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RESEARCH ON THE BIODEGRADABILITY AND ECOTOXICITY OF SOME BIOHYDROGELS

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Abstract

In order to address pressing issues such as persistent drought conditions or limited water availability, researchers have extensively examined hydrogels, which serve as reservoirs for water retention. They have the capacity to store a substantial amount of water and gradually release it in a controlled manner. Biodegradability stands as a main requirement for these polymeric materials, as they can be employed in the field of agriculture and may present a sustainable mechanism. Additionally, hydrogels must exhibit a lack of ecotoxicity, ensuring that no harmful substances are released into the environment following the biodegradation process. The aim of this study was to test 8 different formulations of hydrogels based on acrylic acid, carboxymethyl cellulose and sodium alginate regarding their biodegradability rate and their ecotoxicity potential. After 200 days the tested samples showed a greater rate of biodegradation for the samples containing a higher amount of sodium alginate. The ecotoxicity of the tested biohydrogels, was assessed through the germination rate and rootlet length of the radish seeds measurements. The germination process has been positively influenced by some samples while most of them demonstrated similar behavior with the control.

Key words*: bidegradation, biohydrogel, ecotoxicity.*

INTRODUCTION

Hydrogels play an indispensable role in the field of agriculture due to their outstanding water retention capabilities, which prove to be especially advantageous in arid and semi-arid regions (Saini & Malve, 2023). They possess the ability to increase the moisture content of the soil, reduce the need for irrigation, and improve both the health of the plants and the development of the roots, ultimately leading to increased crop yield (Abobatta, 2018). More specifically, hydrogels based on biopolymers exhibit non-toxicity, biocompatibility, and cost-effectiveness, thereby establishing themselves as a sustainable water management solution in the agricultural sector (Tariq et al., 2023).

Hydrogel biodegradability in agriculture refers to the ability of hydrogels to break down and decompose in the soil, making them environmentally friendly and sustainable for agricultural applications (Sharma et al., 2021). Numerous investigations have been dedicated to the development of biodegradable hydrogels for soil conditioning and the release of nutrients. These hydrogels are typically comprised of natural raw materials, including cellulose derivatives, clay minerals, and biopolymers, which can be interconnected to create three-dimensional structures possessing a high capacity for water absorption (Turioni et al., 2021). The biodegradability of these hydrogels has been assessed through soil burial tests, whereby the generation of carbon dioxide is monitored during the process of degradation. The results demonstrated that these hydrogels are capable of decomposing in the soil, thereby releasing nutrients and enhancing soil fertility, such as the carboxy-methyl tamarind kernel gum based biohydrogel obtained by Malik et al., (2023) which has the potential to be a sustainable and effective substitute for

traditional soil conditioners (bonemeal, peat, manure, vermiculite, chemical fertilizers and sphagnum moss) in agronomy. The synthesis parameters and environmental conditions, such as pH, temperature, and soil properties, have the potential to impact the stability and the biodegradation of these hydrogels within the soil (Durpekova et al., 2021; Rop et al., 2022).

Hydrogel ecotoxicity refers to the potential harmful effects of hydrogels on the environment. Scientific investigations have provided evidence that specific hydrogels can induce acute toxic effects on organisms. To illustrate this aspect, Ramirez et al. (2023) unveiled that a terpolymeric hydrogel crosslinked with modified kraft lignin triggered acute lethal toxic effects in earthworms. Moreover, hydrogels can be utilized for the extraction of water contaminants, such as heavy metals. Halah et al. (2018) discussed the utilization of hydrogels as adsorbent materials for metallic ions, offering a viable remedy for water pollution. In summation, hydrogel ecotoxicity encompasses the potential harmful consequences of certain hydrogels on the natural surroundings, however, not all hydrogels manifest ecotoxic attributes.

A variety of investigations have examined the biodegradability and environmental toxicity of hydrogels.

The biodegradability and ecotoxicity of agricultural hydrogels can be influenced by a variety of factors.

The composition and synthesis parameters of the hydrogels, including the types of natural raw materials used and the degree of crosslinking, have a significant role in determining their stability when placed in soil. By using eco-friendly and biodegradable materials, the hydrogels made from super absorbent polymers (specifically designed for agricultural purposes) are both biodegradable and non-toxic to the soil, crops, and the overall environment (Azeem et al., 2023).

Moreover, environmental conditions, such as the quantity of hydration water and the characteristics of the soil, also have an impact on the degradation process of hydrogels when situated in soil (Vaid & Jindal, 2023).

