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ORIGINAL PAPER

EFFECT OF BIO-FERTILIZER AMENDMENTS ON THE CONCENTRATION OF ELEMENTS IN EDIBLE PARTS OF RADISH AND GREEN BEAN GROWN IN A CROPPING SEQUENCE*

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Abstract

The assumption underlying this study was that bio-fertilizer amendments applied to acid soil would affect the yield of edible parts of vegetables, but would not increase their content of heavy metals. This concept was validated in an artificially prepared, micro-plot experiment, carried out in a cropping sequence of radish-green bean-radish grown in poor loamy sand. Three series of a two-factorial experiment were based on two bio-fertilizers, as the first factor, with different ratios of biomass ash (BA) and solids of biogas digestate (D) (BAD: FE1, 2.2:1; FE2, 1:2.2). The second factor was a dose of the applied BAD: 0.0, 20, 40, 80, 160, and 320 g m^{-2}. The share of digestate in the tested bio-fertilizer was the key factor affecting both the yield and elemental concentration of edible parts of the tested vegetables. FE2, rich in digestate, resulted in an enormous yielding response of plants. The yield of radish was significantly limited by the supply of macronutrients, both directly, such as by Mg affecting the first crop, and indirectly, due to the inefficient action of N towards the third crop in the studied cropping sequence. The yield of green bean grown on the FE1 treated soil followed the model detected for the 1st radish crop. The model of yields from for plants grown on the FE2 treated soil showed an increase reverse to the dose of FE2. Low, single doses of both bio-fertilizers resulted in low concentrations of certain nutrients, including Mg, Ca, Cu (all crops), and K (only green bean). High doses of bio-fertilizers, especially the ones rich in digestate, created a potential threat of excessive accumulation of heavy metals, as demonstrated in roots of the first radish, and pods of green bean. A single dose of any type of bio-fertilizer composed of biomass ash and digestate should be therefore adjusted based on i) grown crop requirements for a particular nutrient, ii) content of key nutrients in the applied fertilizer, iii) the level of respective nutrient content in cropped soil.

Keywords: biogas solids, biomass ash, vegetables, edible parts, nutrients, heavy metals.

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INTRODUCTION

Biomass ash has been used in agriculture for a long time, owing to its unquestionable ability to neutralize soil acidity (Ohno, Erich 1990). Assuming that the elemental content of biomass ash is known, it can serve as a good nutritional source for agricultural plants (Demeyer et al. 2001). Biogas is a product of the decomposition of organic substances under controlled anaerobic fermentation. A by-product of this process is biogas digestate (BD) or simply digestate. This residue contains a range of nutrients, both macro- and microelements. Undisputedly, the kind of substrate used for biogas production has an influence on the digestate's chemical properties. Therefore, inputs that are rich in macroelements (N, P, K) for example, will evidently produce digestate with a generous content of the same elements (Möller and Müller 2012, Nkoa 2014). Among substances found in both bio-ash and digestate which can have a negative influence on crop plants, there are heavy metals, which require a watchful control. An optimal level of an element for the plant growth, as well as its shortage and toxicity range depend on a plant species (Guerra et al. 2012).

Many assessments of quantities of consumed metals by humans confirm that a substantial amount of these elements is provided by vegetables. Because of their short growing season and fast growth, vegetables absorb quite high amounts of macro- and micronutrients (Singh et al. 2012). Lead is one of the most toxic elements for both plants and humans. Despite this, it is relatively easily and intensively absorbed by plants, usually proportionally to its environmental concentration (Musielińska et al. 2016). Regarding cadmium accumulation – another example of the most toxic metals – vegetables can be grouped as follows:

- absorbing very low quantities (transmission factor 0.1÷0.5) – peas, green beans;
- moderate quantities (0.5÷1.0) – carrot, cabbage;
- high quantities (1.0÷3.0) – leek, radish;
- very high quantities (3.0÷6.0) – lettuce, spinach, celery (Gruca-Królikowska 2006).

The maximum acceptable levels of harmful metals in food, including edible parts of vegetables, are the standard that should be used to evaluate the agricultural residue used as a soil amendment (Commission Regulations 2006).

It has been assumed in this study that a bio-fertilizer based on biomass ash and biogas digestate combined in an appropriate ratio can serve as a product of high fertilization value. The validation procedure required an evaluation of an increase in the yield of vegetables and safety of vegetable consumption to human health. The main research objective was therefore to assess the content of macro- and microelements in edible parts of radish and green bean after application of varied doses of two types of bio-fertilizer amendments. The consumption acceptability was inspected in terms of the yield of edible parts and their content of heavy metals relative to the standards.

MATERIAL AND METHODS

The effect of bio-fertilizers based on biomass ash and biogas digestate (BAD) on the content of nutrients and heavy metals in edible parts of selected vegetables was validated in an artificially prepared micro-plot experiment on a plot 1.0 m^2 in size, where vegetables were grown in the sequence: radish, green bean, and radish. Soil used in the study was top-soil characterized by a loamy sand texture, classified as light soil. The agrochemical status of the soil was evaluated based on the Mehlich 3 method (Mehlich 1984). The soil pH was measured in 1:2.5 soil 0.01 M $CaCl_2$ suspension. Detailed description of the soil's chemical properties clearly indicates slightly acidic pH and very low content of available magnesium (Mg) and calcium (Ca), but not of phosphorus (P) and potassium (K) (for details: Przygocka-Cyna et al. 2018). The radish cultivar used here was Scarlet Globe-Rebel. The 1st radish crop was sown at the end of April and harvested on the 10th of June. The 2nd radish crop was sown on the 11th of August and harvested at the end of September. These two radish crops were separated by green bean cv. Sonesta.

