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Study of the Effect of UV Exposure and Soil Type on the Biodegradation of Gelatin-Based Films

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Abstract. This study investigates the degradation behavior of gelatin-based films plasticized with varying concentrations of glycerol (12.5–75.0%) when buried in five distinct soil types - coniferous forest, deciduous forest, cinnamon, black "smolnik", and light sandy soil. Film samples were incubated under both ambient and UV-irradiated conditions for a period of 12 weeks to simulate natural environmental exposure. Soil pH and bulk density were measured and found to influence biodegradation rates. Visual inspection revealed progressive disintegration of the films, with faster degradation in organic-rich soils and under UV exposure. The results demonstrate that both the formulation of gelatin films and the surrounding environmental conditions - particularly soil type and UV irradiation - play crucial roles in determining the rate of degradation.

1 Introduction

The growing global concern over plastic pollution and sustainability has increased the demand for biodegradable alternatives, particularly in the food packaging industry [1]. Traditional polymer packaging poses serious environmental risks due to its persistence and the accumulation of microplastics [2]. As a result, significant effort is being directed toward developing biodegradable materials from renewable sources [3].

Currently, the production of biodegradable films attracts significant attention from both researchers and food manufacturers. Their application in food packaging can partially or completely replace traditional packaging made of synthetic polymers. Among natural biopolymers, gelatin offers several advantages, including excellent film-forming and barrier properties [4], biocompatibility, and non-toxicity. Unlike conventional polymers, gelatin-based films decompose naturally in the environment [5]. They are also cost-effective and provide protection against moisture loss, contributing to improved food safety and extended shelf life [6].

However, the limited mobility of macrochains in the gelatin structure results in inherent brittleness and rigidity. To improve the flexibility and functional properties (mechanical,

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barrier, etc.) of the films, plasticizers are commonly incorporated. Glycerol and sorbitol, due to their stability and edibility, are among the most widely used plasticizers in bioplastic production [3, 7], particularly in gelatin-based films. These additives affect not only the films` physical properties but also their biodegradation behavior [8].

While the mechanical and physicochemical properties of gelatin films have been well studied, fewer investigations have focused on their environmental stability and biodegradation in natural soil environments [9]. Additionally, the influence of specific soil properties, such as texture, pH, organic matter content, and microbial activity, affects the degradation rate and mechanism of gelatin-based materials. These parameters vary widely among different soils and play a key role in the biodegradation process. Furthermore, exposure to environmental stressors such as UV radiation or chemicals can induce significant changes in the structural integrity and mechanical properties of biopolymer films [10].

Understanding the biodegradation of gelatin-based films under realistic environmental conditions is essential for assessing their ecological impact and optimizing formulations for targeted applications. Soil burial tests are widely used to simulate natural degradation, as they reflect the complex interplay of factors such as moisture, temperature, pH, and microbial activity [11]. In parallel, UV exposure mimics outdoor weathering and provides insight into photodegradation pathways.

Gelatin-based films are generally considered highly biodegradable, with complete decomposition reported within 12 to 15 days under favorable soil burial conditions [12-14]. However, degradation rates can vary considerably depending on environmental conditions. The combination of UV exposure and soil bioactivity creates prerequisites for more intensive degradation of biodegradable films, which is of key importance in assessing their environmental safety and applicability under open conditions [15].

The presence of plasticizers further complicates this behavior, as they not only affect the mechanical properties of the films but also alter their affinity for water and their susceptibility to microbial attack. Glycerol, for example, is known to increase water absorption and matrix flexibility, potentially accelerating biodegradation, although excessive amounts may lead to premature disintegration. On the other hand, environmental factors such as UV radiation may enhance degradation through photochemical chain scission, but can also lead to surface crosslinking, which may hinder microbial decomposition.

Despite previous research on the mechanical and barrier properties of gelatin-based films, systematic data on the combined effects of plasticizer concentration, soil physicochemical properties, and UV exposure on their biodegradation behavior remain scarce. Most existing studies have focused on single-factor analyses or artificial composting conditions, which do not accurately represent natural environmental variability. Therefore, the novelty of this study lies in its integrated approach, examining how the interplay among soil type, plasticizer concentration, and UV irradiation influences the degradation rate and mechanism of gelatin-based biopolymers. Understanding these relationships is essential for designing biodegradable materials with predictable and controllable performance in real-world applications, such as biodegradable food packaging and agricultural mulch films.

