EFFECT OF SILVER NANOPARTICLE AND ORGANIC BIOSTIMULATOR NITROZIME OVER MICROPORES AND GERMINATION, GROWTH AND DEVELOPMENT OF SEEDS OF TRITICUM AESTIVUM TO LEVEL OF A BIO ACCUMULATIVE HORIZON TYPE CAMBIC SOIL

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Abstract

The present paper monitors the effect of applying different doses of silver nanoparticles (10 ppm, 15 ppm, 20 ppm), and the effect of organic biostimulator Nitrozimes (2ml/l) on the germination, growing and development of the Triticum aestivum species. The changes suffered by the bioaccumulation horizon of the cambic chernozem at micro porous level is also investigated. The silver nanoparticle solution is obtained through electrolysis technique in miliQ water solvent using Nevoton IS-112 device with silver electrodes of 99.99% purity. The determination of the ppm quantity is realized by using TDS-1. The seed germination is tracked in a controlled environment (Petri dishes) in laboratory conditions for 10 days until germination. After, they are transferred in vegetation dishes in the grow house and tracked in a controlled environment of temperature, humidity and soil pH. Data on the micro porosity was taken and analyzed at I.C.A.M. Laboratories in the V. Adamachi resort using micro computed tomography (Brucker Skyscan 1172). The germination evolution, growth and development of the wheat plants was measured weekly throughout the growing season. The results show a reduction in the diameter of the micropores that is inversely proportional to the concentration of silver ions doses applied. From the germination phase until 2-3 leafs stage, the growth is approximately double than the development of the witness plants (untreated wheat grown in bioaccumulative horizon).

Key words: silver nanoparticles, biostimulator, soil structure.

Silver nanoparticles (AgNPs) are groups of atoms that have sizes between 1 nm and 100 nm. (Williams G. *et al*, 2008). The prefix nano indicates a billionth of an mm or 10^{-9}. The more the particle size is reduced the relation between volume and surface increases. Silver nanoparticles are present in natural ecosystems, including the soil which can penetrate through various channels, with major impact for soil microorganisms and plant organisms, it changes the background of complex chemical and biological nature.

Because bio-solids contaminated by AgNPs may be applied to a variety of soils, understanding the factors that influence Ag NP toxicity will be important in determining the risk that Ag NPs pose to soil systems.

To optimize the performance of target applications is the importance of size, shape, surface and state of aggregation of nanoparticles. (Yeo *et al*, 2003, Zhang *et al*, 2006, Chimentao *et al*, 2004).

It is recommended the use of monodisperse nanoparticles that are free of agglomeration (Sudrik *et al*, 2006, Sun *et al*, 2000, Vilchis N. *et al*, 2008). Nano particle characterization is done by transmission electron microscopy (TEM) analysis of particle size by dynamic light scattering, UV visible spectrum analysis, as well as Zeta potential measurements.

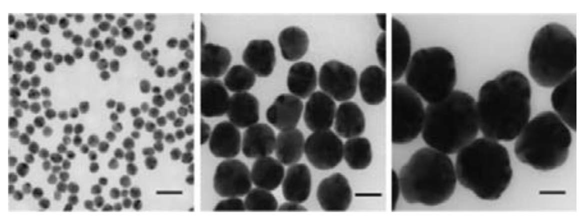

Figure 1 **Transmission electron microscopy (TEM) images of silver nanoparticles with diameters of 20 nm (Aldrich Prod. No. 730793), 60 nm (Aldrich Prod. No. 730815), and 100 nm (Aldrich Prod. No. 730777) respectively. Scale bars are 50 nm.**

Compared to the size of nanoparticles of silver, soil type is a more important factor in accumulating them. (Aaron W. *et al*, 2011).

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At worldwide level, the use of silver as a result of exploitation of this mineral native uncontrolled determine its penetration into the environment, especially in the aquatic systems. Silver nanoparticles are relatively stable in liquid medium, having the opportunity to be present and to accumulate in the soil and in different amounts (ppm). (Korbekandi H. *et al*, 2012).

Systematic application of AgNPs doses at a level horizon bio-accumulative, as do have silver ions to accumulate over time. Extended x-ray absorption fine structure spectroscopy analysis of the horizon indicated that the Ag was approximately 1 to 3% Ag (I), suggesting that Ag ions may be responsible for effects on growth and development caused by exposure to Ag NPs.

