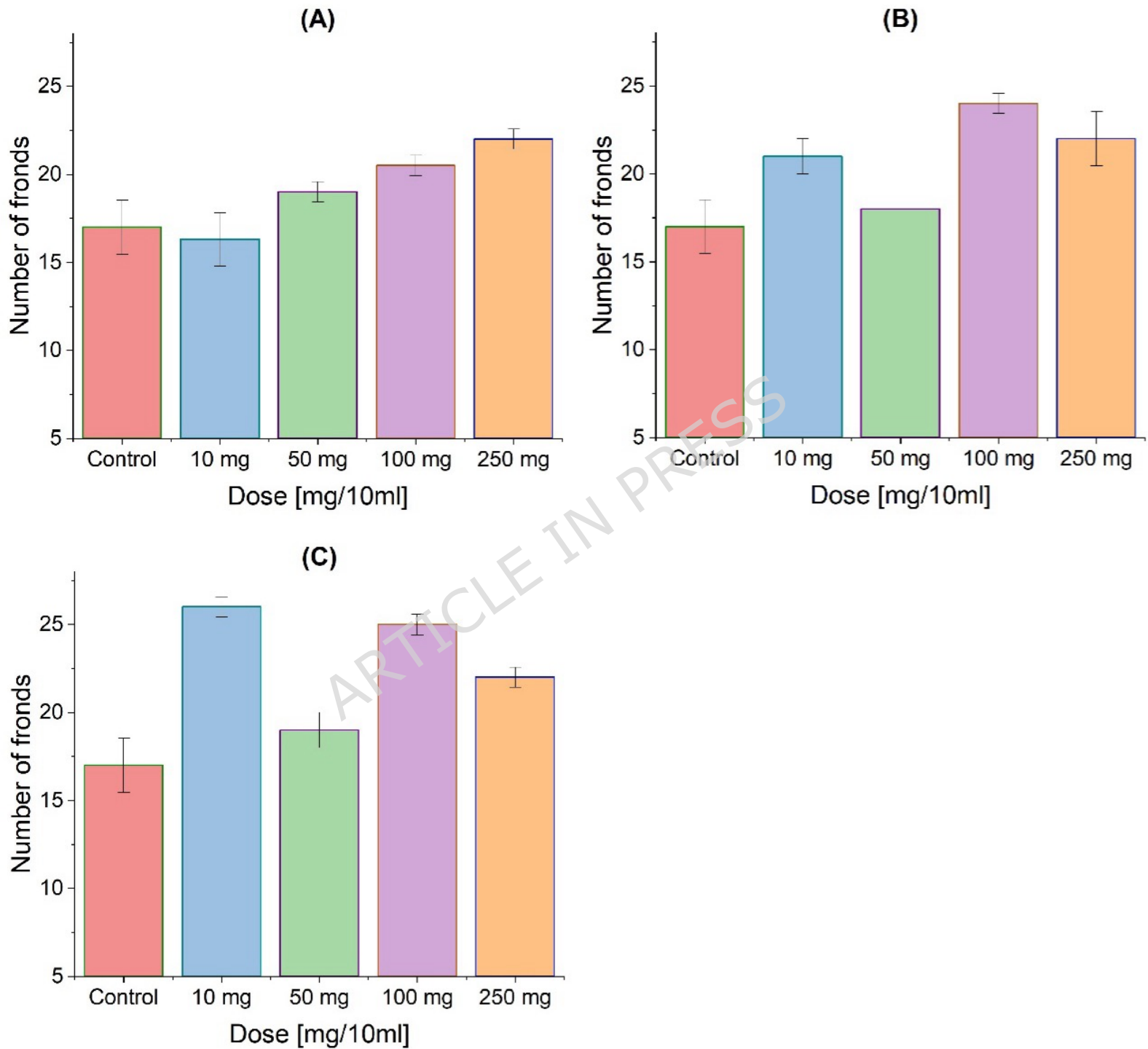
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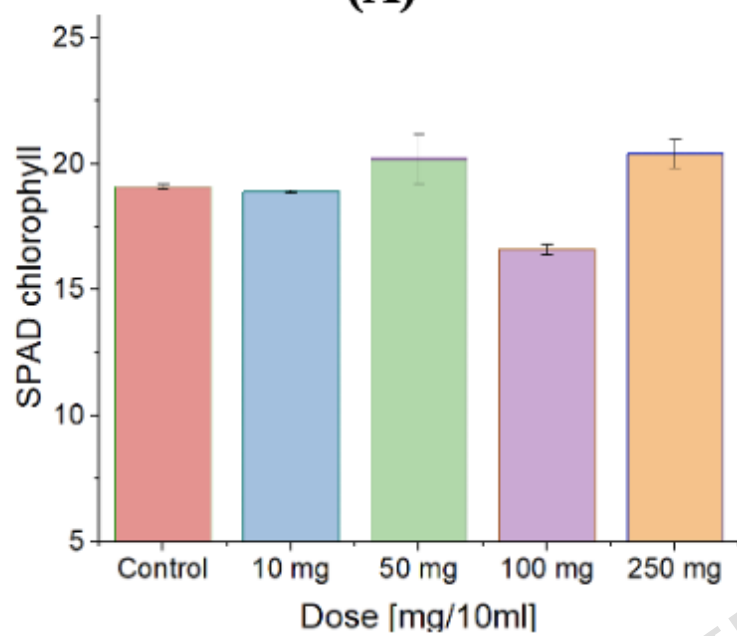
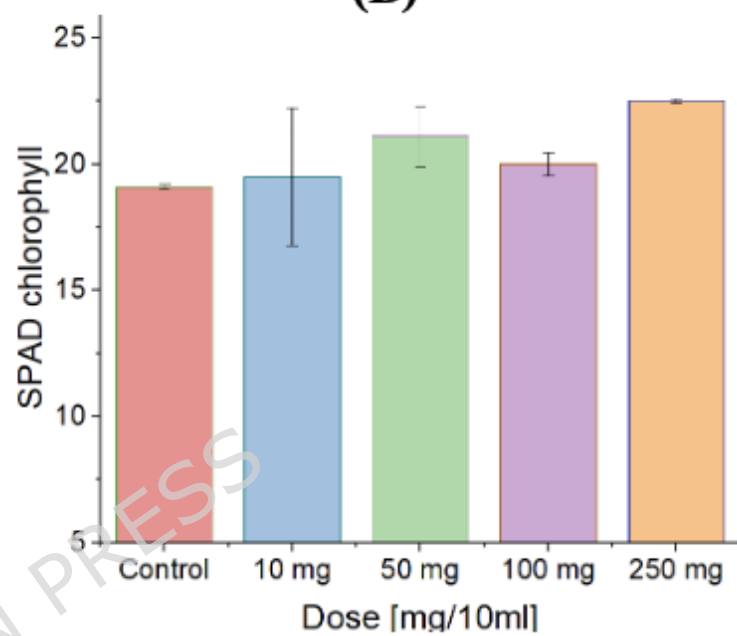
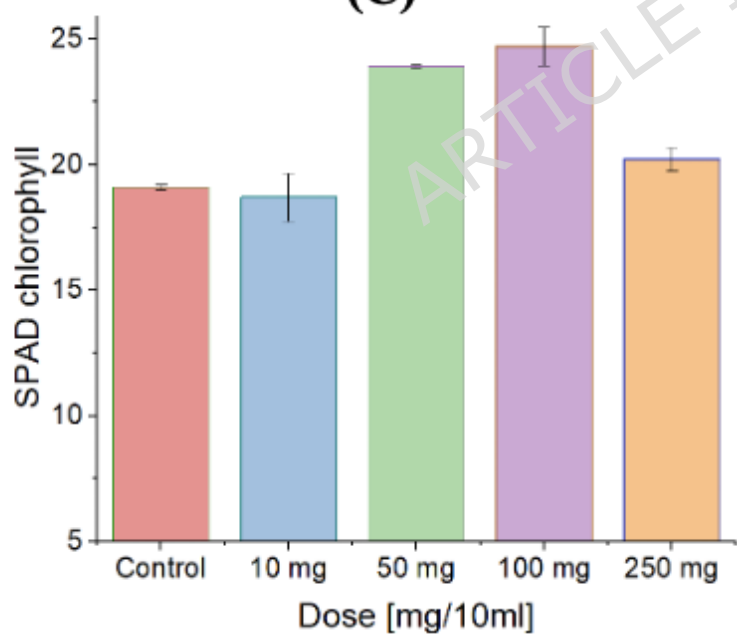


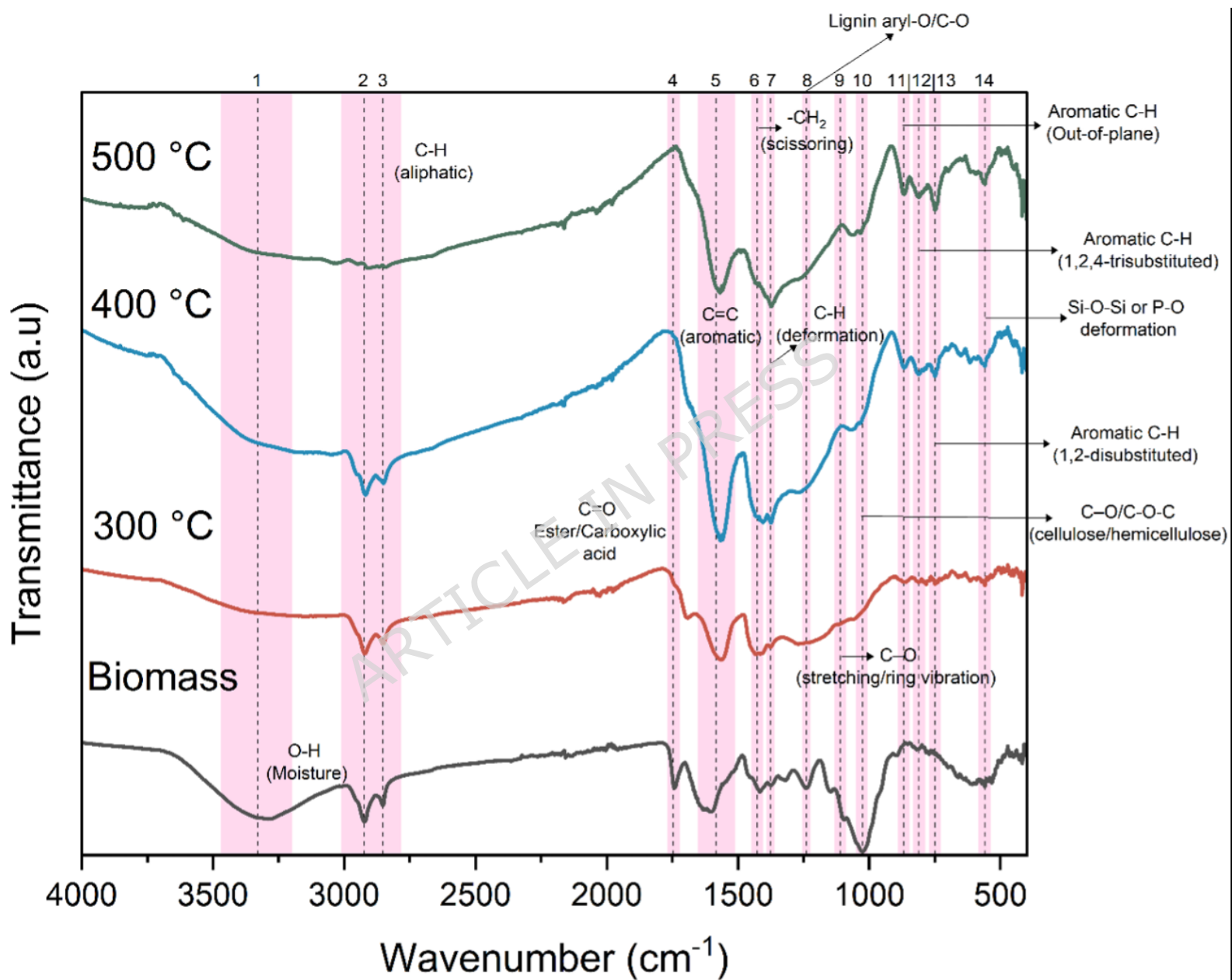
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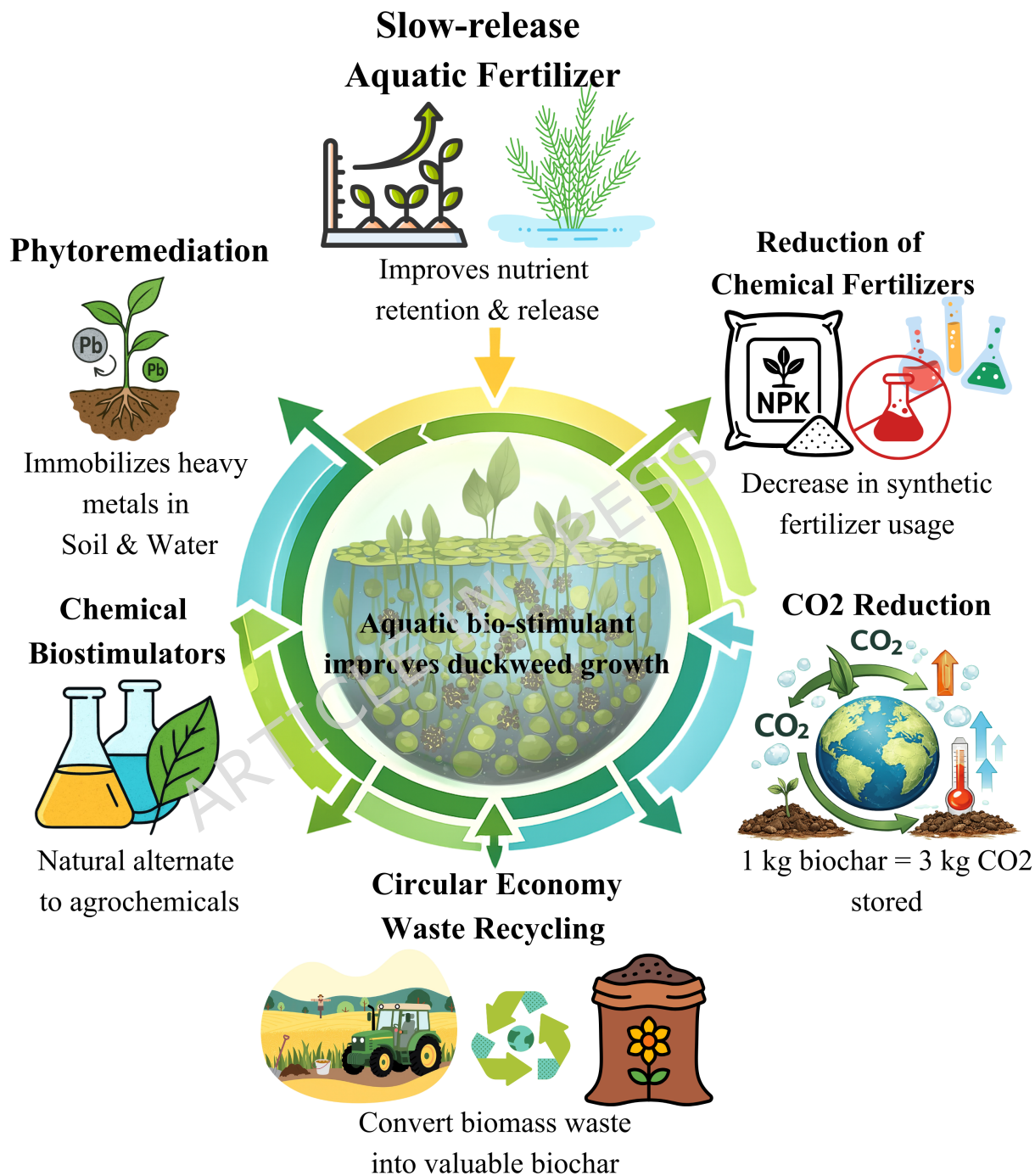


