Original Research

PAN-LIFE-CARPET CONCEPT: INTEGRATING WASTE VALORIZATION AND BIOCRUST ENGINEERING

DOI: 10.5937/EcoTER26022S

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Abstract

Land degradation in arid and semi-arid regions poses a major global threat to ecological stability, food security, and socio-economic resilience. Cyanobacteria-dominated biological soil crusts (biocrusts) play a critical role in soil stabilization and nutrient enrichment, but their early establishment and persistence are frequently limited by severe climatic conditions. This study combines cyanobacterial inoculation (Tolypothrix sp.) with bio-based polysaccharide matrices derived from agro-industrial and marine waste to accelerate biocrust development on loess sediment. Alginate (29.34% yield) and fucoidan (5.18% yield) were successfully isolated from the brown seaweed Laminaria digitata, while native cellulose (30.58% yield) was extracted from plum shells (Prunus domestica). Structural integrity was confirmed via FTIR spectroscopy, followed by an evaluation of their water-holding capacity (WHC) and water swelling capacity (WSC). Alginate exhibited superior hydrocolloid properties, achieving the highest WHC (0.8 g/g) and WSC (2.1 mL/g). During a 30-day laboratory cultivation, the addition of all isolated polysaccharides significantly enhanced biological crust development and biomass accumulation compared to the cyanobacteria-only treatment. The most pronounced stimulatory effect was achieved with 0.3% (m/v) alginate, which induced a ninefold increase in chlorophyll a content (352.46 μg/g) compared to the cyanobacteria control (38.33 μg/g). Fucoidan and cellulose also promoted biocrust growth, yielding significantly higher chlorophyll a value (94.82 μg/g and 77.90 μg/g, respectively) than the polysaccharide-free control. These findings validate the Pan-Life-Carpet framework, demonstrating that valorizing renewable biomass waste into eco-friendly support matrices provides a highly effective, cost-efficient, and scalable strategy for accelerated dryland restoration and soil erosion control.

Keywords: land degradation, biocrusts, cyanobacteria, waste valorization, polysaccharides, soil restoration.

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INTRODUCTION

Arid and semi-arid ecosystems are highly vulnerable to erosion, nutrient loss, and biodiversity decline under climate change, requiring scalable and cost-effective restoration strategies [1,2]. Biological soil crusts (biocrusts), particularly cyanobacteria-dominated early successional stages, are critical for soil stabilization, nutrient enrichment, and water regulation [3]. While artificial inoculation can accelerate crust development, extreme temperatures, irregular precipitation, and poor soil structure often limit establishment and persistence, especially during early colonization [4]. Agro-industrial and marine biomass residues represent promising bio-based supports [5].

In this study, the Pan-Life-Carpet concept is presented: a circular restoration approach in which agro-industrial residues serve as bio-based matrices for cyanobacterial inoculation, combined with physical carriers to enhance soil adhesion, moisture retention, and inoculum stability. The effectiveness of this approach for early-stage soil stabilization under simulated dryland conditions is evaluated, providing insights into scalable and resource-efficient restoration strategies.

MATERIALS AND METHODS

Chemicals and reagents

In this study, 96% (*v/v*) ethanol, concentrated hydrochloric acid, and sodium hydroxide (Zorka Pharma, Šabac, Serbia), as well as potassium bromide (purity ≥98%) (Sigma-Aldrich, St. Louis, MO, USA) were used.

Plant material

Laminaria digitata biomass was purchased from Ozon Way (Belgrade, Serbia), and shells of *Prunus domestica* from Southeastern Serbia (42°53′19″N, 22°01′35″E). The raw materials were air-dried at ambient temperature for seven days. After that, the plant materials were milled and sieved before further use. A 0.5 mm fraction of brown seaweed biomass was used for the isolation of alginate and fucoidan, while a 0.7 mm fraction of plum shells was used for cellulose isolation. The moisture content was found to be 10.6% (*w/w*) for the brown seaweed biomass and 8.17% (*w/w*) for the plum shells.

Extraction of polysaccharides from plant material

Brown seaweed biomass (500 g) was depigmented using 70% (*v/v*) ethanol (5 L) at room temperature (22 ± 2 °C) for 24 h. Subsequently, the pretreated biomass was subjected to ultrasound-assisted extraction using 0.1 mol/L hydrochloric acid at 50 °C and the liquid-to-solid ratio of 15 mL/g for 6 min. Ultrasonic bath (Sonic, Niš, Serbia) operating at a frequency of 40 kHz and a total power of 300 W was used. Alginate was selectively precipitated from the filtrate by adding 2% (*w/v*) calcium chloride ($CaCl_2$) in a 1:1 (*v/v*) ratio. The obtained alginate was bleached twice with 20 mL of acetone at 18 °C. Fucoidan was precipitated from the supernatant remaining after alginate removal by the addition of 96% (*v/v*) ethanol in a 4:1 (*v/v*) ratio. The precipitated alginate and fucoidan were dried, ground in an electric mill, and stored at room temperature.

