DOI: 10.21005/AAPZ2016.38.2.04

FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS
Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 2016, 326(38)2, 47–60

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PRELIMINARY ESTIMATION THE IMPACT OF EFFECTIVE MICROORGANISMS AND FERTILIZATION ON THE YIELD, PHOTOSYNTHETIC ACTIVITY, WATER USE EFFICIENCY OF JAPANESE KNOTWEED (*POLYGONUM CUSPIDATUM* SIEBOLD & ZUCC.) AND CONTENTS OF SELECTED MINERALS IN SOIL

WSTĘPNA OCENA WPŁYWU EFEKTYWNYCH MIKROORGANIZMÓW I NAWOŻENIA NA PLON, AKTYWNOŚĆ FOTOSYNTETYCZNĄ, WSPÓŁCZYNNIK WYKORZYSTANIA WODY RDESTU OSTROKOŃCZYSTEGO (*POLYGONUM CUSPIDATUM* SIEBOLD & ZUCC.) ORAZ ZAWARTOŚĆ WYBRANYCH SKŁADNIKÓW MINERALNYCH W GLEBIE

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Streszczenie. Celem badań była wstępna ocena wpływu efektywnych mikroorganizmów i nawożenia obornikiem na plon, cechy morfologiczne, aktywność fotosyntetyczną, współczynnik wykorzystania wody rdestu ostrokończystego (*Polygonum cuspidatum* Siebold & Zucc.) oraz zawartość wybranych składników mineralnych w glebie. Doświadczenie polowe przeprowadzono w 2014 i 2015 roku w Stacji Doświadczalnej w Lipniku niedaleko Stargardu Szczecińskiego (53º12'N; 14º27'E). Czynnikami doświadczenia były EM – efektywne mikroorganizmy (preparat) i nawożenie obornikiem. Doświadczenie założono w układzie losowym. Obejmowało ono cztery obiekty w czterech replikacjach: 1 – kontrola (bez EM i nawożenia), 2 – preparat EM, 3 – nawożenie obornikiem, 4 – preparat EM i nawożenie obornikiem. Efektywne mikroorganizmy i nawożenie obornikiem nie miało istotnego wpływu na plon, liczbę roślin na poletku, ich wysokość i średnicę łodyg rdestu ostrokończystego. Rośliny nawożone obornikiem oraz obornikiem i preparatem EM zawierały większe ilości makroelementów (magnezu, wapnia, fosforu) i mikroelementów. Większą intensywność asymilacji i transpiracji stwierdzono w roślinach uprawianych na poletkach kontrolnych (bez EM i obornika). Rośliny uprawiane na obiektach nawożonych obornikiem, a także na poletkach z EM i obornikiem lepiej wykorzystywały wodę w okresie jej niedoborów (w 2015 roku).

Key words: manure, effective microorganisms (EM), Japanese knotweed, minerals in soil, morphological traits, photosynthetic activity, water use efficiency (WUE), momentary water use efficiency (WUEI), yield.
Słowa kluczowe: obornik, efektywne mikroorganizmy (EM), rdest ostrokończysty, składniki mineralne w glebie, cechy morfologiczne, aktywność fotosyntetyczna, współczynnik wykorzystania wody (WUE), chwilowy współczynnik wykorzystania wody (WUEI), plon.

INTRODUCTION

The importance of energy crops has increased in recent years, especially with the interest in the production of biofuels. Human civilisation try to find solutions for insufficient energy supply and decreasing emission of green house gases is renewable energy sources

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utilization. Biogas production from biomass is surely one of the possible solutions. Due to the high yields of biomass and a significant calorific value Japanese knotweed (*Polygonum cuspidatum*) seem to be a promising source of renewable energy in appropriate areas (Bernik et al. 2007; Gregorczyk et al. 2012). Japanese knotweed is very expansive and thermophilic perennial plant producing stems that grow up to 2.5−3 m in height. The leaves contain up to 27% protein and 16% fiber in dry matter. As much as 51% fiber can be found in stems of Japanese knotweed (Smith et al. 2007). Due to the presence of organic compounds called stilbenes, it is used in medicine. The root and rhizomae of *Polygonum cuspidatum* sieb.et Zucc., used in many combined decoction, is safe and effective in the treatment of cardiovascular diseases (Du et al. 2009). According Fei et al. (2008) *Polygonum cuspidatum* has an antidiabetic properties and Shan et al. (2008) demonstrated that he crude extract of *Polygonum cuspidatum* roots possessed strong antibacterial activity against five foodborne bacteria. The intake of an extract of *Polygonum cuspidatum* containing resveratrol suppresses oxidative stress and inflammation in normal subjects (Ghanim et al. 2010). In many countries, dietary supplements containing Japanese knotweed rhizome are introduced into the market due to the very high content of resveratrol and other active substances (Spainhour 2008). In China, Japan, and Korea, young above ground shoots and rhizomes are utilized as “wild vegetable” (Jeong et al. 2010).

The soil degradation, including erosion, is a negative effect of agriculture intensification (Souchere et al. 2003) and in consequence, the movement and loss of organic matter, fine particles of minerals and nutrients occurs (Fanglong et al. 2007). Growing perennial biomass crops additionally contributes to other environmental benefits, like a reduction of pesticide and erosion and an increase in biodiversity (Lewandowski and Schmidt 2006).

