

Full Length Research**Leaching and *in vitro* agrochemical screening for new slow release fertilizers containing N, P, Ca, and Mg****Constantin Neamțu*¹, Mariana Popescu², Ștefan-Ovidiu Dima³**^{1,2,3} National Research and Development Institute for Chemistry and Petrochemistry ICECHIM, 202 Splaiul Independentei, 060021 Bucharest, Romania.*Corresponding Author's E-mail: phd.ovidiu.dima@gmail.com

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New slow release fertilizers containing N, P, Ca, and Mg as active nutrients were synthesized by micro-encapsulation in urea-formaldehyde systems. The main objective was to determine the slow-release properties by subjecting these new fertilizers to leaching tests and *in vitro* experiments on *Arabidopsis thaliana*. These incipient results were found to be extremely promising and will be followed by *in vivo* studies on Romanian open fields in order to evaluate the fertilizers' capacity in open environment. This newly developed solid polymer microstructure will facilitate the slow/controlled release of the nutrients and will ensure a total absorption of the active compounds in cultivated plants. It was found that the degree of nitrogen leaching depends on the solubility of controlled fertilizer, being 61.19% for the system with N-P-Ca nutrients, and 31.97% for the system with N-P-Mg nutrients. The soil conductivity variations in time and with the type of fertilizer had a good correlation through second order equation models. The main advantage is that by avoiding the loss of chemicals in soil, water, and air, these new N-P-Ca-Mg fertilizers will ensure also the reducing of the wastes and contaminants (like nitrates, nitrosamines) in agro-systems and also the protection of the biosphere and consumers' health.

Keywords: slow release, fertilizer, urea-formaldehyde, N-P-Ca-Mg, *Arabidopsis thaliana*

INTRODUCTION

Agriculture is one of the major priorities throughout the world (Beer, 2015; Stoett and Temby, 2015; Vaz Patto et al., 2015) and of Romanian economy also, being an essential factor in the national development medium term strategy (Burja, 2014; Dobre and Soare, 2013). That is why the quantitative and qualitative increasing of crop production represents an imperative objective for the sustainable development of the national economy on medium and long term.

The agriculture's recovery involves, along with mechanization and irrigation, the obtaining of new crops'

varieties and their chemical processing, which requires the recovery of fertilizers' industry and of adjacent industries. The agriculture's chemisation is conditioning the increasing of chemical fertilizers production and their diversification.

The use of fertilizers is the decisive factor in agricultural production by increasing soil fertility and increasing crop yields. After approximate calculations, in the last 30-40 years, 50% of the increase in world agricultural production was ensured by using chemical fertilizers. According to Food and Agriculture Organization (FAO)

(Trenkel, 1997), the introduction of 1 kg of nutrient (N, P, K) ensures an average harvest increase of 7.3 kg wheat, 8.5 kg rice, 8.8 kg maize, 5.5 kg soybean, 7 kg cotton, etc (Moore, 1987).

After assessments of American specialists, crop yields are influenced by many factors such as: chemical fertilizers (41%), herbicides (15-20%), soil quality (15%), hybrid seeds (8%), irrigation (5%) and other factors (11-18%).

A number of experts EFMA (European Fertilizers Manufacturers Association) undertook a thorough analysis of the data in order to provide a reliable estimate on the future development of agriculture and the use of fertilizers in the next 10 years in the European Union (EFMA Report 2012). According to the chart below, it can be seen a significant growth estimate (3 to 18%) in the consumption of N, P and K nutrients for next years (until 2020) in the European Union. Figure 1

According to standard EN 13266/2001 (U.S. Patent, 2005), a fertilizer can be described as reduced release or slow-release, if the containing nutrient (or nutrients) meets each of the following three criteria:

- a release of not more than 15% within 24 hours;
- a release of not more than 75% within 28 days;
- a release of 75% for the entire period of use established.

The three main types of fertilizers with low release and controlled release are:

1. Condensation products of urea with various aldehydes materialized as slow-acting fertilizer (urea-formaldehyde fertilizer, urea-crotonaldehyde, isobutylene-dicarbamide, etc.).
2. Encapsulated or coated fertilizers, with a controlled rate of macroelements releasing. These types of fertilizers are obtained, in general, by physical-mechanical coatings of the sulfur containing fertilizer particles, by synthetic coatings, or with additives of nitrification inhibitors.
3. Of a less importance are the forms of conditioning by agglomeration-compaction (like granules, lighters etc.).

