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IMPACT OF EFFECTIVE MICROORGANISMS AND MANURE ON THE CHEMICAL PROPERTIES OF JAPANESE KNOTWEED LEAVES AND THEIR USE IN THE PRODUCTION OF FUNCTIONAL FOODS

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The aim of the study was to determine the effect of the effective organisms (EM) and the granulated manure on the chemical properties of Japanese knotweed (*Reynoutria japonica* Houtt.) and the possibility of using them as a component of functional food. The field experiment was carried out in 2015-16 at the Lipnik Experimental Station (53°12'N; 14°27'E), Poland. The experiment consisted of four objects in four replications: control, effective microorganisms – EM, manure, effective microorganisms + manure. In the experiment, EmFarma Plus™ was used, which contained a mixture of specially selected non-genetically modified strains of microorganisms and their metabolites in the fermented mixture of natural ingredients. The granulated manure contained N – 2.1, P_2O_5 – 1.6, K_2O – 5.9, Ca – 2.0, Mg 0.5 (% DM), organic substances (60% DM), and humic substances (25% DM). Compared with the control the EM, manure, and EM+manure study groups had significantly higher amounts of crude fat, by 17.8, 0.8 and 15.7 $g{\cdot}kg^{-1}$, respectively. The EM and EM+manure groups had significantly higher amounts of crude ash, by 7.4 and 16 $g{\cdot}kg^{-1}$, respectively. The highest phosphorus levels were observed in the EM group by 0.62 $g{\cdot}kg^{-1}$. The EM and EM+manure groups had significantly higher amounts of potassium, by 3.8 $g{\cdot}kg^{-1}$. The higher amounts of calcium (by 1.52 $g{\cdot}kg^{-1}$) had the EM+manure group. The EM, manure and EM+manure study groups had significantly higher amounts of zinc (6.5, 7.2, 17.6 $mg{\cdot}kg^{-1}$, respectively), iron (2.1, 2.0, 14.1 $mg{\cdot}kg^{-1}$, respectively) and manganese (16.4, 49.6, 16.0 $mg{\cdot}kg^{-1}$, respectively). The highest molybdenum levels were found in the group fertilized EM and manure, 4.5 and 5.5 $mg{\cdot}kg^{-1}$, respectively. Due to the high nutrient content, leaves of Japanese knotweed can be used as an alternative source of components in the production of functional foods and pharmaceutical preparations.
Keywords: EM, fiber, manure, macro-microelements, nutritional content, *Reynoutria japonica* Houtt.

INTRODUCTION

Culinary habits are one of the most enduring of social values. However, due to the significant rise in civilizational diseases worldwide, consumers are becoming increasingly interested in healthy and properly balanced diets which in combination with physical activity could effectively prevent many diseases. The importance of diet has been demonstrated in data provided by the National Cancer Institute, according to which one out of every three cancer-related deaths are related to eating habits (Block et al., 1992; Potter and Steinmetz, 1996). One element of this change in nutrition habits is the increasing popularity of bioactive (functional) foods that support mental and physical health and also help to prevent and treat certain diseases.

Bioactive ingredients are defined by the American Dietetic Association as physiologically active components found in food or dietary supplements, both of plant and animal origin, which are necessary to human nutrition and play an important role in sustaining health (Saldanha, 2004). One source of these substances is from plants commonly perceived as weeds, often rich in nutrients and exerting bioactive, pro-health and therapeutic effects. Weeds may contain fiber, vitamins, minerals and essential oils, are low in calories, and their beneficial effects have been known since ancient times.

Japanese knotweed (*Reynoutria japonica*) is a heat-loving perennial plant of the Phyllidae family (*Polygonaceae* Juss.) with shoots reaching 2.5-3 m in length. This plant can tolerate a wide spectrum of soil conditions occurring in both very poor acidic soils and in more neutral and richer soils (Beerling *et al*., 1994). It was imported into Europe and North America in 1825 from Asia as an ornamental plant, and is now found widely across Europe and Great Britain, e.g. on rubble, by rivers, and in gardens (Urgenson *et al*., 2012). It has become a highly prolific and aggressive invasive species in the riparian wetlands of Europe and North America (Topp *et al*., 2008).