To obtain high good-quality yields of crops requires agricultural treatments. Fertilization is very important for the growth, yield and photosynthetic activity of energy crops (Ercoli et. al. 1999). Animal manures may contribute to improving the soil’s physical conditions and are important source of Ca, Mg, S, and micronutrients; they contain only low and highly variable amounts of N, P, and K (Odedina et al. 2011). The increased supply of plants with nitrogen activates growth processes, which is reflected, among others, as the increased number of leaves (Amiri et al. 2012). Nitrogen deficiency inhibits plant growth by reducing the chlorophyll content in leaves, which in turn reduces the efficiency of photosynthesis, and in consequence decreases the yield. Okoroafor et al. (2013) reported that application of organic manure highly increase maize plant height, number of maize leaves, stem girth and yield of maize. All physiological processes like photosynthesis, transpiration, cell turgidity, cell and tissue growth in plants are directly affected by water availability (Sarker et al. 2005). Climate change predictions show clear increases in temperatures (and concomitant increase in potential evapotranspiration) and more frequent episodes of climatic anomalies, such as droughts and heat waves. Consequently, the optimization of water use for crops by improvement of WUE is a challenge for securing agricultural sustainability (IPCC 2013; Medrano et al. 2015). Fertilization phosphorus can improve WUE and helps crops achieve optimal performance under limited moisture conditions. The considerable enhancement in the water use and WUE by the crop could be attributed to the increase in root growth with high P supply (Alkhader and Rayyan 2013). Influence of mycorrhiza on the increased resistance of plants to drought and improvement of nutrients bioavailability is now well known and documented (Ruiz-Sanchez et al. 2010; Rapparini and Peñuelas 2014). Effective microorganisms can be envisaged as an addition to the mycorrhizal helping activity involved in mycorrhizal plant responses (Vivas et al. 2003).

Agricultural practices are important to optimize production and improvement quality yield of plants. However, research on the effect of effective microorganisms and fertilization manure on the yield, growth and physiology of Japanese knotweed is limited. This justifies the tests undertaking which goal was the assessment the impact of effective microorganisms and fertilization manure on the yield, photosynthetic activity, water use efficiency of Japanese knotweed and contents of selected minerals in soil.

MATERIAL AND METHODS

Study sites and plant material

Field experiment was conducted in 2014−2015 on sandy clay soil type at Lipnik Experimental Station (53°12'N; 14°27'E), Poland. The soil on which the experiment was conducted belongs to the typical rusty soils group (Systematyka gleb Polski 2011) and is classified as Haplic Cambisol (IUSS WORKING GROUP WRB 2014). The experiment was set on light, good rye complex soil of a IVa soil class. The soil is classified as brown soil developed from light loamy sands, with acidic pH (pH 1 M KCl = 4.9).

The subject of the study included eight years old plants of Japanese knotweed (*Polygonum cuspidatum* Siebold & Zucc). The outline of the experiment was set according to the factors:

Application of effective microorganisms (EM) by means of soil spraying using EM preparation, which was mixed with the soil to achieve better effects.

Fertilization (granulated manure) – 0.3 kg per circle.

Inoculation was done by product EmFarma Plus ^{TM} (twiece-2 ml per plot). The preparation contains properly selected composition of microorganisms, genetically not modified microorganism strains and their metabolites contained in fermented mixture made of natural components (http://www.probiotics.pl).

Granulated manure contains (minimum): N – 2.1, P_2O_5 – 1.6, K_2O – 5.9, Ca – 2.0, Mg 0.5 (% d.m.), organic substances – 60% d.m., humic substances – 25% d.m.

The experiment was conducted in the totally random system. The circle size was 0.8 m^2.

The experiment consisted of four objects, in four replication: 1 – control, without EM and fertilization; 2 – effective microorganisms, EM; 3 – fertilization granulated manure; 4 – EM and fertilization granulated manure.

Climatic conditions

The atmospheric conditions in the years of the studies are presented in Table 1. In comparison with the mean values of meteorological parameters during the years 1961–2004 the study years were warmer by 1.3°C in first year and by 0.7°C in second year. Of the two years of study in the first year the precipitation was higher than in analogical period of the multiyear (by 131.7 mm), whilst the 2015 year was drier (lower precipitation by 78.1 mm, on average).

Table 1. Sum of rainfall and mean air temperature in 2014–2015 years
Tabela 1. Suma opadów deszczu i średnia temperatura powietrza w latach 2014–2015

Month Miesiąc	Long-term average 1961–2004 Wielolecie 1961–2004		Rain Opady [mm]		Temperature Temperatura [ºC]	
	rain opady [mm]	temperature temperatura [ºC]	2014	2015	2014	2015
IV	34.9	8.9	37.0	15.4	11.1	8.3
V	48.6	13.2	100.5	44.3	14.0	12.3
VI	61.7	16.2	48.5	46.9	16.9	16.5
VII	70.9	18.1	95.0	63.9	21.8	19.4
VIII	54.1	18.1	66.5	19.6	18.2	21.6
IX	51.6	13.6	106.0	53.6	19.9	14.5
IV–IX	321.8	14.7	453.5	243.7	16.0	15.4

Yield, morphological and physiological measurement

The yield of the plants were determined by gravimetric. The measurements of the plants morphological traits: height, diameter of stem, made using a centimeter and caliper.