The obtaining and using of new materials must always take into consideration the environment aspects (Chirag et al., 2015; Dima et al., 2009; Mara et al., 2011; Hadlocon et al., 2015; Dima et al., 2013; Ren et al., 2014). The proposed slow-release fertilizers will contribute to the development of sustainable agricultural systems, diversified and balanced, ensuring the protection of natural resources and of consumers' health, simultaneously with the reducing of wastes and

contaminants from crops and environmental protection by obtaining fertilizing formulations with superior efficacy and dose significantly reduced relative to the classic granulated fertilizers. These fertilizers were subjected to leaching tests and *in vitro* experiments, being known that this kind of experiments are mandatory (Langroudi and Abdossi, 2015; Maitra et al., 2015; Jia et al., 2015).

MATERIALS AND METHODS

This type of fertilizer has as main property a slow and gradually nutrient releasing, preventing the leaching from the 0-60 cm soil layer, where most of the plant roots are spread. That is why it was aimed to determine the degree of leaching in an intensive pluviometer regime. The high content in humus and N, P, K elements make the soil to be reactive, which is why the microencapsulated and coated fertilizers must be tested on a sandy soil with very low humus and nutrients content, ensuring a neutral medium. In order to perform the biological activity and leaching tests, it is necessary for fertilizer's microcapsules to undergo a process of "forming-agglomeration", using a series of specific ingredients.

The preliminary tests were focused on both physical and chemical properties of fertilizers with controlled release properties in order to assess the rate of nutrients' leaching and their agrochemical properties. To assess this, the experiments aimed to determine the total concentration of nutrients in fertilizers and the concentration of mobile species according to standard SR EN 13266/2002 on "slow release fertilizers and nutrients' solubilization". Also it was conducted an agrochemical *in vitro* experiment at laboratory scale in order to follow the growing of *Arabidopsis thaliana* species and to monitor the soil parameters after reaching its maturity.

Determining of leaching degree of N-P-Ca-Mg fertilizers on sandy soil

The determination of nutrients leaching from the obtained slow release fertilizers will be made according to the standard CEN / TR 14405/2009 "Characterization of waste, leaching behavior tests, upflow percolation test (SR CEN/TS 14405, 2009) and through sandy soil leaching columns. More exactly, an amount of 10 g N-P-Ca-Mg fertilizer (crushed to destroy the film), prepared as described in a previous work (Tolescu et. al, 2011) was transferred into 500 ml third quality water, according to EN ISO 3696 standard, and stirred for 24 h at a speed of 300 rot/min. The beaker was placed on a plate with magnetic stirrer and temperature control and was covered with a lid to prevent water evaporation and to keep the temperature at $25 \pm 0.5^\circ\text{C}$. After 24 hours,

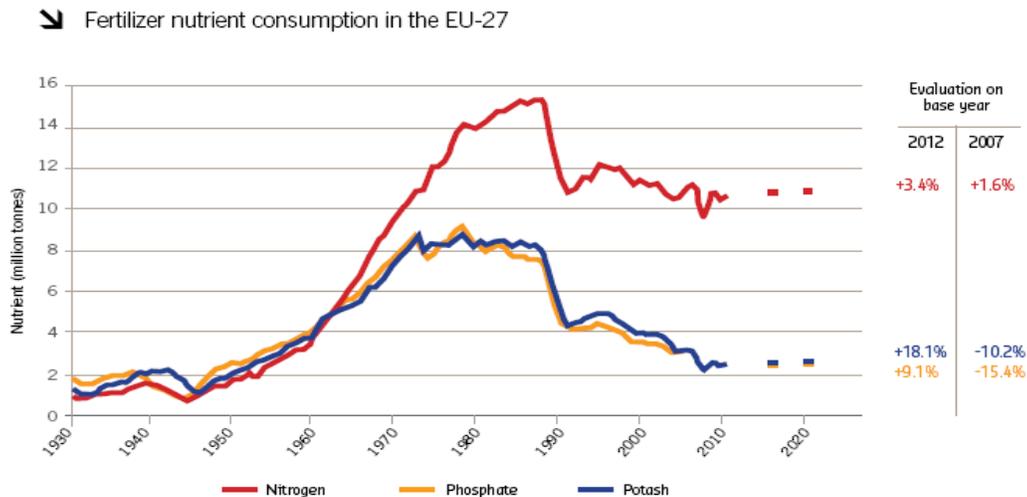


Figure1. Developments and forecasts of fertilizer consumption in the EU (EFMA Report 2012).