A two-factorial experiment, replicated six times, was arranged as follows:

1. The first factor: two types of BAD fertilizers composed of biomass ash (BA) + a solid residue of biogas digestate (BDs) + phosphoric rock (PR) + elemental sulfur (S^0). The tested BAD were composed based on the reverse contribution of the first two components:
   a) FE1: BA-55% + BDs-25% + PR-15% + S^0-5%;
   b) FE2: BA-25% + BDs-55% + PR-15% + S^0-5%;
2. The second factor: five doses of BAD: 0; 20; 40; 80; 160; 320 g m^{-2}.

The BAD fertilizers were applied, irrespectively of a dose, at the beginning of the experiment and mixed into the soil layer of 7 cm. Mineral nitrogen in a dose of 4 g N m^{-2} was applied to the first and the third crop in each plot. The plot without BAD was the nitrogen control plot. P and K fertilizers were not applied. Water moisture during the whole experiment, which lasted for 5.5 months, was kept at the field capacity.

The plant material used for the determination of the fresh yield of edible parts of the investigated vegetables and elemental concentrations were collected from the entire area of the plot. Harvested samples of roots for radish and pods for green bean were first dried (65°C). Nitrogen concentrations were determined using the standard macro-Kjeldahl procedure. Plant material for the determination of elements was mineralized at 600°C, after which the ash was dissolved in 33% HNO_3. The phosphorus concentration was measured by the vanadium-molybdenum method using a Specord 2XX/40 at a wavelength of 436 nm. The content of K, Mg and Ca, Fe, Mn, Zn, Cu, Pb, and Cd was determined using a FAAS technique.

The data were subjected to a conventional analysis of variance using the computer program Statistica 12®. Differences between treatments were evaluated with the Tukey's test. *F* test results (***, **, * indicate significance at the $P < 0.1\%$, 1%, and 5%, respectively) are given in tables, figures and equations.

RESULTS AND DISCUSSION

Radish 1 – yield and macronutrient concentration

The yield of radish roots ranged from 12.2 to 14.8 t ha^{-1} (Table 1). It did not display any significant response to the type of bio-fertilizer amendment. Each dose of the fertilizer, averaged over type, resulted in a yield increase. The maximum root yield was obtained after an application of 80 g m^{-2} (0.8 t ha^{-1}) of BAD and it was around 22% higher than harvested from the N-control

Table 1

The effect of mineral-organic amendments on fresh yield of edible parts of vegetables grown in the sequence: radish - green bean - radish

Factors	Level of factors	Radish 1		Green Bean			Radish 2	
		YR	$R\text{-}DM_c$	TY	YP	$P\text{-}DM_c$	YR	$R\text{-}DM_c$
		(g m^{-2})	(g kg^{-1})	(g m^{-2})		(g kg^{-1})	(g m^{-2})	(g kg^{-1})
BAD	FE1	1360	39^{a}	2167	1291	87.5	922^{a}	39.8
	FE2	1375	42^{b}	2282	1323	83.4	1014^{b}	42.1
F		0.02	62^{*}	2.07	0.48	2.35	7.62^{**}	3.97
Dose (D) (g m^{-2})	0	1218^{a}	37^{a}	2045^{a}	1173^{a}	100.8^{b}	921^{ab}	42.7
	20	1285^{ab}	38^{a}	2238^{ab}	1312^{ab}	92.3^{b}	957^{ab}	41.8
	40	1445^{bc}	42^{b}	2333^{ab}	1363^{b}	70.6^{a}	979^{ab}	40.6
	80	1482^{c}	42^{b}	2336^{ab}	1397^{ab}	89.2^{b}	890^{a}	39.5
	160	1396^{abc}	43^{b}	2400^{b}	1432^{c}	66.3^{a}	963^{ab}	40.5
	320	1380^{abc}	41^{ab}	1997^{a}	1165^{a}	93.5^{b}	1099^{b}	40.6
F		5.54^{***}	48^{***}	2.94^{*}	4.13^{*}	17.1^{***}	5.45^{***}	0.65
F for the interaction								
BAD x D		1.61	0.39	4.76^{*}	5.34^{*}	2.43^{*}	2.70^{**}	0.88

[a] data marked with the same letter are not significantly different; ***. **. * significance at 0.001; 0.01; 0.05. respectively.

