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BIOMASS ASH AND BIOGAS DIGESTATE BIO-FERTILIZERS AS A SOURCE OF NUTRIENTS FOR LIGHT ACID SOIL – AN EXHAUSTION LETTUCE TEST

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ABSTRACT

It has been hypothesized that soil amendments based on biomass ash (BA) and biogas digestate (D) create favourable conditions for vegetable growth and yield on light, acid soil. This assumption was validated in an incubation test, in which six prototypes of bio-fertilizers (BAD) with different BA to D ratios were pre-incubated for six weeks. In the second step, an exhaustion experiment with lettuce was carried out. The incubation test clearly showed that the BA to D ratio is the key factor affecting the content of CaCl_2 extractable soil nutrients such as K, Ca, Mg, Na, Fe, Cu, Zn. The enrichment of BAD with urea resulted in a significant increase in the soil content of N-NH_4 , which in turn affected the content of soil available nutrients, mainly Mn. The yield of lettuce significantly depended on the supply of N-NH_4 . The shortage of N supply as recorded in the soil treated with urea-free bio-fertilizers resulted in some disturbance of the nutrient uptake by lettuce, leading to the shortage of Fe and the concomitant excess of Pb. The high contribution of digestate in the BAD fertilizer coupled with an addition of elemental sulphur resulted in an excessive release of N-NH_4 , which led to a decrease in lettuce yield. The concentration of Pb in lettuce corresponded to the amount of bio-ash incorporated into the soil. The threshold value of $0.2 \text{ mg Pb kg}^{-1} \text{ FW}$ was exceeded in the soil treated with the highest doses of the urea-free bio-fertilizers. The content of Pb can be controlled, provided the tested bio-fertilizers are enriched with magnesium and zinc. The content of cadmium in lettuce was below the threshold value of $0.1 \text{ mg Cd kg}^{-1} \text{ FW}$. The main reason for its low concentration was a sharp decrease of its supply to lettuce on plots treated with BAD bio-fertilizers.

Keywords: nutrients, heavy metals, availability, lettuce, phytotoxicity.

INTRODUCTION

The intensive exploitation of non-renewable resources can be limited by more widespread waste recycling. Agriculture is a sector that has big capacity for successful application of raw bio-waste, especially biologically transformed one. Bio-waste or different composts are a rich source of organic matter and biogenic substances, high in nitrogen and/or phosphorus. This is the biggest advantage of their indirect use as bio-fertilizers (ARTHURSON 2009). The hidden face of raw bio-waste or transformed waste products is a high content of heavy metals (CASTRO et al. 2009). Therefore, all bio-fertilizers based on bio-waste must conform to strict regulations in order to minimize the health risk for humans (KOUPAIE, ESKICIOGLU 2015). This step is unavoidable if any direct negative impact of bio-fertilizers on soil fertility and on edible parts of consumable crops is to be excluded. Lettuce, endive, radish, carrot and many other crops representing various groups of leafy and root vegetables are most sensitive to the excess of heavy metals in the growth medium (SINGH et al. 2012).

The agricultural use of biomass ash (bio-ash, BA) is inherently rooted in the history of human race. The biggest advantage of bio-ash is its high pH, ranging from 11 to 13. As a result, it has been used for centuries as a natural soil liming material for acid soils (SHI et al. 2017). However, as frequent reports show, it also contains a high concentration of heavy metals, such as cadmium, chromium, nickel and lead (CIESIELCZUK et al. 2011). The early 21st century is distinguished by a rapid development of biogas plants, in which different types of organic matter undergo transformation, under strict anaerobic conditions, into a mixture of two CH_4 and CO_2 , termed as bio-gas. The residue left from this process is biogas digestate, frequently called digestate (D). The separated stable part of slurry is used as a single organic bio-fertilizer or as a substrate for production of soil amendments (HOLM-NIELSEN et al. 2009). Like bio-ash, it may contain heavy metals in concentrations posing a risk to the quality and healthfulness of crops grown for humans (BIAN et al. 2015).

A potential risk of heavy metal uptake by crop plants can be directly tested in pot experiments. The main advantage of pot experiments is that manifold doses of the tested bio-fertilizer can be checked, thus facilitating a very quick assessment of the availability of a particular metal to soil or plant tests. The best evaluation procedure is based on repeated measurements of any given metal's content in soil and its subsequent availability to a sensitive crop. Lettuce is known as a reliable test plant used for evaluation of heavy metals in contaminated soil (MELLER et al. 2015, ROBA et al. 2016). Therefore, it is assumed that this crop can be used as a test plant for evaluation of bio-fertilizers based on bio-ash and biogas digestate.

The objectives of this study were as follows: (1) to evaluate the effects of the addition of six types of bio-fertilizers, based on integrated bio-ash and digestate (BAD), on agrochemical characteristics of light acid soil; (2) to compare two types of BAD differing in the presence of urea; and (3) to study the effects of different types and doses of BAD on lettuce yield and concentration of heavy metals.

