

Research Article

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Hydrothermal carbonization of invasive plant biomass as a tool for its safe utilization and production of artificial humic substances

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Abstract

Invasive plants may cause significant environmental risks by affecting biological diversity, ecosystem services, agriculture, and forestry. Safe utilization of invasive plant biomass by obtaining new outcome products useful for bioeconomics is a challenging but promising solution. Hydrothermal carbonization (HTC) presents a sustainable and cost-effective approach to transforming invasive plant biomass into new products while simultaneously supporting carbon capture aims. This study utilized invasive plants such as lupine [*Lupinus polyphyllus* Lindl.], Sosnowsky's hogweed [*Heracleum sosnowskyi* Manden.], and Japanese knotweed [*Polygonum cuspidatum* Siebold & Zucc.] as biomass sources for HTC to produce artificial humic substances (AHS). The greatest impact on the yield of AHS (maximum gained yield of 62%) was the duration of the HTC process (up to 6 h) and temperature of the treatment (from 160 up to 250 °C), as well as the catalyst used (an alkaline medium is preferable). During the HTC treatment, significant changes in the invasive plant biomass composition occur, as indicated by the removal of labile components of organic matter. The AHS obtained are essentially similar to natural humic matter and have biostimulatory properties; thus, they can be applied in agriculture and other areas of bioeconomics.

Introduction

The intensive spread of invasive plants presents a significant environmental challenge. These plants, including herbaceous species, trees, and shrubs, are nonnative species capable of outcompeting local flora. Invasive plants adversely affect biological diversity, ecosystem services, agricultural production, forestry, and even human and animal health (Rai and Singh 2020).

Driven by global trade, transport, and climate change, the proliferation of invasive plants imposes substantial economic costs; for example, in the European Union (EU), these costs amount to tens of billions of euros annually (Haubrock et al. 2021). To mitigate the risks, regulations have been established in the EU to control the spread of invasive plants (EU 2014) and define responsibilities for eradication (Sheppard et al. 2006). Common eradication methods include mechanical or manual removal, the use of herbicides, and biological control measures. These efforts generate large amounts of biomass, making it challenging to safely use biomass from invasive plants. Traditional composting methods are not entirely compelling and safe due to the risk of seed propagation (Albano et al. 2015; Vavrková et al. 2020). Furthermore, composting generates greenhouse gases and is therefore not a climate-positive technology (Nordahl et al. 2023; Sayara and Sánchez 2021). Other methods such as incineration and fermentation also have limitations (Epanchin-Niell et al. 2012; Regan et al. 2006). From a bioeconomics perspective, invasive plant biomass may serve as a valuable resource, and developing safe, effective utilization and valorization methods is crucial (Wei et al. 2018; Wiatrowska et al. 2021; Zihare and Blumberga 2017).

Hydrothermal carbonization (HTC) is a promising technology for biomass waste processing, as it is sustainable and cost-effective and facilitates resource recovery (Nicolae et al. 2020; Zhai et al. 2017). HTC involves treating wet biomass at high temperatures (160–250 °C) and pressures, sometimes in the presence of oxidizing agents or catalysts, leading to complete biomass transformation through thermochemical processes (hydrothermal humification) such as oxidation, hydrolysis, thermal decomposition, and dehydration in subcritical water (Nicolae et al. 2020). Under hydrothermal humification conditions, hydrolysis of lignocellulose takes place, releasing simpler carbohydrates (glucose, xylose, etc.); this is followed by dehydration and combination of lignin fragments and polymerization to form fulvic, humic acids, and humin (Peng et al. 2023; Shao et al. 2024). This process produces hydrochar and artificial humic substances (AHS), transforming biomass carbon into less biologically accessible materials and

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Management Implications

One of the key problems of invasive plant management is the safe utilization of biomass after eradication. Existing approaches (composting, incineration, etc.) have some limitations. Hydrothermal carbonization (HTC) is a sustainable, cost-effective technology that allows the treatment of wet plant biomass at elevated temperatures (160–250 °C) and pressures, producing carbonaceous material such as hydrochar and humic-like substances known as artificial humic substances (AHS). HTC provides possibilities for transforming the carbon of invasive plant biomass into less biologically accessible materials; therefore, the process can be positioned as a carbon capture and storage technology. This article aims to demonstrate the possibilities of safe utilization of invasive plant biomass, admitted as waste material, obtained after invasive plant eradication to produce AHS and subsequently investigates their properties. AHS resemble natural humic substances, the primary organic components in soil, peat, and sedimentary matter, and may find practical application in agriculture and other areas of bioeconomics.

positioning HTC among the carbon capture and storage technologies (Ischia and Fiori 2021; Nicolae et al. 2020). A recent study investigating the hydrothermal humification mechanism (Yang and Antonietti 2020) supports the possibility of increasing AHS yields and designing the HTC process to produce desired HTC products, including AHS. However, to support broader utilization of HTC application, it is important to demonstrate its application to biomass types of bioeconomic interest. In this respect, a particular benefit must be attributed to the processing of invasive plant biomass; in the present case, the primary concern is the safety of the treatment and its efficiency in significantly reducing or eliminating further spread of the invasive plant.

AHS resemble natural humic substances, the primary organic components in soil, peat, and sedimentary matter. AHS hold properties like amphiphilicity and ion-binding capacities (Yang et al. 2019), enhancing water and nutrient retention in soil (Yang and Antonietti 2020). Their potential in various environmental technologies has been studied to some extent (Lan et al. 2021; Yang et al. 2020), but possibilities to use AHS obtained from the processing of invasive plant biomass deserves further research.

This article aims to study the possibilities of safe utilization of invasive plant biomass obtained after plant eradication to produce AHS and to study their properties.

Materials and Methods

Materials

Three invasive plants (alien species), abundant in Europe and included in the list of the Commission Regulation 2022/1203, were chosen for the study: lupine [*Lupinus polyphyllus* Lindl.], Sosnowsky's hogweed [*Heracleum sosnowskyi* Manden.], and Japanese knotweed [*Polygonum cuspidatum* Siebold & Zucc.]. Plants were sampled in the summer/autumn of 2023 in urban territories in the central part of Latvia, Riga and Kekava (56.99576° N, 24.21882° E and 56.813225° N, 24.258978° E, respectively) as composite samples containing leaves, stems, flowers, and seeds; fresh wet plant biomass was stored frozen at –20 °C until analysis. Corresponding plant seeds were used for the early plant

development study implemented alongside the HTC process study. Before analytical characterization, plant biomass was dried overnight at 105 °C in a drying oven (Plus II Oven, Labasco), then milled using a laboratory cutting mill (Fritsch Pulverisette 15, Fritsch) to a particle size <1 mm. The elemental composition and ash content of the biomass samples are provided in Table 1.