The aim of this study is to evaluate the degradation behavior of gelatin-based films containing varying concentrations of glycerol when buried in five soil types with distinct physicochemical characteristics: coniferous forest soil, deciduous forest soil, cinnamon soil, resinous (black) soil, and light sandy soil, typical of Southeastern Bulgaria. The films were incubated under both ambient and UV-irradiated conditions for a period of 12 weeks to simulate environmental weathering. Visual assessments were conducted periodically to analyze the combined effects of soil composition, plasticizer content, and exposure conditions on the biodegradation performance of the films. The results of this work aim to contribute to the growing body of knowledge about biodegradable materials and their environmental behavior, supporting the development of application-specific formulations with optimized degradation profiles for sustainable alternatives to synthetic plastics.

2 Materials and methods

2.1 Soil samples

The soil samples are taken from two areas within the district of Burgas. The main principle of choosing the soil spots was soils from agricultural spots (treated with fertilizers and processed with agricultural machines crop-producing spots) and soils from forest spots, with no treatment. Five types of soil with distinct structural characteristics were selected, as shown in Fig. 1.

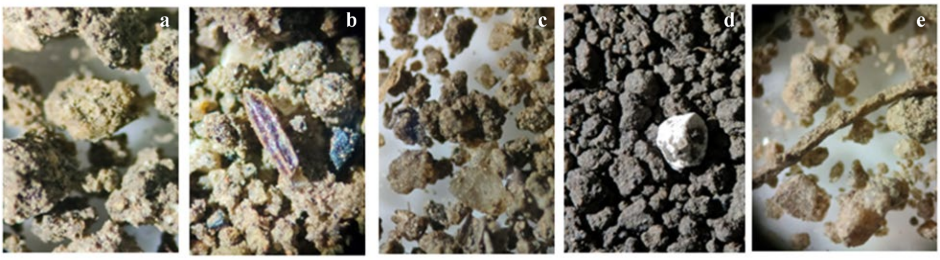

Fig. 1. Optical microscopy for soil samples' surface morphology at 100× magnification, allowing for detailed visualization of soil's features such as granular structures, fiber arrangements, and surface roughness. (a) Soil 1 - coniferous forest soil; (b) Soil 2 - deciduous forest soil; (c) Soil 3 - cinnamon soil; (d) Soil 4 - black soil (smolnik), (e) Soil 5 - light sandy soil.

Soil 1 coniferous forest and Soil 2 deciduous forest belong to the brown mountain-forest soils. They are the main soil type in the mountainous regions of Bulgaria, formed from weathering materials. They are found under coniferous, mixed, and beech forests. They have a light mechanical composition, a thin humus layer, a weak accumulation of clay in the transitional horizons, and the presence of a volcanic layer in the lower part. They can be light and dark.

Soil 3 cinnamon - arable. This type of soil is found in dry to low forests, shrubs, and pastures, with good drainage, formed on carbonate rocks. They are rich in iron compounds, which gives the soil a cinnamon and reddish color.

Soil 4 black "smolnik" arable. Black soils are dark to black colored dense clay soils, which crack strongly during drought. Their name comes from their strong stickiness, like resin, when wet. They have a high clay content and a thick humus layer (60-80 cm), which is crumbly in the upper part and dense below. They are characterized by a high content of carbonates and gypsum.

Soil 5 light sandy. This type of soil is poorly distributed in Bulgaria. Their sandy composition determines their qualities and properties. At a depth of up to 1 m, they contain over 65% coarse sand, have a loose structure, do not form soil horizons, and do not become over-moistened, which is why they lack stratification.