YR – fresh yield of radish roots; $R\text{-}DM_c$ – dry matter content of radish roots; TY. YP. $P\text{-}DM_c$ – total yield, yield of pods, dry matter content of pods, respectively.

plot, which had received only 4 g m^{-2} of N. The highest doses of the amendment applied resulted in a significant decrease of radish yields compared to the archived maximum (Figure 1). The yield of radish did not show any response to the concentration of these key macronutrients in its edible part. The lack of relationships between yield versus N and P can be explained by the fact that both nutrients were added in respective amounts. The concentration of K, despite its low amount incorporated into the soil, was within frequently published ranges (SRIDHAR et al. 2014). The Mg concentration in radish ranged from 1.23 to 1.46 g kg^{-1} and continued to rise up to 80 g m^{-2}, and to 320 g m^{-2} along with the application of FE1 and FE2, respectively (Figure 2). A similar average content level was confirmed in an experiment by BARAN (2011), where it reached 1.35 g kg^{-1}. The stepwise regression performed clearly showed that Mg functioned as a key nutrient, limiting the fresh yield of radish roots (Y_{FW}):

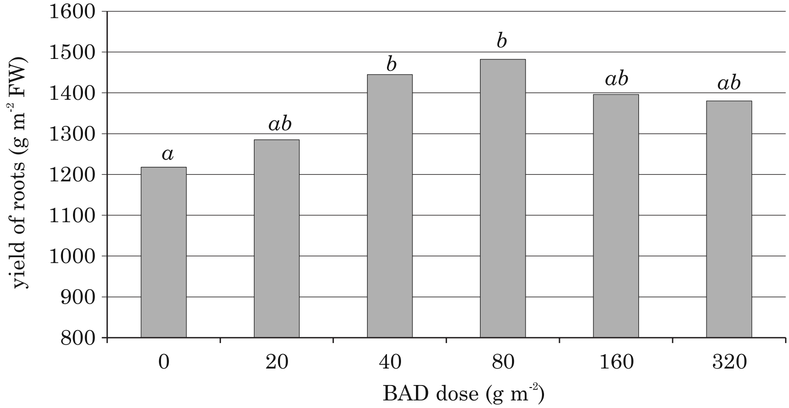

Fig. 1. Yield of radish roots as affected by the dose of BAD (experiment 1)

$$Y_{FW} = 157.8 + 865\ \mathrm{Mg} \text{ for } n = 72, \text{ and } R^2 = 0.37.$$

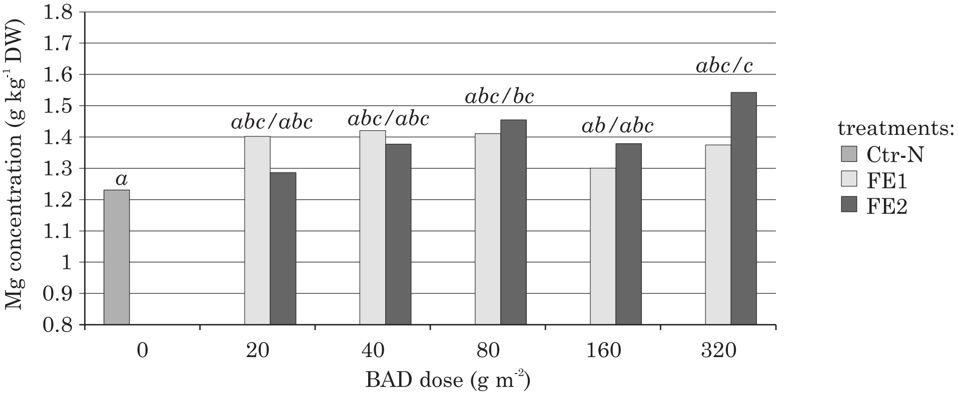

Fig. 2. Magnesium concentration in the radish root as affected by the BAD dose

The optimum concentration of macro-cations, despite their low (Ca, Mg) and moderate (K) soil content, indicates a high uptake potential of radish. As reported by Przygocka-Cyna et al. (2018), the progressive yield of radish resulted in deep exhaustion of phytoavailable K, Ca, and Mg.

Green bean – yield and macronutrient concentration

The experiment showed that the yield of green bean pods ranged between 13.1 and 14.3 t ha^{-1}, irrespectively of the BAD type (Table 1). The yield of pods was within the range considered to be optimal for intensive green bean production (Brown et al. 1993). The effect of BAD as a source of nutrients significantly depended on its type (Figure 3). In the plot with FE1, the lowest dose of BAD, amounting to 20 g m^{-2}, resulted in yield depression. This effect can be partly explained by the exhaustion of plant available K, Ca, Mg by the previously grown crop, i.e. radish (Przygocka-Cyna et al. 2018). Each higher dose of BAD, progressing towards 160 g m^{-2}, resulted in a subsequent yield increase, as compared to the control plot. Quite a different pattern of pod yield development was obtained on soil treated with FE2. The maximum yield was achieved on the plot treated with 20 g m^{-2} of BAD. Then, it decreased in accordance with the increasing BAD dose, but without depression. For both amendments, a significant yield drop with respect to the maximum one was recorded on the plot with 320 g m^{-2}. This response can be explained by the amount of applied Ca, which probably suppressed K uptake, leading to the reduction in bean growth and, consequently, to a drop in pod yield. As suggested by Da Silva Domingues et al. (2016), the K/Ca ratio is an important characteristic of green bean, affecting its yield.

