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BIOTRANSFORMATION OF EXPANDED PERLITE IN ORGANIC-LIKE SUBSTRATE BY CHEMOTROPHIC CONSORTIA

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Abstract

Expanded perlite, resulted by in heating process of a naturally occurring perlite ore, make it an versatile and law-impact material for greenhouse, soil amendment, hydroponics, and which offers advantages such as aeration, drainage, water retention, resistance, reuse. By bioaugmentation with chemotrophic microorganisms that decompose natural / manufactured rocks, expanded perlite can be biotransformed into an organic-like substrate. In the present study, the microorganisms responsible for the biotransformation of expanded perlite into organic-like substrates were analized for compatibility, interspecific synergy (bacteria, fungi, diazotrophs), the organic content by chromatography, humic-like fractions, siderophores, enzyme complex, seed germination and the growing of plantlets. The results reflected the effect of living organisms synergism in the ascendent evolution of organic compounds accumulation. The secondary exomethabolites are involved in humic-like acids fractions formation and biotransformation of the rock, increasing polyphenoloxidase activity, in conversion towards organic-like substrate and in association with colloids. Also, increse in time the siderophores and IAA content in substrate, intensity of physiological and biochemical processess, the seed germination and the plantlets's biomass.

Key words*: expanded perlite, bioaugmentation, biotransformation, organic-like substrate, consortia.*

INTRODUCTION

Bioweathering of natural rocks and biological processes in the rhizosphere improve dissolution due to the intensity of the interactions between bacteria, fungi, diazotrophs, plants and minerals, resulting macro and micronutrients, which are released and accumulate in the substrate solution. Cultivated plants can be important in this dissolution process, acting directly by rooting excretions on the dissolution of minerals in the rock component of the substrate or by supporting in the rhizosphere a biodiversity of microorganisms active in the weathering. These microorganisms act by producing siderophores and organic acids (complexing ligands), exoenzymes, inorganic and organic acids that influence the pH and producing redox reactions, in order to prioritize the process of dissolving minerals in the inorganic substrate. The relatively diverse rhizosphere microflora can also directly promote the weathering activity performed by plants, through a synergistic contribution ensured by their involvement. By using inoculation with bioweathering-specific microbiome, in combination with an inorganic material of natural expanded rocks, used as a substrate, it could favorably influence the fertility, by accumulations of organic and mineral material. The following biological transformations, could improve the quality of the substrate and rhizosphere. Also, the biotransformations can promote the evolution of the initial anorganic substrate chemically fertilized towards generation of an organic-like substrate and cause increasing of the plants.

Expanded perlite has a number of advantages when used in cultures, such as non-toxicity, inert and sterile material, availability of air and moisture, different sizes, availability in different quantities, adaptability to many cultures and culture media, excellent support for immobilization of biocatalysts. Generally, the natural rocks used include a single nutrient

with high solubility or slow nutrient release and with multiple nutrients such as expanded perlite, a silicatic rock (Fayiga & Nwoke, 2016). Because not all nutrients are readily available to the plants, the microorganisms in the consortium intervene to increase solubilization and the effectiveness, both depend on the chemical and mineralogical composition of the rocks, characteristics of the substrate, plants requirements and management.

The chemical composition and physical structure of rock from which the substrate is formed, influences the composition of the initial microbial consortium, where C or N-based compounds are missing (Schulz et al., 2013). The ecto- and endolytic microorganisms attach to the rock and promote a greater dissolution of the elements from the mineral particles (Ahmed & Holmström, 2015). The establishment and activity of the consortium on natural rocks require the formation of microbial biofilm. Humic precursors, organic components derived from the secondary metabolism of the inoculated microbiota, increase water availability and improve the decomposition of rocks. The activity of extracellular enzymes associated with mineral or organic colloids emphasizes the biological ability to convert to an organic substrate and to associate with mineral/organic colloids.

Plants grown on anorganic substrate promote the degradation by improving its basic properties, influencing the water dynamics and the cation cycle, in the fertilization solution. Through roots exudates, plants promote the solubilization of minerals, increase the content of organic acids and chemical ligands. In the rhizosphere, microbiome promotes secretion of siderophores and phytohormones which increase absorption/availability of macro-, micronutrients and plant tolerance to stress conditions (Lopez & Bacilio, 2020).

Research has been carried out to bioaugmentating expanded perlite with a complex microbiome capable of inducing the humification of exometabolized organic matter and the forming of sustainable organo-mineral ligands in the anorganic substrate. Also, compared to conventional fertilization practices, the results can positivelly influence the reducing fertilizer dependence.