The samples were taken manually with a shovel, for each point, single samples were taken from a depth of (0-10) cm. from an area, in the shape of a circle with a radius of 1 m, on a grid principle (through about 0.10 m), which were then homogenized and averaged. Forest soils, due to the coniferous (soil sample 1) and deciduous (soil sample 2) leaf mass they contain, were cleaned of larger particles (leaves, pebbles, tree bark, etc.) manually. For better distribution over the samples, the soils were roughly crushed manually.

2.2 Gelatin film preparation

Animal gelatin powder (type A) was purchased from the local market in Burgas, Bulgaria. Glycerol $C_3H_8O_3$ (molecular weight 92.10 g/mol, density 1.26 g/cm^3) was used as a plasticizer for obtaining the films, purchased from Marvin Ltd, Dimitrovgrad, without further purification. Film-forming solutions were made using distilled water as a solvent. The gelatin-based films were obtained by the traditional solution casting technique: 40 ml of distilled water was mixed with 4 g of gelatin for each film. The resulting solutions were heated at 80°C for 15 min. with stirring. Glycerol plasticizer is added at concentrations of 12.5, 25.0, 37.5, 50.0, and 75.0% (w/w, on a gelatin basis), and the resulting mixtures are stirred for an additional 5 min. at the same temperature. Then the film-making solutions were placed into acrylic mould dishes, kept at room temperature for 24 hours, and dried in an oven at 40°C for a day to dry up properly. After drying, mould dishes were kept at room temperature for another 24 hours. Before being characterized, the dried films were removed from the dishes and kept at room temperature for a week. Specimens prepared were designated as G-12.5 Gly, G-25.0 Gly, G-37.5 Gly, G-50.0 Gly, and G-75.0 Gly, respectively, according to the content of glycerol in the biofilms, listed in Table 1.

Table 1. Composition of gelatin films with different content of glycerol plasticizer.

Films	Gelatin, g	Glycerol, g
G-12.5 Gly	4	0.5
G-25.0 Gly	4	1.0
G-37.5 Gly	4	1.5
G-50.0 Gly	4	2.0
G-75.0 Gly	4	3.0

2.3 Biodegradation tests` experiment

To study the effect of soil type and UV exposure, and to compare it with ambient conditions on the destruction of biodegradable films, was set the experiment. Soil samples from the five soil types were distributed in shallow containers with an area of approximately 20 cm^2. Biopolymer film samples, with a thickness of 250 to 500 µm, were buried at a depth of 1 cm in soil-filled containers and divided into two groups for a period of three months. The first group of soil samples and biopolymer films was placed under ambient conditions (average temperature of 22 - 25°C, relative humidity 42.9%). The second group was irradiated by UV light with wavelengths between 185 and 254 nm, emitted by five 8 W lamps, at room temperature for the same three-month period.

Changes in the appearance of gelatin-based biopolymer films were recorded to assess the degradation process over time. Biopolymer film samples were removed from the soil after 2, 4, and 12 weeks and visually assessed through digital imaging. The results are presented in Figs. 2 - 6. Each experimental condition was conducted in triplicate to ensure reproducibility. In addition, control samples (unburied gelatin films stored under laboratory ambient conditions without soil contact) were used to distinguish the effects of soil and UV exposure.

2.4 Tensile properties

The tensile properties of gelatin-based films prepared were determined at room temperature according to EN ISO 50527-1 using a universal testing machine dynamometer Instron 4203 (England). Samples with dimensions of length 50 mm, width 4 mm, and gauge length 25 mm were cut from the gelatin films. The grip distance was set at 25 mm, and a crosshead speed of 50 mm/min was applied. The samples' thickness was measured before each test.

3 Results and discussion

3.1 Gelatin films properties

Gelatin films prepared with varying concentrations of glycerol (ranging from 0% to 75%) exhibit distinct changes in appearance and transparency. As the glycerol content increases, the films become more elastic and flexible (Table 2), but their optical clarity decreases. Films with low glycerol content (12.5%) are transparent but brittle, whereas those with higher plasticizer concentrations (50.0–75.0%) display increased haze and softness, indicative of over-plasticization. This trend aligns with the well-established role of glycerol as a hydrophilic plasticizer, which reduces internal tension in the polymer matrix but increases susceptibility to environmental factors [12].

