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ORIGINAL PAPER

EVALUATION OF THE POTENTIAL OF BIO-FERTILIZERS AS A SOURCE OF NUTRIENTS AND HEAVY METALS BY MEANS OF THE EXHAUSTION LETTUCE TEST[1]

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Abstract

It has been assumed that lettuce is an appropriate crop to test the potential supply of nutrients and heavy metals (HMs) by bio-fertilizers based on biomass ash (BA) and biogas digestate (D). This hypothesis was validated in an incubation test, in which six prototypes of bio-fertilizers (BAD) with different BA to D ratios (type A without urea: 75:25; 55:25; 25:75; type B with urea: 65:25; 50:25; 45:45) were pre-incubated for six weeks in light soil with neutral pH reaction. Then, lettuce was grown for another five weeks to assess its response to increasing doses of BAD (0, 2, 4, 8 g kg^{-1} soil). The soil test showed a significant, BAD-dependent changes in the content of nutrients and HMs. The key factor impacting the potential N supply to lettuce was urea, whose presence in a given bio-fertilizer led to an extremely large release of N-NH$_4$. The N concentration in lettuce reflected its soil supply. The response of lettuce to N supply was modified by the supply of micronutrients. As a rule, their supply in soils treated with urea-free bio-fertilizers was low, significantly limiting the yield of lettuce. The content of copper (Cu) in soil treated with urea-enriched bio-fertilizers was inversely related to the lettuce yield. Its high content, concomitant with a simultaneously elevated content of lead, was the major cause of reduced lettuce yields. The negative impact of these two elements was overcome by an excess of N-NH$_4$. The decrease in the available Cu content led to a better supply of Zn, which in turn resulted in higher yields of lettuce. The antagonism observed between Mg and Zn versus Pb concentrations in lettuce evidently manifests the importance of the two former elements as necessary inorganic amendments in bio-fertilizer based on bio-ash. The experiment clearly demonstrates that the exhaustion test with lettuce provided data about limiting factors, like copper, and about the ameliorating role of N-NH$_4$.

Keywords: biomass ash, biogas digestate, lettuce, nutrients, heavy metals, availability.

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INTRODUCTION

Two pillars of sustainable agriculture are the increased production of food, fiber and, more recetly, fuel, as well as the effective use of non-renewable resources. The former can be achieved, providing there is an efficient use of fertilizer nitrogen (Woods et al. 2013). Under Polish soil and climate conditions, the extent to which both goals are achieved depends on numerous factors. An example is the effective control of main soil fertility indicators, such as soil pH, content of available phosphorus (P), potassium (K) and magnesium (Mg) (Grzebisz et al. 2010). The *climate package* obligates the UE member countries to increase the proportion of renewable-energy sources up to 20% of the total energy consumption by 2020. In Poland, this target value was fixed at 15% by 2020. The main renewable-energy sources in 2020 are biomass (78.9%), including energy crops (27.9%), biofuels (12.2%), biogas (20.2%) and others (18.6%) (Baum et al. 2013). The cited authors focused on the biomass market management, neglecting the fate of a residual product, i.e. ash. According to Gajewski (2011), the market potential for biomass production for energy in Poland is 15.5 mln t annually. Based on various sources, this equals to 0.4-0.6 mln t of ash, which can be used for bio-fertilizer production.

Biomass transformation, irrespectively of the technology applied, generates both energy and byproducts, which in part can be used as soil amendments. Bio-ash left on the soil surface after primary forest burning was the first source of nutrients for grown crops. Depending on the type of burnt organic material, i.e. woody, annual plants, energy crops, etc., it is rich in Ca, K, P or Si (Demeyer et al. 2001). Another source of valuable agricultural waste is biogas digestate. It is the residue remaining after anaerobic processes applied to convert biological substances into energy. However, its biggest disadvantage is a low content of organic matter, which increases the cost of application. An alternative solution is the separation of raw slurry into solid and liquid parts (Nkoa 2014).

Among substances found both in bio-ash (BA) and, to a lesser extent, in biogas digestate (D) which can have a negative influence on crops there are heavy metals (HMs), which require watchful control (European Commission, 2006). Plant species differ in their ability to absorb desirable amounts of particular nutrients, and these can also vary depending on the weather and soil conditions. The optimal level of a nutrient for plant growth, as well as its shortage and toxicity range, are dependent on a plant species (Borek et al. 2015). Vegetables, especially leafy ones such as cabbage or lettuce, are extremely sensitive to excess of HMs in the growing medium (Singh et al. 2012).

The major purpose of this study has been to evaluate the response of lettuce as a test plant to different types and doses of BAD, based on yield, nutrient content and concentration of heavy metals. Other objectives were to evaluate the effects of six types of bio-fertilizers, based on bio-ash and biogas digestate, on the content of available nutrients and heavy metals in light, acid neutral soil.

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 (Green Block, Energy Plant Połaniec) and biogas digestate (BAD from maize silage). Type A without urea: 75:25, 55:25 and 25:75, and type B with urea: 65:25, 50:25 and 45:45, were tested. More detailed specification is contained in Table 1. Selected physiochemical parameters for the soil were as follows: sand – 670; silt – 140, clay – 190 mg kg^{-1}; C_{org} – 9.0 g kg^{-1} soil; CEC – 5.83 $cmol_{(+)}$ kg^{-1} soil; $pH_{0.01\ M\ CaCl_2}$ – 7.0. 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} soil. The experiment was conducted in 1.0 dm^3 pots, which were filled with 0.75 kg of soil and then left for a 42-day incubation period in a climatic chamber at a temp. of 22±1°C. The constant weight of each pot was maintained by weighing each pot every two days and adding the required amount of distilled water to keep the soil moisture at the level of field capacity. A total of 96 pots (six treatments x four doses, including control, replicated four times) were arranged in a randomized design.