MATERIAL AND METHODS

Soil incubation trial

A bulk sample of loamy sand top soil (Haplic Luvisols) was used to carry out an incubation experiment with six bio-fertilizers based on biomass ash and biogas digestate (BAD). Selected physiochemical parameters for the top soil are as follows: sand – 650; silt – 200, clay – 160 g kg⁻¹; C_{org} – 8.9 g kg⁻¹ soil; CEC – 6.99 cmol₍₊₎ kg⁻¹ soil; pH_{KCl} – 5.28. The componential structure and chemical composition of BAD bio-fertilizers is shown in Table 1. The contribution of bio-ash and digestate were the factors differentiating the manufactured prototypes. The secondary experimental factor was the dose of each bio-fertilizer, incorporated into soil in the amount of 0, 2, 4 or 8 g kg⁻¹

Table 1

Structural composition and content of elements in the tested bio-fertilizers

Components	Types of bio-fertilizers					
	A1	A3	A7	B2	B3	B5
	Contribution of main components (%)					
BA	75	55	25	65	50	45
D	25	25	75	25	25	45
PR	0	15	0	0	15	0
S ⁰	0	5	0	5	5	5
UREA	0	0	0	5	5	5
Organic matter and macronutrients (g kg ⁻¹ DM)						
OM	207	173	600	233	253	407
N	16.0	13.7	37.3	19.8	25.2	38.9
P	5.9	9.5	4.0	5.2	9.1	4.5
K	44.3	33.2	21.8	38.8	30.4	29.8
Ca	84.8	63.9	45.5	74.4	58.7	58.7
Mg	12.9	9.6	5.6	11.2	8.7	8.3
S	0.3	5.1	0.8	5.1	5.1	5.3
Micronutrients and heavy metals (mg kg ⁻¹ DM)						
Fe	18374	13586	7243	15980	12389	11528
Mn	1888	1392	706	1640	1268	1167
Cu	74.9	58.3	59.2	66.6	54.2	60.3
Zn	261.3	205.1	221.2	233.2	191.0	217.1
Pb	28.9	21.8	16.1	25.3	20.1	20.2
Cd	4.8	3.6	2.3	4.2	3.3	3.2

Legend: OM – organic matter; BA – biomass ash; D – biogas digestate; PR – phosphoric rock (Morocco); S⁰ – elemental sulphur.

soil. The experiment was conducted in 1 dm³ pots, which were filled with 0.75 kg of soil and then left for 42-day incubation in the climate chamber at a temp. of about 22±1°C. The constant weight of each pot was adjusted every two days by weighing each pot and adding the required amount of distilled water to maintain the soil moisture at the level of field capacity. Altogether, a total of 96 pots (6 treatments x 4 doses, including control, with 4 replications) were arranged in a completely randomized design.

Soil sampling for determinations of the content of mineral N (N-NO₃; N-NH₄), pH, P, K, Mg, Fe, Mn, Zn, Cu, Pb, and Cd was carried out after 42 days of the experiment. The soil samples were air-dried and crushed to pass through a 2-mm mesh sieve. The soil pH and extractable elements were measured in 1:5 soil 0.01 M CaCl₂ suspension. The content of available P in the extract was determined colorimetrically, while the content of K, Mg and Ca, Fe, Mn, Zn, Cu, Pb, Cd, and Ni was determined using flame AAS.

Exhaustion greenhouse trial

After 42 days, the experimental pots containing 0.5 kg of soil were seeded with lettuce (*Lettuce sativa* L.), which was reduced after emergence to three seedlings per pot. The pots were arranged in a completely randomized block design under glasshouse conditions (day/night period 16/8, room temperature), with regular watering and random rotation. The mean light intensity was 700 μmol m⁻² s⁻¹. The growth trial was terminated after 35 days from emergence, and the fresh mass of the top was harvested and weighed. All samples were then dried at 65°C for 72 h, and the dry mass was determined.

Nitrogen concentrations in plant samples were determined using a standard macro-Kjeldahl procedure, with accuracy of 0.1 mg N. The plant materials for the elements determination were mineralized at 600°C. The ash was then dissolved in 33% HNO₃. 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 flame FAAS.

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

RESULTS AND DISCUSSION

Soil agrochemical status before the exhaustion experiment

The nutrient availability was evaluated immediately before setting out the exhaustion experiment (Table 2). The N-NH₄ content showed a much stronger response to the type of BAD compared to N-NO₃. In soils treated with type A fertilizers, the content of N-NO₃ was as a rule higher than that of N-NH₄. A reverse situation occurred in soil treated with B fertilizers. The N-NH₄ content was much higher in soil treated with type B of fertilizers (Figure 1). The highest increase in both N forms was recorded in soil treated with B5, containing both urea and S⁰. The effect of added phosphorus on inorganic N content was positive but low compared to other treatments. The content of N-NH₄ as the main contributor to N_{min} pool showed a significant dependence on the content of three elements:

$$\text{N-NH}_4 = -177.6 + 51.9\text{Na} + 884\text{Zn} + 26.9\text{Mn} \text{ for } R^2 = 0.82 \text{ and } n = 72 \quad [1]$$

This type of a relationship indicates limiting properties of these three elements with respect to the N-NH₄ content. Sodium was the key factor limiting the N-NH₄ content in soil treated with type B fertilizers ($R^2 = 0.80$, compared to $R^2 = 0.47$ for the entire experiment). Both micronutrients influenced the N-NH₄ content in soils treated with the A fertilizers. This phenomenon was an attribute of soil treated with the A fertilizer, i.e. without added urea.

