Journal of Elementology ISSN 1644-2296

Przygocka-Cyna K., Grzebisz W. 2018. *Effect of biofertilizers on nutrient uptake by vegetables grown in a short cropping sequence*. J. Elem., 23(3): 807-823.
DOI: 10.5601/jelem.2017.22.4.1479

RECEIVED: 11 June 2017
ACCEPTED: 20 November 2017

ORIGINAL PAPER

EFFECT OF BIOFERTILIZERS ON NUTRIENT UPTAKE BY VEGETABLES GROWN IN A SHORT CROPPING SEQUENCE*

Katarzyna Przygocka-Cyna, Witold Grzebisz

Chair of Agricultural Chemistry and Environmental Biogeochemistry
Poznan University of Life Sciences, Poland

Abstract

The assumption underlying this study has been that an application of organic-mineral amendments to acid soil with a moderate content of plant available nutrients can help to meet requirements of more than one crop in a particular cropping sequence. This concept was validated in a micro-plot experiment carried out on light soil. Three series of a two-factorial experiment were based on two biofertilizers as the first factor, with contrary ratios of biomass ash and solids of biogas digestate (BAD: FE1, 2.2:1; FE2, 1:2.2). The second factor was a dose of applied BAD: 0.0, 20, 40, 80, 160 or 320 g m^{-2}. Both biofertilizers, but especially EF2 with a high contribution of digestate, significantly affected the uptake of nutrients by vegetables. The observed patterns of nutrient partition among plants organs were modified by the applied soil amendment. The partition indices for radish showed a predisposition of this crop to accumulating K. Any shortage of macronutrients in the soil medium, as exerted by the low dose of BAD based on bio-ash, resulted in higher accumulation of minerals in the root. Green bean grown on plots fertilized with a biofertilizer rich in digestate (FE2) showed higher content of N, P, K, Na, but also Pb. The high ratio of bio-ash in the biofertilizer (FE1) disturbed the partition of Fe among organs of the radish. The soil amendment based on biogas digestate and biomass ash applied in high doses but without adequate enrichment with K and Mg can create a potential threat to the human health. The shortage of these two nutrients creates favourable conditions for the uptake of heavy metals by edible parts of the radish and green bean. Magnesium can be considered to be a mineral agent decreasing the threat of contamination of vegetables by heavy metals.

Keywords: biogas digestate, biomass ash, vegetables, edible organs, nutrients, heavy metals, uptake, partitioning.

Katarzyna Przygocka-Cyna PhD, Chair of Agricultural Chemistry and Environmental Biogeochemistry, Poznan University of Life Sciences, Wojska Polskiego 71F, 60-625 Poznań, Poland, e-mail: kasiac@up.poznan.pl; wojzu@tlen.pl

* This study is part of the research conducted within the EKO-BIONOM project of the NCBiR titled: *Pro-ecological manufacturing organic-mineral fertilizers based on: byproducts of burning and bio-gasification of biomass*. GEKON; Agreement No. GEKON1/05/214543/38/2015.

INTRODUCTION

Application of biofertilizers based on agricultural waste is considered nowadays as an environment-friendly way of reducing the consumption of non-renewable resources for mineral fertilizer production. The recycled biomass contains nutrients which can decrease the use of fertilizers manufactured from natural, geological deposits. A classical example is the production of nitrogen fertilizers based on natural gas (Odlare et al. 2015, Vaneeckhaute et al. 2013). Agricultural residues used as substrates in biogas plants are rich in carbon, but differ greatly in the content of elements, including nutrients, and heavy metals (Alburquerque et al. 2012). An important feature of biogas residue, i.e. the post-fermentation slurry, also called digestate, is the narrow C:N ratio, which accelerates soil microbial processes (Wentzel, Joergensen 2016). The concentration of elements in raw slurry is low. An alternative in digestate management is to convert digestate into a denser product, i.e. solids (DSs). It is often claimed that DSs manufactured in agricultural biogas plants are not only rich in organic matter, but they also contain sufficient amounts of nutrients, required in substances which are to replace mineral fertilizers (Vaneeckhaute et al. 2013).

Another example of potential soil amendment is wood ash and/or biomass ash (BA). The latter is a residue product from combustion of any organic waste such as agricultural by-products (straw), energy crops, etc. (Nunes et al. 2015). In general, BA is considered as both good liming material and a source of numerous nutrients. The former application arises from the opinion that the pH of biomass ash from wood ranges around 11-12. Thus, its incorporation into acid soils raises the soil pH (Lerner, Utzinger 1986). The latter claim is more controversial. Ash from wood or agricultural residues of crops is considered to be an important source of K, Ca, and P. However, numerous studies have documented high variability of the content of both macro-, and micro-nutrients in wood, straw or energy crops (Piekarczyk et al. 2011, Sander, Andren 1997). The potential value of BA could not be assessed unless its content of trace elements, mostly heavy metals, is determined. Ash from some plants, for example pine, is several times richer in lead and cadmium than ash from cereal straw (Ciesielczuk et al. 2011).

It has been hypothesized that application of organic-mineral amendments to acid soil with a moderate content of available nutrients covers requirements of more than one crop in particular cropping sequences. The basic factor that determined the influence of a specific amendment on a crop's growth and yield is the composition and applied dose of the amending substance. It has also been assumed that the effect of biofertilizer on yield of vegetables extends beyond the yield increase, and therefore investigations should cover the uptake heavy metals.