Sousa et al. (2021) developed superabsorbent hydrogels based on polyacrylamide and cashew tree gum, which showed good swelling capacity and controlled release of nutrients without ecotoxicity. Vaid & Jindal (2023) obtained promising results with their biodegradable hydrogel utilizing natural polysaccharides such as tamarind kernel powder with kappa-carrageenan which has demonstrated a significant level of microbial biodegradability, as evidenced by the degradation rate of 92.6% after a period of 70 days of composting.

In order to evaluate the biodegradability of hydrogels, techniques such as weight loss measurements and microscopy analysis can be employed (Patra et al., 2022). Additionally, the choice of crosslinker type, molecular weight, and diacrylate/amine ratio of the synthesized hydrogels can also influence the biodegradation rate and swelling behaviour. Tamer (2023) incorporated poly (β-amino ester) (PBAE), a biodegradable crosslinker, for the enhancement of the biodegradability rate. The environmental impact cand be thereby reduced by optimizing these parameters.

Furthermore, the development of biodegradable hydrogels utilizing natural raw materials aims to enhance water infiltration, nutrient release, and seed germination in soil (Daour & Bennur, 2022). These hydrogels have been engineered to maintain stability in soil, with their degradation being primarily influenced by environmental factors rather than synthesis parameters, the presence of heavy metals in the surrounding environment can affect the ecotoxicity of hydrogels, as certain hydrogels exhibit a remarkable ability to adsorb heavy metals (Turioni et al., 2021).

Ultimately, a comprehensive understanding of these factors is crucial for the advancement and utilization of biodegradable and environmentally-friendly hydrogels within the field of agriculture (Hu et al., 2021).

These studies collectively underscore the capacity of diverse biodegradable hydrogels in distinct applications, while exhibiting minimal ecotoxicity.

Therefore, the aim of this study was to assess the ecotoxicity potential of the soil resulted from the biodegradation process of 8 different formulations of hydrogels based on sodium alginate, carboxymethyl cellulose and acrylic acid.

MATERIALS AND METHODS

Soil resulted from the biodegradation process of the tested samples

The soil resulted from the biodegradation process of the hydrogels with different composition (Table 1), provided by The National Institute for Laser, Plasma and Radiation Physics Măgurele, was tested for the ecotoxicity assessment. Hydrogels were obtained by using the electron beam radiation technique and are composed of sodium alginate (Alg.), carboxymethyl cellulose (CMC), acrylic acid (AAc.) and potassium persulfate as a catalyst (Figure 1).

Figure 1. Hydrogel samples obtained through electron beam radiation

The eight different hydrogel formulations that were subjected to the biodegradation process are presented in Tabel 1.

Tabel 1. Samples codification used in this study

Sample Code	Materials			
	Alg. (g)	CMC	AAc.	$K_2S_2O_8$
A	1.5	-	+	+
A1	1.5	-	+	-
B	2	-	+	+
B1	2	-	+	-
C	1.5	+	+	+
C1	1.5	+	+	-
D	2	+	+	+
D1	2	+	+	-
M	Control sample (soil without hydrogel)			

\+ presence of the component (same dosage)
\- absence of the component

The biodegradability of the hydrogels was assessed through soil burial method after the standard SR EN ISO 846 which was adapted for the polymeric materials. The hydrogels were buried in the soil under controlled conditions for an extended period of time (Figure 2) and after 100, 200 and 300 days the soil samples were tested for their possible phytotoxic effect.

Figure 2. The biodegradability system which includes two hydrogel samples

Biological material

The radish seeds (*Raphanus sativus*) used in this study were acquired from VDRS Buzău (Figure 3).

Figure 3. Radish seeds (*Raphanus sativus*)

Ecotoxicity assessment

After different periods of time of the biodegradation process of the hydrogels, the soil was tested in order to assess their ecotoxicity on seeds germination and development using the seed germination bioassay method described by Miteluț & Popa (2011).

For the extraction process, the soil samples were mixed with distilled water (1:2 ratio) and the water-soil mixture was shaken for 6h at 25°C, centrifuged at 8000 rpm for 20 min at 20°C, and then filtered. The resulted supernatant was diluted with distilled water to

yield 0, 25, 50, 75 and 100% extract concentration (Figure 4).

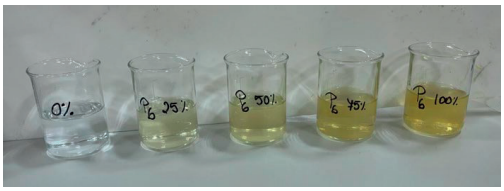

Figure 4. The soil extract at different concentrations

For the seed germination assay, filter paper was added in glass Petri dishes (10 cm diameter), which were further sterilized. In each Petri dish (5 Petri dishes/sample/concentration), 5 ml of extract was added and 10 radish seeds (*Raphanus sativus*) were evenly distributed (Figure 5).

