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The Multifactorial Effect of Digestate on the Availability of Soil Elements and Grain Yield and Its Mineral Profile—The Case of Maize

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Abstract: The fertilizer value of digestate (a biogas plant byproduct) depends on its impact on the availability of soil nutrients and on the concentration of minerals, including heavy metals, in the edible crop parts. This hypothesis was verified in field experiments with maize conducted in the years 2014, 2015, and 2016 in Brody, Poland. The two-factorial experiment consisted of the digestate application method and its rate: 0.2, 0.4, and 0.8 t ha^{-1}. Maize yield in consecutive years fitted the quadratic regression model, reaching a maximum grain yield of 11.5, 10.8, and 9.2 t ha^{-1} for an optimum digestate rate of 0.56, 0.66, and 0.62 t ha^{-1}, respectively. The supply of N-NO_3 to maize, concomitant with a shortage of magnesium and iron, was the key factor limiting the grain yield. Cadmium concentration in maize grain exceeded its threshold content in plants fertilized with digestate. An excessive concentration of lead in grain was recorded in the dry season 2015. Cadmium concentration in grain was controlled by the availability of soil Fe and Pb by a shortage of N-NO_3, zinc, and copper. The negative relationship of Pb with K, Na, Zn, and Fe contents in grain suggests their usefulness as agents to reduce the accumulation of heavy metals.

Keywords: digestate rate; maize; grain yield; yield components; soil elements availability; mineral nitrogen; grain minerals; heavy metals

1. Introduction

The utilization of organic wastes in agriculture has become one of the key ways to decrease the use of nonrenewable resources. This refers not only to the reduction of minerals mined from geological ores, which are necessary for fertilizer production, but also to the lower use of energy carriers (coal, fuel, and methane) [1]. Agriculture is a branch of the economy with a high potential for the effective use of raw biological wastes, as well as those which will eventually be transformed into organic fertilizers [2,3].

The increasing volume of agricultural wastes poses a significant threat to the environment. They refer not only to farmyard manure but also to different types of green wastes from the agro-industry [4,5]. At present, this problem can be solved by transforming them by the use of anaerobic fermentation. The production of biogas is considered in European Union (EU) policy as an important source of renewable energy [6]. The main sources for agricultural biogas plants are animal manures, straw, hay, silage, mostly from maize, wastes from the food industry, or energy crops [7,8].

The main product of anaerobic decomposition of organic matter (20%–95%) is biogas (a mixture containing mainly $CH_4 + CO_2$, small amounts of ammonia (NH_3), and hydrogen sulfide (H_2S)). The byproduct of biogas plants is biogas slurry, known as digestate. It can be used in three different ways. The simplest one is to use it in the same way as liquid organic fertilizers, i.e., to apply it directly to the field. The main advantage of the raw slurry as a fertilizer is its composition, due to macro-, and micronutrients, enzymes, and hormones, which directly or indirectly have an impact on plant growth and yield [9,10]. However, the high variability in the dry matter content, concomitant with broad ranges of nutrient concentration, creates numerous problems in the rational application of digestate to crop plants. As presented by Nkoa [8], the content of total N in biogas slurry ranges from 3.1% to 14% DW. Consequently, the C/N ratio extends from 2:1 to 24.8:1. These values clearly indicate that applied raw digestate is able to accelerate the decomposition of the natural soil organic matter or immobilize the soil N [11,12]. Another disadvantage of digestate is the high cost of its transportation to the field during the growing season. According to EU law, slurry application to arable soils is not allowed during winter months. Therefore, it is necessary to install big tanks or build lagoons for effective slurry storage [8,10]. The content of ammonia ($N\text{-}NH_4$) in the digestate is high, frequently exceeding 60% of total N content. This composition is, however, highly unstable and may subsequently lead to the volatilization of ammonia (NH_3) into the atmosphere [13]. Thus, one of the most important challenges for farmers is to find an optimal solution for application of this fertilizer. The broadcast method of digestate application results in huge losses of N in the form of NH_3, even up to as much as 35% of the N total content [7]. An alternative way is to apply slurry beneath the ground surface, using different injection methods [8,10].

The hidden side of the raw digestate application or even recycled organic wastes for food production is the threat of the uptake of heavy metals by crops. They can accumulate directly in the soil, from where they can be potentially transferred into a plant. There are numerous examples of harmful or even toxic effects of a high heavy metal content in the soil on plant growth and on their concentration in edible plant parts [14]. Biofertilizers based on biowastes undergo a legalization process in order to minimize the health risk for humans [15]. The maximum acceptable levels of harmful metals in food, including edible parts of vegetables, are standardized and subsequently used to evaluate the quality of the applied biofertilizers [16].

Nowadays, maize has become one of the most important crop plants for both feed and food production. Its yielding potential is high, significantly exceeding that of classic cereals [17]. The yielding potential of this crop can be fulfilled as long as there is a good supply of numerous nutrients. This crop is extremely sensitive to the supply of N and Zn, both of which are required in optimal quantities from the beginning of growth until plant maturity [18,19]. The mineralization processes, which can be potentially accelerated in the soil by an application of manures and/or organic fertilizers, are important for exploiting the maize yielding potential [20]. Therefore, maize is a good crop for evaluating the fertilizer value of digestate [21].

At present, there still is a knowledge gap concerning crop plant responses to digestate application. The effects of biogas slurry application to crop plants can be considered on the basis of three mutually complementary criteria. For a farmer, the grain yield is the key evaluator, assuming a higher use efficiency of nitrogen present in the soil/plant system. The second criterion is the content of the soil-available nutrients evaluated just after harvest, assuming a reduced volume of applied key fertilizers like K and P. The third criterion refers to the threat of excessive accumulation of heavy metals in the edible parts of the crop.

The key objective of the study was to evaluate the impact of progressively increased rates of digestate applied to the entire soil surface (broadcast) or alternatively injected into a narrow path of soil beneath the surface (row) on (i) the grain yield, (ii) the post-harvest content of available nutrients and the content of heavy metals, and (iii) the mineral profile of maize grain. Special attention was paid to the content of heavy metals, both in soil and in grain.

2. Materials and Methods

2.1. Study Site

The study on the influence of digestate on the content of soil-available elements and their impact on the mineral profile and yield of maize grain were verified based on a series of field experiments carried out in 2014, 2015, and 2016 at RGD Brody (Poznan University of Life Sciences Experimental Station, 16°28′ E and 52° 44′ N, Poland). According to the FAO/WRB (Food Agriculture World Organization/World Reference Base), the studied soil has been classified as typical Albic Luvisols. The content of soil-available nutrients, as shown in Table 1, was satisfactory for high-yielding maize. The total amount of mineral nitrogen (N_{min}) as measured before maize sowing varied from 46 kg ha^{-1} in 2015 to 61 kg ha^{-1} in 2016. The total sum of precipitation during the growing season (April–September) was 410 mm in 2014, 284 mm in 2015, and 417 mm in 2016, whereas the long-term average was 317 mm. In 2015, maize growth was affected by drought in August with precipitation of 15 mm and temperature higher by 4.5 °C, compared to the long-term average (17.6 °C) (Figure 1).

Table 1. Soil agrochemical properties before maize sowing.

Year	Soil Layer. m	pH [1]	P [2]	K[2]	Mg [2]	N-NO$_3$	N-NH$_4$	N_{min} [3]	N_f [4]
			mg kg^{-1} soil			kg ha^{-1}			
2014	0.0–0.3	5.6	180 [H]	330 [H]	220 [G]	39	10	49	51
	0.3–0.6	5.8	128 [G]	240 [G]	250 [G]	30	10	40	
2015	0.0–0.3	5.7	170 [H]	280 [G]	180 [G]	35	15	50	46
	0.3–0.6	5.9	150 [H]	230 [G]	230 [G]	32	12	44	
2016	0.0–0.3	6.2	149 [H]	220 [G]	172 [G]	25	21	46	61
	0.3–0.6	6.4	110 [G]	210 [G]	122 [L]	22	11	33	

[1] 1 M KCl; [2] Mehlich3 classes: H—high; G—good; L—low; [3] 0.01 M $CaCl_2$. [4] total sum of N_{min} + N_f = 140 kg ha^{-1}.

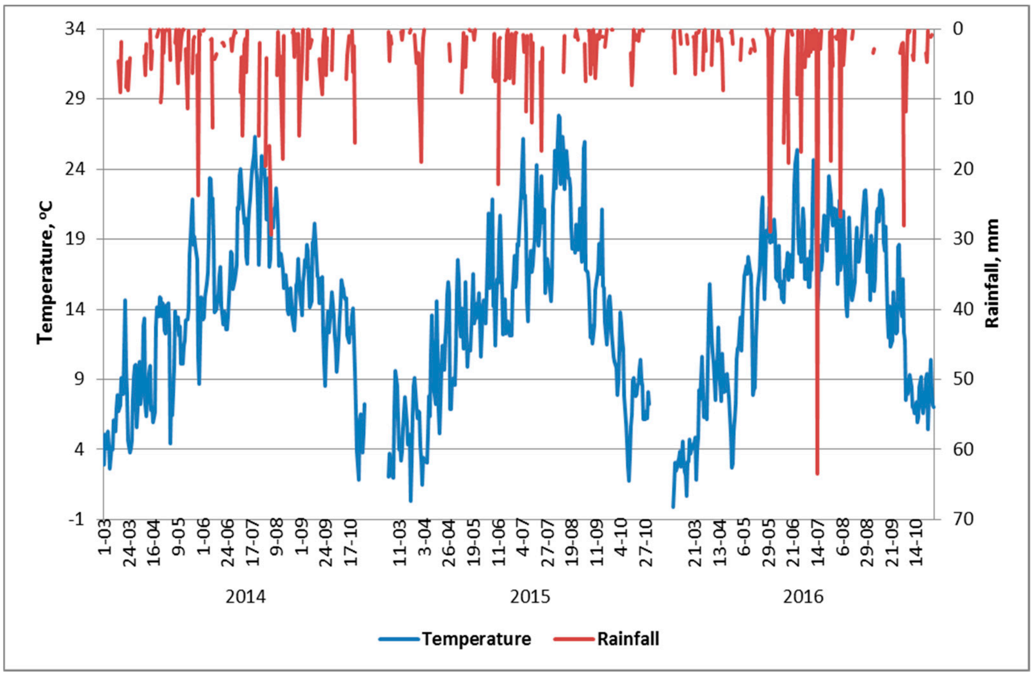

Figure 1. Mean daily air temperature and precipitation during the 2014–2016 growing seasons of maize.

