Using white mustard (*Sinapis alba* L.) in vegetation tests with micronutrient biological fertilizer components based on berries seeds

Mateusz Samoraj[1], **Izabela Michalak**[1], Katarzyna Chojnacka[1]

[1] Department of Advanced Material Technologies, Faculty of Chemistry,

Wrocław University of Technology, Smoluchowskiego 25, 50-372 Wrocław, Poland

Presenting and corresponding author: e-mail: izabela.michalak@pwr.edu.pl (Izabela Michalak)

Abstract

The use of biomass as a fertilizing material has become a popular practice in agriculture. In this study we tested, under controlled and replicated conditions, the effect of inorganic (mineral salt) and biological (post-extraction residues of blackcurrant, raspberry and strawberry seeds) fertilizing compounds on the growth of mustard (*Sinapis* L.). Before pot trials under laboratory condition, biological fertilizing material was additionally enriched with microelement ions: Zn(II), Cu(II) and Mn(II) in the biosorption process. Plants in all groups were additionally fertilized with traditional NPK fertilizer. Plants cultivated only on NPK constituted a control group.

The obtained results revealed that the higher dose of microelements in the fertilizing material, the higher content in the plant. Microelements from the biological form were better bioavailable for plants than from inorganic form. Examined fertilizing materials did not influence the length of the plants. In the case of dry mass, results obtained for enriched post-extraction residues of blackcurrant seeds were 55% higher, than for inorganic salt. Microelements provided in the biological form influenced positively the chlorophyll content in plants. It was higher in all experimental groups when compared to the inorganic salts and the control group.

Fertilization with new micronutrient biological fertilizer components led to biofortification of *Sinapis* L. with Zn, Mn and Cu. New preparations can be used as bioavailable source of nutrients in commercial fertilizers.

Keywords: biosorption, post-extraction residues, berries seeds, fertilizing material, micronutrients, white mustard

INTRODUCTION

The need to feed the planet and to improve agro-efficiency has caused a shift in the fertilizer market. Fertilizers are commercialized as blends of different chemicals (organic and inorganic) and microorganisms. With a changing market, the legal and regulatory framework need to be adopted, too. (Jamers 2015). The European Commission intends to revise Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilizers (the Fertilizer Regulation) pertaining to inorganic fertilizers and to extend its scope to also include: (1) organic fertilizers; (2) soil improvers; (3) liming materials; (4) growing media; (5) plant biostimulants and (6) agronomic fertilizer additives (Traon et al. 2014, Spaey et al. 2012). In the recent years, a special attention has been paid to the following fertilizing materials: growing media, soil improvers, agronomic fertilizer additives and plant biostimulants (Traon et al. 2014).

In this paper, a special attention was paid to the agronomic fertilizer additives. According to the Traon et al. (2014), "an agronomic fertilizer additive is any substance (a chemical element and its compounds, as it occurs naturally or by manufacture) or microorganism, in the form in which it is supplied to the user, added to a fertilizer, soil improver, growing medium with the intention to improve the agronomic efficacy of the final product and/or to modify the environmental fate of the nutrients released by the fertilizer, the soil improver or the growing medium, or any combination of such substances and/or microorganisms intended for this use".

In the present study, post-extraction residues of berries seeds (blackcurrant, raspberry and strawberry seeds) were proposed as a new fertilizer additive. Berries seeds are a by-product in the production of fruit sauces, concentrates, juices, drinks, jellies and jams. These seeds can form 5–70% of total pomace weight depending on the type of dried fruit (Pieszka et al. 2015). This waste residue from the manufacture of juices should be used in a cost-effective and environmentally friendly way. Seeds are known to contain valuable compounds such as linolic and α-linoleic acid (Rój et al. 2009), lignans, polyphenols, vitamin C, *γ*-tocopherol, carotenoids, fibers (Smeds et al. 2012). Therefore, they can be used as a raw material for the extraction of biologically active compounds, for example lipids (especially unsaturated fatty acids) using supercritical fluid extraction with carbon dioxide (Rój et al. 2009). During this process, post-extraction residues of seeds are generated. The new idea involves their application as a fertilizer additive (Chojnacka et al. 2014). Prior the application in the plant cultivation, the biomass is enriched with microelement ions, necessary for proper plant growth. In the previous works, it was found that biomass enriched in the biosorption process acted as an effective fertilizer additive in the cultivation of garden cress (*Lepidium sativum*) under laboratory conditions (Samoraj et al. 2014). The preliminary tests must be confirmed and more advanced experiments must be performed in order to establish the proper ratio of micronutrients in the fertilizer additive, as well as the optimal dose.

The utilitarian properties of the new fertilizing materials are examined in three stages: germination tests under laboratory conditions (Samoraj et al. 2014; 2015), pot trials under laboratory conditions (present study) and finally the field trials which will confirm the efficiency of the new bio-products in real atmospheric conditions. The aim of the present study was to examine the utilitarian properties of the post-extraction residues of blackcurrant, raspberry and strawberry seeds enriched with essential in plant cultivation micronutrients – zinc, manganese and copper. The effect of the new bioproducts on the crop yield and crop quality was assessed.

MATERIALS AND METHODS

Biosorbent and biosorption process

The biomass of post-extraction residues of blackcurrant seeds (blackcurrant, raspberry and strawberry) was obtained from the New Chemical Syntheses Institute – Supercritical Extraction Department in Pulawy. The biomass was enriched with Cu(II), Mn(II) and Zn(II) ions via biosorption in stirred tank reactor (60 L) at 25ºC, pH=5 with the use of custom made pH regulator (Tuhy et al. 2014). Stock solutions of microelement ions (300 mg L^{-1}) were prepared by dissolving $CuSO_4 \cdot 5H_2O$, $MnSO_4 \cdot H_2O$ and $ZnSO_4 \cdot 7H_2O$ in distilled water. The content of the biomass was 1.0 g (d.m.; dry matter) L^{-1}. The mixture was stirred for 90 minutes. The solution was then filtered and the biomass was dried in a laboratory dryer (SUP30, Wamed, Poland).

Vegetation tests

The aim of these experiments was to evaluate the effect of the application of new environmental-friendly fertilizer – post-extraction residues of blackcurrant, raspberry and strawberry seeds enriched with micronutrients, in different doses, in comparison to control inorganic fertilizer (micronutrient salt – $CuSO_4 \cdot 5H_2O$, $MnSO_4 \cdot H_2O$ and $ZnSO_4 \cdot 7H_2O$). The effect of dosage of three micronutrients – Zn(II), Mn(II) and Cu(II) was tested. The micronutrients ratio in applied micronutrient fertilizers was 5:2:1 for Zn, Mn and Cu, respectively. Micronutrients were used in 5 doses, for zinc it was 0.5, 1.25, 2.5, 3.75 and 5.0 mg per pot, for Mn and Cu doses were 2.5 and 5 times lower, respectively (Table 1).