MATERIALS AND METHODS

Bioweathering and biotransformation of expanded perlite experiment. The bioweathering of expanded perlite and biotransformation processes were analyzed in laboratory experiment, in controled conditions. The bioassay pots with bioaugmented expanded perlite were maintained at constant humidity, temperature 27^{o}C, constant photoperiodic lighting (10 hours of light:14 hours of dark), for 61 days. Further, the bioaugmented perlite was sown with 7 cucumbers seeds/pot, in successive series of 30-45days, for 210days, maintaining constant the parameters. The samplings for assessing the evolution of anorganic substrate, the biotransformations /accumulations level of organic matter were made in 4 phases, respectivelly after bioaugmentation and at 61 days after that, and 2 phases after the succesiv sowing, at 136 and 210 days. Microbial activity developed on the surfaces of expanded perlite were observed under microscope and the evolution of the substrate and plants growth was assess by biochemical and biometrics parameters.

Characteristics of expanded perlite. The general characteristics include the appearance of granular product with porous structure, free of impurities, white-gray color, loose and dry density 80-110 Kg/m^3, water absorption 20-35% by volume, granularity 3-5mm, delivery humidity max 1%, neutral pH, free of organic matter. The chemical composition of perlite contains amorphous silicon dioxide, in the proportion of about 74-77% SiO_2, Al_2O_3 12-15%, Fe_2O_3 1.1-1.6%, CaO 1.3-1.7%, MgO 0.1 0.7%, Na_2O, K_2O 5-8%, product manufactured by PROCEMA PERLIT S.R.L.

Consortium preparation. The compatible isolates of bacteria, fungi and diazotrophes were selected, on the basis of their characteristics, for preparation of consortium. The consortium with selected isolates grown on liquid medium was used for bioaugmentating expanded perlite.

Bioaugmentation of expanded perlite. The sterilized perlite granules, with dimensions between 3-5mm, were inoculated with the microbial consortium in the special growth medium, kept under pressure and placed in 250 g bioassay pots. The constant humidity was

maintained by watering with a special water/medium mixture in a ratio of 3:1 and a constant temperature of 27°C.

Quantification of exopolysaccharides. The amount of exopolysaccharides produced by the microorganisms in the consortium was performed using mixed cultures of 24 hours at 28^0C on a special medium. The samples were centrifuged and the exopolysaccharides precipitated from the supernatant with ethanol. These were kept overnight at 4^0C, centrifuged and the precipitate was collected and dried in a Petri dish at 60^0C. The total amount of exopolysaccharide produced was determined gravimetrically. The dry weight of the polymer was reported in one liter, the results obtained represent the average of 3 replicates for each sample. The comparison of the amounts of EPS was performed by statistical analysis.

Siderophores test. In the chrome azurol S reagent (CAS), the iron is bound to hexadecyltrimethylammonium bromide (HDTMA), the ligand extracts the iron from the dye complex, forms a complex and by releasing the dye, the color changes. Thus, 0.5 ml of supernatant was dispersed on blue agar CAS. Non-inoculated medium was used as reference. The positive test was indicated by changing the color of the microbial colonies from blue to orange.

Indole-3-acetic acid (IAA) test. Plate test with JNFb-agar containing 100µg/mL of tryptophan was used to determine IAA production. Samples containing the culture mixture were collected from the perlite substrate after inoculation and at intervals of 61, 136 and 210 days, introduced into the agar wells, grown in culture at 30°C, for 24 h. The cultures were removed from the wells and 200 µL of IAA reagent was added to the wells. A pink halo area appeared around the wells, which was measured to include the entire well. The halo-zone diameter was measured at the time of testing. The diffusion potential of IAA is directly proportional to the concentration, and the diameter of the halo-zone is representative for the amount of IAA produced. A larger size of the halo-zone diameter, in a given time, corresponds to a larger amount of IAA.

Polyphenol oxidase test. Samples extracted from bioaugmented expanded perlite were used to make 30 µl aqueous suspensions which were inoculated into 0.5 mm wells in agar containing bromophenol blue (BFB).

Germination test. The filter paper (Ø15 cm) was used for germination, in the clear glass Petri plate (Ø15 cm) containing distilled water to keep the paper moist. The seeds were placed on the germination paper and introduced in the germination plate. The germination process was performed at 27^0C with a circadian rhythm of 8h light and 16h dark. Each variant had three replicates with 15 seeds per plate. The germination test lasted 7 days.

Plantlets biomass. The 14-days plantlets, with true leaves, were harvested and measured for fresh weight, placed in sachets in an oven at 105^0C for 30 min, then dried at 80^0C for 40h, until a constant weight was reached.

Specific circular chromatogram. The chromatogram involves impregnating a cellulosic support with a heavy metal salt ($AgNO_3$), drying and migrating the extracted supernatant in a basic solution, developing after 24 hours.

RESULTS AND DISCUSSIONS

The use in consortium of pioneering organisms, adhering to perlite, allows the penetration of the rock surface and the fragmentation of minerals.

Also, the microorganisms in the consortium involved in the initial weathering of perlite directly or indirectly cause the disintegration of the primary minerals from which plant nutrients result, except for nitrogen compounds biosynthesized by the action of free diazotrophs, followed by dissolution, hydration and formation of secondary minerals, plant growth and development.