2.2. Trial Establishment

The two-factorial experiment consisted of two methods of digestate application: i) broadcast (Br) and ii) row (Ro). The second factor was the rate of digestate: 0.0, 0.2, 0.4, and 0.8 t ha^{-1}. The characteristics of digestate, containing 10% of DM (dry mass), and the amounts of applied elements are presented in Table 2. The tested digestate was obtained from a biogas plant and used cow slurry and maize silage as raw material. The total amount of N_{min} in spring, including both soil N_{min} resources (0.6 m) and N fertilizer (N_f as ammonium nitrate), was established at 140 kg ha^{-1}. No other nutrients, except N, were applied. The broadcast-applied slurry was incorporated into soil just before maize sowing and mixed at a depth of 7 cm. The row-applied slurry was incorporated into the soil directly after maize sowing. The application path was prepared using the knife method at a distance of 7 cm from the seed row at a depth of 7 cm and 15 cm wide, covering 15% of the surface area. Maize (*Eurostar* variety, FAO 240) sown in a distance of 70 cm was used as the test plant. The individual plot size, replicated four times, was 22.4 m^2. At maturity, maize was harvested from an area of 11.2 m^2. The grain yield was adjusted to 85% of dry matter weight.

Table 2. Chemical composition and rates of elements applied with consecutive rates of digestate.

Rate of Digestate t ha^{-1}	OM[1]	N_t[2]	$N\text{-}NH_4$	P	K	Mg	Cu	Zn	Pb	Cd
	g kg^{-1} DW							mg kg^{-1} DW		
	711	**26.1**	**0.4**	**3.4**	**46.5**	**5.4**	**325**	210	2.80	1.64
	kg ha^{-1}							g ha^{-1}		
0.2	**142**	**5.2**	**0.08**	**0.68**	**9.3**	**1.1**	**65**	42	0.56	0.33
0.4	248	10.4	0.16	1.36	18.6	2.2	130	84	1.12	0.66
0.8	569	20.8	0.32	2.72	37.2	4.4	260	168	2.24	1.32

[1] organic matter, [2] total nitrogen.

2.3. Measurements

2.3.1. Soil

Composite soil samples (0–30; 30–60 cm) for N_{min} determination were collected at the beginning of the growing season and after maize harvest. For N_{min} determination, 20 grams of soil samples were shaken for 1 h with 100 mL of a 0.01 M $CaCl_2$ solution (soil/solution ratio 5:1; m/v). Composite soil samples (0–30 cm) for determination of available forms of nutrients (P, K, Mg, Zn, Cu, Mn, Fe), cadmium (Cd), and lead (Pb) were collected at the beginning of the growing season and after maize harvest. The soil samples were then air-dried and crushed to pass a 2 mm mesh size. The extractable nutrients and heavy metals were determined based on the Mehlich 3 method [22]. The content of available P in the extract was determined calorimetrically, while the content of K, Mg and Ca, Fe, Mn, Zn, Cu, Pb, Cd, and Ni was determined using a flame-type atomic absorption spectrometer (FAAS).

2.3.2. Plant

The harvested samples of maize grain used for the determination of mineral concentration were first dried (65 °C). Nitrogen concentration was determined using a standard macro-Kjeldahl procedure. The plant materials for element determination were mineralized at 600 °C. The obtained ash was then dissolved in 33% HNO_3. Phosphorus concentration was measured by the vanadium–molybdenum method using a Specord 2XX/40 at a wavelength of 436 nm. The concentration of K, Mg, Ca, Fe, Mn, Zn, Cu, Pb, and Cd were determined using FAAS.

2.4. Data Analysis

The experimentally obtained data were subjected to a conventional analysis of variance using the computer program STATISTICA 10®(USA, Statsoft, Electronic Statistics Textbook). The differences between treatments were evaluated with the Tukey's test. The stepwise regression was applied to define the best set of variables for the yield discriminative crop characteristics.

3. Results and Discussion

3.1. Weather Conditions and Grain Yield

The effect of the digestate application method on the grain yield of maize in consecutive years of study was negligible (Table 3). The effect of increasing rates of digestate showed variable but significant trends in the consecutive years of study. The analysis of Figure 2 clearly shows that in all years, the yield response to increasing digestate rates perfectly fitted the quadratic regression model. There are three basic indicators of the patterns obtained. The first one is the yield harvested on the N_f treatment, i.e., on the digestate control. The average yield on this plot increased in the following order: 2016 < 2014 < 2015. This order shows the natural productivity of the soil and the N fertilizer applied (Table 1). The second indicator is the optimal rate of digestate, resulting in the maximum yield of maize grain (GY_{max}), treated as the third indicator of digestate productivity. This amounted to 0.56 t ha^{-1} in 2014; 0.44 t ha^{-1} in 2015, and 0.61 t ha^{-1} in 2016. These values correspond to the GY_{max} of 11.48 t ha^{-1}, 10.76 t ha^{-1}, and 9.996 t ha^{-1} of maize grain, in 2014, 2015, and 2016, respectively. Theoretically, in 2014 and 2016, the net grain yield increase due to digestate application was 2.92 t ha^{-1} and 2.2 t ha^{-1}, respectively. In 2015, it was lower, reaching only 1.03 t ha^{-1}. This finding corroborates the study by Morris and Lathwell [23], who reported a significant increase in the yield of maize fertilized with digested dairy manure.

Table 3. Effect of digestate application method and rate on grain yield and maize yield components in consecutive years of study.

Year	Application method (AM)	RATE t ha^{-1}	GY t ha^{-1}	TGW g	NR	NGR No. cob^{-1}	NGC	HI %
2014	Br	0	8.4	310.7	14.3	30.7	440.7	37.6
		0.2	10.5	301.6	14.3	33.0	473.7	42.2
		0.4	10.7	319.5	13.3	33.0	441.3	43.2
		0.8	10.8	306.8	15.0	33.7	505.3	41.6
	Ro	0	8.4	310.7	14.3	30.7	440.7	37.6
		0.2	11.1	323.0	15.0	33.3	498.7	47.8
		0.4	11.1	298.2	14.3	33.0	475.0	45.0
		0.8	11.2	287.2	15.3	31.0	472.3	42.6
2015	Br	0	9.6	262.2	15.0 bc	30.3	455.0 ab	49.8 ab
		0.2	11.0	271.6	16.0 c	33.3	533.3 c	46.3 ab
		0.4	10.2	271.1	12.7 a	31.3	394.7 a	48.5 ab
		0.8	10.2	275.2	14.0 b	33.3	466.7 bc	43.9 a
	Ro	0	9.6	262.2	15.0 bc	30.3	455.0 ab	49.8 ab
		0.2	10.7	278.0	14.0 b	33.0	462.0 ab	46.9 ab
		0.4	10.7	269.7	14.3 b	32.3	463.7 ab	46.0 ab
		0.8	10.0	267.2	14.0 b	31.3	438.7 ab	50.8 b
2016	Br	0	7.8	272.7	13.0 ab	30.5	397.0	43.7
		0.2	8.6	272.3	12.3 a	30.7	377.3	47.6
		0.4	9.4	272.4	14.7 b	32.0	469.3	49.0
		0.8	10.0	287.6	14.0 ab	32.0	448.0	48.9
	Ro	0	7.8	272.5	13.3 ab	31.0	414.0	45.3
		0.2	8.0	256.3	14.7 b	30.0	440.0	47.2
		0.4	8.1	284.9	12.0 a	30.7	368.0	49.0
		0.8	9.5	284.8	14.7 b	31.3	460.0	51.4
	ANOVA	df						
	Year (Y)	2	66.3 ***	22.5 ***	7.3 **	2.3	8.1 ***	27.2 ***
	Ap. Method (AM)	1	0.2	0.2	0.7	1.2	0.1	3.1
	Rate (R)	3	34.1 ***	0.1	4.4 **	2.4	3.0 *	2.6
	Y x AM	2	4.5 *	0.1	0.7	0.0	0.2	0.4
	Y x R	6	7.4 **	1.1	2.3 *	1.1	1.7	4.1 *
	AM x R	3	0.0	0.4	0.2	0.8	0.2	1.5
	Y x AM x R	6	1.1	0.8	6.8 ***	0.3	4.1 **	1.2

Different letters indicate statistically significant differences between treatments (a, ab, b, bc). F value for different probability level: ***, **, and * significance at $p \leq 0.001$, 0.01, and 0.05, respectively. Legend: GY—grain yield; TGW—thousand kernels weight; NR—number of rows per cob; NGR—number of kernels per row; NGC—number of kernels per cob; HI—harvest index.

