Biofortification of maize with micronutrients by *Spirulina*

DOI: 10.1515/chem-2015-0126
received January 13, 2015; accepted June 16, 2015.

**Abstract:** The aim of the present work was to examine the effect of the application of *Spirulina platensis* post-extraction residues enriched with Zn(II), Mn(II), Cu(II) via biosorption as micronutrient fertilizer for the biofortification of maize grains with micronutrients in field tests. As a nominal dose 2.5 kg ha⁻¹ of zinc, 1.0 kg ha⁻¹ of manganese and 0.5 kg ha⁻¹ of copper were applied. The preparation was applied also in higher doses (150%, 200%) to investigate agronomic biofortification of maize grains.

In field trials, obtained grain yield (7.2 Mg ha⁻¹ for *Spirulina* 100%) was higher than in control group (6.2 Mg ha⁻¹) and commercial reference product (6.6 Mg ha⁻¹). For the same dose of micronutrients, their bioavailability was higher for bio-preparations than for reference fertilizer. The highest content of micronutrients delivered to plants (2.15 mg kg⁻¹ – Cu, 7.07 mg kg⁻¹ – Mn, 29.0 mg kg⁻¹ – Zn) was observed for maize grains fertilized with preparation of *Spirulina* 150%, which signifies that biofortified maize grain was obtained. Corn grains biofortified with micronutrients can be used as staple food or feed preventing from micronutrient malnutrition. The application of micronutrient biocomponents based on *Spirulina* biomass allows to manufacture a valuable fertilizer with bioavailable micronutrients.

**Keywords:** micronutrients fertilization, biomass utilization, biofortification, field tests

1 Introduction

Micronutrient malnutrition is a serious health global problem. The solution seems to be increase of micronutrients density of staple foods. Micronutrient oriented fertilization can be used as a method of biofortification of food, which allows to overcome micronutrient malnutrition.

Intensive exploitation of soils causes many problems – one of them is micronutrient impoverishment [1]. In the developing countries micronutrients malnutrition occurs mainly among women and children. Micronutrient malnutrition is known as hidden hunger [2]. Over 2 billion people in the world suffer from zinc deficiency (more than 30% of the world's population) [3]. Zn deficiency in human causes growth stunting, susceptibility to infectious diseases, and Fe-deficiency anemia [4]. Deficiency of other micronutrients results in slow growth, anemia, decreased immunity and nyctalopia [5].

Micronutrients play an important role in plants nutrition. They are required in small quantities by crops, livestock, and humans [1]. They act as cofactors and participate in many metabolic pathways. Trace elements are also necessary for the proper functioning of the processes of photosynthesis and respiration [6]. Many plants are particularly sensitive to deficiency of trace elements in soil. In maize the consequence is lower crop yield [7]. Micronutrients such as Cu, Zn, Fe and Mn present in grain directly affect food quality [1]. Nonetheless, for years too little attention was paid to micronutrients fertilization practices [5].

Micronutrient-oriented fertilization can be used as a method of biofortification of food, which allows to overcome micronutrient malnutrition. From definition, biofortification is a strategy to increase staple food crops with nutrients by agricultural means, including conventional breeding, agronomic approaches, mutagenesis, genetic engineering [8]. The application of micronutrient fertilizers seems to be the simplest way for biofortification [9]. It was shown that micronutrient fertilizers are effective in increasing micronutrient content in grains of rice, wheat and maize which constitutes important cereal crops because of its economic value in...
livestock nutrition [10]. Agronomic zinc biofortification through fertilizer application is a complementary approach to further increase grain Zn concentration [4].

On the market, there are available different fertilizers such as chelates and technical salts of micronutrients. Chelates are quite expensive but are characterized by high bioavailability. Technical salts are low cost fertilizers but micronutrients are easily eluted by water from soil [11]. What is more, chelating agents such as EDTA are toxic and not biodegradable [12]. New biocomponents with micronutrients basing on waste biomass are an alternative to traditional micronutrient fertilizers [11].

During biosorption, non-living organic matter is enriched with micronutrients in a process based mainly on the sorption and ion exchange [13]. Metal ions can be bound to different functional groups (carboxyl, amine, hydroxyl, etc.) that are exposed on cell walls [14]. Major advantage of using this natural property is low costs of materials and process, easy handling, high efficiency, availability of cheap biosorbents, biodegradability and non-toxicity [15]. Enriched biomass can release micronutrients in equilibrium controlled way. Many of the organic raw materials can be used as biosorbents. One of the types of the biomass which could be enriched with micronutrients and used as a micronutrient fertilizer are residues after supercritical CO₂ extraction of *Spirulina platensis*.