The poikilohydric forms of the consortium form a thin layer of biological activity that takes place on or in the first few centimeters of the surface of the perlite rock.

Although considered of little ecological importance, recent studies (Adhikari et al., 2018) have shown species richness, spread in the most extreme climates, with role in important ecosystem functions, in all biomes, so they can highlight the importance of microbial interactions and disturbances in case of efficient management (Figure 1).

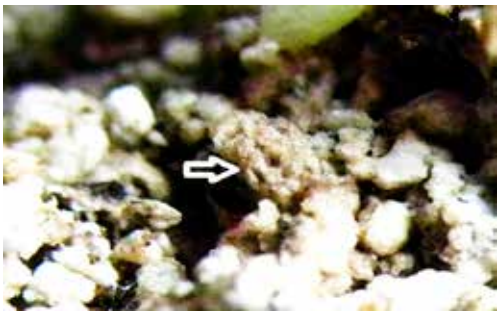

Figure 1. Surface penetration, disintegration and initiation of perlite rock fragmentation by consortium microorganisms

The establishment and activity of the bioweathering consortium on perlite requires, as an essential stage, the formation of the microbial biofilm. The growing biofilm contains an extracellular matrix, represented by a hydrated gel that envelops microorganisms, promotes their adhesion to the surface of perlite granules, supports and protects their activity against drying and external factors, allows the concentration of organic acids, siderophores, of chelating compounds and other weathering agents at the mineral / microorganism interface.
The ecto- and endolytic microorganisms in the consortium attach to the perlite and promote a greater dissolution of the elements in the mineral particles. Also, the biofilm formed in the perlite substrate allows the manifestation of the synergistic effect, regarding the dissolution of minerals, of the different microorganisms in the consortium, of the biological processes, favoring the microbial interactions and the intercellular communication. Biofilms are considered important in the geochemistry of rock bioweathering, and due to recent research indicating their protective roles, highlighted by maintaining the integrity of damaged rock surfaces, by forming rock cover structures (Jackson, 2015; Goswami et al., 2016; Zaharescu et al., 2020). The attachment of microorganisms and the promotion of the dissolution of mineral elements were evidenced in the research studies conducted by Ahmed & Holmström (2015).
The carbon produced is supplied by the exometabolites of the microorganisms in the consortium, by the photosynthetic symbiote and through it the development of microorganisms is promoted but also the secretion of many organic acids, which initiate the intense process of bioweathering.
Generally, biodegradation of rock substrate directly influence water infiltration capacity, substrate moisture, depletion of nutrients, and will determine the availability of water for plants, for optimal growth.
Therefore, the efforts of bioweathering and biotransformation, conservation of the microbiome, use of microorganisms in the perlite substrate and rhizosphere, to stimulate plant roots, could improve the structure of the substrate by aggregating it by microorganisms producing exopolysaccharides in the consortium.
Aggregation involves, in the basic concept, the incorporation of mineral particles with organic and inorganic materials, for the formation of secondary particles.
The dynamics of the process can be influenced by factors such as substrate type, plants, mineral composition, organic carbon concentration, activity of microorganisms, ion exchange, nutrient reserves in the inorganic substrate, humidity and management.
Exopolysaccharides protect the microorganisms in the consortium from a variety of stressors, protect cells from antimicrobial compounds, antibodies, bacteriophages, or ensure the adhesion of other microorganisms or plant tissues.
The mechanism of interaction of microorganisms in the substrate associated with the stability of aggregates and the supply of nutrients to crops is little known.
By using exopolysaccharide-producing microorganisms to aggregate perlite particles, intense activity in the plant rhizosphere is ensured to obtain exopolysaccharide biofilms (Figures 2 and 3).
EPS produced by consortium microorganisms can help to achieve high cell densities, useful in bioweathering and biotransformation, cell-cell recognition and resistance, in case of non-specific and specific host defense.
The presence of the EPS layer around the colonies has an effect on intra-, intercellular diffusion, prevents the access of toxic compounds (toxic metal ions) due to flocculation and their ability to bind metal ions in solution.

Figure 2. EPS layer around the microbial colonies

Figure 3. Microbial biofilm on expanded perlite granules, under the action of the consortium

Thus, through it, increase the immobilization of Pb^{2+} and Cu^{2+} ions, with ecological implications, help organisms to adhere to rock surfaces, can be flocculants that protect hydrophobic membrane areas but also the transitions between hydrophobic and hydrophilic areas.

Consortium biosynthesized EPSs can also act as extracellular storage polymers, in substrate where no other organic source has been identified, such as those encountered in the early stages of expanded perlite. The role of EPS in cellular protection is extensive and could includes bacteriophages, protozoa, phagocytosis processes, anti-cytotoxic activity and plant infection.