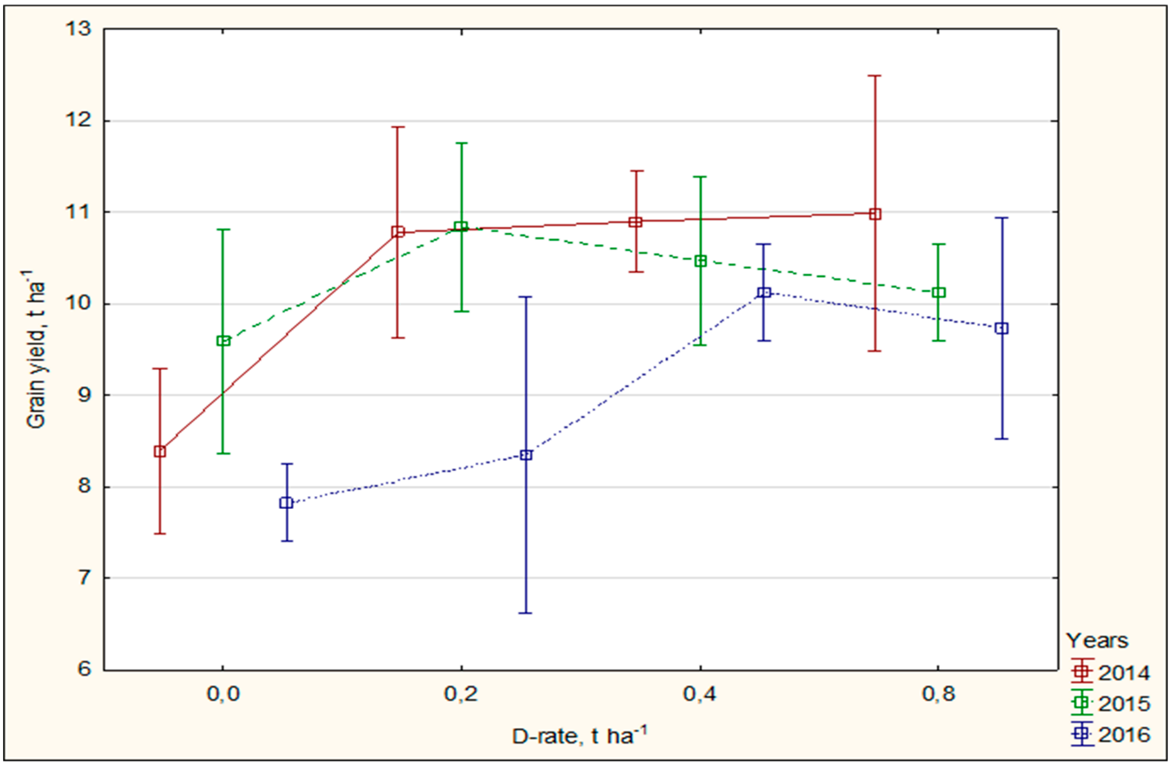

Figure 2. Grain yield of maize response to the increasing rates of digestate in consecutive years. The bars indicate a double standard error of the mean (*n* = 8).

A comparison of yields harvested under the N_f treatments in consecutive years of study with those at the optimal digestate rate clearly indicates the growth disturbance in 2015. The key reason for the much lower yield in this particular year was the severe water shortage which occurred in August and September 2015 (Figure 1). The drought covered the entire postflowering period of maize growth. The total precipitation in August amounted to 15 mm, whereas the average monthly temperature was higher by 4.5 °C compared to the long-term average. The negative impact of environmental factors significantly disturbed the impact of digestate on the number of grain rows per cob (NR), and as a consequence, on the number of grains per cob (NGC). Compared to 2014, there was a sharp decrease in thousand-grain weight (TGW). The yield drop in 2016 cannot be explained by the course of weather because the amount of precipitation during the maize vegetation period was at the same level as in 2014 (around 400 mm). The key reason was probably the shortage of available N, which is required by maize throughout the entire vegetation period [18]. This hypothesis is supported by the low yield of the N_f plot. The limiting effect of N shortage on yield is stressed by the significantly lower NR and NGC. Both components showed a significant dependence on the interaction of fertilization factors and years. The first yield component, i.e., NR, is sensitive to N deficiency during the vegetative period of maize growth, and the second, i.e., NGC, during flowering [19].

3.2. The Post-Harvest Status of Available Elements

The soil pH showed a mild year-to-year variability, mostly due to significantly higher values in 2016 (Table 4). The negative relationship between soil pH and the majority of the examined elements, excluding Zn, Cu, and Mn, indicates a decrease in the content of their available forms in response to increasing pH (Table A1). The postharvest content of nitrate-nitrogen (NO_3-N) was governed by the interaction of both experimental factors and years. As shown in Figure 3, in 2014, the NO_3-N content was 3-fold, and about 7.5-fold higher, with respect to 2015 and 2016. The much lower values in 2015 were due to extended drought in July and August. It is well documented that a severe water shortage results in a drastic reduction in the activity of microorganisms [24]. The effect of the digestate application method and its rate on the NO_3-N content was significant only in 2014. The row method of biogas slurry application (Ro) resulted in a progressive increase in the NO_3-N content up to a rate of 0.4 t ha^{-1}. In other years, this effect was not observed. The mineralization potential of soil to supply N to a plant depends on the amount of ammonium nitrogen (NH_4-N) released during maize growth [20]. This was twice as high in 2015 compared to 2014, but it did not result in an excessive amount of NO_3-N. In 2016, the amount of NH_4-N was several-fold lower compared to 2014, indirectly indicating a much lower mineralization potential of organic N. The low value of the post-harvest NO_3-N content in 2016 indirectly corroborates the presented hypothesis about the shortage of N supply to maize. This was the main reason for a quite different pattern of the grain yield in response to the digestate rates (Figure 2). The NO_3-N content was significantly controlled by the content of soil-available Mg. Any increase in its content resulted in a considerable rise of the NO_3-N content:

$$\text{N-NO}_3 = -88.7 + 0.7\text{Mg for } R^2 = 0.76 \text{ and } n = 72 \quad (1)$$

Table 4. Effect of digestate application method and rate on soil pH, content of inorganic nitrogen, and plant-available macronutrients.

Year.	Application Method (AM)	RATE t ha^{-1}	pH	$N-NO_3$ kg ha^{-1}	$N-NH_4$ kg ha^{-1}	P mg kg^{-1} soil	K mg kg^{-1} soil	Mg mg kg^{-1} soil
2014	Br	0	5.9	60.8 a	23.0 b	180.5	328.3 c	217.0
		0.2	6.2	70.6 a	11.8 a	199.0	218.3 a	237.7
		0.4	6.2	96.7 b	10.4 a	185.7	218.7 a	257.7
		0.8	5.7	73.3 a	9.1 a	198.0	263.3 b	248.7
	Ro	0	5.9	60.8 a	23.0 b	180.5	328.3 c	217.0
		0.2	5.7	107.9 b	23.2 b	204.3	263.0 b	251.7
		0.4	5.5	140.4 c	24.8 b	196.7	244.3 ab	267.0
		0.8	5.4	132.3 c	23.4 b	192.0	254.3 ab	290.0
2015	Br	0	5.8	30.3	35.5	157.8	279.9	180.5
		0.2	5.6	24.0	42.0	249.0	212.7	166.8
		0.4	5.7	42.2	61.6	203.0	220.0	189.2
		0.8	5.6	37.4	44.6	208.3	259.7	182.2
	Ro	0	5.7	39.3	31.8	122.7	278.3	189.6
		0.2	5.9	44.0	30.8	185.3	257.7	189.7
		0.4	5.9	29.1	34.4	201.7	240.0	207.0
		0.8	6.0	30.3	39.0	194.0	218.0	200.9
2016	Br	0	6.0	12.6	6.9	166.9	181.3	148.8
		0.2	6.1	14.3	5.3	177.1	150.5	145.2
		0.4	6.0	12.4	4.8	176.7	150.5	136.8
		0.8	6.1	13.0	4.5	182.8	192.5	157.0
	Ro	0	6.0	12.6	6.9	166.9	181.3	148.8
		0.2	6.1	14.6	6.2	149.4	150.2	137.2
		0.4	6.0	8.6	5.5	146.8	125.7	127.5
		0.8	6.1	11.0	5.3	160.3	188.0	154.3
ANOVA		df						
Year (Y)		2	24.0 ***	497.3 ***	130.4 ***	11.5 ***	81.2 ***	146.3 ***
Ap. Method (AM)		1	3.6	30.8 ***	0.1	9.5 **	0.4	3.6
Rate (R)		3	2.1	13.6 ***	0.8	7.9 ***	17.0 ***	3.9 *
Y x AM		2	24.8 ***	28.9 ***	13.2 ***	3.5 *	0.9	2.1
Y x R		6	4.6 ***	14.9 ***	2.3 *	4.6 ***	2.5 *	2.9 *
AM x R		3	1.4	3.0 *	0.7	0.9	2.2	0.5
Y x AM x R		6	5.6 ***	6.2 ***	1.8	1.2	0.8	0.4

Different letters indicate statistically significant differences between treatments (a, ab, b, c). F value for different probability level: ***, **, and * significance at $p \leq 0.001$, 0.01, and 0.05, respectively.