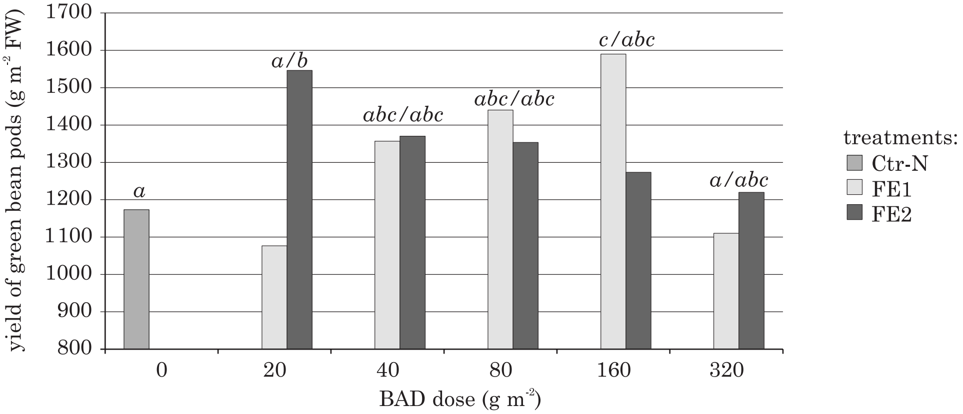

Fig. 3. Yield of green bean as affected by the type and the dose of BAD

Concentrations of all investigated nutrients in pods did not show any response to the type and dose of the applied amendments (Table 2). The content of N, around 26 g kg^{-1} DM of green bean, can be considered as a moderate one compared to other reports (Salinas-Ramirez et al. 2011). The key reason

Table 2

Effect of the BAD type and its dose on the concentration of elements in pods of green bean

Factors	Level of factor	N	P	K	Mg	Ca	Na	Mn	Fe	Zn	Cu	Pb	Cd
		(g kg^{-1} DM)						(mg kg^{-1} DM)					
BAD	FE1	25.8	0.86	10.4	2.02	2.47	0.27	23.9	112.8	29.4	7.67	1.86	0.31
	FE2	26.1	0.87	10.6	2.01	2.36	0.26	22.7	109.1	29.4	7.63	1.79	0.32
F		0.10	0.16	0.33	0.03	2.76	0.29	1.28	0.86	0.00	0.04	0.86	0.57
Dose (D) (g m^{-2})	0	25.3	0.82	10.1	1.98	2.67	0.24	21.3	107.1	28.5	7.68	1.79	0.31
	20	26.4	0.84	11.0	2.05	2.39	0.26	24.9	121.2	29.9	7.80	1.93	0.32
	40	25.8	0.82	10.4	2.03	2.39	0.27	23.4	107.7	29.0	7.33	1.69	0.32
	80	24.5	0.89	10.4	2.10	2.39	0.28	24.4	113.3	30.3	7.65	1.92	0.33
	160	27.7	0.95	10.5	1.99	2.35	0.25	23.4	109.80	30.6	7.98	1.85	0.32
	320	26.2	0.88	10.4	1.95	2.31	0.26	22.3	106.7	28.2	7.48	1.80	0.30
F		1.33	2.21	1.04	0.57	2.29	0.35	0.94	1.3	1.07	1.02	0.97	0.74
F for the interaction													
BAD x D		3.79**	1.48	0.52	1.00	1.40	0.49	0.42	0.50	2.63*	3.21*	0.74	1.64

***. **. * significance at 0.001; 0.01; 0.05. respectively.

was the high yield of pods, resulting in N dilution, which was recorded for plants grown on soil treated with low doses of FE1. This effect did not appear in plants grown on soil treated with FE2, except the plots with 320 g m^{-2} (Figure 4). However, the observed effect can be explained by the action of digestate, which controls the release of inorganic N in response to the growing plant requirement (Lošák et al. 2016). It is necessary to stress that the concentration of N showed significant, negative relationships with concentrations of Mg, Ca, and Na. Thus, the low N content in pods from the FE1 plots can be explained by the insufficient supply of this set of nutrients. The concentration of K in pods was low, but it can be seen as a dietary advantage (Martinzez-Pineda et al. 2016). The content of Ca was low, as compared to the nutritional standard of 5.6 g kg^{-1} DM (Quintana et al. 1999). The results achieved in this study cannot be explained solely by the high yield of pods. Their supply was probably too low for the fast-growing crop. The P content, owing to the application of a relatively high amount of P was above the published values, irrespectively of the BAD type applied (Salinas-Ramirez et al. 2011).

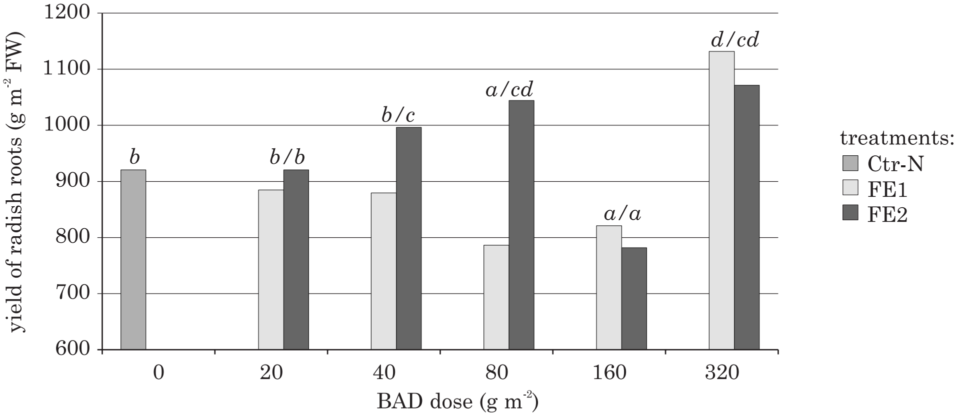

Fig. 4. Yield of radish roots as affected by the interaction of the type and the dose of BAD (experiment 2)

