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# Phosphorus Biofertilizers from Ash and Bones—Agronomic Evaluation of Functional Properties

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## Abstract

Renewable raw materials could be a valuable source of phosphorus for plants. The bioavailability of this element can be enhanced by phosphorus-solubilizing bacteria. Suspension biofertilizers have been produced from sewage sludge ash and animal bones and enriched with the bacteria *Bacillus megaterium*. The functional properties of these preparations were compared in field experiments (northeast Poland, 2014, four replications) on spring wheat (*Triticum aestivum* ssp. *vulgare* Mac Key) to conventional fertilizers (superphosphate, phosphorite), ash-water solution (without microorganisms) and a control treatment without P fertilization. The soil type and cultivation regime were adjusted to the requirements of spring wheat in line with good agricultural practice. The effects of biofertilizers on the following were investigated: wheat yield, ear density, number of grains in the ear, the weight of 1000 grains, harvest index, weed infestation, the weight and structure of crop residues, and the pH of soil. Phosphorus biofertilizers from ash and bones equalled commercial fertilizers in terms of their crop-enhancing efficiency. Biofertilizer from ash, and ash diluted with water reduced weed infestation of the growing crop. Biofertilizer from bones resulted in a greater weight of wheat crop residues. Biofertilizers did not change the pH of soil. It is expected that the production of biofertilizers containing recycled phosphorus will be an alternative to its non-renewable resources and will also contribute to effective waste management.

**Keywords:** animal bones, *Bacillus megaterium*, post-harvest residues, sewage sludge ash, soil pH, spring wheat, weed infestation, yield

## 1. Introduction

Phosphorus is essential for the proper development of plants. In addition to nitrogen and potassium, it is the main component limiting plant growth. Plants need phosphorus in relatively large quantities, and its role cannot be played by any other element (Sultenfuss & Doyle, 1999; Mohammadi, 2012). It performs structural (phospholipids and other phosphorus compounds), reserve (phytin) and regulatory functions (regulation of gene expression). It participates in cell metabolism, both directly (e.g. sugar phosphates) and indirectly (e.g. regulation of enzyme activity through phosphorylation and dephosphorylation). It is involved in processes of transferring genetic information (a component of nucleic acids) and in energy storage (a component of ATP and PPi) (Sultenfuss & Doyle, 1999). The supply of phosphorus determines the development of roots, the condition of the stem, the formation of flowers and fruits, the rate of plant maturation, the efficiency and quality of crops, $N_2$ fixation in legumes, and the resistance to both biotic and abiotic environmental factors (Mohammadi, 2012; Grzebisz, Potarzycki, & Biber, 2003). The shortage of assimilable forms of this component in the environment of plants reduces the yield quantity and biological quality (Grzebisz et al., 2003).

The distinct yield-enhancing action of phosphorus is noticeable in cereals. It appears for the entire growing season, and the critical periods of demand for this element fall into the stages of tillering and grain filling (Grzebisz et al., 2003). In order to produce 1 dt of grain yield with the corresponding straw yield, cereals need from 0.42 to 0.54 kg of phosphorus (Jadczyszyn, 2013). The phosphorus content in mineral soils ranges from 0.009% to 0.15% (Tujaka, 2007). The natural richness of arable soils in this component does not fully provide for the nutritional requirements of plants, especially given that only a small part of the soil pool of this element occurs in a form available to plants (Mohammadi, 2012). Those resources are systematically depleted through the annual removal with the crop as well as leaching to water (Sapek, 2014).

To ensure the continuity of agricultural production and to prevent a decrease in the fertility of soil due to its depletion of nutrients, constant and rational replenishment of nutrients is required (Weigand, Bertau, Hübner, Bohndick, & Bruckert, 2013). A small portion of nutrients remains in the soil with crop plants residues, but the bulk of nutrients reaches the soil with mineral and organic fertilizers. As both agricultural production and consumption increase, the principal source of arable soil enrichment with phosphorus is through phosphorus fertilizers (Sapek, 2014). Production of mineral phosphorus fertilizers is based almost entirely on phosphate rocks (Van Kauwenbergh, Stewart, & Mikkelsen, 2013). Recent estimates of global geological phosphorite resources are over 300 billion tonnes, and their reserves are 67 billion tonnes (United States Geological Survey [USGS], 2015). This has given rise to the commonly held belief that exhaustion of accessible phosphorite deposits is impending, and that would result in the collapse of agricultural production (Korzeniowska & Stanisławska-Glubiak, 2011). Admittedly, natural phosphorites are a non-renewable resource. It should not be expected that the global demand for phosphorus fertilizers will decrease in the future (Tenkorang & Lowenberg-DeBoer, 2009). The growing human population will lead to higher demand for food. The increase in the demand for fertilizers will also result from an increase in the global production of biofuels (Hein & Leemans, 2012). However, at each stage of the man-forced flow of phosphorus from mines, through the field and the table, to the sea, a huge wastage of this component occurs. Only approx. 15% of the mined phosphorus reaches the products we consume (Rosemarin & Jensen, 2013). In this context, recycling of phosphorus from industrial, municipal and animal waste is becoming increasingly important (Weigand et al., 2013; Bierman, Rosen, Bloom, & Nater, 1995; Alotaibi, Schoenau, & Fonstad, 2013), especially that it reponds to the European strategy calling for sustainable phosphorus use (Schröder, Cordell, Smit, & Rosemarin, 2010).