The measurements of the leaves photosynthetic activity in each year were taken in the dynamic using a LCA-4 analyser (ADC Bioscentific LTD, Hoddeson, Great Britain). In the year 2014 the measurements were taken on June 19, July 23 and August 25. In the year 2015 the measurements were taken on June 14, of July 25 and August 27. The leaves chosen for the measurements were well developed, taken from the middle part of one year old shoots on the outer part of plants, in the middle of height. The analysis was conducted on fully developed leaves without any signs of ageing or mechanical damage. The measurements included: NET photosynthesis intensity (Pn), stomatal conductivity (g_c), internal CO_2 concentration (Ci) and leaves transpiration index (T). The photosynthesis water use efficiency (WUE), which mainly depends on the environmental factors, was determined on basis of the ratio of the photosynthesis index to the transpiration (Pn/T). At the same time, the genetically determined momentary water use efficiency (WUEI) was assessed on basis of the ratio of the photosynthesis intensity to stomatal conductivity (Pn/g_c).

Chemical analysis

The soil samples were collected in the spring and autumn from each variant of the experiment with soil layers 0–30 cm. Soil samples were collected using sticks Egner, from several places. From the obtained samples created an average aggregate sample for each experimental variant.

The soil's pH_{KCl} was determined potentiometrically. The amount of organic carbon was determined through oxidation with dichromate (VI) in sulphuric acid (VI). The content of organic matter was assessed through multiplying the organic carbon amount by 1.724 coefficient.

Nitrogen was determined in the solutions derived from mineralization of the soil samples in sulphuric acid (VI) with H_2O_2 – Kjeldahl's method. The content of exchangeable phosphorus and potassium was established by Egner-Riehm method. Extraction with buffered barium chloride solution (pH = 8.1) was applied in order to determine replaceable forms of magnesium and calcium content in the soil.

Total elements (K, Ca, Mg, Fe, Mn, Zn, Pb, Cd, Ni and Cu) were determined in the soil after wet burning in a mixture of nitric (V) and chloric (VII) acids. The analyses were conducted using an Absorption Spectrometer apparatus (Thermo Fisher Scientific, iCE 3000 Series). Phosphorus was determined by calorimetrically with ammonium molybdate, at wavelength 470 nm.

Statistical analysis

The results have been statistically overworked using one-factor analysis of variance (ANOVA) in complete randomized design. Differences between mean values were indicated by least significance difference (LSD), calculated using Tukey test. Statistical significance was declared at $p < 0.05$ level. For calculation was used Statistica 10 version.

RESULTS AND DISCUSSION

The average yield of Japanese knotweed in the years of research (2014−2015) and selected morphological characteristics are shown in Table 2. The statistical analysis indicated that the applied agricultural practices did not significantly differentiate the properties of plants. The average plant yield was 5.2 kg · m^2 fresh mass and the difference in the yield between particular experimental combinations ranged within 0−3%. These findings are confirmed by studies of Van Vliet et al. (2006), who reported ineffectiveness of EM preparation on the manure quality and grass yielding. Although statistically significant effects of tested factors on the height and the number of plants were not proven, they were higher and more numerous on plots where agricultural practices were used. The use of effective microorganisms (EM) in combination with manure fertilization increased the number of plants by 13% as compared to the control. Higher plants were grown on plots with EM preparation and EM preparation and fertilized, respectively by 9%, which confirms the results reported by Minaxi et al. (2013) and Okoroafor et al. (2013). At the same time, the plants were characterized by lower stem diameter, by 15% and 5%, respectively.

Table 2. Yield [kg · m^2 f.m.], the number of plants [per meter^2], height [cm] and diameter of the stalk [mm] of Japanese knotweed. Mean for 2014–2015 years
Tabela 2. Plon [kg · m^2 ś.m.], liczba roślin [na metr^2], wysokość [cm], średnica łodygi [mm] rdestu ostrokończystego. Średnia dla lat 2014–2015

Traits – Cechy	Objects – Obiekty[a]				Mean Średnia	LSD$_{0.05}$ NIR$_{0.05}$
	1	2	3	4		
Yield – Plon	5.12	5.25	5.30	5.15	5.21	n.s.
The number of plants Liczba roślin	26.2	27.7	28.0	29.7	27.9	n.s.
Height – Wysokość	90.1	98.4	92.5	98.1	94.8	n.s.
Diameter – Średnica	11.1	9.5	11.7	10.6	10.7	n.s.

[a] as in methodology – jak w metodyce, n.s. – not significant difference – r.n. – różnica nieistotna.

Environmental conditions, availability of water, minerals, and plant species are key factors determining the process of photosynthesis. The authors (Borde et al. 2010; Nowak and Nowak 2013) inform about elevated photosynthetic activity of mycorrhized plants. Amaya-Carpio et al. (2009) believe that the use of arbuscular mycorrhizal fungi (AMF) increases nutrient uptake from organic fertilizers and consequently the rate of photosynthesis. Analysis of results of own research revealed variable response of Japanese knotweed to the applied agricultural practices (Table 3). Under conditions of periodic water shortages, especially in the second, drier and warmer year of research, the greater intensity of photosynthesis characterized plants growing on the control plots. Assimilation intensity (P_n) – as average within many years – of Japanese knotweed leaves was significantly lower on plots with EM preparation (by 43%), on fertilized (by 45%), and on plots with EM preparation and fertilized (by 32%) as compared to the control. Plants that assimilated less intensively were also characterized by lower levels of transpiration. Plants from plots fertilized with manure (48%), and with EM preparation and fertilized with manure (41%) transpired significantly the least as compared to the control. In the first year of study, more than two-fold significant increase in the leaf stomatal conductance treated of EM preparation and fertilized Japanese knotweed, was recorded. Significantly higher concentration of CO_2 (from 19% in Japanese knotweed fertilized with manure to 39% in treated of EM plants) was found in 2014. In the subsequent year, higher CO_2 levels were recorded only in leaves of fertilized with manure Japanese knotweed (by 24%) as compared to the control. The temperature of leaf in fertilized plants increased significantly by 6%.

