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New phosphorus biofertilizers from renewable raw materials in the aspect of cadmium and lead contents in soil and plants

P-biofertilizers from waste and Cd and Pb in soil and plant

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Abstract: Recycling phosphorus from waste for fertilization purposes appears to be an alternative for non-renewable sources and a solution for managing harmful products of civilisation. Fertilizers from secondary raw materials are considered to be safe to the environment. This study presents an assessment of the effects of five new biofertilizers made from sewage sludge ash and/or animal bones on the content of cadmium and lead in the soil, in wheat grains and straw (test plant), in the mass of the the accompanying weeds and in the post-harvest residues. Biofertilizers were produced in the form of suspension or granules and activated using *Bacillus megaterium* or *Acidithiobacillus ferrooxidans* bacteria. They were tested in four field experiments. The Cd and Pb contents of the soil and plant material were determined using the ICP-MS technique. Similar to superphosphate, new biofertilizers showed no change in the Cd and Pb contents of the soil and plants biomass when applied at amounts up to 80 kg P_2O_5 ha^{-1}. Both Cd and Pb in the soil and plants occurred naturally, and the amounts were within the acceptable standards. Biofertilizers from renewable raw materials, with low toxic element contents, are not thought to pose a hazard to the soil and plants when applied in reasonable amounts. They can be a substitute for conventional phosphorus fertilizers.

Keywords: biofertilizers, sewage sludge ash, animal bones, phosphorus solubilizing bacteria, wheat, weeds.

1 Introduction

Phosphorus is an essential element for the survival of every organism, and its role in the production of food is outright strategic [1]. Unreasonable management of the primary resources of phosphorus has recently been the subject of numerous scientific and political discussions. Concerns for securing the sources of this element for the future usage are one aspect of the problem; another is the fact that the wastage of phosphorus at the stages of sourcing and during its usage results in water and soil contamination [2]. Even though the recent estimates of global geological resources of phosphorus-containing rocks [3] deny the prevailing (until recently) conviction about its impending exhaustion [4], it does not, however, change their status as non-renewable resources. In addition, natural resources of phosphorus are not distributed uniformly throughout the globe [3]. The European Union (EU) has recently categorised phosphorites in the list of 20 critical raw materials [5]. The EU countries, including Poland, are largely dependent on their import.

Closing the phosphorus cycle, i.e. recycling, appears to be a good solution to both agricultural demand for this element [6] and to the issue of managing the growing amount of the wastes of civilisation [7]. Recycling is one of the ideas providing a basis for the European strategy on sustainable use of phosphorus [2]. Modern pro-environmental technologies for fertilizer production enables the usage of waste as secondary raw materials

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[8,9]. Sludge originating from the treatment of municipal wastewater as well as animal bones are considered to be potential renewable phosphorus sources [10,11]. The phosphorus (P) content of the dry mass of sewage sludge or sewage sludge ash ranges from a few to several percent [11,12]. In poultry bones, P_2O_5 content is at a level of 18–20% [13]. Since the 1 of January 2016, recycling of phosphorus from sewage sludge and slaughter waste is mandatory requirement in Switzerland [14]. Germany and Austria are also preparing relevant regulations regarding the recycling process. Since Switzerland banned the direct application of sewage sludge onto the soil in 2006, its regulation has resulted in technical recovery and recycling in the form of inorganic products of phosphorus compounds [14].

The use of bones as a fertilizer dates back to biblical times [15]. In modern times, the management of slaughter waste in the form of bone meal, meat and bone meal and meal ashes to be used for fertilisation is a channel of phosphorus recovery that is an alternative to its use as feed. Meal ash, which used to be widespread, was first banned by the EU [16] (after the occurrence of BSE) and, later on, following the relaxation of relevant regulations, strongly limited [17]. Currently, meals are approved for use in agriculture as soil improvers, which, however, is also subject to numerous limitations aimed at ensuring that livestock have no contact with such preparations. Many authors indicate the good crop-enhancing efficiency of meat and bone meal [18-20] and meat and bone meal ash [21], compare to that of conventional fertilizers.

Management of sewage sludge is becoming a serious problem in the light of EU requirements regarding its storage. In Poland, a legal ban on the storage of sewage sludge containing more than 6% of organic mass has been in force since 2016 [22]. The direct use of sewage sludge for fertilisation purposes is allowed in Poland, provided that the acceptable levels of heavy metals and biogenic compounds are within the level [23]. The presence of harmful pathogens, as well as organic and inorganic contaminants, in sewage sludge prevents the incineration of their biomass [24]. Studies on usage of ashes originated from the incineration of sewage sludge as fertilizers have been carried out in many scientific centres [12,25-27, 28]. The results of many of them are optimistic regarding the crop-enhancing efficiency in relation to various crop plants; however, a relationship with the technology of product manufacturing has also been observed [24,25,27].

A question that arouse is, whether fertilizers from wastes can either change or mitigate the European economy's dependence on the major phosphorus suppliers [29].

Unprocessed phosphoric raw materials originating from both natural resources and secondary sources are characterised by low soluble compounds of phosphorous [10], which determine their limited fertilising efficiency due to poor solubility of the compounds. Solubility issues can be overcome by making use of phosphorus solubilizing microbes (PSMs), which transform phosphorus compounds from hardly available to bioavailable forms for plants consumption [30]. PSMs are abundantly found in agricultural soils. PSM also responsible for other direct and indirect biological mechanisms. They can act as plant-growth-promoting microorganisms (PGPMs) as well [30]. The properties of PSMs have been made use by using them as seed inoculants [31] or applying them along with phosphoric raw materials [32]. Recent research has shown that PSMs efficiently solubilise P from secondary raw materials as well [33].