Examples of waste with an increased content of phosphorus include sludge from municipal waste treatment and animal bones (Saeid, Labuda, Chojnacka, & Górecki, 2012). The biomass of sewage sludge may contain organic and inorganic contaminants as well as harmful pathogens (Severin et al., 2014). Constraints on the direct application of ash from the incineration of sludge as a fertilizer are associated with the presence of toxic metals (Bierman at al., 1995). The use of bones for fertilizing purposes is currently the only way to recycle this burdensome waste, given the EU ban on the use of meat and bone meal in livestock fodder (European Union, 1991). Unprocessed phosphorus raw materials are characterized by the low solubility of phosphorus compounds they contain (Saeid et al., 2012). The transition of phosphorus from a soluble into an insoluble form is the key to both the production of efficient fertilizers and improved bioavailability of its compounds from the soil pool of nutrients.

The phosphorus unavailable to plants may be released from the soil resources by certain microorganisms that secrete specific acids and enzymes (Salimpour, Khavazi, Nadian, Besharati, & Miransari, 2010; Sharma, Sayyed, Trivedi, & Gobi, 2013). Owing to these microbial abilities, as well as other mechanisms, both direct (e.g. production of plant hormones, acceleration of mineralization processes) and indirect (e.g. control of morbid factors through the release of antibiotics and antifungal metabolites), phosphate solubilizing microorganisms (PSMs) may serve as plant growth promoting microorganisms (PGPMs) (Sharma et al., 2013). The relationship between PSMs and the plant is symbiotic, since the former metabolize the organic compounds (mainly sugars) released by plant roots. PSMs may interact with other microorganisms, e.g. mycorrhizal fungi, and produce a synergistic effect on the growth and productivity of plants (Mohammadi, 2012). PSMs may also be useful in the production of phosphorus biofertilizers (Labuda, Saeid, Chojnacka, & Górecki, 2012). *Bacillus megaterium* is indicated as the most efficient PSM (El-Komy, 2003). This strain is also considered to be one of the PGPMs commonly found in soils (Ali, Sabri, Ljung, & Hasnain, 2009). It has recently been demonstrated that these bacteria effectively solubilize phosphorus from waste substances, e.g. from bones (Labuda et al., 2012). This creates a possibility for the inclusion of microbiological methods in the recycling of phosphorus from waste for fertilizing purposes.

At the Department of Advanced Material Technologies of the Wrocław University of Technology (Poland), test batches of phosphorus biofertilizers have been produced on the basis of ash from the incineration of sewage sludge and animal bones, and the phosphorus solubilizing bacteria *Bacillus megaterium*. The performance of these biofertilizers was tested in field experiments by a research team from the Department of Agroecosystems of the University of Warmia and Mazury in Olsztyn (Poland).

The effects of biofertilizers on the efficiency of the test plant, weed infestation of the growing crop, the weight of crop residues and the pH of the soil were compared to those achieved with traditional phosphorus fertilizers and on a plot not fertilized with phosphorus. A research hypothesis was that the performance characteristics of the new generation biofertilizers, as measured in terms of their effect on the above parameters, would equal those of commercial fertilizers.

## 2. Materials and Methods

### *2.1 Fertilizers and Biofertilizers*

In the field experiment, two phosphorus biofertilizers were tested: a biofertilizer from ash from the incineration of biomass of sewage sludge from the 3rd degree wastewater treatment, and a biofertilizer from animal (poultry) bones. They were compared to commercial fertilizers: Fosdar$^{\text{TM}}$ 40 superphosphate and phosphorite Syria.