In general, an increase in the D share in BAD resulted in a decrease of the available content of K, and partly of Na, which were most pronounced for A7 fertilizer. These trends clearly implicate ash as a source of both nutrients. The effect of the BAD doses was in accordance with the experimental design. The concentrations of these three nutrients were significantly mutually correlated. Therefore, the content of K in mineral-organic amendments based on biomass ash can be used as a single predictor of Mg and Na content:

$$\text{Mg} = -0.0027\text{K}^2 + 0.645\text{K} + 0.178 \text{ for } R^2 = 0.91 \text{ and } n = 72 \quad [2]$$

$$\text{Na} = -0.0004\text{K}^2 + 0.077\text{K} - 0.258 \text{ for } R^2 = 0.82 \text{ and } n = 72 \quad [3]$$

These two quadrate regression models clearly show that the linear relationships between the content of plant available K and extracted quantities of the other two nutrients continued to a certain critical value, which equalled 120 and 101 mg K kg⁻¹ soil for Mg and Na, respectively.

The content of soil micronutrients, except Cu, increased in accordance to the contribution of D. The effect of added urea was most pronounced for Mn. One of the most important findings is that an application of BAD to soil resulted in a decrease of plant available content of Zn and Cu. In contrast,

Table 2
Effect of BAD type and rates on the CaCl₂ extractable nutrients; pre-exhaustion test assessment (mg kg⁻¹ soil)

Factor	Level of factor	pH [#]	N-NO ₃	N-NH ₄	N _{min}	K	Na	Mg	Cu	Zn	Mn	Fe	Pb	Cd
BAD	A1	7.22	9.6 ^b	3.6 ^a	13.2 ^a	53.0 ^c	1.5 ^a	20.9 ^b	0.013	0.048 ^{ab}	2.62 ^a	0.41 ^a	0.123	0.014
	A3	6.86	9.1 ^{ab}	5.9 ^a	15.0 ^{ab}	33.7 ^{ab}	1.6 ^a	13.6 ^a	0.012	0.047 ^a	3.33 ^{ab}	0.32 ^a	0.123	0.015
	A7	6.14	6.1 ^{ab}	7.8 ^{ab}	13.9 ^{ab}	27.0 ^a	1.4 ^a	18.9 ^b	0.010	0.055 ^b	3.57 ^{ab}	0.92 ^c	0.127	0.014
	B2	7.15	5.1 ^a	13.5 ^{bc}	18.6 ^{ab}	40.5 ^b	1.7 ^a	19.6 ^b	0.011	0.050 ^{ab}	4.12 ^{bc}	0.40 ^a	0.145	0.015
	B3	6.64	4.7 ^a	16.9 ^c	21.6 ^b	30.5 ^{ab}	1.6 ^a	16.6 ^{ab}	0.011	0.050 ^{ab}	4.80 ^c	0.39 ^a	0.160	0.015
F test	B5	6.12	9.9 ^b	31.5 ^d	41.4 ^c	34.9 ^{ab}	2.4 ^b	17.6 ^{ab}	0.013	0.052 ^{ab}	4.76 ^c	0.58 ^b	0.164	0.016
		6.69 ^f	3.0 [*]	48.0 ^{***}	31.5 ^{***}	10.3 ^{***}	6.7 ^{***}	5.2 ^{***}	0.6	2.5 [*]	12.5 ^{***}	86.3 ^{***}	25.3 ^{***}	0.5
	0	5.44	2.8 [*]	5.8 ^a	8.6 ^a	2.9 ^a	0.1 ^a	2.1 ^a	0.016 ^b	0.161 ^b	1.95 ^b	0.20 ^a	0.130 [*]	0.021 ^b
	2	6.81	7.9 ^b	11.9 ^b	19.8 ^b	26.6 ^b	1.2 ^b	14.4 ^b	0.010 ^a	0.013 ^a	5.58 ^d	0.52 ^b	0.137 ^{ab}	0.013 ^a
	4	7.34	10.3 ^b	14.1 ^b	24.4 ^b	43.4 ^c	2.0 ^c	21.9 ^c	0.009 ^a	0.013 ^b	4.47 ^c	0.61 ^c	0.145 ^{bc}	0.013 ^a
F test	8	7.17	8.6 ^b	22.1 ^c	30.7 ^c	73.5 ^d	3.5 ^d	33.0 ^d	0.010 ^a	0.012 ^b	3.47 ^b	0.68 ^d	0.149 ^c	0.013 ^a
		6.69 ^f	8.2 ^{***}	34.6 ^{***}	39.3 ^{***}	159.9 ^{***}	156.3 ^{***}	197.9 ^{***}	6.7 ^{***}	407.0 ^{***}	61.0 ^{***}	124.1 ^{***}	7.6 ^{***}	23.1 ^{***}
<i>F</i> test for the interaction														
BAD x R		6.69	2.0 ^{***}	9.2 ^{***}	5.4 ^{***}	2.6 ^{**}	2.0 [*]	1.8	0.6	0.7	2.3 [*]	15.4 ^{***}	3.8 ^{***}	0.1

^a numbers marked with the same letter are not significantly different; ***, **, * significance at 0.001, 0.01, 0.05, respectively. # 0.01 M CaCl₂; ¹ average for the respective treatments.

