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A CALCARIC ALLUVIAL SOIL CHARACTERISTICS AT SUBMICROSCOPIC SCALE USING SEM

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Abstract

Soil characteristics play an important role on agricultural crop production, especially in the organic farming system. Increasing the productive potential of organic agroecosystems in the context of climate change is nowadays possible also using soil treatment with microbial bioinoculants. Microbial bioinoculants have the capacity to enhance nutrient availability, nutrients uptake, support the soil and plant health, by controlling the pest and diseases or enhance the plant defence responses. In 2018, at the Vegetable Research and Development Station Buzau, a soil micromorphology study was performed on an Aluviosol Molic Calcaric, in an organic testing field cultivated with vegetables. Soil samples taken at six different depths were analysed using SEM images. The soil micro and macromorphological characteristics were correlated with the analytical data. The results showed the $CaCO_3$ abundance in soil, while at sub-microscopic level it appears as acicular crystals into the efflorescences coatings. The soil skeleton is well represented, the sand-sized mineral grains being abundant. Many skeleton grains (as feldspars and mica flakes) had altered surfaces, being partially covered with secondary decomposed products, whose presence favours the installation of microorganisms.

Key words*: soil micromorphology, SEM image analysis, Buzau, Romania, organic soil.*

INTRODUCTION

At the level of 2017 the total organic area (areas under conversion plus the certified areas) in the EU-28 was 12.6 million hectares (ha) and the trend is still ascendant. The increase in organic area between 2012 and 2017 was with 25%, Bulgaria and Croatia reflecting a growth of over 100%. However, four EU Member States reported reductions in the organic area: Romania (-10.3%), Greece (-11.3%), the United Kingdom (-15.6%) and Poland (-24.5%) (EUROSTAT, 2019). From 2012 to 2017, the share of total organic area in the total used agricultural area (UAA) within the EU rose from 5.6% to 7% (EUROSTAT, 2019). Romania is on the 26 place, of the 28 EU countries, but our organic land under conversion represented in 2017, 42.3%, one of the biggest in Europe. To be able to sustain Romanian farmers in their attempt to produce at high quality standards into organic, in the recent climate change constraints, high support is required from the scientists.

Soil is the most important resource of organic farmers. The attempt of performing in organic crops on calcaric alluvial soils, which are almost like sandy soils, requires high knowledge of crop technologies (Stefan et al., 2018), as usually these soils have less nutrients and low water holding capacity leading to frequent irrigation and fertilizations to meet crop requirements (Alshankiti & Gill, 2016). In studies on soil microbial activity of sandy soils it was found that overall activity was low, due to low organic matter and low rainfall (Unkovich, 2014). Due to lower organic fertility and fewer microsites to protect soil biota, sandy soils have also a lower capacity to suppress pathogens and other pests (Coventry, 1998). Eftene et al. (2014) showed that physical and chemical properties of a soil with sandy texture and low soil organic matter content (due to intense mineralization of organic residues) create conditions for a reduced activity of microflora. Carminati et al. (2008) exploring the soil-water interfaces between isolated heterogeneous aggregates within a soil (controlled by matric tensions) reveal the intimate associations between pore geometry and water.

The physical characteristics of the soils, mainly of the young soils (as Aluviosols) are important for the further evolution of the soils, for the structural and the adjacent poral space genesis that strongly influence the air and soil solution circulation and the biochemical processes. The role and transformations of agricultural inputs, as fertilisers, microbial inoculants, pesticides, etc. are highly dependent on soil characteristics. For a deeper understanding of these characteristics and its interrelations with biotic factors, natural or applied during crop technologies, analysis using Scanning Electron Microscopy (SEM) proved their utility over time. Early SEM observations have been conducted by Barden in 1973 and Grabowska-Olszewska in 1975 on European loess from Belgium and Poland (Delage et al., 2005) and become currently widely used for micromorphological studies. Răducu (2018) showed that soil biota initiated and controlled the important pedogenetic processes, thus soil fauna activity had a major influence on the illuvial process of pedobioplasma, while the activity of microorganisms had a major influence on coatings evolution.