The soil due to his state of dispersion, its components, especially those with colloidal character has the ability to adsorb minerals in different forms: molecular and ionic state of dispersion. Humus and clay soil colloids have respective main character electronegative, thus having the ability to adsorb to the surface layer diffuse different cations present in the soil solution (Puiu S., 1980).

On the horizon bioaccumulate, silver nanoparticles (present following an involuntary anthropogenic pollution or as a result of soil formation and evolution of the deposits of silver) form different combinations of ions Cl, SO_4, NO_3, O, CO_2.

CO_2 conversion rate per unit area is about 10 times larger silver nanoparticles on the support of 5 nm monolithic than in the case of silver. Increasing the concentration of $AgNO_3$ affect nanoparticle form. (Zhu J. *et al*, 2000).

Silver nanoparticles highlights a broad spectrum biocidal all classes of organisms and microorganisms. The toxic effects of silver nanoparticles has been scientifically demonstrated in different species of plant and animal micro-organisms (Popescu M. *et al*, 2010), invertebrates (Baruwati B. *et al*, 2009), plants (Elghanian R. *et al*, 1997), and if human cells (Hurst S.J. *et al*, 2006, Tran Q.H. et al, 2013), because it the fact that silver nanoparticles possess physico-chemical distinctive properties compared to bulk Ag. *(Naser A. et al, 2013)*.

Unstabilized silver nanoparticles suffers a rapid oxidation and a slight aggregation in solution. (Popa I. *et al*, 2015). In presence of soil moisture and oxygen, a process of oxidative degradation silver ions lose an electron, electron actually intervening in inhibiting the multiplication of bacteria and normal development. (Ramyal M. *et al*, 2012). Antifungal and antibacterial characteristics of silver nanoparticles are still poorly clarified.

Stress caused by applying a treatment with AgNPs indicate a nanoparticle accumulation in the soil, roots, as well as a translocation them to the air. (Gorczyca A. *et al*, 2015).

This study material is trying to approach early, multidisciplinary changes, accumulation, toxicity potential and the cumulative impact on the level of state structural horizon bioaccumulative (Am) soil type chernozem cambic, as well as on the physiology of the species *Triticum aestivum*, following by applying a voluntary human intervention during germination and along the course of vegetation doses of silver nanoparticles and organic bio-stimulator Nitrozime.

MATERIAL AND METHOD

We choosed a representative location (V.Adamachi farm), with soil profile depth 1.5-2m on which we made the description of the indicators presented in the methodology of writing pedomorfological soil studies. The sample of soil for laboratory analysis (soil texture, morphological type of structure, size of the structural elements, formations of chemical and biological nature, content of organic matter calcium carbonate) was prelevated from each horizon bottom on top to avoid contamination of soil material collected.

The silver nanoparticle solution is obtained through electrolysis technique in miliQ water solvent using Nevoton IS-112 device with silver electrodes of 99.99% purity. The determination of the ppm quantity is realized by using TDS-1. The seed germination is tracked in a controlled environment (Petri vessels) in laboratory conditions for 10 days until germination. After, they are transferred in vegetation vessels in the grow house and tracked in a controlled environment of temperature, humidity and soil pH. Data on the micro porosity was taken and analysed using micro computed tomography (Brucker Skyscan 1172).

REZULTS AND DISCUSSIONS

The soil contents of humus accumulation horizon (Am), with high thickness (45 cm) followed by Bv color cambic horizon and C horizon with accumulation of calcium carbonate.

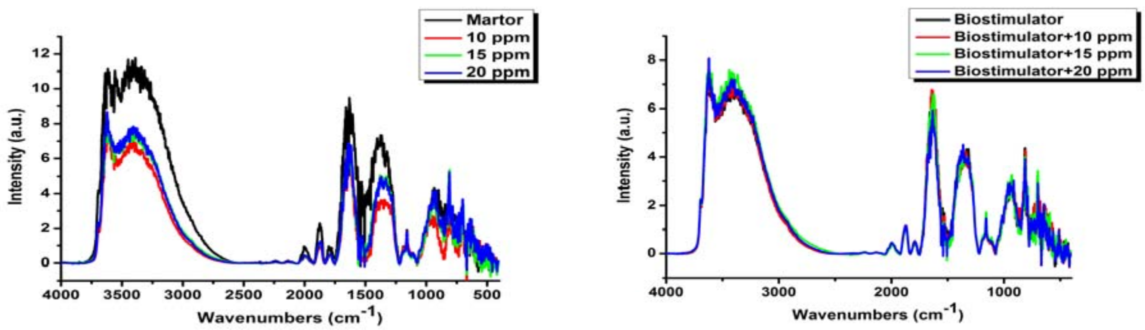
Reserve humus and nutrients is high (3.57g/%) with H/F = 2.4, the maximum content of calcium carbonate is recorded in C horizon. 15.7 g/%, CaCO3 ground presence weak to moderately alkaline reaction (pH – 7.98).

