Abstract: Effect of the application of blackcurrant seed post-extraction residues (BS) enriched via biosorption with Zn(II), Mn(II) and Cu(II) was examined in field tests on maize. As a nominal dose (100%), 2.5 kg of zinc, 1 kg of manganese and 0.5 kg of copper per hectare, were applied. The preparation was applied, also, in higher doses (150%, 200%).

Crop yield and quality were assessed and multielemental analysis of grains was conducted. Grain yield obtained for maize treated with different doses of micronutrients (7.3 and 7.2 Mg ha\(^{-1}\) for BS 100% and BS 200%, respectively) was higher than in control group (6.2 Mg ha\(^{-1}\)) and similar to a commercial reference product (7.1 Mg ha\(^{-1}\)).

Bioavailability of micronutrients from BS was shown to be higher than from reference commercial fertilizer. The highest content of micronutrients delivered to plants was observed for groups fertilized with BS in nominal dose of micronutrients (1.79, 7.08 and 28.55 mg kg\(^{-1}\) for Cu, Mn and Zn, respectively). The content of each micronutrient was 5.6% (Cu), 12.1% (Mn) and 12.6% (Zn) higher than in untreated group and 8.9% (Cu), 9.7% (Mn) and 8.7% (Zn) higher than commercial reference micronutrient fertilizer. New biocomponents are cheap and biodegradable carriers of nutrients which can be released in controlled way.

Keywords: biosorption, utilization, field test, microelement fertilization, biocomponent

1 Introduction

The deficiency of micronutrients in cultivated lands affects micronutrient deficiency in plants, disrupting, directly or indirectly, many biochemical pathways [1]. The shortage of microelements in crop plants can also lead to hidden hunger being a cause of serious health problems in humans. Over 2 billion people in the world suffer from zinc deficiency [2]. It was reported that one of the methods of overcoming micronutrient malnutrition is biofortification of plants.

Biofortification is the term characterizing four main strategies of fortification of grains with nutrients: genetic modifications, conventional breeding, mutagenesis and fertilizer application of which fertilization is the most readily achievable [3]. It was shown that cereal crops can suffer from the low content of micronutrients [4]. Micronutrient fertilizers were proved to be a sufficient tool in increasing micronutrient content in grains of rice and wheat [5]. Also biofortification of maize was the aim of many experiments, mainly, because maize constitutes one of the most important cereal crops due to its economic value in livestock nutrition [6]. Micronutrient fertilization was shown to be the most common and most efficient way of micronutrient application to plants, and it was proved to be an effective tool for the agronomic biofortification of grains of maize, wheat etc. [7]. Increased content of micronutrients in grains was shown after soil application, foliar spraying as well as the combination of these two ways of fertilization [8].

Among different types of micronutrient fertilizers, the application of inorganic salts and chelates seems to be the most widespread and most effective. Inorganic salts are cheap, but micronutrients delivered with this type of fertilizer are released quickly; and the bioavailability of microelement ions is low [9]. Chelate fertilizers are characterized by controlled release of nutrients and hence, high bioavailability; but they are more expensive than inorganic salts and can cause toxic effects in plants. A toxic effect can be observed due to the chelating agents
which are not biodegradable [10]. As an alternative for traditional micronutrient fertilizers, unconventional fertilizers basing on waste biomass and enriched with micronutrients in biosorption process can be used. It was also reported that a combination of organic and mineral fertilization gave positive results on crop yield [11].

Biosorption is a simple, quick and cheap reversible process in which the different types of biomass are able to bind ions due to the presence of functional groups [12]. Biosorption is widely described in literature as the method of wastewater treatment [13-15]. There are also some works describing the application of the process in the production of micronutrient feed additives and fertilizer components [16-18]. Micronutrient feed and fertilizer components produced in biosorption process are characterized by controlled release of nutrients that is controlled by equilibrium [18]. High bioavailability of nutrients adsorbed to the surface of the biomass was proved in experiments carried out in laboratory and application tests [19]. One of the types of biomass used in plant fertilization are residues after supercritical CO2 extraction of berry seeds.

Blackcurrant seeds constitute a waste from the production of jams and juices. Blackcurrant production in Poland is about 200,000 Mg year1 and blackberries seeds, as a waste, constitute 15–30%. Blackcurrant seeds are subjected to supercritical CO2 extraction for the production of proteins and polyphenols used in pharmaceutical or cosmetic industry [20,21]. Post-extraction residues constitute a waste; the storage of which costs about 100–200 $ Mg1. The possibility of its application in biosorption process, and hence, in plant fertilization can solve the problem of waste application and reduce costs of its storage. Biomass as a material of biological origin is totally biodegradable and if it contains no toxic elements, can be used in plant cultivation.