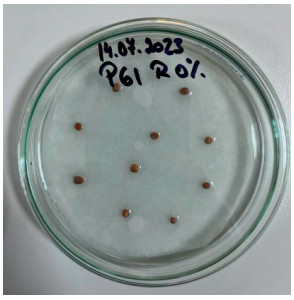

Figure 5. The radish seeds (*Raphanus sativus*) evenly distributed in Petri dish

After incubation for 72h in the dark at 25°C, germinated seeds were counted and the root length (cm) was measured (Figure 6).

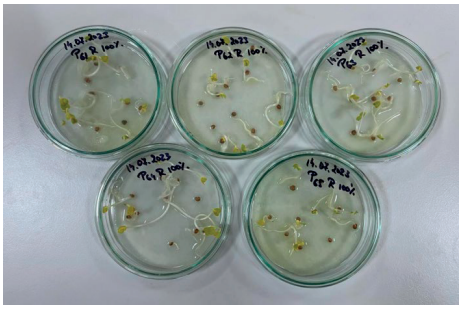

Figure 6. Radish seeds germination after 72 h incubation in the dark

The Germination index (Gi) of the samples for each concentration was calculated according to the formula:

$$Gi = \frac{G}{G_0} \times \frac{L}{L_0} \times 100$$

where G_0 and L_0 represent the germination percentage and rootlet growth of the 100% distilled water control (0% supernatant).

Based on the Gi, The Global Index of Germination (GI) was calculated for each sample, which represents the average of 50 and 75% dilution treatments. According to Tiqua (1996), the soil has no phytotoxic effects when the GI values are over 80%.

RESULTS AND DISCUSSIONS

The biodegradability test show after 300 days of soil burial that the samples containing the highest dosage of Alg. and CMC demonstrated the highest rate of biodegradability while the lowest rate was recorded for the samples with low concentration in Alg. (1.5 g) and no CMC.

The germination capacity of the radish seeds (Table 2), for the 100% supernatant concentration of the tested soils, after 100 days, proved to be over 80% for the control sample (M) as well as samples A, B1, C1 and D. The lowest percentage of germination was registered for the samples A1, B, C and D1.

After 200 days, for the 100% supernatant concentration of the tested soils, results proved to be 66% for the control sample. Higher percentage of germination capacity was recorded for the samples A, A1, B and B1 (biodegraded hydrogels with no CMC) while the samples C, C1, D and D1 (biodegraded hydrogels with CMC) registered lower values than the control sample.

The germination capacity of the control sample after 300 days of controlled biodegradation, for the 100% supernatant concentration of the tested soils, proved to be 78% similar to sample C1. Higher values were recorded for the samples C and D1, while the samples A, A1, B, B1 and D demonstrated lower values compared to the control sample.

The germination process has therefore been positively influenced by some samples while most of them demonstrated similar behaviour with the control.

Table 2. Germination capacity of radish seeds (%) (mean values of 50 seeds with standard deviation)

Incubation period	Supernatant concentration of the soil samples	Samples								
		M	A	A1	B	B1	C	C1	D	D1
100 DAYS	25%	92 ± 8.37	88 ± 8.37	80 ± 7.07	78 ± 13.04	82 ± 4.47	90 ± 7.07	82 ± 8.37	80 ± 12.25	78 ± 16.43
	50%	76 ± 19.49	82 ± 19.24	86 ± 11.40	76 ± 8.94	84 ± 8.94	88 ± 13.04	80 ± 14.14	82 ± 13.04	84 ± 11.40
	75%	94 ± 5.48	76 ± 18.17	84 ± 11.40	74 ± 11.40	84 ± 5.48	90 ± 12.25	82 ± 8.37	72 ± 16.43	78 ± 8.37
	100%	**82 ± 8.37**	**80 ± 12.25**	**76 ± 11.40**	**76 ± 15.17**	**88 ± 8.37**	**74 ± 15.17**	**84 ± 15.17**	**92 ± 8.37**	**78 ± 8.37**
200 DAYS	25%	68 ± 13.04	72 ± 14.83	70 ± 15.81	90 ± 10.00	90 ± 10.00	64 ± 16.73	66 ± 23.02	60 ± 18.71	46 ± 20.74
	50%	62 ± 16.43	74 ± 15.17	68 ± 19.24	92 ± 8.37	90 ± 12.25	52 ± 14.83	76 ± 8.94	54 ± 11.40	70 ± 10.00
	75%	58 ± 8.37	76 ± 11.40	72 ± 24.90	86 ± 11.40	84 ± 5.48	50 ± 17.32	56 ± 8.94	70 ± 15.81	52 ± 17.89
	100%	**66 ± 16.73**	**70 ± 14.14**	**80 ± 15.81**	**76 ± 13.42**	**78 ± 17.89**	**50 ± 15.81**	**52 ± 13.04**	**58 ± 22.80**	**58 ± 8.37**
300 DAYS	25%	80 ± 7.07	64 ± 15.17	62 ± 10.95	68 ± 14.83	66 ± 27.93	74 ± 11.40	66 ± 19.49	88 ± 8.37	70 ± 7.07
	50%	92 ± 8.37	52 ± 21.68	56 ± 13.42	56 ± 19.49	60 ± 15.81	76 ± 21.91	72 ± 16.43	84 ± 11.40	84 ± 8.94
	75%	84 ± 8.94	66 ± 8.94	54 ± 11.40	64 ± 15.17	60 ± 12.25	72 ± 10.95	72 ± 14.83	78 ± 14.83	72 ± 13.04
	100%	**78 ± 10.95**	**62 ± 16.43**	**62 ± 10.95**	**58 ± 16.43**	**60 ± 14.14**	**80 ± 15.81**	**78 ± 8.37**	**72 ± 8.37**	**80 ± 15.81**