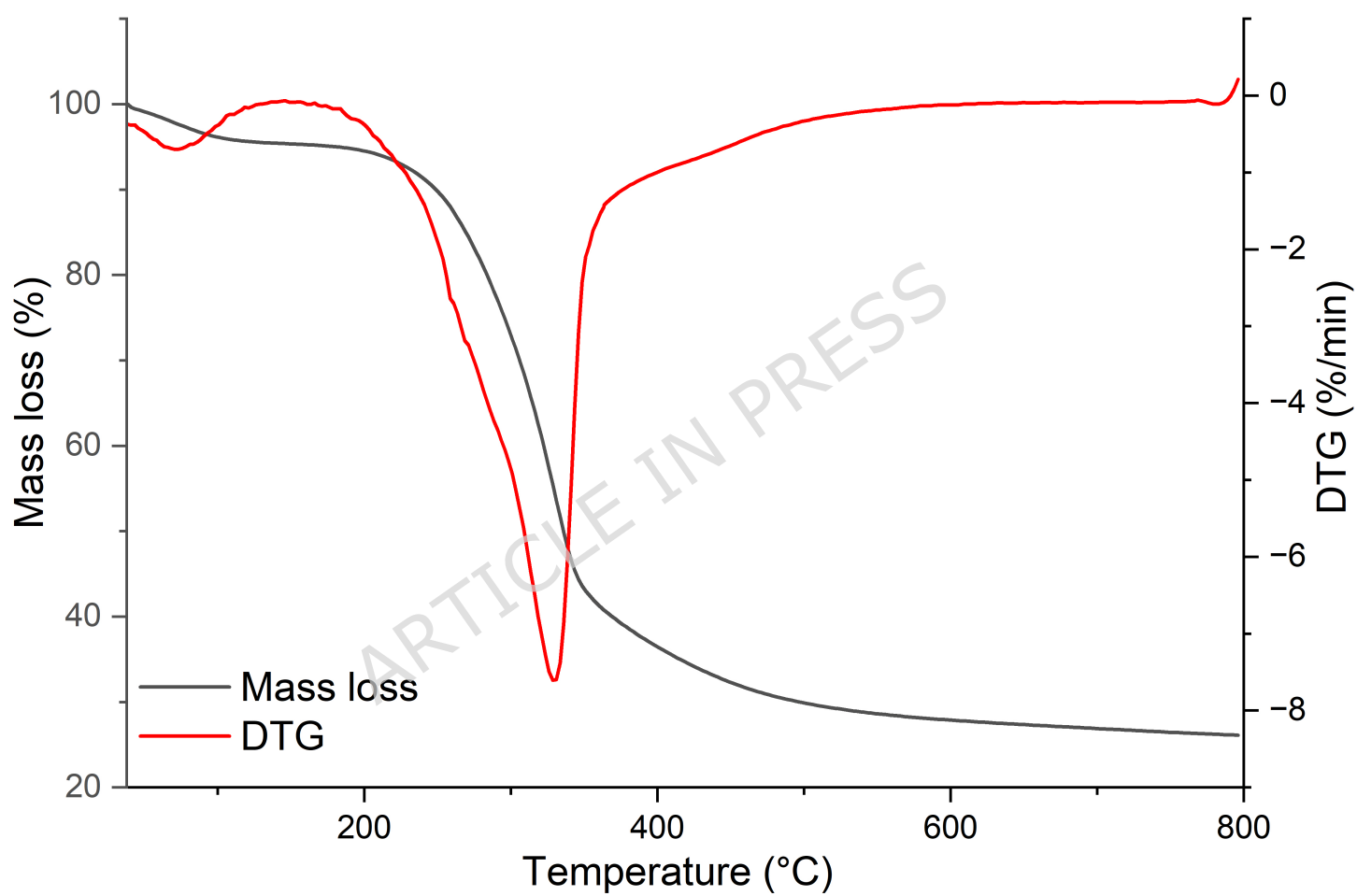


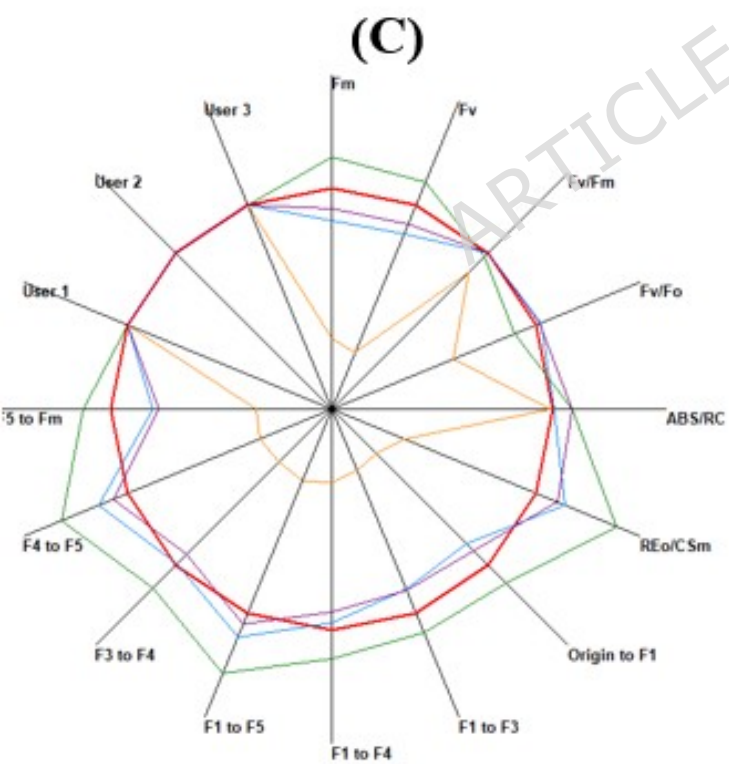
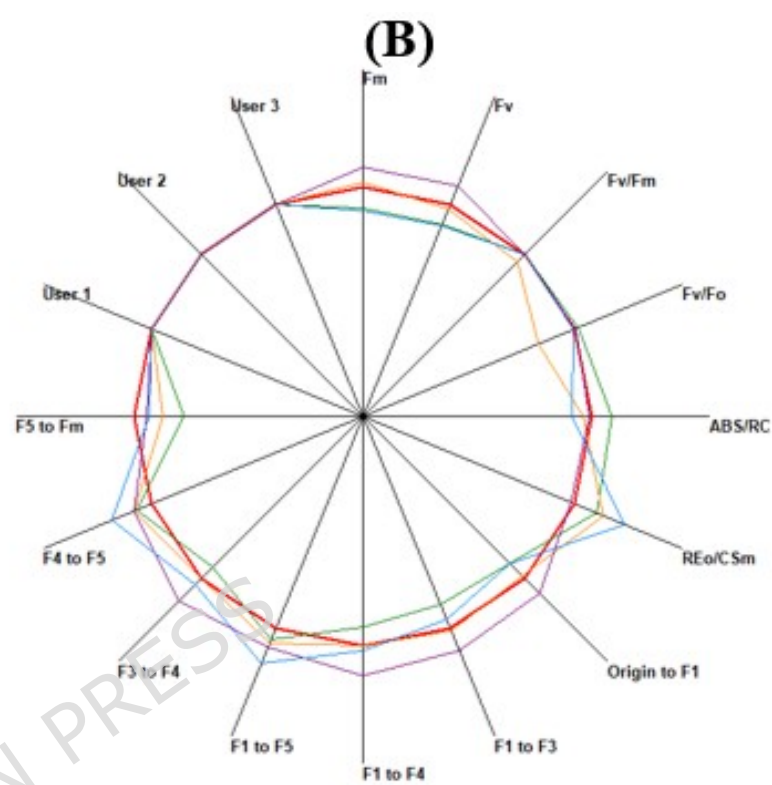
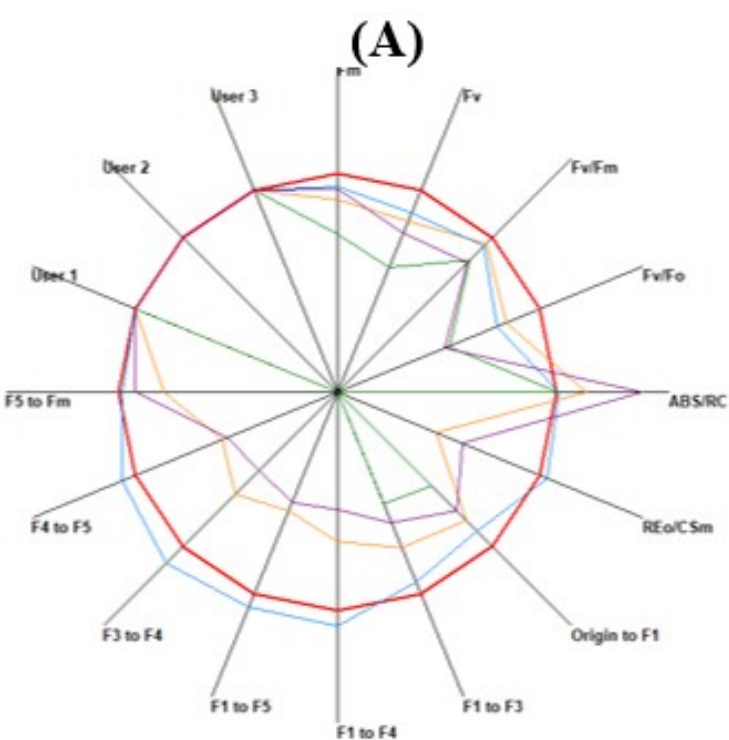


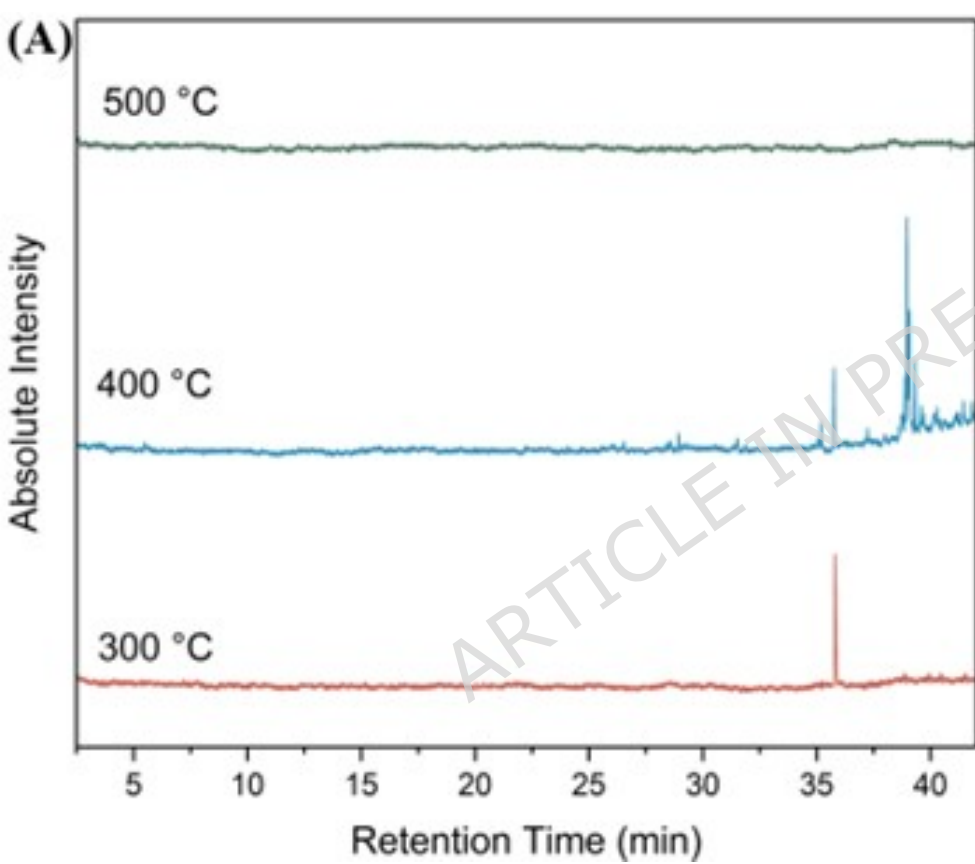
(A)**(B)****(C)**











(B)

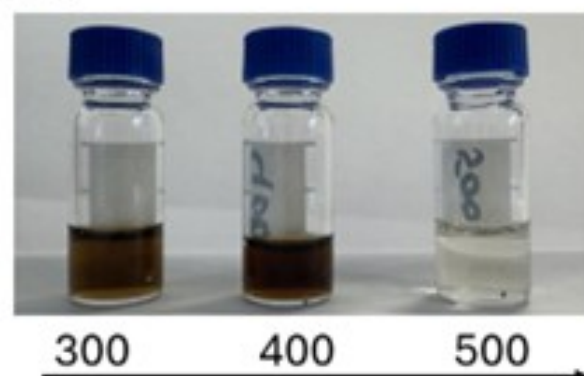


Table 1 Composition of growth media liquid