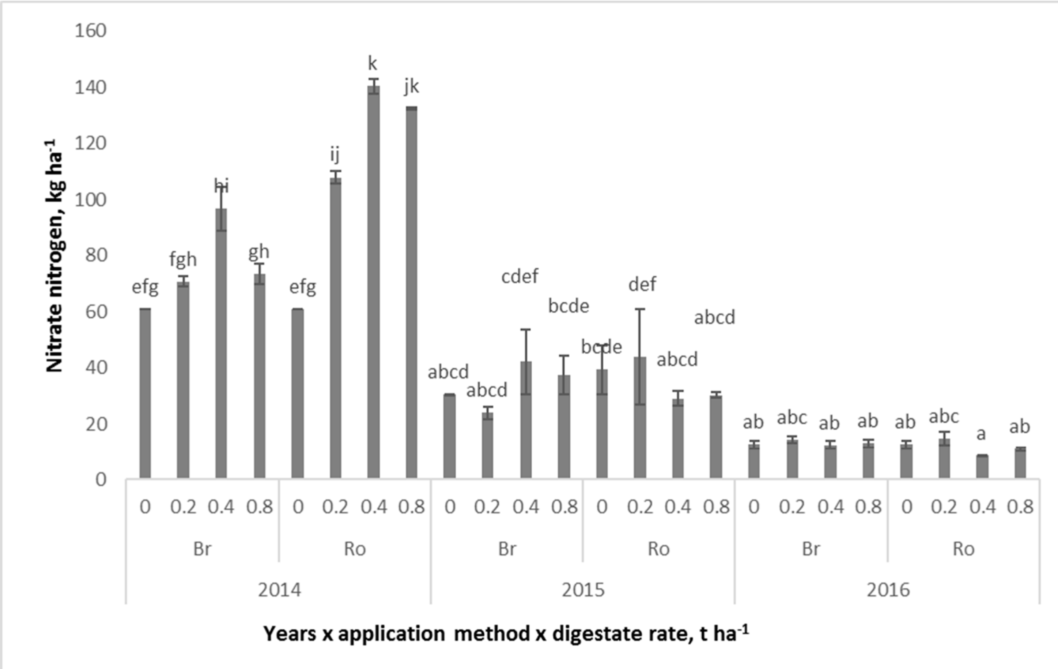

Figure 3. Effect of the method and rate of digestate on the post-harvest nitrate–nitrogen content in soil in consecutive years. [a] Numbers marked with the same letter are not significantly different; the bars indicate a double standard error of the mean ($n = 4$).

The impact of soil-available Mg on the NO_3-N content can be explained in two ways. Firstly, the amount of postharvest N-NO_3 showed a declining yearly trend, being in accordance with the declining content of available Mg (Table 4). Only in 2014, was there a linear increase in the content of N-NO_3 in response to the amount of Mg added in the digestate. The observed phenomena, i.e., drop in postharvest N-NO_3 content in the dry season 2015, can also be explained by the shortage of water, but in 2016 by the shortage of a readily available N pool (Table 1). The second explanation corroborates the hypothesis on the required synchrony between N release from organic fertilizers and maize growth [20]. The lack of this synchrony in 2015 and 2016 was probably the key reason for the fall in grain yield.

The postharvest content of the soil-available P showed a remarkable increase as compared to its presowing status. However, its content was much lower in 2016 compared to 2014 and 2015. In each year, the content of available P did not respond to experimental factors. Nevertheless, taking into account the interaction of experimental factors and years, a significantly higher content of available P was recorded in the soil with broadcast application of digestate. The effect of the digestate rate on the P content was positive with respect to the N_f control, and its highest availability was recorded in soil treated with 0.2 t ha^{-1} digestate. The content of the soil-available K showed the same year-to-year variability as found for P. Its postharvest content was much lower compared to that observed in spring, i.e., before maize sowing. The effect of digestate resulted in a significantly lower K content in treatments fertilized with digestate. This finding was a result of the higher grain yield harvested on plots receiving digestate. It is well documented that maize is a crop with a high requirement for potassium (Szczepaniak at al.) [25].

The third group of studied nutrients refers to micronutrients (Table 5). The effect of fertilization factors on the content of micronutrients was significant for Mn in 2014 and for Fe in 2016. Contents of available forms of all four micronutrients such as Zn, Cu, Mn, and Fe showed a significant year-to-year variability. The lowest impact of the course of weather was observed for Zn and Mn. The contents of these two nutrients were significantly lower in the dry 2015. For Cu, a reverse response to the experimental factors was recorded. The content of the soil-available Fe showed a different pattern.

It was very similar to those observed for macronutrients. This close relationship was corroborated by high values of correlation coefficients between Fe and K, as well as Mg, followed by inorganic N forms (Table A1). The content of available Fe followed the same trend as recorded for N-NO_3 in 2016 compared to 2014 (Figure 3). As shown in Figure 4, contents of both nitrogen mineral forms increased exponentially with the content of available Fe. The optimum range of the soil-available Fe for the elevated content of both mineral N forms was 900 mg kg^{-1} soil. This relationship clearly indicates that the N-NO_3 content, as a yield-driving factor, significantly controlled the amount of available Fe. Despite favorable weather conditions in 2016, its shortage resulted in the yield decrease. As reported by Ali et al. [26], the application of biofertilizer leads to a drop in soil pH, subsequently resulting in an increase of available Fe and inorganic N. Our study clearly showed that the contents of both N mineral forms and Fe were negatively correlated with the soil pH (Table A1).

Table 5. Effect of digestate application method and rate on soil pH, content of inorganic nitrogen and plant-available micronutrients and trace elements.

Year	Application	RATE	Zn	Cu	Mn	Fe	Cd	Pb
	Method (AM)	t ha^{-1}			mg kg^{-1} soil			
2014	Br	0	50.0	11.4	112.4 ^{a}	987.9	0.22	4.31
		0.2	52.3	12.0	125.3 ^{a}	924.5	0.25	4.61
		0.4	50.7	11.3	120.4 ^{ab}	993.6	0.26	4.58
		0.8	50.0	11.1	113.7 ^{ab}	967.3	0.26	4.52
	Ro	0	50.0	11.4	112.4 ^{ab}	987.9	0.22	4.31
		0.2	43.0	11.0	117.5 ^{ab}	1023.4	0.27	4.50
		0.4	42.3	10.5	119.5 ^{ab}	969.9	0.29	4.64
		0.8	40.0	11.1	129.5 ^{b}	1074.1	0.27	4.74
2015	Br	0	43.8	21.6	113.4	981.4	0.25	4.51
		0.2	43.0	20.7	112.7	946.9	0.24	4.39
		0.4	43.7	21.7	109.4	1016.5	0.24	4.37
		0.8	42.3	20.7	112.8	950.5	0.24	4.37
	Ro	0	43.0	21.3	107.8	975.3	0.26	4.44
		0.2	41.0	21.0	112.9	957.4	0.25	4.39
		0.4	42.3	22.0	109.8	967.1	0.27	4.49
		0.8	42.0	20.7	106.2	1000.5	0.26	4.33
2016	Br	0	54.5	18.4	123.8 ^{bc}	619.6 ^{b}	0.15	4.81
		0.2	42.8	17.7	115.5 ^{ab}	562.9 ^{ab}	0.12	3.60
		0.4	48.4	18.3	132.4 ^{c}	682.2 ^{b}	0.12	3.67
		0.8	49.9	16.9	135.3 ^{c}	587.9 ^{ab}	0.15	4.77
	Ro	0	54.5	18.4	123.8 ^{bc}	619.6 ^{b}	0.15	4.81
		0.2	44.3	16.7	133.8 ^{c}	691.9 ^{b}	0.12	3.45
		0.4	37.5	13.0	93.5 ^{a}	341.2 ^{a}	0.10	3.28
		0.8	47.8	16.0	118.2 ^{b}	572.2 ^{ab}	0.12	3.54
ANOVA		df						
Year (Y)		2	5.0 *	274.5 ***	5.3 **	237.3 ***	291.0 ***	8.1 ***
Ap. Method (AM)		1	6.5 *	4.1 *	1.4	0.0	2.6	1.3
Rate (R.)		3	2.8 *	1.8	0.8	0.8	0.6	2.4
Y x AM		2	1.5	2.6	1.2	3.0	3.3 *	1.9
Y x R		6	1.4	1.5	0.7	1.6	5.5 **	4.1 **
AM x R		3	0.9	1.4	1.4	7.7 ***	0.3	0.4
Y x AM x R		6	0.6	1.3	2.7 *	2.6 *	0.5	0.8

Different letters indicate statistically significant differences between treatments (a, ab, b, bc, c). F value for different probability level: ***, **, and * significance at $p \leq 0.001$, 0.01, and 0.05, respectively.

The content of the available Mn responded well to the interaction of experimental factors and years. In contrast to Fe, the impact of the digestate rate depended on the method of application in the two contrastive years, i.e., 2014 and 2016. In 2016, the Mn content increased in accordance with the progressive digestate rates in the broadcast treatment (Br). A reverse situation was observed in the treatment with the row-applied digestate. The content of Mn was negatively correlated with NH_4-N and positively with Zn (Table A1). Averaged over years, the content of the soil-available Zn was significantly higher in the Br treatment. It showed a decreasing trend in response to digestate application, irrespective of its rate. The most interesting nutrient, due to its significant impact on the grain yield, is Cu, but it did not respond to any experimental factors. Averaged over factors, its content was much higher in 2015 and 2016 compared to the high-yielding 2014. It was negatively correlated with N-NO_3 and Mg, but positively with N-NH_4 (Table A1).