Soil sampling, to determine soil pH and the content of mineral N ($N\text{-}NO_3$; $N\text{-}NH_4$), K, Na, Mg, Mn, Fe, Zn, Cu, Pb and Cd, were carried out at the end of the pre-incubation experiment and after the lettuce exhaustion tests. Soil samples were then air-dried, crushed and sifted through a 2-mm mesh sieve. The soil pH and extractable elements were measured in 1:5 soil 0.01 M $CaCl_2$ suspension. The content of available K, Na, Mg, Mn, Fe, Zn, Cu, Pb and Cd was determined using FAAS (SpectrAA 250 Plus, Varian).

Greenhouse exhaustion trial

After 42 days of the incubation test, a pot containing 0.5 kg of soil was planted with lettuce (*Lettuce sativa* L.), which was thinned to three seedlings after one week. The pots were arranged using a random complete block design and placed under glasshouse conditions (day/night period 16/8, room temperature), with regular watering and variable rotation. The mean light intensity was 700 μmol m^{-2} s^{-1}. The growth trial was terminated after 35 days following germination, 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 the standard macro-Kjeldahl procedure, at 0.1 mg N accuracy. The plant material for elemental determinations was mineralized 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/40 at a wavelength of 436 nm. The content of K, Ca, Mg and Mn, Fe, Zn, Cu, Mn, Fe, Pb and Cd was determined using FAAS (SpectrAA 250 Plus, Varian).

The experimental data were subjected to conventional variance analysis supported by the computer program STATISTICA 12®. Differences between treatments were evaluated with the Tukey's test. The results are presented in tables, figures and as *F* test equations (***, **, * indicate significance at the $P \leq 0.001$; 0.01; 0,05 respectively).

RESULTS AND DISCUSSION

Availability of soil elements – pre-exhaustion soil evaluation

The effect of bio-fertilizers on soil pH was low (Table 1), mostly because of the initial pH of 7.0 (0.01 M $CaCl_2$). The reaction was slightly lower in soil treated with urea-enriched amendments. The content of mineral nitrogen (N_{min}) measured just before the exhaustion test setup was influenced by the variability of its inorganic form content. $N\text{-}NO_3$ significantly affected the N_{min} content ($r = 0.42$, $n = 72$), but to a much lesser extent compared to $N\text{-}NH_4$ ($r = 0.75$, $n = 72$). In general, $N\text{-}NH_4$ increased linearly in accordance to the bio-fertilizer doses. A high increase was noted in B treatments, especially in B5 one (Figure 1). It is well-documented that an increase in soil pH with the concomitant presence of easily available N results in a massive rise in the soil microbial activity (Abubaker et al. 2015).

The content of K, Na, Mg showed very similar responses to both experimental factors. The effect of the progressively higher BAD doses, averaged over the fertilizer type, was linear, showing strong resemblance to the variability pattern of the $N\text{-}NH_4$ content. However, no significant relationship was determined between the content of $N\text{-}NH_4$ and the above set of nutrients. The main mode of nitrogen's response to experimental factors was the lack of the *urea effect*. In general, the nitrogen content corresponded to the contribution of bio-ash irrespectively of the fertilizer group (Table 2). Based on the K content, it is possible to evaluate the content of the other two nutrients:

$$Mg = 0.003K^2 + 0.67K - 3.04; \quad R^2 = 0.91 \text{ and } n = 72 \qquad [1]$$

$$Na = 0.0005K^2 + 0.098K - 0.95; \quad R^2 = 0.86 \text{ and } n = 72 \qquad [2]$$

These two regression models clearly show that both Mg and Na concentrations were synergistically released from bio-fertilizers linearly up to the K content of 120 and 98 mg kg^{-1} soil, respectively.

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	18 374	13 586	7243	15 980	12 389	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: BA – biomas ash; D – biogas digestate; PR – phosphoric rock; S^0 – elemental sulphur

The third group of elements consists of micronutrients such as manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn). Manganese deserves special attention because its content increased in all A and B2 fertilizer treatments in response to the first fertilizer dose, afterwards either decreasing sharply or stabilizing, as seen for the other two B fertilizers. A very similar pattern of response was observed for Fe in soils fertilized with A1 and A3. A progressive pattern of its response to increasing doses of BAD was the attribute of soil treated with A7 and B5. A third model was identified, characterized by an extremely small Fe content in pots with the soils treated with B2 and B3 fertilizers. Special attention should be paid to the patterns of Cu response to the experimental factors (Figure 2). The content of copper on average was lower in soils treated with A7, B3 and B5. Its response, irrespective of the type of bio-fertilizer, was specific, rising up to the 1st or 2nd BAD dose and decreasing afterwards. The decline in the Cu content in the soil treated with