aliquots were carefully taken to avoid any undissolved particles and to determine the nutrient concentration following standard methods of analysis. The leaching degree of N-P-Ca-Mg fertilizers on sandy soil columns was determined by following the next steps. It was prepared an installation consisting of glass cylinders, having the dimensions of length x diameter LxD 60x20 cm, with an internal area of 314 cm² and a collector at the base. The cylinders were filled with sandy soil and 20 g of fertilizers were placed at the top and spread up to a depth of 20 cm. After that, the soil was gradually washed and the percolation water was collected and analyzed. Weakly acid, with high permeability and very low humus content and nutrients, sandy soil meets the optimum qualities for determining the leaching degree of synthesized fertilizers. The irrigation that produces the leaching of nutrients was reproduced at lab scale taking into account the maximum norms specified for main crops, meaning 4000-5000 m³ water / ha in 6-10 sprays of 500-800 m³/ha or 60 L/m² in 8 sprays. And since the cylinder's area is approx. 0.031 m², it was considered that a quantity of 1.8 L demineralized water, sprayed 8 times during 8 days, will reproduce well the field conditions. At the end of each eight days cycle, the water that percolated the sand layer was measured and analyzed to determine the degree of leaching expressed by percentage as the amount of leachate element in grams divided at the initial amount, in grams.

Experimental set up for *in vitro* tests

The main property of slow release fertilizers is to ensure the plant nutrients through a gradually and slow leaching

on a period significantly longer than conventional mineral fertilizers. In this way the plants benefit from an improved nutrition on a longer cycle and provide higher production increases due to a higher coefficient of nutrients' recovery. This property should be assessed by agrochemical *in vitro* and *in vivo* experiments, meaning in a vegetation house and on the field, together with leaching tests, solubility tests, and physical-chemical analyzes of the fertilizers. Also, amounts of nutrients from slow release fertilizers are leached (in the case of N), downgraded by reactions with Ca in soil (especially P) or degraded by absorption in soil.

The reducing of wastes and contaminants in agro-systems represents a never ending and crucial objective (Hadlocon et al., 2015; Mehta et al., 2015). With these new slow release fertilizers, the generally recommended doses of fertilizers can be reduced without impact on the productivity and, subsequently, the chemical pollution of the soil due to fertilization is substantially reduced.

To organize the agrochemical *in vitro* experiments, *Arabidopsis thaliana* was chosen because this plant is one of the most important models in biology research. It was first used in the early 40's for genetic studies because of the ease with which mutant plants could be created. Later in the 80's began to be used on further studies of physiology and biochemistry. The main reasons for which this plant gained a huge expansion in biological studies are:

- Has a life cycle relatively short, of about 8 weeks;
- It produces many seeds;
- Has a small genome, which has been completely sequenced, allowing researchers to

Table 1. Determined physical properties for the experimental fertilizers.

Fertilizer	pH	Conductivity, μS/cm	N, %	P ₂ O ₅ (%)	Mg (%)	Ca (%)
SR-N	7.24	1422	26.31			
SR-N-P-Mg	8.32	1619	6.00	1.920	0.152	
SR-N-P-Ca	7.98	7590	6.89	0.220		1.420

isolate sequences of interest;

- It has detailed genetic maps of each chromosome, which allows the isolation of various genes for various studies;
- There are numerous lines of mutants, allowing the study of the effect of lack or excess of certain genes;
- The plant can be easily transformed by infection with *Agrobacterium tumefaciens*, which allows easy introduction of new genes in the genome.

From the structural point of view, *Arabidopsis* is a very simple plant. The seeds have about 1 mm in diameter and 50 seeds weigh about 1 mg. Their color varies from light brown to dark brown. After germination, it initially produces a rod with two cotyledon leaves, and then forms a rosette of about 20 leaves near to the ground. From this rosette, the plant forms more flower stems, 20-25 cm tall, which at the base can still have leaves. The flowers are small, white, commonly grouped in the top of the flower stem. The plants have usually 10 to 12 flowers. *Arabidopsis*'s fruit is a capsule, inside of which there are the seeds. Depending on the plant, it can produce up to 600 seeds.