Pedogenetical horizons sequence shown is specific soil type. Edaphic large volume useful and good aero-hydric regime.

Relatively uniform soil matrix color indicates that the soil is not affected by stagnant excess moisture. Angular polyhedral structure and the uneven distribution of roots that are located preferential faces structural elements

Concerning the chemical properties of structural horizon bioaccumulative (Am) soil type chernozem cambic, the FTIR spectra shows the presence of OH group in range of 3000–3700 cm^{-1}, C=O group at 1869 and 1790 cm^{-1}, C=C at 1624 cm^{-1}, C-H, C-N and N-H in range of 1227-1500 cm^{-1} and C-O at 1161 and 953 cm^{-1} (Figure 2 (a) and (b)).

All the identified peaks are presents in martor soil and also in martor plus biostimulator. After the introducing the AgNPs, at different concentrations, for the martor soil, the intensity for all beaks are decreasing, which means the AgNPs create chemical reactions with molecules presented in soil (Figure 2 (a)). For the soil treated also wit Nitrozime biostimulator, the intensity of line are in the same range, which means that the biostimolator molecules are covered the AgNPs, but does not affesct the chemical structure of soil (Figure 2 (b)).



(a) (b)

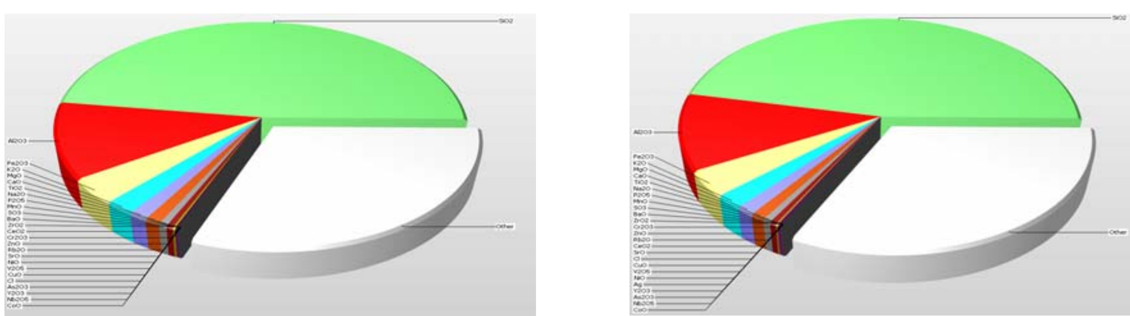
Figure 2 **Typical FTIR spectrum of martor soil and martor soil plus silver nanoparticles (a) martor pus bitstimulator soil and martor soil plus bitstimulator and silver nanoparticles (b)**

Also, this effect was demonstrated with X-ray fluorescence spectroscopy, where the concentrations of minor and major elements remains at the same range (Figure 3 (a) and (b)).

In terms of Ag^{2+} nanoparticles, the concentration are grows and accumulates in soil when the silver solution is added. The maximum concentration of silver is approximatively 50 ppm, for martor soil when added 15 and 20 ppm silver solution and 40 ppm for martor plus Nitrozime biostimulator (2ml/l) soil when added 15 and 20 ppm silver solution every week (Figure 4).

The micro porosity results show a reduction with more than 50% in the diameter of the micropores that is inversely proportional to the concentration of silver ions doses applied (Figure 5 (a) and (b)).

Concerning the total porosity of soil, this are decreasing also when the soil are treated with silver nanoparticles and Nitrozime biostimulator (2ml/l) plus biostimulator. This can be explained by reducing or blocking of the soil pores by the silver nanoparticles and biostimulator aggregates (Figure 6 and Figure 7).