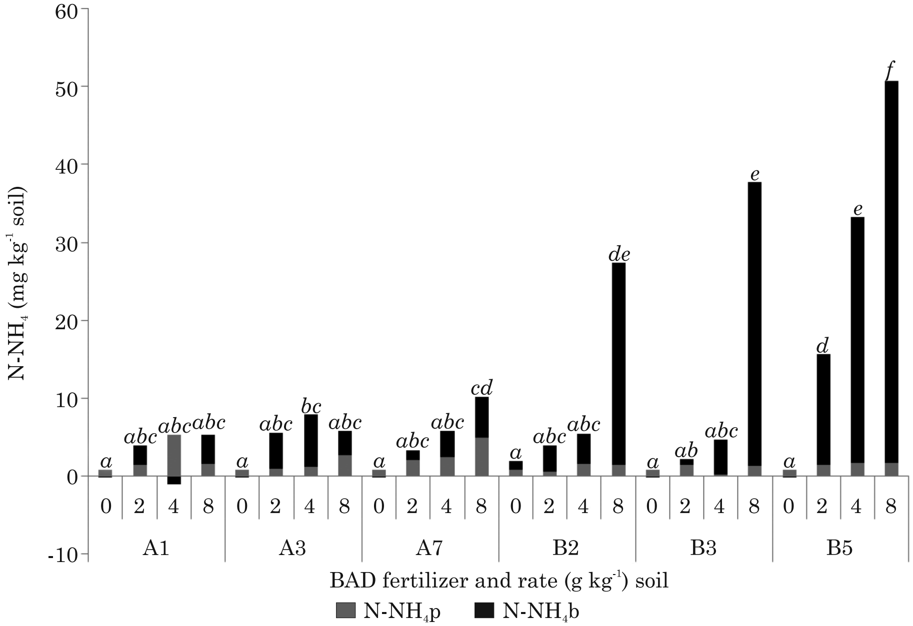

Fig. 1. Structure of the change in N-NH_4 during the exhaustion lettuce test: N-NH_4 p – before the exhaustion experiment, N-NH_4 b – balance

the A bio-fertilizers probably resulted from the rising soil pH in response to the amount of applied ash (Demeyer et al. 2001). It is almost certain that Cu^{2+} ions were complexed by NH_4^+ ions (Tills, Alloway 1981). The content of zinc did not respond to the type of applied bio-fertilizer, but sharply decreased following the first dose of fertilizer. It was the only case of a negative impact of increasing soil pH on the content of the micronutrients.

The content of both heavy metals, lead (Pb) and cadmium (Cd), showed a weak response to the experimental factors. The content of Pb was slightly lower in soil treated with the lower amount of ash. The content of Cd was affected by both factors, but their effect did not show any constant trend, except in the B3 treatment.

Exhaustion lettuce test

Lettuce proved to be a crop highly sensitive to both the type and dose of a particular bio-fertilizer (Figure 3). The average yields of plants grown in pots treated with the A type of fertilizers showed a descending trend with an increasing contribution of digestate. Even more interesting is the fact that the pattern of yield response to the BAD doses underwent a change from a positive one, as recorded for A1 and A3, to the negative response to A7. The pattern noticed in A1 was intensified on B3 and B5 treatments. This trend can be explained by the presence of urea in the tested bio-fertilizers. At the

Table 2

Effect of BAD type and doses on the 0.01 M $CaCl_2$ 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	Mn	Fe	Cu	Zn	Pb	Cd
BAD	A1	7.56	23.4^{b}	3.6^{a}	27.0^{c}	55.9^{b}	1.5^{a}	20.5^{d}	3.2^{a}	0.35^{c}	0.011^{b}	0.03	0.19^{ab}	0.014
	A3	7.31	6.4^{a}	5.0^{a}	11.3^{a}	38.9^{a}	1.7^{ab}	14.1^{a}	4.3^{ab}	0.22^{ab}	0.011^{b}	0.02	0.19^{b}	0.014
	A7	7.20	7.2^{a}	5.0^{a}	12.2^{ab}	36.7^{a}	1.6^{ab}	19.5^{cd}	4.4^{b}	0.28^{bc}	0.008^{ab}	0.03	0.18^{ab}	0.013
	B2	7.26	13.9^{ab}	17.4^{bc}	30.8^{d}	50.0^{ab}	2.3^{c}	24.8^{d}	5.8^{bc}	0.49^{d}	0.013^{c}	0.01	0.18^{ab}	0.014
	B3	7.10	6.1^{a}	11.3^{b}	17.4^{b}	36.9^{a}	1.8^{b}	16.9^{bc}	3.9^{ab}	0.12^{a}	0.008^{ab}	0.02	0.18^{ab}	0.014
	B5	6.80	3.6^{a}	25.1^{c}	28.6^{cd}	36.8^{a}	2.2^{c}	16.4^{ab}	6.1^{c}	0.29^{bc}	0.006^{a}	0.02	0.18^{ab}	0.014
F		-	37.8^{***}	21.5^{***}	33.0^{***}	36.5^{***}	17.2^{***}	14.7^{***}	14.4^{***}	11.3^{***}	8.50^{***}	0.05	4.35^{**}	1.99
Dose (D) (g kg^{-1})	0	6.90	8.0	0.6^{a}	8.5^{a}	10.1^{a}	0.1^{a}	3.2^{a}	0.8^{a}	0.13^{a}	0.007^{a}	0.07^{b}	0.18	0.013^{a}
	2	7.20	8.1	6.2^{b}	14.3^{b}	27.8^{b}	1.1^{b}	13.5^{b}	6.5^{c}	0.28^{b}	0.010^{b}	0.01^{a}	0.18	0.014^{ab}
	4	7.40	11.2	11.2^{c}	22.5^{c}	45.0^{c}	2.1^{c}	21.1^{c}	5.8^{c}	0.29^{b}	0.010^{b}	0.01^{a}	0.18	0.014^{ab}
	8	7.30	10.0	22.0^{d}	31.9^{d}	81.3^{d}	3.7^{c}	32.0^{d}	4.4^{b}	0.31^{b}	0.008^{ab}	0.01^{a}	0.18	0.014^{b}
F		-	1.84	76.8^{***}	64.7^{***}	276.8^{***}	128.5^{***}	119.4^{***}	102.8^{***}	12.5^{***}	6.48^{**}	4.71^{**}	0.28	3.41^{*}
F for interaction of factors														
BAD x D		7.20^{2}	8.67^{***}	67.4^{***}	13.6^{***}	60.5^{***}	3.42^{**}	5.23^{***}	2.74^{**}	6.98^{***}	2.44^{*}	1.0	1.40	4.10^{***}