The microbial cells in the consortium produce EPS, respectively homo- and hetero-EPS, depending on the monosaccharide content, which they secrete in the rock substrate, through the action of extracellular enzymes. Biosynthesized EPS ensures resistance to various stressors and bacteriophage attack, toxic metal action, dehydration, the role of coating agents, conjugation, but also, matrix for microflora (Grosu-Tudor & Zamfir, 2014).

The screening performed at intervals of 61, 136 and 210 days after the bioaugmentation of the expanded perlite showed the presence of polysaccharide material, in different quantities, which increase progressively, until the end of the experiment.

These results are supported by data from the literature, which showed dependence of the amount of EPS produced by a number of selected microorganisms, on the composition of the substrate, the microbial strain, the nature of the source and the growing conditions, respectivelly temperature, pH, oxygen. (Patel et al., 2013).

The experiment examined the ability of the selected consortium to produce exopolysaccharides in the perlite substrate at certain time periods.

Under the influence of controlled experimental parameters at 61 days after expanded perlite inoculation, EPS yield of consortium strains was 47.5 mg/l EPS (Figure 4 b).

EPS production increased under second cucumber culture at 136 days after bioaugmenting expanded perlite, to 84.7 mg/l EPS and respectivelly, at 144.3 mg/l EPS, at the end of the experiment (Figure 4 c and d).

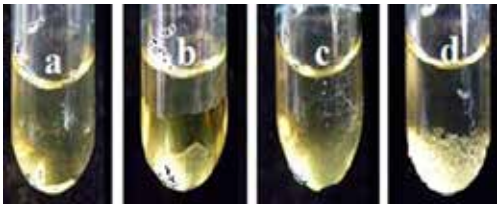

Figure 4. a) EPS in bioaugmented expanded perlite, b) EPS produced at 61 days, (c) EPS at 136 days under plants culture (d) EPS at 210 days under plants culture

The data are consistent with the observations of other authors who point out that the production of EPS is dependent on the microorganisms tested, but also on environmental factors. Thus, Madhuri & Prabhakar (2014) highlighted the dependence of the amount of EPS on the microorganism species. Vamanu et al. (2007) highlighted the influence of pH, carbon source and temperature on the formation of EPS in probiotic strains and the biosynthesis of EPS could be considered a mechanism of microbial

self-protection against unfavorable conditions. (Lin & Chang Chien, 2007).

Living microorganisms from the expanded perlite need iron for vital processes and for cell growth.

They receive iron by direct diffusion through cell membranes or by the synthesis/secretion of siderophores, avoiding its lack of bioavailability, manifested in low concentrations of Fe (III), below 10^{-18} M.

Basically, the role of siderophores biosynthesized by the consortium is bringing insoluble iron from the inorganic substrate into the cell.

Siderophores, in addition to affinity for Fe (III), also form complexes with other metal ions, such as Fe^{2+}, Mg^{2+}, Ca^{2+} and Al^{3+}, which can be bioweathered from the expanded perlite substrate by the microorganisms from the consortium. In the competitive medium of bioaugmented expanded perlite, there is initially a deficiency in soluble iron and, to ensure the survival and growth of plants and of the microbial isolates from the consortium, iron increases its availability in the substrate and in the roots zone of cultivated plants. The intensity of siderophores biosynthesis increased over time, starting from their absence in the inorganic substrate of expanded perlite, after inoculation of the consortium (Figure 5a) and until the evidence of a generalized biosynthesis of siderophores by the microbiome developed in the substrate, at the end of the experimental period (Figure 5d).

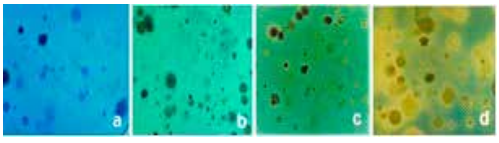

Figure 5. a) Siderophores presence in bioaugmented expanded perlite, (b) siderophores after 61 days, (c) siderophores after 136 days under culture, (d) siderophores after 210 days under culture

Iron is essential for the processes of respiration and DNA synthesis, which is why most microbial species have developed a high-affinity iron (Fe^{3+}) transport system, based on siderophores, to avoid the very low solubility of these ions at neutral pH.

Soluble Fe^{3+} complexes can be taken up by active transport mechanisms by microorganisms/plants, become available to microbial cells by dissolving these ions, by siderophore. Also, the siderophores produced inhibit the colonization of roots by plant/ pathogens (Alexander & Zuberer, 1991; Khan et al., 2006).

In the substrate, siderophore functional groups have interacted with iron through negatively charged oxygen atoms, but sometimes distortions can occur when they can be replaced with nitrogen, potassium or magnesium from bioweathering of the substrate, which reduces the affinity for Fe(III).

The natural siderophores in the substrate can be arranged in a superior chelating structure, highlighted over time by the generalization and intensification of their biosynthesis (Figure 5c and 5d).