(a) (b)

Figure 3 **XRF results of martor soil and martor soil plus silver nanoparticles (a) martor pus bitstimulator soil and martor soil plus bitstimulator and silver nanoparticles (b)**

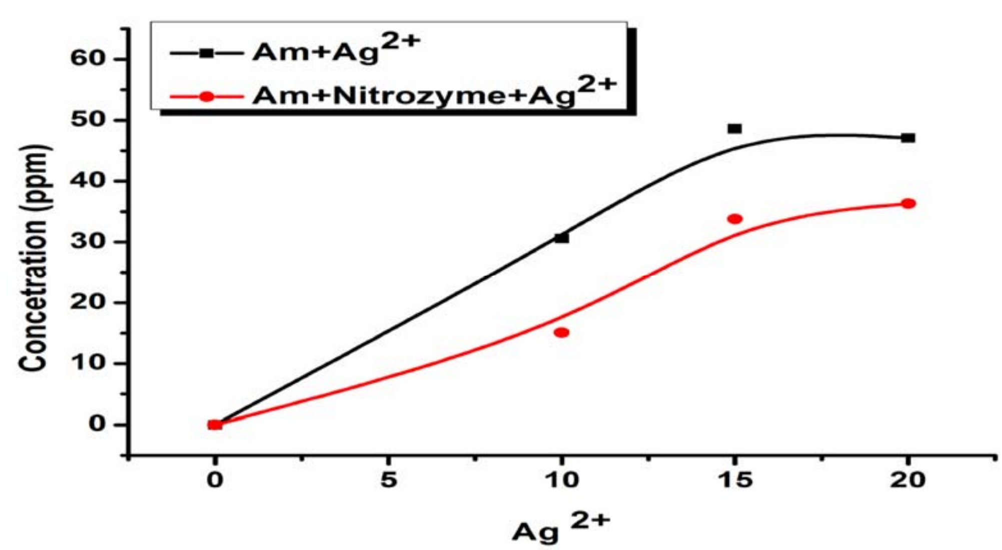
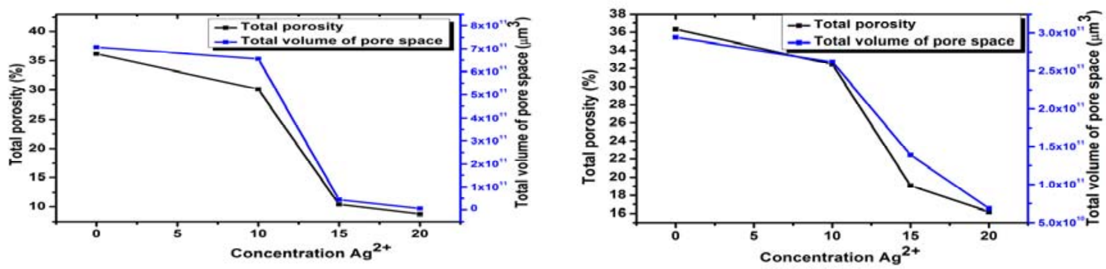


Figure 4 **XRF results of silver nanoparticles concentration martor pus bitstimulator soil and martor soil plus bitstimulator and silver nanoparticles at different concentration (b)**



(a) (b)

Figure 5 **Micro porosity results for martor soil and martor soil plus silver nanoparticles at different concentration (a) martor pus bitstimulator soil and martor soil plus bitstimulator and silver nanoparticles at different concentration (b)**