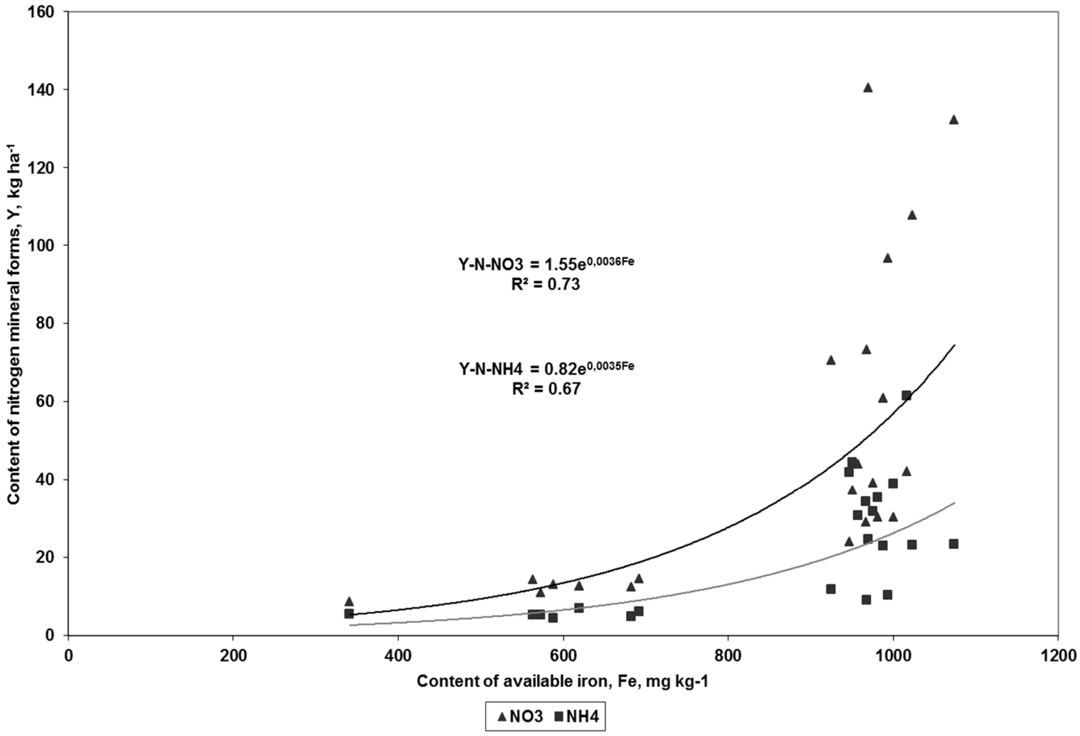

Figure 4. The content of mineral nitrogen forms as a function of the content of available iron.

The stepwise regression analysis showed that the grain yield (GY) of maize significantly depended on the soil content of three nutrients:

$$GY = 4.29 + 0.02N\text{-}NO_3 + 0.016P + 0.1Cu \text{ for } R^2 = 0.56 \text{ and } n = 72 \quad (2)$$

The significant effect of N-NO_3 was revealed due to its shortage in two out of the three years of study. The shortage of P seems to be controversial because its postharvest content was significantly higher on plots with digestate. The limiting effect of Cu occurred as a result of its shortage in 2014, the year with the highest yield.

3.3. Mineral Profile of Maize Grain

The concentration of nitrogen (N_c) in maize grain was, in general, around 15 g kg^{-1} DW. The N_c, as reported by Tenorio et al. [27] for the US North–Central region ranged from 7.6 to 16.6 g kg^{-1}. Therefore, the value obtained can be considered as in the optimal range for maize grain. This level of N_c indicates the high efficiency of soil and fertilizer N on plots with digestate. This finding is in agreement with the work of Sieling et al. [21], who showed a much higher nitrogen use efficiency (NUE) for maize fertilized with digestate. The N_c was significantly higher in 2014, and especially in the dry 2015, compared to 2016 (Table 6). The observed N_c decrease in 2016 supports the presented hypothesis about the shortage of N supply to maize in this particular year (Figure 2). The effect of the digestate application method on N_c was significant only in 2015. The first rate of biogas slurry significantly increased N_c as compared to the N_f control. A positive increase in phosphorus concentration (P_c) was recorded in 2014 and 2015. Significantly higher P_c was associated with the broadcast method (Br) of slurry application. This clearly supports the conclusion that P was not, in fact, a nutritional factor limiting the yield of maize in the studied case. This opinion is also supported by the lack of P_c correlation with N_c (Table A2). It can be explained by the fact that maize develops an extensive root system and subsequently exploring, very efficiently, the soil enriched by easily available phosphorus [28]. The third main nutrient, i.e., potassium concentration in maize grain (K_c), followed quite a different trend in consecutive years of the study. It decreased in the following order: 2014 > 2015 > 2016. This order is concomitant with the initial content of available K in the top layer (Table 2). The effect of the experimental treatments on K_c was not observed in particular years of the study. However, averaged over years, a significantly higher K_c was recorded in treatments with the row method (Ro) of applied digestate. This trend does not suggest any shortage of K supply to maize during plant growth, although its concentration correlated significantly with N_c (Table A2). K_c showed a marked response to the digestate rate, decreasing in accordance with the increase of its rates on plots with Br, but stabilized on plots with the Ro method.

Table 6. Response of macronutrient concentration in maize grain to digestate application method and rate.

Year	Application Method (AM)	RATE t ha^{-1}	N	P	K	Mg	Ca	Na
			g kg^{-1} DW					
2014	Br	0	15.1	1.8 [ab]	3.5	1.06 [a]	2.4	1.2
		0.2	15.6	2.7 [c]	3.5	1.22 [abc]	2.6	1.8
		0.4	15.0	2.3 [b]	3.6	1.53 [b]	2.6	1.5
		0.8	15.4	1.6 [a]	3.5	1.4 [abc]	2.6	1.4
	Ro	0	15.1	1.8 [ab]	3.3	1.42 [bc]	2.6	1.2
		0.2	15.4	1.8 [ab]	3.8	1.18 [abc]	2.6	1.5
		0.4	14.8	1.5 [a]	3.2	1.08 [ab]	2.7	1.5
		0.8	15.2	1.5 [a]	3.9	1.45 [b]	2.6	1.5
2015	Br	0	15.0 [a]	1.8 [ab]	2.5	1.1	3.1	0.2 [a]
		0.2	17.1 [b]	2.8 [c]	3.3	1.7	3.9	1.1 [d]
		0.4	15.3 [b]	2.3 [bc]	2.8	1.4	3.5	0.5 [c]
		0.8	15.2 [b]	1.5 [a]	2.1	1.2	3.3	0.4 [bc]
	Ro	0	15.0 [b]	1.8 [ab]	2.5	1.1	3.1	0.2 [a]
		0.2	15.4 [b]	1.8 [ab]	3.4	1.3	3.3	0.2 [a]
		0.4	15.5 [b]	1.8 [ab]	3.4	1.2	3.2	0.3 [ab]
		0.8	15.4 [b]	1.5 [a]	3.5	1.1	3.1	0.3 [ab]
2016	Br	0	13.8	2.3	1.8	1.7	2.5	1.2
		0.2	14.8	2.1	2.2	1.5	3.0	1.2
		0.4	15.9	2.5	2.2	1.8	2.5	1.2
		0.8	14.7	2.2	1.6	1.5	2.9	1.2
	Ro	0	13.8	2.3	1.8	1.7	2.5	1.2
		0.2	14.8	2.4	2.1	1.9	2.7	1.3
		0.4	15.2	2.1	2.8	1.9	2.4	1.1
		0.8	15.6	2.0	2.4	1.9	2.5	1.2
ANOVA		df						
Year (Y)		2	3.8 *	15.5 ***	56.1 ***	36.7 ***	51.8 ***	219.3 ***
Ap. Method (AM)		1	0.5	17.4 ***	6.3 *	0.1	4.6 *	7.5 **
Rate (R.)		3	3.8 *	10.4 ***	3.5 *	1.0	3.6 *	9.8 ***
Y x AM		2	0.3	4.7 *	1.9	4.6 *	2.5 **	6.1 **
Y x R		6	2.2	2.7 *	1.4	1.9	1.1	1.8
AM x R		3	1.0	5.7 **	3.0 *	1.8	1.2	4.2 *
Y x AM x R		6	0.9	2.1	0.8	2.1	0.4	2.6 *

[a] Numbers marked with the same letter in each column are not significantly different (a, ab, b, bc, c). F value for different probability level: ***, **, and * significance at $p \leq 0.001$, 0.01, and 0.05, respectively.

The second group of macronutrients comprises Mg, Ca, and Na. The Mg concentration (Mg_c) followed the same trend as recorded for P. The higher values of both nutrients in grain harvested in 201, indirectly corroborated a disturbance of the N supply to maize, subsequently leading to a yield decrease (Equation (2); Figure 3). The negative relationship between Mg_c and yield, concomitant with its higher concentration in 2016, clearly indicates that the Mg accumulated in grains was not fully exploited by the crop. The main reason was a shortage of soil N supply to the growing crop (Equation (2)). Ca concentration (Ca_c) in maize grain had much higher values in the dry 2015. The observed increase can be treated as an indicator of water stress [29]. No effect of fertilization treatments on Ca_c was observed in any particular year of the study. Averaged over years, a notably higher Ca_c in maize grain was recorded for plants grown on Br plots. The effect of digestate rate was significant, but an increase of Ca_c was recorded only for plants fertilized with 0.2 t ha^{-1}. The concentration of Na (Na_c) showed quite an opposite trend to Ca_c, underlined by a negative relationship with this nutrient. (Table A2). In 2015, Na_c was almost 4-fold lower compared to 2014. This huge drop in its concentration can be considered as an indicator of water shortage [30]. In 2015, a significantly higher Na_c was recorded in plants grown on the Br plot. The effect of the digestate rate, averaged over years, was the same as that observed for Ca_c, reaching the highest concentration on the plot fertilized with 0.2 t ha^{-1} of digestate.