The natural habitat range of *Reynoutria japonica* includes Japan, Korea, the Kuril Islands, South-West China, Taiwan, Vietnam and Sakhalin (Albertenst and Bohmer, 2011). In

Asia, the rootstock of Japanese knotweed is dried and used as an infusion, and has been applied for centuries to treat many conditions including heart disease and stroke (Burns *et al*., 2002). In Japan and China, young shoots and rhizomes are used as a 'wild vegetable' (Jeong *et al*., 2010). In South Korea, *Reynoutria japonica* was officially classified as a functional food in 2012 (Kim *et al*., 2012). On Sakhalin and the Kuril Islands, Russians use this plant as a replacement for sorrel in soups, or consume it as a jelly. In England and the United States, young shoots of Japanese knotweed are cooked in a similar way to rhubarb (Pirożnikow, 2012). Its rhizomes contain large amounts of resveratrol, which is recommended as an antioxidant that inhibits the growth of fungi and bacteria, and can even prevent cancer (Kimura and Okuda, 2001). According to Burns *et al*. (2002) the stalks and leaves of the Japanese knotweed are one of the richest sources of natural resveratrol.

Producers of functional foods are usually interested in high-quality components made from special crops and cultures. This often requires environmentally friendly production, where traditional chemical protection and mineral fertilizers are replaced by natural ones, such as manure; and by organic fertilizers, such as composts, crop residues and green manures. Traditional fertilizers can also be substituted with biological preparations such as effective microorganisms (EM), fermented mixed cultures of naturally occurring species of coexisting microorganisms in an acidic medium. The main microorganisms in EM cultures include species of photosynthetic bacteria (*Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*), yeasts (*Saccharomyces* spp.), lactobacilli (*Lactobacillus plantarum*, *L. casei*, and *Streptococcus lactis*), and actinomycetes (*Streptomyces* spp.) (Javaid and Bajwa, 2011). In many cases it exists a symbiosis among different micro-organisms caused by synergies of their different enzymatic systems and metabolic pathways (Yara *et al*., 2006). Adding photosynthetic bacteria in the soil enhances other effective microorganisms. Bioactive substances such as hormones and enzymes produced by yeasts promote active cell and root division. Their secretions are useful substrates for effective microorganisms such as lactic acid bacteria and actinomycetes. Actinomycetes can coexist with photosynthetic bacteria. Both species enhance the quality of the soil environment, by increasing the antimicrobial activity of the soil (Golec *et al*., 2007). The microorganisms in EM cultures improve crop health and yield by increasing photosynthesis, controlling soil-borne diseases, accelerating decomposition of organic materials and producing bioactive substances such as hormones and enzymes (Hussain *et al*., 2002).

Previous studies (Yamada and Xu, 2000; Khaliq *et al*., 2006) confirm the competitiveness of EM in relation to conventional fertilization. According to Singh (2007), this kind of microbiological preparation increased the bioavailability of nitrogen in soybeans and yardlong beans, the levels of proteins and fats in soybean seeds, and seed yield. Xu *et al*. (2001) found better quality and increased yields in tomatoes, increased vitamin C and sugars in its fruit, and stimulated photosynthesis.

Despite the growing popularity of Japanese knotweed as a source of resveratrol and its culinary and therapeutic use in many regions of the world, there is still little research on the chemical composition of its leaves. The aim of this research was to verify the hypotheses that effective organisms (EM) and granulated manure differentiate the chemical composition of the Japanese knotweed (*Reynoutria japonica* Houtt.) leaves, and Japanese knotweed leaves may serve as a good component in functional food. The aim of the study was to determine the effect of the effective organisms (EM) and granulated manure on the chemical properties of the Japanese knotweed (*Reynoutria japonica* Houtt.), and the possibility of using them as a component of functional food.

MATERIALS AND METHODS

***Study sites and plant material*:** The field experiment was carried out in 2015 and 2016 at the Lipnik Experimental Station (53°12'N; 14°27'E), Poland. The effects of effective microorganisms (EM) and fertilization with granulated manure on the content of basic nutrients (protein, carbohydrates, fiber, fat, ash, NFE), fiber fractions and minerals in leaves of Japanese knotweed (*Reynoutria japonica* Houtt.) were analyzed.