The key objective of this study was to assess the impact of two biofertilizers, differing in the type of biogas digestate, biomass ash ratio and applied doses, on the uptake of nutrients and heavy metals and their partitioning among organs of vegetables grown in the cropping sequence: radish, green bean, radish.

MATERIAL AND METHODS

The effect of biofertilizers manufactured from biomass ash and digestate (BAD) on the supply of nutrients and heavy metals to plants has been tested in an artificially prepared micro-plot experiment, with the plot size of 1.0 m^2, involving vegetables grown in a cropping sequence: radish, green bean, and radish. Soil used in the study was the top-soil characterized by a loamy sand texture and classified as light soil. A detailed description of chemical properties of the soil reveales its slightly acid pH and very low content of available magnesium (Mg) and calcium (Ca), but a high level of phosphorus (P) and a good supply of K (K) (for details: Przygocka-Cyna et al. 2018).

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 BADs were composed with different contributions 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 and 320 g m^{-2}.

The BAD fertilizers were applied in a single dose at the beginning of the experiment, by mixing them within the upper 7-cm soil layer. Mineral nitrogen in a dose of 4 g N m^{-2} was applied to all plots under both radish crops. P and K fertilizers were not applied there. The plot where only N was added served as the BAD control. The content and quantities of nutrients and heavy metals applied with the BAD fertilizers are presented in Table 1. The water moisture regime during the whole experiment, which continued for 5.5 months, was kept at the field capacity. The radish cultivar used here was Scarlet Globe-Rebel. The 1st radish crop was sown at the end of April and harvested on 10th of June. The 2nd radish crop was sown on 11th of August and harvested at the end of September. These two radish crops were separated by green bean, cultivar Sonesta. The incubation period for the applied BAD, preceding the first crop's cultivation, lasted for 14 days.

Plant samples used for the determination of the dry matter and element content were collected from the entire area of each plot. Harvested sample were partitioned into subsamples of roots and leaves (tops) for radish and pods and leaves for green bean, and then dried (65°C). The nitrogen content was determined using the standard macro-Kjeldahl procedure, at accuracy of 0.1 mg N. The plant samples for the elemental determinations were minera-

Table 1

Chemical composition and the amount of elements incorporated into the soil with BAD fertilizers

Fertilizer type	Content of the element	Fertilizer dose (kg ha^{-1})				
		200	400	800	1600	3200
FE1						
Element	(g kg^{-1})	Amount of the element, kg ha^{-1}				
P	36.8	7.5	15.0	30.0	60.1	120.1
K	31.1	6.6	13.3	26.6	53.1	106.3
Ca	57.4	12.8	25.6	51.1	102.3	204.5
Mg	9.1	1.0	3.8	7.7	15.3	30.6
S	48.0	9.6	19.2	38.4	76.8	153.6
	(mg kg^{-1})	Amount of the element (g ha^{-1})				
Fe	13586	2717	5434	10867	21738	43475
Mn	1392.4	278.5	557.0	113.9	2227.8	4455.7
Zn	205.1	41.0	82.0	164.0	328.1	656.2
Cu	58.3	11.7	23.3	46.7	93.3	186.6
Pb	21.8	4.4	8.7	17.5	34.9	69.8
Cd	3.6	0.7	1.4	2.9	5.8	11.5
Ni	14.9	3.0	6.0	11.9	23.8	47.7
FE2						
	(g kg^{-1})	Amount of the element (kg ha^{-1})				
P	36.4	7.3	14.6	29.1	58.2	116.5
K	19.7	3.9	7.9	15.8	31.5	63.1
Ca	40.4	8.1	16.1	32.3	64.6	129.1
Mg	5.2	1.0	2,1	4.2	8.4	16.8
S	48.0	9.6	19.2	38.4	76.8	153.6
	(mg kg^{-1})	Amount of the element (g ha^{-1})				
Fe	6907.6	1382	2763	5526	11052	22104
Mn	682.7	136.5	273.1	546.1	1092.3	2184.6
Zn	181.0	36.2	72.4	144.8	289.6	579.1
Cu	49.0	9.8	19.6	39.2	78.3	156.6
Pb	14.1	2.8	5.6	11.3	22.6	45.2
Cd	2.1	0.4	0.8	1.7	3.3	6.7
Ni	16.0	3.2	6.4	12.8	25.6	51.1

lized at 600°C. The ash was then dissolved in 33% HNO$_3$. The phosphorus concentration was measured by the vanadium-molybdenum method using a Specord 2XX. The concentrations of K, Mg and Ca, Fe, Mn, Zn, Cu, Pb, and

Cd were determined by FAAS (Varian 250 Spectra Plus). The amount of a particular element taken up by the plants was calculated by multiplying its content and the plant's respective biomass. The partition index (PI) of a particular element (E) was calculated from the general formula:

PI = amount of E in the edible part/amount of E in crop residues.

The experimental data were subjected to an analysis of variance supported by the computer software programme Statistica 12®. The significance of differences between means was evaluated with the Tukey's test. In the tables, figures, and equation *F* test results, the symbols ***, **, * indicate significance at the $P < 0.1\%$, 1%, and 5%, respectively.