The aim of the present work was to examine the effect of the application of blackcurrant seed post-extraction residues enriched with micronutrients in the biofortification of maize grain with zinc, copper and manganese in comparison with commercial micronutrient fertilizer.

2 Experimental procedure

2.1 Micronutrient additives production

Micronutrient additives were produced by biosorption process. For the experiments, post-extraction residues, after supercritical CO2 extraction conducted on blackcurrant seeds, were used. The biosorption of zinc(II), copper(II) and manganese(II) ions by biological materials was conducted in a fixed bed reactor (200 L), separately, for each micronutrient for 6 hours. The concentration of Zn(II) (ZnSO4 × 7H2O), Cu(II) (CuSO4 × 5H2O), Mn(II) (MnSO4 × 1H2O) ions in the solution was 500 mg L1, pH was controlled by pH-stat as 5, measured at 25°C. In each process, 15 kg of biosorbents were used. After the process, the solution was filtered on sieves and then on a filtered press for biomass recovery. The product was dried in industrial dryer (Hajnowka, Poland) at 50°C for 24 hours. The content of elements in the enriched biomass was examined by ICP-OES after mineralization according to PN-EN ISO17025 [22].

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). Soil characteristics were tested: sandy loam, IIIb quality class, 2.2% of organic matter, pH 7.2. Each plot size was 21 m2, blocks were randomized according to EPPO PP 1/152 (3), intervals between rows of plants was 75 cm, between plants was 16 cm. Planting density was 85000 pcs of seeds ha1. Each combination was carried out in 4 replications. The experiment was conducted for 6 months (from May to October 2013) with an average temperature of 15.2 ± 4.3°C (n = 198) and average month total rainfall was 68.7 mm (n = 198). Raw weather data were taken from meteorological station.

2.3 Fertilization

For each prepared combination of micronutrient fertilizer 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. 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. For comparison of fertilizer results, there were 3 control combinations – untreated, Polifoska 4 (only NPK fertilizer) and NPK(Mgs) with micronutrients (technical salts of Zn, Cu, Mn).

The quantitative description of fertilization within tested combinations is listed in Table 1. The dose of commercial products are in accordance with manufacturer recommendation for maize fertilization. The dose of
bio-components (100%) are equivalent to commercial products.

During the 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).

### 2.4 Mineralization

Materials (1 g) were digested with nitric acid – 69% m/m (5 mL), spectrally pure *(Suprapur, Merck, USA)* in teflon bombs in a 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 introduced with ultrasonic nebulizer CETAC U5000AT+. The analyses were carried out in the 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 the Combined Quality Control Standard from ULTRA SCIENTIFIC, USA. All samples were analyzed in three repeats (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, a statistical test was selected, which was used to investigate the significance of differences between the groups. The differences between the 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

#### 3.1 Multielemental analysis of micronutrient fertilizer additives.

ICP-OES results of multielemental 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 zinc was $6475\, \text{mg kg}^{-1}$, manganese – $5875\, \text{mg kg}^{-1}$ and copper – $13875\, \text{mg kg}^{-1}$. Blackcurrant post-extraction residues constitute, also, a source of macronutrients essential in plants cultivation. Apart from phosphorus and potassium, the biopreparations contains about 0.5% of calcium and 0.3%
Blackcurrant seeds biomass as new micronutrient fertilizer of sulfur. The content of toxic elements was below the level defined in the Act of fertilizer and fertilization (18 June 2008) approved by the Polish Ministry of Agriculture and Rural Development.

### 3.2 Yield properties

Phytotoxicity was not observed in all experimental groups. Plant parameters (vigour ratio, height, number of plant and cob number) were presented in Table 3.

Statistically, significant differences in the plant vigour between all treated groups and the untreated group were observed. The highest number of plants (8.3 per m$^2$) and cobs (8.5 per m$^2$) was obtained for BS 150%. Also, plant height was the highest for BS150% treated group. Results in case of plant height, plant number and cob number were not statistically significant ($p < 0.05$).