In the experiment, EmFarma Plus^{TM} was used, which contained a mixture of specially selected non-genetically modified strains of microorganisms and their metabolites in the fermented mixture of natural ingredients (http://www.probiotics.pl).

This was applied by spraying the soil with a preparation (2 ml per circle) which, in order to achieve a better effect, was mixed with soil, or by adding granulated manure (0.3 kg per circle) containing N – 2.1, P_2O_5 – 1.6, K_2O – 5.9, Ca – 2.0, Mg 0.5 (% DM), organic substances (60% DM) and humic substances (25% DM). The manure was fertilized and the effective microorganisms applied in early spring.

The plants grew in concrete circles with a single pot covering an area of 0.8 m^2. The experiment was conducted in a totally random system. The experiment consisted of four objects, in four replications: control (without effective microorganisms and fertilization); EM (effective microorganisms), manure (fertilization with granulated manure); EM+manure (effective microorganisms and fertilization with granulated manure).

The leaves were collected before the flowering of the plants (the third week of July). The leaves collected displayed no signs of aging or mechanical damage.

***Soil conditions*:** The experiment was conducted on a light, good rye complex soil of class IV. The soil is classified as

Table 1. Basic properties of the soil.

Specification[1]	pH_{kcl}	$C_{org.}$	N	P	K	Mg	C_{org}:N
	(g·100g⁻¹ d. m.)			(mg·100g⁻¹ DM[2])			
Control	4.70	0.63	0.07	5.34	22.8	4.67	9:1
EM[3]	4.44	0.63	0.03	3.24	11.2	3.76	21:1
Manure	5.32	0.69	0.04	5.28	20.5	9.99	17:1
EM+manure	5.25	0.67	0.04	6.00	20.1	3.69	17:1
	Fe	**Zn**	**Mn**	**Cu**	**Cd**	**Pb**	**Ni**
	(mg·100g⁻¹ DM)						
Control	45.3	2.96	9.92	0.34	0.35	0.58	1.15
EM	42.9	2.64	8.69	0.28	0.33	0.78	1.36
Manure	46.3	2.97	14.7	0.29	0.38	0.60	1.62
EM+manure	43.1	3.04	13.3	0.30	0.43	0.74	1.63

[1]C_{org} = organic carbon, N = total nitrogen, P = available phosphorus, K = available potassium Mg = exchangeable magnesium, Ca = exchangeable calcium. [2]DM = dry matter. [3]EM = effective microorganisms.

brown soil developed from light loamy sands. The plants were grown in four different soil conditions (Table 1).

1. Control – acidic soil reaction (pH = 4.70); content of soil minerals (mg·100g^{-1} DM): low levels of phosphorus (5.34), moderate levels of magnesium (4.67) and very high levels of potassium (22.8).
2. EM – very acidic soil reaction (pH = 4.44); content of soil minerals (mg·100g^{-1} DM): very low levels of phosphorus (3.24), moderate levels of magnesium (3.76) and potassium (11.2),
3. Manure fertilization – acidic soil reaction (pH = 5.32); content of soil minerals (mg·100g^{-1} DM): low levels of phosphorus (5.28) and very high levels of magnesium (9.99) and potassium (20.5),
4. EM + manure fertilization – acidic soil reaction (pH = 5.25); content of soil minerals (mg·100g^{-1} DM): low levels of phosphorus (6.00), moderate levels of magnesium (3.69) and very high levels of potassium (20.1).

The levels of metals in the soil did not exceed permissible limits (Ordinance of the Ministry of the Environment, Journal of Laws no 165, item 1359, 2002).

***Climatic conditions*:** The meteorological conditions for the 2015 and 2016 periods are shown in Table 2. The second year of the research, 2016, was warmer and more humid, with precipitation higher by 14.7 mm and temperature higher by 0.4°C in comparison to 2015. With regard to the multi-annual period 1961-2004, the period in which the experiment was conducted was drier and warmer. Total precipitation amounts in 2015 and 2016 were 78.1 and 63.4 mm, lower than the long-term averages. The air temperatures were higher by 0.7°C and 1.1°C, respectively.