Radish 2 – yield and macronutrient concentration

The yield of radish following green bean was much lower compared to the first crop in the crop rotation (Table 1). Figure 5 shows the difference between the effects of the BAD fertilizers. Regarding the FE2 fertilizer, a significant difference in relation to the N control was already recorded at the dose of 40 g m^{-2}, increasing progressively with its higher doses. Quite a different model was observed for the FE1 fertilizer. The decreasing trend of root yield increased parallel to the FE1 doses up to 160 g m^{-2}. The key reason of yield depression, confirmed by its recovery on the plot with 320 g m^{-2}, was the exhaustion of macronutrients such as K, Mg, and Ca (Przygocka-Cyna et al. 2018). The progressive effect of FE2 on radish yield can only be explained

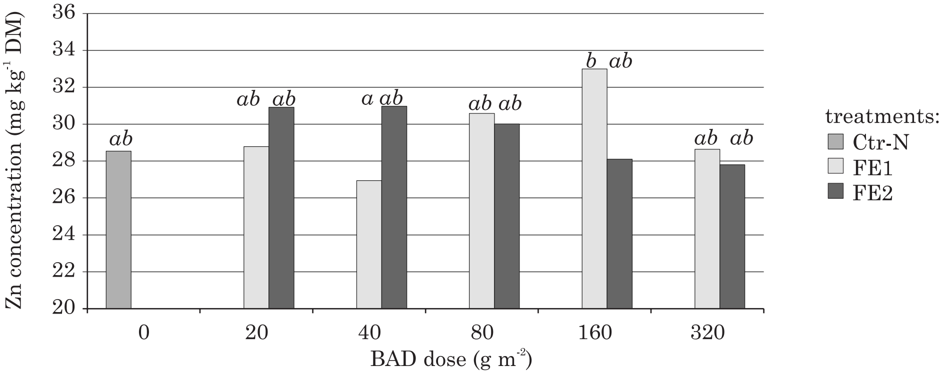

Fig. 5. Zinc concentration in green bean pods as affected by the type of BAD

by a specific effect of biogas solids. It can be related to the accelerated rate of mineralization processes, leading to a more rapid mineralization of organic residues (Grigatti et al. 2011).

The level of macronutrient concentrations in edible plant parts indicates good P nutrition, but poorer N nutrition (compared to the spring radish) (Table 3). All plots fertilized with BAD fertilizers demonstrated a higher N content in plant in comparison with the control, which had no correspondence to the dose of a fertilizer applied. Before the crop sowing, mineral N was applied at a dose of 4 g m^{-2}. Hence, radish should not suffer the shortage of this particular nutrient but could have been affected by an insufficient supply of other macronutrients. The experiment did not prove any influence of the fertilizer dose on the concentration of the remaining macronutrients in the radish roots (Table 3). In general, the N concentration significantly depended on the content of P, Mg, Ca, and Na. The positive signs of all these interactions, except Na, indicate that the key reason for N and P inefficiency was the shortage of macronutrients, with the exception of Na (data not shown, but available from the authors).

Evaluation of microelements concentration

Qualitative and quantitative concentration of elements in any crop part to a large extent depends on the primary composition of a given bio-fertilizer (Sridhar et al. 2014). When applying 200 kg ha^{-1} of BAD, 2717 g Fe becomes incorporated into soil (Przygocka-Cyna et al. 2018). In the analysis of the Fe concentration in plants, an increased quantity of that element has been noticed in all plants as compared with the control, but not in a linear relation to the dose. In both radish crops, a significant difference was recorded between the Fe concentration in the radish root and the control after an application of the lowest fertilizer dose of 20 g m^{-2} for the 1st radish crop and 80 g m^{-2} for the 2nd one. The content of Mn in the tested plants grown on plots treated with BAD prototypes ranged from 12.4 to 24.9 mg kg^{-1}.

Table 3

Effect of the BAD type and its dose on the concentration of elements in edible part of radish (2^{nd} experiment)