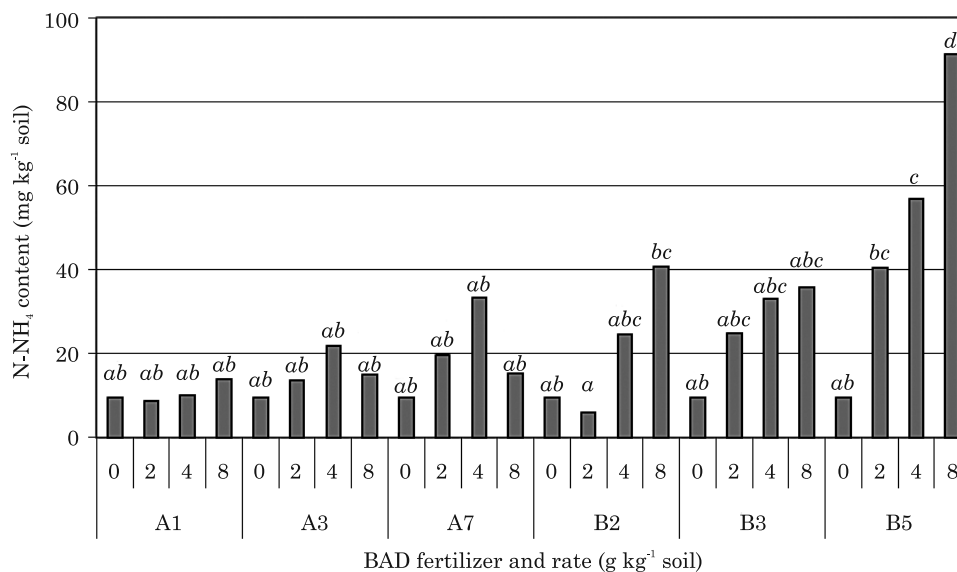


Fig. 1. The content of soil ammonium nitrogen before the exhaustion lettuce test

the content of Mn and Fe increased progressively with the higher BAD doses. In addition, any increase in the content of plant available Mg resulted in a decrease in the content of both Zn, and Cu, but not of Fe.

The pattern of Pb response to the tested amendments followed the same pattern as observed for Mn, showing a much higher content in soil treated with B fertilizers. This dependence was corroborated by a significant relationship between both elements ($r = 0.50$). The content of Cd did not respond to the type of BAD fertilizer, but it was positively correlated with the content of Cu ($r = 0.85$) and Zn ($r = 0.84$), while being negatively correlated with the content of the other nutrients, including Mn, Fe and K ($r = -0.59$).

The exhaustion lettuce test

The fresh yield of lettuce, harvested at the seedling stage, showed a distinctive response to the type of BAD and its dose (Figure 2). Plants grown on soil treated with the A fertilizers produced half the yields harvested from lettuce grown in the fertilizer B treatments. The highest average yield of lettuce seedlings was harvested from the B3 treatment, which had a biomass ash and D ratio equal 2:1, and also contained phosphoric rock (Pc), elemental sulphur (S⁰) and urea. The highest yield obtained in both main plots was probably the effect of the applied Pc, as a similar effect was achieved in the A3 treatment. The lowest yield of lettuce seedlings was harvested from the A7 treatment, where the soil amending substance was composed of only biomass ash and D in a 1:3 ratio. The observed yield decrease, as compared to the control plot, suggests N shortage. However, the analysis of data collated

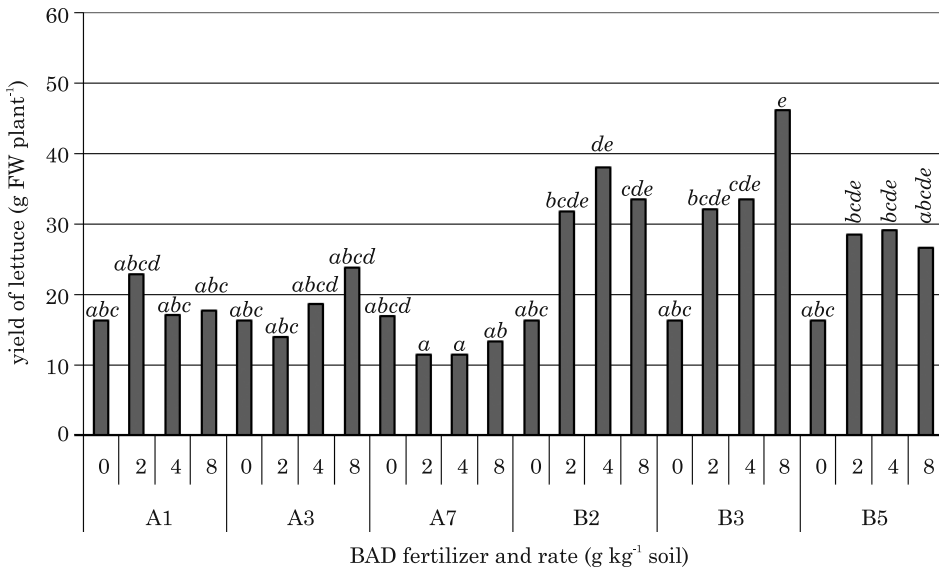


Fig. 2. Yield of lettuce seedling in response to the type and dose of bio-fertilizers

in Table 3 did not indicate that N or P concentrations in lettuce leaves limited its yield. The set of yield-indicating elements, based on their concentrations in lettuce, comprises of Mn, Fe and Pb. The model obtained is as follows:

$$Y = 11.8 + 0.025\text{Mn} + 0.028\text{Fe} - 3.03\text{Pb} \text{ for } R^2 = 0.88 \text{ and } n = 48 \quad [4]$$

The positive signs of the first two nutrients in this stepwise regression model indicate their insufficient supply to growing plants, whereas the concentration of Pb was excessive. The Fe content in plants grown in soil treated with the A type of BAD was nearly half of its content recorded in plants grown on soil with the B type of BAD. The shortage of Mn and Fe seems surprising, too, as the content of nutrients in soil was high, especially in the A treatments (Table 2). A possible cause was insufficient microbial activity due to the N shortage (LOVLEY 1995). This suggestion is entirely corroborated by a significant increase in the content of both metals in soil treated with type B fertilizers containing urea. The concentration of Pb in lettuce was governed by the following set of nutrients:

$$\text{Pb} = 0.17 + 0.11\text{N} + 0.59\text{P} + 0.07\text{K} - 0.51\text{Mg} - 0.007 \text{ Zn} \\ \text{for } R^2 = 0.80 \text{ and } n = 48 \quad [5]$$

The most important finding from this model is the relationship of Pb with Mg and Zn. It is well documented that Pb impairs the uptake of Mg and activity of many Mg-dependent plant enzymes (POURRUT et al. 2011). Therefore, the noticed negative impact of both nutrients on the Pb concentra-

tion in lettuce suggests that an increase in the K and Mg supply leads to a decrease in the Pb concentration.