***Proximate composition analyses*:** The samples chemical compositions were determined according to the procedures of the Association of Official Analytical Chemists (AOAC, 2012): dry matter was determined by drying at 105°C to a constant weight, crude fat by Soxhlet extraction with diethyl ether, and crude ash by incineration in a muffle furnace at 580°C for 8 h. Crude protein (N × 6.25) was established by the Kjeldahl method using a Büchi B-324 Distillation Unit. Crude fibre was determined in an ANKOM 220 fibre analyzer. Nitrogen-free extract (NFE) was calculated as: NFE = 100 − (moisture + crude protein + crude fat + crude ash + crude fibre).

***Analyses of the fiber components*:** The fiber components were determined using the detergent method with the ANKOM 220 fibre analyzer, according to Van Soest *et al*. (1991). The neutral detergent fibre (NDF) was calculated on an ash-free basis and included sodium dodecyl sulphate (Merc 822050). The acid detergent fibre (ADF) level included hexadecyl-trimethyl-ammonium bromide (Merc 102342), while the acid detergent lignin (ADL) was

Table 2. Sums of monthly precipitation (mm) and mean monthly air temperature (°C) in the years of research in comparison to long-term averages for 1961-2004.

Month	Long-term averages 1961-2004		Precipitation		Air temperature	
	Precipitation	Temperature	2015	2016	2015	2016
IV	34.9	8.9	15.4	19.7	8.3	8.1
V	48.6	13.2	44.3	41.2	12.3	15.6
VI	61.7	16.2	46.9	79.9	16.5	17.9
VII	70.9	18.1	63.9	70.8	19.4	18.5
VIII	54.1	18.1	19.6	33.5	21.6	17.4
IX	51.6	13.6	53.6	13.3	14.5	16.6
IV-IX	321.8	14.7	243.7	258.4	15.4	15.8

determined by the hydrolysis of ADF samples in 72% sulphuric acid. The hemicellulose content was calculated as the difference between NDF and ADF, and the cellulose content as the difference between ADF and ADL.

***Analyses of the mineral compounds*:** The material for analyses of the major dietary element concentrations was subjected to mineralization in concentrated H_2SO_4 and $HClO_4$ acids. The material for analyses of the micro-compound was subjected to mineralization in a mixture of HNO_3 and $HClO_4$. The concentration of phosphorus (P) was determined by the colorimetric method using a Specol 221 apparatus. An Atomic Absorption Spectrometer apparatus (iCE 3000 Series, Thermo Fisher Scientific) was used to determine potassium (K), sodium (Na) and calcium (Ca) by means of emulsion flame spectroscopy, whilst magnesium (Mg), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Mo), copper (Cu), cadmium (Cd), lead (Pb) and nickel (Ni) were established by means of absorption flame spectroscopy.

***Soil analyses*:** The soil pH_{KCl} was determined potentiometrically according to the ISO 10390/1997 norm. The amount of organic carbon was determined by Westerhoff's colorimetric method. Concentrations of total nitrogen (N) were determined in samples digested in sulfuric acid (VI) with H_2O_2 by the Kjeldahl method. The content of available phosphorus (P) and potassium (K) was determined by the Egner-Riehm method (DL) (Egner *et al*., 1960). Extraction with a buffered barium chloride solution pH = 8.1 (ISO 13536: 2002P) was used to determine the amount in the exchangeable magnesium (Mg).

The total metal contents of iron, zinc, manganese, copper, cadmium, lead and nickel (Fe, Zn, Mn, Cu, Cd, Pb and Ni) were determined after wet combustion in a mixture of nitric (V) and chloric (VII) acids (ISO 11047: 2001). The analyses were carried out using an Atomic Absorption Spectrometer (iCE 3000 Series, Thermo Fisher Scientific).

***Statistical analysis*:** The results of experiment were statistically analyzed, using a one-factor analysis of variance (ANOVA), after assessing the normality and homogeneity of variance. The significance of differences between means was compared by Tukey multiple-range tests ($P<0.05$). The admissible error for determinations of chemical components was 5%. All samples were analyzed in triplicate. The results are presented as mean ± SD (standard deviation) from two years of experimentation (no significant differences were found between the years of researches). A Statistica 12. version software (StatSoft, Poland) was used for calculation.