Plum shell powder (50 g) was suspended in 500 mL of 18% (*w/v*) NaOH and subjected to alkaline extraction at 70 °C for 90 min. The solid residue was washed with distilled water to pH neutral. The obtained material was then bleached with 500 mL of 30% (*v/v*) H_2O_2 until complete whitening was achieved. The isolated native cellulose was air-dried and stored in a sealed glass container at 25 ± 2 °C.

Fourier transform infrared spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) was used for structural characterization of isolated polysaccharides. Spectra of KBr pellets were recorded in the wavenumber range of 4000–400 cm^{-1} at a resolution of 2 cm^{-1} on a BOMEM Hartmann & Braun MB-series spectrophotometer (Quebec, Canada). The recorded spectra were processed using Win-BOMEM Easy software (Galactic Industries, Salem, New Hampshire, USA).

Hydration properties of isolated polysaccharides

To measure water-holding capacity (*WHC*) and water swelling capacity (*WSC*), 0.5 g of the polysaccharide was added to 20 mL of distilled water at room temperature. After centrifuging the hydrated samples at 6000 rpm for 10 min, the *WHC* was calculated according to Eq. 1. The *WSC* determination involved dispersing the samples for 24 h at room temperature. After measuring the volume of the swollen polysaccharide, the *WSC* was calculated using Eq. 2.

$$WHC\left(\frac{g}{g}\right) = \frac{m_2 - m_1}{m_1} \quad (1)$$

where, m_1 is a dry sample mass, m_2 is a sample mass after immersion in water and centrifugation.

$$WSC\left(\frac{mL}{g}\right) = \frac{V_1 - V_0}{m_1} \quad (2)$$

where, V_1 is a volume of swollen sample (mL), V_0 is a volume of dry sample (mL), and m_1 is a mass of dry sample (g).

Biocrust development and growth assessment

The potential of isolated polysaccharides, including alginate, fucoidan, and cellulose, to promote cyanobacterial biocrust establishment was evaluated on loess sediment under controlled laboratory conditions. Cyanobacterial inocula (*Tolypothrix* sp.) obtained from the Novi Sad Cyanobacterial Culture Collection (NSCCC, Serbia) were cultivated in nitrogen-free BG11 medium at 25 ± 2 °C under daylight conditions. Experiments were conducted in Petri dishes filled with homogenized loess sediment. Cyanobacterial inoculum (0.494 mg DW cm^{-2}) was applied alone and combined with isolated polysaccharides, namely alginate, fucoidan, and cellulose at concentrations of 0.3%, 0.5% and 1% (*m/v*), respectively. Non-inoculated sediment treated only with water served as the control. Application of cyanobacterial inoculum was performed manually using a Pasteur pipette. Biocrust were cultivated under daylight at room temperature (25 ± 2 °C) for 30 days, while irrigation was carried out on weekdays using about 30–50 mL of water per container. Following cultivation, developed biocrust material was collected, homogenized, and prepared for chlorophyll *a* analyses. Chlorophyll *a* extraction and quantification were performed according to Park et al. [6] and Chamizo *et al*. [7]. Homogenized biocrust material was extracted in 96% ethanol,

vortexed and incubated at 80 °C for 5 min, after which the incubation was continued at 4 °C for 24 h. The samples were vortexed, sonicated for 10 min, and centrifuged at 3000 rpm for 10 min (Beckman Coulter Avanti J-15). The obtained supernatants were analyzed spectrophotometrically at 665 and 750 nm using a SPECTROstar Nano spectrophotometer (BMG LABTECH, Germany), while absorbance at 665 nm (A_{665}) was corrected by subtracting the absorbance measured at 750 nm. Chlorophyll *a* concentration, expressed in μg/g, were calculated according to Ritchie [8] and Castle *et al*. [9] (Eq. 3):

$$\text{Clorophyll } a\left(\frac{\mu g}{g}\right) = \frac{11.9035 \times A_{665} \times V}{m \times L} \quad (3)$$

where, V is the solvent volume (mL), m is the dry mass of the sample (g), and L is the cuvette path length (cm).

Statistical analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation. Statistical analysis was conducted using GraphPad Prism 9 (San Diego, CA, USA). Statistical differences between groups and treatments were evaluated via one-way ANOVA (Tukey's and Dunnett's post hoc tests) and Student's t-test at a 95% confidence level ($p < 0.05$).