*Spirulina platensis* is a photosynthetic, blue-green microalgae. This is a rich source of vitamins, essential minerals, amino acids, essential fatty acids and antioxidants [16]. It was proved that high yields can be produced using desalinated wastewater and animal manure [17]. Annual worldwide production is about 2000 tons [18]. Algal biomass can be used as a biosorbent material because is available in large quantities, its processing is relatively cheap and have gives good performance at low cost [19]. The dried biomass has excellent capability to bind cations of chromium, copper, nickel, zinc and other micronutrients because the cell wall possesses functional groups (carboxyl, phosphoryl, amino, thiol, sulphhydryl) potentially available for metal ions [14,20].

The aim of the present work was to examine the effect of the application of *Spirulina platensis* post-extraction residues enriched with zinc, manganese and copper ions via biosorption as micronutrient fertilizer for the biofortification of maize grain with micronutrients in comparison with the reference commercial micronutrient fertilizer.

2 Experimental procedure

2.1 Micronutrient bio-components production

Micronutrient fertilizer bio-components were produced in biosorption process. *Spirulina platensis* post-texttraction residues after supercritical CO₂ extraction were taken for the experiment. The biosorption of zinc(II), copper(II) and manganese(II) ions by biological materials was conducted in batch reactor (1000 L) separately for each micronutrient for 2 h. The concentration of Zn(II) (ZnSO₄ · 7H₂O, POCH, Poland), Cu(II) (CuSO₄ · 5H₂O, POCH, Poland) and Mn(II) (MnSO₄ · H₂O, POCH, Poland) ions in the solution was 500 mg L⁻¹, pH was controlled by pH regulator as 5, measured at 25°C. In each process 10 kg of biosorbent were used. After the process, the suspension was filtered and each type of enriched biomass was dried in industrial dryer (Hajnowka, Poland) at 50°C for 24 h. The content of elements in enriched biomass was examined by ICP-OES after mineralization [21].

2.2 Field trials

Field trials were conducted on maize (KOSMO 230) at the Plant Breeding and Acclimatization Experimental Station in Olesnica Mala (Lower-Silesia, South-western Poland). The characteristics of soil were as follows: sandy loam, IIIb quality class, 2.2% of organic matter, pH 7.2. The experimental area was divided into 21 m² plots. The bloks were randomized. The interval between rows of plants were 75 cm, between plants – 16 cm. Planting density was 85000 pcs of corn seeds/ha. Each combination was carried in 4 replication. Experiment was conducted during 6 months (from May to October). Average temperature was 15.2 ± 4.3°C, average month total rainfall was 68.7 mm. During experiment all plant parameters were examined according to the guidelines EPPO PP 1/144 (3), EPPO PP 1/135(3), EPPO PP 1/152 (3), EPPO PP 1/181(3).

2.3 Fertilization

For the experiment, bio-components with zinc, manganese and copper based on the biomass of *Spirulina* were prepared. For each combination of micronutrient fertilizer with bio-components an additional NPK fertilizer was used – Polifoska 4 NPK(MgS) (4%-N, 12%-P, 32%-K, 2%-Mg, 9%-S), delivered by Grupa Azoty Z. Ch. “Police” S.A., Poland. For comparison of fertility results, 3 control
combinations were used – untreated, NPK(MgS) (only NPK fertilizer) and NPK(MgS) with micronutrients (technical salts of Zn, Cu, Mn, POCH, Poland). The micronutrients ratio in applied micronutrient bio-components and NPK(MgS) fertilizer with micronutrients was 1:0.4:0.2 for Zn, Mn and Cu, respectively. The quantitative description of fertilization within tested combinations is listed in Table 1, dose of commercial products are in accordance with manufacturer recommendation for maize fertilization. Dose of bio-components (100%) are equivalent to commercial products.

2.4 Mineralization

Each material (1 g) was digested with nitric acid – 69% m/m (5 mL), spectrally pure (Suprapur, Merck, USA) in teflon bombs in microwave system Milestone Start D (USA). Parameters of the mineralization process were selected to assure complete digestion of samples. Samples were diluted 10 times with ultrapure water (Millipore Simplicity) to perform multielemental ICP-OES analysis.