RESULTS AND DISCUSSION

Uptake of elements by radish – first crop in the cropping sequence

The type of the mineral-organic fertilizer (BAD) affected significantly the uptake of six out of the twelve investigated elements such as nitrogen (N), phosphorus (P), potassium (K), iron, (Fe), manganese (Mn) and zinc (Zn) (Table 2). As a rule, higher uptake of all these nutrients appeared in the radish grown on plots fertilized with FE2. A slightly stronger response to this fertilizer was shown by micronutrients (≈14%) compared to macronutrients (< 10%). The partition of a particular element among organs of vegetable crops is one of its most important traits. As presented in Figure 1, leaves were the key allocation organ of all the investigated elements. The highest share of elements absorbed by roots, but not exceeding 40% of the total uptake, appeared in the case of K, Zn, and Cu, whereas the lowest one was for Ca (< 5%). The values of the partition indices of elements found in roots over all the treatments were as follows:

– macronutrients:

K (0.6) > Na (0.4) = N (0.4) > P (0.3) = Mg (0.3) > Ca (0.02);

– trace elements:

Cu (0.6) = Zn (0.6) > Pb (0.4) > Cd (0.3) > Fe (0.2) = Mn (0.2).

Roots were the richest in K followed by N, whereas tops accumulated almost equal amounts of N, Ca and K. The partition indices (PIs) clearly corroborate the advantage of tops over roots in macronutrient accumulation. The ratio of micronutrients in tops to roots was several times higher for all macronutrients, but only doubled for zinc. Special attention should be devoted to Fe, which accumulated in high amounts, exceeding Mn several times, but the partition indices for both elements were the same.

The significant effect of the BAD dose was observed for N, P, Mg, Fe and Zn. The first dose of the applied fertilizer, i.e. 20 g m^{-2}, resulted in a considerable increase in the uptake of nutrients with respect to the N-plot. The ef-

Table 2

The uptake of elements by radish grown as the first crop in the sequence: radish – green bean – radish

Factors	Level of factor	Macronutrients (g m^{-2})						Trace elements (mg m^{-2})					
		N	P	K	Mg	Ca	Na	Fe	Mn	Zn	Cu	Pb	Cd
BAD	FE1	6.35^{a}	0.25^{a}	5.98^{a}	0.37	4.39	0.36	54,9^{a}	7.06^{a}	3.04^{a}	0.60	0.55	0.06
	FE2	6.86^{b}	0.27^{b}	6.43^{b}	0.39	4.74	0.37	62.7^{b}	8.04^{b}	3.49^{b}	0.62	0.52	0.06
F test		6.52*	5.79*	7.20**	1.20	2.51	0.62	8.57**	5.69*	10.49**	1.08	0.68	0.32
Dose (D) (g m^{-2})	0	5.95^{a}	0.22^{a}	5.72	0.30	4.61	0.29	55.8^{ab}	6.75	2.53	0.52	0.53	0.07
	20	6.45^{ab}	0.26^{b}	6.01	0.39	4.50	0.39	65.2^{b}	8.20	3.20	0.60	0.53	0.06
	40	6.47^{ab}	0.26^{b}	6.33	0.36	4.11	0.42	50.2^{a}	7.31	3.31	0.64	0.55	0.07
	80	6.90^{ab}	0.27^{b}	6.49	0.41	4.63	0.41	63.0^{ab}	7.81	3.29	0.67	0.54	0.06
	160	6.79^{ab}	0.27^{b}	6.25	0.40	4.63	0.32	59.3^{ab}	7.51	3.38	0.60	0.54	0.06
	320	7.07^{b}	0.28^{b}	6.42	0.43	4.91	0.35	59.2^{ab}	7.69	3.86	0.63	0.53	0.06
F test		2.65*	4.63**	2.03	2.86*	0.97	0.97	2.63*	0.96	6.41***	2.91*	0.02	0.53
F test for the interaction													
BAD x D		0.98	1.14	0.88	0.79	0.66	0.66	2.64*	0.83	1.71	1.21	0.26	0.33

^{a} numbers marked with the same letter are not significantly different; ***, **, * significance at $P < 0.001$; 0.01; 0.05, respectively.