For comparison purposes, reference compounds such as standardized humic acids (Humic acid, technical, Sigma-Aldrich) and humic acids isolated from peat in our previous studies (Klavins and Purmalis 2013; Krumins and Klavins 2022) were used.

HTC

HTC primarily was performed in an alkaline medium. About 20 g of finely ground biomass was weighed into a Teflon™ capsule, to which 220 ml of 0.65 M KOH (analytical grade, Sigma-Aldrich) were added. The capsule was then inserted into a stainless-steel container and sealed. The reaction mixture was heated at 200 °C for 24 h in an oven, then slowly cooled and filtered to separate hydrochar from the AHS solution. To evaluate the effects of temperature and reaction time, similar experiments were performed with invasive plant biomass heated at 200 °C and 220 °C for durations of 4, 6, 8, 12, 24, and 48 h. To assess the impact of the catalyst on the process, alkaline medium was replaced with acidic medium (4% H₂SO₄, analytical grade, Sigma-Aldrich) and neutral medium by deionized water (<0.1 μS, Millipore Elix-3, Millipore), maintaining the same biomass mass-to-volume ratio. The HTC process was conducted for 24 h at 200 °C for both the neutral and acidic environments. After the reaction, the solid phase containing hydrochar was filtered off, extensively washed with deionized water, treated with 25 ml of ethanol, and dried in a desiccator to a constant weight. All tests were conducted in triplicate (*n* = 3) to ensure data reliability. The yield (%) of hydrochar was calculated using Equation 1:

$$Y_{\text{HC}} = (M_{\text{HC}}/M_{\text{Raw}}) \times 100 \quad [1]$$

where Y_{HC} is the hydrochar yield, M_{HC} is the hydrochar weight, and M_{Raw} is the biomass weight.

Isolation of Artificial Humic Acids

In the liquid phase remaining after the HTC process, total organic carbon (TOC) was analyzed using a total carbon analyzer (TOC-VCSN, Shimadzu). The content of artificial humic acids (AHA) in the liquid product was determined gravimetrically after acidification with 6 N HCl to pH = 1. Solid AHA were separated from the liquid by vacuum filtration, then washed with deionized water and dried in a desiccator to a constant weight to determine the yield relative to the weight of biomass. TOC of the liquid phase, consisting of fulvic acids and low-molecular-weight organic compounds (LMWOC), was also determined using a total carbon analyzer (TOC-VCSN, Shimadzu).

Characterization of Biomass and AHA

The elemental analysis to detect the C, H, N, S, and O content was conducted using an elemental analyzer equipment (EA-1108, Carlo Erba Instruments). The obtained values were normalized with respect to ash content. The ash content was measured by heating 50 mg of each sample at 750 °C for 8 h.

Table 1. Elemental composition of investigated invasive plant biomass

Plant species in biomass	Elemental composition				
	C	H	N	O	S
	% of dry mass				
<i>Heracleum sosnowskyi</i>	44.54 ± 0.41	5.62 ± 0.04	0.36 ± 0.02	49.16 ± 0.24	0.32 ± 0.03
<i>Lupinus polyphyllus</i>	39.42 ± 0.12	5.54 ± 0.09	0.75 ± 0.03	53.64 ± 0.85	0.65 ± 0.03
<i>Polygonum cuspidatum</i>	45.63 ± 0.21	5.23 ± 0.05	1.23 ± 0.05	47.06 ± 0.74	0.85 ± 0.04

Fourier-transform infrared (FTIR) spectra were obtained using an FTIR spectrophotometer (IR-Tracer 100, Shimadzu) at a wavenumber interval of 4,000 to 400 cm^{-1} , with a resolution of 4 cm^{-1} and 10 scans.

Ultraviolet-visible (UV/Vis) spectra were recorded using a γ UV spectrophotometer (UV-1800, Shimadzu) in a 1-cm quartz cuvette.

Thermogravimetric (TG) analysis was performed using a TG analyzer (SDT-Q600, TA Instruments) at a heating rate of 20 C min^{-1} under a dynamic flow of nitrogen (100 ml min^{-1}) with 10 mg of a solid powder sample, recording TG curves.

Fluorescence spectra were acquired using a spectrofluorometer (Aqualog VS 140, Horiba). Emission wavelengths were set between 210 nm and 620 nm, and excitation wavelengths ranged from 200 nm to 600 nm. Data were captured at 1-nm intervals for both excitation and emission spectra. Raw fluorescence matrices were imported into the MATLAB R2020a (Mathworks) tool for preprocessing and analysis. Operations were performed using the N-way toolbox and the DOMFluor package (Chemometrics Research). A three-way data array was prepared for parallel factor analysis. Exploratory analyses, including visual inspection and principal component analysis, were used to determine the number of components for the subsequent Tucker decomposition model.

Early Plant Development Tests

Early plant development tests were conducted to evaluate the growth-stimulating activity of the studied humic substances, following the previously applied method (Ali et al. 2014). The tests were performed using solutions of AHS and natural humic substances to assess their ability to stimulate plant development and growth through hormone-like and fertilizer-like activities.

