Evaluation of nutrients bioavailability from fertilizers in *in vivo* tests

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Abstract: *Bioavailability of nutrients is crucial in obtaining high crop yields and influences the possible pollution of groundwater. The paper presents in vivo methods (seed testing, pot trials, field trials) of the evaluation of micronutrients bioavailability from fertilizers which enable selection of the most efficient way of micronutrient fertilization by determination of bioavailability of micronutrients, plant height and crop yields.*

Key words: *fertilizers, micronutrients, bioavailability, in vivo*

1. Introduction

In response to global micronutrients deficiency in plants and in soil, micronutrient fertilizers are used [1]. Micronutrients in fertilizers are characterized by different bioavailability to plants [2]. Very often micronutrients delivered with fertilizers are leached to groundwater causing its contamination [3]. The excess of micronutrients can be also dangerous to plants what was found for crops overfertilized with micronutrients [4]. Nutrients bioavailability is one of the most important parameters characterizing fertilizers efficiency. For quantative description of micronutrients bioavailability from fertilizer to plant, transfer factor (TF) (1) which expresses the ratio of the ammount of nutrient in plant to the amount of nutrient delivered with fertilizer can be used [5].

$$\mathrm{TF} = \frac{m_{plant}^{nutrient}}{m_{fertilizer}^{nutrient}} \quad (1)$$

Among different fertilizers, the most desired products are those characterized by the highest TF.

The bioavailability of nutrients and thereby the efficiency and usefullness of fertilizer can be estimated in *in vivo* tests designed to examine the possibility of practical application of new fertilizers. The most widespread *in vivo* methods of the evaluation of nutrients bioavailability from fertilizers enabling selection of the most effective way of fertilization are germination tests, pot trials and field trials [6].

2. Germination tests

Germination tests are preliminary experiments carried out in laboratory scale which aim to evaluate whether examined material can be concerned as micronutrient fertilizer. Germination tests are performed on different seeds i.e. garden cress *Lepidium sativum*, radish *Raphanus sativus*, bean *Phaseolus vulgaris*, maize *Zea mays* or rice *Oryza sativa* according to the international norm (the International Seed Testing Association) [7]. Plastic Petri dishes covered with cotton (approximately 5.0 g) soaked with deionized water are prepared. On each dish, one hundread seeds are placed in rows at equal distances from each other (10x10). In the next step seeds are subjected to the stratification in 1˚C for three days. After stratification appropriate amounts of particular fertilizer are spread evenly on Petri dishes and germination is observed in seed germinator in 25˚C for 10

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days [8]. All probes are taken in triplets or sextuplicates for greater statistical significance of obtained results. Plates to which fertilizer was not applied constitute control groups. On the 3rd and 6th day of experiment the counting of germinated seeds is done. Different fertilizer materials as well as different doses of micronutrients or bioavailability of different nutrients can be compared. After the experiment the number of germinated seeds is counted again and the plant height is determined. Moreover, the content of chlorophyll in leaves is analyzed. The yield from each plate is dried to the constant weight, mineralized and multielemental analysis by ICP-OES is carried out.

Germination tests give many important informations about fertilizing efficiency of examined material. The test gives the possibility to compare bioavailability of nutrients from different fertilizers. Furthermore, obtained results inform about possible toxic effect of nutrients and correlations between different nutrients. Influence of tested fertilizer on crop yield, mass of plant and chlorophyll content is also examined.

Germination tests give enough information to decide whether a new material is worth to be concerned as fertilizer and tested in larger scale.

3. Pot trials

Pot trials conducted in phytotrons usually constitute preliminary studies to field trials. Phytotrons are special buildings (green houses) which provide suitable conditions (temperature, humidity, illuminance) for plant growth and development. Plants are usually sown in pots filled with about 7 kg of soil [9]. Type of soil depends on the experiment but most commonly soil from arable land is used. Generally doses of macronutrients are c.a. N: 1.6 g, P: 0.40 g, K: 1.72 g and Mg: 0.32 g while doses of micronutrients are 3.2 mg (B), 16 mg (Cu, Zn), 32 mg (Mn, Fe) although different doses of micro-and macronutrients can be examined. Moisture of subsoil is kept on the constant level 60% during experiment [10]. The number of sown seeds is determined for a given species. Usually in pot trials 50-200% excess of sown seeds is applied. The number of plants left in pot after germination strongly depends on the species and vary from 3-20 per pot [11]. Root crops should be tested in larger pots (one plant per pot). The duration of pot trials usually varies from few months to two years and depends on characteristic of plant [10, 12]. Every probe is taken in 3 to 6 replicates for making experiments more reliable and for achieving higher significance of results. In pot trials, the effect of different conditions is controlled by monitoring plants response to the exposure to different factors (temperature, humidity, soil richness, fertilization). The results obtained in tests conducted on different doses of micronutrient fertilizers suggest optimal intensity of fertilization and give informations about bioavailability of micronutrients delivered with fertilizers. The effect of several nutrients on plant can be determined at the same time. Pot trials are very important because bioavailability of nutrients can be examined in any conditions (i.e. characteristic for specific region) and, what is very convenient, can be conducted regardless of the season. Described tests are also useful in examination of possible interdependences between nutrients (synergism or antagonism) affecting bioavailability of the ions of interest [13]. Taking into account the simplicity of these trials and low costs pot trials are carried out prior field trials however field tests can not be substituted by pot trials.