Table 1. Dosing scheme

Dose	Zn(II) dose per pot (mg)	Mn(II) dose per pot (mg)	Cu(II) dose per pot (mg)
0.5	0.5	0.2	0.1
1.25	1.25	0.5	0.25
2.5	2.5	1.0	0.5
3.75	3.75	1.5	0.75
5.0	5.0	2.0	1.0

All treatment groups contained a fixed level of macronutrients because the biomass cannot provide NPK in sufficient amount. NPK conventional fertilizer (Polifoska 4, Police, Poland) was added – optimal dosage was chosen in preliminary studies where plants length and chlorophyll content were determined. Taking into account these parameters, optimal dosage of NPK fertilizer was 250 mg per pot, in further tests this dosage was applied.

Vegetation tests were performed on white mustard (*Sinapis alba* L.) with methodology adopted from international norm (the International Seed Testing Association). On each pot, 25 seeds were placed at equal distances from each other. Before adding fertilizing material, seeds were subjected to the stratification in 2 °C for 2 days. After stratification, fertilizer materials in known amount were spread evenly on soil on each pot, then thin layer of soil was added. Each group was performed in triplicates at 25 °C for 2 months. Watering with 50 ml of deionized water for each pot was used every three days during the experiment.

Crop yield and crop quality were assessed – micro- and macronutrients content, micronutrients Transfer Factor (TF defined as a ratio between delivered micronutrient and assimilated plants). Micro- and macronutrient content in fertilizer materials and plants was determined using Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) method. The mass and length of plants were measured after the end of the experiment. The content of chlorophyll was measured with SPAD 502 plus chlorophyll meter (readings were taken from the fully expanded functional leaves) and with spectrophotometric method.

Analytical methods

Multielemental ICP-OES analysis

Before multielemental analysis, each material (0.5 g) was digested in microwave system Milestone Start D (USA) with nitric acid – 69% m/m (5 ml), spectrally pure (Suprapur, Merck, USA) in teflon bombs. Samples were diluted 10 times with ultrapure water (Millipore Simplicity) to perform multielemental

ICP-OES analysis (Varian–Vista MPX, Australia). All mineralization parameters were matched to assure complete digestion of samples.

Determination of element content in natural and metal-loaded biomass was examined using ICP–OES. 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 repeats (results of analyses were arithmetic mean, the relative standard deviation was <5%).

Chlorophyll content analysis

Chlorophyll content measured with SPAD 502 plus chlorophyll meter and with spectrophotometric method. For SPAD 502 plus measurements six plants for each pot were taken, washed and leaves were tested with SPAD 502 plus in triplicate. For spectrophotometric determination of chlorophyll content about 0.2 g of fresh leaves were taken from each pot. Leaves were washed and homogenized in methanol. After homogenization samples were centrifuged in order to obtain clear chlorophyll solution. Absorbance of each solution was measured at 663, 645 and 665 nm. For calculations methodology from work of Marr et al. (1995) was used. The concentration of total chlorophyll (Total Chl), Chl(a) and Chl(b) was determined from the equations:

$$\text{Total chlorophyll (mg L}^{-1}\text{)} = 20.2A_{645} + 8.02A_{663} \quad (1)$$

$$\text{Chlorophyll a – Chl(a) (mg L}^{-1}\text{)} = 12.7A_{663} - 2.69A_{645} \quad (2)$$

$$\text{Chlorophyll b – Chl(b) (mg L}^{-1}\text{)} = 22.9A_{645} - 4.68A_{663} \quad (3)$$

In the second method, the absorbance of the solution was measured at 665 nm (independent measurement of chlorophyll – Ind. Chl(a).

$$\text{Ind. Chl(a)} = 10.81\ A_{665} \quad (4)$$

Statistical Analysis

Obtained results were statistically elaborated using *Statistica* 10 software. For all obtained results, the distribution was tested for normality test (Shapiro–Wilk). For normal distribution, homogeneity of variance was checked using Brown–Forsyth test. 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 $p<0.05$ and $p<0.1$.

RESULTS AND DISCUSSION

In the present study, the effect of the new bioproducts – post-extraction residues of blackcurrant, raspberry and strawberry seeds enriched with micronutrients (Mn, Zn and Cu) on the crop yield and crop quality was assessed. As a model plant, mustard (*Sinapis alba* L.) was chosen. Mustard is an annual plant of the family *Brassicaceae* (Vaughan & Hemingway 1959). Mustard is cultivated for its seeds, condiment, as fodder crop or as a green manure. White mustard seeds, in addition to high-fat, are rich in proteins, essential amino acids (Downey et al. 1980). This plant is widespread worldwide. It is probably originated from the Mediterranean region (Vaughan & Hemingway 1959). Mustard is an undemanding plant model. Another advantages of mustard as a test plant is the ease of measuring the plant quality parameters.

Characteristics of the fertilizing material

The content of the micro- and macroelements in the enriched post-extraction residues of blackcurrant, raspberry and strawberry seeds is presented in Table 2. From the examined biosorbents, the best biosorption properties shown post-extraction residues of blackcurrant seeds. The content of Cu in the enriched biomass increased 1041 times, Zn – 328 and Mn – 105, when compared to the control group. In the case of raspberry, the increase was as follows: Cu – 1406, Zn – 138 and Mn – 33. For strawberry residue: Cu – 710, Zn – 115 and Mn – 60. It can be concluded that all types of the examined biomasses showed the highest affinity for Cu(II) ions, then Zn(II) and finally Mn(II) ions. This results from the ionic characteristics of sorbed metal ions, for example: atomic weight, atomic radius, ionization potential, electronegativity (Can & Jianlong 2007). As it can be seen from Table 2, most macroelements (especially K, Mg) were released from the biomass of post-extraction residues during biosorption process. The content of the main macronutrients was insufficient in the examined materials, therefore they cannot be treated as a fertilizer, rather as a fertilizing material. The exemplary effect of the bio-products on the growth of mustard is presented in Figure 1.

Figure 1. The effect of enriched post-extraction residues on the growth of mustard in the pot trials

Table 2. Micro- and macronutrients content in the tested materials (N=3) (Samoraj et al. 2015)

Post-extraction residues of seeds	Content in the biomass (mg kg^{-1} of d.m.)									
	Zn	**Cu**	**Mn**	**Fe**	**P**	**K**	**S**	**Ca**	**Mg**	**Na**
Blackcurrant	32.9 ±6.6	12.3 ±2.5	30.6 ±6.1	131 ±26	3240 ±648	7730 ±1546	2060 ±412	5630 ±1126	2260 ±452	< 0.05
Blackcurrant Zn	**10800 ±2161**	11.7 ±2.3	30.7 ±6.1	118 ±24	9710 ±1940	1950 ±391	2630 ±527	6210 ±1242	1700 ±340	51.2 ±10.2
Blackcurrant Cu	241 ±48	**12800 ±2550**	29.8 ±6.0	107 ±21	3790 ±758	2010 ±403	3360 ±671	5580 ±1115	2140 ±428	345 ±69
Blackcurrant Mn	18.2 ±3.6	36.6 ±7.3	**3210 ±641**	44.4 ±8.9	1970 ±395	951 ±190	1230 ±245	2610 ±522	893 ±179	136 ±27
Strawberry	43.6 ±8.7	13.5 ±2.7	85.8 ±17.2	152 ±30	2780 ±556	3350 ±671	2200 ±440	5590 ±1119	2460 ±493	615 ±123
Strawberry Zn	**5030 ±1010**	12.9 ±2.6	32.8 ±6.6	131 ±26	1430 ±287	672 ±134	1480 ±296	5120 ±1025	915 ±183	674 ±135
Strawberry Cu	151 ±30	**9580 ±1920**	25.6 ±5.1	118 ±24	1300 ±260	555 ±111	1450 ±290	4900 ±980	791 ±158	333 ±67
Strawberry Mn	59.1 ±11.8	87.3 ±17.5	**5130 ±1027**	117 ±23	1780 ±357	1720 ±343	1360 ±272	5420 ±1085	505 ±101	349 ±70
Raspberry	34.6 ±6.9	8.96 ±1.79	75.9 ±15.2	122 ±24	1550 ±310	2770 ±553	1410 ±281	2500 ±500	1800 ±360	472 ±94
Raspberry Zn	**4780 ±955**	9.09 ±1.82	18.8 ±3.8	101 ±20	757 ±151	290 ±58	1090 ±218	1120 ±225	339 ±68	622 ±124
Raspberry Cu	171 ±34	**12600 ±2520**	14.0 ±2.8	184 ±37	797 ±159	156 ±31	1370 ±274	585 ±117	170 ±34	630 ±126
Raspberry Mn	26.1 ±5.2	71.5 ±14.3	**2480 ±497**	113 ±23	1060 ±211	295 ±59	1010 ±202	1520 ±304	354 ±71	787 ±157