Three plant species were used for the tests: common wheat (*Triticum aestivum* L.), white mustard (*Sinapis alba* L.), and gardencress pepperweed (watercress; *Lepidium sativum* L.). Seeds were obtained from local suppliers and tested using five different concentrations (50, 250, 500, and 1,000 mg L^{-1}) for each solution of humic substances, with deionized water serving as a control. The early plant development tests were carried out on Phytotestkit (Microbiotests) plates, using polyester cloth and filter paper as substrate replacements. Before planting, wheat seeds were surface sterilized in a 1:1 solution of chlorine-containing bleach (Ace, Procter & Gamble) and carefully rinsed with deionized water for 7 min, then washed with at least 2 L of deionized water, placed between damp filter papers in petri dishes, and kept in the dark at room temperature (21 C) for 2 d to germinate. Only seeds that had begun to germinate (germination rate about 50%) were used for further tests. Seeds of white mustard and gardencress pepperweed were soaked in deionized water for half an hour before being placed on the plates. The Phytotestkit plates were prepared by first covering them with a polyester cloth dampened with 7 ml of the

appropriate test solution, and then covering the cloth with filter paper. The next step involved placing 10 seeds of each sample on the filter paper, which was then covered with a transparent plastic film. Each treatment was replicated twice. The plates were placed vertically in a growth chamber (Phytotestkit, Microbiotests) in the dark at 25 C . Seeds of white mustard and gardencress pepperweed were grown for 3 d, but wheat seeds were grown for 5 d. At the end of the plant development tests, root and shoot lengths were measured, and the samples were separated and dried at 50 C for 12 h in a drying oven (Plus II Oven, Labasco) to obtain the dry mass of the samples. For comparison, the concentration-response dependence was calculated from the initial measurements (shoot or root length) as a percentage increase over the respective values of untreated control samples. The data obtained were subjected to one-way ANOVA tests (at a 95% confidence level), with a post hoc LSD test to differentiate treatments, using SPSS (IBM) and MS Excel (Microsoft) software.

Results and Discussion

Outcome of Synthesized AHS

Conditions of the HTC process applied for invasive plant biomass were selected based on results of previous studies on the use of HTC for biomass waste (Lan et al. 2021; Yang et al. 2019, 2020). However, the HTC process was further optimized to achieve the highest possible yields of AHS. The obtained results indicate a slight impact of the type of plant biomass on the yield of AHS. Significant increase of AHS yields in comparison with initial conditions was achieved, reaching the maximum yield up to 62% with respect to biomass carbon transformation. The yield of AHS and the differences among the main types of HTC products (hydrochar, AHA, fulvic acids, and LMWOC) varied depending on the pH applied in the HTC process (Figure 1A and 1B). The highest yield of AHS, on average 50% of biomass carbon, was obtained when the HTC process was performed using an alkaline medium. Complete transformation of invasive plant biomass was achieved, resulting in synthesis of humic acids, fulvic acids, LMWOC, and hydrochar, thus obtaining the compounds applicable in agriculture and environmental technologies (Nicolae et al. 2020; Yang et al. 2019).

The transformation of invasive plant biomass is significantly influenced by the duration of the process (Figure 1C). An optimal duration of approximately 3 h was found to be the most effective, as humic acids formed in a strong alkaline medium are hydrolyzed into LMWOC. Another factor affecting the HTC process is the temperature. An increase in temperature up to 250 C can elevate the yield of AHS up to 62% (Figure 1D). However, further temperature increase is limited due to the rise in pressure in the reaction vessel and the dominance of other carbonaceous matter transformation processes (González-Arias et al. 2022).

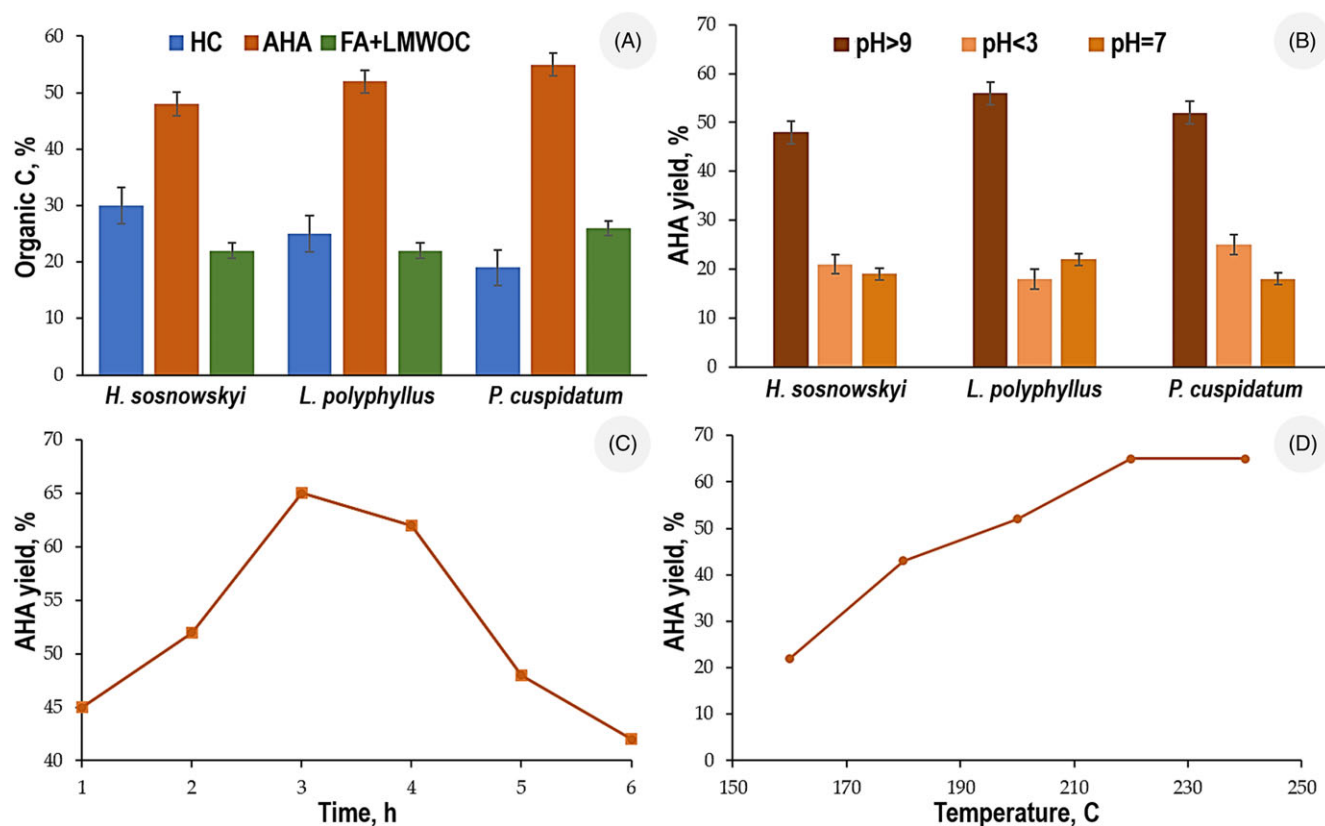


Figure 1. Characterization of the hydrothermal carbonization (HTC) process applied to biomass of invasive plants (*Heracleum sosnowskyi*, *Lupinus polyphyllus*, and *Polygonum cuspidatum*): (A) organic carbon content in artificial humic acids (AHA), hydrochar (HC), and fulvic acids and low-molecular-weight organic compounds (FA+LMWOC); (B) the effect of pH on the yield of AHA; (C) the impact of the reaction duration time on the yield of AHA; (D) the effect of temperature on the yield of AHA.