Mineral fertilizers, particularly phosphorus fertilizers, are generally regarded as one of the major sources of heavy metals and other elements that have adverse effects on the environment [34]. On the other hand, they can affect the phytoavailability of heavy metals through modifying the properties of soil [34]. With a high content of readily soluble phosphates, hardly soluble cadmium phosphates and lead phosphates may precipitate in the soil.

The degree of heavy metal contamination of fertilizers is most largely affected by the raw material from which they are produced, and by the technological process used in their production [34,35]. Phosphorus-containing rocks are naturally contaminated with heavy metals, yet the level of these elements changes depending on the source of the raw material [36,37]. European markets primarily import phosphorites from North and East Africa and from the Middle East, which are characterised by relatively high cadmium content [36]. The tightening of the limits on Cd content to a value of 1 kg of P_2O_5, planned by the EU, has caused concern among fertilizer producers and farmers [37].

Heavy metals in soils may be a potential source of hazard to plants and groundwater [38]. The ability to take up and accumulate heavy metals, as well as tolerance to their presence in plant tissues, are genetic traits [39]. The uptake of trace elements from contaminated soils by plants often exceeds their physiological demand, which leads to phytotoxic effects [40]. After being incorporated into the trophic chain, they can contribute to maintaining the proper development of organisms in each of its links [38]. Significantly, heavy metals accumulate in the soil, and the consequences of soil contamination with those heavy metals, while being seldom visible in a short term, may lead to dangerous environmental changes in a long term [41]. An important characteristic that distinguishes

Table 1: Biofertilizers tested in the experiments.

Experiment	Year	Test plant	Raw material	Bacteria	Form	Symbol
I	2014	spring wheat	ash	*Bacillus megaterium*	suspension	$\mathrm{A_s}$*Bm*
			bones	*Bacillus megaterium*	suspension	$\mathrm{B_s}$*Bm*
II	2015	spring wheat	ash	*Bacillus megaterium*	suspension	$\mathrm{A_s}$*Bm*
III	2015	winter wheat	ash	*Acidithiobacillus ferrooxidans*	granules	$\mathrm{A_g}$*Af*
			ash + bones	*Acidithiobacillus ferrooxidans*	granules	$\mathrm{AB_g}$*Af*
IV	2016	winter wheat	ash + bones	*Bacillus megaterium*	granules	$\mathrm{AB_g}$*Bm*

heavy metals from other undesirable substances is that they are not biodegradable but only biotransformable, due to the complex physicochemical and biological processes occurring in the soil. These processes determine the mobility and bioavailability of heavy metals in the soil-plant system [34,38,41]. The uptake and accumulation of heavy metals in plants are determined by the soil properties, particularly its pH, granulometric composition and organic matter content [41]. Their mobility is also affected by the substances excreted by plant roots and soil microorganisms [42].

Bone waste contains no heavy metals [43], but these elements quite considerably restrict the use of sewage sludge ashes [26]. Many scientific centres are conducting studies on new technological solutions that can decrease the contents of toxic compounds in fertilizers which can be obtained from ashes and are within the acceptable level of heavy metals and other toxic elements [12,24-27]. In response to the need for phosphorus recycling, scientists from the Department of Advanced Material Technologies of the Wrocław Institute of Science and Technology have developed a technology of producing phosphorus biofertilizers from cheap renewable raw materials and PSM based on the previous studies on phosphorous solubilisation from secondary raw materials by the PSMs. It is expected that these innovative biofertilizers will not only be efficient in terms of crop-enhancing at a level comparable to conventional phosphorus fertilizers, but also ensure safety for consumers of field crops and for the environment. This study addresses the issue of the presence of cadmium and lead in the soil, crop plants, the accompanying weeds and post-harvest residues in the context of the application of biofertilizers originating from renewable raw materials. It was assumed that the biofertilizers would not elevate the level of these toxic elements in the soil and plant biomass compared with the commercial fertilizers, i.e. superphosphate.

2 Experimental procedures

2.1 Field experiments

In the years 2014-2016, four field experiments involving common wheat (*Triticum aestivum* ssp. *vulgare* Mac Key, winter or spring) were conducted, which tested five various biofertilizers produced on the basis of secondary raw materials, i.e. ash from the incineration of sewage sludge biomass from 3rd degree wastewater treatment and from poultry bones and phosphorus-solubilising bacteria of the *Bacillus megaterium* and *Acidithiobacillus ferrooxidans* strains (Table 1). The ash from sewage sludge was obtained from the municipal wastewater treatment plant ‘Łyna’ in Olsztyn and the bones from households or from the Firma Handlowo-Produkcyjna ‘Podolski’ (with registered office in Lutome, Wielkopolskie Voivodeship, Poland). The bacteria strains were obtained from the Polish Collection of Microorganisms at the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences in Wrocław (Poland). Phosphorus solubilisation involving *Bacillus megaterium* occurs due to the production of weak organic acids (citric, propionic) [10]. In the presence of sulphur, *Acidithiobacillus ferrooxidans* produce sulphuric acid, thus increasing the intensity of the digestion of the phosphorus raw material [10]. The procedure of obtaining fertilizer formulations were described elsewhere [44, 45]. The elemental composition of biofertilizers is provided in the Table 2.