The percentage increase or decrease in the tensile strength, elongation at break, and Young`s modulus of gelatin-based biofilms, depending on the glycerol plasticizer content added, is placed in Table 2.

Table 2. Percentage change in tensile properties of gelatin-based biofilms with the addition of glycerol plasticizer.

Concentration of glycerol, %	Tensile strength, %	Elongation at break, %	Young’s modulus, %
12.5	–67.94	23.86	–71.24
25.0	–71.39	2864.02	–92.61
37.5	–86.25	4619.70	–98.46
50.0	–87.47	10793.90	–99.83
75.0	–90.54	14983.30	–99.95

It can be concluded that the use of plasticizer has a beneficial effect on the elongation at break, especially for the films based on gelatin with a glycerol content above 25.0%, compared to the same samples without glycerol. At the same time, a percentage decrease in tensile strength by up to about 90% and Young`s modulus by up to almost 100% is observed for all films with an increase in plasticizer to 75.0%.

3.2 Soil samples’ pH

The pH was measured in aqueous extract of soil samples at a 1:5 soil-to-water ratio using the REVio Multiparameter portable Kit with a DHS electrode. As shown in Table 3, four of the five soils exhibited pH values between 7.21 and 7.37, indicating near-neutral conditions. Only the light sandy soil had a lower pH of 6.25, suggesting a slightly acidic nature.

3.3 Soil bulk density

The bulk density of each soil was determined by freely and uniformly filling a 25 cm^3 cylinder and subsequently weighing. The mass-to-volume ratio of the sample (g/cm^3) provides insights into soil porosity and compaction - critical parameters that influence microbial access, oxygen diffusion, and ultimately, the biodegradation rate of the embedded gelatin-based materials.

Table 3. Parameters of the studied soil types.

Parameter	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5
pH	7.21	7.37	7.31	7.24	6.25
Bulk density, g/cm^3	1.25	1.29	1.45	1.17	1.67

Table 3 shows the determined bulk density values for the five soil types. As can be seen, the bulk density of coniferous forest soil is relatively low due to the high humus content, with a value of 1.25 g/cm^3. The bulk density of soil 2 – deciduous forest soil is 1.29 g/cm^3. Soil 3 – cinnamon-brown soil has a balanced ratio of sand, silt, and clay, which allows for stable aeration and water retention. Its bulk density value is 1.45 g/cm^3. “Smolnik” - soil 4, due to its high organic matter content and fine texture, has a bulk density of 1.17 g/cm^3. The bulk density of soil 5 is higher (1.67 g/cm^3), with large particle sizes and low clay or organic matter content. This soil is highly permeable, but has low nutrient and water retention. This differentiation is confirmed in Fig. 1, where microscopic images show a coarser and looser structure in soil 5 and a compact, well-aggregated structure in soil 4. This topology directly influences moisture retention and the development of soil microbiota, which in turn determines the rate of biofilm degradation.

3.4 Degradation rate of gelatin-based films after burial in soils under ambient and UV irradiation conditions

When the biopolymer is buried in the five types of soil, the gelatin films are biologically degraded, since the soil contains numerous microorganisms (bacteria, fungi) that can degrade gelatin to amino acids. The degradation time depends on: the type of soil (clay, sandy, humus, etc.), temperature and humidity, the presence of oxygen, and pH of the soil. In this case, chemical reactions may occur between the organic acids and salts contained in the soil and the components of the film, in which hydrolysis or even a change in the structure of gelatin can be observed. On the other hand, irradiation with UV light can lead to photodegradation of gelatin films, due to the rupture of peptide bonds in its structure. The process is accelerated in the presence of moisture or oxygen.

Five different soil types were selected to evaluate the influence of soil composition on the degradation behavior of gelatin-based films. Figs 2 to 6 illustrate the visual changes in gelatin-based films containing various concentrations of plasticizer (12.5% - 75.0%) after burial in each soil type. Comparisons were made between samples exposed to ambient conditions (left) and those subjected to UV irradiation (right), evaluated at 0, 2, 4, and 12 weeks. The biodegradation experiments reveal regular differences between the individual soil environments and exposure conditions.