Properties of AHS Produced

During the HTC process, significant changes occur in the elemental composition of invasive plant biomass, as evidenced by the differences between the plants and the AHA produced. The C and N content in the plant biomass ranges from 37% to 46% and from 0.5% to 2.2%, respectively. In contrast, AHA produced from *L. polyphyllus* has $60.34 \pm 0.28\%$ C content and $2.78 \pm 0.08\%$ nitrogen; from *H. sosnowskyi*, $68.94 \pm 0.32\%$ C content and $1.83 \pm 0.08\%$ N content; and from *P. cuspidatum*, $63.25 \pm 0.32\%$ C content and $3.15 \pm 0.09\%$ N content. Therefore, the elemental composition of AHA is within the range of values for naturally occurring soil humic acids (Rashad et al. 2022) and humic acids isolated from peat (Klavins and Purmalis 2013; Krumins and Klavins 2022). The analysis further indicates the removal of oxygen-containing moieties in AHS obtained from invasive plants (Table 2).

The UV-Vis spectra of both AHA and reference humic acids, as revealed in Figure 2A, are featureless and show a monotonic decrease with increasing wavelength. However, in comparison to AHS derived from invasive plants, reference and peat humic acids demonstrated higher absorption capacity in the region around 280 nm, which is associated with the presence of aromatic structures in the molecules. Despite this, the UV-Vis spectra of both groups of humic acids are similar, differing only slightly in optical density.

Analysis of obtained FTIR spectra provides the characterization of functional groups of AHS (Figure 2B). In the wavenumber range from $3,500$ to $3,300\text{ cm}^{-1}$, a broad hydroxyl group signal was observed, indicating an H-bonded OH stretch, which can be attributed to aliphatic hydroxyl bonds for bound water. Signals

detected at $2,914\text{ cm}^{-1}$ and $2,850\text{ cm}^{-1}$ are attributed to the methylene ($>\text{CH}_2$) C–H asymmetric/symmetric stretch of aliphatic hydrocarbons. In AHS obtained from *H. sosnowskyi* and *P. cuspidatum*, these signals are significantly more intense than in other humic substances studied. A characteristic carboxylic acid C=O signal was observed at $1,725$ to $1,735\text{ cm}^{-1}$ for all substances. Alkenyl C=C stretch and aromatic ring stretch were observed at $1,650\text{ cm}^{-1}$ and $1,590\text{ cm}^{-1}$, respectively. Signals in the range of $1,490$ to $1,500\text{ cm}^{-1}$ can be attributed to the ketone group C=O. A signal for aromatic acids was observed at $1,360$ to $1,390\text{ cm}^{-1}$, while arylalkyl ethers, common in lignin $-\text{OCH}_3$ groups, were identified at $1,280$ to $1,260\text{ cm}^{-1}$. Glycosidic bonds, C–O–C, were detected at $1,200$ to $1,180\text{ cm}^{-1}$. A peak in the wavenumber interval of $1,050$ to $1,040\text{ cm}^{-1}$ corresponds to alcohol groups, C–OH, but aromatic carbon-hydrogen bonds, C–H, were observed at 910 to 900 cm^{-1} . Generally, it can be concluded that after the HTC process, the quantity of oxygen-containing functional groups decreased due to dehydration and decarboxylation reactions, whereas the number of aromatic structures increased. This finding led to the corresponding characteristic peaks of aromatic structures in the range from $1,650$ to $1,450\text{ cm}^{-1}$ becoming stronger and broader.

Advanced spectroscopic techniques, including fluorescence spectroscopy, were utilized to characterize the structural and functional aspects of AHA produced from invasive plants and reference humic acids. The excitation–emission matrices (EEMs) of the two types revealed evident similarities. EEMs for all samples (Figure 3) displayed two common peaks at EEM wavelengths of approximately $350/450\text{ nm}$ and $450/550\text{ nm}$, with varying fluorescence intensities. Among the plant-derived AHA,

Table 2. Elemental composition of artificial humic acids (AHA) produced by hydrothermal carbonization (HTC) from invasive plant biomass during this study and humic acids obtained from peat (Klavins and Pūrmalis 2013) and soil (Rashad *et al.* 2022) for reference

Origin of humic acids	Elemental composition				
	C	H	N	O	S
	% of dry mass				
<i>Heracleum sosnowskyi</i>	68.94 ± 0.32	5.62 ± 0.04	1.83 ± 0.08	23.29 ± 0.24	0.32 ± 0.03
<i>Lupinus polyphyllus</i>	60.34 ± 0.28	5.54 ± 0.09	2.78 ± 0.08	30.69 ± 0.85	0.65 ± 0.03
<i>Polygonum cuspidatum</i>	63.25 ± 0.32	5.23 ± 0.05	3.15 ± 0.09	27.52 ± 0.74	0.85 ± 0.04
Peat	48–64	3.0–6.0	0.5–4.0	48–25	0.2–1.1
Soil	52–62	3.0–5.5	3.5–5.0	30–33	—