Table 3. Photosynthetic activity of Japanese knotweed
Tabela 3. Aktywność fotosyntetyczna rdestu ostrokończystego

Traits Cechy	Years – Lata	Objects – Obiekty[a]				Mean Średnia	$LSD_{0.05}$ $NIR_{0,05}$
		1	2	3	4		
P_n	2014	16.0	7.33	10.8	9.57	10.9	3.80
	2015	14.4	10.1	8.90	11.1	11.1	5.28
	2014–2015	15.2	8.7	9.86	10.3	11.0	3.38
T	2014	2.20	2.01	2.44	2.05	2.17	n.s.
	2015	2.97	2.61	1.52	1.71	2.20	1.08
	2014–2015	2.58	2.31	1.98	1.87	2.19	n.s.
g_c	2014	0.19	0.17	0.16	0.69	0.30	0.31
	2015	0.17	0.14	0.19	0.21	0.18	n.s.
	2014–2015	0.18	0.16	0.17	0.45	0.24	0.24
C_i	2014	238.3	332.0	285.0	307.3	290.7	57.4
	2015	272.0	257.0	338.7	227.0	273.7	85.9
	2014–2015	255.2	294.5	311.8	267.2	282.2	n.s.
T_{leaf}	2014	26.0	26.7	27.7	27.0	26.8	1.07
	2015	24.7	24.3	25.0	24.0	24.5	n.s.
	2014–2015	25.3	25.5	26.3	25.5	25.7	n.s.

[a] as in methodology – jak w metodyce; n.s. – not significant difference – r.n. – różnica nieistotna,
P_n [umol $CO_2 \cdot m^{-2} \cdot s^{-1}$] – the intensity of assimilation – intensywność asymilacji,
T [m mol $H_2O \cdot m^{-2} \cdot s^{-1}$] – intensity of transpiration – intensywność transpiracji,
g_c [m mol $H_2O \cdot m^{-2} \cdot s^{-1}$] – stomatal conductance – przewodność szparkowa,
C_i [umol $CO_2 \cdot mol^{-1}$air] – the concentration of CO_2 into the distance intercellular – stężenie międzykomórkowe CO_2,
T_{leaf} [°C] – temperature – temperatura.

Water use efficiency (WUE), dependent on the environmental conditions and transpiration intensity, very well defines the plant's water balance during the vegetation. In the experiment, the WUE did not significantly depend on tested experimental factors (Table 4). However, it is worth underlining that on objects fertilized with manure as well as EM preparation and manure higher values of water use efficiency (WUE) were determined in 2015 (drier and warmer year) suggesting better utilization and management of water by plants under conditions of water deficiency or its considerable limitations within the soil (Blum 2005).

Candido et al. (2015) received similar results. It also confirms that WUE is closely associated with transpiration: the lower transpiration intensity, the better utilization of water by plants (higher WUE values) (Jaroszewska 2015). Values of momentary water use efficiency (WUEI), that is genetically determined, was significantly higher for plants growing on the control object in the first year of study (Table 4). A similar trend was noted in the subsequent season, although no statistically significant effects of the studied factors on WUEI were observed.

Table 4. Water use efficiency (WUE) [μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ m mol $H_2O \cdot m^{-2} \cdot s^{-1}$] and momentary water use efficiency (WUEI) [μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ m mol $H_2O \cdot m^{-2} \cdot s^{-1}$] of Japanese knotweed
Tabela 4. Współczynnik wykorzystania wody (WUE) [μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ m mol $H_2O \cdot m^{-2} \cdot s^{-1}$] i chwilowy współczynnik wykorzystania wody (WUEI) [μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ m mol $H_2O \cdot m^{-2} \cdot s^{-1}$] rdestu ostrokończystego

Traits − Cechy	Years − Lata	Objects – Obiekty[a]				Mean Średnia	$LSD_{0.05}$ $NIR_{0,05}$
		1	2	3	4		
WUE	2014	7.67	3.93	4.45	5.04	5.27	n.s.
	2015	4.87	3.99	6.76	6.79	6.60	n.s.
	2014−2015	6.27	3.96	5.61	5.91	5.44	n.s.
WUEI	2014	80.0	62.7	68.5	15.6	56.7	42.7
	2015	96.0	84.0	44.5	55.3	69.9	n.s
	2014−2015	88.0	73.3	56.5	35.5	63.3	45.7

[a] as in methodology – jak w metodyce, n.s. – not significant difference – r.n. – różnica nieistotna.

The pH of the soil largely determines the solubility of minerals, affects the appropriate development of plants and soil microorganisms. Japanese knotweed has a high resistance to habitat conditions and low soil pH. In this study, there was no significant effect of agronomic treatments on the value of this characteristic. The pH of the topsoil layer (0−30 cm) was similar in all experimental combinations and formed within the range from 4.4 to 5.3 (Table 5), which means that alkaline reaction was not present on any plot.