The third analyzed group of nutrients refers to micronutrients, such as Zn, Cu, Mn, and Fe (Table 7). The trend of Zn concentration (Zn_c) in maize grain followed the same pattern as observed for Na_c, but differences between the years were much lower. This observation is corroborated by the significant relationships of Zn with Na, followed by Mg and P, but negative with Ca (Table A2). The effect of the digestate rate on Zn_c depended on the method of the fertilizer application. A considerably higher Zn_c was recorded for plants grown on Br plots, where its concentration increased up to a digestate rate of 0.4 ha^{-1}, whereas on the Ro up to 0.8 t ha^{-1}. The exception was 2015, when no effect of the studied factors on Zn_c was observed. The concentration of Cu (Cu_c) reacted in the same way to variable weather conditions as observed for Zn but without any marked differences between 2015 and 2016. This was confirmed by the positive relationships between Cu and Zn. No significant variability in Cu_c in response to the experimental factors within a particular year of the study was noted. The effect of digestate rates, averaged over years, on Cu_c depended on the digestate application method. For this particular nutrient, a positive impact of the row method was observed up to a digestate rate of 0.4 t ha^{-1}. The yearly pattern of Mn concentration in maize grain (Mn_c) was the same as observed for Ca. A much higher Mn_c was recorded in the dry season of 2015. This was corroborated by a significant relationship between both elements. A negative relationship was revealed between Mn_c and Mg_c (Table A2). The effect of the method of digestate application, averaged over years, was the same as recorded for P, Ca, Na, and Zn. The effect of the digestate rate on Mn_c in maize grain depended on the method of its application. An increase in Mn_c was recorded only in grain from the Br treatment with 0.2 t digestate ha^{-1}. For all other cases, a significant drop was observed with respect to the N_f control. Each year, the pattern of Fe concentration (Fe_c) in maize grain was significantly governed by experimental factors. The Fe_c showed a negative relationship with Mn, followed by Ca and K. At the same time, positive relationships were found between Fe and Zn, and also with Mg and Na. A significantly higher Fe_c was recorded for plants grown on the Br plot. Each year, the effect of digestate rates was considerably modified by the method of application. In 2015, a progressive increase in Fe_c was recorded for plants grown on the Ro plots. The same trend, but irrespective of the digestate application method, was demonstrated in 2016.

The yield of maize grain showed positive relationships with N, K, Ca, and Mn concentrations, indicating a shortage of these nutrients. A negative relationship was found only for Mg. The applied stepwise regression model indicated that N, Mg, and Cu as a set of nutrients significantly affected the maize yield (GY):

$$GY = 3.5 + 0.5N - 1.8Mg + 0.5Cu \text{ for } R^2 = 0.44 \text{ and } n = 72 \quad (3)$$

This equation corroborates the hypothesis about the shortage of N supply to maize during the growing season. Maize is a crop that shows high synchrony between N requirements during growth on the one hand and its net release from organic fertilizers on the other [20]. The equation developed also stresses the importance of Mg as a factor controlling N management by a maize crop [31]. This negative value for Mg seems to be confusing. It can be assumed that the amount of Mg accumulated in maize grains during the grain-filling period was not diluted. This would indicate a shortage of N supply to the enlarging grains during the grain-filling period, which significantly reduces the TGW, as recorded in 2015 and in 2016. The first case refers to the environmental conditions, which were unfavorable for N uptake by maize during the postflowering period [32]. The second case, revealed in 2016, was due to N shortage in the soil (Tables 1 and 4) (Equation (1)).

Table 7. Response of micronutrient and heavy metals concentration in maize grain to digestate application method and rate.

Year	Application Method (AM)	RATE $t\ ha^{-1}$	Zn	Cu	Mn	Fe	Cd	Pb
			mg kg^{-1} DW					
2014	Br	0	15.5 ^{abc}	1.33	4.03	29.3 ^{abc}	0.07 ^{a}	0.14 ^{a}
		0.2	19.0 ^{d}	2.40	5.27	23.5 ^{a}	0.14 ^{ab}	0.24 ^{b}
		0.4	22.5 ^{d}	2.73	3.20	25.2 ^{a}	0.19 ^{c}	0.16 ^{a}
		0.8	17.9 ^{abcd}	2.80	5.37	50.4 ^{d}	0.15 ^{bc}	0.15 ^{a}
	Ro	0	13.4 ^{a}	2.13	3.43	23.7 ^{a}	0.11 ^{a}	0.14 ^{a}
		0.2	14.1 ^{bc}	2.47	2.53	27.5 ^{ab}	0.14 ^{bc}	0.14 ^{a}
		0.4	19.5 ^{d}	3.37	2.57	38.6 ^{bc}	0.16 ^{c}	0.14 ^{a}
		0.8	21.6 ^{d}	2.93	4.00	39.1 ^{c}	0.14 ^{bc}	0.16 ^{a}
2015	Br	0	11.5	1.93	4.20	18.3 ^{ab}	0.15^{abc}	0.20
		0.2	15.1	3.03	5.57	14.4 ^{a}	0.18^{bcd}	0.24
		0.4	14.6	1.93	5.23	16.1 ^{a}	0.12^{a}	0.22
		0.8	10.4	1.63	3.67	17.1 ^{ab}	0.20^{d}	0.21
	Ro	0	11.5	1.93	4.20	18.3 ^{ab}	0.15^{abc}	0.20
		0.2	11.2	1.70	3.60	13.6 ^{a}	0.18^{bcd}	0.26
		0.4	10.8	2.17	3.87	13.1 ^{a}	0.16^{abcd}	0.18
		0.8	9.6	1.77	2.53	22.4 ^{b}	0.14^{ab}	0.25
2016	Br	0	13.5 ^{ab}	2.30	3.33	17.6 ^{a}	0.05 ^{a}	0.18 ^{ab}
		0.2	14.9 ^{ab}	1.33	2.13	51.8 ^{b}	0.15 ^{c}	0.19 ^{b}
		0.4	23.9 ^{c}	1.97	2.23	69.7 ^{c}	0.15 ^{c}	0.16 ^{ab}
		0.8	14.0 ^{ab}	1.47	1.47	81.7 ^{c}	0.10 ^{b}	0.12 ^{ab}
	Ro	0	13.5 ^{ab}	2.30	3.33	17.6 ^{a}	0.05 ^{a}	0.18 ^{ab}
		0.2	13.1 ^{a}	1.80	2.00	45.7 ^{b}	0.14 ^{c}	0.12 ^{a}
		0.4	17.7 ^{abc}	3.70	2.03	52.9 ^{b}	0.10 ^{b}	0.16 ^{ab}
		0.8	23.2 ^{bc}	2.25	2.15	68.8 ^{c}	0.12 ^{bc}	0.14 ^{ab}
ANOVA		df						
Year (Y)		2	46.2 ***	5.9 **	45.1 ***	408.2 ***	60.9 ***	57.6 ***
Ap. Method (AM)		1	4.7 *	6.0 *	23.1 ***	11.1 **	0.7	3.2
Rate (R.)		3	15.3 ***	5.2 **	2.7	121.5 ***	48.4 ***	5.8 **
Y x AM		2	1.5	5.4 **	8.1 ***	12.9 ***	0.6	3.1
Y x R		6	5.2 ***	5.0 ***	8.1 ***	58.3 ***	15.9 ***	5.3 ***
AM x R		3	11.2 ***	3.4 *	3.5 *	2.6	3.0 *	8.3 ***
Y x AM x R		6	2.1	1.5	1.3	7.9 ***	10.1 ***	3.2 *

Different letters indicate statistically significant differences between treatments (a, ab, abc, abcd, b, bc, bcd, c, d). F value for different probability level: ***, **, and * significance at $p \leq 0.001$, 0.01, and 0.05, respectively.

3.4. Availability of Heavy Metals in Soil

The content of soil-available cadmium (Cd), as well as lead (Pb), showed year-to-year variability, but no response to the experimental factors was observed in any particular year of the study (Table 5). The contents of both metals were governed by the interaction of digestate rates with years. The source of plant-available heavy metals can be both digestate (Table 2) and also their inherent soil resources [33]. In the past, phosphorus fertilizers were one of the most important sources of both trace elements in arable soils [34].

For Cd, its content was significantly lower in 2016, showing a high resemblance to the patterns described for macronutrients and Fe. This fact was corroborated by crucial positive relationships with all macronutrients and both inorganic N forms (Table A1). It is necessary to stress that the content of Cd showed the strongest and most positive relationship with Fe. The content of soil-available Fe explains 75% of the variability in the Cd soil-available content:

$$Cd = -0.018 + 0.0003Fe \text{ for } R^2 = 0.75 \text{ and } n = 72 \quad (4)$$

This strong dependence clearly shows that favorable conditions for the soil-available Fe release also resulted in an increased release of soil-available Cd. The high Fe soil content was due to a high rate of nitrification, as shown in Figure 4. It is well documented that oxidation of the NH_4^+ ion leads to the release of H^+ ions, which in turn are responsible for soil acidification [35]. Consequently, this process results, as accelerated by digestate application, in the release of cations of different metals, including both Fe and heavy metals, into soil solution [26].