[a] data marked with the same letter are not significantly different; ***, **, * significance at 0.001, 0.01, 0.05, respectively. [#] pH in 0.01 M $CaCl_2$

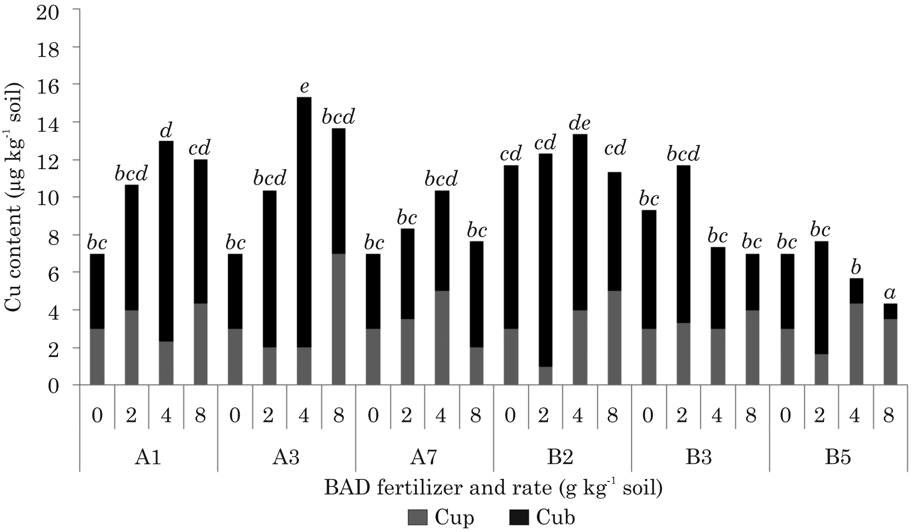

Fig. 2. Structure of the change in available copper during the exhaustion lettuce test: Cu p – before the exhaustion experiment, Cu b – balance

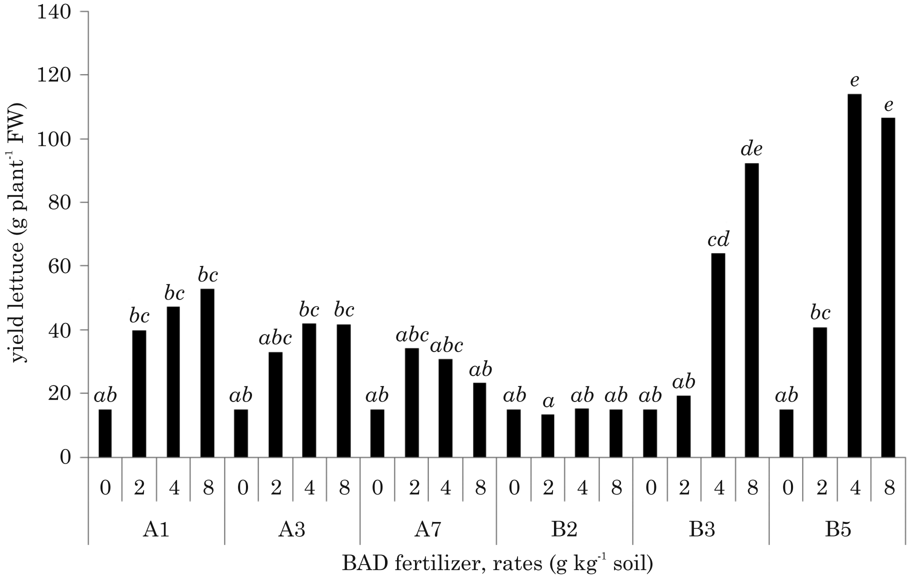

Fig. 3. Yield of lettuce in response to BAD fertilizers and incremental doses: FW – fresh weight

same time, yield stagnation was recorded in the whole B2 treatment. Therefore, the variability of lettuce yields in soil with the initial high pH level can be largely explained by the supply of nitrogen, as supported by the model:

$$Y = 1.979\text{N-NH}_4 + 21.1 \text{ for } R^2 = 0.77 \text{ and } n = 72 \qquad [3]$$

This model clearly indicates that the supply of $N\text{-}NH_4$ measured before the experiment setup was low, thus being a factor limiting yield of lettuce. This conclusion is supported by the concentration of N in lettuce (Table 3). It was lower, on average, in plants grown on soil treated with urea-free bio-fertilizers and increased in response to its progressive doses. This pattern was mostly observed on B3 and B5 pots. In these two treatments, the N concentration reached the level recorded for lettuce fertilized with sewage sludge (Castro et al. 2009).

The concentration of P showed high variability, achieving the highest values in pots with the lowest yields (A7, B2), yet remaining within the ranges recorded for lettuce fertilized with sewage sludge (Castro et al. 2009). The same pattern was observed for potassium (K), whose concentration was much below the frequently recorded data, but within those reported by Pinto et al (2014). The concentration of Ca and Mg was in ranges reported for organically fertilized lettuce (Castro et al. 2009).