Biofertilizers were tested and compared with no phosphorus fertilisation (No P – no phosphorus fertilization – a control object in each experiment) and superphosphate Fosdar™ 40 (SP). This all-purpose phosphorus fertilizer contains 40% P_2O_5, 10% CaO, 5% SO_3, and microelements (B, Co, Cu, Fe, Mn, Mo, and Zn) according to the commercial information provided on the label.

Table 2: Elemental composition of biofertilizers.

Element	**Unit**	$\mathbf{A_s}$*Bm*	$\mathbf{B_s}$*Bm*	$\mathbf{A_g}$*Af*	$\mathbf{AB_g}$*Af*	$\mathbf{AB_g}$*Bm*
P_2O_5	% mass.	0.406	0.595	21.2	17.2	13.5
N		0.255	0.350	0.530	3.190	3.14
K_2O		0.587	0.262	1.16	0.876	0.93
CaO		0.970	0.521	17.5	16.8	15.0
MgO		0.198	0.015	4.05	1.95	1.97
S (SO_3)		0.055 (0.137)	0.046 (0.115)	1.38 (3.45)	1.50 (3.75)	1.91 (4.78)
Na_2O		0.0663	0.0494	0.455	0.767	0.493
C		0.590	1.650	6.71	23.6	16.1
Fe	g kg^{-1}	1.679	0.022	33.0	15.1	14.4
Al		1.774	0.008	24.4	13.9	11.3
Zn		0.117	0.007	2.00	1.18	1.56
As	mg kg^{-1}	< LD[a]	< LD[a]	4.35	2.55	< LD[a]
Cd		0.274	0.01	0.43	0.24	0.84
Cr		5.94	0.218	135	60.0	94.7
Cu		55.0	0.433	880	398	444
Ni		2.45	0.212	57.7	25.1	44.3
Pb		10.4	1.04	21.6	11.2	14.5

[a] level of detection

In experiment I, phosphorus fertilizers were applied to the wheat crop at a P_2O_5 dose of 48 kg ha^{-1}, and in experiments II-IV, the P_2O_5 doses were applied at 3 different levels: 40, 60, and 80 kg ha^{-1}. Fertilizer suspensions were applied by coarse droplet spraying and solid fertilizers were sown. In experiment I, the plants were not protected against pests (–PP), in experiment II, two variants of plant protection were adopted: without the protection (–PP) and with full protection (+PP), and in experiments III and IV, full protection (+PP) was applied.

Wheat was cultivated annually in a soil adjusted to its species requirements (Table 3). Cd and Pb contents of the soil before the start of each experiment fell within a range considered to be natural, and did not exceed the acceptable standards for agricultural use (Table 4). Basic agricultural data for the experiments are provided in Table 5. The precipitation and thermal conditions during the growing seasons were different from the values typical of the region and were not favourable to the cultivated plants (Table 6). In experiments I-III, the growing seasons for spring and winter wheat proved to be very dry, while in experiment IV, winter wheat developed under wet season conditions. The water content of the soil may change the availability of metals to plants [54]. Heavy precipitation may also result in the rain-out of metals from the surface layers of the soil and in their accumulation in the depressions of the ground [55].

Experiments I, III, and IV were established in a randomised block design and experiment II in a parallel strip design. In each experiment, particular experimental variants were performed in 4 replications.

2.2 Soil and plant sampling

The soil was sampled using a core soil sampler from the 0-30 cm layer from each plot from evenly spaced points. A total of approx. 1 kg of soil was collected from an individual plot. The soil was dried at room temperature for several days, thoroughly mixed and sieved, and then separated into portions of approx. 300 g which were subjected to chemical analyses.

The mass of grains obtained during the combine harvesting from each plot was thoroughly mixed and samples weighing approx. 1 kg were taken. From each sample, approx. 200 g of grains were weighed, cleaned to get rid of impurities and weed seeds and then subjected to chemical analyses.

Just before the harvesting of wheat, plants were collected manually from two 1-metre-long rows of each plot and the spikes and roots were then cut off at a height corresponding to the height of mowing by a combine harvester. Straw (i.e. stems with leaves) was cut into pieces of approx. 10 cm in length. The samples were dried at room temperature for several days, and then a portion of 50 g was weighed from each sample and subjected to chemical analyses.

Table 3: Soil characteristic before the start of the experiments.

Properties		Experiment			
		I	II	III	IV
Soil type		Luvisols	Luvisols	Luvisols	Luvisols
Soil texture		sandy clay loam	sandy loam	sandy loam	sandy loam
pH in KCl		6.23	5.32	5.51	5.23
Total	C, g kg^{-1}	8.31	8.90	8.87	7.15
	N, g kg^{-1}	1.30	1.35	1.36	1.09
	P, mg kg^{-1}	574	566	433	548
	K, mg kg^{-1}	2979	2895	3210	3304
	Mg, mg kg^{-1}	2070	2007	2253	2195
	Cd, mg kg^{-1}				
	– average	0.025	0.292	0.309	0.133
	– max	0.365	0.827	1.083	0.553
	Pb, mg kg^{-1}				
	– average	4.855	7.959	7.059	2.297
	– max	13.198	25.099	22.895	18.180

Table 4: Reference values for cadmium and lead contents (mg kg^{-1}) of the soil and plants, according to various sources.