RESULTS AND DISCUSSION

In the present study, brown seaweed biomass was utilized as a sustainable source of alginate and fucoidan. Plum shells were selected as an alternative lignocellulosic material for cellulose isolation. The valorization of these by-products is significant from both environmental and economic perspectives. The obtained alginate yield (29.34 ± 0.45%) falls within the range commonly reported for brown seaweeds (15%–40%) [10]. In contrast, the fucoidan yield (5.18 ± 0.09%) is consistent with previously reported values (1%–10%) [11]. Cellulose was isolated from plum shell, which matches the high cellulose content of 30.58 ± 0.79% typically reported for lignocellulosic materials [12].

Structural characterization of isolated polysaccharides

FTIR spectra confirmed the structural integrity and successful isolation of all three polysaccharides (Figure 1). In the spectrum of alginate (Figure 1a), characteristic bands of a uronic acid-rich structures were identified through O–H and C–H stretching at 3385 cm^{-1} and 2930 cm^{-1}, carboxylate ($–COO^–$) asymmetric and symmetric stretching at 1617 cm^{-1} and 1417 cm^{-1}, and glycosidic bond and C–O–C ring vibrations between 1046 cm^{-1} and 1130 cm^{-1}. For fucoidan (Figure 1b), successful isolation of the sulfated polysaccharide was validated by characteristic O–H and C–H bands (3360 cm^{-1} and 2927 cm^{-1}), alongside prominent sulfate group indicators at 1240 cm^{-1} (S=O) and 851 cm^{-1} (C–O–S), reflecting equatorial substitution at the C-4 position in accordance with literature reports [13]. The cellulose spectrum (Figure 1c) displayed a broad O–H band at 3307 cm^{-1} indicating extensive hydrogen bonding, a C–H stretching band at 2886 cm^{-1}, and an absorbed water bending peak at 1652 cm^{-1} [14]. Crystalline cellulose features were evident from peaks at 1425 cm^{-1} and 1374 cm^{-1}, while the absorption at 896 cm^{-1} confirmed the presence of β-glycosidic linkages. Ultimately, these differing chemical functionalities suggest complementary mechanisms that

could be exploited to enhance cyanobacterial growth and productivity within the cultivation systems.

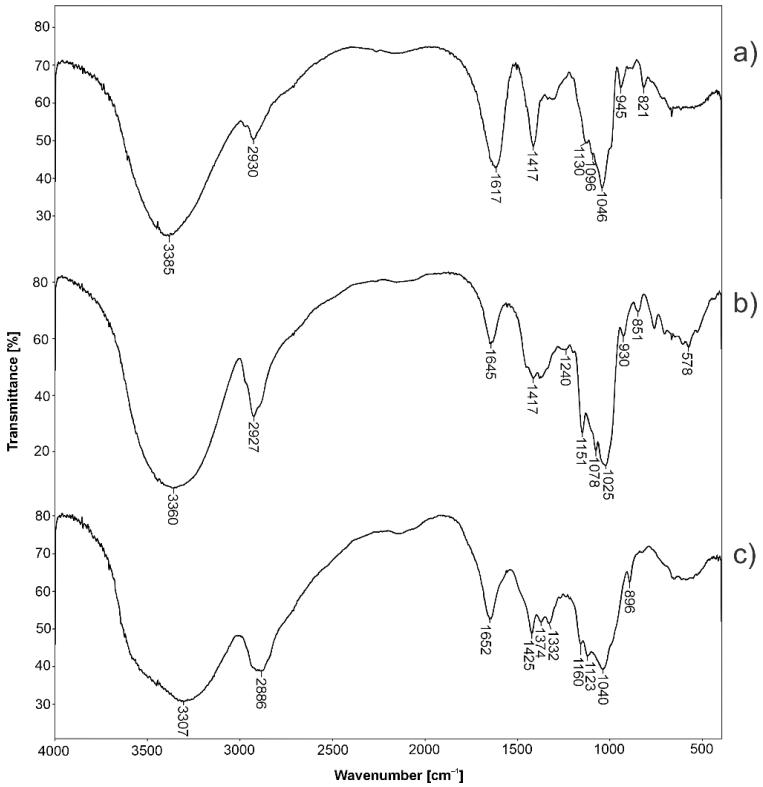

Figure 1 *FTIR spectra of isolated: a) alginate; b) fucoidan; and (c) cellulose*

Hydration properties of isolated polysaccharides

The *WHC* and *WSC* are key functional parameters that reflect the hydration behavior and structural organization of polysaccharides. They depend on the molecular architecture, crystallinity, and porosity, as well as the availability and accessibility of hydrophilic functional groups capable of forming hydrogen bonds with water [15]. The obtained results indicate different hydration properties among the analyzed polysaccharides (Table 1).