Bold – the content of a given microelement in the enriched biomass

Crop quality

Content of microelements in the cultivated plants

Crop quality was assessed for the content of examined microelements (Zn, Cu and Mn) in the cultivated plants (Figure 2 (a) – Zn, (b) – Cu, (c) – Mn). For this reason, different combinations were applied: group watered only with deionized water (untreated), group which was fertilized with NPK fertilizer (control) and experimental groups in which a given microelement was supplied in different doses and from different sources (inorganic salts and biological materials). The basis in all the experimental groups constituted traditional NPK fertilizer.

In the case of natural fertilizing materials, the degree of micronutrients availability in sources derived from various industrial or agricultural by-products is related to the manufacturing process, the source of complexing or chelating agents (organic sources) and the original product used as the microelement source (Gangloff et al. 2012).

In the case of Zn, the addition of Zn in the organic or inorganic form increased its content in the plant biomass. The higher dose, the higher content in the biomass (Table 3). For all applied doses of the post-extraction residues of blackcurrant and raspberry seeds, the content of Zn in plants was higher than in the case of the application of inorganic salts. The strawberry experimental group was the weakest from all the examined. The best results were obtained for post-extraction residues of blackcurrant seeds – dose 5.0. The content of Zn in the plant was 25% higher than for inorganic salts supplied in the same dose. It can be concluded that the biological form of microelements was characterized with higher bioavailability than inorganic form. Main statistically significant differences ($p<0.05$) were between: blackcurrant seeds (doses 3.75 and 5.0) and the control group and raspberry seeds (dose 5.0) and the control group (NPK fertilizer).

In the case of Cu, the highest dose of the fertilizing material – 5.0 (both inorganic and organic form) provided the highest content of Cu in the plant. The content of Cu in the cultivated plants was influenced in the highest extent by the the post-extraction residues of strawberry seeds in the highest dose – 5.0. It was 65% higher than in the case of inorganic salts supplied in the same dose. The lower doses (0.5, 1.25 and 2.5) in all the experimental groups slightly influenced the content of Cu in the cultivated plants. Main statistically significant differences ($p<0.05$) were observed between control group and: blackcurrant seeds (dose 5.0), and the strawberry seeds (dose 5.0).

For Mn, its content was higher for all doses and for all biological fertilizing materials when compared with the inorganic salt. Generally, the higher dose, the higher content of Mn in plant (with the exception of post-extraction residues of blackcurrant seeds for the dose 3.75). The best group was post-extraction residues of raspberry seeds for the dose 5.0. The content of Mn in plant was 49% higher than for inorganic salts. Main statistically significant differences ($p<0.05$) were observed between control group and: blackcurrant seeds (doses 2.5 and 3.75), raspberry seeds (dose 5.0) and the strawberry seeds (dose 5.0).

Overall, enriched post-extraction residues of berries seeds were the most effective source of Zn, Mn and Cu when the content of these micronutrients in plant tissue was used as the criterion. Comparing individual fertilizing materials, the post-extraction residue of blackcurrant seeds proved to be the best. These observations are confirmed by the literature data – in the work of Gangloff et al. (2012) it was found that Zn-Lignosulfonate and Zn-EDTA (in the highest applied dose – 8.0 g per pot) were always the most effective materials in supplying the plant’s needs when compared to inorganic Zn sources. The content of Zn in the biomass of corn increased with the dose (from 0.5-8.0 g per pot) and the best results were obtained for Zn-EDTA – the content of Zn was 37.6 mg kg^{-1} which was 2.5 times higher than for $ZnSO_4$. Tuhy et al. (2014) also examined the effect of different forms of Zn (peat, bark, seaweeds, seaweed post-extraction residues and spent mushroom substrate enriched via biosorption with Zn(II) ions and conventional fertilizers: inorganic salt and chelate) on its content in the plants – *Lepidium sativum*. It was shown that the content of Zn in the cultivated plants was higher in the groups with seaweeds, seaweed post-extraction residues and spent mushroom substrate when compared with the control group (differences statistically significant), as well as with zinc sulfate and even with Zn-EDTA.

On the basis of the conducted experiments it can be concluded, that the higher dose of the fertilizing material added to the standard NPK fertilizer, the higher content of a given element in the cultivated plants. The highest applied dose of microelements – 5.0 was not toxic to the plants. The obtained results are in agreement with the previous observations recorded during the germination test on *Lepidium sativum*. Post-extraction residues of blackcurrant, raspberry and strawberry seeds enriched with zinc, manganese and copper were used as a fertilizing material in which the ratio of Zn:Mn:Cu was the same as in the present study – 5:2:1. The biomass was applied in the following doses: 0.1, 0.25, 0.5, 0.75, 1.0. It was noted that with the increase of dose, the content of micronutrients increased in the edible parts of plants (Samoraj et al. 2015).

Additionally in the present study it was found that biological fertilizing materials were a better source in terms of bioavailability of microelements to plants than inorganic form. In some cases, it is claimed that organic complexes have greater micronutrient availability (e.g. Zn) than inorganic salts and require lower application rates to satisfy plant needs. Producers of organic sources generally claim a 10:1 advantage of organic sources vs. inorganic sources (zinc sulfate) to satisfy the agronomic demand (Gangloff et al. 2012).

Moreover, the effect of the biofortification of plants with microelements in all experimental groups was achieved. However, the content of examined microelements in plants was higher in the case of application of biological fertilizing material rather than for inorganic salt. For the plants in the group with post-extraction residues of blackcurrant seeds, the content of Zn, Mn and Cu was higher than in the group: NPK + Inorganic salt (for the best dose – 5.0) by: 25%, 18% and 38%, respectively. For plants in the group with the post-extraction residues of raspberry seeds was higher by: 11%, 48% and 19%, respectively. In the case of post-extraction residues of strawberry seeds, the content of Zn in plants was higher than in the control group (NPK fertilizer) by 50%, but slightly lower than in the case of group – NPK + Inorganic salt. The content of Mn in plants cultivated with post-extraction residues of strawberry seeds was 26% higher than in the group NPK + Inorganic salt and the content of Cu by 65%.