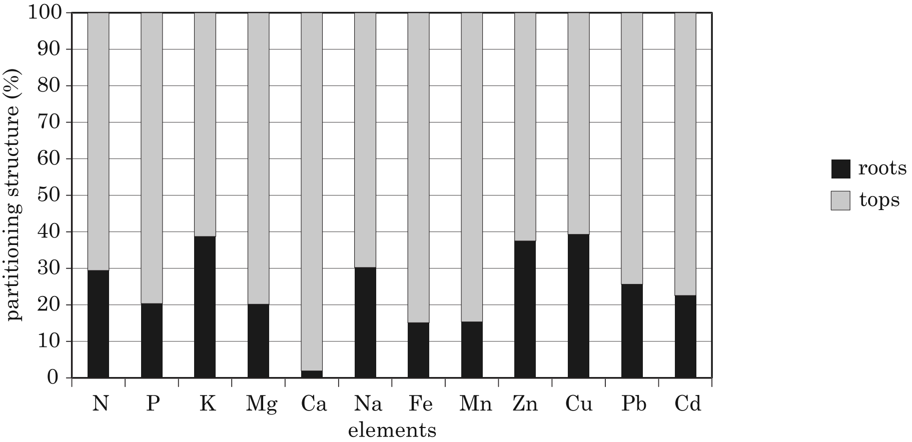

Fig. 1. Structure of elements partitioning among the radish 1st organs

fect of higher doses of BAD was revealed incidentally for N, Mg and Zn uptake by radish on the plot with the highest dose of BAD (320 g m^{-2}). The interaction effect of both experimental factors was observed only in the case of Fe. The results presented in Figure 2 clearly show that both biofertilizers resulted in a significant increase in the uptake of Fe by radish. This observation is important for consumers suffering from iron deficiency, e.g. pregnant women (Bojar et al. 2012). The maximum uptake was recorded in the entire crop biomass under the influence of the FE1 dose of 40 g m^{-2}, and the FE2 dose of 80 g m^{-2}. The decrease in Fe concentrations in radish treated with BAD doses above 80 g m^{-2}, especially deep in the soil treated with FE1, was probably due to the high dose of applied ash, resulting in a sudden increase in soil pH, as observed just before the sowing of radish (Przygocka-Cyna et al. 2018). A higher uptake of Fe by plants grown on soil treated with FE2 doses above 80 g m^{-2} was very likely because of the specific effect of digestate on soil processes, overcoming the negative impact of soil pH (Diacono, Montemurro 2010).

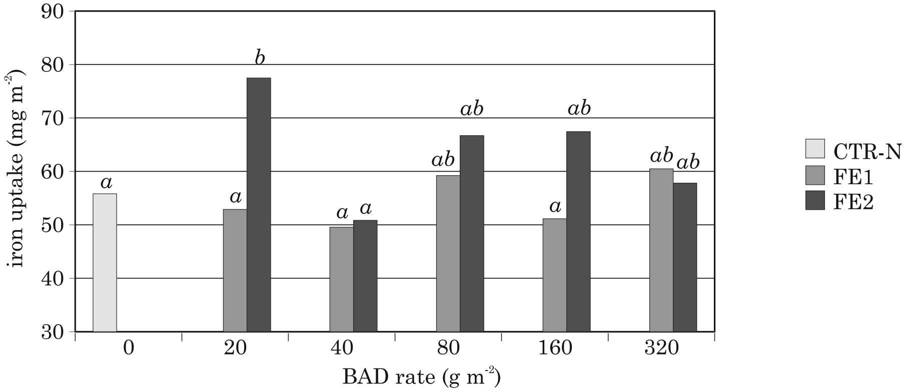

Fig. 2. The effect of BAD type on the total iron uptake by the 1st radish crop

Heavy metals are a particularly important issue in vegetable production, especially when biomass ash or digestate are used as soil amendments (Kupper et al. 2014). The stepwise analysis conducted in this research showed that the total amount of Pb and Cd in radish depended significantly on the content of Mn. The regression models obtained are as follows:

– lead:

$Pb = 137.3 + 53.0\ Mn$ for $n = 72$, $R^2 = 0.41$ and $P \leq 0.001$;

– cadmium:

$Cd = 26.3 + 4.72\ Mn$ for $n = 72$, $R^2 = 0.32$, and $P \leq 0.001$.

These two relationships were BAD specific, being much higher for radish grown on soil amended with FE1 (Pb for FE1: $R^2 = 0.72$; FE2: 0.29; Cd for FE1: $R^2 = 0.62$, FE2: 0.16). The Mn content was significantly correlated with the amount of K, which was the biomass driving factor. These two dependences clearly point to biomass ash as a key carrier of nutrients in the studied organic-mineral biofertilizers, including the supply of heavy metals to growing plants.

Uptake of elements by green bean – the second crop in the cropping sequence

The structure of elements absorbed by green bean, as the second crop in the studied cropping sequence, was different than in radish (Table 3). As a rule, lower amounts N, Ca, as well as for Pb, and Cd were absorbed by bean. The biggest difference between both crops appeared with respect to sodium (Na), whose total uptake by bean was several times lower than by radish. The same level of uptake for both crops was recorded for K. Quantities of other nutrients taken up by green bean, especially P, Mg and the micronutrients, were much higher compared to radish. Another important matter with respect to the nutritional value of vegetables is the amount of any particular nutrient and the structure of its partitioning among organs of a vegetable. The partition indices of elements for pods averaged over treatments were as follows:

– macronutrients:

Ca (5.82) > P (1.96) > K (0.82) ≥ N (0.82) > Mg (0.69) = Na (0.68);

– trace elements:

Fe (2.03) > Mn (1.97) > Cu (0.49) ≥ Zn (0.42) = Pb (0.42) > Cd (0.19).

The partitioning of each nutrient among green bean organs is presented in Figure 3. The PI equal to 1.0 indicates that 50% of any given element is accumulated in pods. This plant part proved to be a strong accumulator of Ca and, to a much lower degree, of Fe, Mn and P. These results are in agreement withreported by other authors (Sandberg 2002, Timoracka et al. 2011), who stress a high nutritional value of green bean with respect to these four nutrients. Despite the high PI value for Ca, the accumulation of this element in pods did not respond to the experimental factors. The same conc-

Table 3

The uptake of elements by green bean grown as the second crop in the sequence: radish – green bean – radish