The gelatin-based films showed noticeable degradation in coniferous forest soil over the 12-week testing period – Fig. 2. Samples exposed to UV light showed faster and more pronounced structural degradation compared to those stored under ambient conditions. Films with lower plasticizer content (e.g., 12.5%) and under UV radiation were more prone to early fragmentation and loss of structural integrity. These observations indicate that both plasticizer concentration and environmental factors significantly influence the rate of biodegradation in forest soil conditions.

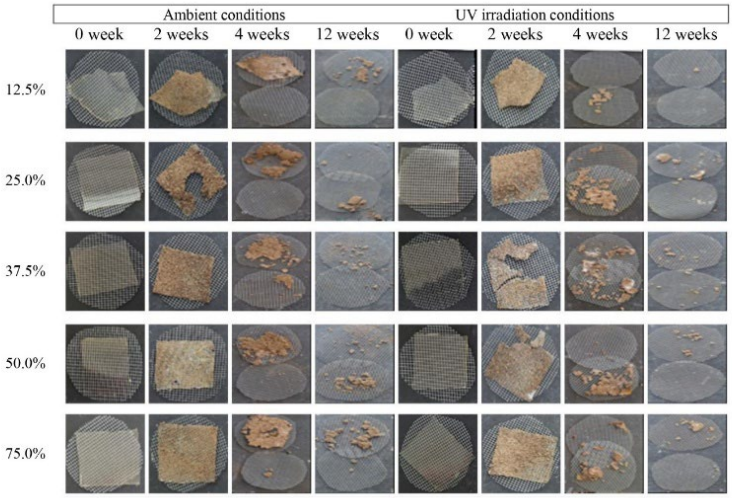

Fig. 2. Visual changes of gelatin films with different plasticizer concentrations after burial in coniferous forest soil (soil 1) under ambient (left) and UV irradiation (right) conditions.

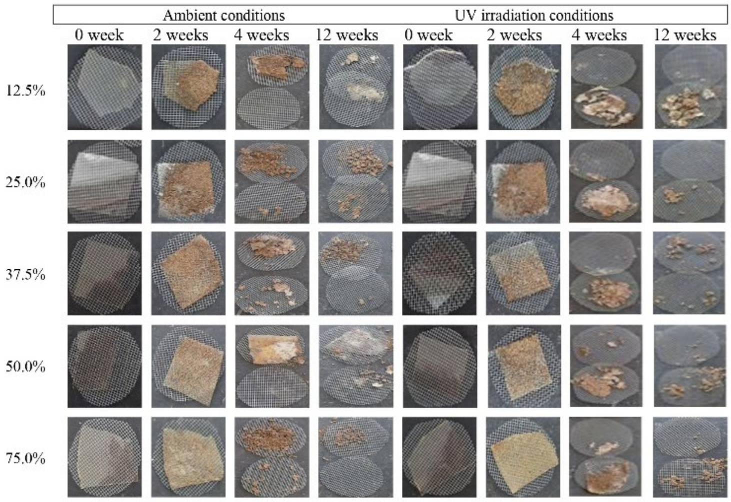

Fig. 3. Visual changes of gelatin films with different plasticizer concentrations after burial in deciduous forest soil (soil 2) under ambient (left) and UV irradiation (right) conditions.

In deciduous forest soil (soil 2) – Fig. 3, degradation proceeds moderately. Under atmospheric conditions, degradation is observed, with a significant increase in the degree of destruction at glycerol content ≥ 37.5%. The reason for this is the good retention capacity of these soils, combined with moderately developed microbiological activity, which determines slow but progressive hydrolytic and microbial degradation. However, under UV irradiation conditions, even samples with lower glycerol content begin to show signs of photodegradation after the second week, due to the presence of moisture and a closed environment that promotes photochemical processes.

Visual changes in the films over time using cinnamon soil (soil 3) are shown in Fig. 4. In cinnamon soil, gelatin films showed an increased rate of biodegradation compared to forest soils. The presence of iron-rich minerals, pH neutral, medium organic matter content, and good drainage, favors the activity of soil microflora and leads to accelerated degradation of samples with ≥ 25% glycerol by week 4. UV irradiation accelerates this process, leading to further destruction of the film surface, especially at plasticizer contents above 50%, where the softened and hydrophilic structure of the material facilitates the penetration of UV light and oxygen.