Preparing the *in vitro* experiments

30 flower pots were filled with 400 g soil for which it was determined a field capacity of approx. 20-22%. At approx. 1 cm depth were applied the experimental fertilizers in amount of approx. 100 mg, after which they were watered to field capacity.

On filter paper discs placed in sterilized Petri dishes were put for germination seeds of *Arabidopsis thaliana* and then moistened with a solution of liquid fertilizer 0.01% Plantfert U, covered with filter paper rings and left in darkness at normal temperature (25 – 27°C).

After 5 days, the sprouted seeds were transferred to pots ready for growing, according to a randomization scheme in triplicates, including a control sample with unfertilized soil. The root of approx. 1 cm is placed on the moist soil surface, on which were added a few drops of distilled water, then a layer of dry soil of approx. 1-1.5 mm, easily moisten afterwards.

The vegetation conditions were: temperature 20.6°C –

23.6°C, relative humidity 36-41%, luminosity over 6000 lux for 12 h/day. The plants were watered at a 48 h interval.

In the growing pots, a multiparameter sensor was installed to monitor and record with a frequency of one record / min the values of temperature, humidity, and electrical conductivity.

RESULTS AND DISCUSSIONS

The percentage content of the N-P-Ca fertilizer was: N 6.89%, P 0.22%, Ca 1.42%, pH 7.98, conductivity 7590 μS/cm. The evolution of the mobile forms, meaning the total content of extractable nutrients is strongly influenced by the nature of fertilizer's matrix and less by the method of obtaining controlled release fertilizer. Extractable forms ranged from 6 to 26.31% for N, 0.22 to 1.92% in the case of phosphorus (calculated as P₂O₅), 0.15 to 1.42% for Mg and Ca. Iron concentration was below the detection limit.

For experimental fertilizers were determined the physical properties, namely pH and electrical conductivity, important features in the subsequent evolution of fertilizer incorporated into the soil. The results are shown in Table 1. The obtained data allowed the calculation and prediction of nutrients solubility of the tested slow release fertilizers.

The nitrogen leaching degree ranged from 31.97% to 65.51% for SR-N-P-Mg and SR-N-P-Ca. The degree of nitrogen leaching depends on the solubility of controlled fertilizer, being 65.51% for SR-N, 61.19% for SR-N-P-Ca, and 31.97% SR-N-P-Mg. The degree of phosphorus leaching ranged between 0.78% in SR-N-P-Ca and 5.88 in SR-N-P-Mg (Figure 2 and 3). The meso-elements calcium and magnesium showed a low leaching rate, of 11.3% for Ca and 1.7% for Mg.

To realize both the agrochemical experiments and the preliminary leaching tests, preluvosoil reddish clay loam was used, having the characteristics listed in Tables 2-4.

To achieve the preliminary leaching tests of nutrients from the tested fertilizers, the soil was dried at room temperature, grinded and passed through a sieve of 0.2 mm. Amounts of 25 g of soil for each fraction were weighed into 12 vials, special for centrifugation, and afterwards were added 12 g of water, keeping the soil: water ratio 1:0.5, and 0.125 g experimental fertilizer. Additionally was prepared a control sample without

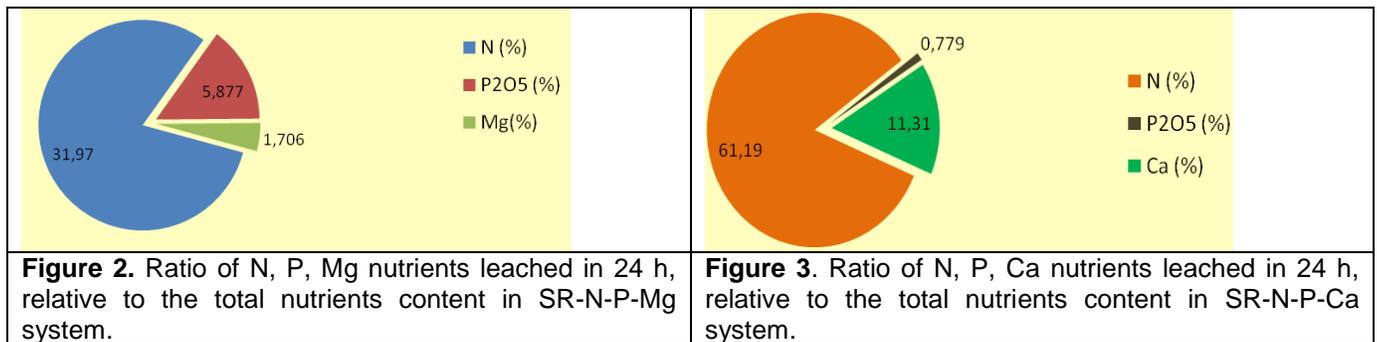


Table 2. Analytical results for tested soil.

