

Effects of serpentinite fertilizer on the chemical properties and enzyme activity of young spruce soils**

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A b s t r a c t. The experimental plots used in the study were located in the middle forest zone (elevation: 900-950 m a.s.l.) on two nappes of the flysch Carpathians in southern Poland. The aim of this study was to assess the effects of serpentinite in combination with nitrogen, phosphorus, and potassium fertilizers on selected chemical properties of the soil and activity of dehydrogenase and urease in the studied soils. All fertilizer treatments significantly enriched the tested soils in magnesium. The use of serpentinite as a fertilizer reduced the molar ratio of exchangeable calcium to magnesium, which facilitated the uptake of magnesium by tree roots due to competition between calcium and magnesium. After one year of fertilization on the Wisła experimental plot, the pH of the Ofh horizon increased, while the pH of the mineral horizons significantly decreased. Enrichment of serpentinite with nitrogen, phosphorus, and potassium fertilizers stimulated the dehydrogenase activity in the studied organic horizon. The lack of a negative effect of the serpentinite fertilizer on enzyme activity in the spruce stand soil showed that the concentrations of the heavy metals added to the soil were not high enough to be toxic and indicated the feasibility of using this fertilizer in forestry.

K e y w o r d s: spruce stand soil, fertilizer use, chemical properties, dehydrogenase activity, urease activity

INTRODUCTION

In recent years in Europe, spruce mortality has been a serious problem in forestry and this key problem occurs on both sides of Poland southern border. The highest mortality rates have been recorded in the Silesian Beskid Range

and Żywiec Beskid Range (Błońska *et al.*, 2015; Januszek *et al.*, 2011; Małek *et al.*, 2012); they were related to magnesium deficiency in the soil and tree needles (Januszek, 2006; Małek, 2010; Šrámek *et al.*, 2010). Magnesium deficit symptoms have also been noted in other forested areas (Jandl *et al.*, 2001; Landmann *et al.*, 1997). The importance of Mg in crop production has been underestimated in the last decades (Cakman and Yazici, 2010; Gransee and Führs, 2013). Dolomite fertilization of spruce soils in the Höglwald in southern Bavaria increased significantly and permanently only the Ca concentration in needles, but not so much the concentration of Mg, although the amounts of Ca and Mg in the dolomite were the same (Huber *et al.*, 2006). The ‘antagonism’ between Ca and Mg explains the negative effect of liming on plant growth. The harmful effects of high doses of lime on the plant yield cause imbalance between Ca and Mg in the soil (Gorlach and Gorlach, 1983). The correct ratio of Ca:Mg in the soil and in plants may be an important factor for optimal plant growth. Serpentinite rock is mainly composed of Mg silicate, but it also contains heavy metals, mainly nickel and chromium. Compared with carbonate rocks such as dolomite, serpentinite reacts more slowly. Serpentinite fertilization with N, P, and K fertilizer may improve the condition of dying spruce stands (Małek *et al.*, 2011), as it enriches the soil and needles with Mg, P, K, and N; with an increase in organic matter mineralization and plant yield, it also affects the chemical and biological properties of the soil (Melero *et al.*, 2006). The assessment of soil conditions is essential to monitor the stability of forest ecosystems.

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Soil chemical properties change slowly over time with drastic environmental changes, whereas biochemical properties such as enzyme activity react quickly to changes in the environment, as they are directly related to the number and activity of soil microorganisms (Trasar-Cepeda *et al.*, 2000; Zhang *et al.*, 2015). Studies have shown that soil enzyme activity decreases significantly with increasing contamination with heavy metals, and this is especially true of dehydrogenase and urease activity (Chen *et al.*, 2005). In addition, enzyme activity is sensitive to soil changes due to tillage, use of cropping systems, and land use (Acosta-Martínez *et al.*, 2007). The determination of soil enzyme activity can also provide insight into potential nutrient transformation (Tabatabai and Dick, 2002). Many researchers have studied the effects of chemical-type fertilization on overall soil fertility by investigating the level of soil enzyme activity (Liu 2004; Gianfreda and Ruggiero, 2006; Nannipieri *et al.*, 2012). Mijangos *et al.* (2006) and Ajwa *et al.* (1999) showed that biological parameters were sensitive to early changes in soil properties induced by different land management strategies and types of fertilization. The same pattern of sensitivity was not observed for conventional chemical parameters. Dehydrogenase activity is an indication of microbial activity in soil and has been used to assess the impact of management on soil quality, and the degree of recovery of degraded soils (Gil-Sotres *et al.*, 2005). These researchers argue that urease activity should be used as an indicator of changes in soil quality due to fertilization.