The yield of lettuce decreased, and its depression as recorded in the A7 treatment can be explained based on the N-NH_4 content in soil just before the exhaustion test. As presented in Table 2, the N-NH_4 content in soil treated with fertilizers A was low, resulting in a low yield (Figure 2). In the case of B plots, the N-NH_4 content of up to 37.4 mg kg^{-1} soil limited the growth of lettuce, but above that level it resulted in its decrease (Figure 3). It is

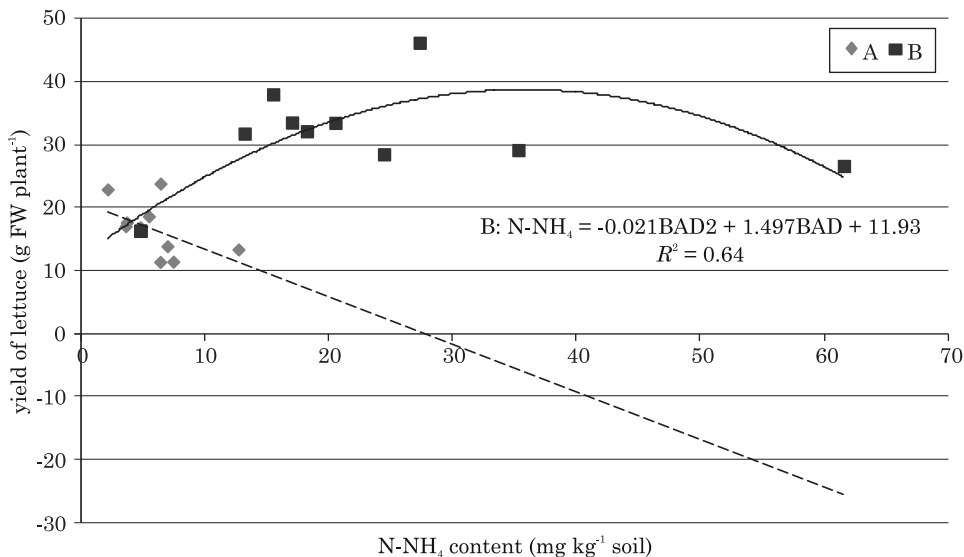


Fig. 3. Yield of lettuce seedlings as a function of the ammonium nitrogen content in soil prior the exhaustion experiment

much more difficult to explain the limiting effect of Fe and Mn on the yield of lettuce. The analysis of lettuce yield dependence on the supply of nutrients based on their content just before the experiment clearly showed an excess of K and Fe coinciding with the shortage of Mg and Mn:

$$Y = 15.6 - 0.71K + 2.47Mg + 2.52Mn - 41.2Fe \text{ for } R^2 = 90 \text{ and } n = 24 \quad [6]$$

The equations 4 and 6 confirm the critical role of Mn in lettuce yielding. In addition, they stress the insufficient supply of Mg from both soil and fertilizer resources for yield development. The insufficient concentration of Fe in lettuce confronted with its high amounts incorporated into the soil seems confusing. The key reason for the disturbance of Fe and Mn uptake from abundant soil resources was probably soil pH, raised up in response to the applied ash (Table 1). The addition of urea to BAD resulted in a decrease in the soil pH. There are numerous reasons behind this process, but the most

important one seems to be ammonium ion oxidation, responsible for a pH decrease, followed by an increased activity of trace elements (BOLAN et al. 2003).

The content of N in lettuce is highly sensitive to the supply of fertilizer nitrogen. As reported by PITURA and MICHAŁOJĆ (2015), the N concentration in lettuce leaves reflects fairly well its supply from the growth medium. The N concentration in lettuce in the exhaustion experiment was low, ranging from 6 to 18 g kg⁻¹ DM, irrespectively of the fertilizer type. The difference in the N supply between treatments was the key factor affecting the yield of lettuce. Phosphorus concentration showed high variability, varying from 1.0 to 4.5 mg kg⁻¹ DM, which fits within the published ranges (MELLER et al. 2015, PITURA, MICHAŁOJĆ 2015). The variability in the K content was even greater, ranging from 7 to 42 g kg⁻¹ DM. The lettuce seedlings grown in soils treated with the B fertilizers showed a decrease in the K content. A similar trend was observed for calcium and magnesium, but their concentrations were within the published ranges (MELLER et al. 2015). It is well documented that an excess of NH₄⁺ ions in the growth medium reduces the uptake of other cations, including K⁺, thus decreasing the plant growth rate (WEISE et al. 2013).