The third group of elements are micronutrients. As a rule, their concentrations increased in accordance to the dose of applied bio-fertilizers. This type of response indicates bio-ash to be the key carrier of micronutrients to lettuce. The concentration of Zn on average was higher in plants grown on soil treated with urea-enriched fertilizers and with a concomitant high contribution of digestate. The observed patterns are described by the following regression models:

All B pots: $Y = 0.63Zn - 32 \quad R^2 = 0.63$, for $n = 36$, and $P \leq 0.001$ [4]

B3, B5 pots: $Y = 0.67Zn - 26.1 \quad R^2 = 0.86$, for $n = 24$, and $P \leq 0.001$ [5]

It is necessary to stress that Zn concentration in lettuce showed a high relationship with N concentration in lettuce ($r = 0.85$ for $P \leq 0.001$). Hence, the presence of urea and concomitant increase of digestate content in the B fertilizer, except B2, resulted in better Zn supply to lettuce, leading to higher its yield. This phenomenon can be compared to the response of maize to fertilization with Zn (Grzebisz et al. 2008). The key question, however, is why the yield in the B2 treatment staganted. The answer can be found in Figure 4. For plants grown in soil treated with type A of BAD, a quadrate response model was recorded, with the optimum Cu concentration of 13.4 μg kg^{-1} soil as measured before the test setup, and the respective yield maximum of 46.4 g $plant^{-1}$ FW. In contrast, plants grown in the B pots followed a powerful regression model, reaching the maximum yield of 90 g $plant^{-1}$ at the Cu content of around 6 μg kg^{-1} soil. The highest Cu concentration in lettuce leaves was recorded in A7 and B2 treatments. Therefore, the yield decline can be explained by an excessive supply of copper, responsible for yield depression. The same level of Cu concentration in lettuce was recorded by Castro et al. (2009) on soil fertilized with municipal solid waste compost, although it led to a yield drop as compared to the NPK fertilized control.

Table 3

Yield and nutrient concentration in lettuce – an exhaustive test

Factor	Level of factor	N	P	K	Ca	Mg	Mn	Fe	Zn	Cu	Pb	Cd	Pb_c	Cd_c	Y
		(g kg^{-1} DM)					(mg kg^{-1} DM)						(mg kg^{-1} FM)		(g plant^{-1})
BAD	A1	20.8^{a}	1.9^{a}	17.9^{a}	9.7^{a}	2.2^{a}	179^{b}	27.7^{ab}	57.8^{a}	5.7^{a}	2.16^{ab}	0.28^{a}	0.19^{ab}	0.03^{a}	31.3^{b}
	A3	23.8^{ab}	2.5^{ab}	19.0^{ab}	12.3^{a}	2.4^{ab}	159^{ab}	42.2^{c}	91.9^{b}	6.8^{ab}	1.87^{a}	0.54^{c}	0.16^{a}	0.05^{bc}	33.0^{bc}
	A7	24.1^{ab}	3.7^{c}	33.7^{d}	18.5^{c}	3.8^{c}	134^{ab}	41.5^{c}	86.0^{ab}	11.0^{c}	2.72^{b}	0.50^{bc}	0.26^{c}	0.05^{c}	25.9^{ab}
	B2	29.2^{abc}	3.0^{bc}	32.7^{cd}	17.3^{bc}	2.9^{abc}	189^{bc}	38.9^{bc}	107.0^{bc}	8.8^{bc}	1.95^{a}	0.31^{a}	0.22^{bc}	0.04^{ab}	14.7^{a}
	B3	33.2^{c}	2.5^{ab}	24.9^{abc}	13.1^{ab}	2.3^{a}	110^{a}	24.9^{a}	114.3^{bc}	6.7^{ab}	1.73^{a}	0.31^{a}	0.14^{a}	0.03^{a}	47.7^{c}
	B5	32.2^{bc}	2.9^{bc}	27.0^{bcd}	19.0^{c}	3.3^{bc}	240^{c}	29.6^{ab}	138.8^{c}	7.9^{ab}	2.33^{ab}	0.37^{ab}	0.15^{a}	0.03^{a}	69.1^{d}
F		6.40***	7.86***	11.5***	11.6***	8.71***	10.88***	8.50***	12.8***	8.90***	5.24**	11.7***	14.1***	19.4***	31.9***
Dose (D) (g kg^{-1})	0	23.2^{a}	1.7^{a}	16.2^{a}	15.5	2.5	59^{a}	26.8^{a}	59.6^{a}	4.5^{a}	1.97^{ab}	0.35^{ab}	0.22^{b}	0.04^{b}	15.1^{a}
	2	27.5^{ab}	2.8^{ab}	29.7^{b}	14.0	2.7	182^{b}	36.2^{b}	91.9^{b}	7.5^{b}	1.66^{a}	0.25^{a}	0.16^{a}	0.02^{a}	25.1^{a}
	4	28.1^{ab}	3.0^{ab}	29.3^{b}	14.7	2.8	193^{b}	37.4^{b}	108.3^{b}	8.8^{bc}	2.27^{bc}	0.49^{c}	0.17^{a}	0.04^{b}	52.2^{b}
	8	30.0^{b}	3.6^{b}	28.2^{b}	15.8	3.1	241^{c}	36.3^{b}	137.4^{c}	10.5^{c}	2.61^{c}	0.45^{bc}	0.21^{b}	0.04^{b}	55.3^{b}
F		3.12*	19.2***	16.2***	0.81	2.19	47.4***	5.43**	26.5***	24.0***	9.90***	16.2***	10.1***	14.3***	52.3***
F for interaction of factors															
BAD x D		0.74	6.75***	2.38*	2.63*	6.56***	5.85***	5.84***	2.65*	9.50***	2.51*	13.7***	5.4***	21.2***	9.2***