4. Field trials

The most advanced and most accurate of plant experiments are field trials, conducted as annual crops, multiannual periodical and static [14]. Field experiments are the best way to define fertilizer requirements because they suggest which nutrients and in what doses are suitable for plants. Duration of tests varies from few months to few years because sometimes only after two or three years crucial observations can be made [15]. Thanks to this type of research it is possible to observe efficiency of fertilizers and reaction of plants to fertilization in field conditions. Plants are sown on fields characterized with the smallest possible variability of soil conditions. Experimental area is divided into equal plots ranging from few to a few hundread square meters [16]. Size of plots and selection of plants mainly depends on the aim of experiment and local conditions. The smaller plot the greater influence of adjacent crops is observed. Each experiment is taken in at least three replicates for minimization of unwanted effect of uncontrolled factors. There are several systems of dividing area into plots and depends on the direction of soil diversity [17]. Very often the influence of different doses of nutrients is tested in long term experminents. Obtained results are often compared with control groups without fertilization, and with plants fertilized with traditional product available on the market. Selection of plants is dependent on the reaction of plant to micronutrient excess or shortage, i.e. corn is sensitive to zinc level variables. After trial, the group of several parameters is measured, mainly Transfer Factor, gluten and protein content, moisture and crop yield. At the end of experiment, also the content of interesting nutrient in different part of plant, mainly in edible parts is determined. Enriching edible parts of plants in micronutrients is particularly important for producers of biofortified food. This is one of the reasons why the field trials focusing on micronutrient bioavailability are usually conducted on cultivated plants and cereals. Tests give straight answer whether the new fertilizer can be considered as efficient and perspective nutrient fertilizer. The main advantage of field trials is that they are carried out in natural conditions and it can be expected that obtained results will be repetitive in practice.

5. Summary

Micronutrients defficiency and its negative effects results in the need to search for the most efficient instruments for overcomming this global problem. Fertilizers market seems to be the most developing branch in this field. Many laboratories conduct researches aimed at new micronutrient fertilizers. New formulations differ from each other in its influence on plant growth and development and in bioavailability of nutrients. Evaluation of nutrients bioavailability on three stages of *in vivo* tests (germination tests, pot and field trials) gives us possibility to estimate effectiveness at the very beginning of research and reject it or shift to more advanced experiments. Huge advantage of *in vivo* tests is simplicity and the fact that obtained results can be transformed to environmental conditions. Unfortunately duration of experiments is long and costs of trials (mainly field) trials is high. Due to this fact, wide range of quick and cheap *in vitro* tests become more and more popular and start being rapidly growing area of research. However these *in vitro* tests are required to be verified by *in vivo* experiments.

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OCENA BIODOSTĘPNOŚCI SKŁADNIKÓW ODŻYWCZYCH Z NAWOZÓW W BADANIACH *IN VIVO*

Biodostępność składników odżywczych dla roślin jest istotna z punktu widzenia osiągniętych plonów oraz możliwego wpływu na zanieczyszczenie wód gruntowych jonami metali dostarczanych roślinom wraz z nawozem. Metody określania biodostępności mikroelementów *in vivo* (testy kiełkowania, doświadczenia wazonowe oraz polowe) umożliwiają wybór nawozu mikroelementowego charakteryzującego się najwyższą wydajnością nawożenia na podstawie uzyskanych wyników biodostępności oraz plonowania roślin. Niewątpliwą zaletą badań *in vivo* jest łatwość przeniesienia otrzymanych wyników na warunki środowiskowe. Niestety, czas trwania doświadczeń oraz ich wysoki koszt (przede wszystkim testów polowych) sprawiają, że wciąż poszukuje się alternatywnych rozwiązań w postaci testów *in vitro*. Istnieje jednak konieczność potwierdzenia rezultatów otrzymanych w badaniach *in vitro* badaniami *in vivo*.