pH	Humu s (%)	Nt (%)	C/N	P _{AL} (mg*hg ⁻¹)	K _{AL} (mg*hg ⁻¹)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)
6.65	2.70	0.148	12.3	62	151	8.52	9.07	56	47

Table 3. Properties of cationic exchange.

pH	SB (me/100g sol)	Ah (me/100g sol)	T=SB+Ah (me/100g sol)	V _{Ah} %	CTSS* (mg/100g sol)
6.65	20.33	1.94	22.27	91.3	56

*CTSS- content of total soluble salts

Table 4. Analytical results regarding the soil texture.

Granulometric fractions (mm), (% from mineral content of soil)											
Coarse sand				Fine sand			Dust	Clay		Carbonates	
2.0-0.2	2.0-1.0	1.0-0.5	0.5-0.2	0.2-0.1	0.1-0.05	0.05-0.02	0.02-0.002	<0.002	<0.01	%	
2.2	0.8	0.7	0.7	34.6	0.9	0.0	33.7	32.3	30.9	47.0	0.0

fertilizer. The samples were stirred and homogenized, and afterwards the components were extracted in aqueous solution by stirring using a rotating stirrer at a speed of 50 rot / min. Fractions were collected from the soil solution at intervals of one hour for a period of 7 hours. The fractions were separated by centrifugation at 3000 rot / min for 5 min, and the resulting solution was filtered on a blue band filter paper. The filtrate was submitted to physical-chemical analysis of pH, electrical conductivity, and elements content (N, P, Ca, and Mg). The results and the evolution of physical parameters are shown in Figures 4 and 5.

Referring to Figure 4, the conductivity variation of extraction solutions for the control sample, containing only demineralized water and soil, increase with the time

of extraction, except the extract obtained after 4 hours which has a value slightly lower than the previous one.

For the extracted sample containing the experimental fertilizer type SR-N it is observed in Figure 5 an increasing evolution of the conductivity value based on the extraction time due to solubilization and leaching of nutrients from fertilizer and because of physical-chemical processes at the level of clay-humic complex.

For the soil with SR-N-P-Mg slow-release fertilizers, the tendency is of conductivity increasing versus time. The physical and chemical phenomena that occur in soil lead to an increase in conductivity up to 4 hours of extraction, followed by a slight decrease after 5 to 6 hours, the highest value being obtained after 7 hours (Figure 6).

The conductivity variation in extracts from fertilizer SR-

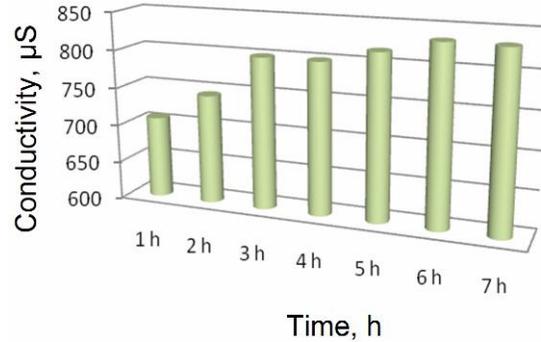


Fig. 4. The variation of electric conductivity in control sample.