Factor	Level of factor	N	P	K	Mg	Ca	Na	Fe	Mn	Zn	Cu	Pb	Cd	Ni
		($g\ kg^{-1}$ DM)					($mg\ kg^{-1}$ DM)							
BAD	1	26.4	2.63	39.3	0.93	1.64	1.65	114.7	15.2	12.09	2.62	2.40	0.28	0.59
	2	25.3	2.49	39.2	0.86	1.54	1.54	100.2	13.4	15.85	2.45	2.33	0.29	0.52
F		1.22	3.43	0.24	3.05	1.76	2.20	2.42	2.05	1.95	2.15	0.88	1.35	3.66
Dose (D) ($g\ m^{-2}$)	0	24.6	2.55	39.3	0.84	1.48	1.49^{ab}	83.0^{a}	14.0	14.77	2.33	2.15	0.24^{a}	0.60
	20	25.9	2.66	38.7	0.89	1.58	1.69^{ab}	116.0^{ab}	15.5	12.24	2.71	2.43	0.29^{b}	0.59
	40	26.3	2.60	40.4	0.96	1.63	1.78^{ab}	138.7^{b}	16.2	18.40	2.56	2.36	0.30^{b}	0.59
	80	26.2	2.58	39.2	0.94	1.58	1.82^{b}	118.5^{ab}	14.9	17.31	2.76	2.46	0.30^{b}	0.60
	160	26.1	2.42	38.7	0.87	1.61	1.44^{ab}	98.7^{ab}	12.9	14.63	2.38	2.44	0.29^{b}	0.48
	320	26.2	2.53	39.2	0.85	1.65	1.35^{a}	90.0^{a}	12.4	6.46	2.45	2.35	0.28^{b}	0.48
F		0.32	0.74	1.93	1.16	0.42	4.49^{**}	3.31^{*}	0.92	1.68	1.51	1.35	6.11^{***}	1.40
F for the interaction														
BAD x R		0.92	0.87	0.80	0.43	0.39	1.37	0.64	0.70	1.26	0.34	0.90	0.00	0.44

[a] data marked with the same letter are not significantly different; ***. **. * significance at 0.001; 0.01; 0.05. respectively.

A higher level of Mn was detected in pods of green beans compared to radish. In the 1st radish, the Mn concentration in roots was higher than in the 2nd one. In the studies presented by Baran (2011), the Mn concentration, similarly to Fe, was higher in leaves, whereas in radish roots it was approximately 20 mg kg^{-1}. Therefore, a large quantity of Mn administered into soil with bio-fertilizer did not have any influence on the uptake or the concentration of these two nutrients in the edible parts of radish and green beans.

The average Zn content in bean pods did not depend on the fertilizer dose and equalled 29 mg kg^{-1}. In general, a higher Zn content was determined in the 1st radish (22 mg kg^{-1}) than in the 2nd one (14 mg kg^{-1}), not only in the plants where BAD fertilizers were applied, but also in the controls. The Zn concentration in bean pods was 29.4 mg kg^{-1} and depended on the interaction of both the type and dose of a fertilizer. A comparable level of Zn content was determined in an experiment carried out by Tariq (2006), where in the control plots the Zn content in radish tubers was 18 mg kg^{-1}, while in roots it amounted to 20 mg kg^{-1}. As presented in Figure 5, the highest yield of pods was concurrent with the highest Zn concentration in this part of bean plant. Noteworthy, Zn was positively correlated with the pods yield. As determined by the equation presented below, the pods yield (PY) depends on Zn concentration (Zn) in this plant part, provided it is grown on the FE2 treated plots:

$$\mathrm{PY} = 41.8\ \mathrm{Zn} + 94.4 \text{ for } R^2 = 0.38,\ n = 36 \text{ and } P \leq 0.001.$$

This phenomenon can be partly explained by the soil content of plant available Zn, which for the FE2 reached the maximum in soil treated with 20 g m^{-2}, and then decreased with increasing doses of the fertilizer (data not shown, but available from the authors).

Copper concentration in the 1st radish reached 4.3 mg kg^{-1} on average, while in 2nd one, it was much lower, achieving only 2.5 mg kg^{-1}. In both experiments, the dose of BAD fertilizers did not affect the Cu content in radish roots, but in the late-summer experiment a negative yield dependency on the Cu content was noticed. The values obtained were even lower compared to data reported by Sridhar et al. (2014) for radish treated with bio-solids. The Cu content in bean pods was 7.4 mg kg^{-1} and depended, in similar way as for Zn, on the interaction of both the type and fertilizer dose.

Evaluation of the content of heavy metals

The acceptable level of Cd and Pb for radish roots as well as pods of green bean is 0.1 mg of Pb and Cd kg^{-1} fresh matter (Commission Regulations 2006). This threshold value was exceeded for roots of the 1st radish, and for pods of green bean (Tables 2, 4, 5). As shown in Figure 6, the Pb concentration in roots was significantly affected by the interaction of the BAD type and its dose. The threshold value of 0.1 mg kg^{-1} FW was reached and/or exceeded in radish roots harvested from soil treated with a dose of FE1 above

Table 4

Effect of the BAD type and its dose on the content of elements in edible part of radish
(1^{st} experiment)