The aim of this study was to assess the effect of serpentinite fertilization enriched with N, P, and K fertilizers on selected chemical properties of the soil and the activity of dehydrogenase and urease in the soil. Another goal was to evaluate the impact of heavy metals introduced with the fertilizer on soil properties and the feasibility of using serpentinite as a magnesium fertilizer. In the study, we have hypothesized that: 1) fertilization improves the chemical properties as well as the level of enzyme activity of the soil 2) heavy metals, especially nickel, introduced with the fertilizer, do not produce a negative impact on the level of enzyme activity.

MATERIAL AND METHODS

The study was conducted in southern Poland in the Ujsoły and Wisła Forest Districts (Coordinates: Wisła – 49°38'12.92 N 18°58'56.36 E; Ujsoły – 49°24'58.02 N 19°10'18.18 E). Experimental plots were selected following an analysis of site conditions including geology, soil type, and forest stand characteristics. The plots were set up in two Carpathian areas:

- Magura Nappe region, on thin-bedded sandstone with some clayey-marly slate inclusions and a clayey waste-mantle with meso/eutrophic soils, characterized by a high Ca to Mg ratio – also known as the Ujsoły Forest District;

- Silesian Nappe on Barania Góra Mountain, on lower Istebna layers, consisting of thick-bedded sandstone and conglomerate and a sandy-clayey waste-mantle with oligotrophic soils, characterized by Mg deficiency – also known as the Wisła Forest District.

Haplic Podzol soils formed of very stony, coarse sandy loam stratiform of very cobbly sandy loam with mor humus was noted on the experimental plots in Wisła. The Endoeutric Cambisol built of loam deposition on very cobbly clay loam and very cobbly silty-clay loam with the moder type of humus was noted on the experimental plots in Ujsoły.

The experimental plots were located in the middle forest zone among spruce stands in the 2nd age category (21-40 years). The experiment was carried out on 10x10 m plots, with 5 m insulating strips between the plots. The plots were treated with six different types of fertilizer and each treatment was repeated three times. Serpentinite was added in the autumn of 2008, and the other fertilizers in the spring of 2009. The six fertilizer treatments used were as follows: (C) control plot – no fertilizer, (S) ground serpentinite (2000 kg ha⁻¹ in the Ujsoły Forest District, 4 000 kg ha⁻¹ in the Wisła Forest District), (SN) serpentinite + N (ammonium nitrate: 150 kg N ha⁻¹), (SP) ground serpentinite + P (400 kg of granulated triple superphosphate), (SNP) ground serpentinite + NP (ammonium nitrate ha⁻¹: 150 kg N ha⁻¹ and granulated triple superphosphate ha⁻¹: 80 kg P ha⁻¹), (SNPK) ground serpentinite + NP (formulated above) + K (potassium sulphate: 110 kg K ha⁻¹). The serpentinite used in the study contained the following macro- (%) and micro-elements (mg kg⁻¹): C: 1.67; N: 0.001; S: 0.011; Na: 0.009; K: 0.006; Ca: 0.39; Mg: 18.10; Fe: 5.77; Mn: 621.5; Zn: 38.4; Cu: 11.5; Cd: 1.96; Ni: 1,673.21; Pb: 1.25; Cr: 131.5.