Factor	Level of factor	Macronutrients (g m^{-2})						Trace elements (mg m^{-2})					
		N	P	K	Mg	Ca	Na	Fe	Mn	Zn	Cu	Pb	Cd
BAD	FE1	3.57^{a}	0.83^{a}	5.47^{a}	0.46	2.82	0.07^{a}	66.1	11.1	6.51	1.78	0.38^{a}	0.04
	FE2	4.16^{b}	0.99^{b}	6.15^{b}	0.50	3.04	0.08^{b}	71.8	12.0	7.01	1.94	0.42^{b}	0.04
F test		8.99^{**}	11.67^{**}	8.73^{**}	3.57	1.57	6.07^{*}	3.52	1.93	3.18	3.96	5.87^{*}	0.65
Dose (D) (g m^{-1})	0	4.30^{b}	0.89^{ab}	5.78	0.50^{ab}	3.21	0.07^{a}	69.5^{ab}	11.4	6.80^{ab}	1.96^{ab}	0.40^{abc}	0.05^{ab}
	20	4.12^{ab}	0.97^{ab}	6.26	0.53^{b}	3.07	0.08^{ab}	75.6^{b}	13.1	7.38^{b}	2.03^{b}	0.44^{bc}	0.05^{ab}
	40	3.81^{ab}	0.90^{ab}	5.77	0.45^{a}	2.78	0.07^{a}	65.6^{ab}	11.2	6.44^{ab}	1.77^{ab}	0.37^{ab}	0.04^{a}
	80	3.77^{ab}	1.04^{b}	6.05	0.54^{b}	3.22	0.09^{b}	74.3^{ab}	12.2	7.38^{b}	2.02^{b}	0.47^{c}	0.05^{b}
	160	4.03^{ab}	0.91^{ab}	5.80	0.44^{a}	2.82	0.08^{ab}	70.0^{ab}	11.6	6.48^{ab}	1.75^{ab}	0.36^{ab}	0.04^{a}
	320	3.17^{a}	0.75^{a}	5.18	0.44^{a}	2.47	0.07^{a}	58.7^{a}	9.9	6.07^{a}	1.64^{a}	0.35^{a}	0.04^{ab}
F test		2.70^{*}	2.83^{*}	1.67	3.16^{*}	1.79	2.55^{*}	2.68^{*}	1.93	2.39^{*}	2.58^{*}	5.08^{***}	3.79^{**}
F test for the interaction													
BAD x D		2.47^{*}	3.44^{**}	5.04^{***}	3.98^{**}	2.00	4.84^{***}	5.04^{***}	3.76^{**}	5.88^{***}	3.80^{**}	3.12^{*}	3.94^{**}

[a] numbers marked with the same letter are not significantly different; ***, **, * significance at $P < 0.001$; 0.01; 0.05, respectively.

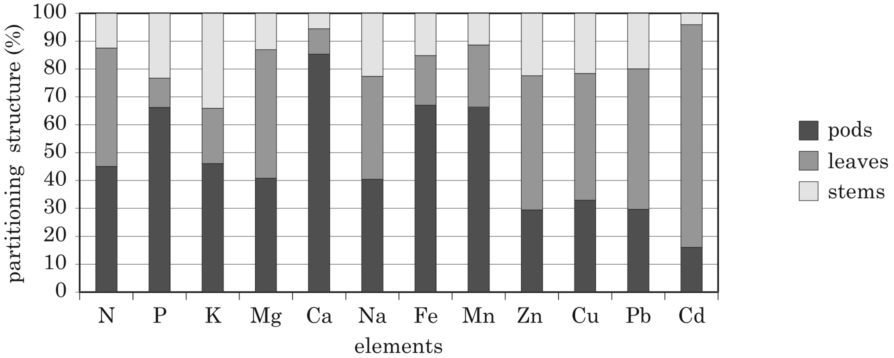

Fig. 3. Structure of elements partitioning among green bean organs

lusion was drawn by Quintana et al. (1999), who tested the response of four snap bean cultivars to mineral Ca fertilizers. The P accumulation in pods was affected by both BAD fertilizers, exceeding the N-variant in two cases for both FE1 (80 and 160 g m^{-2}), and FE2 (20 and 80 g m^{-2}). The accumulation patterns of Fe and Mn in pods showed the same response to both biofertilizers. The amounts of Fe and Mn accumulated in pods with respect to the N-variant were higher in plots fertilized with 20 and 80 g m^{-2} of both BADs, and with 160 g m^{-2} treated with FE1. There are no consistent data in the current literature concerning the response of these nutrients in pods of the legume crop to organic or mineral-organic amendments (Eldin-Mekki 2016). The data clearly show that biofortification of green been by means of soil amendments with micronutrients, or even with P, depend on numerous factors, including the type of an applied biofertilizer and its dose.

The partitioning of heavy metals among green bean organs is important. A high concentration of Cd in leaves (> 70%) was detected, followed by Pb, Zn and Cu (≈ 50%). The The PIs for Fe and Mn point to the pod as a strong sink of both nutrients, thus decreasing the uptake of heavy metals. These results clearly show that application of low doses of biofertilizers to radish and green bean can significantly improve their nutritional value with respect to nutrients such as iron and zinc, which can be deficient for sensitive groups of humans (Cakmak 2002).

In general, plants grown on plots fertilized with FE2 showed a higher content of all the studied elements, but a significant response was recorded for N, P, K, Na and Pb (Table 3). The significant effect of a BAD dose was confirmed for most of the studied elements except K, Ca and Mn. However, it was only Ca that did not respond to both experimental factors.

The total amounts of Pb and Cd accumulated by green bean showed positive interactions with the content of all other elements. According to the results from the stepwise analysis, the amount of Pb in the edible part of green bean can be evaluated on the basis of the content of Mg in pods:

$$Pb = 22.2 + 0.81\ Mg \text{ for } n = 72,\ R^2 = 0.68, \text{ and } P \leq 0.001.$$

The number of variables describing the content of Cd in pods was much higher, comprising N, Mg, Mn and Zn. However, Mn was the only element that showed a negative impact on the Cd content in pods:

$$Cd = -0.88 + 0.003\ N + 0.84\ Mg - 0.0023\ Mn + 0.004\ Zn$$
$$\text{for } n = 72,\ R^2 = 0.94, \text{ and } P \leq 0.001.$$

The presence of Mg as an antagonist of the uptake of heavy metals is an indirect indicator of its shortage, creating conditions for their uptake by the crop (Hermans et al. 2011).