Possibly, over time, hexadentate and tetradentate structures become predominant, more stable, and less labile, as the iron-bound ligand causes the entropy to change. In addition, in the substrate, the aqueous solution causes the siderophores to surround the natural complex $Fe(H_2O)_6^{3+}$, and the water to be completely replaced in a favorable manner, from an entropic point of view. Also, for the formation of the iron complex, siderophores could donate more oxygen atoms, so they form weak field donors (σ and π). The combination of complex geometry and configuration it possible may not generate enough energy to stabilize the ligand field, either due to ligand dentition or multiple interactions between strong acids and bases. A negative charge corresponds to the accumulation of bases such as catecholates/carboxylates which, due to negatively charged oxygen atoms/functional group, can perform closer interactions with Fe (III) in the perlite substrate or can achieve a higher negative charge density, respectively a higher affinity for iron, shown in Figure 5c-5d.

Also, the Fe(III)-siderophore complexes that form in the inorganic substrate could be sensitive to pH values, so that in a substrate with a low pH, intense competition for siderophores can be triggered, due to the increase of the proton concentration solvates or free iron.

Immediately after the inoculation of the consortium in the substrate, it was observed that the test applied to the samples collected

from the bioaugmented expanded perlite did not allow the highlighting of a pink area around the well, which suggested the absence of IAA in the substrate. Over time, the samplings carried out at different time intervals, revealed changes in the diameter of the area with pink halo.

Thus, 61 days after inoculation of the expanded perlite, the diameter of the pink halo was 2 mm. In the case of the other samples (at 136 and, respectively 210 days), the additional influences mediated by the succesiv cultivated plants intervene, also. After the evaluation of the IAA synthesis capacity of the consortium, pink areas around the cavity were highlighted, with various diameters, respectively by 4mm and 7mm, probably due to the higher amounts of IAA produced by the consortium in the perlite substrate (Figure 6).

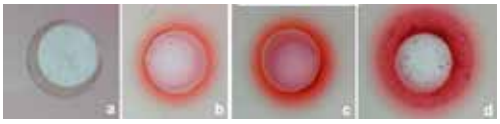

Figure 6. IAA diffusion in plate assay. a) no IAA synthesis in bioaugmented expanded perlite, b) IAA halo-zone after 61 days to the bioaugmented expanded perlite, c) IAA halo-zone after 136 days under plants culture, d) IAA halo-zone after 210 days under plants culture

Selected consortium produce indole-3-acetic acid (IAA) to solubilize different toxic metal (Al) containing minerals and for metal tolerance. In addition, the metal tolerance /solubilizing activities of consortium, vary for different minerals/microbial species in a inverse report between solubilization and the concentration of metal. În experiment, the highest IAA concentration was obtained after 210 days from the perlit bioaugmentation, under plants culture.

The germination index, determined at the established time intervals, was 23.7% higher at 61 days than at the control, after germination. In particular, the germination rate at the end of the experimental period (210 days) was 66.2% higher than that obtained in the perlite substrate before the introduction of the cultures (61 days), and respectively by 25.7% higher than that obtained in the bioaugmented perlite substrate, for perlite under the second culture (136 days).

IAA production varies between different species and is influenced by growing conditions, growth stage and substrate availability. Many plant-associated organisms produce related auxin and indole compounds. Microorganisms living in the substrate and rhizosphere of plants use rich sources, from the substrate and from root exudates, biosynthesize and release auxins, as secondary metabolites.

Silicon (Si) is abundant in the perlite substrate, in the form of insoluble silicates, and is a quasi-essential element for plants, which can be absorbed only in the soluble form of monosilicic acid. Due to the bio-weather of silicate rocks, its production depends on temperature, pH, redox potential, water content and the level of microbial activities. Numerous studies have shown the role of indole-3-acetic acid (IAA) and organic acids produced by silicate solubilizing microorganisms. IAA, a product of L-tryptophan metabolism, is produced by the consorțium microorganisms, not only by plants (Apine & Jadhav, 2011).

In the experiment, it was observed that priming with IAA, derived from microbial biosynthesis and root exudation, improves seed germination, by regulating the content of IAA, and growing seedlings. The favorable response of these parameters depends on the species and the optimal concentration of IAA present in the substrate. The results also showed that by showing with IAA, germination can be regulated positively, by the synthesis of favorable phytohormones and negatively, by inhibiting unfavorable phytohormones (ABA).

During the germination stage, the IAA priming caused an increase in weight and water absorption, but the dry weight remained constant. After analyzing the effect of seed priming on seedling growth, it was concluded that different chemicals can induce seedling growth through different mechanisms. Thus, as in melatonin initiation, IAA increased the weight of the plant root under stress, possibly by counteracting the effects on metabolites and by increasing the antioxidant capacity (Bahcesular et al., 2020).