Content		Cd	Pb	Ref.
In soil				
Average in soils		0.01-3	0.1-200	[46]
Geochemical background for Poland		0.18 (0.03-1.00)	9.8 (5.0-59.0)	[47]
In surface level of Polish soils	– mineral soils	0.08-1.6	5-50	[48]
	– organic soils	0.01-0.1	18-85	
In surface level of World soils	– mineral soils	0.01-1.61	1.5-70	[48]
	– organic soils	0.19-2.2	1.5-176	
Permissible in Poland in arable land	– I[a]	2	100	[49]
	– II	3	250	
	– III	5	500	
Monitoring of arable soils	– Poland – min-max	0.02-67.98	4.5-856.6	[50]
	– average	0.59	22.5	
	– median	0.15	11.8	
	– region[b]	0.07-0.18	7.6-14.4	
In plant				
Average in plants		0.03-0.5	0.1-5.0	[46]
Sufficient or normal		0.05-0.2	5-10	[51]
Excessive or toxic		5-30	30-300	
Average in cereal grain		0.03-0.5	0.2-0.5	[48]
Permissible in Poland	– in wheat grain	0.20	0.20	[52]
	– in grain of other cereals	0.10	0.20	[52]
	– in feed materials of plant origin	1	10	[53]

[a] Sub-groups of arable land depending on soil compaction and pH, [b] range for 4 measurement points (villages) located geographically closest to the test site (Bałcyny)

Table 5: Basic agricultural data for the experiments.

Item	Experiment I	II	III	IV
Wheat cultivar	Trappe	Monsun	Julius	Julius
Previous crop	spring barley	cereal-legume mixture	winter rape	winter rape
Soil tillage system	plough tillage	plough tillage	plough tillage	plough tillage
Fertilization				
– K_2O, kg ha^{-1}	120	100	100	100
	potassium chloride	potassium chloride	potassium chloride	potassium chloride
– N, kg ha^{-1}	100	110	130	120
	ammonium sulphate	ammonium sulphate	ammonium sulphate	ammonium sulphate
Plant protection	–PP	–PP or +PP	+PP	+PP
– herbicides		MCPA	2.4-D + florasulam	2.4-D + florasulam
– fungicides		azoxystrobin + propiconazole	fenpropimorph + epoxiconazole + metrafenon	fenpropimorph + epoxiconazole + metrafenon
		cyproconazole	fluksapyroksad + piraklostrobina + epoxiconazole	fluksapyroksad + piraklostrobina + epoxiconazole
– insecticides		lambda-cyhalothrin	deltamethrin	deltamethrin
– growth regulators			trinexapac ethyl	trinexapac ethyl
Sowing date	25.04.2014	9.04.2015	2.10.2014	15.10.2015
Harvest date	11.08.2014	11.08.2015	5.08.2015	9.08.2016

Table 6: Precipitations and air temperatures during the period of study according to the Meteorological Station in Bałcyny.

Month	Precipitations, mm				Air temperatures, °C			
	2014	2014/ 2015	2015/ 2016	1981-2010	2014	2014/ 2015	2015/ 2016	1981-2010
IX		30.8	51.2	56.2		14.5	14.2	13.0
X		21.3	20.8	51.2		9.5	6.6	8.1
XI		21.2	80.8	46.1		4.4	5.1	2.8
XII		56.6	80.4	42.6		-0.6	3.8	-1.0
I		28.5	28.7	30.1		0.6	-3.8	-2.4
II		8.8	50.5	23.1		0.3	2.7	-1.6
III		46.0	20.5	30.7		4.6	3.6	1.8
IV	26.1	23.4	33.1	29.8	9.5	7.2	8.8	7.7
V	34.9	25.4	70.8	62.3	13.3	12.1	14.9	13.2
VI	72.2	43.0	66.3	72.9	14.8	15.7	18.0	15.8
VII	20.4	71.0	138.6	81.2	21.0	18.0	18.5	18.3
VIII	59.2	13.0	71.9	70.6	17.9	21.3	17.6	17.7
Total/average for IX-VIII		389.0	713.6	596.8		9.0	9.2	7.8
Total/average for IV-VIII	212.8	175.8	380.7	316.8	15.3	14.9	15.6	14.5

Weeds were sampled from a randomly determined area of 0.25 m^2 (0.5 m x 0.5 m frame) from each plot prior to harvesting the test plant. After the separation of the roots, the above-ground biomass of weeds was dried at room temperature for several days and the samples in their entirety (different mass) were then forwarded for chemical analyses.

Post-harvest residues were sampled from an area of 0.40 m^2 and from a depth of 0.30 m. After washing the collected mass on sieves and removing earthy portions, the roots, stubble and weed residues were separated and dried at room temperature for several days and were then forwarded for chemical analyses.

2.3 Analyses

The Cd and Pb contents of the soil and plant material were determined using a inductively coupled plasma-optical emission spectrometer (ICP-OES). The appropriate mass (0.5 g) of biological sample materials (plant and soil samples) was digested in Teflon vessels (microwave oven Milestone MLS-1200) with 5 mL of concentrated 65 mg kg^{-1} HNO_3 suprapur grade from Merck in the case of plant samples, and with 10 mL of aqua regia in the case of soil samples. After mineralization, all samples were diluted to 50 mL. The ICP-OES with an pneumatic nebulizer with axial view (iCAP Duo Thermo Scientific, USA) was used to determine the concentration of elements in all digested and diluted biological samples. Determination of content of toxic metal ions was carried out with all the principles of measurement traceability. Determination of content of toxic metal ions was carried out with all the principles of measurement traceability. What is more, certificated reference materials were used to check the quality and metrological traceability. The detection levels for Cd and Pb for the soil material were 0.01 and 0.15 mg kg^{-1}, respectively, and for the plant material they were 0.001 and 0.01 mg kg^{-1}, respectively.