The biofertilizers were produced at the Department of Advanced Material Technologies of the Wrocław University of Technology. They are products of the microbial decomposition of ash or animal bones, and have the form of suspension which, in addition to $P_2O_5$ originating from the decomposition of raw materials, also contains proliferated *Bacillus megaterium* bacteria. The ash from sewage sludge was obtained from the Municipal Wastewater Treatment Plant in Olsztyn, and the bones originated from households.

Fosdar$^{\text{TM}}$ 40 Superphosphate (made at the Gdańsk Phosphorus Fertilizer Plant 'Fosfory' Sp. z o.o.) was purchased on the market. Phosphorite Syria (bought at the Lubena S.A. plant in Luboń) was supplied by the Institute of New Chemical Synthesis in Puławy. The chemical composition of the fertilizers and biofertilizers used is presented in Table 1.

Table 1. Elemental composition of applied fertilizers and bioferilizers

| Element | Unit | Superphosphate* | Phosphoryte** | Biofertilizer from ash*** | Biofertilizer from bones*** |
|---|---|---|---|---|---|
| N | % | | | 0.255 | 0.350 |
| $P_2O_5$ | | 40.0 | 27.8 | 0.406 | 0.595 |
| $K_2O$ | | | | 0.587 | 0.262 |
| CaO | | 10.0 | 49.4 | 0.970 | 0.521 |
| MgO | | | | 0.198 | 0.015 |
| S ($SO_3$) | | 2.00 (5.00) | 0.404 (1.01) | 0.055 (0.137) | 0.046 (0.115) |
| $Na_2O$ | | | | 0.0663 | 0.0494 |
| $SiO_2$ | | | 5.96 | | |
| C | | | | 0.590 | 1.650 |
| Fe | mg/kg | + | 902.2 | 1679 | 21.5 |
| Al | | | 794.1 | 1774 | 8.36 |
| As | | | 0.447 | < LD | < LD |
| Cd | | | 7.39 | 0.274 | 0.00965 |
| Cr | | | 115 | 5.94 | 0.218 |
| Cu | | + | | 55.0 | 0.433 |
| Ni | | | | 2.45 | 0.212 |
| Pb | | | 3.23 | 10.4 | 1.04 |
| Zn | | + | | 117 | 6.85 |
| Hg | | | 0.023 | | |
| B, Co, Mn, Mo | | + | | | |

*Note*. *: according to label, **: according to the New Chemical Syntheses Institute in Puławy, ***: according to the Department of Advanced Material Technologies of the Wrocław University of Technology; +: trace presence; < LD: below the level of detection.

### *2.2 Experimental Designs and Agronomic Management*

A controlled field experiment was established in the spring of 2014, on fields of the Production and Experimental Plant "Bałcyny" Sp. z o.o. in Bałcyny, near Ostróda (Warmińsko-Mazurskie Province, Poland, 53.60°N, 19.85°E). Spring common wheat (*Triticum aestivum* ssp. *vulgare* Mac Key) of the cultivar Trappe was a test plant.

The following variants with phosphorus fertilizers were compared in the experiment:

(1) control–without phosphorus fertilization;

(2) superphosphate;

(3) phosphorite;

(4) ash-water solution;

(5) biofertilizer from ash;

(6) biofertilizer from bones.

The field experiment was set in a completely random design with four replications. The size of a single experimental plot was 20 $m^2$ (2 m × 10 m), and its area to be harvested was 15 $m^2$. Spring barley was grown as a preceding crop to spring wheat. The tillage was performed under the ploughing system.

A dose of $P_2O_5$ at 48 kg/ha was used on all experimental plots (except for the control), assuming that it would ensure a yield at a level of 4 t/ha. Fertilization with nitrogen and potassium was the same throughout the experiment, at the following amounts per 1 ha: 100 kg of N (ammonium nitrate 34%), and 120 kg of $K_2O$ (potassium salt 60%). The total dose of potassium was applied pre-sowing, and that of nitrogen was divided into two doses: 50% was introduced as pre-sowing fertilizer, and another 50% for top dressing, at the stem elongation stage.

Whole doses of solid phosphorus fertilizers (superphosphate and phosphorite) were applied pre-sowing along with the potassium fertilizers and the first dose of nitrogen fertilizers, and then mixed with the soil using a combined cultivator and a medium harrow. To monitor the course of adaptation of the *Bacillus megaterium* strain introduced with the biofertilizers, doses of suspension biofertilizers from ash and bones as well as the ash water solution were divided into 3 equal portions and applied on three dates determined according to the wheat development stages and meteorological conditions:

- Pre-sowing (April 25)–by large-drop spraying of the soil, and then mixed with the soil using a harrow;
- At the 3 leaves unfolded stage for wheat (May 15)–to the soil, into the wheat interrows (at a depth of approx. 5 cm);
- At the beginning of tillering (June 5)–to the soil, into the interrows.