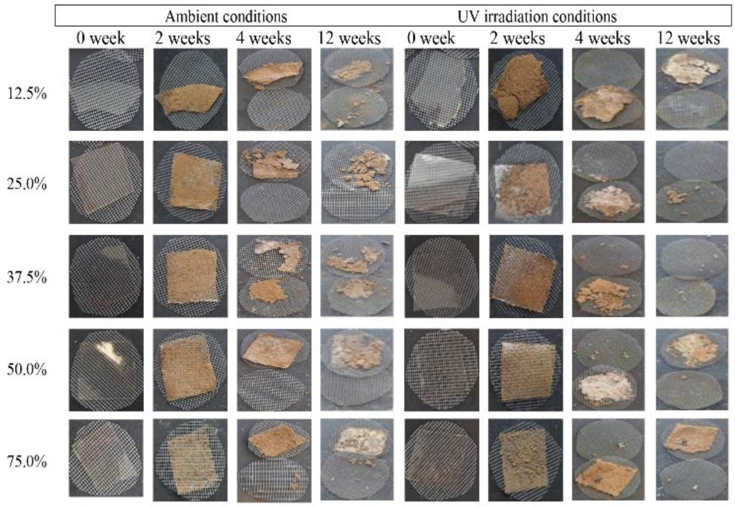

Fig. 4. Visual changes of gelatin films with different plasticizer concentrations after burial in cinnamon soil (soil 3) under ambient (left) and UV irradiation (right) conditions.

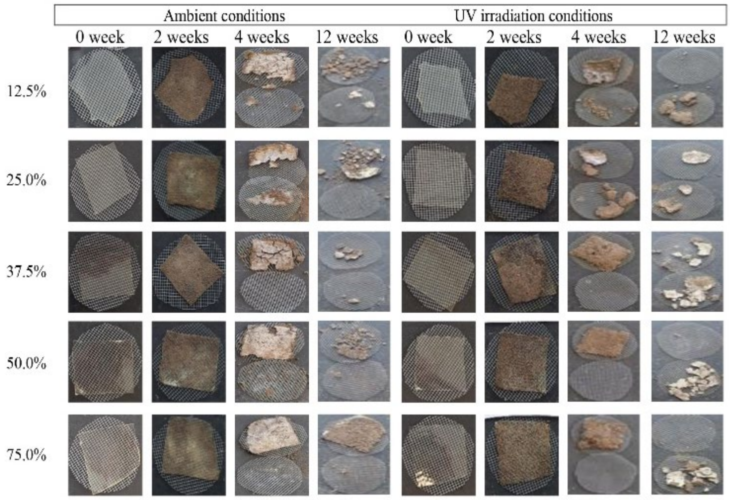

Fig. 5. Visual changes of gelatin films with different plasticizer concentrations after burial in black soil “smolnik” (soil 4) under ambient (left) and UV irradiation (right) conditions.

Fig. 5 shows that soil 4 - “smolnik” creates the most favorable conditions for the biological degradation of gelatin films. This is due to the high content of organic matter, its moisture-retaining ability, and the presence of active microflora (visible from the microstructure in Fig. 1d). Already at week 2, clear signs of degradation are observed even in samples with the lowest glycerol content (12.5%), and the process is almost complete by week 12. Under UV irradiation conditions, the process is further accelerated, and degradation of films with ≥ 37.5% glycerol occurs as early as week 4, due to the increased photosensitivity of glycerol-plasticized matrices. By week 12, almost all samples are destroyed.