2.5 Multielemental ICP-OES analysis

The concentration of elements in digested biomass was determined by ICP-OES Varian-Vista MPX, Australia. Samples were supplied with ultrasonic nebulizer CETAC U5000AT+. The analyses were carried out in Laboratory Accredited by Polish Centre of Accreditation (PCA) according to PN-EN ISO/IEC 17025:2005. Quality assurance of the test results was achieved by using Combined Quality Control Standard from ULTRA SCIENTIFIC, USA. All samples were analyzed in three replicate (results of analyses were arithmetic mean, the relative standard deviation was < 5%).

2.6 Statistical analysis

The results were elaborated statistically by Statistica ver. 10. Descriptive statistics (means, standard deviations) were reported. Normality of distribution of experimental results was assessed by Shapiro-Wilk test. On this basis, statistical test for the evaluation of the significance of differences between the groups was selected. The differences between groups were investigated with (RIR) Tukey test which compares all pairs of means following one-way ANOVA. Results were considered significantly different when p < 0.05.

3 Results and discussion

3.1 Multielemental analysis of micronutrient fertilizer additives.

Inductively coupled plasma-optical emission spectrometry (ICP-OES) results of multielement content of new biocomponents are presented in Table 2. The biosorption process led to the significant enrichment of the biomass with micronutrients, the content of which was 21260 mg kg⁻¹, 11180 mg kg⁻¹ and 39910 mg kg⁻¹ for zinc, manganese and copper, respectively. *Spirulina* constitutes also a source of macronutrients essential in plants cultivation. Apart from phosphorus and potassium, the biopreparations contains about 0.5% of calcium and magnesium. The content of toxic elements was below

| Group | Fertilizer | Product | Dose (kg ha⁻¹) | Additional fertilizer |
|-------|------------|---------|----------------|-----------------------|
| 1     | Spirulina 100% | Spirulina + Zn | 117.6 | + NPK(MgS) (500 kg ha⁻¹) |
|       |            | Spirulina + Mn | 89.3 |  |
|       |            | Spirulina + Cu | 12.5 |  |
| 2     | Spirulina 150% | Spirulina + Zn | 176.4 | + NPK(MgS) (500 kg ha⁻¹) |
|       |            | Spirulina + Mn | 134 |  |
|       |            | Spirulina + Cu | 18.8 |  |
| 3     | Spirulina 200% | Spirulina + Zn | 235.2 | + NPK(MgS) (500 kg ha⁻¹) |
|       |            | Spirulina + Mn | 178.6 |  |
|       |            | Spirulina + Cu | 25.0 |  |
| 4     | Untreated  | - | - | - |
| 5     | NPK        | Polifoska 4 | 500 | - |
| 6     | NPK + Zn, Mn, Cu | NPK(MgS) + Zn, Mn, Cu | 500 | - |
the level defined in the Act of fertilizer and fertilization (18 June 2008) approved by Polish Ministry of Agriculture and Rural Development. The main advantage of the biomass of Spirulina, as a carrier of biologically bound micronutrients are: good biosorption properties and content of other micro and macronutrients, important for plants (i.e., potassium, phosphorus, sulfur, calcium, iron, etc.)

3.2 Yield properties

For the qualitative description of plant yield, plant vigour, height, number of plants and cobs number were examined and presented in Table 3. Phytotoxicity was not observed for all experimental groups. Statistically significant differences in the plant vigour between all groups and untreated group were observed. Plants after micronutrient fertilization with the use of commercial reference fertilizer by bio-preparations were higher than plants fertilized with NPK fertilizer or untreated. The number of cobs was higher for plants after micronutrient fertilization (instead of Spirulina 200%). In both cases, the best results were obtained for Spirulina 150% (better or comparable to commercial product). Results in case of plant height and cob number were not statistically significant.

During experiment, the positive influence of micronutrients fertilization on crop yield was noticed, that confirmed observations reported by other researchers [22,23]. Pagani and co-workers (2012) [24] reported that one of the major macronutrients responsible for plant yield of corn is sulfur, which is also present in new biocomponents in large quantities (about 10 mg g⁻¹, Table 2). Plants and cobs number per 1m² of the fertilized and untreated plots were significantly not different

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Table 2: Multielement content of new biocomponents.