RESULT AND DISCUSSION

***Proximate composition*:** The levels of essential nutrients in the leaf samples are presented in Table 3. The chemical composition of the Japanese knotweed leaves depended on the agrotechnical agent used. Although it is most beneficial to use the herbs when they are fresh, the limited life of the plants after harvesting necessitated the use of different technologies for fixation. Drying eliminated the growth of microorganisms and limited biochemical reactions in the dried leaves (Di Cesare *et al*., 2004).

In fresh herbal samples, the lowest level of dry matter was found in the leaves of plants grown in soils where effective microorganisms (EM) had been used (22.7%), and the highest level where manure fertilization had been used (about 30.3%).

Drying produced a dry mass of material between 91.9 and 92.3%. The fertilization choice did not significantly differentiate the dry matter content of the dried material, nor did it affect the level of protein in the examined leaves ($P>0.05$), which ranged 130.1-134.8 $g{\cdot}kg^{-1}$ DM. The highest amount of protein found in the Japanese knotweed leaves was after the use of effective microorganisms (EM) (1.8% vs. control, $P>0.05$). Hameed *et al*. (2008), in their investigation of selected medicinal plants, found only about 92 g protein per kg of dry mass in *Persicaria maculosa* S.F. Gray leaves. Our research results corroborate Khaliq *et al*. (2006), who found a positive effect of EM on plant nitrogen nutrition.

The fertilization factor also had an effect on the lipid content in the Japanese knotweed leaves, with much higher levels in plants growing on EM and EM+manure (59.5 and 52.5% higher than control, respectively).

Crude fiber is the part of the fiber containing cellulose, lignin and some hemicellulose (Selvendran and MacDougall, 1995). The control leaves had the highest levels of raw fiber

Table 3. Mean (±SD[1]) chemical composition in the tested leaves of Japanese knotweed ($g{\cdot}kg^{-1}$ DM[2]).

Specification	Control	EM[3]	Manure	EM+manure
Moisture ($g{\cdot}kg^{-1}$ FM[4])	741.4±0.02c	772.8±0.02a	696.7±0.02d	770.7±0.02b
Dry matter ($g{\cdot}kg^{-1}$ dried material)	919.5±0.07a	920.0±0.07a	921.0±0.07a	923.4±0.07a
Crude protein	132.4±2.03a	134.8±5.24a	130.1±10.2a	130.6±9.02a
Crude fat	29.9±0.46b	47.7±0.46a	30.7±1.07b	45.6±1.22a
Crude fiber	200.3±0.75a	194.9±0.90b	181.2±1.52d	190.4±0.75c
Crude ash	75.5±0.77c	82.9±0.53b	75.5±0.07c	91.5±0.15a
NFE[5]	531.9±44.0a	539.6±3.34a	582.4±12.9a	541.9±11.1a

[1]±SD = standard deviation. [2]DM = dry matter.[3]EM = effective microorganisms. [4]FM = fresh matter. [5]NFE = nitrogen free extract. [abcd] Mean values with the same letter in each row are not significantly different at $P \leq 0.05$.

Table 4. Mean (± [1]SD) mineral compounds in the tested leaves of Japanese knotweed.

Specification	Control	EM[3]	Manure	EM+manure
Macroelements ($g \cdot kg^{-1}$ DM[2])				
P	5.19±0.10b	5.81±0.10a	4.06±0.10c	5.10±0.20b
K	16.7±0.10b	20.5±0.15a	15.7±0.15c	20.5±0.10a
Ca	5.90±0.12b	5.86±0.10b	5.26±0.02c	7.42±0.23a
Mg	2.89±0.01c	3.02±0.01b	3.52±0.01a	2.99±0.01b
Na	0.31±0.00a	0.30±0.00ab	0.28±0.00b	0.30±0.00ab
Ca:Mg	2.04	1.94	1.49	2.48
Ca:P	1.14	1.01	1.29	1.45
Na:K	0.02	0.01	0.02	0.01
Microelements ($mg \cdot kg^{-1}$ DM)				
Zn	29.1±0.10d	35.6±0.15c	36.3±0.10b	46.7±0.10a
Fe	46.3±0.38c	48.4±0.10b	48.3±0.38b	60.4±0.00a
Mn	177.3±0.70b	193.7±0.32b	226.9±1.32b	337.3±35.7a
Mo	62.2±3.08a	66.7±1.76a	67.7±3.83a	61.7±2.37a
Cu	1.80±0.10ab	1.85±0.02a	1.14±0.05c	1.58±0.08b
Cd	nd[4]	nd	nd	nd
Pb	0.24±0.01b	0.40±0.01a	nd	nd
Ni	nd	nd	nd	1.17±0.01