Factor	Level of factor	N	P	K	Mg	Ca	Na	Fe	Mn	Zn	Cu	Pb	Cd
		(g kg^{-1} DM)						(mg kg^{-1} DM)					
BAD	FE1	34.42	0.96	43.0	1.36	1.53	1.97	155.3	20.1	21.7	4.32	2.58	0.26
	FE2	34.84	0.94	43.2	1.38	1.57	1.94	162.7	21.4	22.2	4.28	2.40	0.24
F		0.17	0.64	0.17	0.90	0.60	0.10	2.44	3.34	0.34	0.05	0.92	2.39
Dose (D) (g m^{-2})	0	33.3^{ab}	0.92	43.8	1.23^{a}	1.43	1.86^{ab}	145.7^{a}	19.5	20.6	4.15	2.58	0.27
	20	36.6^{b}	0.94	43.0	1.34^{abc}	1.48	1.91^{ab}	175.2^{b}	22.3	22.5	4.26	2.46	0.26
	40	31.7^{a}	0.99	42.9	1.40^{bc}	1.59	2.17^{b}	160.4^{ab}	21.6	22.4	4.43	2.29	0.26
	80	36.2^{b}	1.00	43.4	1.43^{bc}	1.56	2.08^{ab}	169.4^{ab}	20.2	22.3	4.62	2.56	0.25
	160	33.2^{ab}	0.91	42.3	1.34^{abc}	1.59	1.60^{a}	149.9^{a}	19.3	21.2	4.26	2.37	0.24
	320	36.8^{b}	0.94	43.6	1.46^{c}	1.62	2.09^{b}	153.4^{ab}	21.6	22.8	4.08	2.69	0.23
F		2.96^{*}	1.14	1.02	8.43^{***}	1.40	3.18^{*}	3.93^{**}	1.21	0.93	0.92	0.69	0.83
F for the interaction													
BAD x D		0.72	2.6^{*}	0.35	3.07^{*}	0.79	2.14	1.22	0.18	0.47	1.71	0.98	0.47

[a] data marked with the same letter are not significantly different; ***. **. * significance at 0.001; 0.01; 0.05. respectively.

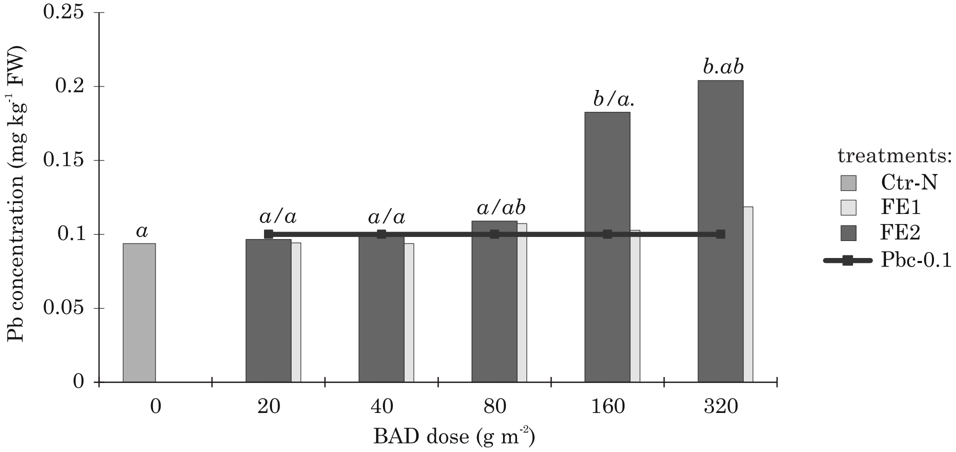

Fig. 6. Lead concentration in the radish root as affected by the types and doses of BAD against the background of the Pb treshold value

Table 5

The content of heavy metals in edible parts of tested plants (mg kg^{-1} FW)

Factor	Level of factor	Radish 1		Green bean		Radish 2	
Heavy metals		Pb	Cd	Pb	Cd	Pb	Cd
Threshold value$^{\#}$		**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**
BAD	FE1	0.10	0.010	0.16	0.028	0.09	0.010
	FE2	0.13	0.010	0.15	0.027	0.09	0.011
F		13.91***	0.31	3.04	0.62	0.34	0.84
Dose (D) (g m^{-2})	0	0.10	0.010	**0.18**^{b}	0.032^{b}	0.09	0.009
	20	0.09	0.010	**0.18**^{b}	0.029^{b}	0.09	0.011
	40	0.10	0.011	**0.12**^{a}	0.022^{a}	0.09	0.011
	80	**0.11**	0.010	**0.17**^{b}	0.029^{b}	0.09	0.011
	160	**0.14**	0.010	**0.12**^{a}	0.021^{a}	0.09	0.010
	320	**0.16**	0.009	**0.17**^{b}	0.029^{b}	0.08	0.009
F		13.02***	0.46	8.37***	8.75***	0.78	1.35
Significance of interaction							
BAD x D		4.73**	0.63	2.05	2.26	0.15	1.74

$^{\#}$ EU (2006); bold – values exceeding the permissible level.

40 g m^{-2}. This borderline value was determined in roots taken from all plots fertilized with FE2. The Pb content almost doubled in roots harvested from plots fertilized with 160 and 320 g m^{-2} The threshold values for both heavy metals were also exceeded in pods of green bean but not in roots of radish grown as the third crop in the sequence. The increased concentration of Pb

in pods of green bean can be explained by the ability of this plant to acidify the root rhizosphere, thus increasing solubility of heavy metals. It is worth emphasizing that the Pb concentration in pods decreased in the plot with a low dose of bio-fertilizer. It is necessary to underline the controlling function of K, which was negatively correlated with the content of both heavy metals. It confirms the anti-stress function of this nutrient as reported by Nazar et al. (2012). However, this means that any decrease in the K supply may result in the concurrent increase in the heavy metal concentration in green bean pods.