Wheat was sown on April 25, at a depth of 3-4 cm, in a spacing of 15 cm. The delayed sowing was routinely compensated for by increasing the amount of seeds to 200 kg/ha. No chemical protection of wheat against pests was provided so as to observe the natural defence efficiency of wheat against diseases and pests, as well as its competitive potential against weeds, when supported only by the fertilizers. Combine harvesting was performed on August 11.

### *2.3 Soil and Meteorological Conditions*

Wheat was cultivated on grey-brown podzolic soil developed from medium loam overlying light loam. The soil contained 7.09-9.46 g/kg C and 1.07-1.56 g/kg N, and 394.6-704.4 mg/kg P, 2202-3871 mg/kg K, 1713-2410 mg/kg Mg (total contents). The soil composition was determined at the accredited Chemical Laboratory for Multi-Elemental Analyses at the University of Technology in Wrocław. The arable layer of soil had slightly acid reaction (pH in KCl was 5.96-6.38).

The weather conditions from April to August 2014 are shown in Table 2. They were not beneficial to either the initial development of the plants of spring wheat or the edaphon. Drought occurred during wheat emergence and was accompanied by ground frosts in May. Throughout the period of intensive growth of wheat, i.e. the stages of stem elongation and heading, favourable humidity and thermal conditions prevailed. Precipitation was deficient at the end of July, which accelerated the ripening of plants.

Table 2. Atmospheric precipitations and air temperatures during the period of study according to the Meteorological Station in Bałcyny

| Month | Period of ten days | | | Total or average | Total or average 1981-2010 |
|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | | |
| *Atmospheric precipitations (mm)* | | | | | |
| April | 16.7 | 5.6 | 3.8 | $26.1^{M*}$ | 29.8 |
| May | 15.0 | 2.3 | 17.6 | $34.9^{D}$ | 62.3 |
| June | 15.7 | 21.5 | 35.0 | $72.2^{M}$ | 72.9 |
| July | 11.8 | 8.6 | 0.0 | $20.4^{VD}$ | 81.2 |
| August | 37.3 | 6.8 | 15.1 | $59.2^{M}$ | 70.6 |
| Total for April-August | | | | $212.8^{VD}$ | 316.8 |
| *Air temperatures (℃)* | | | | | |
| April | 7.0 | 8.5 | 12.9 | 9.5 | 7.7 |
| May | 8.9 | 13.3 | 17.1 | 13.3 | 13.2 |
| June | 16.5 | 14.2 | 13.8 | 14.8 | 15.8 |
| July | 20.5 | 19.6 | 22.8 | 21.0 | 18.3 |
| August | 22.2 | 17.2 | 14.6 | 17.9 | 17.7 |
| Average for April-August | | | | 15.3 | 14.5 |

*Note*. *: assessment of precipitations according to Grabowska, Banaszkiewicz, and Szwejkowski (2004): season, month: M: moderate; D: dry; VD: very dry.

### *2.4 Plant and Soil Sampling*

#### 2.4.1 Grain Yield and Yield Structure Components

The yield was assessed on the basis of the amount of grain obtained from individual plots. The results were calculated per 1 ha and at the grain moisture of 12%. The following yield structure components were determined: ear density (the density of productive tillers per 1 $m^2$)–prior to wheat harvest, with the frame method (dimensions of a frame 0.50 m × 0.50 m); the number of grains per ear–based on the measurements on 25 plants sampled from each plot; the weight of 1000 grains–based on grain samples taken during the combine harvesting.

#### 2.4.2 Harvest Index

The harvest index (HI) was calculated as a ratio of the wheat grain yield to the total biological yield (grain + straw).

#### 2.4.3 Weed Infestation of the Growing Crop

The assessment was performed at the wheat ripening stage (BBCH 87-89) by the frame method (a frame sized 0.50 m × 0.50 m). The density and aerial biomass of weeds were determined per area unit of 1 $m^2$.

#### 2.4.4 Crop Residues

An analysis of the wheat crop residues was performed after the harvest. Soil samples were taken (to the depth of 30 cm) along with the stubble mulch, using a special cylinder (surface area of 400 $cm^2$). After rinsing the collected mass on sieves and removing soil debris, the roots, stubble mulch and weed residues were separated, dried (to the air-dry weight) and weighed, converting the results to an area of 1 ha. On this basis the structure of residues was determined (in %).