In the soil environment, the microbial phase development (on the mineral and organic constituents), usually represent the constituents transformation and weathering/decomposition (according to their mineral or organic status). Mica destruction was detected by SEM, due to the growth and metabolic activities of bacteria (*Bacillus cereus)*, which cause the extraction of iron atoms from the octahedral position in mica from the kaolin sample (Štyriakov et al., 2003). Also, Balogh-Brunstad et al. (2008) found, by the aim of SEM images, a biotite surface covered with biofilms containing bacteria and fungal hypha. Often the concept of soil macro-aggregates (>200 mm) and micro-aggregates (50-200 mm) is invoked in an attempt to understand the importance of physical protection of organic matter isolated from microbial and chemical degradation (Young et al., 2008).

Many Alluvial soils have calcium carbonate accumulations, either inherited from the parent material or accumulate under water table influence.

Based on the results obtained following the investigation of a pedochronosequence, Kuznetsova et Khokhlova (2012) conclude that the sub-micromorphology (by the aim of SEM) of the carbonate accumulations may be useful for reconstruction of recent climatic conditions and allow to estimate the general trend of soil evolution, and even the rate and dynamics of soil formation processes.

Heberling et al. (2016) showed that calcite based environmental remediation strategies rely on the fast recrystallization of calcite and the concurrent uptake and immobilization of pollutants. Young et al. (2008) showed that it is need to bring together a new discipline that combines the biology and physics of the soil ecosystem. Thus, the biophysical approach, combined, where required, with important mineral-microbe knowledge is needed to help us understand the mechanisms by which soils remain productive, and to identify the tipping-points at which there may be no return to sustainability. Manea et al. (2016) studying a Fluvisols with neutral - slightly alkaline reaction, concluded that the correlations between soil fungal number and soil chemical properties are generally positive and significant, especially for soil reaction, the degree of soil base saturation, the sum of cation exchange capacity, as well as the calcium contents.

It is proven that beneficial microorganisms have the ability to improve yield quality and soil fertility. For organic crops, the use of plant resistance enhancers started to become very popular and appreciated by the market.

In a climate change perspective, a different distribution of microbial organisms is expected and that the progressive increase of air temperature and reduced cold shocks on microbial cells may lead to spring water pollution also during winter, differently from the actual observations Naclerio et al. (2012). Therefore, a throughout research of soil - microorganisms - plants interactions is needed for each soil type.

The aim of the present study was to emphasize the main characteristics of an Aluviosol Molic Calcaric from an organic agroecosystem, before the treatment with microbial bioinoculants, using scanning electron microscope (SEM), in order to better understand the changes that occur in the soil (open field) after application of such microbial inoculants.

MATERIALS AND METHODS

The study was carried out in the Vegetable Research and Development Station Buzău (V.R.D.S. Buzău), Romania (lat.: 45.16108714 N and long: 26.82423914 E, alt: 92 m) in 2018, in the organic farming research plot, having an area of 1.8 ha. The studied area it is situated inside the Buzău city, in Buzău county, at around 1 km distance of the river. V.R.D.S. Buzau is well known for tomato production and breeding, being obtained here, over time, valuable varieties appreciated by both consumers and growers (Zamfir et al., 2018).
The soil is Aluviosol Molic Calcaric (according to SRTS-2012 and Mollic Fluvisol Calcaric - according to WRB-SR-2006) formed in calcaric fluvial deposits.
For the detailed study of the soil characteristics, it was used the scanning electron microscopy (SEM) technique, to obtain details of soil samples at very small scale. The SEM analysis of the Aluviosol Molic Calcaric was carried out with FEI Inspect S50 model, in the Laboratory of Microscopy and Plant Anatomy, of the Research Center for Study of Food and Agricultural Products Quality, inside the University of Agronomic Sciences and Veterinary Medicine of Bucharest.
The terminology used for description at microscopic level was according to Bullock et al. (1985).