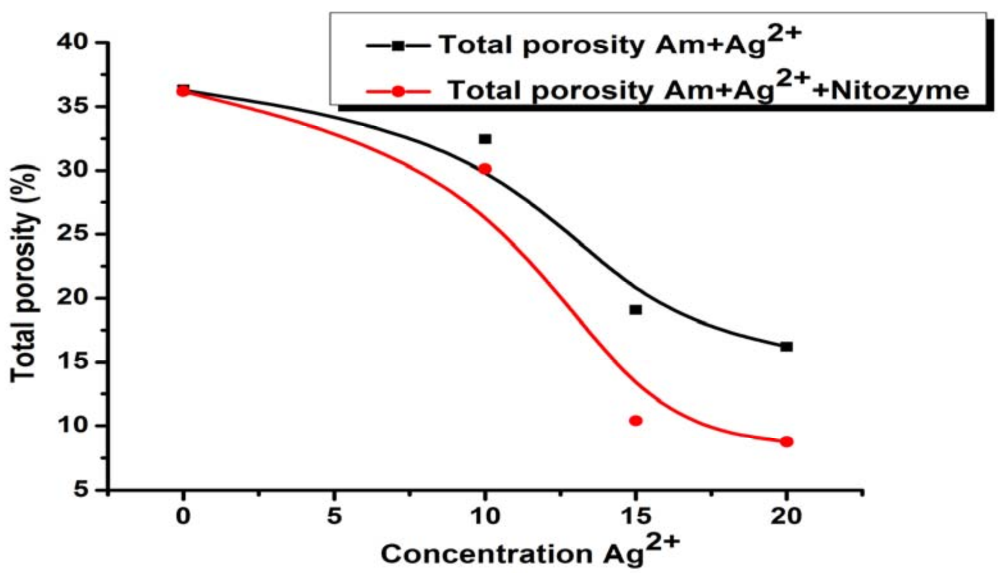
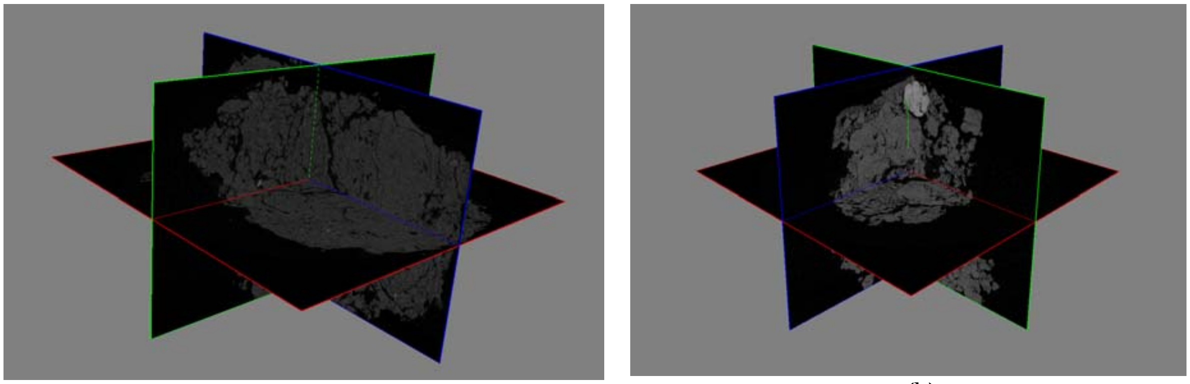


Figure 6 **Micro porosity results for martor soil and martor soil plus silver nanoparticles and bitstimulator at different concentration**



(a) (b)

Figure 7 **Micro CT image for martor soil (a) and martor soil plus silver nanoparticles at concentration 20 ppm (b)**

Figure 8 **Germination process in Petri dishes**

The effect of different doses of AgNPs (ppm) Ag electrodes produced by electrolysis of purity 99.9% with a voltage of 48.5 V with a current intensity of 100 mA continuous, on germination and growth of the species Triticum aestivum was analysed. Germination analysis indicates a value of 90% in the contextual variant, which was not intervened with anthropogenic AgNPs, through a strong saprophyte attack which was approximately 95%. Comparing versions AgNPs, is highlighted keep the germination of 90-95% dose for 15 ppm, with the presence of a small green fungal disease. Germination decreases with about 35% in solution with 20 ppm, while the dose with 10 ppm mentions a germination of 60% with the presence of a small indigo fungal disease. Germinated embryos were transferred into pots vegetation, continues the weekly treatment with AgNPs (*figure 8*).

CONCLUSIONS

Treatment with nanoparticles produced a major disintegration of cell membranes made of seeds germinated in Petri dishes, an effect emphasized by their low rate of germination. Contextually transfer germinated seeds in vegetation vessel and continued treatment with AgNPs vegetation, affect plant height and a quicker maturity with a decrease in production, reflected in lower thousand grain weight.

The applied of different AgNPS doses, lead to an increase of 47% AgNPs concentration, concentration which decrease to 33% after applying of organic biostimulator Nitrozime.

The stress can be counteract on the mass of microorganisms after applying voluntary AgNPs may be diminished in weakly alkaline conditions of neutral pH and the presence of a calcic mull type humus quality in quantity at least 3-4 g% g soil, a question supply of 160-200 t / ha on a depth of 0 -50 cm.

The stress applying AgNPs treatment indicate an accumulation of nanoparticles in soil, roots, and how their translocation to the airline.

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