Researches showed that the germination rate improves significantly after initiation with IAA. Our results are in line with previous researches on seed priming with chemicals and promoting germination. Thus, the priming of

Cuminum syminum L. seeds with polyethylene glycol favors the germination of seeds in conditions of temperature and water stress, and the priming of *Nigella sativa* L. seeds with KH_2PO_4 increased the vigor of the seeds (Min et al., 2014; Seyyedi et. al., 2015; Espanany et al., 2016).

In addition, additional silicon priming could improve biomass and plant yield by reducing oxidative stress (Hussain et al., 2019). Also, results were obtained regarding the priming with melatonin in corn, in order to improve the germination of the seeds under low thermal stress. The selected consortium which can produce indole-3-acetic acid (IAA) could solubilize and different toxic metal (Co, Cd, Cu, Pb, Zn) containing minerals, or induce metal tolerance.

Microorganisms are able to produce IAA in the liquid medium, from the expanded perlite substrate, obtaining the highest IAA yield after 210 days, under plants culture. So that, by the biological activity of consortium, IAA produced can stimulate increase seed germination, hypocotile elongation and root length of plants.

Also, the metal tolerance/solubilizing activities of consortium possibly vary, for the different minerals or microbial species, in a inverse report between solubilization and concentration of metal, aspect that could sugest a low tolerance to metals.

Different types of microorganisms, according to recent studies, are involved in the solubilization of the unavailable form of Si (Li et al., 2018). Mineral and rock bioweathering are some of the beneficial processes in which microorganisms play an important role in promoting dissolution. Different types of rocks are susceptible to bioweathering and may also include siliceous rocks (silicates, silica, aluminosilicates). So that, microorganisms form geochemical agents and act on insoluble forms of Si, transforming them into soluble orthosilicic acid, usable and available to plants (Sheng et al., 2008).

Phenotypes of *Cucumis sativus* L. seedlings, after the germination test of IAA-treated seeds, were investigated 14 days after emergence. The characteristics of *Cucumis sativus* L. seedlings, determined at the beginning of each established time interval, according to Table 1, allowed the evaluation of some biometric parameters, of plant growth and development under experimental conditions, following the analysis which found that some parameters such as hypocotyl length, roots length, fresh biomass and dry biomass of plants increased.

Table 1. Biometric parameters of *Cucumis sativus* L. plants, under experimental conditions

Time periods	Hypocotyls length (cm)	Root Length (cm)	Biomass Fresh weight (g/plant)	Biomass Dry weight (g/plant)
Initial	1.46 ± 0.21 d	2.28 ± 1.07 d	0.328 ± 0.025 d	0.085 ± 0.004 d
61days	2.76 ± 0.32 c	3.12 ± 1.32 c	0.512 ± 0.032 c	0.102 ± 0.006 c
136 days	3.28 ± 0.33 b	6.48 ± 1.44 b	1.057 ± 0.045 b	0.135 ± 0.006 b
226 days	5.37 ± 0.39 a	9.12 ± 1.52 a	1.262 ± 0.052 a	0.147 ± 0.007 a

Values are mean ± standard deviation (SD), Letters behind the values in the same column indicate significant difference at different periods of times, $p < 0.05$.

Based on the direct and indirect action of microbial biosynthesized IAA and IAA from roots exudates, some plants parameters have improved, which over time have induced significant differences between the values determined for the same parameters. The direct /indirect action of microbial biosynthesized IAA and from roots exudates, improves the level of microbial activity and the organic content of the perlite substrate. It is possible that *Cucumis sativus* roots exudates released in expanded perlite have the ability to restructure microbial communities, reduce substrate infestation, significantly reduce phytopathogen favorability and improve microbial activity.

IAA priming could have a positive impact on seedling germination and growth. Compared to germination, biomass, including fresh and dried weight, accumulated significantly compared to the control, especially at the end of the experiment, because the initiation of IAA activated photosynthesis, also.

Given the multitude of processes in which biosynthesized exoenzymes by microorganisms consortium and plants may be involved, the assessment of the presence and activity of these exoenzymes, respectivelly of the polyphenol oxidases in the substrate is important because it allow microorganisms to interact effectively with the expanded perlite substrate, on direct contact level with the anorganic substrate.

Also, polyphenol oxidases as exoenzymes, causes degradation of the plant organic matter, that begins to accumulate after the first culture, catalyzes fragmentation of high molecular

weight biopolymers from the environment into simpler forms that can then be easily assimilated and used, helps the microorganisms în metabolisation/using the fractions and subfractions of organic matter, intervene in the processes of stimulating the growth/microbial activity in the substrate.
Qualitative tests on the presence and activity of polyphenol oxidase were performed at the same time intervals established for the initial phase, respectively 61 days after the bioaugmentation of the expanded perlite, when the diameter of the halo was Ø=0.92mm. Under successive cultures, the level of enzyme activity increases and determines at 136 days a halo diameter of Ø=1.24cm and at the end of the experiment Ø=2.3cm (Figure 7).