RESULTS AND DISCUSSIONS

Physical and chemical characteristics

On the general background of a soil with specific characteristics for an Aluviosol Molic Calcaric (Table 1), the granulometric data showed a medium sandy-loam texture into the top Am horizon, and a fine to coarse sandy-loam texture into the deeper horizons.
The colloidal clay (<0.002 mm) is very low, ranging between 18.5% in the surface Am horizon and 10.3% in the deeper analysed horizon (Ck_2).

Table 1. Morphological characteristics of the Aluviosol Molic Calcaric

Am (0-37 cm)	**Ck_1 (37-96 cm)**	**Ck_2 (96-124 cm)**
Medium sandy-loam; crumb structure weak developed; moderate biological activity; frequent fine pores; weak-moderate effervescence (locally); very frequent thin roots from cultivated vegetation.	Fine sandy-loam; massive; frequent thick pores; frequent fine roots; moderate-strong effervescence.	Coarse sandy-loam; massive; very friable; coarse mineral grains (visible by the necked eye); frequent $CaCO_3$ pseudomycelium; strong effervescence.

The physical characteristics data shows that total porosity is very high (52-56% v/v), while the bulk density is very low (1.13-1.22 g/cm^3).
The chemical characteristics data showed that studied soil is moderate alkaline, the pH values ranging between 8.06 in the surface horizon and 8.3 in the deeper analyzed horizon.
The base saturation degree is very high (96-100%) as a result of the calcium carbonate presence into the soil profile.
The organic matter content is medium. However, being highly humified and predominating the calcium humates, the colour of the surface horizon is very dark (under 10YR 5/3 on dry), which classified the studied soil to the mollic subtype.
The nitrogen index (calculated according to the humus content and the saturation degree at hydrolytic acidity) is medium (3.45-2.00) in the most part of the soil profile, except the deeper horizon, where the value decrease drastically (to 1.4).
The potassium content decreased with the increasing depth, ranging between medium (123-162 ppm) in the upper horizons and low (13 ppm) in deeper ones.
Very significant exponential relationship was established, by Manea et al. (2011) researches, between mobile phosphorus and soil reaction.
In the studied Aluviosol Molic Calcaric, the mobile phosphorus decreased with the soil profile depth, from the higher value (46 ppm) in the surface to the lowest value (13 ppm) in the deeper analyzed horizon.

Soil characteristics at submicroscopic scale

The microscopic observations by the aim of SEM were performed on the freshly broken surfaces of the soil sampled from each pedological horizon.

In the top soil, developed an A mollic horizon (Am 0-37 cm) having crumb structure (Figure 1). The spatial organization of the structural elements generated a relatively high porosity composed predominantly by the interconnected pores.

The spatial organization of the soil structural elements is also emphasized by the analytical data of total porosity.

Detailing to a higher scale of observation (Figure 2) it became obvious that although the clay content is low (18.5%), the soil constituents are relatively well structured, the skeleton grains and the colloidal constituents are binding together relatively tight.

The content of the organic matter (an important binding agent of the soil) is ranging from 3.6% to 1.4% (decreasing from the surface to the deeper horizons). At sub-microscopic scale, the organic matter appears only as humified substances, embedded into the soil matrix together with the clay particles.

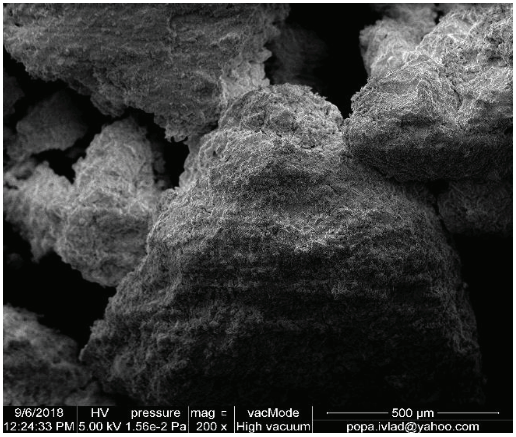

Figure 1. SEM image of Am (0-37 cm) horizon: crumby structure with interconnected pores (200 X)

