

Evaluation of nutrients bioavailability from fertilizers in *in vitro* tests.

Mateusz Samoraj¹, Łukasz Tuhy¹, Katarzyna Chojnacka¹

Abstract: Modern biological fertilizers and biological fertilizers components can release nutrients in bioavailable forms in a controlled manner. Quick evaluation of nutrient bioavailability, essential in the evaluation of quality of fertilizers, is possible to use *in vitro* extraction tests (conventional, microwave, ultrasonic), described in this article. These tests enable not only total content of nutrients in fertilizer, but also their bioavailability, through simulation of condition occurring in soil solution (e.g. citrate solution).

Key words: *in vitro* bioavailability, fertilizers, nutrient extraction.

1. Introduction

Nowadays, microelements deficiency in soil is one of the agricultural problems. The proper growth of plants without trace elements is impossible [1]. Microelements (Fe, Mn, Zn, Cu) are cofactors and participate in various metabolic pathways. Therefore it is necessary to provide them in available form such as divalent ions or chelates.

Currently used fertilizers are characterized by different bioavailability. Bioavailability is defined as the amount of nutrients that is available to plant in a useful form. It is an important factor in fertilization [2, 3]. Bioavailability depends on soil type, content of organic matter, pH, adsorptive surface and other chemical, physical and biological conditions [2]. The bioavailability can be assessed in *in vitro* tests described in this paper.

Trace elements play an important role in nutrition of livestock, and humans as well. Nutrient malnutrition is also a global problem (e.g. Mn, Zn, Cu) affecting over 3 billion people - over half of the world's population. Therefore, it is important to serve bioavailable forms of micronutrients [4].

Assessment of metals concentration in soil and fertilizers is a subject of many multidisciplinary researches - from agronomists to environmental chemists [5, 6]. Soil and fertilizers are difficult matrices. Determination of metals concentration requires high purity reagents - to avoid contamination [5]. Determination of micronutrient quantities in natural products required the material digestion [7]. This forced development of quick screening bioavailability tests.

2. Bioavailability *in vitro* tests

The bioavailability of nutrients to plants depends on many factors such as plant genotype, agronomic management, climatic conditions etc. The bioavailability can be measured *in vivo* by direct uptake experiments by plants and *in vitro* (laboratory tests). With the exception of a few methods, most of techniques required sample preparation. Sample must be converted from a solid to a liquid form.

A liquid extraction methods are the most popular. Important is, to select extractant (or extractants) adequate to sample, numbers of stages, temperature and time. Methods described in this article are showed in Figure 1 [5, 8].

¹ Instytut Technologii Nieorganicznej i Nawozów Mineralnych, ul. Smoluchowskiego 21, Budynek B-1, 50-372 Wrocław, mateusz.samoraj@pwr.wroc.pl

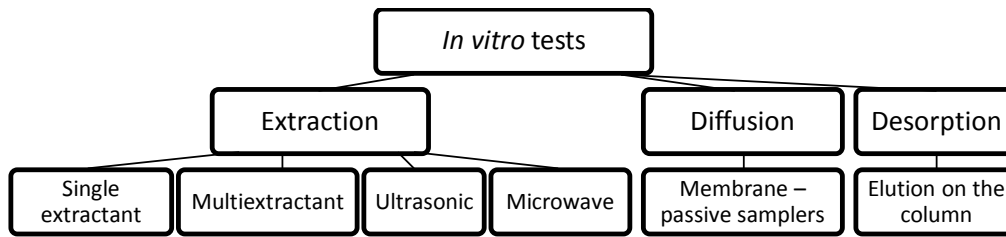


Fig. 1. *In vitro* bioavailability assessment methods.

There are several possibilities of metal binding to biomass or soil. The main are showed in Figure 2 and characterized in Figure 3.

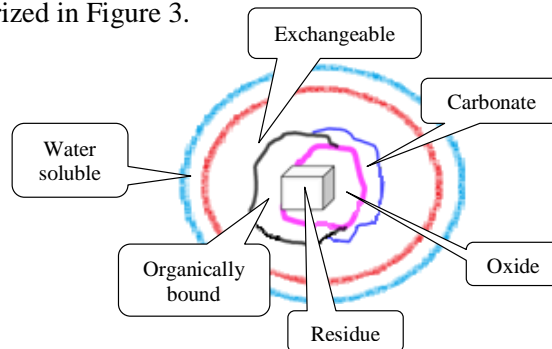


Fig. 2. Model of metal fractions in soils and biomass.

Metal ions can be loosely bound in a water-soluble form. Alkaline earth metal ions prefer to form electrostatic interactions with surface (negative charged). These metal ions belong to exchangeable fractions. In specific condition ions could be precipitated i.e. as carbonate compounds. Adsorption of metals may occur due to interaction with metal oxides and organic matter. Residues are bound by strong interactions – covalent bonds [5, 9].