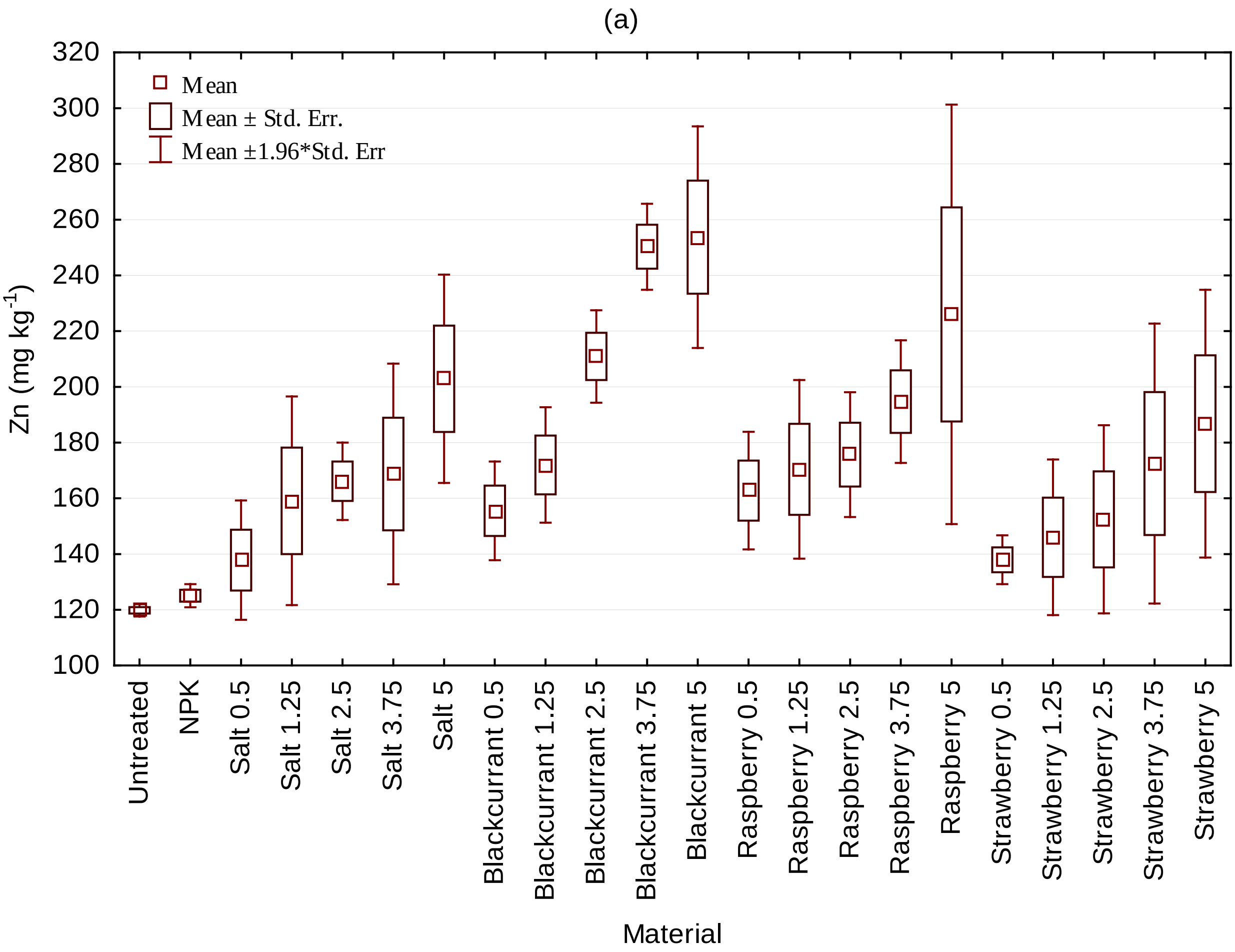
Table 3. Micronutrient content in plants after vegetation tests (N=3)