The comparison of grain yield and moisture between experimental and control groups is presented in Table 4 and Fig. 2. Grain yield obtained for maize treated with different doses of micronutrients delivered with blackcurrant seeds (7.3 and 7.2 Mg ha$^{-1}$ for BS 100% and BS 200%, respectively) was higher than in case of the control group (6.2 Mg/ha) and similar to the group treated with commercial product (7.1 Mg ha$^{-1}$).
3.3 Micronutrient content in maize grain determined by ICP-OES.

Micronutrient content in maize grain was determined by ICP-OES, results are presented in Table 5 and Figs. 4-6. Transfer Factor (TF) of micronutrients was presented in Table 5. Transfer Factor describes bioavailability of nutrients to plants [24]. Statistically significant differences between experimental groups were found only in the case of Zn and Mn (p < 0.05), thus Transfer Factor for these micronutrients was calculated and presented in Fig. 2 and Fig. 3, respectively. The differences in micronutrient content in grain between groups are presented in Figs. 4-6. The highest content of three micronutrients delivered to plants was observed for maize grains fertilized with preparation BS 100% (1.79, 7.08 and 28.55 mg kg\(^{-1}\) for Cu, Mn and Zn, respectively). The content of each micronutrient was 5.6% (Cu) 12.1% (Mn) and 12.6% (Zn) higher than in the untreated group, and 8.9% (Cu) 9.7% (Mn) and 8.7% (Zn) higher than commercial reference micronutrient fertilizer.

4 Discussion

The experiments carried out on the blackcurrant seed post-extraction residues showed that the biomass can constitute a carrier of metal ions bound to its surface in the biosorption process. It was shown that the biomass is characterized by good biosorption capacity for three micronutrients essential in plant cultivation – zinc, copper and manganese. Moreover, blackcurrant seeds with micronutrients were shown as a source of other micro- and macronutrients important for plants, i.e. potassium, phosphorus, sulfur, calcium, iron etc. (Table 2).

Table 4: Effect of fertilization on yield properties – grain yield [kg plot\(^{-1}\), Mg ha\(^{-1}\)], grain moisture [%]. Results with standard deviations.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fertilizer</th>
<th>Grain yield [kg plot(^{-1})]</th>
<th>Grain moisture [%]</th>
<th>Grain yield [Mg ha(^{-1})]</th>
<th>Grain yield (15% moisture) [Mg ha(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BS 100%</td>
<td>15.4 ± 2.7</td>
<td>21.8 ± 0.3</td>
<td>7.3 ± 1.3</td>
<td>6.7 ± 1.1</td>
</tr>
<tr>
<td>2</td>
<td>BS 150%</td>
<td>13.1 ± 1.7</td>
<td>21.7 ± 0.2</td>
<td>6.2 ± 0.8</td>
<td>5.7 ± 0.8</td>
</tr>
<tr>
<td>3</td>
<td>BS 200%</td>
<td>15.2 ± 1.6</td>
<td>21.8 ± 0.2</td>
<td>7.2 ± 0.7</td>
<td>6.7 ± 0.7</td>
</tr>
<tr>
<td>4</td>
<td>Untreated</td>
<td>13.0 ± 1.2</td>
<td>22.0 ± 0.2</td>
<td>6.2 ± 0.6</td>
<td>5.7 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>NPK(MgS)</td>
<td>14.1 ± 1.9</td>
<td>21.7 ± 0.1</td>
<td>6.7 ± 0.9</td>
<td>6.2 ± 0.8</td>
</tr>
<tr>
<td>6</td>
<td>NPK+ Zn Mn Cu</td>
<td>15.0 ± 2.9</td>
<td>21.8 ± 0.2</td>
<td>7.1 ± 1.4</td>
<td>6.6 ± 1.3</td>
</tr>
</tbody>
</table>

Table 5: Effect of fertilization on the micronutrients content and TF for maize grains.