The rootlet length of the germinated radish seeds was measured, and is presented in Table 3. It was observed that after 100 days, for 100% supernatant concentration, the lowest values were obtained for the samples A, A1 and C1 compared to the control sample (2 cm). The samples B, B1, C, D and D1 registered close or higher values than the control.

After 200 days, the rootlet length of the germinated seeds for the control sample for 100% supernatant concentration, was registered at 1.67 cm. Samples resulted from hydrogels containing Alg. and no CMC (A, A1, B and B1) demonstrated similar or higher values while samples C, C1, D and D1 (hydrogels containing Alg. and CMC) measured lower values compared to the control.

After 300 days, for the 100% supernatant concentration, the rootlet length of the control sample was registered at 2 cm. Samples resulted from hydrogels containing Alg. and CMC demonstrated similar or higher values than the control (C, C1, D and D1), while the lowest values were registered for the samples resulted from hydrogels containing Alg. and no CMC (A, A1, B and B1).

Table 3. Rootlet length of radish seeds (cm) (mean values of 50 seeds with standard deviation)

Incubation period	Supernatant concentration of the soil sample	Samples								
		M	A	A1	B	B1	C	C1	D	D1
100 DAYS	25%	2.83 ± 0.35	1.47 ± 0.30	1.67 ± 0.31	1.64 ± 0.56	1.52 ± 0.35	3.48 ± 0.52	1.82 ± 0.38	1.80 ± 0.74	1.99 ± 0.47
	50%	1.85 ± 0.77	1.52 ± 0.70	2.59 ± 1.07	1.92 ± 0.37	2.26 ± 0.98	3.29 ± 0.97	1.74 ± 0.43	1.99 ± 0.63	2.03 ± 0.56
	75%	3.34 ± 0.47	1.29 ± 0.37	2.02 ± 0.76	2.04 ± 0.42	1.75 ± 0.34	2.91 ± 0.76	1.88 ± 0.29	1.62 ± 0.35	2.14 ± 0.63
	100%	**2.26 ± 0.63**	**1.98 ± 0.38**	**1.60 ± 0.54**	**2.07 ± 0.41**	**2.61 ± 0.56**	**2.02 ± 0.93**	**1.78 ± 0.55**	**2.22 ± 0.43**	**2.18 ± 0.12**
200 DAYS	25%	1.57 ± 0.51	1.18 ± 0.66	1.29 ± 1.23	3.03 ± 0.83	2.47 ± 0.54	1.15 ± 0.54	1.68 ± 0.73	1.60 ± 0.52	0.75 ± 0.67
	50%	1.85 ± 0.89	1.25 ± 0.35	1.21 ± 0.83	2.72 ± 0.54	3.06 ± 1.00	0.99 ± 0.50	1.97 ± 0.32	1.17 ± 0.50	1.58 ± 0.41
	75%	1.53 ± 0.66	1.54 ± 0.52	1.69 ± 1.02	3.16 ± 0.96	2.56 ± 0.63	1.42 ± 0.73	0.99 ± 0.48	1.66 ± 0.63	1.78 ± 0.93
	100%	**1.67 ± 0.91**	**1.67 ± 1.10**	**2.77 ± 0.90**	**2.10 ± 0.85**	**2.42 ± 0.90**	**1.01 ± 0.36**	**0.78 ± 0.40**	**1.03 ± 0.56**	**1.18 ± 0.60**
300 DAYS	25%	2.37 ± 0.93	1.39 ± 0.48	1.74 ± 0.54	1.53 ± 0.60	1.49 ± 0.82	2.37 ± 0.61	1.62 ± 0.46	2.83 ± 0.53	1.81 ± 0.66
	50%	2.86 ± 0.59	1.19 ± 0.53	1.64 ± 0.70	1.18 ± 0.52	1.72 ± 0.65	2.37 ± 0.95	1.99 ± 0.55	2.26 ± 0.37	2.46 ± 0.04
	75%	2.66 ± 0.58	1.67 ± 0.59	1.38 ± 0.34	1.42 ± 0.55	1.64 ± 0.37	2.04 ± 0.55	2.11 ± 0.65	2.10 ± 0.58	1.89 ± 0.45
	100%	**1.97 ± 0.49**	**1.56 ± 0.37**	**1.17 ± 0.19**	**1.19 ± 0.43**	**1.23 ± 0.31**	**2.71 ± 0.57**	**1.75 ± 0.55**	**1.98 ± 0.39**	**2.36 ± 0.46**