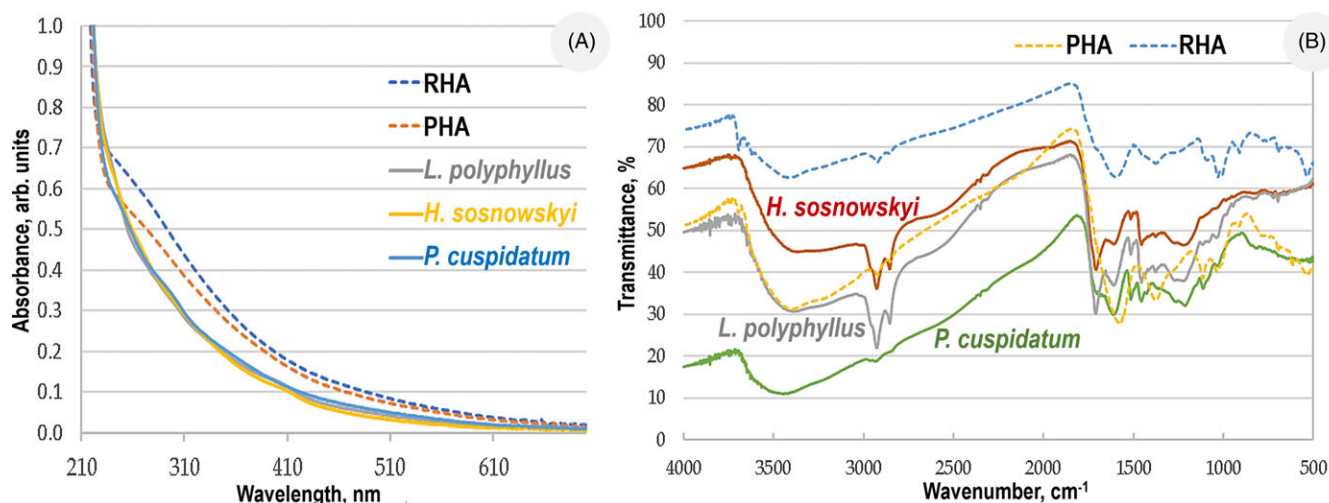


Figure 2. Ultraviolet-visible (UV/Vis) spectra (A) and Fourier-transform infrared (FTIR) spectra (B) of artificial humic acids (AHA) obtained by hydrothermal carbonization (HTC) from biomass of invasive plants (*Lupinus polyphyllus*, *Heracleum sosnowskyi*, and *Polygonum cuspidatum*) compared with peat-based humic acid (PHA) and standardized reference humic acid (RHA; Sigma-Aldrich).

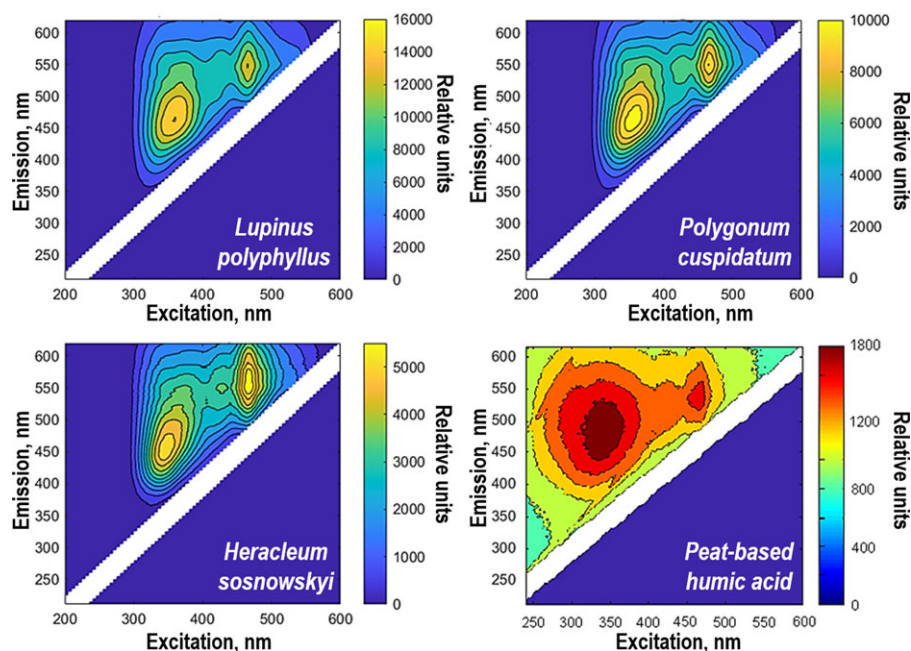


Figure 3. Excitation–emission matrices of artificial humic acids (AHA) obtained by hydrothermal carbonization (HTC) from biomass of invasive plants (*Lupinus polyphyllus*, *Heracleum sosnowskyi*, and *Polygonum cuspidatum*) compared with reference peat-based humic acid.

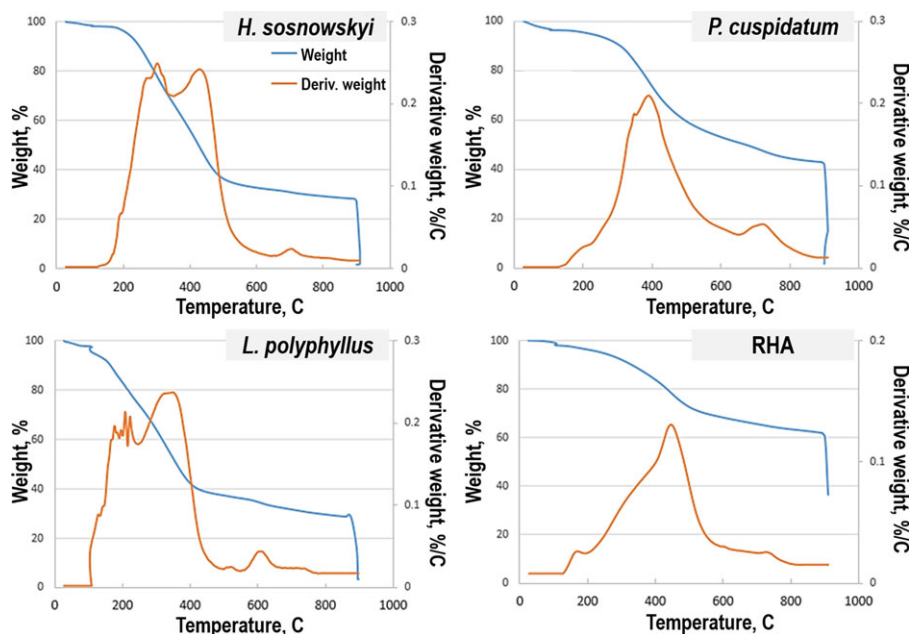


Figure 4. Thermogravimetric analysis of artificial humic acids (AHA) obtained by hydrothermal carbonization (HTC) from biomass of invasive plants (*Heracleum sosnowskyi*, *Lupinus polyphyllus*, and *Polygonum cuspidatum*) compared with standardized reference humic acid (RHA; Sigma-Aldrich).

compounds from *L. polyphyllus* exhibited the highest fluorescence intensity, followed by AHA derived from *P. cuspidatum* and *H. sosnowskyi*. Additionally, the EEMs of the latter showed greater complexity compared with AHA derived from *L. polyphyllus*, indicating potential differences in their molecular structures.