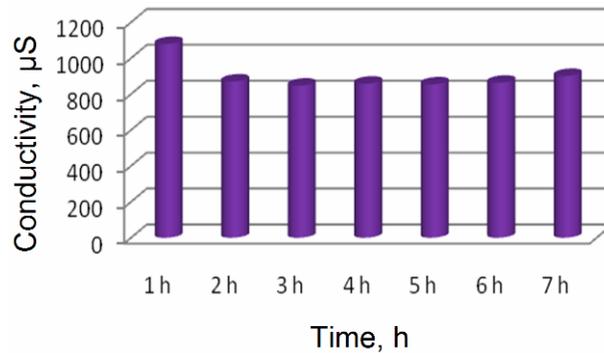
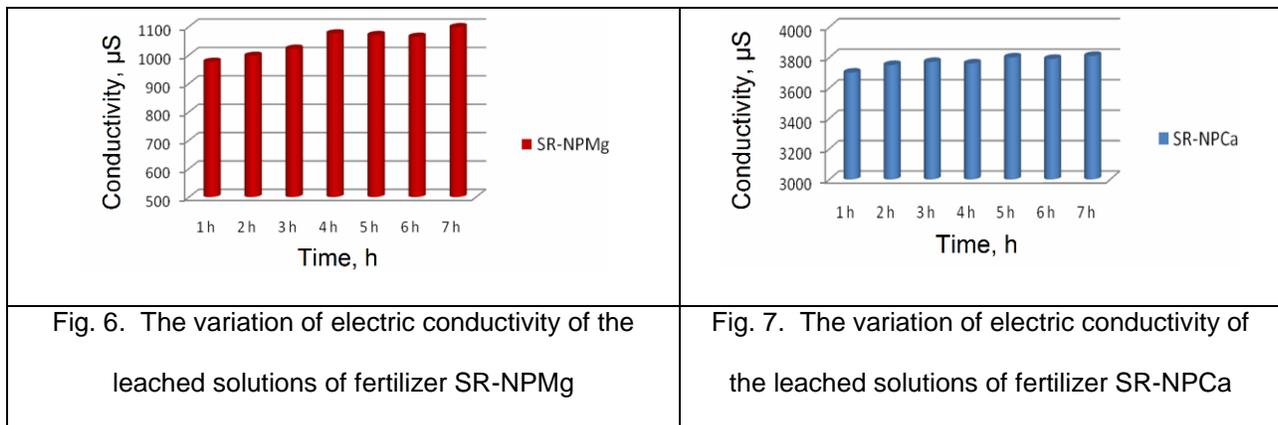


Fig. 5. The variation of electric conductivity in fertilizer SR-N.



N-P-Ca is very small and is around 3700 $\mu\text{S} / \text{cm}$ for the extract obtained after 1 h and 3800 $\mu\text{S} / \text{cm}$ for the one obtained after 7 hours of extraction. The values at times 5, 6 and 7 had minor variations, only 10 $\mu\text{S} / \text{cm}$ (Figure 7).

From the analyzes performed on the soil used in this experiment it can be deduced that the soil is poorly feed

in macro and micronutrients. Therefore, to observe the contribution due to fertilizers solubilization, the differences between fertilizers and compared with the control sample were determined.

Looking at Figure 8 and 9 it can be seen that the biggest difference between the conductivity of the solution (maximum value - minimum value) after

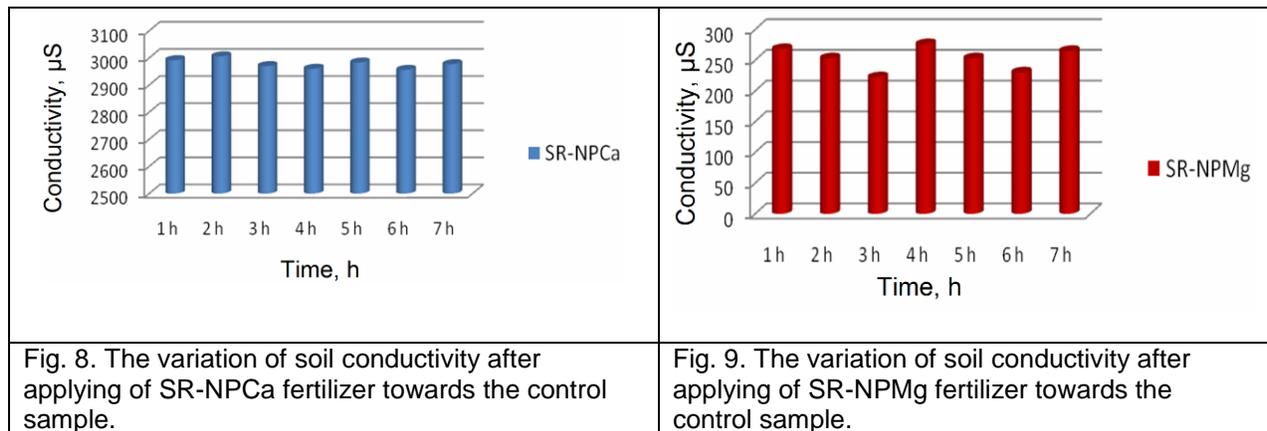


Fig. 8. The variation of soil conductivity after applying of SR-NPCa fertilizer towards the control sample.