| Material          | Macronutrients (mg kg⁻¹) | Micronutrients (mg kg⁻¹) | Toxic elements (mg kg⁻¹) |
|-------------------|--------------------------|--------------------------|-------------------------|
|                   | P | K | S | Ca | Mg | Na | Zn | Mn | Cu | Fe | Cd | Ni | As | Pb | Cr |
| Spirulina +Zn 8058| 852| 9487| 5788| 935| 596| 21260| 28.7| 253| 1072| 3.44| 3.07| 0.187| 7.21| 3.85|
| ±1611            | ±128| ±1897| ±1158| ±140| ±89| ±2763| ±3.7| ±33| ±140| ±0.44| ±0.40| ±0.04| ±0.94| ±0.50|
| Spirulina +Mn 4518| 942| 9546| 5049| 766| 537| 11180| 32.67| 1386| 1.67| 12.3| 12.3| 4.88| 8.42| 3.28|
| ±903             | ±141| ±1909| ±1010| ±115| ±80| ±1653| ±4| ±180| ±0.22| ±1.6| ±1.6| ±0.63| ±1.09| 3.36|
| Spirulina +Cu 11610| 456| 10340| 3437| 632| 655| 404| 20.7| 3991| 3022| 0.178| 3.14| 0.187| 11.2| 16.7|
| ±2322            | ±68| 2068| ±2068| ±95| ±98| ±52| ±2.7| ±5188| ±393| ±0.04| ±0.4| ±0.04| ±1.5| ±2.2|

Limit*                                   5  60  50  140  100

Table 3: Plant vigour, height, number and cob number per 1m².

| No | Fertilizer | BBCH crop growth stage | 12-13 | 13-14 | 63 | 89 (assessment performed before harvest) |
|----|------------|------------------------|-------|-------|----|------------------------------------------|
|    |            |                        | Plant vigour* | Plant height (cm) | Plant (number m⁻²) | Cob (number m⁻²) |
| 1  | Spirulina 100% | 5.0±0 | 5.0±0 | 6.0±0 | 6.0±0 | 224.5±15 | 8.0±0 | 8.3±0.5 |
| 2  | Spirulina 150% | 5.0±0 | 5.0±0 | 6.0±0 | 6.0±0 | 226.1±14 | 8.5±1 | 8.3±0.5 |
| 3  | Spirulina 200% | 5.0±0 | 5.0±0 | 6.0±0 | 6.0±0 | 224.2±12 | 8.0±0 | 8.0±0 |
| 4  | Untreated    | 5.0±0 | 5.0±0 | 5.0±0 | 5.0±0 | 222.2±12 | 8.0±1 | 8.0±1 |
| 5  | NPK         | 5.0±0 | 5.0±0 | 6.3±0.5 | 6.0±0 | 222.7±10 | 8.0±0 | 8.0±0 |
| 6  | NPK + Zn, Mn, Cu | 5.0±0 | 5.0±0 | 6.3±0.5 | 6.3±0.5 | 225.1±12 | 8.3±0.5 | 8.3±0.5 |

* vigour on a 0-10 scale: 0 – plant death, 5 – optimum vigour (Untreated), 10 – most vigorous plants
Tukey test: statistically significant differences for a given element between materials, a,b... (p<0.05)

* Act of fertilizer and fertilization, 18 June 2008, approved by Polish Ministry of Agriculture and Rural Development.
(Table 3). Also, no differences between groups were found for root length and plant height. For all groups treated with micronutrient fertilizers, in biological and mineral form, better plant vigour was observed. This confirmed that increased micronutrient fertilization (particularly with Zn(II)) can affect seed vitality and vigour of plants [25].

### 3.3 Micronutrient content in corn grain

The content of zinc, manganese and copper in maize grain after field trials was determined by ICP-OES and the results are presented in Table 5. Transfer Factor (TF), defined as a nutrient mass in grain to nutrient mass delivered with fertilizer ratio [26]:

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TF = \frac{M_{\text{nutrient in grain}}}{M_{\text{delivered nutrient}}} \times 100\%.
\]

describing bioavailability of nutrients to plants, was presented in Table 5. The differences in micronutrient content of grain between groups are presented in Fig.1. The highest mass of zinc and manganese in grain was observed for Spirulina 100% and it was 12.5% and 12.7% higher than for plants fertilized with micronutrient reference fertilizer for zinc and manganese, respectively. Furthermore, the content of these nutrients in grains for Spirulina 150% was 20.0% − for Zn(II), 12.0% − for Mn(II) and 21.4% − for Cu(II) higher than plants from untreated group. It was also reported that increasing doses of micronutrients led to decrease in bioavailability of zinc, manganese and copper from micronutrient fertilizers – that occurs in decreasing of TF with the dose (Table 5). In addition, grain yield decrease with increasing dose was observed (Table 4). Result of the application of Spirulina platensis post-extraction residues enriched with micronutrients on mineral content of grain in comparison with commercial reference fertilizer and control group was shown in Table 5. The highest content of three micronutrients in grain (2.15 mg kg\(^{-1}\) – Cu, 7.07 mg kg\(^{-1}\) – Mn and 29.0 mg kg\(^{-1}\) – Zn) was observed for maize grains fertilized with preparation Spirulina 150%. These results are higher than...
in case of fertilization with other biopreparations and with commercial micronutrient fertilizer. Furthermore, the content of nutrients in grains for *Spirulina* 150% was 20.0% – for Zn(II), 12.0 % – for Mn(II) and 21.4% – for Cu(II) higher than plants from untreated group (Fig. 1).