[a] data marked with the same letter are not significantly different; ***, **, * significance at 0.001, 0.01, 0.05, respectively; Pb_c, Cd_c – standardized content based on the fresh matter (FM) content

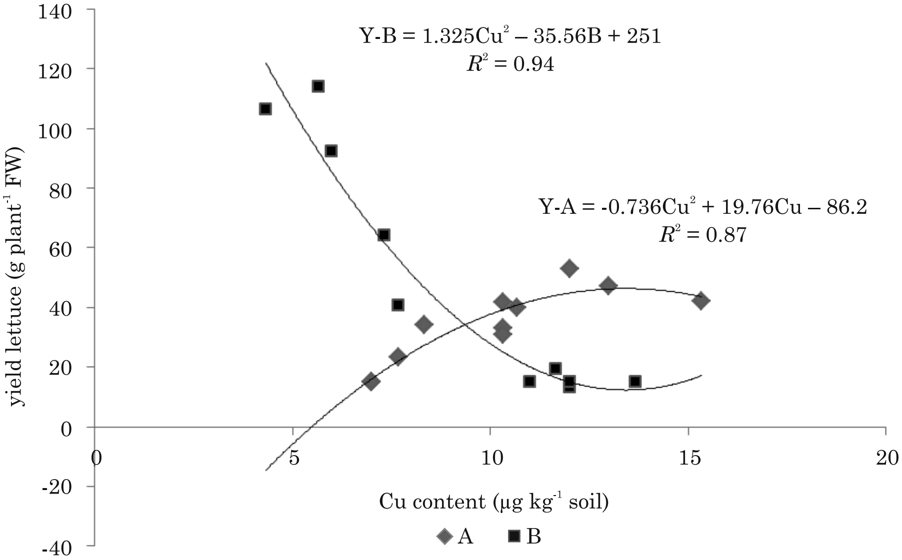

Fig. 4. Yield of lettuce as a function of available copper at the exhaustion test start: A – urea free BAD, B – urea enriched BAD

Patterns of Mn concentrations in lettuce show high resemblance to those recorded for Zn. Its shortage was a constraining growth factor for plants grown in the A pots, i.e. fertilized by the urea-free amendments. It was also the limiting growth factor for plants from the B pots, excluding the B2 treatment. This model clearly demonstrates that the Mn concentration of 407 mg kg^{-1} DM in B pots was the highest maximum yield of lettuce:

A: $Y = 0.14Mn + 10.9$ for $R^2 = 0.61$; $n = 36$, and $P \leq 0.001$ [6]

B3+B5: $Y = -0.0008Mn^2 + 0.655Mn - 16.55$ for $R^2 = 0.72$, $n = 24$ [7]

$Mn_{op} = 407$ mg kg DM and $Y_{max} = 116.4$ g plant^{-1}.

The patterns of Fe concentrations in lettuce were largely consistent with those found for Cu. The highest iron concentration was in the low yielding pots, i.e. in B2, A7, and A3 treatments.

The fourth group of elements comprises two heavy metals, i.e. lead (Pb) and cadmium (Cd), whose content was evaluated based on the dry and fresh matter content. The concentration of Pb based on a DM content showed high sensitivity to the type of bio-fertilizer, but the highest values were the attribute of plants grown in pots that had received the highest amount of digestate (A7, B5). On the contrary, the concentration of Cd declined in plants treated with urea-enriched bio-fertilizers. An evaluation of HMs concentration carried out based on FM content clearly indicates *the dilution effect* due to the presence of urea in applied bio-fertilizers. The effect of the BAD doses was inconsistent. As presented in Figure 5, the tested bio-fertilizers, exclu-

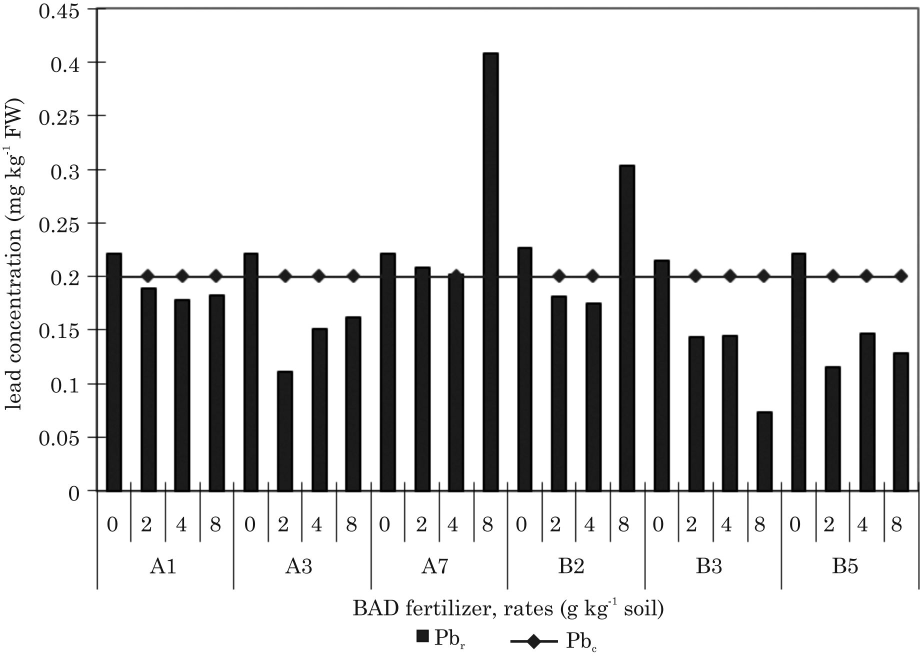

Fig. 5. The concentration of lead in lettuce on the background of standardized norms: Pb_r – real concentration, Pb_c – critical concentration

ding the whole A7 and B2 treatments, resulted in a concentration decrease with respect to the control. The concentration of Pb_c in lettuce can be explained by the following set of concomitant elements:

$$Pb_c = 0.146 + 0.014Ca - 0.075Mg - 0.001Zn + 0.021\ Cu \text{ for } R^2 = 0.55 \qquad [8]$$

The presence of calcium in this model clearly confirms that bio-ash as the key source of Pb. Most importantly, iron is affected negatively by magnesium and zinc. This means that the two elements are antagonists to lead. The conclusion is indirectly supported by studies showing the necessity for an increase of in the Mg concentration (Rosanoff 2013).