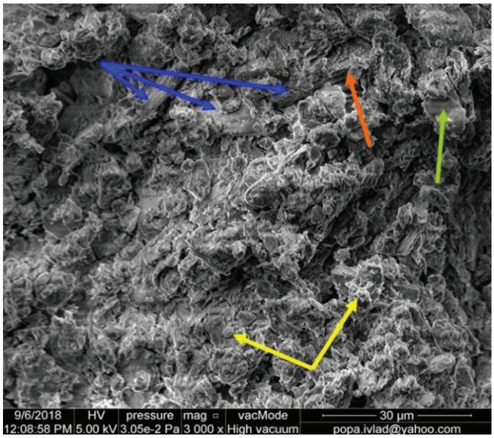

Figure 2. SEM image of Am (0-37 cm) horizon: mineral skeleton grains (→); microorganisms (→); plagioclase feldspars (→); mica flakes (→) (3000 X)

The soil skeleton is well represented mainly by the fine sand-size and silt-size mineral grains. Quartz dominates the coarse fraction of the horizon.

Many fine sand-size plagioclase feldspars were observed among the skeleton grains (with polysynthetic twinning - Figure 2) together with the silt-size mica flakes (emphasised by their foliated texture - Figure 2).

These mineral grains being poorly weathered, their surfaces are partially covered with secondary alteration products (secondary clays and/or Fe oxy-hydroxides etc.).

The presence of the weathered products, favour the microorganism development on the mineral grains surfaces (Figure 4).

Another important aspect, related to the structuring process and consequently to the building of a favourable environment for plant development, is the presence of microorganisms (Figure 2) which, by generating "organic cement" (their metabolic products dominated by the presence of mucilaginous polysaccharides) increase the particle cementation and consequently the soil structuring process.

The high interconnected porosity (Figure 3) reveals excessive drainage, and therefore low water retention capacity of the soil, which negatively affects the plant water supply.

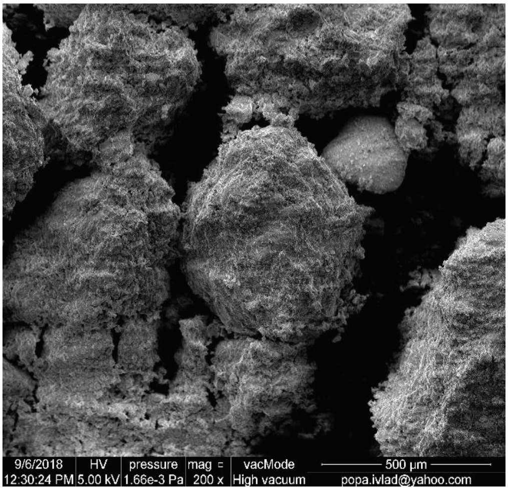

Figure 3. SEM image of Am (20-37 cm) horizon: crumby structure with interconnected pores (200 X)

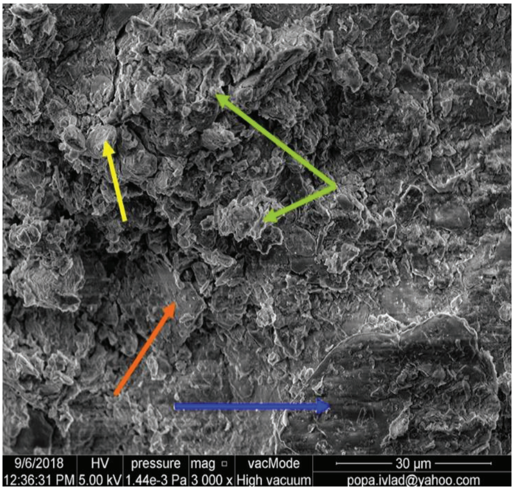

Figure 4. SEM image of Am (20-37 cm) horizon: mineral skeleton grains (→); calcite (→); clay particles binding together (→); mica flakes (→) (3000 X)

The SEM emphasised the organization of the soil constituents at micro-scale, their aggregation into the peds with the formation of the adjacent micro-pores, represent the bricks of the next levels of organization (until the macro-scale) and induce the physical and chemical characteristics of the soil, and further the health of the organic agroecosystem environment.