2.4 Calculations

The results were processed using an analysis of variance, or the alternative Kruskal-Wallis test if the analysis of variance assumptions were not met. The normality of variable distribution was checked using the Shapiro–Wilk W-test and the homogeneity of variance was checked using Levene's test. The differences between objects were evaluated using Duncan's test or a multiple comparison test. In statistical calculations, the samples with element contents above the detection level (LD) were replaced by values equal to the LD. For each experiment, the median and the maximum value were determined. The relationships between the analysed variables were expressed using simple correlation coefficients determined based on the data from all experiments. In all cases, p=0.05 was assumed. The calculations were performed using *Statistica* 12.0 software.

Ethical approval: The conducted research is not related to either human or animals use.

3 Results and Discussion

3.1 Cadmium (Cd)

The Cd content of the soil in the experiments (Table 7) fell within the range regarded as natural under Polish conditions and did not exceed allowable levels for arable land (Table 4). The average values in the experiments did not differ from the average national and regional values and even the highest values determined (0.642 mg kg^{-1} in 2016) fell within the range of the geochemical background (Tables 4 and 7).

No effect of the fertilisation factor on the Cd content of the topsoil layer was found in any of the experiments (Table 7). Biofertilizers from renewable raw materials, similar to superphosphate, irrespective of the P_2O_5 dose, did not significantly change the content of this element as compared to the control. In experiments I and IV, no significant change occurred in relation to the baseline state of the soil (prior to the start of the experiments) and in experiments II and III, the level of Cd in the soil in relation to the baseline state decreased with no relation to the phosphorus fertilisation applied. The application of the protection of plants in comparison with the lack of plant protection treatments (experiment II) did not change the Cd content of the soil either. The medians in the experiments were usually at a level below the detection level (an exception was experiment IV).

Previous studies indicated that feeding the plants with either mineral or organic fertilizers of primary or secondary origin, which are rich in Cd, may result in a significant increase in the content of this element in the soil [56,57], even during one growing season [56] and contribute to an increase in its more or less intense uptake and accumulation in plants [57-60]. Many reports have been published, according to which the secondary sources of nutrients, such as hard coal ash [60], sewage sludge and a mixture with brown coal and ashes [61], sheep manure

Table 7: Cadmium content in soil and plant biomass from wheat field, mg kg^{-1} (dry matter).

Exp.	Phosphorus fertilizer	P_2O_5, kg ha^{-1}	Plant protection	Soil	Wheat grain	Straw	Weeds (above-ground biomass)	Post-harvest residues
I	No P	0	–PP	0.042	0.092	0.070	0.164	0.127
	SP	48		< LD*	0.089	0.051	0.129	0.156
	A_s*Bm*	48		0.035	0.095	0.051	0.140	0.133
	B_s*Bm*	48		0.034	0.094	0.065	0.134	0.151
	average			0.029	0.093	0.059	0.142	0.142
	median			<LD*	0.083	0.055	0.135	0.138
	max			0.159	0.138	0.101	0.269	0.197
II	No P	0	–PP	< LD	0.040	0.192	0.312	0.150
	SP	40		0.127	0.037	0.186	0.310	0.158
		60		< LD*	0.047	0.188	0.312	0.154
		80		0.106	0.039	0.199	0.358	0.140
	A_s*Bm*	40		0.035	0.044	0.162	0.345	0.164
		60		0.063	0.041	0.130	0.390	0.139
		80		< LD*	0.040	0.121	0.335	0.142
			average	0.052	0.041	0.168	0.340[a]	0.146
	No P	0	+PP	< LD*	0.034	0.132	0.279	0.135
	SP	40		0.018	0.040	0.132	0.208	0.146
		60		0.115	0.038	0.140	0.136	0.119
		80		< LD*	0.030	0.123	0.158	0.144
	A_s*Bm*	40		0.097	0.043	0.155	0.228	0.141
		60		0.031	0.036	0.099	0.314	0.127
		80		0.126	0.040	0.193	0.271	0.142
			average	0.058	0.037	0.139	0.242[b]	0.134
	average			0.055↓	0.039	0.154	0.283	0.143
	median			< LD*	0.041	0.155	0.544	0.155
	max			0.360	0.052	0.353	0.287	0.209
III	No P	0	+PP	< LD*	0.015	0.030	1.659	0.212
	SP	40		0.018	0.009	0.034	1.733	0.166
		60		0.050	0.008	0.039	1.720	0.158
		80		0.039	0.014	0.028	1.673	0.204
	A_g*Af*	40		0.092	0.012	0.029	1.651	0.287
		60		< LD*	0.008	0.036	1.168	0.190
		80		< LD*	0.020	0.033	1.812	0.210
	AB_g*Af*	40		< LD*	0.016	0.033	1.579	0.255
		60		< LD*	0.009	0.035	1.471	0.153
		80		0.041	0.010	0.023	1.631	0.175
	average			0.029↓	0.012	0.032	1.610	0.201
	median			< LD*	0.011	0.032	1.690	0.179
	max			0.340	0.030	0.052	2.606	0.558
IV	No P	0	+PP	0.045	0.008	0.049	0.325	no data
	SP	40		0.250	0.015	0.046	0.314	
		60		0.339	0.024	0.049	0.341	
		80		0.307	0.023	0.039	0.289	
	AB_g*Bm*	40		0.229	0.016	0.042	0.348	
		60		< LD*	0.011	0.049	0.317	
		80		0.293	0.014	0.062	0.369	
	average			0.210	0.016	0.048	0.329	
	median			0.143	0.016	0.045	0.311	
	max			0.642	0.039	0.092	0.539	

* – level of detection; ↓ - significant decrease in relation to the baseline state of the soil; a, b – different letters indicate significant differences between values

compost [62], poultry manure [63], did not contribute to increase the Cd content of the soil [61-63] or of the crop plant growing on this soil [60,62,63].