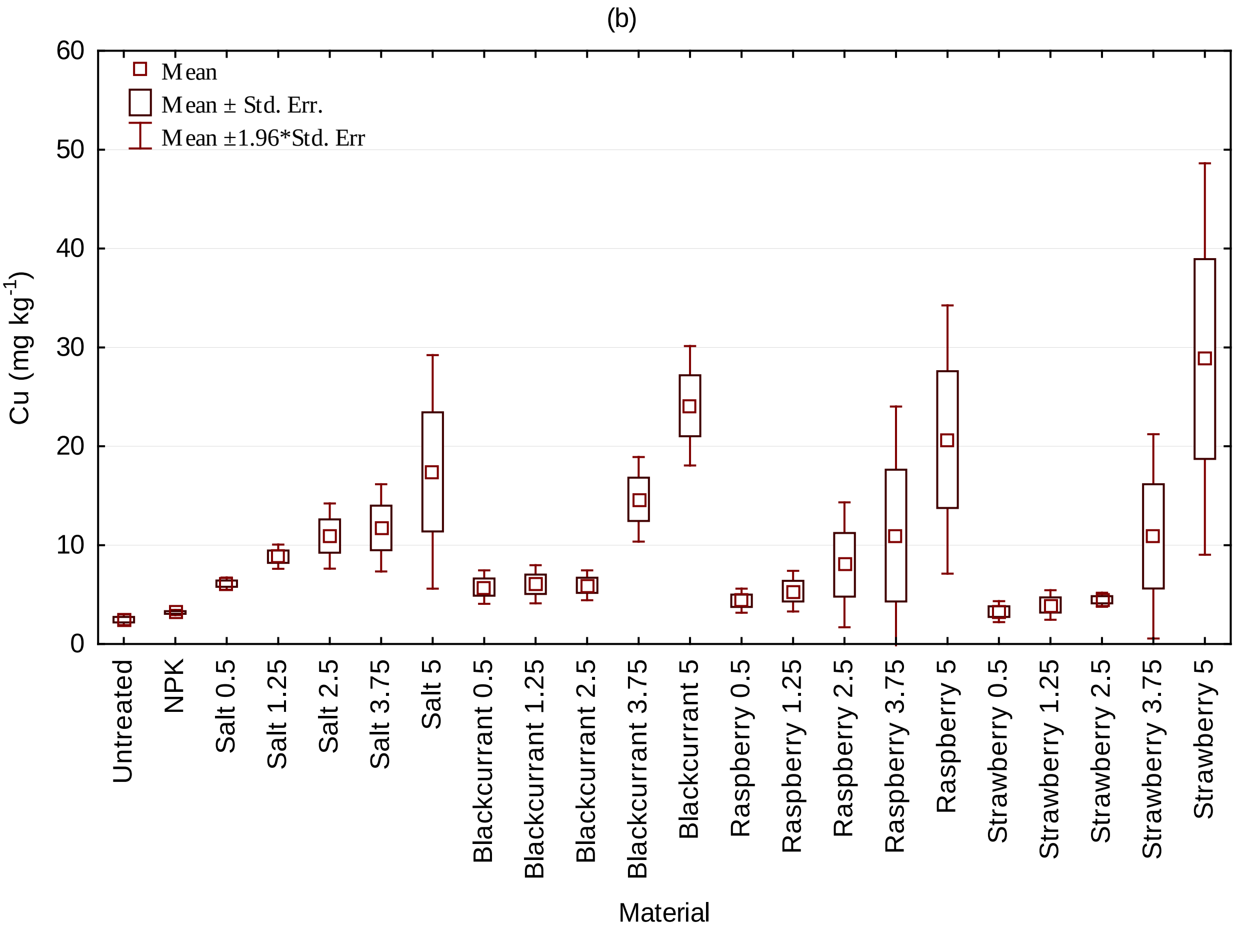
Content of microelements in the cultivated plants after application of examined materials in different doses 0.5, 1.25, 2.5, 3.75 and 5.0 (mg kg^{-1})																						
Element	Untreated	NPK (Control)	NPK + Inorganic salts					NPK + post-extraction residues of blackcurrant seeds					NPK + post-extraction residues of raspberry seeds					NPK + post-extraction residues of strawberry seeds				
			0.5	1.25	2.5	3.75	5.0	0.5	1.25	2.5	3.75	5.0	0.5	1.25	2.5	3.75	5.0	0.5	1.25	2.5	3.75	5.0
Zn	120 ±2 a,b,c,d,A	125 ±4 e,f,g,B	138 ±19 h,i,C	159 ±33 j,k	166 ±12 D,E	169 ±35 F	203 ±33 A	156 ±16 l,m	172 ±18	211 ±15 a,B	250 ±14 b,e,h,j,l,,n,o,p,D	254 ±35 c,f,i,k,m,q,r,s,t,E,F,G	163 ±19 q	170 ±28 G	176 ±20	195 ±19	226 ±67 d,g,C	138 ±8 n,r	146 ±25 o,s	152 ±30 p,t	172 ±44	187 ±42
Mn	57.0 ±12.4 a,b,c,d,e,f,g,A,B,C	70.0 ±1.0 h,i,j,k	70.7 ±1.9 l,m,n,D	81.7 ±21.8 o,p	79.5 ±2.6 q,r	91.9 ±5.2 s	97.7 ±14.6	94.1 ±4.1 t	101.7 ±6.2	121 ±21 a,h	135 ±32 b,i,l,o,q	115 ±11 c	93.0 ±22.9 u	97.0 ±13.5 E	110 ±26 d,D	114 ±4 e	145 ±3 f,j,m,p,r,s,t,u,E,F	94.3 ±10.7 F	105 ±5 A	107 ±15 B	104 ±16 C	123 ±35 g,k,n
Cu	2.46 ±0.48 a,n	3.20 ±0.23 b,o	6.11 ±0.54 c	8.84 ±1.08 d	10.9 ±2.9	11.8 ±3.9	17.4 ±10.4	5.76 ±1.50 e	6.05 ±1.71 f	5.94 ±1.33 g	14.6 ±3.8	24.1 ±5.3 n,o,p,q,A,B,C	4.38 ±1.08 h,A	5.35 ±1.82 i,B	8.02 ±5.58 j	11.0 ±11.5	20.7 ±12.0	3.28 ±0.93 k,p	3.95 ±1.32 l,q	4.48 ±0.63 m,C	10.9 ±9.1	28.8 ±17.5 a,b,c,d,e,f,g,h,i,j,k,l,m

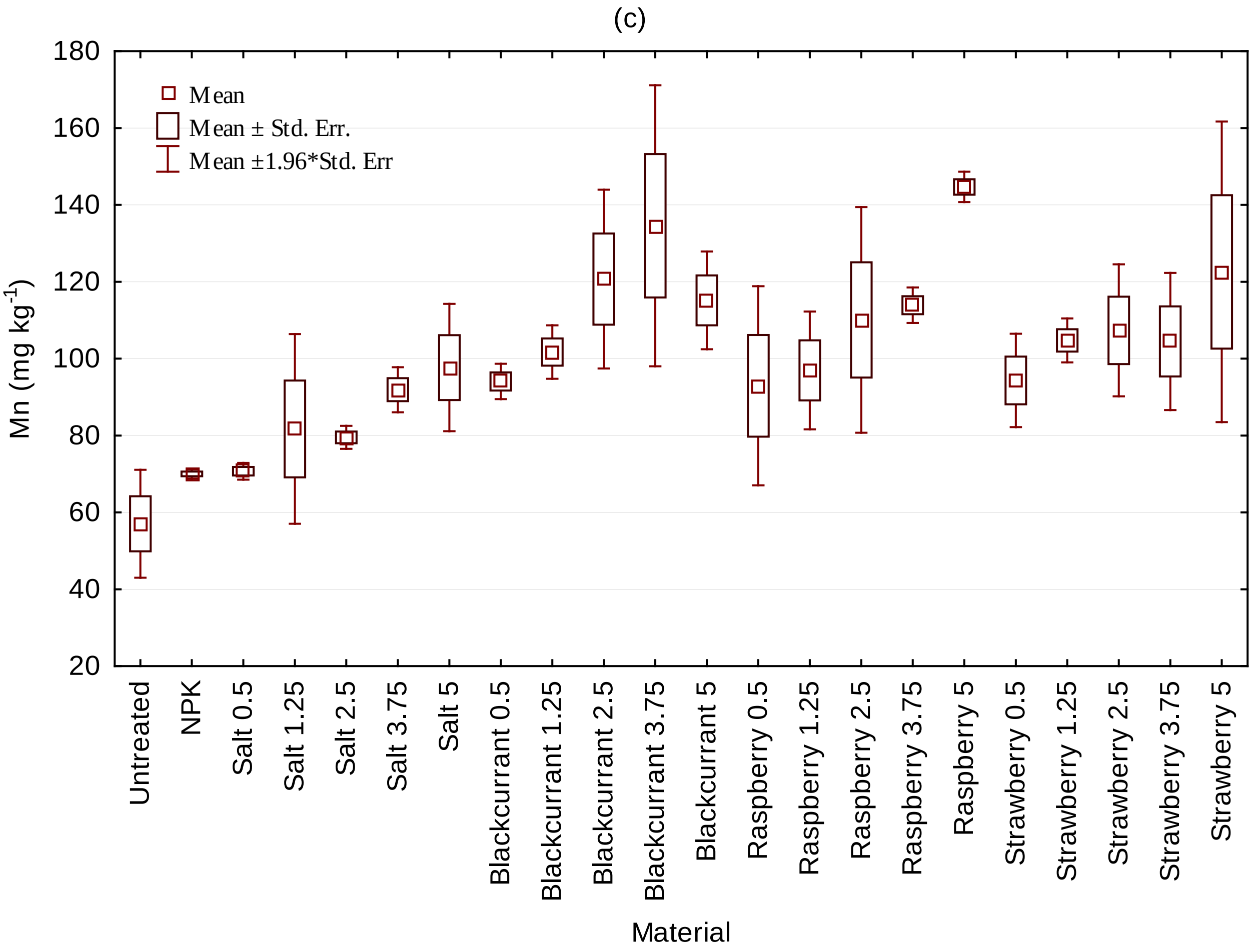
a,b,c,… statistically significant differences found with Tukey test (p<0.05)