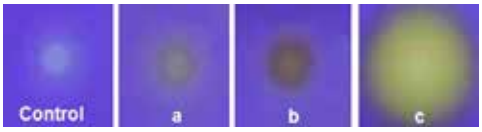

Figure 7. a) Polyphenol oxidase activity in bioaugmented expanded perlite, after 61 days, b) polyphenol oxidase activity after 136 days under plants culture, c) polyphenol oxidase activity after 226 days under plants culture.

The yellow halo indicates the presence of polyphenol oxidases in the substrate, highlighted by the change from blue to yellow. The diameter of the yellow halo could be a semi-quantitative measure of the level of enzyme activity. Also, due to the directly proportional relationship between the amount of enzyme present in the sample and the size of the halo, could determine the biosynthesis and presence level in substrate. The ever-increasing level of polyphenol oxidase activity also reflects the fact that there are favorable conditions for the accumulation and intensification in the perlite substrate of enzyme-mediated biochemical processes.
The microorganisms in the consortium and the plants used released extracellular polyphenol oxidases into the perlite substrate, which possible are involved in the formation of organic compounds, secondary metabolites, melanic protective compounds. The exoenzymes intervene in the biosynthesis/ degradation of organic matter and humus, in obtaining carbon and nutrients, in attenuation of the toxicity of phenolic molecules and metal ions from the anorganic substrate and in antimicrobial defense. Also, the exoenzymes biosynthesized and released into substrate are involved in the oxidation of a wide range of small molecules, including prehumic or humic, in the formation of stable radicals, in the polymerization of soluble phenols and in humification, function as proteolytic/ chitinolytic enzymes or are able to extract nitrogen from humic complexes.
The specific circular chromatograms highlight the evolution and structure of the organic matter biosynthesized by the microbial consortium and by the root exudates of the seedlings in the expanded perlite.
Specific chromatography also provided the opportunity to distinguish different characteristics, from the point of view of expanded perlite or from the point of view of the consortium, as well as indications of the progress of humification, the likely effect of bioweathering and biotransformation induced by the consortium on the substrate.
Biosynthesis and biotransformation of organic matter can also be considered effective in retaining carbon, because it, in dissolved form, will react rapidly with minerals in the substrate for stabilization. Thus, the high values of pH in the substrate, associated with those of ammonium, potassium, sodium, bring the necessary solubility to the synthesized organic matter.
The specific circular chromatograms made it possible to obtain information on the evolution of the biological quality of the bioaugmented and cultured substrate, through analytical separations and the formation of images whose pattern of uniformity, shape, size, color, texture indicates soil health, vitality, fertility, intensity of biotic activity, substrate conditions, complexity of organic matter and the presence of stable humus (Figure 8).
The images of bioaugmented expanded perlite assay pots, correspond to a-d chromatograms images, showed in Figure 9 a-d.

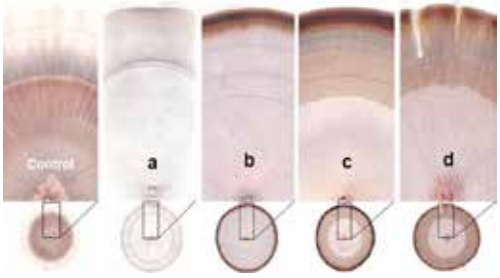

Figure 8. Control-standard humic acid chromatogram and a) organic matter in bioaugmentated expanded perlite, b) organic matter accumulation în expanded perlite after 61 days of consorțium influence, c) organic matter accumulation în expanded perlite, after 136 days of consorțium and plants influence, d) organic matter evolution în expanded perlite, under consorțium and plants influence, at 210 days

In the peripheral area, chromatogram a highlights organic matter content under bioaugmentated expanded perlite with consortium. Enzymatic activity is reduced to 61 days (chromatogram b) since the introduction of the consortium, without a nutritional variety, without stable structures over time, and relatively weak microbial activity, not diversified compared to 136 days (chromatogram c), where the activity appears more diversified and the enzymatic activity becomes more intense, the nutritional variety is constantly growing and humic structures begin to stand out. At 210 days (chromatogram d) enzymatic processes appear relatively diversified and more intense, there is nutritional variety and the tendency to accumulate organic melanic structures, more intense microbial activity, but insufficiently diversified.

The external area is of particular importance in the analysis of chromatograms. The standard humic acid chromatogram was used as a control for highlighting the main fractions of structured humic compounds, condensed, highly mobile, migrating compounds.

The strong coloration and thickness of the outer area suggest an intense biosynthesis of mobile organic precursors. At the same time, through the processes of biotransformation, after a period of time, the changes can be observed on the chromatogram by the loss of mobile compounds, which indicates an association with other compounds, the formation of complexes with inorganic constituents and therefore more insoluble.