Table 5. pH_{KCl} and the content of organic carbon ($C_{org.}$) and organic matter [$g \cdot kg^{-1}$ d.m.] nitrogen [$g \cdot kg^{-1}$ d.m.], phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca) [$mg \cdot kg^{-1}$ d.m.] in soil. Mean for 2014–2015 years
Tabela 5. $pH_{KCl,}$ zawartość węgla organicznego i materii organicznej ($C_{org.}$) [$g \cdot kg^{-1}$ s.m.], azotu [$g \cdot kg^{-1}$ s.m.], fosforu (P), potasu (K), magnezu (Mg), wapnia (Ca) [$mg \cdot kg^{-1}$ s. m.] w glebie. Średnia z lat 2014–2015

Traits − Cechy	Objects – Obiekty[a]				Mean Średnia	$LSD_{0.05}$ $NIR_{0,05}$
	1	2	3	4		
pH	4.71	4.40	5.31	5.20	4.90	n.s.
Organic matter Materia organiczna	15.0	16.0	15.0	17.0	15.7	n.s.
$C_{org.}$	6.30	6.31	6.90	6.72	6.55	n.s.
N	0.67	0.34	0.36	0.37	0.43	0.12
P	53.4	32.4	52.8	60.0	49.6	3.80
K	228.0	112.0	205.4	205.6	187.8	2.73
Mg	46.7	37.6	99.9	36.9	55.3	2.32
Ca	337.0	317.4	342.1	323.7	330.1	6.64

[a] as in methodology – jak w metodyce, n.s. – not significant difference – r.n. – różnica nieistotna.

Organic matter occurring in soil plays a very important role in the nutrient circulation. Its presence determines the nutrient profiles, soil structure, water holding capacity, and pH (Dai 2011), and its content can be increased by fertilizers application (Quintern et al. 2006). Some positive impact of manure treatment on organic carbon content in soil was reported by Siwik-Ziomek and Lemanowicz (2014) as well as Cvetkov and Tajnšek (2009). In own studies, no significant differences in organic matter and organic carbon contents in compared objects were observed. Slightly higher levels of organic matter (by 13%) were recorded in treated of EM preparation soil in combination with manure fertilization than in soil treated with manure (by 9%) as compared to the control (Table 5).

According to Sanchez (2002), the lack of some macronutrients in the soil is a fundamental factor that limits the yields of worldwide crops. Plant's reaction to their deficiency varies and results from the role they play. One of the basic functions is participation in building the important organic compounds present in a plant. Agricultural practices significantly differentiated the content of selected macronutrients in soil (Table 5). Nitrogen limitation is associated with decreased enzyme activities that are required for energy metabolism such as photosynthesis and respiration (Shin et al. 2005). The largest amounts of nitrogen were observed in the soil from control plots. Soil fertilized with manure contained its lowest quantities (0.3 g · kg^{-1}, i.e. 57%). The lack of significant influence of animal-origin manure on nitrogen content in soils was proven by Odedina et al. (2011). Plants require adequate phosphorus from the very early stages of growth for optimum crop production. Restricted early-season phosphorus supply frequently limits crop production. Long-term input of P through fertilizers and manures can influence the amount and phytoavailability of phosphorus in the system and the development of mycorrhizal associations (Grant et al. 2005), which is in part confirmed in own study. Application of EM preparation with manure increased the content of phosphorus in soil by 12%. The objects with EM preparation revealed by about 39% less phosphorus content than objects without EM preparation (control). Potassium is essential in all cell metabolic processes. Potassium deficiencies in the soil can thus become a serious problem, because its amounts are easily reduced due to the uptake by plants, runoff, leaching, and soil erosion (Abou-El-Seoud and Abdel-Megeed 2012). According to Bohrer et al. (2003), bio-inoculants efficiently enhance this element concentrations in the soil. In own study, potassium content in all compared experimental objects was significantly lower than in the control. The lowest amounts of potassium were found in treated of EM preparation soil (112.0 mg · kg^{-1} d.m., i.e. 51%). Achieved results did not confirm those published by Ramakrishnaiah and Vijaya (2013), who showed a positive impact of VAM fungi, Azotobacter and PSB on nitrogen, phosphorus, and potassium contents in the soil. Magnesium is involved in the processes of photosynthesis, respiration, and phosphorus uptake by plants. The least magnesium was contained in soil from EM preparation plots (by 20%), the highest – soil fertilized with manure (by 114%) as compared to the control. Concentration of calcium is inseparably related to the soil pH, and it was significantly higher on the plots, where higher pH was recorded. The own study results correspond with those achieved by Odedine et al. (2011) and Mucheru-Muna et al. (2007), who reported the increase in magnesium and calcium contents in manure-treated soils.

Heavy metals in small doses are microelements necessary for a proper plant development and course of many metabolic processes. The presence of metals in the soil in excessive doses can, however, be a potential hazard to plants and underground water due to the excessive, often exceeding the physiological demands, uptake by plants from contaminated soils, which in consequence can be phytotoxic. Thirteen trace metals and metalloids (Ag, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Sb, Se, Tl, Zn) are considered priority pollutants (Sparks 2005; Gadd 2010). Excessive amounts of copper, nickel, cadmium, lead, or zinc negatively affect the growth and development of plants, disturb the photosynthesis processes, cell division, water balance within plants. In our study, the concentration of metals did not exceed the limits in any of the studied experimental objects (Regulation of the Minister of the Environment 2002) – Table 6.

Table 6. The content of selected micronutrients [mg · kg^{-1} d.m.] in soil. Mean for 2014–2015 years
Tabela 6. Zawartość wybranych mikroskładników [mg · kg^{-1} s.m.] w glebie. Średnia z lat 2014–2015

Traits – Cechy	Objects – Obiekty[a]				Mean Średnia	LSD$_{0.05}$ NIR$_{0,05}$
	1	2	3	4		
Cu	3.43	2.84	2.94	2.97	3.40	0.19
Ni	15.4	13.6	16.2	16.3	15.4	1.36
Cd	3.47	3.27	3.82	4.28	3.65	0.37
Zn	29.6	26.4	29.7	30.4	29.0	0.83
Pb	5.83	7.85	6.05	7.36	6.76	0.67
Mn	99.2	86.9	146.9	132.9	116.5	11.1
Fe	4528.6	4288.2	4634.8	4312.9	4441.1	n.s.