#### 2.4.5 pH of Soil

Soil samples for pH analyses were taken from the soil layers 0-10, 10-20, and 20-30 cm (using core soil samplers) at the following times: (1) before the application of fertilizers and wheat sowing, (2) after the application of the third portion of phosphorus suspension fertilizers, (3) following the harvest of spring wheat. The pH in 1 M KCl was determined at the Chemical and Agricultural Research Laboratory in Olsztyn, in accordance with the standard methods.

### *2.5 Statistics*

The results were processed statistically using the one-way analysis of variance. Differences between plots were determined using the Duncan test. The relationship between the grain yield and the element of yield structure was determined using simple correlation coefficients. In all cases, P = 0.05 was assumed.

## 3. Results and Discussion

### *3.1 Grain Yield and the Elements of Yield Structure*

The delayed sowing and rather unfavourable weather conditions during the growing period resulted in the yield of wheat that was only close to the average wheat yield in Poland (Statistical Yearbook of Agriculture, 2014). As compared to the control plot (without phosphorus fertilization), the applied phosphorus fertilizers and biofertilizers had significant yield-enhancing effects (Table 3). The highest yield was obtained under the superphosphate fertilization treatment. The yield of wheat fertilized with the biofertilizers from ash and bones did not differ from the yield achieved with the commercial fertilizers, although it was slightly lower than that harvested from plots treated with superphosphate, and slightly higher than that under the phosphorite fertilization treatment. Comparably good yield-stimulating efficiency was also demonstrated by ash diluted with water (without bacteria).

Table 3. Grain yield, elements of yield structure and harvest index of spring wheat

| Treatment | Grain yield (t/ha) | Ears density per 1 m$^2$ | Grains per ear | Weight of 1000 grains (g) | Harvest index |
|---|---|---|---|---|---|
| Control | 4.18 b | 557 b | 31.8 b | 32.1 b | 0.415 c |
| Superphosphate | 5.40 a | 609 ab | 35.6 a | 34.4 a | 0.468. a |
| Phosphoryte | 4.77 ab | 634 ab | 34.3 ab | 33.4 ab | 0.445 b |
| Ash-water solution | 5.00 a | 612 ab | 35.9 a | 33.6 a | 0.445 b |
| Biofertilizer from ash | 5.26 a | 593 ab | 36.7 a | 34.3 a | 0.458 ab |
| Biofertilizer from bones | 4.89 ab | 637 a | 37.0 a | 34.3 a | 0.448 b |

*Note*. a, b, c: values in column followed by the same letter do not differ significantly at P = 0.05.

The results of other experiments on the use of ash from the incineration of sewage sludge for fertilization purposes are also quite promising, but a relationship with the product manufacturing technology is noted. Franz (2008) confirms that the yield-enhancing action of fertilizer from ash on the formation of biomass of 3 plant species (kohlrabi, Swiss chard and maize) was comparable with and even slightly better than the effects of commercial fertilizers. Fertilizer from sewage sludge ash proved to be as effective in influencing the yield of crop plants as triple superphosphate in the studies by Bierman and Rosen (1994) on maize, and by Weigand et al. (2013) on oilseed rape. In turn, in the study by Severin et al. (2014), preparations from sewage sludge, depending on further treatment (including enrichment with various components) or the lack thereof, increased the yield of maize as much as superphosphate did or had no effect on it (yield at the level of control, without P fertilization). The yield-enhancing efficiency of meat and bone meal, being comparable with that of commercial fertilizers, was indicated by Jeng, Haraldsen, Grønlund, and Pedersen (2006), and of ash from meat and bone meal, by Alotaibi et al. (2013).

The unit efficiency of cereal grains is the resultant of 3 factors: the number of productive tillers per area unit (ear density), the number of grains formed in the ear, and the weight of a thousand grains (Kuś & Jończyk, 1997). As compared to the control (without P fertilization), the applied fertilizers and biofertilizers improved the values of wheat yield structure components (Table 3). The biofertilizers influenced the above traits in a manner similar to superphosphate and phosphorite. As regards the yield structure components, the yielding level was primarily determined by the weight of 1000 grains ($r = 0.7349$; $p = 0.000$), then by the number of grains in the ear ($r = 0.5443$; $p = 0.006$); no relationship with ear density was found ($r = 0.1251$; $p = 0.560$). In literature, opinions vary as regards the involvement of the above structural components in influencing the yield of particular cereal species (Rymuza, Turska, Wielogórska, Wyrzykowska, & Bombik, 2012). Kuś and Jończyk (1997) state that the unambiguous determination of the effects of the yield structure components on the yield is difficult because the efficiency largely depends on both the variety and environmental conditions.