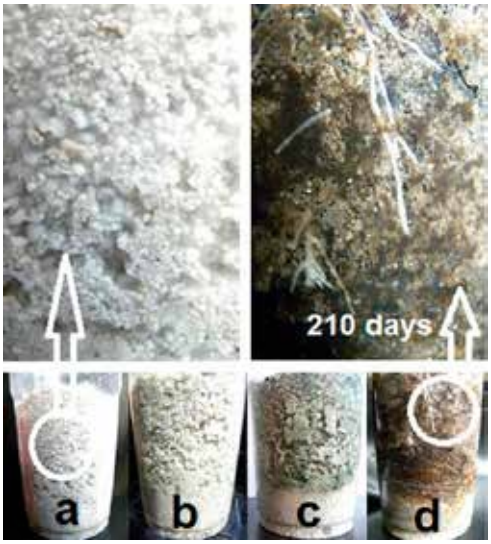

Figure 9. Bioassay pots with (a) bioaugmented expanded perlite; b) bioaugmented expanded perlite, after 61 days; c) bioaugmented expanded perlite and plants influence, after 136 days, and d) after 210 days

If the relative concentration of fulvic compounds is high in the extract, a thick front of motion appears, forming a dark outer zone, as seen in chromatograms b and c. Due to the biotransformation processes, the proportion of these mobile compounds changes. It becomes considerably lighter, more fragmented, or may disappear altogether if the mobile compounds are missing. Also, few humic substances are formed, mainly acidic; nutrient content and low organic content, colloidal substances are present (chromatogram a). Complex humic substances are formed, mainly in chromatogram d, more than in chromatograms b and c. More intense accumulations of humic substances appear in chromatogram d. The presence of colloidal substances increases, the acidic character of the organic material is progressively reduced from chromatogram b-d.

The intermediate zone of the chromatograms progressively shows an increase of the mineral diversity relatively integrated in the organic material, a rich content of protein material, their structural complexity increases, and the level of microbial activity is highest in the chromatogram d. Intermediate development processes are observed with tendencies of accumulation and integration in the substrate of the little organized material. There is a good

connection between the different components, aggregates are present in the solution. There are still unfavorable conditions and deficiencies. Accumulations of humic compounds appear in chromatograms c and d but are not sufficiently integrated (chromatogram c). The microbial activity increases in intensity, the tendencies of accumulation and integration of the organic material synthesized in the substrate are maintained (chromatogram d). There are no differences between chromatograms c and d regarding the presence of aggregates in the solution, and from the analysis of the uniformity model and the chromatogram texture, the existence of unfavorable conditions is observed. The model appears more evolved in chromatogram d, without major separations or sub-average parameters of the soil processes.
In the expanded perlite substrate, intermediate development processes are highlighted, a weakly structured organic material appears, characteristics of a healthy evolution state, more intense releases of molecular aggregates in chromatogram c. Unlike the situation in chromatogram b, in chromatograms c and d no more signs of such intense mineralization appear, so that the equilibrium can be considered to be in favor of organicization. The interactions of organic carbon are more intense with polysaccharide complexes and with the diversified mineral component in chromategram d. In the inner and central area, biotic conditions become predominant in chromatograms c and d, and small non-flocculating inorganic molecules appear in the solution. There are increased amounts of accessible salts, of nitrogenous compounds, which, in comparison, have a higher weight in the chromatogram d over time.

CONCLUSIONS

The ecto- and endolytic microorganisms from consortium attach to the perlite and promote surface penetration, disintegration and initiation of perlite rock fragmentation.
The biofilm formed in the perlite substrate allows the manifestation of the synergistic effect and favoring the microbial interactions and the intercellular communication.
The selected consortium produced exopolysaccharides in the perlite substrate, under controlled experimental conditions, from 47.5 mg/l EPS at 61 days after bioaugmentation to the 144.3 mg/l EPS under plants culture.
Siderophores biosynthesis increased in intensity all over the time, starting from a low presence after inoculation of the consortium, to a high presence, after succesive plants cultures, at the end of the experiment.
Substrate bioaugmention produced IAA, so that at 61 days after inoculation, the diameter of the pink halo was at 2 mm and under additional influences mediated by plants, the higher amounts of IAA produced a pink halo of 7 mm diameter.
The germination index was 23.7% higher at 61 days than at the control and at the end of the experiment was 25.7% higher than that obtained in the perlite substrate, under the second succesion of plants culture.
IAA priming had a positive impact on plants growth, so that the biomass (fresh and dried) accumulation increased significantly compared to the control, especially at the end of the experiment.
The presence and activity of polyphenol oxidase tested at 61 days after the bioaugmentation of perlite, was highlight by the yellow halo with Ø 0.92 mm and under the successive plants cultures, the level of enzyme activity increased to Ø 2.3 cm, at the end of experiment.
The chromatograms highlights organic matter content evolution under bioaugmentated expanded perlite, a nutritional variety in a constantly growing, the accumulation of organic structures, intensification of microbial activity but insufficiently diversified and prehumic and humic structures begin to stand out.

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