Table 1 *Water-holding capacity (WHC) and water swelling capacity (WSC) of analyzed polysaccharides*

Sample	*WHC* (g/g)	*WSC* (mL/g)
Alginate	0.8 ± 0.02^{c}	2.1 ± 0.12^{c}
Fucoidan	0.2 ± 0.01^{a}	0.46 ± 0.08^{a}
Cellulose	0.4 ± 0.01^{b}	0.84 ± 0.02^{b}

Different lowercase letters (a, b, c) in the same column indicate statistically significant differences between groups, as determined by Tukey's post hoc test at the 95% confidence level ($p < 0.05$).

Alginate exhibited the highest hydration values, confirming its superior ability to hold and absorb water, which is consistent with literature describing alginate as a highly tunable hydrocolloid [16]. The linear structure of alginate, composed of uronic acid residues, provides a high density of carboxyl groups that strongly interact with water molecules. Ca^{2+}-mediated junction zones enable the formation of a network capable of significant swelling without

structural collapse. Such systems achieve optimal water holding only when a balance is maintained between the crosslinking density and the available free volume within the gel network. The moderate WHC and lower WSC of cellulose can be attributed to its highly ordered and semi-crystalline structure, where extensive intra- and intermolecular hydrogen bonds within cellulose microfibrils limit the accessibility of hydroxyl groups and restrict water diffusion pathways [17]. In contrast, fucoidan exhibited the lowest hydration capacity despite the presence of highly hydrophilic sulfate groups. The hydration behavior of fucoidan is highly dependent on the degree of branching, sulfate distribution, and molecular weight heterogeneity [18]. The observed low swelling implies that strong intramolecular interactions and possible aggregation effects dominate over simple hydrophilicity. Specifically, electrostatic repulsion between sulfate groups can cause conformational packing under aqueous conditions, thus reducing the available hydration volume.

Biocrust development

Chlorophyll *a* content is commonly used as an indicator of biocrust development [6,19,20]. Cyanobacterial inoculation with *Tolypothrix* sp. successfully initiated biocrust formation on loess sediment (Table 2). The addition of isolated polysaccharides further stimulated biocrust development and biomass accumulation. Among the tested polysaccharides, alginate showed the most pronounced enhancement, which is likely related to its high-water retention capacity, supporting the importance of moisture availability [21]. Other studies also showed that natural polysaccharides significantly promote biocrust growth [22,23]. However, the chlorophyll *a* concentrations obtained here were multiple times higher, supporting the applicability of the Pan-Life-Carpet concept for ecosystem restoration [24].

Table 2 *Chlorophyll a content of developed crusts on loess sediment*

Sample	Chlorophyll *a* (μg/g)
Uninoculated sediment	0.18 ± 0.06^{a}
Cyanobacteria	$38.33 \pm 5.10^{*, b}$
Cyanobacteria + Alginate (0.3%)	$352.46 \pm 28.67^{*, c}$
Cyanobacteria + Fucoidan (0.5%)	$94.82 \pm 6.44^{*, d}$
Cyanobacteria + Cellulose (1%)	$77.90 \pm 4.38^{*, e}$

Asterisks indicate statistically significant differences compared to the control according to Dunnett's post hoc test at the 95% confidence level ($p < 0.05$). Different letters (a, b, c, d, e) indicate statistically significant pairwise differences among treatments according to the unpaired t-test at the 95% confidence level ($p < 0.05$).

CONCLUSION

This study successfully demonstrates the potential of utilizing natural polysaccharides isolated from marine biomass and agro-industrial by-products as sustainable bio-matrices to accelerate the establishment of cyanobacterial biocrusts. Alginate stood out with the highest water-holding and swelling capacities, which proved to be the critical driving factor for inoculum survival and proliferation. Biological assays on loess sediment confirmed that the application of all tested polysaccharides significantly enhances early-stage biocrust development, with the 0.3% (*m/v*) alginate treatment inducing the highest biomass accumulation and a more than ninefold increase in chlorophyll a concentration. The proposed Pan-Life-Carpet concept provides an innovative, ecologically safe, and resource-efficient

strategy capable of broad application in combating land degradation, enhancing sand stabilization, and restoring degraded dryland ecosystems worldwide.

ACKNOWLEDGEMENT

This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-34/2026-03/200133, 451-03-33/2026-03/ 200125, and 451-03-34/2026-03/ 200125) and the Science Fund of the Republic of Serbia, #17807, The Loess Plateau Margins: Towards Innovative Sustainable Conservation – LAMINATION.

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