[a] as in methodology – jak w metodyce, n.s. – not significant difference – r.n. – różnica nieistotna.

According to many researches (Gadd 2007, 2010), microbial activities frequently result in immobilization or mobilization of metals, depending on the specific organisms and mechanisms involved, as well as the microenvironment, in which these processes are taking place. The EM preparation used in the experiment significantly reduced the contents of copper, nickel, cadmium, and zinc in the test soil, respectively by 18%, 12%, 6%, and 11%. The content of these metals in soil sampled from fertilized and EM preparation and fertilized plots, was significantly more than in the soil of the control. Increasing the content of copper and zinc in soil under the influence of manure was confirmed by studies carried out by Zhan et al. (2014). Bioavailability of lead in the soil depends largely on the soil pH, organic compounds, and phosphorus content. The amount of soluble lead and the solubility percentage is even greater, when the soil is more acidic. In our study, in the treated of EM preparation soil with very acidic reaction, the largest amount of lead was recorded among all compared objects (by 34% as compared to the control). Manganese is an activator of numerous enzymatic processes and a growth stimulator. Toxicity of manganese, according to Marschner (1988), for plants depends on the transformation of the compounds of this element in the soil and the plant characteristics (species and genotype). As with other micronutrients, the limit between deficiency or excess of manganese is very narrow. The largest amounts of manganese in the soil were recorded on fertilized as well as EM preparation and EM preparation and fertilized plots, respectively by 48% and 34% more than on control plots. The least contents of manganese were found in mycorrhized soil (by 12%), which according to Olszewska and Grzegorczyk (2008), can be a result of worse assimilation and transpiration intensity in leaves of Japanese knotweed grown in that experimental combination (Table 3). Iron excess causes blocking the phosphorus uptake, and in combination with other minerals, it has a toxic effect on plants. No significant effect of EM preparation and manure treatment on the iron content in the soil was observed. However, among compared objects, soil fertilized with manure contained the largest quantities of iron, which was confirmed by Brzeziński and Sosulski (2009).

CONCLUSION

Summing up the results of research, it can be concluded that the use of EM preparation and manure fertilization had no significant effect on the yield of Japanese knotweed. There were neither significantly differentiated the number of plants per plot, their height, and diameter of stems. Plants fertilized with manure and EM preparation with manure treatment revealed higher concentrations of studied macronutrients (magnesium, calcium, phosphorus) and microelements, which did not affect the increase in the photosynthetic activity in leaves. The EM preparation used in the experiment reduced the contents of copper, nickel, cadmium, and zinc in the soil. Greater intensity of assimilation and transpiration was observed in plants grown in the control plots. The coefficient of water use was dependent on the transpiration rate. The higher transpiration coefficient, the lower WUE. Plant grown on objects fertilized with manure as well as EM preparation and manure-treated better utilized water during its shortages (in 2015 year). Availability of heavy metals to plants is determined by means of the so-called bio-availability coefficient (ratio of the metal content in the plant to its content in the soil), which means that necessity to expand the research upon the analysis of the plant material and to continue in the subsequent years due to the relatively short period of measurements and analyzes.

REFERENCES

Abou-El-Seoud I.I., Abdel-Megeed A. 2012. Impact of rock materials and biofertilizations on P and K availability for maize (*Zea Maize*) under calcareous soil conditions. Saudi J. Biol. Sci. 19(1), 55−63.

Alkhader A.M.F., Rayyan A.M.A. 2013. Improving water use efficiency of lettuce (*Lactuca sativa* L.) using phosphorous fertilizers. Springerplus 2, 563.

Amaya-Carpio L., Davies F.T. jr, Fox T., He C. 2009. Arbuscular mycorrhizal fungi and organic fertilizer influence photosynthesis, root phosphatase activity, nutrition, and growth of *Ipomoea carnea ssp. Fistulosa.* Photosynthetica 47(1), 1−10.

Amiri E., Gohari A.A., Esmailian Y. 2012. Effect of irrigation and nitrogen on yield, yield components and water use efficiency of eggplant. Afr. J. Biotechnol. 11(13), 3070−3079.

Bernik R., Tušar R., Zver A. 2007. Japanese knotweed as renewable energy source, in: Proceedings 35th International Symposium 'Actual tasks on agricultural engineering', Opatija, Croatia, Veljače 2007. [b.w.], 347−352.

Blum A. 2005. Drought resistance, water-use efficiency, and yield potential-are they compatible, dissonant, or mutually exclusive? Aust. J. Agric. Res. 56, 1159−1168.

Bohrer G., Kagan-Zur V., Roth-Bejerano N., Ward D., Beck G., Bonifacio E. 2003. Effects of different kalahari-desert VA mycorrhizal communities on mineral acquisition and depletion from the soil by host plants. J. Arid. Environ. 55(2), 193−208.

Borde M., Dudhane M., Jite P.K. 2010. AM Fungi influences the photosynthetic activity, growth and antioxidant enzymes in *Allium sativum* L. under salinity condition. Not. Sci. Biol. 2(4), 64−71.

Brzeziński M., Sosulski T. 2009. Wpływ wieloletniego nawożenia na zawartość ruchomych form manganu i żelaza w glebie lekkiej [The influence of long-term fertilization on the content of mobile forms of manganese and iron in the light soil]. Zesz. Probl. Post. Nauk Rol. 541, 73−79. [in Polish]

Candido V., Campanelli G., D'Addabbo T., Castronuovo D., Perniola M., Camele I. 2015. Growth and yield promoting effect of artificial mycorrhization on field tomato at different irrigation regimes. Sci. Hortic. 187, 35−43.