A,B,C... statistically significant differences found with Tukey test (p<0.1)

Figure 2. The effect of different doses and materials on the content of (a) Zn, (b) Cu and (c) Mn in the cultivated plants




(b)
Mean
Mean ± Std. Err.
Mean ±1.96*Std. Err
Cu (mg kg-1)
0
10
20
30
40
50
60
Untreated
NPK
Salt 0.5
Salt 1.25
Salt 2.5
Salt 3.75
Salt 5
Blackcurrant 0.5
Blackcurrant 1.25
Blackcurrant 2.5
Blackcurrant 3.75
Blackcurrant 5
Raspberry 0.5
Raspberry 1.25
Raspberry 2.5
Raspberry 3.75
Raspberry 5
Strawberry 0.5
Strawberry 1.25
Strawberry 2.5
Strawberry 3.75
Strawberry 5
Material



Transfer Factor

For all examined microelements, Transfer Factor (TF) was determined (Table 4). TF is defined as a ratio between micronutrient assimilated by plant and delivered micronutrient. Generally, the higher dose of fertilizing material with microelements, the higher transfer factor. These results correspond with the observation for the multielemental composition of the cultivated plants. For Zn, the highest TF was obtained for post-extraction residues of blackcurrant seeds, for Cu for post-extraction residues of strawberry seeds and for Mn for post-extraction residues of raspberry seeds. In all cases, the highest TF was for the highest dose of the microelements (5.0) supplied in different forms of fertilizing material. Generally, TF was also higher in the case of the application of the biological source of microelements than for inorganic salt.

Growth parameters of the cultivated plants

In the present paper, it was found that the addition of fertilizing materials with microelements (regardless of the form – organic or inorganic) to the traditional fertilization influenced positively the length, as well as the mass of the cultivated plants. The application of organic and inorganic fertilizer to the soil is considered as good agricultural practice because it improves the fertility of the soil and plant quality (Mary & Nithiya 2015).

Length of the cultivated plants

Length of the cultivated plants was comparable in all examined experimental groups, as well as for different doses within the same group. The average length of plants for all examined doses for all groups was as follows: inorganic salt 10.2±0.3 cm; post-extraction residues of blackcurrant seeds 10.3±0.3 cm; post-extraction residues of raspberry seeds 10.0±0.5 cm and for post-extraction residues of strawberry seeds 10.1±0.3 cm. Statistically significant differences were not observed.

Dry mass of the cultivated plants

Table 4 shows also the average mass of dry plants cultivated with the use of the examined fertilizing materials with different doses of microelements. The applied doses did not influence the mass of plants in high extent. It was comparable within examined group for all tested doses. The highest average mass for all doses was obtained for post-extraction residues of blackcurrant seeds 0.0856±0.0069 g; then for post-extraction residues of raspberry seeds 0.0728±0.0067 g, of strawberry seeds 0.0690±0.0028 g and finally for inorganic salt 0.0554±0.0047 g. Biological fertilizing material had a beneficial influence on the mass of plants. The statistically significant difference ($p<0.05$) was observed between group with blackcurrant seeds – dose 2.5 and the control group – with NPK fertilizer. The dry mass of the cultivated plants in the experimental group was 2.5 times higher than in the control group. In the work of Gangloff et al. (2012) it was found that Zn supplied in different doses (from 0.5-8.0 g per pot) as $ZnSO_4$ (for dose 8.0 g per pot), Zn-Lignosulfonate (for doses 2.0 and 4.0 g per pot) and Zn-EDTA (for doses 0.5 and 1.0 g per pot) produced the largest increases in dry matter production (15-21%) when compared to the control. Organic sources were better source of Zn in lower doses, whereas inorganic in the highest applied dose.

Chlorophyll content in plants

Chlorophyll content in the cultivated plants in all examined groups was determined using two methods: extraction with methanol and direct measurement with the use of SPAD 502 plus. There was no direct influence of the dose of the fertilizing material on the chlorophyll content in the cultivated plants (Table 5). SPAD measurement showed that the content of chlorophyll in plants fertilized with biological materials was higher than in the case of the application of the fertilizer alone and for inorganic salts as fertilizing material. The highest statistically significant difference for the chlorophyll content was between group with inorganic salt – dose 3.75 (24.2) and group with raspberry seeds – dose 3.75 (33.4) and was 38% higher. As it was shown in Table 6, measurements of the chlorophyll content performed with the use of SPAD 502 plus were correlated positively with the content of chlorophyll determined spectrophotometrically.

It was also found that the content of chlorophyll measured spectrophotometrically at two wavelengths: 665 nm (Ind. Chl.(a)) and at 663 and 645 nm (total chlorophyll) was highly correlated. Generally, the addition of fertilizing material (in inorganic or organic form) to NPK fertilizer in the cultivation of white mustard increased the content of chlorophyll. In the group NPK+ Inorganic salt (the best dose 0.5) it was 30% higher than in the control group (NPK fertilizer), in the group: NPK + Blackcurrant seeds (2.5) it was 95% higher, in the group: NPK + Raspberry seeds (5.0) it was 90% higher and in the group: NPK + Strawberry seeds it was 95% higher. The advantage of biological form of microelements over inorganic form is significant. These results are in agreement with observations reported by other author, for example: Amujoyegbe et al. (2007) examined the effect of the type of nutrient source (inorganic fertilizer (IF), mixture of inorganic fertilizer and poultry manure (IFPM), poultry manure (PM) and control (C) (no fertilizer or manure treatment)) on the chlorophyll content in maize and sorghum. It was found that the highest content of chlorophyll for both crops was determined for IFPM, then for PM, IF and finally for control group. It was suggested that the nutrients were released by IFPM and PM toward the post-anthesis stage. Therefore, the nutrients were available to develop the site of photosynthesis, thereby aiding yield development of the two crops.

Correlations

Four experimental groups (all the preparations in five doses and the control group with NPK fertilizer, as well as untreated group) were tested in triplicates. The biomass of white mustard was collected from each pot separately and multielemental composition was analyzed, also in triplicates. Additionally, other parameters were also measured: effect if dose, dry mass (d.m.), fresh mass (f.m.), length (cm), chlorophyll (SPAD), independent chlorophyll a, total chlorophyll, chlorophyll a, chlorophyll b, transfer factor. Basing on this analysis, correlation matrix was plotted and presented in Table 6.

The most important correlations concerned for example: the content of chlorophyll (measured by SPAD, as well as spectrophotometrically) was positively correlated with the type of fertilizing material used. Results obtained for chlorophyll measured by SPAD were positively correlated with the chlorophyll measured with the use of spectrophotometric method. Very high correlations were observed for the total chlorophyll, chlorophyll a and b.

The content of Zn, Mn, Cu and P in plant, as well as transfer factor of Zn, Mn and Cu was positively correlated with the dose of fertilizing material with micronutrients as it was expected. It should be noted that the transfer factor of a given microelement was correlated with its content in the plant biomass. This correlation confirms our results, the higher dose of the fertilizer, the higher content of nutrients in plants, as well as the higher transfer factor. It was also found that Zn was correlated positively with Cu (0.42). According to the literature data, Cu and Zn are presumably absorbed through the same mechanism, therefore each competitively inhibits the uptake of the other (Bowen 1979). In the present study, the synergism between these two micronutrients can be explained by the doses applied – in the fertilizing material, the content of Cu was 5 times lower than the content of Zn.