According to the GI values (Figure 7), the tested soil that resulted after the biodegradation process of the hydrogels after 100 days presented values over 80% for all the samples, most of them recording values over 105% demonstrating a non-toxic effect over the radish seeds.
After 200 days, most of the samples, including the control, had values under 80%. These results could be explained by the possibility of the carbon mineralization decreasing with increasing water salinity during the controlled incubation conditions (Mancer & Bouhoun, 2018), which could impact soil health from the ecotoxicity point of view. Another possible explanation for the results obtained could be the reduced availability of CO_2 in soil resulting in limiting the nitrification process (Azam et al., 2005).
After 300 days of biodegradation, the GI of the samples varied depending of the composition of the hydrogels. The GI presented increased values for the control sample as well as for the samples containing CMC (C, C1, D, D1) as opposed to the no CMC hydrogels (A, A1, B, B1) which are very much below to the control and below the GI limit of toxicity (80%).

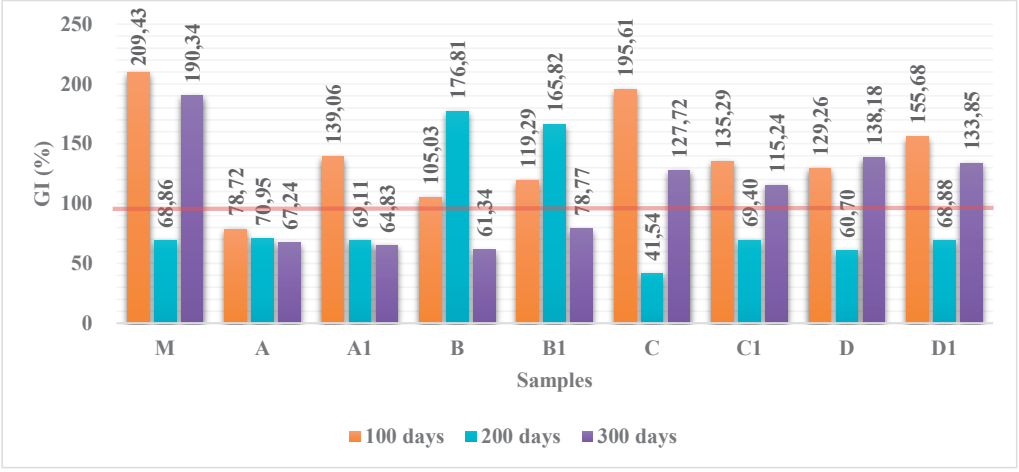

Figure 7. Global Index of Germination GI (%) of the radish seeds

CONCLUSIONS

The ecotoxicity effect of the soils resulted after 300 days of biodegradation of eight different formulations of hydrogels were studied using the bioassay germination method.
For the soil resulted after 100 days of hydrogels soil burial, no toxic effect related to the biodegradation of the materials was registered on radish seeds.
The results obtained for the samples collected after 200 days of hydrogels soil burial process, were not conclusive because all the samples tested, including the control (without hydrogels), registered values under the GI limit of ecotoxicity. Further analysis of soil samples are required to better understand this occurrence.
In the case of the samples collected after 300 days of hydrogels soil burial process, the samples resulted from hydrogels containing Alg. and CMC (C, C1, D, D1), demonstrated higher values than the GI limit of ecotoxicity.

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