Given the reports on positive results of the application of PSMs as biofertilizers (Galavi, Yosefi, & Ramrodi, 2011), including *Bacillus megaterium*, in wheat cultivation (El-Komy, 2005), and on the synergistic action of PSMs and mineral fertilizers (Galavi et al., 2011; Beigzade, Maleki, Siaddat, & Malek-Mohammadi, 2013), the authors of the current study also expected an additional bio-stimulating effect of the bacteria *Bacillus megaterium* on the wheat yield and yield structure components. However, no such effect was noted. Indeed, the biofertilizer from ash slightly increased the yield of wheat as compared to the action of ash with water, although the effect was not statistically verified. The activity of bacteria could have been affected by the precipitation and thermal conditions during the growing season (ground frosts, drought), which deviated from the ecological requirements of this species (Gibson & Gordon, 1974). However, Madani, Malboobi, Bakhshkelarestaghi, and Stoklosa (2011) found no significant effect of the interaction between the phosphorus solubilizing bacteria (PSB) and ammonium phosphate on the height of plants, biomass, the number of silicles on the plant, the content of oil, and the yield of oilseed rape seeds. In turn, Salimpur et al. (2010) reported that the yield of oilseed rape seeds under phosphorite fertilization with the addition of PSB and organic matter only slightly (insignificantly) exceeded the control yield (without P fertilization), and was lower than that following the application of triple superphosphate (TSP). Mahanta et al. (2014) noted that the addition of PSB to single superphosphate only slightly (insignificantly) increased the yield of soybean and wheat.

### *3.2 Harvest Index*

Phosphorus fertilization, as compared to its absence, resulted in an increased HI for wheat (Table 3). Usually, such an effect is associated with an increase in the grain yield, and its more favourable relation to the straw yield (Sinclair, 1998). The highest HI was obtained under superphosphate fertilization treatment. The same index level was determined for the application of the biofertilizer from ash. As compared to the plot under superphosphate, the HI for the wheat fertilized with biofertilizer from bones was lower, albeit comparable, with the index achieved under the phosphorite and ash-and-water fertilization treatments. The lower HI on the indicated plots is associated with the more intensive unproductive tillering and greater biomass accumulation in the stems.

For comparison, in the experiment by Alam and Shah (Alam & Shah, 2002), the HI for wheat was the same irrespectively of the applied phosphorus sources, at the same dose of pure ingredient, and higher than that for the control. In turn, Heitholt and Sloan (2006) demonstrated that the HI for soya beans and sweet corn increased more following the application of waste water residuals or biosolids than after the application of municipal yard waste compost, as compared to the control without fertilization. Studies with the use of biological factors also provide diverse data. Hussein and Radwan (2001) report that the biofertilizer (PSB and *Azospirillum* sp.) increased the HI for wheat. Yosefi, Galavi, Ramrodi, and Mousavi (2011) demonstrated that the application of biofertilizer (Bio-phosphate) with mineral fertilizer increased the HI for sweet corn relative to the application of a two-fold higher dose of mineral fertilizer as well as to the biofertilizer applied alone. In turn, in the experiment by Agraval and Pathak (2011) with the application of PSB with phosphorus mineral fertilizers, the HI for wheat indicated a downward trend (insignificant differences).

### *3.3 Weed Infestation of the Growing Crop*

Not only crop plants but also weeds use the resources of soil phosphorus. It follows from earlier reports that the richness of soil in this component may be even more important to weeds than nitrogen (N) or potassium (K), and that the response to the availability of phosphorus depends on the species (Blackshaw & Brandt, 2009). Weeds respond to the dose of fertilizer (Blackshaw, Brandt, Janzen, & Entz, 2004), the method of application (Blackshaw & Molnar, 2009), and the type and form of fertilizer, due to additional components (Lundy et al., 2010; Tang et al., 2014). In our study, the applied fertilizers and biofertilizers had no strong effect on the weed infestation of wheat (Table 4). Phosphorite tended to stimulate weed development, which may be associated with an increased content of Ca in the fertilizer (Lundy et al., 2010). It is also worth noting that the biofertilizer from bones did not change the density and biomass of weeds as compared to the plot without phosphorus fertilization and with superphosphate fertilization, while sewage sludge ash (in the biofertilizer and in the solution with water) showed a tendency towards reducing the weed infestation of wheat. As compared to the plot with phosphorite fertilization, the change was significant. Most importantly, the density and biomass of the dominant species population (*Chenopodium album*, *Fallopia convolvulus* and *Veronica persica*) were reduced.