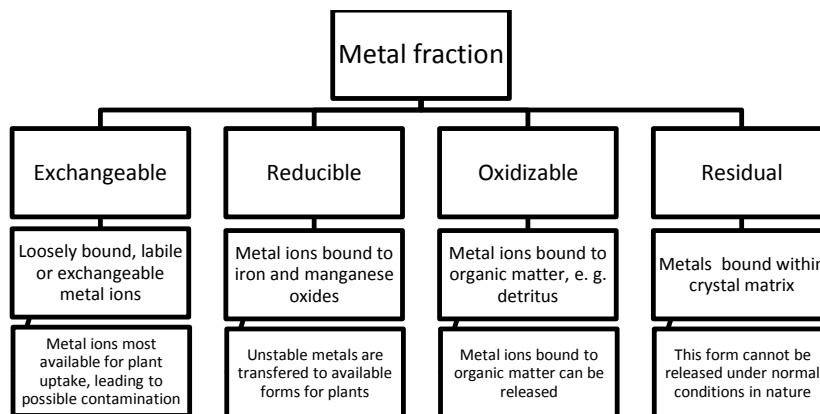


Fig. 3. Metal ions fraction in soils and biomass [9].

Humic substances are the dominant organic matter in soils. They react with biomass due to several kinds of reactions – e.g. due to weak ionic bonds and strong covalent bonds controlled by carboxylic, phenolic, and amino groups (pH dependent). Metal affinities depend upon electronegativity and charge/radius ratios [5, 8].

Soil solution (soil liquid phase) contains dissolved metal ions in free and complexed forms which are mostly bioavailable. In this medium, nutrients can move among gas, solid and organic soil compartments. Therefore, the analysis of soil solution gives information about bioavailable forms of ions [8].

The idea of *in vitro* bioavailability studies of fertilizers and modern fertilizer additives is to simulate conditions which occur in soil solution [5].

Ions concentration of specific metal ions in prepared samples (without organic matter) can be measured directly i.e. using ICP-OES technique. Samples with organic matter need digestion – widely used is microwave digestion, but some matrices need the use of wet acidic digestion.

2.1. Extraction based methods

In single extractant method mainly used are chelating agents such as DTPA acid, EDTA acid, NTA acid, neutral ammonium citrate. The last one is currently often used to determine bioavailable phosphorus [10]. At present, there is conducted a research on the assessment of the bioavailability of other nutrients with the use of this extractant (Fig. 4) [5].



Fig. 4. Carboxyl groups of citrate can react with metal ions.

Single extraction procedures are the major methods for *in vitro* bioavailability assessment. These methods are designed to provide a speciation analysis. Alkaline metal salt solutions such as 0.05M CaCl₂, 0.05 M MgCl₂, 0.1 M NaNO₃, are used to extract weakly bound metal ions (electrostatic interactions, Van der Waals, Hydrogen bond) to surface of soil. These salt extractants allows to detect low levels of metal ions i.e. Cu, Mn, and Zn. Chelating agents in low concentration are stronger extractants. 0.05 M EDTA and 0.005 M DTPA are good extractants for organically bound metals. These methods provide information about difference in concentration between two important metal fractions: adsorbed metals and geological metals in mineral matrices, but very long time is needed to achieve complete extraction for these fractions. Multi stage extraction with the same extractant gives better results but is also time-consuming. Multi stage extraction, with usage of different, selective extractants provides information about total metal concentration in shorter time [5, 11]. Examples of extractants used in selective extraction are shown in Table 1:

Tab. 1. Selective extractants [5].

Fraction	Extractants
Water soluble	Water
Exchangeable	0.05 M CaCl ₂ , 1 M KNO ₃ , 1 M MgCl ₂ , 1 M NaOAc (pH 8)
Carbonate	1M NaOAc /HOAc(pH 5)
Organically bound	0.005 M DTPA, 0.05 M EDTA, NH ₄ -citrate (pH 7)
Bound, occluded in oxides	NH ₂ OH·HCl/HOAc, Na ₂ S ₂ O ₄ /Na-citrate/H-citrate, Acid (NH ₄)C ₃ O ₄ ,
Residual/primary	HNO ₃ /HF, HClO ₄ /HF

To reduce time of extraction, intensification technique which uses external energy sources for example ultrasonic extraction and microwave extraction may be used. In the first there is

produced ultrasonic field which causes micro-cavitations in the liquid surrounding biomass. In the result of this process cell wall is disrupted and liquid is locally heated. By ultrasonic extraction it is possible to increase diffusion of solvent into cellular membrane or wall of cells. This results in a better mass transfer in the solution.

Second method uses microwave energy which makes extraction more efficient in shorter time. The main advantage of this method is to reduce thermal gradients, also increase energy transfer. Biomass is selectively heated because microwave energy is converted into kinetic energy. While microwaves are absorbed by liquid phase, the diffusion rate increases. This is due to an increase in the kinetic energy of the liquid [12].