The **Ck$_1$ (60-96 cm)** horizon, being dominated by the characteristics inherited from the parent material, is low structured, with subangular blocky structure.

The friability of the horizon (Figures 5 and 6) is the result of the granulometry dominated by the fine sand-size and loam-size particles (Table 1 and Figure 6).

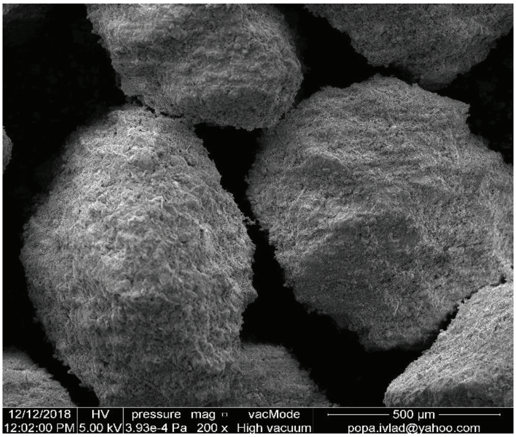

Figure 5. SEM image of Ck$_1$ (60-96 cm) horizon: crumby structure with interconnected pores (200 X)

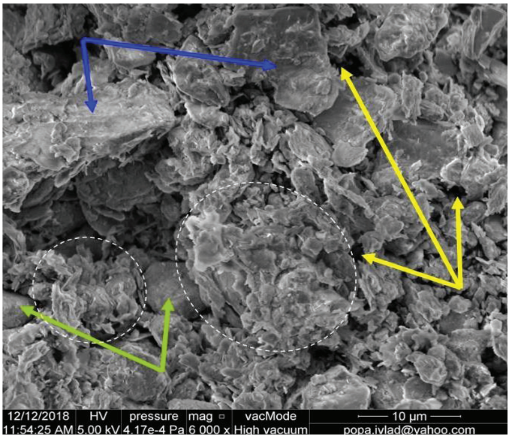

Figure 6. SEM image of Ck$_1$ (60-96 cm) horizon: mineral skeleton grains (→); silt-size micro-aggregates (◌); intrapedal packed pores (→); calcit crystals (→) (6000 X)

The clay particles formed micro-aggregates of silt-size by binding together face-face and/or edge-edge, and also embedding calcite crystals (Figure 6). The small intrapedal packed pores also formed by the spatial organization of the micro-aggregates.

The **Ck$_2$ (96-124 cm)** horizon is very friable (Figures 7 and 8), showed also by the granulometric data that highlighted a coarse sandy texture.

The main part of the horizon material is un-aggregated. The aggregates are small and poorly formed (Figure 7).

The high structural friability that characterizes this horizon is also given by the abundance of calcium carbonate which, on the one hand, impregnates the horizon matrix, and on the other hand it appears as efflorescences (coatings) on the walls of the poral space.

The presence of calcium carbonate was also reported by the macroscopic description in the field, where frequent $CaCO_3$ pseudomycelium has been observed.

At the sub-microscopic level (Figure 8) it can be noted that these efflorescences coatings are made up of acicular crystals (needle from calcite), also called *lublinite*. This type of acicular calcite grows up from the saturated solutions, when the soil moisture rapidly changes.

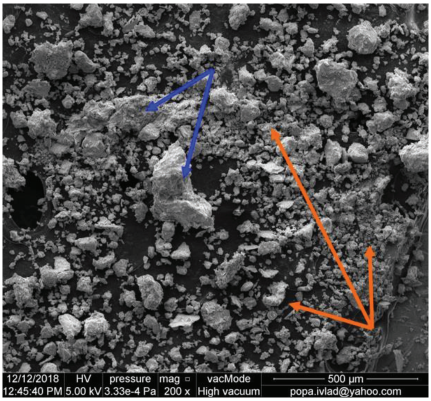

Figure 7. SEM image of Ck$_2$ (96-124 cm) horizon: poorly developed and non-durable structural elements (→) by the side of un-aggregated coarse material (→) (200 X)