Uptake of elements by radish – the third crop in the cropping sequence

The uptake of elements by the 2nd radish crop in the studied cropping sequence was inferior compared to the 1st one (Table 4). The key reason was a considerably lower biomass of tops produced by this crop. Regardless of big quantitative differences between both radish crops, the partitioning of elements among plant organs appeared very similar. The relative contribution of roots in the K uptake increased up to above 60%, whereas that of Zn decreased below 10%. The relative amounts of P and Mg in roots almost doubled compared to the first radish crop (Figures 1 and 4). The average values of the partition index for radish were as follows:

– macronutrients:
  K (1.6) > P (1.1) > Na (0.9) > N (0.8) > N (0.8) > Mg (0.6) > Ca (0.1);
– trace elements:
  Cu (0.7) > Pb (0.6) > Cd (0.4) > Fe (0.3) = Mn (0.3) > Zn (0.1).

The comparison of the above data with those for the 1st radish in the studied cropping sequence clearly shows that under a shortage of macronutrients the PIs increased dramatically. This was observed for all macronutrients, but the highest relative increase was recorded for P (PI increased two-fold). In contrast, the PIs for trace elements, except Zn, where it decreased six-fold, did not show great variability.

The advantage of FE2 over FE1 in terms of the uptake of elements was observed for most of the elements studied, excluding Fe and Mn. However, a significant effect of the BAD type was observed only for K, Na and Fe. This can be related to the impact of digestate as the key ingredient of FE2. The effect of a BAD dose on the amount of elements taken up by radish was significant for Na and Mn. For both nutrients, the maximum content was recorded in plants grown on the plot fertilized with 40 g m^{-2}.

Potassium was demonstrated to be a single variable in determining the Pb uptake in radish roots, but the optimal set of variables was much broader in the case of tops:

Table 4

The uptake of elements by radish grown as the third crop in the sequence: radish – green bean – radish

Factors	Level of factors	Macronutrients (g m^{-2})						Trace elements (mg m^{-2})					
		N	P	K	Mg	Ca	Na	Fe	Mn	Zn	Cu	Pb	Cd
BAD	FE1	2.25	0.19	2.40^{a}	0.09	1.21	0.13^{a}	17.8^{b}	2.46	5.86	0.24	0.24	0.042
	FE2	2.38	0.20	2.62^{b}	0.10	1.29	0.14^{b}	15.6^{a}	2.43	7.15	0.25	0.27	0.043
F test		2.71	1.28	12.2^{***}	1.51	5.04^{*}	4.49	6.94^{*}	0.07	0.96	2.59	2.55	0.20
Dose (D) (g m^{-2})	0	2.26	0.19	2.51	0.09	1.19	0.13^{abc}	14.3	2.22^{a}	6.20	0.24	0.22	0.043
	20	2.25	0.20	2.47	0.09	1.18	0.13^{abc}	16.7	2.47^{ab}	6.53	0.24	0.25	0.042
	40	2.40	0.20	2.60	0.10	1.32	0.15^{c}	18.1	2.79^{b}	8.27	0.26	0.27	0.045
	80	2.22	0.19	2.35	0.10	1.31	0.14^{bc}	17.8	2.46^{ab}	6.87	0.25	0.26	0.041
	160	2.37	0.19	2.47	0.09	1.29	0.12^{ab}	16.1	2.38^{ab}	7.10	0.25	0.26	0.042
	320	2.37	0.20	2.67	0.09	1.23	0.11^{a}	17.3	2.34^{ab}	4.04	0.25	0.28	0.042
F test		0.69	0.62	2.15	1.71	1.78	4.78^{***}	1.99	2.71^{*}	0.75	0.45	1.14	0.48
F test for the interaction													
BAD x D		0.29	0.31	0.75	0.25	1.94	1.33	1.32	1.12	0.90	0.13	0.40	0.57

[a] numbers marked with the same letter are not significantly different; ***, **, * significance at $P < 0.001$; 0.01; 0.05, respectively.

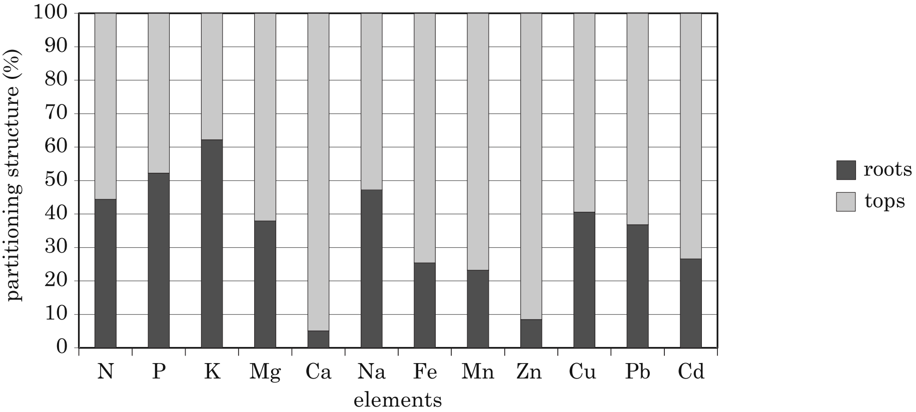

Fig. 4. Structure of elements partitioning among the radish 2nd organs

– roots:

Pb = 2.33 + 0.06 K for $n = 72$, $R^2 = 0.42$, and $P \leq 0.001$;

– tops:

Pb = 0.62 - 2.34 Mg + 0.17 Ca + 0.05 Mn

for $n = 72$, $R^2 = 0.71$, and $P \leq 0.001$.