The concentration of Cd in lettuce was low, ranging from 0.03 to 0.05 mg kg^{-1} FM. It is even below the limit proposed by Simmons et al. (2005) of 0.05 mg kg^{-1} FW or 4 mg kg^{-1} DW. The urea-free fertilizers induced a significantly higher concentration of Cd, but still far below the threshold value. Based on the stepwise regression, the concentration of Cd in lettuce leaves can be said to be driven by the following set of elements:

$$Cd_c = -0.006 - 0.00008Mn + 0.0009Fe + 0.011Pb \text{ for } R^2 = 0.51 \qquad [9]$$

The model clearly points to bio-ash as the source of Cd. However, its supply to lettuce can be ameliorated, at least partly, by increasing the concentration of Mn in the growing solution. This can be achieved simply by enriching the bio-fertilizer composition based on bio-ash and digestate with urea, as shown in equation No. 7. Application of $MnSO_4$ salts to soils treated with waste rich in Cd is also suggested (Huang et al. 2017). However, this solution will not be successful in soil with neutral pH. The reason is the instability of Mn^{2+}, which undergoes transformation into a higher valent form (Mn^{3+} and Mn^{4+}) under alkaline conditions (Bolan et al. 2003).

Availability of soil elements – a post-test evaluation

The content of $N\text{-}NO_3$ after lettuce harvest was low (Tables 1, 4). The pool of $N\text{-}NH_4$ was also base, depleted in all BAD treated soils (Figure 1). As a rule, much deeper depletion was the attribute of urea-enriched treatments, exceeding for most treatments 90% of the $N\text{-}NH_4$ pre-test value. However, the yield of lettuce responded significantly to the $N\text{-}NH_4$ balance (Δ) only for B3 and B5 treatments, considered together:

$$Y = 1.95\Delta N\text{-}NH_4 + 25.2 \quad R^2 = 0.81, \text{ for } n = 24, P \leq 0.001 \qquad [10]$$

A question remains about the N_{min} content and structure in the B2 treatment and the yield of lettuce, which did not exceeded the control level. The first hypothesis can be related to the N_{min} structure, because $N\text{-}NH_4$ prevailed (> 95%) over $N\text{-}NO_3$. It is well documented that an excess of NH_4^+ ions disturbs the uptake of other cations, especially K^+, by a plant (Esteban et al. 2016). However, the K balance and its depletion level did not show high discrepancy between the treatments studied and the K content increased parallel to the K supply (Tables 1 and 4). A very similar pattern was recorded for Mg.

The second hypothesis refers to copper. As presented in Figure 4, the excess of available Cu just before the exhaustion experiment resulted in a lettuce yield depression in the B2 pot. This treatment showed the highest level of Cu depletion, which could not be related to its uptake by lettuce. The most conspicuous is the fact that the degree of Cu depletion decreased in the order: B2 < B3 < B5, i.e. in accordance with the rise of the digestate content in a particular fertilizer. The model presented below suggests that the decrease in the Cu content was the result of the increasing content of $N\text{-}NH_4$, following the power function:

$$Cu = 14.46 N\text{-}NH_4^{-0,23} \text{ for } R^2 = 0.54 \qquad [11]$$

The complexing effect of ammonium ions on the availability of copper is a well-recognized fact (Tills, Alloway 1981).

The balance of Zn during the lettuce exhaustion test was positive for the control and A1 pot, but negative in other pots with BAD applied in higher doses (Tables 1 and 4). The recorded post-harvest increase in the available Zn content revealed in pots fertilized with urea-enriched fertilizers correlated with both the contribution of digestate and its dose (Figure 6). A change in

Table 4

Effect of BAD type and its rates on the 0.01 M $CaCl_2$ extractable nutrients after exhaustive cropping (mg kg^{-1} soil)