No significant differences were found between Pb content in the digestate control plot and plots fertilized with increased Pb rates. The simple balance between the Pb content in the soil and its concentration in grain indicates a net uptake by maize. This element cannot be leached because it is strongly fixed by organic matter or other compounds. As reported by Brennan et al. [36], Pb in soil rich in phosphorus undergoes precipitation as Pb–phosphate. The content of soil-available Pb, averaged over experimental treatments, was significantly higher in the dry season 2015 compared to other years of the study. These differences were much smaller compared to those observed for Cd. The Pb content was positively correlated with the contents of the most-studied elements, excluding $N\text{-}NH_4$, Mn, and Cu. The strongest relationship was, however, recorded with Cd. The applied stepwise regression analysis indicated three elements as those governing the content of Pb:

$$Pb = 1.25 + 0.009NO_3\text{-}N + 0.037Zn + 0.06Cu \text{ for } R_2 = 0.34 \text{ and } n = 72 \quad (5)$$

This finding confirms the opinion that application of digestate into arable soil accelerates the rate of both N and trace elements release, irrespective of the species [11,12]. The obtained regression model clearly shows that the relationships between available forms of Pb and other elements were much weaker compared to those recorded for Cd. The only negative, but significant, impact on the content of soil-available Cd and Pb was exerted by soil pH, which also had a negative impact on the content of available Fe (Table A1).

3.5. Heavy Metals Concentration in Maize Grain

Maize is a plant with a high potential for heavy metals uptake from soil treated with amendments containing available forms of these elements. The metals absorbed by maize are relatively easily transferred to the edible parts, i.e., to grains [37,38]. The threshold level of Cd concentration (Cd_c) in grain used for consumption is 0.1 mg kg DW [16]. The recorded content of this element was significantly affected by the course of the weather. In 2016, it was more than 20% lower than in 2014 (Table 7). The average Cd_c in grains increased by 60% on plots fertilized with digestate with respect to the digestate control (N_f only), significantly exceeding the threshold value of 0.1 mg kg^{-1} DW. The Cd_c patterns were differently affected by the interaction of the experimental factors and years (Figure 5). In 2014, irrespective of the application method, the highest values were recorded in plants on plots fertilized with 0.4 t digestate ha^{-1}. In 2015, the Cd_c was highly variable between treatments. For the Br treatment, its highest value was recorded on the plot with 0.8 t ha^{-1}, and for Ro on the plot with 0.2 t ha^{-1} of digestate. In 2016, the pattern of Cd_c was very similar to that observed in 2014, but the digestate control plants showed much lower values. The Cd_c was significantly correlated with concentrations of N, K, and Ca (Table A2). The first two elements were in shortage, but the Ca was in excess as a result of the drought in 2015 and due to N shortage in 2016. No simple relationship was found between the content of soil-available Cd and its concentration in maize grain, but it was much higher in the years with a high content of N_{min} in the soil. These results indicate that the supply of Cd to maize plants is a very complex phenomenon, resulting from the mineralization processes induced by the addition of digestate into the soil (Table 5).

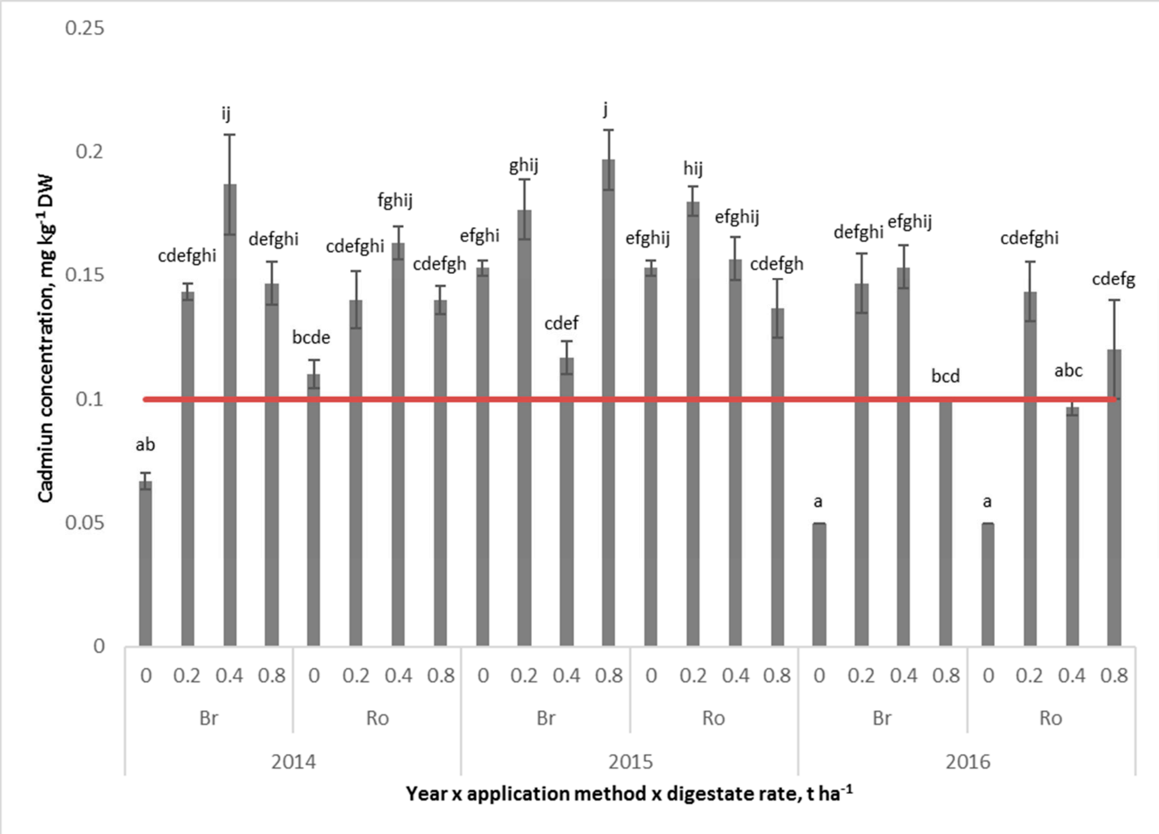

Figure 5. Effect of the method and rate of digestate on the cadmium concentration in maize grain in consecutive years on the background of the threshold Cd concentration. [a] Numbers marked with the same letter are not significantly different; the bars indicate a double standard error of the mean (*n* = 4).

The analysis of Table 7 and A2 shows that any increase in Fe concentration in maize grain resulted in a simultaneous decrease in Cd concentration. This was the most visible when comparing 2015 and 2016. He et al. [39] showed that Cd uptake by a model plant, i.e., *Arabidopsis thaliana* L., was governed by two mechanisms:

1. an enhanced Fe → Cd antagonism: an increased exogenous supply of Fe results in decreased Cd uptake by plant roots;
2. an inhibition *IRT1*, a divalent cation transporter of numerous cations, including Fe and Cd → an increased Fe supply in the growth medium inhibits *IRT1* expression, lowering Cd uptake by plant roots.

Maize, as a mocotydenelous plant, takes Fe as Fe^{3+} ions from soil solution [40]. It is probable that similar mechanisms to those proposed by He et al. [39] for *Arabidopsis thaliana* L. are also present in maize.

The threshold level of Pb concentration (Pb_c) in consumption grain is 0.2 mg kg DW [16]. Its concentration in maize grain was significantly driven by the interaction of all factors (Figure 6). The key factor impacting Pb_c was the annual course of weather. On average, the highest Pb_c was recorded in 2015, a year with a severe drought. As a result, the Pb_c increased and at the same time exceeded, irrespective of the application method, the threshold Pb on plots fertilized with digestate. The observed phenomenon can be explained in two completely different ways. Three elements, i.e., Ca, Mn, and Pb, showed higher accumulation in the dry 2015 season as compared to other years. As reported by Rose et al. [41], over 90% of Ca accumulates in wheat grain during the first 14 days of grain growth. Ca concentration in a plant cell increases significantly in response to water stress [29].

At the same time, drought stress reduces the accumulation of starch in the developing grain [42]. As a result of these two processes, the concentration of Ca in grain increases. In our study, concentrations of Ca and Pb were significantly correlated (r = 0.48 ***) and thousand-grain weight was significantly lower in 2015 as compared to 2014, indirectly corroborating divagations presented above. The observed phenomenon can also be explained by the fact that plants under drought increase the amount of released exudates to the rhizosphere. Plant root exudates consist of numerous organic compounds, including phytosidorephores, which solubilize unavailable soil minerals, including Ca, Fe, and others (Dakora et. al.) [43]. In addition, digestate also consists of a wide range of natural ligands, containing minerals in plant-available forms [37,38]. In 2014, with the exception of the plot with 0.2 t ha^{-1}, it was much lower than the standard. In 2016, Pb concentration was beneath its threshold. The Pb_c was negatively correlated with Fe, Na, Zn, and Mg, but positively with Ca and Mn (Table A2). Therefore, any increase in the concentration of elements from the first group resulted in Pb_c decrease and vice versa. The best example was Fe. Its low content in 2015 was due to an extreme drought, subsequently resulting in the elevated concentration of Pb. On the other hand, the Pb_c increased in accordance with the increasing Ca content. On this basis, it can be concluded that Pb concentration in maize kernels can be used as an indicator of water stress. In practice, the Pb_c can be efficiently controlled by nutrients from the first of the above-mentioned groups. The control of Pb concentration in maize grain through the content of N-NO_3 can be explained by the enhanced content of the soil-available Fe (Table A1; Figure 4). An enhanced supply of Fe can decrease the net uptake of Pb ions, probably by the same mechanism as proposed by He et al. [39] for Cd.