These findings suggest that the plant biomass source influences the properties of AHA. The fact that all three plant-derived AHA exhibit peaks at wavelengths similar to those produced by peat-based humic acids indicates similarities in core molecular structures and common functional groups. However, the differences in fluorescence intensity and the additional complexities in the EEMs of AHA derived from *P. cuspidatum* and *H. sosnowskyi*, as well as in peat-based humic acids, point to variations in the degree of conjugation, the presence of different side chains, or additional functional groups not present in AHA derived from *L. polyphyllus*. For instance, increased complexity in the EEMs could arise from molecular heterogeneity, suggesting a broader range of molecular sizes or a higher degree of aromaticity. Such complexity indicates that AHA derived from *P. cuspidatum* and *H. sosnowskyi* possess a greater array or concentration of specific functional groups, such as carboxyl or phenolic groups. Therefore, the more complex EEM patterns for AHA derived from *P. cuspidatum* and *H. sosnowskyi* imply a different or more diverse set of molecular characteristics, which in turn may affect their role in the soil environment, including their reactivity and solubility.

In general, fluorescence peaks in the regions around (1) 450-nm emission with 350-nm excitation and (2) 550-nm emission with 450-nm excitation indicate the presence of aromatic and conjugated systems, which are typically responsible for the fluorescence of AHA. The complexities in the EEMs of AHA derived from *P. cuspidatum* and *H. sosnowskyi* suggest that these AHA contain a more diverse set of functional groups, each contributing differently to the overall fluorescence pattern. Higher emission wavelengths, as observed in the second peak, imply that the AHA molecules have an extended system of conjugated double

bonds or larger aromatic networks, which tend to emit at longer wavelengths following excitation. This second peak could be indicative of the presence of polycyclic aromatic hydrocarbons being larger and more complex aromatic structures.

The EEM profiles of peat-based humic acids derived after long-term anaerobic decomposition tend to be more stable and contain a higher proportion of oxygen-containing functional groups. In contrast, plant-derived AHA are relatively less mature and exhibit different sets of secondary metabolites. Nevertheless, their EEM profiles were found to be notably similar to those of peat-based humic acids, suggesting a level of structural and functional resilience that extends across their varying maturities and history of decomposition.

Thermogravimetric analysis (TGA) of AHA obtained from invasive plants reflects differences in the composition of the precursor biomass and its transformation process during carbonization (Figure 4). The first thermal degradation step determines the amount of water (physically as well as constitutionally bound water molecules) and gas particles that detach up to 115 C. The second mass loss region is associated with significant mass loss (20% to 35%) at 280 to 295 C and is the result of the thermal decomposition of polysaccharides, decarboxylation of carboxylic acids, and dehydration of hydroxyl group-containing moieties (Dell'Abate et al. 2002; Francioso et al. 2005). For most humic acids, TGA analysis revealed the major weight loss at 320 to 400 C, which could be attributed to the removal of the majority of functional groups as well as condensation reactions of unsaturated aliphatic structures to form polyaromatic structures (Peuravuori et al. 1999).

This thermal decomposition mechanism is common for the lignocellulose feedstock of invasive plant biomass, resulting in the development of highly condensed aromatic structures. There is a significant difference in the thermal transformation process of AHA obtained from *L. polyphyllus* and *H. sosnowskyi* on one hand, and AHA derived from *P. cuspidatum* and peat-based humic acids