Factor	Level of factor	$N\text{-}NO_3$	$N\text{-}NH_4$	N_{min}	K	Na	Mg	Mn	Fe	Cu	Zn	Pb	Cd
BAD	A1	4.2	10.4^{b}	14.6^{b}	15.3^{ab}	3.9^{a}	10.5^{ab}	0.71^{ab}	0.083^{ab}	0.003^{a}	0.011^{ab}	0.025^{ab}	0.012
	A3	3.2	6.4^{a}	9.6^{a}	31.5^{c}	9.6^{b}	15.7^{c}	0.62^{ab}	0.070^{a}	0.006^{b}	0.013^{b}	0.042^{b}	0.014
	A7	3.2	11.4^{b}	14.6^{b}	19.2^{b}	8.1^{ab}	16.8^{c}	0.30^{a}	0.058^{a}	0.003^{a}	0.011^{a}	0.020^{a}	0.012
	B2	2.4	5.0^{a}	7.4^{a}	13.6^{a}	5.1^{a}	8.5^{a}	1.41^{bc}	0.086^{ab}	0.003^{a}	0.012^{ab}	0.043^{b}	0.013
	B3	2.8	4.2^{a}	7.1^{a}	16.2^{ab}	7.6^{bc}	11.3^{b}	1.17^{abc}	0.069^{a}	0.003^{a}	0.011^{ab}	0.041^{b}	0.013
	B5	3.1	6.3^{a}	9.4^{a}	16.4^{ab}	7.5^{b}	11.4^{b}	1.66^{c}	0.112^{b}	0.003^{a}	0.012^{ab}	0.040^{b}	0.013
F		0.83	31.8^{***}	31.0^{***}	37.5^{***}	18.2^{***}	25.1^{***}	6.0^{***}	5.6^{***}	5.3^{***}	3.2^{*}	4.1^{**}	3.1^{*}
Dose (R) g kg^{-1}	0	5.0^{b}	3.7^{a}	8.6^{a}	7.5^{a}	5.4^{a}	7.6^{a}	0.03^{a}	0.068^{a}	0.003	0.012	0.035^{a}	0.013^{b}
	2	2.5^{a}	5.9^{b}	8.3^{a}	11.3^{b}	6.4^{ab}	8.7^{a}	1.04^{b}	0.067^{a}	0.003	0.011	0.023^{a}	0.011^{a}
	4	2.9^{a}	9.3^{c}	12.3^{b}	16.8^{c}	7.2^{b}	12.0^{b}	1.10^{ab}	0.079^{a}	0.003	0.011	0.031^{a}	0.013^{b}
	8	2.2^{a}	10.3^{c}	12.6^{b}	39.3^{d}	8.8^{c}	21.3^{c}	1.74^{c}	0.105^{b}	0.006	0.012	0.052^{b}	0.014^{c}
F		5.80^{**}	53.1^{***}	26.3^{***}	66.5^{***}	12.9^{***}	41.1^{***}	16.6^{***}	7.2^{***}	15.1^{***}	1.77	8.9^{***}	15.0^{***}
F for interaction of factors													
BAD x D		1.35	22.5^{***}	43.8^{***}	50.4^{***}	8.81^{***}	27.9^{***}	2.84^{**}	2.38^{*}	10.2^{***}	2.53^{**}	2.70^{**}	3.97^{***}

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

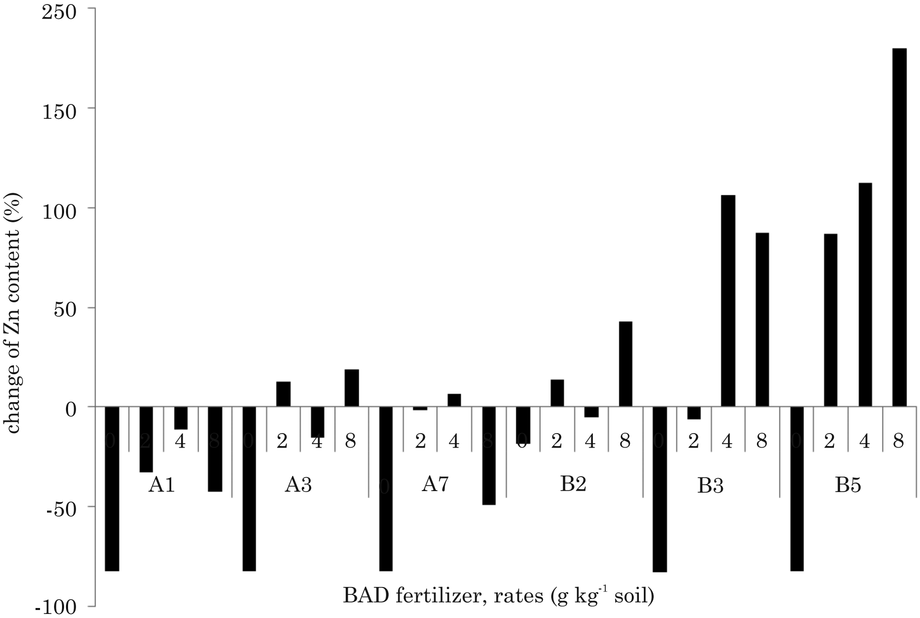

Fig. 6. The relative change in available zinc content during the exhaustion test

the Zn balance was inversely related with a change in the Cu content. Thus, it can be hypothesized that the complexing of Cu by NH_4^+ ions resulted in a higher supply of zinc, in turn increasing the yield of lettuce. This hypothesis explains the effect of the Zn concentration in lettuce plants fertilized with B type of BAD on the yield of lettuce, as presented in equations nos 4 and 5.

The content of Pb decreased substantially during the growth of lettuce, except for two pots with the highest B2 doses. The degree of Pb depletion was very stable, ranging from 58% to 95%. In contrast, the degree of the initial Cd pool depletion was below 30%. For the B2 treatment, its balance was positive. These two facts can provide additional explanation why yield were depressed in this pot.

CONLUSIONS

1. The concentration of N in lettuce corroborates its shortage in plants fertilized with the urea-free bio-fertilizers, resulting in low lettuce yields.

2. The supply of copper to lettuce was demonstrated to be a key factor depressing the yield of lettuce.

3. The content of available form of copper and lead can be ameliorated by simultaneous addition of digestate and urea.

4. The decreased content of copper during the lettuce test resulted in the concomitant increase of available zinc content, stimulating the lettuce yield.

5. The antagonism observed between Mg and Zn versus Pb concentrations in lettuce manifests the importance of the two former elements as necessary inorganic amendments in bio-fertilizer based on bio-ash.

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