Wierzbowska et al. [64], while using ash from the incineration of sewage sludge (wastewater treatment plant Łyna, Olsztyn) as a phosphorus fertilizer in the cultivation of *Virgina fanpetals*, noted no differences in its effect on the Cd content of the soil, but the Cd content of plants increased.

In our experiments, the applied biofertilizers (similar to superphosphate) irrespective of the dose of the pure component, did not significantly change the Cd content of wheat grains or straw, in the accompanying weeds or in post-harvest residues.

In the plant material, Cd was usually present in amounts regarded as natural, and none of the experiments approached a level indicating an excessive or toxic content of Cd (Tables 4 and 7).

In the grains, Cd was usually present in amounts far below the acceptability threshold for wheat, which is indicated not only by the low median values but also by the maximum values noted in experiments II-IV. The relatively highest amounts of Cd in the grains were determined in 2014 (experiment I); however, even in this case, the maximum value (0.138 mg kg^{-1}) was below the acceptability threshold.

Greater contents of Cd were usually found in wheat straw than in the grains, which is consistent with the conclusions of previously reported studies [65,66]. An exception was observed in experiment I in which the content of Cd in the straw was lower than that in the grains. This may be due to the relatively low Cd content of the soil and the highest pH value of the soil. It is known that under conditions of a higher pH value, the uptake of Cd by plants is blocked [34,67]. This hypothesis was also confirmed by the fact that the relatively lowest Cd content of the weed biomass was observed in experiment I. The relatively highest Cd content of the straw was determined in experiment II. This level, however, does not disqualify it as a feed stuff (Table 4). A higher content in straw could have been associated with the more intense uptake of this element by plants on the soil with a lower pH value (in relation to experiment I). In this experiment, a higher Cd content as compared to that in experiment I was also observed in the weeds.

Plant protection against pests (experiment II) had no effect on the Cd content of wheat grains and straw. Similarly, Wołejko et al. [42] found protection treatments with fungicide and herbicide conducted during the growing season did not result in exceeding the significant concentration of Cd in grain or leaves of wheat growing on soil enriched with granular sludge.

The Cd content of weeds in experiment II was differentiated by the protection of plants; specifically, its effect on the species structure of the weeds. In the biomass of weeds from the unprotected wheat field, *Chenopodium album* and *Fallopia convolvulus*, which accumulated more Cd than other species, were predominant (unpublished data). The limitation of their share in the community thanks to the herbicide (protected wheat field) contributed to a decrease in the Cd content of the overall mass of weeds and was manifested by a significant difference in relation to the un-weeded wheat fields. The ability of these species to accumulate Cd was also documented by Stupnicka-Rodzynkiewicz et al. [68,69], who also indicated the differentiation of the Cd content depending on the soil properties. Relatively high Cd contents of the weeds were observed in experiment III: the average values and the median exceeded 1 mg kg^{-1}, and the maximum value amounted to 2.606 mg kg^{-1}. In the community concerned, the predominant species was *Viola arvensis*, which proved to be quite resistant to the applied herbicide and accumulated large amounts of Cd in its mass. It appears probable that the accumulation of Cd by the weeds restricted the uptake and accumulation of Cd in wheat plants [70]. *Violaceae* exhibit high tolerance to heavy metals [71]; the significant ability of *Viola arvensis* to accumulate Cd was reported by Stupnicka-Rodzynkiewicz et al. [68,69].

The Cd content of post-harvest residues was at a similar level in experiments I and II, and slightly elevated in experiment III. The level of Cd in their mass is determined by the ratio between the residues of the roots, stubble and weeds. Cd has a high bioaccumulation coefficient [42], is relatively intensely taken up by plants and is easily transported by the root system to all organs, but usually the roots of cereals accumulate more Cd than the stubble [72,73], which was confirmed by our own study. In turn, the accumulation in weed residues depends on the weed species being part of the mass of post-harvest residues as well as of their organs [74]. The percentage of *V. arvensis* in post-harvest residues in experiment III appears to be responsible for the slightly elevated Cd level in their overall biomass.

The Cd content of the soil demonstrated no significant relationship with its accumulation in plants (Table 9), while an increase in Cd accumulated in weeds and post-harvest residues resulted in less of it being found in wheat grains and straw. However, the Cd contents of weeds and post-harvest residues were convergent. Ociepa et al. [61],

Table 8: Lead content in soil and plant biomass from wheat field, mg kg^{-1} (dry matter).