Fig. 9. The variation of soil conductivity after applying of SR-NPMg fertilizer towards the control sample.

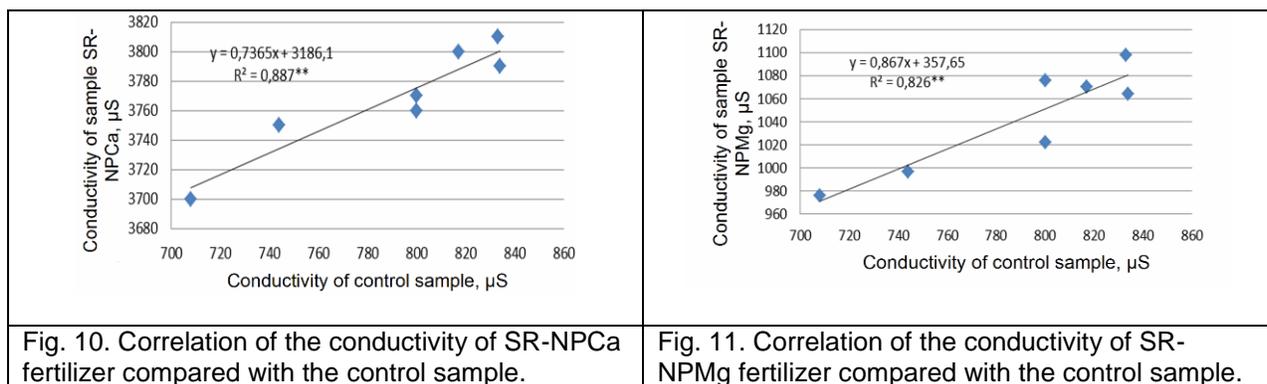


Fig. 10. Correlation of the conductivity of SR-NPCa fertilizer compared with the control sample.

Fig. 11. Correlation of the conductivity of SR-NPMg fertilizer compared with the control sample.

subtracting the contribution of control sample was obtained for sample containing the fertilizer SR-N-P-Ca (50 μS / cm).

From the collected experimental data it can be seen that both before and after subtracting the contribution of control sample, the solutions' conductivities have the same variation. The correlation of conductivity evolution for control and sample solutions containing fertilizers SR-N-P-Ca and SR-N-P-Mg for 7 different times of extraction is described by a second degree equation, as can be seen in Figures 10 and 11.

The obtained correlation coefficients for conductivity values determined every hour during 7 hours for the experimental fertilizers and control sample are significant for all pairs analyzed separately. Thus, it can be seen that the mean values of conductivity for the collected extracts corresponding for the experimental fertilizers are in a very wide range (900-3800 μS / cm). Through physical and chemical mechanisms, the clay-humic soil has the ability to regulate these phenomena. As for the conductivity variations in time and with the type of fertilizer the variation of this indicator shows a good

correlation in time.

In Figure 12 are shown some aspects of the work done for growing the culture of *Arabidopsis thaliana*. The *in vitro* results were promising, demonstrating good slow-release properties for both macro- and meso-nutrients.

CONCLUSIONS

The study aimed the developing and implementing of innovative technologies for the production and application in organic farms of microencapsulated slow release fertilizer (nanosuspensions or dry granules) with a high efficiency at lower doses compared to conventional fertilizers. The main objective was to present and test a new type of N-P-Ca-Mg encapsulated fertilizers, with a slow release of the active substances, such fertilizing formulations generating a higher bioavailability of active substances in plant tissues, even if lower doses than conventional granular fertilizers are being applied. The leaching tests, meaning the fertilizer's gradual degradation with the release of nutrients was done