2.2. Ion diffusion based methods

Generally ion diffusion methods are basing on equilibrium processes. Passive samplers are devices that rely on diffusion of the ions to the sampler to reach and maintain equilibrium with the soil solution (which is in equilibrium with the solid soil). Ions concentration in samples represent conditions at the sampling point (during the last few days). The passive samplers based on variety types of membranes i.e.: Polyethylene Diffusion Bag Samplers (PDBs), Nylon-Screen Passive Diffusion Samplers (NSPDS), Regenerated-Cellulose Dialysis Membrane Samplers, Rigid Porous Polyethylene Samplers (RPPS) [13, 14].

2.3. Desorption based methods

One of desorption based method in bioavailability assessment is elution on the column. These method based on continuous desorption process with using extractants i.e. ammonium citrate or other chelating agents. Concentration of ions in eluate decreased in time. Comparison of elution curves give useful information about time depending elution of bioavailable forms of ions. These data are important in designing modern fertilizers, able to release nutrients in controlled pattern.



Fig. 5. Continuous desorption column

3. Determination of the total metal content in samples

Determination of the total metal content in samples may be conducted by few methods i.e. alkaline, acidic (wet) digestion and microwave digestion. Matrices type and sample mass determine the method which can be used in analysis.

3.1. Alkaline digestion

Alkaline digestion destroys structure of organic matter material, in mild conditions, take place degradation into smaller molecules. All proteins are destroyed during the process. Generally bases are water solutions of alkali metal hydroxides i.e sodium hydroxide or potassium hydroxide. Combined method with heating or microwave source dramatically accelerate the mineralization reaction [15].

3.2 Acidic (wet) digestion

Acidic (wet) digestion is often used when an unknown sample has to be analyzed. This technique is preferred for large masses of samples (5-10 g) [7]. Commonly used are mixtures of strong inorganic acids such as: HNO_3 , H_2SO_4 , HClO_4 , HCl , depending on the metal under consideration and on the material type. Digestion of silicate compounds may require the use of hydrofluoric acid (HF). The digestion is performed at temperatures close to the boiling point of the acid. A hot plate is often used as a heat source (hot acid digestion technique). During the process, some analytes are lost through volatilization [7]. High concentrations of inorganic acids used for mineralization mostly cause total dissolution of matrices. The extracted mixture is filtered or centrifuged to remove contaminants. This provides lower selectivity, but is used for multi trace element analysis [5].

3.3 Microwave digestion

Microwave digestion is based on acid digestion combined with microwaves as a source of energy. It is excellent method for routine analysis of well-known samples – trace elements content. There are several methods which provide rapid sample dissolution. Some (EPA-3051) uses only concentrated HNO_3 to digest matrices. EPA-3051A method used HNO_3 - HCl (3:1), hydrochloric acid helps to improve the recoveries and stabilize some analytes i.e.: Fe, Ag, Al, Sb, Ba. Other methods (EPA-3052) is designed for total dissolution of samples, HNO_3 -HF is used to dissolve organically based matrices and silicate containing materials. To measure the concentration of analytes ICP-OES, atomic absorption spectrometry (GFAAS), ICP-AES, or ICP-MS are used [5, 7]. The main disadvantage of this technique is that only 0.1-0.5 g sample can be digested, thus final volume and the concentrations will be low [7].

4. Summary

In vitro assessment of bioavailability methods was studied. The main idea of the use of *in vitro* tests is shortening the time of bioavailability evaluations. The most valuable results give *in vivo* tests. The simplest methods, with using single extractant could give quick and valuable results, but firstly correlation between *in vitro* and *in vivo* TF's (Transfer Factors) would be confirmed. Transfer Factor is the ratio of the amount of nutrient in plant or extract to the amount of nutrient delivered with fertilizer. Wide variety of biosorbents causes the need for development short screening *in vitro* tests, to choose the best biosorbent characterized by high bioavailability. Digestion methods help to assess non available forms of metals. These methods are also helpful in balancing concentration of metals.

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OCENA BIODOSTĘPNOŚCI SKŁADNIKÓW ODŻYWCZYCH Z NAWOZÓW W BADANIACH *IN VITRO*.

Nowoczesne nawozy biologiczne oraz biologiczne dodatki nawozowe posiadają zdolność kontrolowanego uwalniania składników odżywczych. Szybka ocena biodostępności składników odżywczych, istotna w ocenie jakości nawozów, możliwa jest dzięki testom ekstrakcyjnym *in vitro* (konwencjonalnym, mikrofalowym, ultradźwiękowym), które opisano w artykule. Testy te są tańsze od badań *in vivo*, dając możliwość określenia nie tylko całkowitej zawartości składników odżywczych, lecz także ich biodostępności, przez odzwierciedlenie warunków panujących w roztworze glebowym. Głównym celem stosowania testów *in vitro* jest skrócenie czasu oceny biodostępności. Ogromna różnorodność materiałów biologicznych, potencjalnych biosorbentów, wymaga rozwoju szybkich testów przesiewowych do oceny biodostępności, nad którymi trwają obecnie intensywne badania.