Among the measured micronutrients, the highest impact on lettuce yield was exerted by manganese. As a rule, a distinctly higher Mn concentration was recorded in plants grown in soil treated with B fertilizers. A drop in the Mn concentration in lettuce grown in treatment A7 could be the cause of yield decline. In the other A treatments, its increase was progressive with the BAD rate. The same pattern of response to both experimental factors was noticed for Fe. The concentrations of both micronutrients in lettuce were much higher than usually reported (PITURA, MICHAŁOJĆ 2015). The concentration of Zn was high, above the published ranges (PITURA, MICHAŁOJĆ 2015, ROBA et al. 2016). The main reason for the recorded values was its positive response to increasing doses of BAD fertilizer. In the control plants, it did not exceed the published ranges. Zinc was a nutrient which exerted a negative impact on lead concentration, as presented in eq. 5. The concentration of copper was within the published ranges (MELLER et al. 2015), showing a great variability in its response to the type of BAD and its dose.

The fourth group of measured elements is composed of heavy metals, represented by lead and cadmium. Their concentrations were evaluated based on both dry and fresh matter content. The concentration of Pb in lettuce dry matter showed a significant dependence on the type of BAD and its dose. A much higher concentration was recorded in lettuce grown on soil treated with the A type of BAD amendments. The Pb content decreased in plants grown in soil enriched with type B amendments. This response clearly indicates that biomass ash is a key source of lead for growing plants. The variability pattern of Pb concentrations based on the fresh matter content is even more interesting (Figure 4). The threshold value of 0.2 mg kg⁻¹ FM was exceeded in A treatments with the highest doses of the BAD fertilizer. Addition

Table 3

The effect of type and doses of bio-fertilizers on the nutrient concentration in lettuce – an exhaustion test

Factor	Level of factor	(g kg ⁻¹ DM)										(mg kg ⁻¹ DM)				(mg kg ⁻¹ FM)	
		N	P	K	Ca	Mg	Na	Mn	Fe	Zn	Cu	Pb	Cd	Pb _c	Cd _c		
BAD	A1	12.1 ^b	2.4 ^{ab}	14.1 ^{ab}	9.8 ^a	1.9 ^a	2.4 ^a	152 ^a	209 ^a	137 ^{ab}	6.3 ^a	2.0 ^c	0.24 ^b	0.18 ^b	0.022 ^b		
	A3	8.9 ^{ab}	2.0 ^a	18.2 ^{bc}	14.1 ^b	2.3 ^{ab}	2.8 ^{ab}	282 ^b	226 ^a	124 ^a	6.5 ^a	2.1 ^c	0.33 ^c	0.20 ^b	0.031 ^c		
	A7	9.9 ^{ab}	2.9 ^b	23.9 ^c	12.2 ^{ab}	2.8 ^{bc}	3.1 ^{ab}	143 ^a	262 ^a	176 ^{abc}	8.4 ^a	1.6 ^{bc}	0.25 ^b	0.17 ^b	0.025 ^b		
	B2	8.6 ^a	2.8 ^b	21.1 ^{bc}	11.1 ^{ab}	3.1 ^c	2.5 ^a	300 ^{bc}	487 ^b	195 ^c	11.6 ^b	1.2 ^{ab}	0.22 ^{ab}	0.09 ^a	0.016 ^a		
	B3	9.9 ^{ab}	2.0 ^a	16.9 ^{bc}	11.0 ^{ab}	2.3 ^{abc}	2.9 ^{ab}	342 ^{bc}	382 ^b	207 ^c	6.7 ^a	0.8 ^a	0.16 ^a	0.06 ^a	0.012 ^a		
F test	B5	11.1 ^{ab}	1.9 ^a	9.5 ^a	9.8 ^a	2.3 ^{abc}	3.4 ^b	413 ^c	267 ^a	188 ^{bc}	6.2 ^a	0.7 ^a	0.16 ^a	0.06 ^a	0.013 ^a		
	0	2.86 [*]	6.51 ^{***}	9.88 ^{***}	3.77 [*]	5.26 ^{**}	3.40 [*]	16.5 ^{***}	18.2 ^{***}	6.57 ^{***}	12.9 ^{***}	23.8 ^{***}	12.8 ^{***}	64.9 ^{***}	44.7 ^{***}		
	2	7.6 ^a	1.7 ^a	9.6 ^a	9.4 ^a	1.8 ^a	2.6	119 ^a	172 ^a	93 ^a	5.3 ^a	0.8 ^a	0.17 ^a	0.07 ^a	0.016 ^a		
	4	10.1 ^b	1.9 ^a	17.0 ^b	9.5 ^a	2.8 ^b	2.9	257 ^b	284 ^b	156 ^b	7.2 ^b	1.2 ^b	0.22 ^{ab}	0.11 ^b	0.020 ^b		
	8	11.2 ^b	2.5 ^b	21.6 ^b	13.0 ^b	2.4 ^b	2.7	359 ^c	379 ^c	210 ^c	9.2 ^c	1.9 ^c	0.25 ^b	0.17 ^c	0.022 ^b		
F test	7.50 ^{**}	18.4 ^{***}	17.1 ^{***}	10.2 ^{***}	9.1 ^{***}	2.15	27.7 ^{***}	23.6 ^{***}	31.9 ^{**}	13.4 ^{***}	23.3 ^{***}	8.1 ^{***}	40.3 ^{***}	8.9 ^{***}			
<i>F</i> for the interaction																	
BAD x D		3.58 [*]	4.46 ^{***}	9.83 ^{***}	4.32 ^{***}	9.88 ^{***}	3.93 ^{**}	6.08 ^{***}	7.7 ^{***}	2.65 ^{**}	7.65 ^{***}	5.97 ^{***}	7.43 ^{***}	13.7 ^{***}	13.4 ^{***}		

^a numbers marked with the same letter are not significantly different; ^{***}, ^{**}, ^{*} significance at 0.001; 0.01; 0.05, respectively.Legend: DM – dry matter; FM – fresh matter; Pb_c, Cd_c – concentration of Pb and Cd assessed based on the FM content.