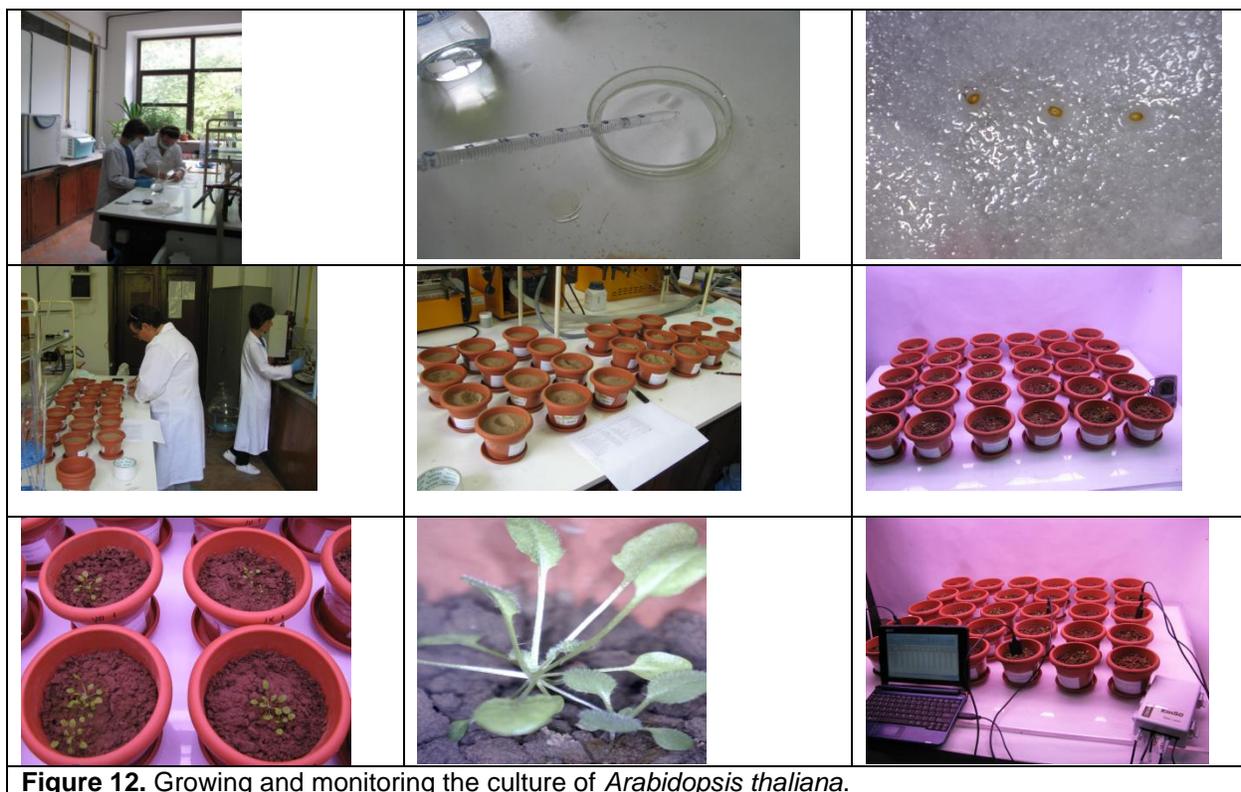


Figure 12. Growing and monitoring the culture of *Arabidopsis thaliana*.

according to the methodology imposed by the standard EN 13266/2002. The *in vitro* tests were focused on both physical and chemical properties of fertilizers in order to assess the leaching rate of nutrients and their agrochemical properties. It was determined the total concentration of nutrients in fertilizers and also the concentration of mobile species according to standard EN 13266/2002, highlighting also the evolution in time of nutrients' concentrations. *Arabidopsis thaliana* species was used to agrochemically test these new slow-release fertilizers and complex analyzes of soil and plant were performed after the plant reached its maturity. For the soil with N-P-Mg slow-release fertilizers, the tendency is of conductivity increasing versus time. The *in vitro* results were good, demonstrating good slow-release properties for both macro- and mesonutrients. The main advantage of these N-P-Ca-Mg fertilizers is the release of active ingredients through a progressive solubilization compared to conventional fertilizers usual.

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ABBREVIATIONS

SR-N – slow release (SR) fertilizer with nitrogen (N) as nutrient
 SR-N-P-Ca – slow release (SR) fertilizer with nitrogen (N), phosphorous (P), and calcium (Ca) as nutrients
 SR-N-P-Mg - slow release (SR) fertilizer with nitrogen (N), phosphorous (P), and magnesium (Mg) as nutrients

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