Exp.	Phosphorus fertilizer	P_2O_5, kg ha^{-1}	Plant protection	Soil	Wheat grain	Straw	Weeds (above-ground biomass)	Post-harvest residues
I	No P	0	–PP	2.121	0.018	0.159	0.136	2.050
	SP	48		7.714	0.012	0.192	0.114	2.074
	A_s*Bm*	48		7.326	0.017	0.122	0.132	2.210
	B_s*Bm*	48		6.999	0.013	0.047	0.238	2.613
	average			6.040	0.015	0.130	0.155	2.237
	median			5.955	0.012	0.125	0.134	2.129
	max			14.484	0.035	0.404	0.481	3.512
II	No P	0	–PP	< LD[*]	0.025	2.749	0.672	2.197
	SP	40		0.902	0.067	1.708	0.653	1.737
		60		2.103	0.020	2.510	0.490	1.900
		80		< LD	0.041	2.663	0.796	2.073
	A_s*Bm*	40		4.065	0.019	1.461	0.503	1.934
		60		< LD[*]	0.082	0.996	0.927	2.620
		80		2.438	0.017	1.032	0.987	1.651
			average	1.423	0.039	1.874	0.717^b	1.981^b
	No P	0	+PP	2.104	0.019	1.692	1.072	1.698
	SP	40		0.150	0.052	2.366	0.785	2.447
		60		2.533	0.046	1.430	0.738	1.920
		80		2.456	0.037	1.199	0.846	2.351
	A_s*Bm*	40		< LD[*]	0.055	2.341	1.050	2.459
		60		0.854	0.014	0.716	1.210	2.106
		80		3.223	0.066	2.222	1.142	2.281
			average	1.639	0.041	1.709	1.017^a	2.266^a
	average			1.523↓	0.040	1.797	0.839	2.090
	median			< LD[*]	0.023	1.867	0.812	2.052
	max			11.895	0.164	5.520	2.151	3.228
III	No P	0	+PP	1.682	0.062	0.219	0.539	3.282
	SP	40		1.765	0.088	0.241	0.751	3.043
		60		0.684	0.040	0.193	0.629	3.048
		80		2.648	0.106	0.299	0.639	3.410
	A_g*Af*	40		3.000	0.056	0.131	0.858	3.410
		60		3.459	0.109	0.347	0.465	3.649
		80		1.748	0.069	0.163	0.543	3.026
	AB_g*Af*	40		0.630	0.104	0.237	0.749	2.605
		60		2.211	0.076	0.258	0.605	4.165
		80		2.128	0.067	0.168	0.663	3.387
	average			1.996↓	0.078	0.226	0.644	3.303
	median			1.607	0.041	0.190	0.585	3.352
	max			8.136	0.194	0.666	1.375	5.450
IV	No P	0	+PP	1.750	0.023	0.095	0.100	no data
	SP	40		< LD[*]	< LD[*]	0.020	0.108	
		60		3.208	0.026	0.108	0.157	
		80		0.150	< LD[*]	0.053	0.488	
	AB_g*Bm*	40		0.896	0.030	0.025	0.284	
		60		2.547	0.017	0.088	0.351	
		80		3.020	0.014	0.015	0.146	
	average			1.674	0.019	0.058	0.233	
	median			< LD[*]	< LD[*]	< LD[*]	0.061	
	max			9.272	0.092	0.323	1.749	

[*] – level of detection; ↓ - significant decrease in relation to the baseline state of the soil; a, b – different letters indicate significant differences between values

Table 9: Simple correlation coefficients calculated between the cadmium and lead content in soil and plant biomass.

Content in	Soil	Grain	Straw	Weeds
Cd				
Grain	n.s.			
Straw	n.s.	n.s.		
Weeds	n.s.	−0.68	−0.56	
Post-harvest residues	n.s.	−0.29	−0.21	0.43
Pb				
Grain	n.s.			
Straw	−0.28	n.s.		
Weeds	−0.23	n.s.	0.32	
Post-harvest residues	n.s.	0.32	−0.31	n.s.

n.s. – no significant at p=0.05

concluded that the heavy metal content of the aboveground parts of prairie cordgrass (*Spartina pectinata*) were not dependent on the total metal content of the soil. Hirzel et al. [73] reported that Cd accumulation in grains, straw, and roots of durum wheat was significantly affected by increasing Cd rates, and Wang et al. [75] reported that Cd content of wheat grains was positively correlated with its content in the roots. In a study by Rogóż [74], the Cd content of plants of certain weed species exhibited dependence on the content of this element in the soil and decreased with an increase in the pH value above 5.5.

3.2 Lead (Pb)

In the experiments, Pb (similarly to Cd) was found in soils (Table 8) in concentration regarded as natural in Poland and did not exceed levels acceptable for agricultural use (Table 4). Referring to the geochemical background for Poland, the Pb content of soils under experimental conditions must be regarded as low. Only the maximum values determined in particular experiments (the highest value of 14.484 mg kg^{-1} in 2014) were at a level of national (average and median) and regional statistics (Table 4).

In none of the experiments the variants of phosphorus fertilisation changed the Pb content of the topsoil layer; the use of phosphorus in relation to the omission of fertilisation or the type and dose of fertilizer did not differentiate this characteristic (Table 8). In experiment I, only a trend toward increasing the Pb content of the soil after the application of superphosphate and biofertilizers from ash and bones was observed, but no statistical confirmation given and, considering the context of results of the remaining experiments, this should not raise concerns.

Similar to Cd, no significant change occurred in experiments I and IV in relation to the baseline state of the soil (prior to the start of the experiments) and in experiments II and III, the level of Pb in the soil in relation to the baseline state decreased with no relation to the phosphorus fertilisation applied. The protection of plants in comparison with the absence (experiment II) also did not change the Pb content of the soil.