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>Parameter</th>
<th>Fertilizer</th>
<th>BS 100%</th>
<th>BS 150%</th>
<th>BS 200%</th>
<th>Untreated</th>
<th>NPK(MgS)</th>
<th>NPK+ Zn Mn Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>content [mg kg(^{-1})]</td>
<td>28.55 ± 3.47</td>
<td>24.52 ± 3.56</td>
<td>26.62 ± 0.93</td>
<td>24.95 ± 1.96</td>
<td>23.78 ± 1.60</td>
<td>26.06 ± 1.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TF [%]</td>
<td>7.60(^{a,b}) ± 0.65</td>
<td>3.72(^{a}) ± 0.46</td>
<td>3.56(^{a,b}) ± 0.26</td>
<td>-</td>
<td>-</td>
<td>6.83(^{a,d}) ± 2.01</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>content [mg kg(^{-1})]</td>
<td>7.08 ± 0.80(^{a})</td>
<td>5.80 ± 0.61(^{a})</td>
<td>6.25 ± 0.40</td>
<td>6.22 ± 0.37</td>
<td>6.40 ± 0.55</td>
<td>6.39 ± 0.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TF [%]</td>
<td>4.74(^{a,b}) ± 0.62</td>
<td>2.21(^{a}) ± 0.19</td>
<td>2.10(^{a,b}) ± 0.29</td>
<td>-</td>
<td>-</td>
<td>4.13(^{a,d}) ± 0.89</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>content [mg kg(^{-1})]</td>
<td>1.79 ± 0.34</td>
<td>1.52 ± 0.25</td>
<td>1.61 ± 0.20</td>
<td>1.69 ± 0.07</td>
<td>1.63 ± 0.26</td>
<td>1.63 ± 0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TF [%]</td>
<td>2.36 ± 0.24</td>
<td>1.15 ± 0.18</td>
<td>1.07 ± 0.07</td>
<td>-</td>
<td>-</td>
<td>2.25 ± 0.61</td>
<td></td>
</tr>
</tbody>
</table>

a, b, c, d, e, f – Means within the same row with different letters are significantly different (p < 0.05).
The multielemental analysis of produced components showed that the content of toxic elements was below the limit approved by the Polish Ministry of Agriculture and Rural Development [23]. Furthermore, according to the same act, micronutrient components basing on the biomass can be mixed with traditional NPK fertilizer and used as organic-mineral fertilizers.

The evaluation of utilitarian properties of new preparations was examined in field trials carried out on maize. The best crop yield properties (number of plants 8.3 per m$^2$, number of cobs − 8.5 per m$^2$, plant height − 225.4 cm) were obtained for BS150% (better or comparable to commercial reference product) (Table 3). Experiments showed that grain yield obtained for maize treated with different doses of micronutrients delivered with blackcurrant seeds (7.3 and 7.2 Mg ha$^{-1}$ for BS 100% and BS 200%, respectively) was higher than in the case of the control group (6.2 Mg ha$^{-1}$) and similar to the group treated with commercial product (7.1 Mg ha$^{-1}$) (Table 4). Obtained results confirmed observations reported by Bameri [25] that, not only macronutrients, but, also, micronutrient fertilization influenced crop yield. The influence of the micronutrient application on plant yield was also examined by Rahmann [26]. The explanation of obtained results can, also, influence other micro- and macronutrients delivered with new preparations found in the biomass, especially sulfur. It would confirm the thesis proposed by Pagani [27] focusing on sulfur as the major macronutrient responsible for plant yield of corn. It would also suggest that biomass constitutes a source of a bioavailable form of this element.

Field tests showed that increased doses of micronutrients delivered with the enriched biomass did not affect crop yield. It was also observed that the plant and cob number per 1 m$^2$ on the fertilized and untreated plots did not differ significantly. While no differences between groups were reported for plant height and root length, the analysis of the vigor of plants showed that plants treated with any type of micronutrient fertilizers were characterized by

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![Figure 3: Transfer Factor for Mn.](image-url)

![Figure 4: Copper content [mg kg$^{-1}$] in grain vs. fertilizer.](image-url)

![Figure 5: Manganese content [mg kg$^{-1}$] in grain vs. fertilizer.](image-url)

![Figure 6: Zinc content [mg kg$^{-1}$] in grain vs. fertilizer.](image-url)
better vigor. This observation confirmed the statement that increased micronutrient fertilization (especially zinc fertilization), positively, affected seed vitality and vigor of plants [7].

The effect of the application of the blackcurrant seeds enriched with micronutrients on mineral content of corn in comparison with the control group and the commercial reference product was presented in Table 5. The highest content of three micronutrients delivered to plants was observed for maize grains fertilized with preparation BS 100% (1.79, 7.08 and 28.55 mg kg\(^{-1}\) for Cu, Mn and Zn, respectively). The content of each micronutrient was 5.6\% (Cu) 12.1\% (Mn) and 12.6\% (Zn) higher than in untreated group, and 8.9\% (Cu) 9.7\% (Mn) and 8.7\% (Zn) higher than commercial reference micronutrient fertilizer. In similar tests on maize, Lungu [28] observed 6−15\% increase of Zn in grain of maize, with soil application of 50 kg ha\(^{-1}\) \(\text{ZnSO}_4 \times 7\text{H}_2\text{O}\), Menzeke [29] obtained comparable results (7.2−18\%) with cattle manure application with Zn. Cakmak [3] reported that a 44.4\% increase of Zn in grain of maize is possible in soil application of micronutrient fertilizer (23 kg Zn per ha).