The amount of Cd in roots showed significant relationships with five elements, such as K, Ca, Na, Fe and Mn. However, only Mg exerted a negative impact on the Cd accumulation in the radish root. In tops, the amount of Cd depended on the amount of accumulated magnesium:

Cd = 8.87 + 0.38 Mg for $n = 72$, $R^2 = 0.40$, and $P \leq 0.001$.

These three equations presented above indirectly corroborate the hypothesis by Hermans et al. (2011) that a shortage of Mg leads to an increasing uptake of heavy metals by plants.

The total element uptake by the cropping sequence

The differences presented above can be explained, at least partly, by patterns of the total nutrient uptake by plants growing in the cropping sequence: radish - green bean - radish in response to the type and dose of a biofertilizer based on bio-ash and digestate (Table 5). Only five of the 12 studied elements significantly responded to the BAD type, clearly stressing the advantage of FE2 over FE1. The soil amendment with the higher ratio of digestate to bio-ash (FE2) effected a significant increase in the total uptake of N, P, K, Ca, and Cu. All these nutrients were added into the soil in much lower amounts with FE2 than with FE1, characterized by a much higher content of biomass ash. The stronger uptake of any of these nutrients from soil treated with FE2 with respect to FE1 indicates a specific impact of FE2 on soil properties. The observed response agrees with data presented in the review by Diacono and Montemurro (2010). They reported that digestate in-

Table 5

The total uptake of elements by plants grown in the sequence: radish – green bean – radish

Factors	Level of factor	Macronutrients (g m^{-2})						Micronutrients (mg m^{-2})					
		N	P	K	Mg	Ca	Na	Fe	Mn	Zn	Cu	Pb	Cd
BAD	FE1	9.92^{a}	1.27^{a}	13.85	0.92	8.42^{a}	0.56	83.9	13.6	12.4	2.02^{a}	1.18	0.085
	FE2	11.02^{b}	1.45^{b}	15.20	0.99	9.07^{b}	0.59	87.5	14.4	14.2	2.19^{b}	1.21	0.087
F test		13.7^{***}	14.6^{***}	16.3^{***}	3.98	4.47^{*}	3.17	1.36	1.76	1.75	4.33^{*}	0.81	0.82
Dose (D) (g m^{-2})	0	10.25	1.30^{ab}	14.01	0.88	9.00	0.49^{a}	83.9^{ab}	13.6	13.0	2.19	1.16	0.090
	20	10.57	1.43^{ab}	14.75	1.00	8.75	0.61^{bc}	92.4^{b}	15.6	13.9	2.27	1.22	0.090
	40	10.27	1.36^{ab}	14.70	0.91	8.21	0.64^{c}	83.7^{ab}	14.0	14.7	2.02	1.19	0.084
	80	10.68	1.49^{b}	14.88	1,05	9.16	0.64^{c}	92.1^{b}	14.6	14.2	2.27	1.27	0.091
	160	10,82	1.36^{ab}	14.53	0.94	8.74	0.52^{ab}	86.1^{ab}	14.0	13.6	2.00	1.16	0.081
	320	10.25	1.23^{b}	14.28	0.96	8.61	0.53^{ab}	76.1^{a}	12.2	10.1	1.88	1.16	0.082
F test		0.47	2.50^{*}	0.64	2.15	0.76	8.42^{***}	2.56^{*}	2.00	0.99	2.31	1.02	1.94
F test for the interaction													
BAD x D		2.40^{*}	3.31^{*}	3.45^{**}	2.59^{*}	1.10	3.13^{*}	5.45^{***}	3.92^{**}	0.76	3.60^{**}	1.43	2.39^{*}

^{a} numbers marked with the same letter are not significantly different; ***, **, * significance at $P < 0.001$; 0.01; 0.05, respectively.

corporated into soil induces a series of processes, thus increasing the content of plant available nutrients.

The effect of a BAD dose on the total element uptake was recorded only for P, Na and Fe. For each of these elements, the uptake pattern was very similar. In general, the maximum uptake occurred on the plot fertilized with 80 g m^{-2}, decreasing afterwards in parallel to the increasing doses of BAD. The interaction effect of both experimental factors on element uptake was unobserved only for Ca, Zn and Pb.

The amount of Pb accumulated in the total crop biomass during the entire rotation cycle can be predicted based on a single variable, i.e. the amount of accumulated Mg:

$$\mathrm{Pb} = 459.7 + 0.77\ \mathrm{Mg} \text{ for } n = 72,\ R^2 = 0.59, \text{ and } P \leq 0.001.$$

The prediction of the total cadmium uptake by crops requires data on the total amounts of Mg and Cu taken up by plants grown in the studied sequence:

$$\mathrm{Cd} = 33.6 + 0.03\ \mathrm{Mg} + 0.01\ \mathrm{Cu} \text{ for } n = 72,\ R^2 = 0.49, \text{ and } P \leq 0.001.$$

These two equations clearly indicate that Mg can be considered as a critical element for heavy metal accumulation in edible parts of the studied vegetables. The doses of BAD above 80 g m^{-2} (0.8 t ha^{-1}) should be applied to vegetables with caution due to a potential threat of heavy metals accumulating in edible parts of sensitive crops, like radish. According to ASDEO and LOONKER (2011) radish leaves are a great accumulator of cadmium and lead. Therefore, Mg can be considered as a mineral agent decreasing the threat of contamination of vegetables by heavy metals.