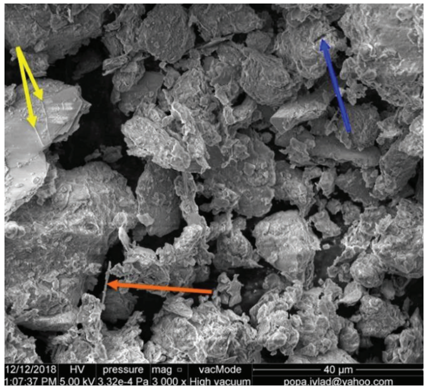

Figure 8. SEM image of Ck$_2$ (96-124 cm) horizon: acicular calcite crystal (→); fungi mycelium on a platy mica grain (→); fissured calcite and with local recrystallizations (→) (3000 X)

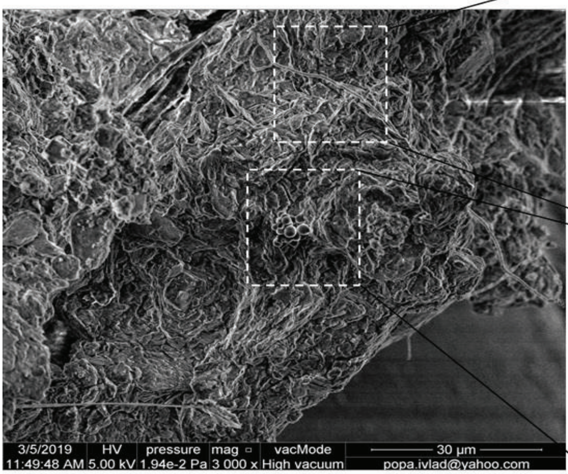

Figure 9. SEM image of Am (0-37 cm) horizon: a highly decomposed vegetal residue (3000 X)

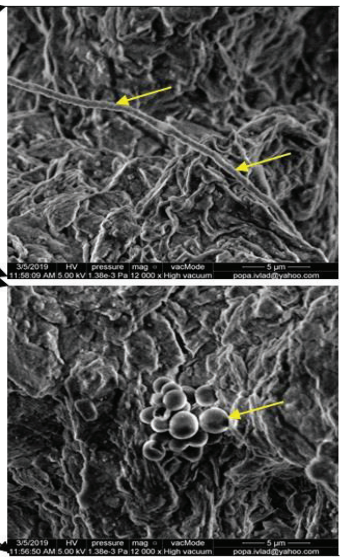

Figure 10. Details of figure 9: microorganisms (→) on the decomposed vegetal residue (12000 X)

Into the soil profile the microorganism activity was also detected, mainly on highly decomposed vegetal remains (Figures 9 and 10).

The presence of different species of microorganisms, showed the biological diversity of the soil and their high activity, which leads the decomposition processes, as well as the release of the nutrients into the soil and the humic substances accumulation.

The data of the physical and chemical characteristics, together with the detail observations of the soil constituent fabric at sub-microscopic scale reveal that Aluviosol Molic Calcaric environment is favorable to the development of the microbial bioinoculants.

CONCLUSIONS

The results obtained with the help of the scanning electron microscope (SEM) following the study of an Aluviosol Molic Calcaric from an organic agroecosystems before the treatment with microbial bioinoculants revealed detailed aspects related to the spatial organization of the soil at sub-microscale.

The high magnification (until 6000 X) allowed the visualization of the soil elementary fabric into the peds.

Even if the SEM images are in the greyscale, the skeleton grains were detected according their specific characteristics (type of twinning, foliated texture etc.).

Many skeleton grains are weathered and partially covered with secondary alteration products (clay, Fe etc.), whose presence favours the development of microorganisms on the faces of the mineral grains.

The study of the behaviour of $CaCO_3$ in the soil pedological horizons showed the presence of the different types of calcite crystals: crypto-crystals (embedded into the soil matrix), acicular crystals (on the pore walls) etc.

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