It was also found that dry mass of the cultivated plants was correlated with the fresh mass and the average length of the plants.

Table 4. Parameters of the crop after application of examined materials in different doses

	Crop quality after application of examined materials in different doses (0.5, 1.25, 2.5, 3.75 and 5.0)																					
Parameter	**Un-treated**	**NPK Control**	**NPK + Inorganic salts**					**NPK + post-extraction residues of blackcurrant seeds**					**NPK + post-extraction residues of raspberry seeds**					**NPK + post-extraction residues of strawberry seeds**				
			0.5	**1.25**	**2.5**	**3.75**	**5.0**	**0.5**	**1.25**	**2.5**	**3.75**	**5.0**	**0.5**	**1.25**	**2.5**	**3.75**	**5.0**	**0.5**	**1.25**	**2.5**	**3.75**	**5.0**
Length (cm)	9.69 ± 0.34	8.86 ± 0.77	10.5 ± 0.3	10.4 ± 0.7	9.81 ± 0.65	10.5 ± 0.8	10.0 ± 0.8	10.4 ± 0.3	10.2 ± 0.9	10.5 ± 1.0	10.6 ± 1.0	9.86 ± 0.32	10.7 ± 0.9	10.2 ± 0.5	10.1 ± 0.7	9.33 ± 0.10	9.81 ± 0.92	10.0 ± 0.6	9.58 ± 1.42	10.2 ± 1.0	10.4 ± 0.4	10.3 ± 0.4
Fresh plant mass (f.m.) (g)	5.59 ± 0.68	4.70 ± 2.74	5.84 ± 0.78	6.70 ± 1.02	6.49 ± 0.32	5.67 ± 0.68	5.35 ± 2.05	7.09 ± 0.48	5.99 ± 0.67	5.78 ± 2.11	8.41 ± 0.19	6.76 ± 1.41	6.03 ± 1.93	5.67 ± 1.00	5.65 ± 2.07	5.39 ± 0.46	4.78 ± 0.91	5.74 ± 0.71	5.80 ± 1.70	6.07 ± 1.09	6.09 ± 1.01	6.72 ± 0.18
Dry plant mass (d.m.) (g)	0.0479 ± 0.0051	0.0355 ± 0.0288^{a,A}	0.0481 ± 0.0096	0.0597 ± 0.0223	0.0589 ± 0.0101	0.0536 ± 0.0127	0.0568 ± 0.0103	0.0898 ± 0.0238^{A}	0.0824 ± 0.0194	0.0958 ± 0.0275^{a}	0.0801 ± 0.0287	0.0801 ± 0.0117	0.0785 ± 0.0230	0.0710 ± 0.0102	0.0659 ± 0.0154	0.0811 ± 0.0167	0.0673 ± 0.0187	0.0664 ± 0.0073	0.0715 ± 0.0239	0.0710 ± 0.0097	0.0655 ± 0.0218	0.0704 ± 0.0206
Zn TF	-	-	16.3 ± 2.2^{a,b,A}	18.8 ± 3.9^{c,B}	19.6 ± 1.4^{C}	19.9 ± 4.1	23.9 ± 3.9	18.4 ± 1.8^{d,e}	20.3 ± 2.2	24.9 ± 1.7	29.5 ± 1.6^{a,d,f,g,h,B,D}	29.9 ± 4.1^{b,c,e,i,j,k,C}	19.2 ± 2.2^{D}	20.1 ± 3.3	20.7 ± 2.3	23.0 ± 2.3	26.7 ± 7.8^{A,E}	16.3 ± 0.9^{f,i,E}	17.2 ± 2.9^{g,j}	18.0 ± 3.5^{h,k}	20.4 ± 5.2	22.0 ± 5.0
Mn TF	-	-	20.9 ± 0.6^{a,d,h,A}	24.1 ± 6.4^{b,e}	23.5 ± 0.8^{c,f}	27.1 ± 1.5^{g}	28.8 ± 4.3	27.8 ± 1.2^{B}	30.0 ± 1.8	35.6 ± 6.1^{A}	39.7 ± 9.55^{a,b,c}	34.0 ± 3.3	27.4 ± 6.8^{C}	28.6 ± 4.0	32.5 ± 7.7	33.6 ± 1.2^{B}	42.7 ± 1.0^{d,e,f,g,C}	27.8 ± 3.2	30.9 ± 1.5	31.7 ± 4.5	30.8 ± 4.7	36.2 ± 10.2^{h}
Cu TF	-	-	3.60 ± 0.32^{a}	5.22 ± 0.64	6.45 ± 1.72	6.93 ± 2.30	10.3 ± 6.2	3.40 ± 0.89^{b}	3.57 ± 1.01^{c}	3.51 ± 0.79^{d}	8.64 ± 2.23	14.2 ± 3.1^{k,A,B}	2.58 ± 0.63^{e}	3.16 ± 1.07^{f}	4.73 ± 3.29^{g}	6.47 ± 6.80	12.2 ± 7.1	1.93 ± 0.55^{k,h}	2.33 ± 0.78^{i,A}	2.64 ± 0.37^{j,B}	6.43 ± 5.39	17.0 ± 10.3^{a,b,c,d,e,f,g,h,i,j,k}

a,b,c… statistically significant differences found with Tukey test (p<0.05)

A,B,C... statistically significant differences found with Tukey test (p<0.1)

Table 5. Chlorophyll content in plants after application of examined materials in different doses