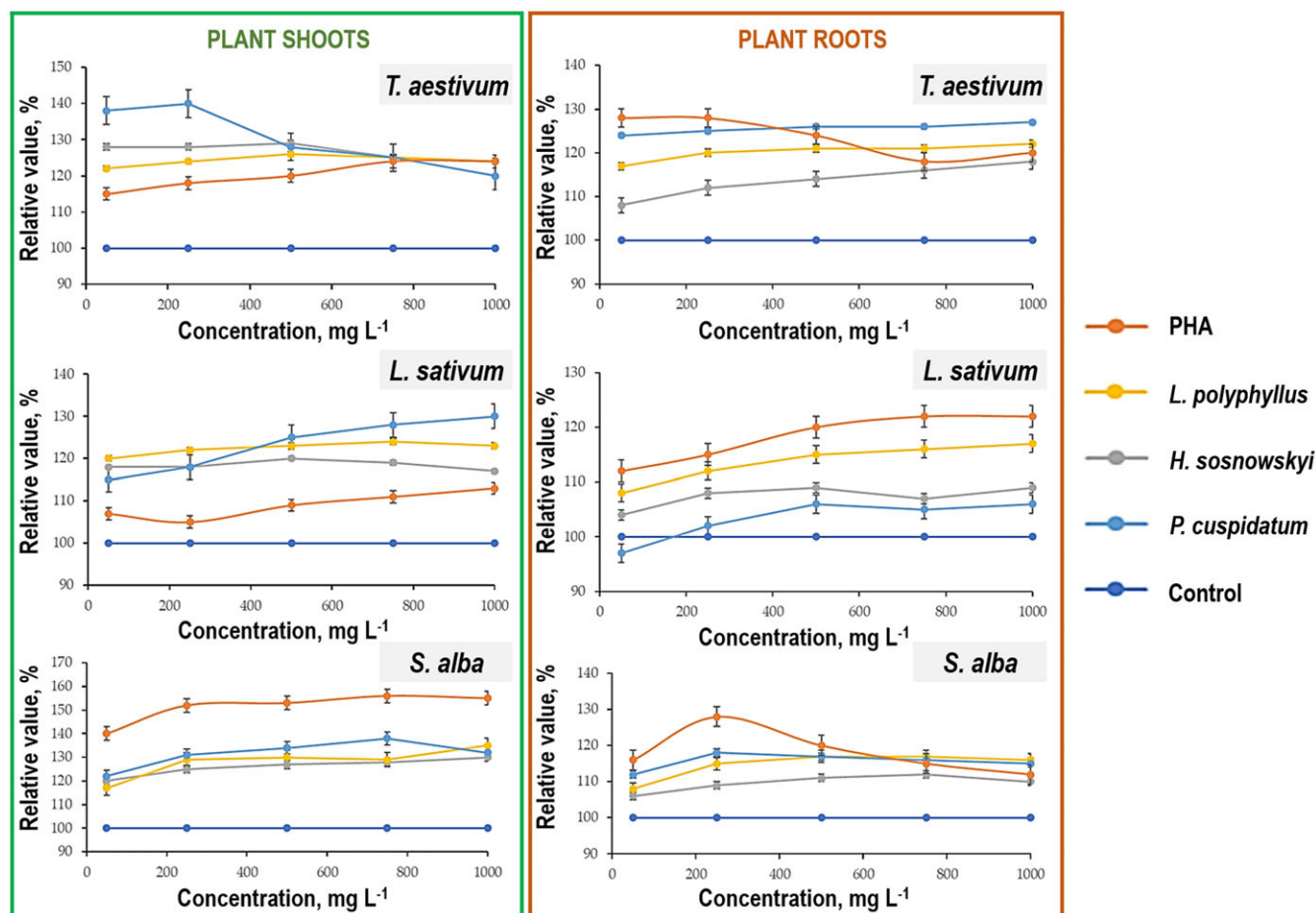


Figure 5. Relative changes in early plant development of tested species (white mustard [*Sinapis alba*], gardencress pepperweed [*Lepidium sativum*] and wheat [*Triticum aestivum*]) expressed as shoot and root length across a concentration range of applied solution of artificial humic substances (AHS) obtained during the hydrothermal carbonization (HTC) from invasive plants (*Lupinus polyphyllus*, *Heracleum sosnowskyi*, and *Polygonum cuspidatum*) compared with reference peat-based humic acid (PHA) solutions, ranging from 25 mg L⁻¹ to 1,000 mg L⁻¹.

on the other. AHA derived from *L. polyphyllus* and *H. sosnowskyi* exhibit more intensive decomposition peaks at 280 to 295 °C, indicating the presence of labile structures.

Applicability of AHS Produced from Invasive Plant Biomass in Agriculture

Humic substances of natural origin such as soil humic substances are essential for plant development, and humic acids isolated from them have biostimulatory properties (Conselvan et al. 2017; da Silva et al. 2019; Traversa et al. 2014; Vieira et al. 2018).

AHS resulting from the HTC processing of invasive plant biomass have demonstrated potential in stimulating seed germination, early plant development and growth in three tested plants, white mustard, gardencress pepperweed, and wheat (Figure 5). The stimulatory effect varies depending on the plant species, the type of humic substance, and its concentration. The greatest impact on the development of wheat leaves was observed applying AHS obtained from *P. cuspidatum* at a concentration of 250 mg L⁻¹; however, the plant development stimulatory effect decreases with increasing concentration. The impact of other AHS and peat-based reference humic acids did not vary much with concentration. The stimulatory effect on wheat root development is generally higher than that of peat-based humic acid and did not

vary significantly with AHS concentration. Both AHS and peat-based humic acids stimulate the development of gardencress pepperweed leaves and roots, with the exception of AHS obtained from *P. cuspidatum* at a concentration of 25 mg L⁻¹, and the stimulatory effect increases with concentration. The impact on white mustard development (both roots and leaves) is relatively independent of AHS and peat-based humic acids concentration but is higher than for the other test plants.

The observed biostimulatory impact of AHS and naturally occurring humic acids aligns with the results of other studies, in which the biostimulatory effect on plant growth of humic substances isolated from peat (Conselvan et al. 2017; Traversa et al. 2014), composts (da Silva et al. 2019), coal (Vieira et al. 2018), and industrially produced humic substances (Klavins et al. 2021) was demonstrated. Differences in the stimulatory effect on plant development and growth can be attributed to varying humification conditions and varying composition of humic matter precursors. For example, peat-based humic acids are formed from lower plants such as mosses, while compost-based humic acids originate from higher vegetation. Variations in the N content of the studied AHS may also contribute to the differences in their biostimulatory activity on plant development. These findings add significant support to the potential application of AHS derived from invasive plants in agriculture.

HTC, a climate-neutral technology, effectively transforms invasive plant biomass through the chain of reactions called hydrothermal humification into refractory material, namely AHS, involving fulvic and humic acids. This transformation was demonstrated in three highly invasive and widespread plants, *L. polyphyllus*, *H. sosnowskyi*, and *P. cuspidatum*. The obtained AHS yield, which can reach up to 62%, primarily depends on pH, temperature, and the duration of the HTC process. Additionally, this process produces a solid carbonaceous material known as hydrochar. The elemental composition, spectral characteristics, and thermal decomposition parameters of AHS are generally similar to those of naturally occurring humic substances. Plant early development tests indicated that AHS, like naturally occurring humic substances, support plant development and can be applied as biostimulatory agents in agriculture and other areas of bioeconomics. During the HTC process, seeds of invasive plants lose their germination capacity and subsequent propagation ability. Therefore, HTC can be applied as a promising and economically valuable option in the reduction of invasive plants with supplementary acquisition of the substances valuable for bioeconomics.