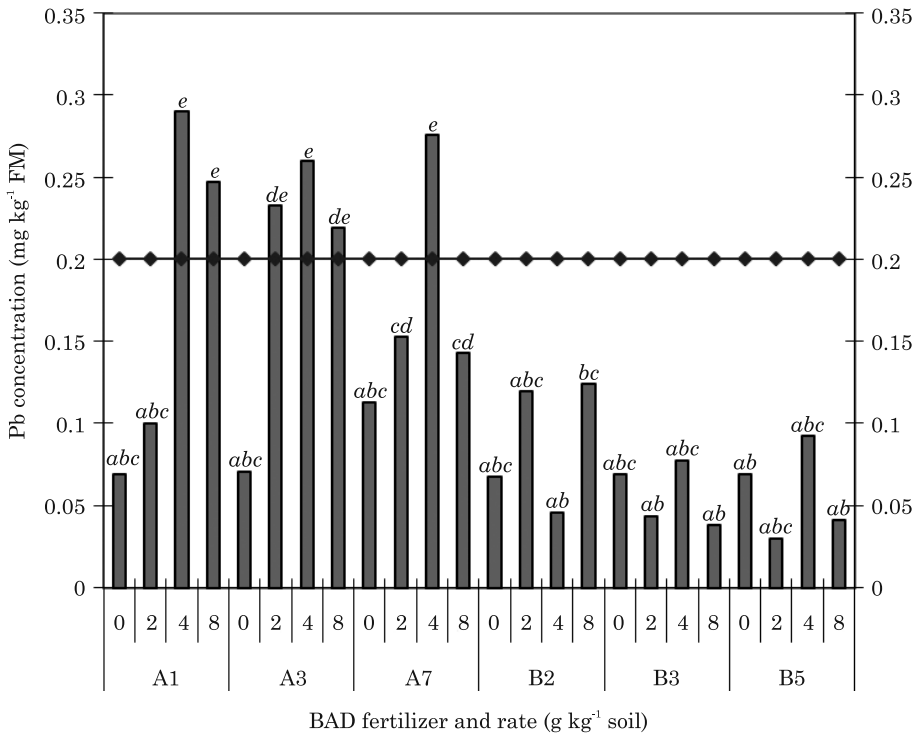


Fig. 4. The effect of different types and doses of bio-fertilizers on the lead content in lettuce seedlings

of urea to the mineral-organic amendment based on biomass ash and digestate resulted in a significant decrease in the Pb concentration in lettuce. The key reason was probably the phenomenon termed as *nutrient dilution* (MARLES 2017), but in this case it refers to the non-essential element, which creates a health risk. Another reason for a much lower Pb concentration was the antagonistic effect of magnesium and zinc, as documented in equation 5. The concentration of Cd in lettuce seedlings was low, typical for data from uncontaminated soils (ROBA et al., 2016). The threshold value of 0.1 mg kg⁻¹ FW was far below the maximum values, reaching 0.045 mg kg⁻¹ FW. The concentration of Cd in lettuce seedlings was governed by the following set of elements:

$$\text{Cd} = -0.003 + 0.057\text{P} + 0.019\text{Ca} - 0.0007\text{Zn} \text{ for } R^2 = 0.76 \text{ and } n = 48 \quad [7]$$

This equation indicates that biomass ash is a key source of Cd. It also points to the ameliorating function of Zn. This equation clearly indicates Zn should be a very important additive in any soil amendments based on biomass ash.

Status of soil extractable nutrients after the exhaustive test

The content of most CaCl_2 extractable elements changed dramatically after the exhaustive test with lettuce (Table 4). The yield of lettuce seedlings (Y), in the light of a stepwise regression model, depended on the following set of plant available elements:

$$Y = 57.2 + 40.9\text{Fe} - 21.9\text{N-NO}_3 + 44.2\text{N-NH}_4 - 1533\text{Cd} \quad [8]$$

for $R^2 = 0.76$ and $n = 24$

Being the key nutrient, responsible for plant growth and yield, nitrogen should be considered first. The content of nitrate nitrogen (N-NO_3) was completely depleted, irrespectively of the BAD type and dose. The negative sign of this form of N is due to the low yield harvested from soil fertilized with type A of BAD amendments. A slightly different relationship was observed for the N-NH_4 content. Its resources were fully depleted in soils treated with type B of fertilizers. The key reason for this lettuce yield decline was the shortage of N-NH_4 in soil before the experiment, which was demonstrated in all plots fertilized with urea-free fertilizers (A) – Table 2, Figure 3). In plots with urea-enriched (B) substances, the yield of lettuce (Y) showed a curvilinear dependence on the N-NH_4 content before the experiment. It has been also documented that the yield of lettuce responded to the N-NH_4 net balance ($\Delta\text{N-NH}_4$), clearly indicating its shortage and excess, implicating the value of $32.5 \text{ g } \Delta\text{N-NH}_4 \text{ m}^{-2}$ as the critical one:

$$Y = 0.02(\Delta\text{N-NH}_4)^2 + 1.3 \Delta\text{N-NH}_4 + 16.9 \text{ for } R^2 = 0.65 \text{ and } n = 24 \quad [9]$$

The excess of N-NH_4 revealed in the soil treated with B5 bio-fertilizer, whose distinguishing feature was a double content of digestate compared to other B fertilizers. The high share of D together with the presence of S^0 may have accelerated the release of N-NH_4 from both applied digestates. The excess of N-NH_4 in the growth medium as the principal N source can depress the growth of plants (CHAILLOU et al. 1986). This was observed in treatment B5.