CONCLUSIONS

1. The biofertilizers based on bio-ash and biogas digestate significantly affected uptake of nutrients by the tested vegetables. The biofertilizer rich in digestate (FE2) in comparison to the bio-ash rich one (FE1) significantly impacted the accumulation of elements in edible parts of vegetables.

2. The partition indices showed preference of radish roots to accumulate K, Cu and Zn, but not Fe and Mn, in spite of the high quantities of both elements in roots.

3. The high ratio of bio-ash in biofertilizer can disturb the uptake and partitioning of Fe between organs of vegetables. This negative effect can be overcome by increasing the contribution of digestate in a particular biofertilizer.

4. Green bean plants grown on plots fertilized with the biofertilizer rich in digestate (FE2) showed higher uptake of all the studied elements; a significant response was recorded for N, P, K, Na, but also Pb.

5. The mineral-organic soil amendment based on biogas digestate and bio-ash applied in elevated doses can create a potential health threat due to their high uptake by grown vegetables.

6. The shortage of Mg and K in soil treated with the studied biofertilizers can create favourable conditions for the uptake and accumulation of heavy metals in edible parts of radish and green bean.

7. This study clearly suggests that biofertilizers based on bio-ash should be enriched with magnesium and/or zinc.

REFERENCES

Alburquerque J.A., De la Guente C., Ferrer-Costa A., Carrasco L., Cegarra J., Abad M. & Bernal M.P. 2012. *Assessment of the fertiliser potential of digestates from farm and agroindustrial residues*. Biomass Bioenergy, 40: 181-189.

Asdeo A., Loonker A. 2011. *A comparative analysis of trace metals in vegetables*. Res. J. Environ. Toxicol., 5(2): 125-32.

Bojar I., Owoc A., Humeniuk E., Wierzba W., Fronczak A. 2012. *Inappropriate consumption of vitamins and minerals by pregnant women in Poland*. Ann. Agric. Environ. Med., 19(2): 263-266.

Cakmak I. 2002. *Plant nutrition research: priorities to meet human needs for food in sustainable ways*. Plant Soil, 247: 3-24.

Ciesielczuk T., Kusza G., Nemś A. 2011. *Fertilization with biomass ashes as a source of trace elements for soils*. Ochr. Środ. Zas. Nat., 49: 219-227. (in Polish with English summary)

Diacono M., Montemurro F. 2010. *Long-term effects of organic amendments on soil fertility. A review*. Agron. Sustain. Develop., 30(2): 401-422.

El-Din Mekki B.2016. *Effect of bioorganic, chemical fertilizers and their combination on growth, yield and some macro and micronutrients contents of faba bean* (*Vicia faba* L.). Biosci. Res., 3(1):8-14.

Hermans CH., Chen J., Coppens F., Inze D., Verbruggen N. 2011. *Low magnesium status in plants enhance tolerance cadmium exposure*. New Phytologist, 192: 428-436.

Kupper T., Bürge D., Bachmann H.J., Güsewell D., Mayer J. 2014. *Heavy metals in source-separated compost and digestates*. Waste Manage., 34: 867-874.

Lerner R.B., Utzinger J.D. 1986. *Wood as as soil liming material*. HortSci., 21: 76-78.

Nunes L.J.R., Matias J.C.O., Catalao J.P.S. 2016. *Biomass combustion systems: A review on the physical and chemical properties of the ashes*. Renew. Sustain. Energy. Rev., 53: 235-242.

Odlare M., Lindmark J., Ericsson A., Pell M. 2015. *Use of organic wastes in agriculture*. Energy Procedia, 75: 2472-2476.

Piekarczyk M., Kotwicka K., Jaskulski D. 2011. *The elemental composition of ash from straw and hay in the context of their agricultural utilization*. Acta Sci. Pol., Agric., 10(2): 97-104.

Przygocka-Cyna K., Grzebisz W., Łukowiak R. 2018. *The effect of mineral-organic amendments based on biomass ash and biogas digestate on agrochemical properties of soil cropped with vegetables*. J. Elem., 23(1): 163-177. DOI: 100.5601/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*. HortSci., 34(4): 646-647.

Sandberg A.S. 2002. *Bioavailability of minerals in legumes*. Brit. J. Nutrit., 88, Suppl. 3, S281-S285.

Timoracka M., Vollmannova A., Ismael D.S. 2011. *Minerals, trace elements and flavonoids content in white coloured kidney bean*. Potravinárstvo, 5(1): 56-60.

Vaneeckhaute C., Meers E., Michels E., Buysse J., Tack F.M.G. 2013. *Ecological and economic benefits of the application of bio-based mineral fertilizers in modern agriculture.* Biomass Bioenergy, 49: 239-248.

Wentzel S., Joergensen R.G. 2016. *Effect of biogas and raw slurries on grass growth an soil microbial indices.* J. Plant Nutr. Soil Sci., 179: 215-222.