For this preparation (*Spirulina* 150%) the biofortification effect was visible. In all experimental groups, the highest value of Transfer Factor was obtained for most important micronutrient – Zn(II), the lowest value was observed for Cu(II). It was observed that the use of reference commercial micronutrient fertilizer resulted in lower content of all tested micronutrients in comparison with biocomponents (in the same dose) – for *Spirulina* 100% treatment groups the content of each micronutrient was about 5% higher (4.5% – Cu, 5.5% – Mn and 6.4% – Zn) than in untreated group. Also, higher content of micronutrients in grains of plants fertilized with biocomponents was observed in comparison with plants treated only with NPK fertilizer. That eliminates the possibility of dominant influence of NPK fertilization on crop yield.

As it was shown in this study and reported by Hossain et al., (2012) and Zhang et al., (2013), soil application of micronutrients can lead to the biofortification of staple food. Field trials proved that increase of micronutrient dose resulted in decrease of micronutrient content in grains [10,27]. Optimal dose of micronutrients for maize fertilization was delivered with *Spirulina* 100% (characterized by the highest transfer factor), while the highest degree of biofortification was obtained for preparation *Spirulina* 150% and the grain yield was comparable with the reference product. Zinc content in grains, for plants treated with *Spirulina* 150% was 17.9% higher than for plants fertilized only by NPK fertilizer. In similar tests on maize, Menzeke and coworkers (2014) obtained comparable results (72–18%) with cattle manure application with Zn [28]. Lungu and coworkers (2011) observed 6–15% increase of Zn in grain of maize, with soil application of 50 kg ha$^{-1}$ ZnSO$_4$ × 7H$_2$O (11.4 kg ha$^{-1}$ of pure Zn) [29]. Cakmak (2008) reported that even 44.4% increase of Zn in grain of maize is possible in soil application of micronutrient fertilizer (23 kg Zn per ha) [9].

The new micronutrient biocomponents can be used as an alternative to commercial micronutrient fertilizers, often used in biofortification of grains, which could be too toxic or too quickly leachable. New biocomponents are competitive for inorganic salts, which affect soil organic carbon pool and agronomic yield, as was reported by Ortas and Lal (2014) [30].

Low cost and simple in production micronutrient biocomponents, from spent biomass of *Spirulina platensis*, produced by biosorption are an alternative to conventional fertilizers. The new product has several advantages: biodegradability, easy dosing, low content of toxic elements, high content of micro- and macronutrients in bioavailable form.

During the experiment, no phytotoxic symptoms were observed in the crop of maize for all tested groups and the plants did not lodge. For groups fertilized by new biocomponents, slightly better vigour of plants was noticed, in comparison with unfertilized plots and the reference fertilizer. There were no statistically significant differences in the crop quantity, harvested from the untreated plots and fertilized plots, nevertheless mean content of micronutrients in grain was about 11.2% – for Zn, 10.6% – for Mn and 5.5% – for Cu higher than in groups fertilized with reference commercial fertilizer.

### 4 Conclusions

The biomass of *Spirulina platensis* post-extraction residues was shown to be a good biosorbent for micronutrient ions and efficient micronutrient fertilizer bio-component. Zinc, manganese and copper ions delivered to plants with enriched *Spirulina* sp. were characterized by higher bioavailability to plants than micronutrients delivered with
conventional fertilizer. The application of micronutrient fertilizer biocomponents led to the biofortification of maize grains with microelements essential for plants, animals and humans. Biofortified maize grain can be used as components of animal feed or the component of a human diet, useful in prevention from microelement deficiencies in livestock and human.

Acknowledgments: The work was financed by National Science Center Poland, project No 2012/05/E/ST8/03055. This project was financed in the framework of grant entitled “Innovative technology of seaweed extracts – components of fertilizers, feed and cosmetics” attributed by The National Centre for Research and Development in Poland, project No PBS/1/A1/2/2012

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