Cadmium appeared as the second element negatively impacting the yield of lettuce seedlings. This effect seems to be confusing, because all BAD amendments resulted in a strong depletion of plant available Cd. The same trends were noticed for lead as well as for copper and zinc. It means that all these three elements are significantly absorbed by plants from soil treated with mineral-organic amendments based on biomass ash and digestate. The content of iron, despite its excess in soil just before the exhaustion experiment presented itself as a growth limiting factor. The extent of Fe exhaustion was significantly higher in soil treated with type A of BAD. Its post-exhaustion level decreased by 6% to 33% of the initial values, compared to 14 to 71% under the impact of B-BAD. The shortage of Fe appeared in treatments with a high contribution of ash in particular fertilizers (A1, A3, B2, B3). In the remaining two treatments, the content of Fe was excessive, thus reducing the yield of lettuce. It was particularly evident in treatment A7.

Table 4
Effect of BAD type and doses on the CaCl₂ extractable nutrients after exhaustive cropping

Factor	Level of factor	(mg kg ⁻¹)											
		NO ₃	NH ₄	N _{min}	K	Na	Mg	Cu	Zn	Mn	Fe	Pb	Cd
BAD	A1	1.9	4.6	6.5	9.3	4.5	8.4 ^a	0.013	0.015	1.48 ^b	0.05 ^a	0.108 ^b	0.017
	A3	2.1	5.3	7.4	8.8	4.0	8.1 ^a	0.011	0.015	0.85 ^{ab}	0.08 ^a	0.090 ^{ab}	0.016
	A7	2.0	6.8	8.8	10.0	5.2	14.0 ^b	0.010	0.013	0.61 ^a	0.12 ^{ab}	0.080 ^a	0.016
	B2	1.9	3.8	5.7	8.0	4.0	5.6 ^a	0.010	0.016	0.98 ^{ab}	0.12 ^{ab}	0.101 ^{ab}	0.017
	B3	2.2	4.6	6.8	15.2	7.3	8.9 ^a	0.012	0.019	0.88 ^{ab}	0.17 ^{ab}	0.095 ^{ab}	0.016
F test	B5	1.9	4.7	6.7	11.1	6.5	6.5 ^a	0.012	0.020	0.83 ^{ab}	0.28 ^b	0.103 ^b	0.018
	0	0.79	2.32	2.33	2.08	3.32	8.98 ^{***}	0.56	2.33	3.34 [*]	5.09 ^{***}	3.37 [*]	0.18
	2	2.9 ^c	2.0 ^a	4.9 ^a	2.4 ^a	4.3	5.2 ^a	0.024 ^b	0.028 ^b	0.55 ^a	0.05 ^a	0.140 ^b	0.031 ^b
	4	1.5 ^a	5.7 ^b	7.2 ^b	12.4 ^{bc}	5.8	8.7 ^b	0.007 ^a	0.011 ^a	0.98 ^{ab}	0.09 ^b	0.075 ^a	0.012 ^a
	8	1.6 ^{ab}	6.1 ^b	7.7 ^b	16.4 ^c	5.7	11.9 ^a	0.006 ^a	0.011 ^a	1.16 ^b	0.18 ^c	0.078 ^a	0.012 ^a
F test	28.2 ^{***}	13.8 ^{***}	6.50 ^{***}	2.05 [*]	1.21	11.94 ^{***}	47.8 ^{***}	29.8 ^{***}	4.27 ^{**}	7.74 ^{***}	45.3 ^{***}	43.1 ^{***}	
<i>F</i> test for the interaction													
BAD x D	1.53	1.10	1.34	2.06 [*]	1.21	3.29 ^{**}	0.67	1.16	2.74 ^{**}	1.57	2.57 ^{**}	0.12	

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

CONCLUSIONS

1. Bio-fertilizers based on biomass ash and digestate are a poor source of nutrients, except iron, and the ultimate effect significantly depends on the contribution of ash.

2. The content of ammonium nitrogen in soil treated with the tested prototype fertilizers based on bio-ash and biogas digestate was the key indicator of their different composition due to the presence or absence of urea.

3. Concentrations of soil potassium, magnesium and sodium, which were significantly mutually correlated, showed a strong dependence on the applied doses of bio-ash. An increase in the soil content of bio-ash, in response to higher doses of the tested bio-fertilizers, was also noted for inorganic nitrogen as well as the levels of available manganese and iron.

4. The yield of lettuce was twice as high on soils treated with urea-enriched bio-fertilizers. Lettuce yield depression was due to the shortage of nitrogen supply from soil, but it was not explained directly by the content of N in lettuce.

5. The concentration of lead in lettuce corresponded to the amount of bio-ash incorporated into soil. The threshold value of 0.2 mg kg⁻¹ FW was exceeded in the soil treated with the highest doses of urea-free bio-fertilizers. The enrichment of bio-fertilizer in magnesium and zinc can be seen as a measure controlling the uptake of lead by crops.

6. The content of cadmium in lettuce was far below the threshold value of 0.1 mg kg⁻¹ FW. The main reason was a strong decrease of its supply to lettuce in pots treated with BAD bio-fertilizers.

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