[1]±SD = standard deviation. [2]DM = dry matter. [3]EM = effective microorganisms. [4]nd = not detected. [abcd] Mean values with the same letter in each row are not significantly different at $P \leq 0.05$.

(200.3 g), followed by the leaves of plants growing with effective microorganisms (EM) (194.9 g), and then those growing on soil fertilized by manure (9.5% less than control). These results show that Japanese knotweed leaves are a good source of raw fiber, with much higher levels than in the selected medicinal plants investigated by Hameed *et al*. (2008).

The Japanese knotweed leaves examined were markedly different in their ash mineral content, with the significantly highest levels in the plants growing on the combination of effective microorganisms and granulated manure (91.5 $g \cdot kg^{-1}$ DM). The main component of total dry matter was nitrogen-free extract (NFE), which ranged from 531.9 to 582.4 g; it was not significantly affected by the factor tested.

***Fiber components*:** The main source of dietary fiber in the diet is cereal products, which provide about 50% of this ingredient, as well as fruits and vegetables (Lambo *et al*., 2005). The impact of dietary fiber in the human body is related not only to its amount in the diet, but also to its fractional composition, which may vary depending on the plant species, degree of maturity, anatomical part, and the technological process used (Pastuszewska *et al*., 2009; Dhingra *et al*., 2012).

The largest proportion of the dietary fiber fraction was neutral detergent fiber (NDF), followed by acid detergent fiber (ADF) which contains lignin and cellulose (Fig. 1). The mean NDF varied from 392.1 to 399.2 $g \cdot kg^{-1}$ DM. ADF ranged from 300.1 to 338.6 $g \cdot kg^{-1}$ DM. These two fractions had the highest levels in the control group, and the lowest in the EM+manure group (P<0.05).

The content of cellulose in plants increases during their maturation and aging. Lignins are deposited in the cell walls at the end of cell growth after the complete formation of the polysaccharide wall skeleton. The greatest number of CELs and ADLs were found in the leaves of control plants and those fertilized with manure (52.9 and 52.3 g, and 150.4 and 147.3 $g \cdot kg^{-1}$ DM, respectively). Both fractions are important in supporting intestinal peristalsis (Fuller *et al*., 2016). The lowest levels of the other fiber fraction, hemicellulose, which best binds heavy metal ions, was found in control plants (60.6 g) and the highest in three experimental groups, ranging 88.9-91.9 $g \cdot kg^{-1}$ DM. The literature lacks data concerning fiber fractions in Japanese knotweed.

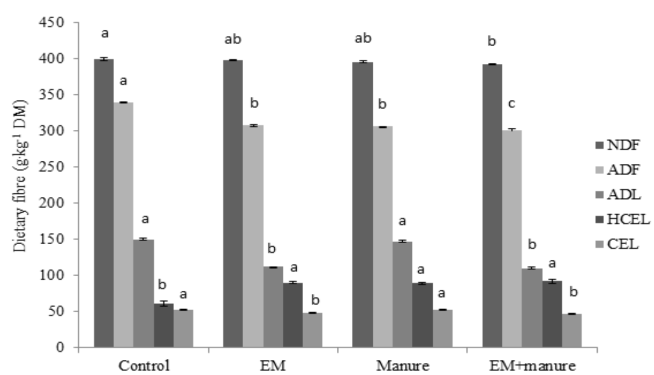

Figure 1. The tested leaves of Japanese knotweed dietary fiber ($g \cdot kg^{-1}$ DM). DM = dry matter. EM = effective microorganisms. NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin, HCEL = hemicelluloses, CEL = cellulose.