Table 4. Weed infestation of spring wheat

| Treatment | Weed density (No./m$^2$) | Weed biomass (g/m$^2$) |
|---|---|---|
| Control | 104 ab | 62.5 ab |
| Superphosphate | 106 ab | 44.8 ab |
| Phosphoryte | 147 a | 76.8 a |
| Ash-water solution | 85 b | 27.1 b |
| Biofertilizer from ash | 70 b | 22.6 b |
| Biofertilizer from bones | 96 ab | 56.8 ab |

*Note*. a, b: values in column followed by the same letter do not differ significantly at P = 0.05.

Some authors emphaisize changes in the diversity and productivity of phytocoenoses in response to changes in the pH of soil, antagonisms between certain elements and also elevated levels of heavy metals contained in fertilizers (Vasseur, Cloutier, & Ansseau, 2000; Čiuberkis & Končius, 2006). However, it is difficult to refer those factors to our studies. Weed infestation of crop plants is still a rare subject of studies on the functional properties of biofertilizers, including those produced on the basis of renewable raw materials. Hussain and Radwan (2001) noticed no changes in the weight of weeds in wheat fields induced by the introduction of PSB and *Azospirillum* ssp. to the traditional nitrogen and phosphorus fertilization. Some interesting findings in our study as well as the scarcity of information contained in the available literature suggest that this issue needs further experimental exploration.

### *3.4 Crop Residues*

The residues of roots, stubble mulch and weeds remaining after the harvest are a valuable source of organic matter and nutrients (Tujaka, 2007). Fertilization, one of the critical factors influencing the biological yield (Nowicki & Marks, 1994), also has a significant effect on the yield weight. There were more crop residues from wheat on the plot fertilized with the biofertilizer from bones than on the other plots either fertilized or without phosphorus fertilization (Table 5). This difference was caused by the greater weight of both the roots and stubble mulch. The higher weight of stubble mulch may be associated with more abundant unproductive tillering and the formation of more numerous short productive tillers. In turn, the notion that a good supply of phosphorus favours the growth of roots (Grzebisz et al., 2003) contrasts with the opinion that an increase in the weight and length of the underground parts of plants, an increase in the number of branch roots, and the elongation of root hairs are a response to the phosphorus deficit (Belemi & Negisho 2012). The relatively smallest weight of roots on the control plot (without P fertilization) in our study seems to support the latter opinion. An increase in the weight of roots may be associated with the stimulation by PSB, which is confirmed by the study of Mahanta et al. (2014) on wheat and soybean. However, it is puzzling that no such effect concerned was obtained under the second biofertilizer (i.e. that from ash) treatment.

Table 5. Crop residues of spring wheat

| Treatment | Total t/ha (%) | Roots t/ha (%) | Stubble t/ha (%) | Weeds t/ha (%) |
|---|---|---|---|---|
| Control | 3.13b (100) | 1.34b (42.9) | 1.75b (55.9) | 0.04d (1.2) |
| Superphosphate | 3.35b (100) | 1.46b (43.5) | 1.69b (50.6) | 0.20b (5.9) |
| Phosphoryte | 4.44ab (100) | 1.96ab (44.1) | 2.18ab (49.1) | 0.30a (6.8) |
| Ash-water solution | 3.80b (100) | 1.81b (47.6) | 1.97b (51.7) | 0.03d (0.7) |
| Biofertilizer from ash | 4.01b (100) | 1.77b (44.1) | 2.01b (50.2) | 0.23ab (5.8) |
| Biofertilizer from bones | 5.46a (100) | 2.52a (46.1) | 2.84a (51.9) | 0.11c (2.0) |

*Note*. a, b, c: values in column followed by the same letter do not differ significantly at P = 0.05.

No differences in the structure of residues were found on the plots under analysis. Generally, there was a greater proportion of stubble mulch in the total weight of crop residues than that of the roots. The contribution of weeds to the weight of residues was small. The slightly greater weight and share of mulch in the total weight of residues on certain plots is a result of either their generally greater weight (phosphorite), or the occupation of lower layers in the growing crops competing well with them (biofertilizer from ash).