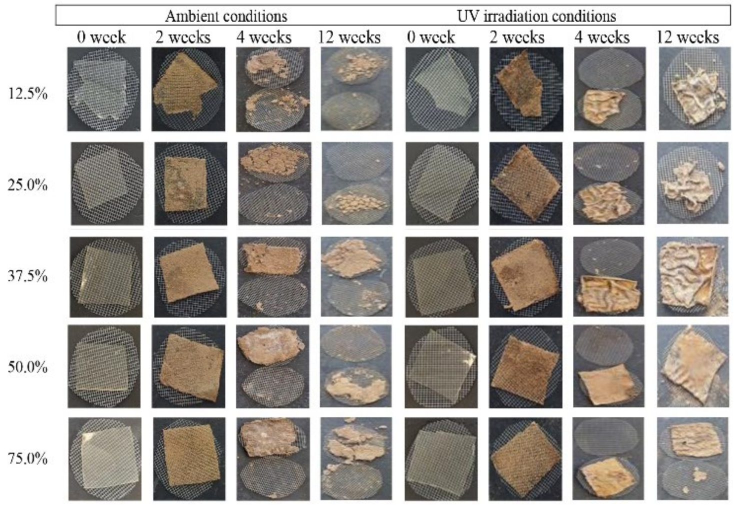

Fig. 6. Visual changes of gelatin films with different plasticizer concentrations after burial in light sandy soil (soil 5) under ambient (left) and UV irradiation (right) conditions.

In soil 5 - light sandy soil (Fig. 6), due to the loose structure, low organic matter content (Fig. 1e), poor moisture retention capacity, low acidity of the medium, and limited microbiological activity, the biodegradation of gelatin films proceeds the slowest. Even after 12 weeks, some of the samples (especially those with low glycerol content) retained a significant portion of their integrity. The UV aging process was also limited by dry conditions, which slowed down hydrolytic degradation and limited UV penetration into the microporous structure of the soil. As a result, a much slower degradation of the gelatin films, almost dry “mummification”, was observed.

The observed differences in degradation among the soil types can be attributed to their distinct microbial activity, moisture retention, and mineral composition. In particular, the enhanced biodegradation in “smolnik” soil is consistent with previous studies reporting accelerated gelatin hydrolysis in humus-rich environments [9, 14]. Conversely, the slower degradation in sandy soils corresponds to their low microbial density and poor moisture-holding capacity [11]. Moreover, the acceleration under UV irradiation suggests that photo-induced peptide bond cleavage facilitates subsequent microbial assimilation. Similar synergistic effects between photodegradation and biodegradation have been observed in other protein-based films [13]. These findings highlight that the degradation process is governed by a combination of physicochemical (UV-induced oxidation, hydrolysis) and biological (enzymatic activity, microbial colonization) mechanisms, the relative contributions of which depend on the surrounding soil conditions and film formulation.

4 Conclusions

Based on the results obtained in the present study, it was found that increasing the glycerol content significantly accelerates the degradation of biofilms, regardless of the soil type and exposure conditions. Black soil "smolnik" - soil 4 creates the most intense conditions for degradation both under atmospheric conditions and under UV irradiation, and gelatin films demonstrate excellent biodegradation potential in this soil environment. Soil 5, light sandy soil due to poor moisture retention and low biological activity, slows down the degradation process, almost dry "mummification", which leads to partial preservation of the film structure even after 12 weeks. UV irradiation increases the degradation rate in all soils, the effect being more pronounced in samples with a high glycerol content and in soils with good moisture and microbiological activity. The observed differences highlight the synergistic effects of soil microbial activity and UV-induced photodegradation, which vary depending on soil composition, pH, bulk density, and plasticizer content in the films. These findings confirm the complex interaction between film composition and environmental conditions in determining the degradation ability of gelatin-based biodegradable materials. This is essential for the development of environmentally friendly alternatives to synthetic plastics in the packaging and related industries.

Future research should focus on quantitative measurements of degradation (mass loss, carbon dioxide evolution, microbial counts) to better correlate physicochemical and biological processes. In addition, exploring the incorporation of natural additives or crosslinking agents could enhance the mechanical stability–biodegradability balance of gelatin-based films. The present results indicate strong potential for their application in biodegradable packaging and controlled-release agricultural films, where soil and UV conditions vary widely. A broader understanding of these degradation dynamics will support the design of next-generation biopolymer systems aligned with circular economy principles.

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