***Mineral compounds*:** The investigated leaves had on average 5.04 g·kg^{-1} phosphorus, i.e. 30% more than *Corylus avellana L*., and more than twice the amount in the study by Rahmanov *et al.* (2014) on Japanese knotweed (*Reynoutria japonica*), 1.24 to 2.10 g·kg^{-1} depending on the habitat. In our experiment, the highest amount of phosphorus (12%, i.e. 0.62 g·kg^{-1} more than control) was found in the leaves collected from plants in which EM was used.

The Japanese knotweed leaves analyzed contained an average of 18.3 g·kg^{-1} of potassium, i.e. more than 4 times the amount of the potassium-rich banana (*Musa* ssp.) (3.85 g·kg^{-1}) (Anyasi *et al*., 2013). According to Rahmanov *et al*. (2014) the content of potassium in the leaves of Japanese knotweed ranged 6.27-15.5 g·kg^{-1}, i.e. from 18% to 190% lower than in our study. Significantly higher amounts of potassium were found in the leaves in the EM and EM+manure groups (20.5 g·kg^{-1}, 3.8 g·kg^{-1} higher than in controls, i.e. by 23%), with no statistically significant differences between these two groups.

In this study, average calcium levels in Japanese knotweed were 6.11 g·kg^{-1}, much more than the 0.81 g·kg^{-1} in soybean (*Glycine max*) (Plaza *et al*., 2003). Similar levels for Japanese knotweed are given by Sirka *et al*. (2016), who reported 2.75 to 5.10 g·kg^{-1}. In our study, the highest Ca levels were found in the EM+manure group (26%, i.e. 1.52 g·kg^{-1}, higher than control).

Its average level magnesium in the leaves studied was 3.10 g·kg^{-1}, much higher than in *Reynoutria japonica* (2.0 g·kg^{-1}) in the study Strasil and Kara (2010). The highest Mg levels were found in the group fertilized with manure (22%, i.e. 0.63 g·kg^{-1}, higher than control), probably due to the higher uptake of this element from the fertilizer, which is known to significantly increase Mg levels in the soil.

The mean concentration of sodium in the Japanese knotweed leaves (0.30 g·kg^{-1}) was several times lower than in sodium-rich alfalfa seeds (*Medicago sativa*) and soybeans (*Glycine max*), containing from 1.70 to 2.61 g·kg^{-1} (Plaza *et al*., 2003). According to Rahmanov *et al*. (2014) sodium levels in Japanese knotweed leaves varied from 0.07 to 2.72 g·kg^{-1}, which is confirmed by our research. Fertilizer factors significantly reduced the concentration of sodium in the leaves studied, with the lowest in plants fertilized with manure (10%, i.e. 0.03 g·kg^{-1}, lower than control).

The quality of food depends on the correct proportions of macro- and micronutrients. Calcium intake depends primarily on its ratio to phosphorus and magnesium. A Ca:P ratio higher than one indicates good food quality, while lower than 0.5 indicates poor quality (Ihedioha and Okoye, 2011). When the Ca:Mg ratio in the mammalian diet is higher than 3, it indicates a deficiency of phosphorus and magnesium in food (Majkowska-Gadomska and Wierzbicka, 2008). A Na:K ratio lower than 1 helps to protect health, especially that of the heart and vascular system, and helps control blood pressure (Yusuf *et al*., 2007). All the Japanese knotweed leaves tested met the above-mentioned conditions as good-quality food, and so Japanese knotweed can be considered to be an excellent source of calcium, phosphorus and magnesium in the human diet.

Metals such as zinc, iron, manganese, molybdenum and copper in adequate concentrations are essential for healthy bodily functions, but at high concentrations are likely to act as toxins (Nkansah and Opoku Amoako, 2010). Slightly and moderately acidic soils resulted in an increase in concentrations of the bioavailable and mobile forms of these heavy metals. In our experiment, the concentration of metals in the leaves of Japanese knotweed (apart from manganese) did not exceed WHO limits for food (Nkansah and Opoku Amoako, 2010).