Chlorophyll content in plants after application of examined materials in different doses (0.5, 1.25, 2.5, 3.75 and 5.0)																						
Parameter	**Un-treated**	**NPK Control**	**NPK + Inorganic salts**					**NPK + post-extraction residues of blackcurrant seeds**					**NPK + post-extraction residues of raspberry seeds**					**NPK + post-extraction residues of strawberry seeds**				
			0.5	**1.25**	**2.5**	**3.75**	**5.0**	**0.5**	**1.25**	**2.5**	**3.75**	**5.0**	**0.5**	**1.25**	**2.5**	**3.75**	**5.0**	**0.5**	**1.25**	**2.5**	**3.75**	**5.0**
Chlorophyll (Spad 502 units)	28.8 ± 0.9	26.7 ± 2.0	25.2 ± 1.1 ^{a}	24.4 ± 0.2 ^{b,c}	27.2 ± 1.8	24.2 ± 2.8 ^{d,e}	31.0 ± 3.6	28.8 ± 1.8	29.5 ± 0.7	32.3 ± 1.9 ^{b,d}	31.3 ± 2.1	28.8 ± 1.1	28.3 ± 3.6	31.3 ± 2.9	29.9 ± 3.2	33.4 ± 3.2 ^{a,c,e}	30.4 ± 2.1	30.7 ± 3.3	31.1 ± 2.0	30.3 ± 3.1	30.9 ± 3.7	27.9 ± 3.8
Ind. Chlorophyll a (mg kg^{-1})	607 ± 401	492 ± 79	642 ± 91	546 ± 241	552 ± 253	386 ± 70	604 ± 269	664 ± 207	749 ± 204	978 ± 166	961 ± 289	851 ± 286	660 ± 232	708 ± 288	669 ± 79	652 ± 276	949 ± 366	974 ± 353	868 ± 153	752 ± 199	963 ± 213	657 ± 131
Total chlorophyll (mg kg^{-1})	885 ± 579	717 ± 110	930 ± 124	801 ± 358	811 ± 368	563 ± 104	880 ± 386	959 ± 287	1081 ± 297	1404 ± 233	1383 ± 407	1241 ± 417	957 ± 340	1013 ± 401	959 ± 120	925 ± 390	1365 ± 526	1401 ± 503	1254 ± 226	1081 ± 290	1377 ± 297	952 ± 194
Chlorophyll a (mg kg^{-1})	639 ± 423	518 ± 82	678 ± 96	574 ± 252	581 ± 266	407 ± 75	636 ± 283	697 ± 216	789 ± 216	1028 ± 172	1013 ± 306	895 ± 300	693 ± 243	747 ± 305	703 ± 83	684 ± 290	1000 ± 387	1026 ± 372	915 ± 161	793 ± 209	1012 ± 223	693 ± 138
Chlorophyll b (mg kg^{-1})	246 ± 156	200 ± 28	252 ± 29	228 ± 106	230 ± 102	157 ± 29	245 ± 103	262 ± 71	292 ± 82	376 ± 63	371 ± 101	347 ± 117	264 ± 98	266 ± 96	257 ± 38	241 ± 100	365 ± 139	375 ± 131	339 ± 68	289 ± 81	365 ± 74	259 ± 58

a,b,c… statistically significant differences found with Tukey test ($p<0.05$)

Table 6. Correlation matrix for measured parameters

		Mat.	Dose	d.m. (g)	f.m. (g)	Length (cm)	Chl. (Spad)	Ind. Chl(a)	Total Chl.	Chl(a)	Chl(b)	Zn	Mn	Cu	P	K	S	Na	Mg	TF Zn (%)	TF Mn (%)	TF Cu (%)
												Content in plant (mg kg^{-1} d.m.)										
Material		1.00																				
Dose		0.00	1.00																			
d.m. (g)		0.16	-0.04	1.00																		
f.m. (g)		-0.10	-0.02	0.39	1.00																	
Length (cm)		-0.11	-0.15	0.32	0.50	1.00																
Chl. (Spad)		0.41	0.14	0.35	-0.27	-0.21	1.00															
Ind. Chl(a)		0.35	0.05	0.21	-0.06	0.09	0.41	1.00														
Total chl.		0.34	0.05	0.21	-0.06	0.09	0.41	1.00	1.00													
Chl(a)		0.35	0.05	0.21	-0.06	0.09	0.41	1.00	1.00	1.00												
Chl(b)		0.30	0.05	0.21	-0.05	0.09	0.39	0.99	1.00	0.99	1.00											
Zn	**Content in plant (mg kg^{-1} d.m.)**	-0.13	0.60	0.03	0.08	-0.19	0.07	0.12	0.12	0.12	0.13	1.00										
Mn		0.33	0.50	0.26	0.00	0.07	0.43	0.30	0.30	0.30	0.29	0.38	1.00									
Cu		-0.05	0.68	0.04	0.16	-0.08	-0.04	0.01	0.02	0.01	0.02	0.42	0.19	1.00								
P		-0.20	0.30	-0.02	-0.07	0.00	0.07	-0.11	-0.11	-0.11	-0.09	0.36	-0.05	0.07	1.00							
K		0.39	0.15	-0.12	-0.17	0.04	0.22	0.03	0.03	0.03	0.02	-0.01	0.00	-0.05	0.38	1.00						
S		-0.11	0.04	0.19	0.10	-0.01	-0.11	-0.13	-0.13	-0.13	-0.11	0.15	-0.07	0.08	0.47	0.05	1.00					
Na		-0.22	0.15	-0.27	-0.16	-0.16	-0.20	-0.04	-0.02	-0.04	0.01	0.29	-0.26	0.21	0.27	0.24	0.20	1.00				
Mg		-0.08	0.11	-0.14	-0.14	-0.19	-0.11	-0.13	-0.12	-0.13	-0.10	0.19	-0.22	0.14	0.51	0.26	0.36	0.49	1.00			
TF Zn (%)		-0.13	0.60	0.03	0.08	-0.19	0.07	0.12	0.12	0.12	0.13	1.00	0.38	0.42	0.36	-0.01	0.15	0.29	0.19	1.00		
TF Mn (%)		0.33	0.50	0.26	0.00	0.07	0.43	0.30	0.30	0.30	0.29	0.38	1.00	0.19	-0.05	0.00	-0.07	-0.26	-0.22	0.38	1.00	
TF Cu (%)		-0.05	0.68	0.04	0.16	-0.08	-0.04	0.01	0.02	0.01	0.02	0.42	0.19	1.00	0.07	-0.05	0.08	0.21	0.14	0.42	0.19	1.00

CONCLUSIONS

The objective of the present study was to estimate the effect of fertilizing materials (both natural, biological and inorganic) added to the traditionally used NPK fertilizers on the growth and quality of white mustard (*Sinapis alba* L.) in pot trials. The bioavailability for plants of three main micronutrients (Zn, Mn, Cu) derived from post-extraction residues of berries seeds was compared to the mineral salts ($ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $MnSO_4 \cdot H_2O$) commonly used to correct microelemental deficiencies in soils. The effect of examined fertilizing material on the content of elements in the biomass, transfer factor, length, mass and chlorophyll content was elaborated.

It was found that in the case of all microelements studied, their addition in the biological or inorganic form increased its content in the plant biomass. The higher dose, the higher content in the biomass. However, the biological fertilizing materials were a better source in terms of bioavailability of microelements to plants than inorganic form. It means that lower application rates of the enriched biomass will satisfy plant needs. With the increase of the dose of fertilizing material with microelements in all experimental groups, the transfer factor also increased. The transfer factor of a given microelement was correlated with its content in the plant biomass.

The effect of the biofortification of plants with microelements in all experimental groups was achieved (especially in the case of the application of biological fertilizing materials). The highest content of Zn in plants was for the group with post-extraction residues of blackcurrant seeds, Mn for the group with post-extraction residues of raspberry seeds and Cu for post-extraction residues of strawberry seeds.

The examined fertilizing materials with microelements did not influence the length of the cultivated plants, however they affected the mass of plants. The best results were obtained in the group with post-extraction residues of blackcurrant seeds – dry mass of the cultivated plants in this group was 2.5 times higher than in the control group.

The addition of fertilizing material (in inorganic or organic form) to NPK fertilizer in the cultivation of white mustard increased also the content of chlorophyll. A visible advantage of biological forms of microelements over inorganic was observed.

Concluding – taking into account all the examined parameters (content of microelements, chlorophyll in the cultivated plants, as well as the length and mass of the plants) the best fertilizing material was chosen and it was post-extraction residues of blackcurrant seeds. The obtained results also showed, that the addition of microelemental fertilizing materials to traditionally used mineral fertilizers is beneficial to increase the crop yield and its quality and should become a common practise.

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