Atafar et al. [56] reported a dramatic increase in Pb content of the soil during the growing season (from the state before fertilisation to the state after harvesting the crop plant) under the influence of mineral fertilizers rich in Pb. Numerous authors have also noted an increase in the total content of Pb or its forms in the soil as a result of the use of fertilizers from secondary sources [57,62,76,77]; however, opinions on the lack of effect of fertilizers on the level of Pb in soil have also been raised [63,64]. Moreover, results of studies into the effects of fertilizers on Pb content of plants also provide divergent information [57,60,62,63,78,79].

It follows from the studies carried out so far that the uptake of Pb from the soil by plant roots is low, but its bioavailability to plants can be changed due to a change in the pH value, organic compound content, iron oxides and the amount of phosphorus which has an antagonistic effect on the uptake of Pb [67]. Guo et al. [72] decreased, even in contaminated soil, the content of available forms of Cd and Pb as well as the content of these elements in wheat grains through the use of phosphorus fertilizers (sepiolite, single superphosphate, triple super phosphate, calcium magnesium phosphate). In general, the cultivation of plants on soils whose Pb content does not exceed 300 mg kg^{-1} is regarded as safe [38].

In our study, biofertilizers from secondary raw materials (just like superphosphate) irrespective of the dose of a pure component, did not significantly change the Pb contents of wheat grains or straw, the weeds growing in a corn field or in the post-harvest plant residues in any of the experiments (Table 8).

In the tested plant biomass, Pb was usually found in concentration accepted as natural, satisfactory or normal. The contents were far from levels regarded as excessive or toxic to plants in all of the experiments (Table 4).

In the grains, Pb was usually found in small amounts far from the acceptability threshold (Table 4), which is demonstrated by the values of medians in the experiments (incidentally, higher contents approaching the threshold value were also determined in experiments II and III).

This should not, however, raise concerns since as elevated values were also noted in control samples and they should be considered in terms of an experimental error.

The protection of plants in comparison with an absence (experiment II) did not change the Pb content of wheat grains. Wołejko et al. [42] reported that the concentration of Pb in wheat grain protected by MCPA and 2,4-D with thiophanate-methyl and azoxystrobin was higher than the maximum levels of 0.20 mg kg^{-1} D.M.

Generally, in our study, Pb accumulated in greater amounts in wheat straw than in its grains, which is consistent with other reports [38]. The relatively highest level of Pb content of the straw was noted in experiment II. Significantly, the average values were approx. 10-fold higher than those in straw from the other experiments (exceeding 1 mg kg^{-1}) and the maximum content was determined to be 5.520 mg kg^{-1}. This content does not exceed the level of acceptable content in feed materials of plant origin (Table 4). This could be associated with the pH value of the soil, since in more acidic soils the uptake of this element by plants increases [38]. In experiment III (a similar pH level), the Pb content of post-harvest residues suggests that the metal was retained in the roots to a greater extent than it was retained in straw. In experiment IV, the uptake and distribution of Pb in plants could have been determined by the relatively lowest Pb content of the soil. In experiment II, the protection of plants in comparison with the absence of protection did not change the straw Pb content.

The overall Pb content of the weed biomass in particular experiments was primarily determined by the species structure of the community and by the species' suitability for the accumulation of this element. This is particularly evident in experiment II in which the protection of plants, while reducing the percentage of dominant weed species (*Chenopodium album* and *Fallopia convolvulus*) accumulating smaller amounts of Pb (unlike for Cd), contributed to an increase in the Pb content of the weed mass due to increasing the proportion of other species accumulating greater amounts of Pb. *Viola arvensis* (primarily in experiment III) proved to be a poorer accumulator of Pb than of Cd, which supports the results of Stupnicka-Rodzynkiewicz et al. [68].

The mobility of Pb in plants is very much limited and most of this element is accumulated in the roots [38,57,67], hence the Pb content of post-harvest residues was mainly determined by the percentage of Pb within the entire mass as well as by its percentage and the species structure of the weed remnants. In experiment II, a higher percentage of weeds in the mass of residues from the unprotected wheat field determined the reduction in Pb content of these residues in relation to the residues originating from the weeded wheat field, in whose residues the roots had a relatively higher percentage.

The lead content of the soil demonstrated an inverse correlation with the content in straw and in the weeds, with no relation to the grains and residues (Table 9). Other studies confirm that the seeds are the organs which are most protected by plants against the excessive accumulation of toxic elements [38].

Findings in our study are in alignment with the conclusion drawn by previously reported studies [80,81] that explains the reasonable use of fertilizers from renewable raw materials, with low toxic element contents, are not considered to pose a hazard to the soil or to food chains.

4 Conclusion

Phosphorus biofertilizers from sewage sludge ash and from bones, microbiologically activated and applied in amounts of P_2O_5 up to 80 kg ha^{-1}, did not change the Cd and Pb contents of the soil, grain or straw of the tested plants, or the mass of accompanying weeds or of post-harvest residues, similar to superphosphate. Both Cd and Pb in the soil and plant biomass occurred in amounts regarded as natural and are within the acceptable standards.

Reasonably applied biofertilizers from renewable raw materials, with low toxic element contents, are not believed to pose a hazard to the soil or plants and may be used as a substitute for conventional, phosphorite-based fertilizers.

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