CONCLUSIONS

1. A bio-fertilizer rich in digestate creates favourable growth conditions for vegetables grown in an intensive cropping sequence, but its dose should be adjusted to both the requirements of cultivated vegetables and the content of their available soil pools.
2. Yield of radish was significantly limited by the supply of macronutrients, either directly, e.g. by magnesium for the first crop, or indirectly, through the effect of nitrogen on the third crop in the studied cropping sequence.
3. The yield of green bean grown on the FE1 treated plots followed the growth model for radish. The yield model for plants grown on the FE2 treated soil suggests its dependence on the K/Ca supply ratio.
4. A high, single dose of a bio-fertilizer applied to vegetables can maintain high yields of crops grown in a sequence, but it creates a health risk due to an excessive supply of heavy metals.

REFERENCES

Baran A., 2011. *Content of selected minerals in radish purchased on street markets in Kraków.* Bromat. Chem. Toksykol., 44(1): 25-31. (in Polish)

Brown J.E., Gilliam C.H., Schumack R.L., Porch D.W. 1993. *Commercial snap bean response to fertilization with broiler litter*. HortScience, 28(1): 29-31.

Da Silva Domingues L., Dalfollo Ribeiro N., Andriollo J.L., Della Flora Possobom M.T., Mezzomo Zemolin A.E. 2016. *Growth, grain yield and calcium, potassium, and magnesium accumulation in common bean as related to calcium nutrition.* Acta Scien. Agron., 38(2): 207-217.

Demeyer A., Voundi Nkana J.C., Verloo M.G. 2001. *Characteristics of wood ash and influence on soil properties and nutrient uptake: an overview.* Biores. Technol., 77: 287-295. European Union 2006. *Setting maximum levels for certain contaminants in foodstuffs*. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 Off. J. Eur. Union L. 364, 5-24.

Grigatti M., Di Girolamo G., Chincarini R., Ciavatta C., Barbanti L. 2011. *Potential nitrogen mineralization, plant utilization efficiency and soil* CO_2 *emissions following the addition of anaerobic digested slurries*. Biomass Bioenergy, 35: 4616-4629.

Gruca-Królikowska S., Wacławek W. 2006. *Metals in the environment*. Part II. *Effect of heavy metals on plants*. Chemia Dydaktyka Ekologia Metrologia, 11: 1-2, 41-56. (in Polish)

Guerra F., Trevizam A.R., Muraoka T., Marcante N.Ch., Canniatti-Lošák T., Hlušek J., **Válka** T., Elbl J., **Vítěz** T., **Bělíková** H., Von Bennewitz E. 2016. *The effect of fertilisation with digestate on kohlrabi yields and quality*. Plant Soil Environ., 62: 274-278.

Martinez-Pineda M., Yagűe-Ruiz C., Caverni-Muňoz A., Vercet-Tormo A. 2016. *Reduction of potassium content of green bean pods and chard by culinary processing. Tools for chronic kidney disease*. Nefrologia, 36(4): 427-432.

Mehlich A. 1984. *Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant*. Commun. Soil. Sci. Plant. Anal., 15: 1409-1416.

Musielińska R., Kowol J., Kwapuliński J., Rochel R. 2016. *Antagonism between lead and zinc ions in plants*. Arch. Environ. Protect, 42(2): 78-91.

Möller K., Müller T. 2012. *Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review*. Eng. Life Sci., 3: 242-257.

Nazar R., Iqbal N., Masood A., Khan M.I., Syeed S., Khan N.A. 2012. *Cadmium toxicity in plants and role of mineral nutrients and its alleviation*. Am. J. Plant Sci., 3: 1476-1489.

Nkoa R. 2014. *Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: A review*. Agr. Sust. Devel., 34: 473-492.

Ohno T., Erich M.S. 1990. *Effect of wood ash application on soil pH and soil test nutrient levels*. Agric. Ecosyst. Environ., 32: 223-239.

Przygocka-Cyna K., Grzebisz W., Łukowiak R. 2018. *The effect of bio-fertilizer amendments on agrochemical properties of soil cropped with vegetables*. J. Elem., 23(1): 163-177. DOI: 10.5601/jelem.2017.22.3.1476

Quintana J.M., Harrison H.C., Palta J.P., Nienhuis J., Kmiecik K. 1999. *Calcium fertilizers fail to affect pod calcium concentration and yield of four snap bean cultivars*. HortScience, 34(4): 646-647.

Salinas-Ramirez N., Escalante-Estrada J.A., Rodriguez-Gonzalez M.T., Sosa-Montez E. 2011. *Yield and nutritional quality of snap bean in terms of biofertilization*. Trop. Subtr. Agroecosyst., 13(3): 347-355.

Singh S., Zacharis M., Kalpana S., Mishra S. 2012. *Heavy metals accumulation and distribution pattern in different vegetable crops*. J. Environ. Chem. Ecotoxic, 4(10): 170-177.

Sridhar B.B., Witter J.D., Wu Ch., Spongberg A.L., Vincent R.K. 2014. *Effect of biosolids amendments on the metal and nutrient uptake and spectral characteristics of five vegetable crops*. Water, Air Soil Pollut., 225: 2092.

Tariq M., Mott C.J.B. 2006. *Effect of boron supply on the uptake of micronutrients by radish*. J. Agric. Biol. Sci., 1(2): 2-8