In all experimental groups, the highest value of Transfer Factor was obtained for Cu(II), the lowest value was observed for Zn(II). It was also observed that increasing the dose of micronutrients affected their bioavailability to plants (decreasing of TF). TF of micronutrients delivered with BS 100\% were higher than in the commercial reference group and other experimental groups and differences between groups were statistically significant for zinc and manganese (Table. 5). Furthermore, the standard error was higher in case of results obtained for commercial fertilizer probably due to the easier repetitive dosing. Obtained results confirmed an observation reported by Zhang [30] that micronutrient fertilization lead to the biofortification of maize with these essential micronutrients. It was also observed, that providing plants with commercial micronutrient fertilizer resulted in lower content of Cu, Mn and Zn in comparison with the group fertilized with the same dose of micronutrients in the form of biological fertilizer components. Higher content of micronutrients in corn grains in plants fertilized with blackcurrant seeds with micronutrients than for plants treated only with NPK fertilizer allowed elimination of the possibility of positive and dominant influence of NPK fertilization on micronutrient content of plant. It was shown that higher levels of zinc, manganese and copper in corn grain mainly result from the fertilization with the micronutrient components. The high content of micronutrients compared with control groups, proved that soil application of biological fertilizer components with micronutrients lead to the biofortification of maize grains. It confirmed results obtained by Hussain [8] who showed that soil application can also lead to the biofortification of staple food. Field trials proved that an increase of micronutrient dose did not result in increase of micronutrient content in grains which suggests that the proposed micronutrient dosage was the best for micronutrient fertilization of maize.

Obtained results showed that micronutrient fertilization with the use of biological fertilizer components can be applied in micronutrient fertilization of plants instead of inorganic salts which, as it was reported by Ortas and Lal [31], affect the soil’s organic carbon pool and agronomic yield.

There is a need to study new techniques enabling overcoming micronutrient deficiency. One of the methods is agricultural biofortification of food. Commercial micronutrient fertilizers are often used in biofortification of grains, but sometimes, the application of traditional micronutrient fertilizers is associated with toxic effect towards soils or plant yields and high leachability of nutrients to groundwater [24].

Low cost and simplicity of production of new micronutrient fertilizer components from biomass of blackcurrant seeds produced by biosorption, constitutes a good alternative to conventional fertilizers. Blackcurrant seed residues constitute waste material after production of seed oils, and the only cost of acquisition is connected with transport. During the experiment, several advantages of new product were underlined such as: ease of dosing, biodegradability, low content of toxic elements, macronutrients and micronutrients which are supplied in bioavailable form. No phytotoxic symptoms were observed in the crop of maize (cv. Kosmo 230) either from the tested products or from the reference fertilizer NPK(MgS) \((\text{Polifoska 4})\). A slightly better plant vigor compared to the not fertilized plots was assessed on all the plots fertilized with the tested products and the reference fertilizer. The plants did not vary. No significant differences were noted in the yield quantity harvested from the untreated plots and fertilized plots, but the content of micronutrients was about 7.60\% higher for Zn and 4.74 for Mn, for Cu – not statistically important.

### 5 Conclusions

The biomass of blackcurrant seeds enriched with micronutrients in a biosorption process can be used as micronutrient fertilizer component instead of inorganic salts. Micronutrients delivered with new bio-preparations
are characterized by high bioavailability, and its application led to the biofortification of maize grain with essential micronutrients. Due to the fact that BS100% was proved to give the best results in grain yield and micronutrient content, the advisable dosage would be as follows: 386.1 kg ha$^{-1}$ of Blackcurrant seeds + Zn, 170.2 kg ha$^{-1}$ of Blackcurrant seeds + Mn, 36.0 kg ha$^{-1}$ of Blackcurrant seeds + Cu and 500 kg ha$^{-1}$ of NPK (Polifoska). The application of micronutrient bio-preparations based on waste biomass allows the use of fertilization material characterized by controlled release of nutrients which are cheap and the carrier of micronutrients which is fully biodegradable. The new preparations can be a source of other micro- and macronutrients in bioavailable forms. Fertilization of corn with the use of bio-preparations resulted in the biofortification of grains with micronutrients which can be used as staple food or feed supporting overcoming micronutrient malnutrition.

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