Mineral component	Value	Unit	Mineral component	Value	Unit
Calcium (Ca ²⁺)	72-76	mg/L	Aluminum (Al ³⁺)	<20	µg/L
Magnesium (Mg ²⁺)	1.70-10.40	mg/L	Zinc (Zn ²⁺)	0.003-0.009	mg/L
Sodium (Na ⁺)	5.08-6.40	mg/L	Copper (Cu ²⁺)	<0.004	mg/L
Potassium (K ⁺)	0.57-1.13	mg/L	Nickel (Ni ²⁺)	<4.0	µg/L
Chloride (Cl ⁻)	11.00-12.00	mg/L	Lead (Pb ²⁺)	<2.0	µg/L
Sulfate (SO ₄ ²⁻)	21-24	mg/L	Chromium (Cr ³⁺ /Cr ⁶⁺)	<2.0	µg/L
Nitrate (NO ₃ ⁻)	0.61-2.60	mg/L	Selenium (Se)	<1.0	µg/L
Nitrite (NO ₂ ⁻)	<0.04	mg/L	Boron (B)	<0.05	mg/L
Fluoride (F ⁻)	0.10-0.14	mg/L	Manganese (Mn ²⁺)	6-28	µg/L
Iron (Fe)	20-35	µg/L			

Table 2 Quantitative results for X-ray Fluorescence Spectrometer Analysis

Quantitative Result	Raw biomass	300 °C	400 °C	500 °C