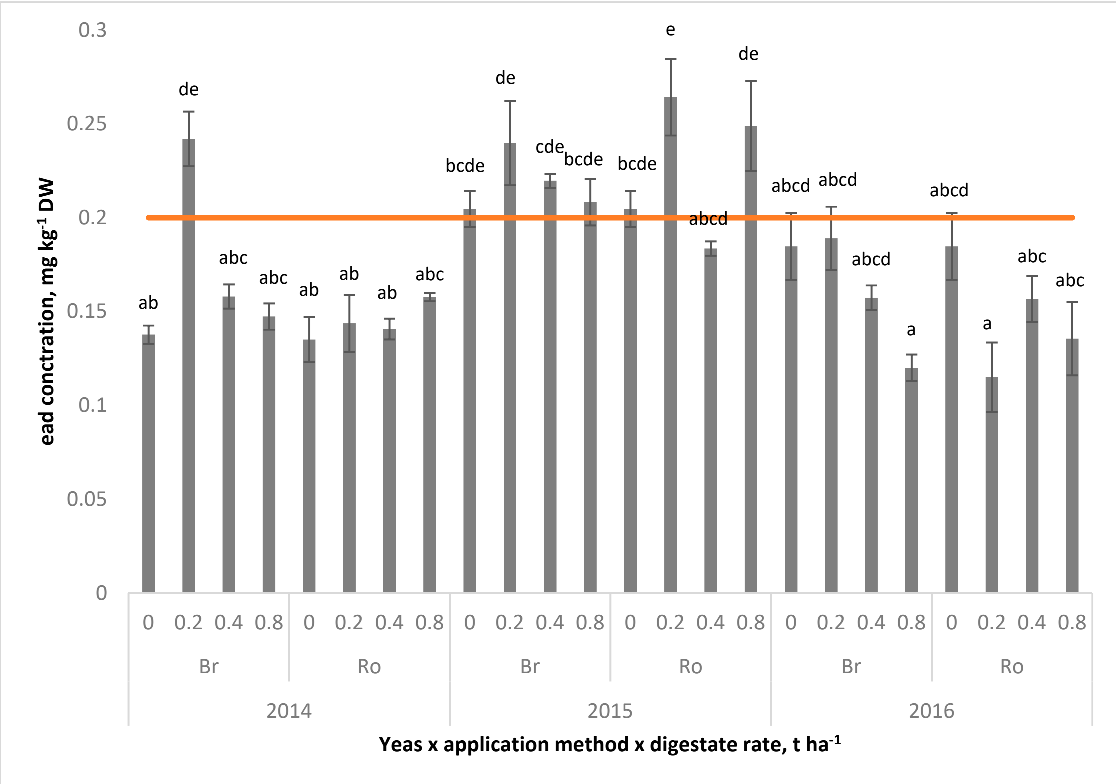

Figure 6. Effect of the method and rate of digestate on the lead concentration in maize grain in consecutive years on the background of the threshold Pb concentration. [a] Numbers marked with the same letter are not significantly different; the bars indicate a double standard error of the mean (n = 4).

4. Conclusions

The application of digestate to maize resulted in a considerable increase in the grain yield. The pattern of its response to the biogas slurry rate significantly depended on the supply of N, being modified by the course of weather. The method of slurry application was negligible, indicating the high capacity of maize for N uptake, irrespective of the method of its application. The maximum yield of 11.5, 10.8, and 9.2 t ha^{-1} was obtained by applying 0.56, 0.66, and 0.62 t ha^{-1}, respectively of digestate. The net NUE increase in response to biogas slurry application is important for both farmers and the environment. The gross productivity of fertilizer N, applied in consecutive years at rate of 51 kg ha^{-1}, 46 kg ha^{-1}, and 61 kg ha^{-1}, increased with respect to the digestate control plot (N fertilizer applied alone) by 60 kg grain kg^{-1} N (165 → 225) in 2014, by 28 kg grain kg^{-1} N (208 → 236) in 2015, and by 16 kg grain kg^{-1} N (130 → 146) in 2016. The lower amount of applied fertilizer N indicates that digestate can offer an efficient production means in sustainability-oriented agriculture. The supply of N to maize was the key factor driving the grain yield, which subsequently depended on the availability of other nutrients, such as Mg, Fe, and also K. This type of response suggests that application of digestate to maize is an efficient production means, provided there is a sufficiently high level of soil fertility. The postharvest evaluation of the impact of digestate on soil fertility was positive for the content of available P, which showed a net increase up to its rate of 0.2 t ha^{-1}. A negative trend was observed for K, whose content decreased in accordance with the increased digestate rates. Mg content showed a slight increase in response to applied digestate, but only in 2014. Magnesium availability was revealed as the key factor controlling the amount of released $N\text{-}NO_3$. Strong relationships were found between soil-available Fe and the contents of both N mineral forms, but especially with $N\text{-}NO_3$. Consequently, a shortage of available $N\text{-}NO_3$ led to a low concentration of N in maize grain, finally resulting in a lower grain yield. Contents of soil-available micronutrients, averaged over years, did not show any significant decrease in response to increased rates of applied digestate. The observed stability suggests that their efficient uptake by maize, as indirectly indicated by the trend in available Zn, decreased in response to digestate application.

A nutrient dilution phenomena in grain was observed for N, P, K, Ca, and Na present on plots fertilized with a digestate rate of above 0.2 t ha^{-1}, and for Zn and Cu, above 0.4 t ha^{-1}. The Fe concentration in grain increased in accordance with the digestate rate, thus stressing its high availability to maize. The application of digestate did not affect the content of soil-available Cd or Pb. The variability in the soil-available Cd content was in accordance with the changes in the content of available Fe. Cd concentration in maize grain increased, however, in response to the rate of applied digestate, exceeding its threshold value. It showed a positive relationship with the concentration of both N and Ca. The content of available Pb in the soil was seriously limited by the supply of $N\text{-}NO_3$, and also by Zn and Cu. Pb concentration in maize grain was highest in the dry 2015, exceeding its threshold limit. The Pb_c was negatively correlated with K, Na, Zn, and especially Fe, which showed a strong negative relationship with Pb only. Therefore, the high increase in the content of soil-available Fe as a result of a high nitrification rate, as indicated by the $N\text{-}NO^{-3}$ content increase, can be treated as a mechanism controlling the uptake of soil Pb. This set of relationships obtained suggests the use of this set of nutrients as agents, decreasing the accumulation of heavy metals in maize grains.

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Appendix A

Table A1. Correlation matrix of relationships between the contents of nitrogen and Mehlich3 available elements, *n* = 72.

	$N\text{-}NH_4$	P	K	Mg	Zn	Cu	Mn	Fe	Cd	Pb	pH	GY
$N\text{-}NO_3$	0.13	0.24 *	0.46 ***	0.87 ***	−0.04	−0.62 ***	0.07	0.63 ***	0.67 ***	0.32 **	−0.42 ***	0.55 ***
$N\text{-}NH_4$	1.00	0.37 **	0.44 ***	0.19	−0.35 **	0.41 ***	−0.33 **	0.59 ***	0.58 ***	0.22	−0.52 ***	0.38 **
P		1.00	0.10	0.30 *	0.04	−0.03	0.07	0.35 **	0.41 ***	0.35 **	−0.26 *	0.55 ***
K			1.00	0.57 ***	0.05	−0.14	−0.05	0.73 ***	0.67 ***	0.38 ***	−0.40 ***	0.27 *
Mg				1.00	−0.03	−0.51 ***	0.02	0.71 ***	0.74 ***	0.39 **	−0.32 **	0.60 ***
Zn					1.00	−0.03	0.42 ***	−0.03	−0.10	0.38 **	0.28 *	−0.18
Cu						1.00	−0.08	−0.03	−0.08	0.04	0.04	−0.08
Mn							1.00	−0.03	−0.08	0.31	0.09	−0.06
Fe								1.00	0.86 ***	0.34 **	−0.45 ***	0.55 ***
Cd									1.00	0.56 ***	−0.49 ***	0.68 ***
Pb										1.00	−0.25 *	0.36 **
pH											1.00	−0.32 **

***, **, and * significance at 0.001, 0.01, and 0.05, respectively.

Table A2. Correlation matrix of relationships between elements in maize kernels and grain yield, *n* = 72.

	P	K	Mg	Ca	Na	Zn	Cu	Mn	Fe	Cd	Pb	GY
N	0.2	0.33 **	−0.04	0.26 *	0.00	0.13	0.07	0.27 *	−0.07	0.35 *	0.20	0.42 ***
P	1.00	−0.21	0.46 ***	−0.02	0.27 *	0.30 *	0.14	0.13	0.09	−0.15	0.09	−0.19
K		1.00	−0.35 **	0.002	0.11	0.18	0.24 *	0.38 **	−0.30 *	0.28 *	0.14	0.49 ***
Mg			1.00	−0.15	0.31 **	0.33 **	0.23	−0.25 *	0.39 **	−0.19	−0.30 **	−0.43 ***
Ca				1.00	−0.53 ***	−0.45 ***	−0.21	0.30 *	−0.39 **	0.46 ***	0.47 ***	0.30 *
Na					1.00	0.59 ***	0.31 **	−0.12	0.40 **	−0.19	−0.48 ***	−0.02
Zn						1.00	0.38 **	−0.04	0.48 ***	0.02	−0.32 **	0.11
Cu							1.00	0.19	0.02	0.01	−0.11	0.22
Mn								1.00	−0.53 ***	0.11	0.39 **	0.33 **
Fe									1.00	−0.11	−0.56 ***	−0.12
Cd										1.00	0.28 *	0.60 ***
Pb											1.00	0.26 *

***, **, and * significance at 0.001, 0.01, and 0.05, respectively.

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