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References

- Albano PE, Ruiz TT, Ramos MS, Casero LPJ, Vázquez PFM, Rodríguez MPL, Moreno JL, Gallego FL, Cortes JG, Sánchez GJM (2015) Seed germination and risks of using the invasive plant *Eichhornia crassipes* (Mart.) Solms-Laub. (water hyacinth) for composting, ovine feeding and biogas production. *Acta Bot Gallica* 162:203–214
- Ali H, Akbar Y, Razaq A, Muhammad D (2014) Effect of humic acid on root elongation and percent seed germination of wheat seeds. *Int J Agric Crop Sci* 7:196–201
- Conservan GB, Pizzeghello D, Francioso O, Foggia MD, Nardi S, Carletti P (2017) Biostimulant activity of humic substances extracted from leonardites. *Plant Soil* 420:119–134
- Da Silva RR, Botero WG, de Lima RSN, dos Santos CL, de Oliveira LC (2019) Evaluation of root growth of coriander (*Coriandrum sativum* L.) by humic substances. *J Environ Sci Toxicol Food Technol* 13:15–19
- Dell'Abate MT, Benedetti A, Trinchera A, Dazzi C (2002) Humic substances along the profile of two Typic Haploxerert. *Geoderma* 107:281–296
- Epanchin-Niell RS, Haight RG, Berc L, Kean JM, Liebhold AM (2012) Optimal surveillance and eradication of invasive species in heterogeneous landscapes. *Ecol Lett* 15:803–812
- [EU] European Union (2014) Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. *Offic J EU* L317:35–55
- Francioso O, Montecchio D, Gioacchini P, Ciavatta C (2005) Thermal analysis (TG–DTA) and isotopic characterization (^{13}C – ^{15}N) of humic acids from different origins. *Appl Geochem* 20:537–544
- González-Arias J, Sánchez ME, Cara-Jiménez J, Baena-Moreno FM, Zhang Z (2022) Hydrothermal carbonization of biomass and waste: a review. *Environ Chem Lett* 20:211–221
- Haubrock PJ, Turbelin AJ, Cuthbert RN, Novoa A, Taylor NG, Angulo E, Ballesteros-Mejía L, Bodey TW, Capinha C, Diagne C, Essl F, Golivets M, Kirichenko N, Kourantidou M, Leroy B, et al. (2021) Economic costs of invasive alien species across Europe. Pages 153–190 in Zenni RD, McDermott S, García Berthou E, Essl F, eds. *The Economic Costs of Biological Invasions around the World*. *NeoBiota* 67. <https://doi.org/10.3897/neobiota.67.69971>
- Ischia G, Fiori L (2021) Hydrothermal carbonization of organic waste and biomass: a review on process, reactor, and plant modeling. *Waste Biomass Valoriz* 12:2797–2824
- Klavins M, Grandovska S, Obuka V, Ievinsh G (2021) Comparative study of biostimulant properties of industrially and experimentally produced humic substances. *Agronomy* 11:1250
- Klavins M, Purmalis O (2013) Properties and structure of raised bog peat humic acids. *J Mol Struct* 1050:103–113
- Krumins J, Klavins M (2022) Characterization of humic acids in boreal mires depending on a peat type. *Boreal Environ Res* 27(1–6):1–31
- Lan Y, Du Q, Tang C, Cheng K, Yang F (2021) Application of typical artificial carbon materials from biomass in environmental remediation and improvement: a review. *J Environ Manag* 296:113340
- Nicolae SA, Au H, Modugno P, Luo H, Szego AE, Qiao M, Li L, Yin W, Heeres HJ, Berge N, Titirici MM (2020) Recent advances in hydrothermal carbonization: from tailored carbon materials and biochemicals to applications and bioenergy. *Green Chem* 22:4747–4800
- Nordahl SL, Preble CV, Kirchstetter TW, Scown CD (2023) Greenhouse gas and air pollutant emissions from composting. *Environ Sci Technol* 57:2235–2247
- Peng X, Gai S, Cheng K, Yang F (2023) Hydrothermal humification mechanism of typical agricultural waste biomass: a case study of corn straw. *Green Chem* 25:1503–1512
- Peuravuori J, Paaso N, Pihlaja K (1999) Kinetic study of the thermal degradation of lake aquatic humic matter by thermogravimetric analysis. *Thermochim Acta* 325:181–193
- Rai PK, Singh JS (2020) Invasive alien plant species: their impact on environment, ecosystem services and human health. *Ecol Indic* 111: 106020
- Rashad M, Hafez M, Popov AI (2022) Humic substances composition and properties as an environmentally sustainable system: a review and way forward to soil conservation. *J Plant Nutr* 45:1072–1122
- Regan TJ, McCarthy MA, Baxter PW, Dane Panetta F, Possingham HP (2006) Optimal eradication: When to stop looking for an invasive plant. *Ecol Lett* 9:759–766
- Sayara T, Sánchez A (2021) Gaseous emissions from the composting process: controlling parameters and strategies of mitigation. *Processes* 9:1844
- Shao Y, Li Z, Long Y, Zhao J, Huo W, Luo Z, Lu W (2024) Direct humification of biowaste with hydrothermal technology: a review. *Sci Total Environ* 908:168232
- Sheppard AW, Shaw RH, Sforza R (2006) Top 20 environmental weeds for classical biological control in Europe: a review of opportunities, regulations and other barriers to adoption. *Weed Res* 46:93–117
- Traversa A, Loffredo E, Gattullo CE, Palazzo AJ, Bashore TL, Senesi N (2014) Comparative evaluation of compost humic acids and their effects on the germination of switchgrass (*Panicum virgatum* L.). *J Soils Sedim* 14:432–440
- Vaverková MD, Adamcová D, Winkler J, Koda E, Petrželová L, Maxianová A (2020) Alternative method of composting on a reclaimed municipal waste landfill in accordance with the circular economy: benefits and risks. *Sci Total Environ* 723:137971
- Vieira J, Kledson L, Oliveira LC, De Braga J, Rosa T, Botero WG (2018) Evaluation of germination of chili pepper using humic substances and humic acids. *J Environ Sci Toxicol Food Technol* 12:33–39
- Wei H, Huang M, Quan G, Zhang J, Liu Z, Ma R (2018) Turn bane into a boon: application of invasive plant species to remedy soil cadmium contamination. *Chemosphere* 210:1013–1020

- Wiatrowska BM, Wawro A, Gieparda W, Waliszewska B (2021) Bioethanol production potential and other biomass energy properties of invasive *Reynoutria*, *Solidago*, and *Spiraea* plants. *Forests* 13:1582
- Yang F, Antonietti M (2020) Artificial humic acids: sustainable materials against climate change. *Adv Sci* 7:1–7
- Yang F, Zhang S, Cheng K, Antonietti M (2019) A hydrothermal process to turn waste biomass into artificial fulvic and humic acids for soil remediation. *Sci Total Environ* 686:1140–1151
- Yang F, Zhang S, Fu Q, Antonietti M (2020) Conjugation of artificial humic acids with inorganic soil matter to restore land for improved conservation of water and nutrients. *Land Degrad Develop* 31:884–893
- Zhai Y, Peng C, Xu B, Wang T, Li C, Zeng G, Zhu Y (2017) Hydrothermal carbonization of sewage sludge for char production with different waste biomass: effects of reaction temperature and energy recycling. *Energy* 127:167–174
- Zihare L, Blumberga D (2017) Invasive species application in bioeconomy: case study *Heracleum sosnowskyi* Manden in Latvia. *Energy Proc* 113:238–243