Compared to the metal levels in *Reynouti x bohemica* (zinc 21.2-49.7 mg·kg^{-1}, iron 191-493.3 mg·kg^{-1}, and manganese 39.6-68.9 mg·kg^{-1}) (Širka *et al*., 2016), the Japanese knotweed leaves studied had similar Zn (36.9 mg·kg^{-1}), lower Fe (50.8 mg·kg^{-1}) and higher Mn (233.8 mg·kg^{-1}) levels.

Compared to *Corylus avellana* L. nuts, which are considered a good source of micronutrients (Zn – 24 mg·kg^{-1}, Fe – 75.3 mg·kg^{-1}, and Mn – 127.2 mg·kg^{-1}), the Japanese knotweed leaves studied had 34% less Zn, 48% more Fe and 45% less Mn (Cosmulescu *et al*., 2013). The highest micronutrient levels were observed in the EM+manure group. The level of zinc was higher by 17.6 mg·kg^{-1} (60%), of Fe by 14.1 mg·kg^{-1} (30%) and of Mn by 160 mg·kg^{-1} (90%) than in the control. The Mn levels in the leaves of plants grown on EM and EM+manure exceeded the WHO limit for manganese (200 ppm) in medical plants (Raouf *et al*., 2014).

The plants' molybdenum content depends primarily on the soil properties and the genotype. We found no significant effect of the applied agrotechnical factors on molybdenum content in the Japanese knotweed leaves studied.

The average concentration of copper in the Japanese knotweed leaves was 1.59 mg·kg^{-1} (below the WHO limit of 50 mg·kg^{-1}), which is not confirmed in the studies Dassonville *et al*. (2007) who found a concentration of Cu several times higher in the leaves of *Fallopia japonica*. In our study, the highest Cu levels were found in the leaves of the plants grown on EM (3%, i.e. 0.05 mg·kg^{-1} higher than control).

The uptake of metals by plants depends on a number of factors, including the chemical and physical properties of the soil, plant genotype, and the humidity and climatic conditions in the area. Rahmanov *et al*. (2014) showed that *Reynoutria japonica* leaves contained from 0.17 to 5.28 mg·kg^{-1} cadmium, 0.87 to 9.77 mg·kg^{-1} lead, and 0.47 to 1.64 mg·kg^{-1} nickel. No traces of cadmium were found in the samples. We did find small amounts of lead in the control and EM leaves, but they did not exceed WHO food limits (100 mg·kg^{-1}) (Nkansah and Opoku Amoako, 2010). The leaves of plants growing in soil fertilized with EM had 67%

of the lead in the control (i.e. 0.16 mg·kg^{-1}). According to WHO, the limit for nickel in food is 50 mg·kg^{-1} (Nkansah and Opoku Amoako, 2010). In the leaves of the plants fertilized with manure and EM, the nickel level was 1.17 mg·kg^{-1}. The remaining samples did not show any traces of this metal.

The increased concentrations of phosphorus, potassium, calcium, zinc, iron, manganese and copper in the leaves of plants fertilized with EM or EM+manure were probably due to the beneficial effect of the added microorganisms, resulting in the accelerated decomposition of the organic matter and enhanced access to nutrients (Hussain *et al*., 2002). This confirms Javaid and Bajwa (2011) who reported that EM application with manure increased the concentration of minerals in mung beans. Similarly, Kleiber *et al.* (2014) recorded a statistically significant tendency to increased potassium and calcium concentrations in tomato leaves after EM application. Similar effects of EM application were also found for cotton, soybean and apple (Khaliq *et al*., 2006; Sahain *et al*., 2007; Singh, 2007).

***Conclusions*:** The use of manure and effective microorganisms (EM) had a beneficial effect on the chemical composition of Japanese knotweed leaves. The EM, manure, and EM+manure study groups had significantly higher levels of crude fat, crude ash, phosphorus, potassium, calcium, sodium, zinc, iron, manganese, and molybdenum. This opens up a possibility of reducing the use of artificial fertilizers in agriculture, especially in the cultivation of plants for food and the pharmaceutical industry. Due to the high content of nutrients, Japanese knotweed leaves can be used as an alternative source of components used in the production of functional foods and pharmaceutical preparations.

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