Cvetkov M., Tajnšek A. 2009. Soil organic matter changes according to the application of organic and mineral fertilizers within long-term experiments. Acta Agric. Slov. 93(3), 311−320.

Dai O., Singh R.K., Nimasow G. 2011. Effect of arbuscular mycorrhizal (AM) inoculation on growth of Chili plant in organic manure amended soil. Afr. J. Microbiol. Res. 5(28), 5004−5012.

Du J., Sun L.N., Xing W.W., Huang B.K., Jia M., Wu J.Z., Zhang H., Qin L.P. 2009. Lipid-lowering effects of polydatin from *Polygonum cuspidatum* in hyper lipidemic hamsters. Phytomedicine 16(6−7), 652−658.

Ercoli L., Mariotti M., Masoni A., Bonari E. 1999. Effect of irrigation and nitrogen fertilization on biomass yield and efficiency of energy use in crop production of *Miscanthus*. Field Crops Res. 63(1), 3−11.

Fanglong G., Jianhui Z., Zhengan S., Xiaojun N. 2007. Response of changes in soil nutrients to soil erosion on a purple soil of cultivated sloping land. Acta Ecol. Sin. 27, 459−464.

Fei H.H., Xiao Z.D., Gao W. 2008. Effects of giant knotweed rhizome medicine on the expressions of RAGE and VEGF of rats with diabetic nephropathy. Health Sci. 46, 43−47.

Gadd G.M. 2007. Geomycology: biogeochmical transformations of rocks, minerals, metals, and radionuclides by fungi, bioweathering and bioremediation. Mycol. Res. 111, 3−49.

Gadd G.M. 2010. Metals, minerals, and microbes: geomicrobiology and bioremediation. Microbiology 156, 609−643.

Ghanim H., Sia C. L., Abuaysheh S., Korzeniewski K., Patnaik P., Marumganti A., Chaudhuri A., Dandona P. 2010. An antiinflammatory and reactive oxygen species suppressive effects of an extract of *Polygonum Cuspidatum* containing resveratrol. J. Clinic. Endocrinol. Metab. 95(9), 1−8.

Grant C., Bittman S., Montreal M., Plenchette Ch., Morel Ch. 2005. Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. Can. J. Plant. Sci. 85(1), 3−14.

Gregorczyk A., Wereszczaka J., Stankowski S. 2012. Wykorzystanie biomasy rdestowca ostrokończystego (*Polygonum cuspidatum* Siebold & Zucc.) do celów energetycznych [Utilisation of biomass of Japanese knotweed (*Polygonum cuspidatum* Siebold & Zucc.) for energy purposes]. Folia Univ. Agric. Stetin., Agricultura 293(21), 35−40. [in Polish]

IPCC. 2013. Climate change 2013, in: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Eds. T.F. Stocker, D. Qin, G.K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, P.M. Midgley. Cambridge, Cambridge University Press, 1535.

IUSS Working Group WRB. 2014. World reference base for soil resources. World Soil Resources Reports. Rome, FAO, 106.

Jaroszewska A. 2015. The effect of irrigation and mineral fertilization on the photosynthetic activity and water use in respect of cherry cv. 'Kelleris 16' yielding. Acta Sci. Pol. Hort. Cult. 14(5), 109−120.

Jeong E.T., Jin M.H., Kim M.S., Chang Y.H., Park S.G. 2010. Inhibition of melanogenesis by piceid isolated from *Polygonum cuspidatum*. Arch. Pharm. Res. 33(9), 1331−1338.

Lewandowski I., Schmidt U. 2006. Nitrogen, energy and land use efficiencies of miscanthus, reed canary grass and triticale as determined by the boundary line approach. Agric. Ecosyst. Environ. 112(4), 335−346.

Marschner H. 1988. Mechanism of manganese acquisition by roots from soils, in: Manganese in soils and plants. Eds. R.D. Graham, R.J. Hannam, E.C. Uren. Dordrecht, Kluver Academic Publishers, 191−204.

Medrano H., Tomás M., Martorell S., Flexas J., Hernández E., Rosselló J., Pou A., Escalona J M., Bota J. 2015. From leaf to whole-plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as a selection target. Crop J. 3(3), 220−228.

Minaxi Saxena J., Chandra S., Nain L. 2013. Synergistic effect of phosphate solubilizing rhizobacteria and arbuscular mycorrhiza on growth and yield of wheat plants. J. Soil Sci. Plant. Nutr. 13(2), 511−525.

Mucheru-Muna M., Mugendi D., Kung'u J., Mugwe J., Bation A. 2007. Effects of organic and mineral fertilizer inputs on maize yield and soil chemical properties in a maize cropping system in Meru South District, Kenya. Agrofor. Syst. 69, 189−197.

Nowak J., Nowak J.S. 2013. CO_2 enrichment and mycorrhizal effects on cutting growth and some physiological traits of cuttings during rooting. Acta Sci. Pol. Hort. Cult. 12(6), 67−75.

Odedina J.N., Odedina S.A., Ojeniyi S.O. 2011. Effect of types of manure on growth and yield of cassava (*Manihotesculenta*, Crantz). Researcher 3(5), 1−8.

Okoroafor I.B., Okelola E.O., Edeh O.N., Emehute V.C., Onu C.N., Nwaneri T.C., Chinaka G.I. 2013. Effect of organic manure on the growth and yield performance of maize in Ishiagu, Ebonyi State. J. Agric. Vet. Sci. 5(4), 28−31.