K (Potassium)	1.494%	3.467%	4.099%	6.098%
Mg (Magnesium)	0.269%	0.551%	0.645%	2.475%
Ca (Calcium)	0.217%	0.849%	0.996%	1.503%
P (Phosphorus)	0.083%	0.429%	0.433%	0.744%
S (Sulfur)	0.150%	0.243%	0.226%	0.263%
Si (Silicon)	0.026%	0.179%	0.185%	0.108%
Cl (Chlorine)	0.084%	0.239%	0.278%	0.441%
Fe (Iron)	0.003%	0.222%	0.504%	0.344%
Mn (Manganese)	0.004%	0.004%	0.007%	0.008%
Zn (Zinc)	0.004%	0.005%	0.089%	0.138%
Cu (Copper)	0.001%	0.003%	0.003%	0.005%
Rb	0.001%	0.002%	0.003%	0.004%
Br	0.001%	0.001%	0.002%	0.003%
Sr	-	0.001%	0.002%	0.003%

Material		Surface area (m ² /g)	BJH Surface area (m ² /g)	Pore Volume (cm ³ /g)	Pore Diameter D _v (d) (nm)
Raw biomass		0	0.756	0.001	3.070
Biochar 300 °C	0.849		4.923	0.0188	3.088
Biochar 400 °C	0.577		3.914	0.013	3.049
Biochar 500 °C	1.124		19.603	0.118	24.114

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Retention time (min)	Compounds	Normalised Peak Areas (%)
300 °C		
35.8	Hexadecanoic acid, methyl ester	100
400 °C		
35.2	2-Pentadecanone, 6,10,14-trimethyl-	4.43
35.8	Hexadecanoic acid, methyl ester	13.30
38.9	1-Tetradecyne	7.39
39.0	11-Octadecenoic acid, methyl ester	29.56
39.1	11-Octadecenoic acid, methyl ester, (Z)-	25.62
39.3	Methyl stearate	9.36
39.6	o-Dodecylphenol	2.22
41.5	Docosane	1.48
42.3	Eicosanoic acid, methyl ester	2.96
42.5	Octadecane, 1-iodo-	1.72
43.8	Docosanoic acid, methyl ester	1.97
500 °C		
None		