### *3.5 pH of Soil*

The soil under analysis was characterised by pH in the range corresponding to the greatest bioavailability of phosphorus (5.5-7.0) (Tujaka, 2007). The applied fertilization treatment did not produce significant and long-term changes to this characteristic (Table 6). At the time (2) following the application of fertilizers (the last dose of biofertilizers), no changes in the pH level affected by the type of the fertiilizer were demonstrated. However, as compared to the baseline, in the layer of 0-10 cm following the application of phosphorite and water solution of ash, the pH value was lowered, same as on the plot without phosphorus fertilization. However, no significant reduction was noted due to the application of biofertilizers and superphosphate. A slight reduction in the pH value on the mentioned plots might have been a response of wheat plants to the deficit of available phosphorus in the soil either in the absence of fertilization with this component (control) or when it was applied in the form of less soluble compounds (phosphorite, fertilizer from sewage sludge). Under phosphorus stress conditions, at the stages of a significant demand for this component (Grzebisz et al., 2003) the plants change the pH value of the substrate through the release of organic acids which dissolve poorly soluble phosphates (Belemi & Negisho, 2012). Such change *did* occur only in the 0–10 cm layer, in which the majority of the root system of young plants is found during the period under analysis. This may be suggestive of high bioavailability of phosphorus in the applied biofertilizers, similar to that of superphosphate. The study by Labuda et al. (2012) demonstrated that the solubilization of phosphorus from bones, with the involvement of *Bacillus megaterium*, was significantly more efficient than that from phosphorite. After the harvest of wheat, the soil pH value increased and was the same on all the plots. It was probably a result of the uptake of nutrients by plants, or leaching of anionic forms of nutrients, that is the onset of N-$NH_4$ release from dying roots (Miller & Cramer, 2004). The buffer properties of soil also play a specific role (Bednarek, Dziadowiec, Pokojska, & Prusinkiewicz, 2004).

Bierman et al. (1995) explains that the reduction in the soil pH value after four-year application of sewage sludge ash and TSP results from the acidifying action of nitrogen fertilizers, the removal with the crop, or the leaching of cations. The same authors associate lesser reduction in the pH value following the application of ash than of superphosphate with stronger buffer properties of ash. In the study by Rosyadi (2004), the pH of soil increased in a pot experiment with various renewable raw materials, and was significantly higher following the application of ashes from various secondary raw materials (sewage sludge ash, SSA, bone meal ash, BMA, meat and bone meal ash, MBMA) than following the application of their raw equivalents (sewage sludge, SS, bone meal, BM, meat and bone meal, MBM), TSP, and on the control plot. The author attributes these differences to a higher load of Ca in these materials (SSA, BMA, MBMA), and the nature of the ashing process in which $CO_2$ is removed and the alkaline action of CaO is generated, as well as to a higher uptake of Ca with MBM, and the potential release of protons during the TSP hydrolysis. Codling, Chaney, and Sherwell (2002) report an increase in the pH of soil under the influence of poultry litter ash, while Yusiharni, Ziadi, and Gilkes (2007) observed differences resulting from the application of wood ash and chicken litter ash. On the other hand, Alotaibi et al. (2013) noted a decrease in the pH value for soil fertilized with MBMA.

Table 6. Soil pH (in 1 M KCl)

| Treatment | Soil layer depth (cm) | Time of analysis | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| Control | 0-10 | 6.20 | 6.03↓ | 6.32↑ |
| | 10-20 | 6.21 | 6.16 | |
| | 20-30 | 6.28 | 6.31 | |
| Superphosphate | 0-10 | 6.14 | 6.27 | 6.32 |
| | 10-20 | 6.22 | 6.19 | |
| | 20-30 | 6.47 | 6.28 | |
| Phosphoryte | 0-10 | 6.21 | 6.02↓ | 6.35↑ |
| | 10-20 | 6.21 | 6.13 | |
| | 20-30 | 6.30 | 6.28 | |
| Ash-water solution | 0-10 | 6.16 | 5.99↓ | 6.48↑ |
| | 10-20 | 6.19 | 6.14 | |
| | 20-30 | 6.22 | 6.12 | |
| Biofertilizer from ash | 0-10 | 6.24 | 6.16 | 6.40↑ |
| | 10-20 | 6.22 | 6.29 | |
| | 20-30 | 6.24 | 6.14 | |
| Biofertilizer from bones | 0-10 | 6.16 | 6.09 | 6.39↑ |
| | 10-20 | 6.15 | 6.18 | |
| | 20-30 | 6.37 | 6.30 | |

*Note*. No significant difference between values within the same soil layer and time of analysis; ↓↑: significant decrease or increase in relation to the previous time o analysis.

Inoculation of soil with *Thiobacillus* bacteria in the study by Rosyadi (2004) significantly decreased the pH of soil. No such effect was observed in our field experiments with biofertilizers containing *Bacillus megaterium*.

## 4. Conclusions

Phosphorus biofertilizers from ash and bones equalled commercial fertilizers in terms of crop-enhancing efficiency. Biofertilizer from ash and ash diluted with water tended to reduce weed infestation. Biofertilizer from bones resulted in a greater weight of wheat crop residues, particularly roots. The biofertilizers did not change the pH of soil.

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