Olszewska M., Grzegorczyk S. 2008. Effect of manganese deficiency on gas exchange parameters, leaf greenness (spad) and yield of perennial ryegrass (*lolium perenel.*) and orchard grass (*dactylis glomeratal.*). J. Elem. 13(4), 589−596.

Quintern M., Lein M., Joergensen R.G. 2006. Changes in soil-biological quality indices after long-term addition of shredded shrubs and biogenic waste compost. J. Plant. Nutr. Soil Sci. 169(4), 488−493.

Ramakrishnaiah G., Vijaya T. 2013. Influence of VAM fungi, *Azotobacter* sp. and PSB on soil phosphatase activity and nutrients (N, P, K, Cu, Zn, Fe and Mn) status in the rhizosphere of *Stevia rebaudiana* (Bert.) plants. Am. J. Plant. Sci. 4, 1443−1447.

Rapparini F., Peñuelas J. 2014. Mycorrhizal fungi to alleviate drought stress on plant growth, in: Use of microbes for the alleviation of soil stresses. Ed. M. Miransari. New York, Springer Science+Business Media. DOI: 10.1007/978-1-4614-9466-9_2.

Regulation of the Minister of the Environment. 2002. Regarding in soil quality standards and earth quality standards. J. Laws. 165.

Ruiz-Sánchez M., Aroca R., Muñoz Y., Armada E., Polón R., Ruiz-Lozano J.M. 2010. The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the antioxidative response of rice plants subjected to drought stress. J. Plant. Physiol. 167, 862−869.

Sanchez P.A. 2002. Soil fertility and hunger in Africa. Science 295, 2019−2020.

Sarker B.C., Hara M., Uemura M. 2005. Proline synthesis, physiological responses and biomass, yield of eggplants during and after repetitive soil moisture stress. Sci. Hortic. 103, 387−402.

Shan B., Cai Y.Z., Brooks J.D., Corke H. 2008. Antibacterial properties of *Polygonum cuspidatum* roots and their major bioactive constituents. Food Chem. 109, 530−537.

Shin R., Berg R.H., Schachtman D.P. 2005. Reactive oxygen species and root hairs in arabidopsis root response to nitrogen, phosphorus and potassium deficiency. Plant Cell Physiol. 46(8), 1350−1357.

Siwik-Ziomek A., Lemanowicz J. 2014. The content of carbon, nitrogen, phosphorus and sulphur in soil against the activity of selected hydrolases as affected by crop rotation and fertilisation. Zemdirbyste 101(4), 367−372.

Smith J.M.D., Ward J.P., Child L.E., Owen M.R. 2007. A simulation model of rhizome networks for Fallopia japonica (*Japanese knotweed*) in the United Kingdom. Ecol. Model. 200, 421−432.

Souchere V., King C., Dubreuil N., Lecomte-Morel V., Le Bissonnais Y., Chalat M. 2003. Grassland and crop trends: role of the European Union Common Agricultural Policy and consequences for runoff and soil erosion. Environ. Sci. Pollut. Res. 6, 7−16.

Spainhour J. 2008. Medical attributes of *Polygonum cuspidatum* – Japanese knotweed, www.klemow..wilkes.edu/Polygonum.html - 16k, access: 22.10.2012.

Sparks D.L. 2005. Toxic metals in the environment: the role of surfaces. Elements 1, 193−196.

Systematyka gleb Polski [The classification of Polish soils]. 2011. Soil Sci. Ann. 62(3), 1−193. [in Polish]

Van Vliet P.C.J., Bloem J., Goede R.G.M. de. 2006. Microbial diversity, nitrogen loss and grass production after addition of effective microorganisms (R) (EM) to slurry manure. Appl. Soil Ecol. 32,188−198.

Vivas A., Marulanda A., Ruı´z-Lozano J.M., Barea J.M., Azco´n R. 2003. Influence of a *Bacillus* sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PE Ginduced drought stress. Mycorrhiza 13, 249−256.

Zhan Y., Luo W., Jia J., Kong P., Tong X., Lu Y., Xie L., Ma F., Giesy J.P. 2014. Effects of pig manure containing copper and zinc on microbial community assessed via phospholipids in soils. Environ. Monit. Assess. 186, 5297−5306.

Abstract. The aim of research was preliminary assessment the influence of effective microorganisms and fertilization manure on the yield, morphological traits, photosynthetic activity, water use efficiency of Japanese knotweed (*Polygonum Cuspidatum* Siebold & Zucc.) and on amount of selected minerals in soil. Field experiment was conducted in 2014 and 2015 in Experimental Station in Lipnik near Stargard Szczeciński (53º12'N; 14º27'E), Poland. Experimental factors were EM – effective microorganisms (preparation) and fertilization manure. The experiment was conducted in the totally random system. The experiment consisted of four objects, in four replication: 1 – control (without EM preparation and fertilization); 2 – EM preparation; 3 – fertilization granulated manure; 4 – EM preparation and fertilization granulated manure. Effective microorganisms and manure fertilization had no significant effect on the yield, the number of plants per plot, their height, and diameter of stems of Japanese knotweed. Plants fertilized with manure and EM preparation with manure had higher concentrations of studied macronutrients (magnesium, calcium, phosphorus) and microelements. Greater intensity of assimilation and transpiration was observed in plants grown in the control plots (without EM and manure). Plant grown on objects fertilized with manure as well as EM preparation and manure--treated better utilized water during its shortages (in 2015 year).