Samples for laboratory testing were collected from the organic horizon, the first mineral horizon down to 20 cm (AE or AB), and the second mineral horizon (from 20 to 35 cm – horizon B). Four subsamples were collected from each plot and were thoroughly mixed to give a composite soil sample. The samples were collected in August in 2009 and 2010. Enzyme activity was then determined for samples taken from the first two horizons. Dehydrogenase and urease activity was determined for samples collected in August 2009 and August 2010. Enzyme activity was determined by taking a naturally moist sample, sieving it through a sieve (Ø 2 mm), and storing it at 4°C prior to analysis. Samples designated for the analysis of chemical properties were first dried at room temperature to an air-dry condition, and were then sieved. The analysis of these samples included (Carter and Gregorich, 2006; Ostrowska *et al.*, 1991):

- soil pH in H₂O and 1M KCl solution, determined potentiometrically;
- exchangeable aluminium (H_{Al}) determined with the Sokołow method;

- hydrolytic acidity (Y) determined using the Kappen method;
- total nitrogen and carbon content determined using a LECO analyser, which calculates the C/N ratio;
- alkaline cation content in 1M ammonium acetate with calculation of effective cation exchange capacity (T_e) and the degree of soil saturation with alkaline cations ($V\%$);
- available phosphorus with the Bray-Kurtz method;
- Cd, Cr, Cu, Ni, Pb, and Zn content in 1M HCl solution (ICP OES iCAP 6000 DUO, Thermo Fisher Scientific, Cambridge, UK);
- manual calculation of (Ca+Mg+K):Al and Ca:Mg ratios.

Dehydrogenase activity was determined according to the Casida procedure (Alef and Nannipieri, 1995) and expressed in mg of triphenyl formazan (TPF) on 100 g of soil released per 24 h. The TTC method uses 3% of triphenyl-tetrazolium chloride (TTC) as a substrate. The resulting formazan was extracted from the soil with ethyl alcohol and methanol (Alef and Nannipieri, 1995). Urease activity was determined using the Tabatabai and Bremner method (1972) with water solution as a substrate. The level of activity was determined by the amount of NH_4^+ that was released after 2h at 37°C (Alef and Nannipieri, 1995). Each determination of the level of enzyme activity was repeated three times.

Statistical data analysis was performed using Statistica 10 software. In order to reduce the number of variables in the statistical dataset and visualize the multivariate dataset, principal component analysis (PCA) was used in the process. PCA was also used to interpret factors dependent on the dataset type. Chemical properties and enzyme activity levels were used as inputs in the PCA analysis. Differences between mean values were evaluated using the nonparametric Kruskal-Wallis test. The statistical significance of the results was verified at a significance level of $\alpha = 0.05$.

RESULTS

The pH of organic horizons (Ofh and Oh) was increased by the fertilizer treatments (S, SN, SP, SNPK, SNP) compared with the control sample (C) one and two years following the fertilization (Tables 1 and 2). The maximum difference of 0.82 pH units in the mean pH in H_2O was observed for the Oh horizon of soil in the Ujsoly plot between the SNP variants and control sample (C) treatments in 2009. In the B horizon of soils obtained from the Wisła plot, pH was lower in the fertilizer variants in relation to the control sample. The pH in H_2O of the B horizon with the S, SP, SNP, and SNPK treatments applied to the Ujsoly plot was higher than that for the control sample. The concentrations of exchangeable aluminium in the Ofh horizon of fertilized soil sampled at the Wisła plot and in all the horizons of most fertilized soils sampled at the Ujsoly plot were lower compared with the Al^{3+} concentration noted

for respective control soil samples (Tables 1 and 2). The largest decrease was noted for the Al^{3+} concentration in the AB horizon of the SP fertilized plots in the Ujsoly study area in 2009 ($7.57 \text{ cmol}(+) \text{ kg soil}^{-1}$) (Table 1). In the AE and B horizons of all fertilized soils from Wisła in 2009-2010, except those with SN, the Al^{3+} concentrations were higher than those for the AE and B horizons of control soils (Tables 1 and 2). The Al concentration more than doubled in the B horizon with the S treatments in 2009 compared with that in the B horizon of the control soils (Table 1). In less than one year following the application of serpentinite, the Al concentration increased about $7.14 \text{ cmol}(+) \text{ kg soil}^{-1}$. The difference in the concentration of exchangeable Al in the B horizon between the S and C treatments in 2010 was smaller at $3.78 \text{ cmol}(+) \text{ kg soil}^{-1}$. The tested soils were significantly enriched in Mg following the fertilization (Table 3); thus, the molar ratio of exchangeable Ca to Mg decreased. The mean value of the ratio of Ca, Mg, K : Al increased following the fertilization, particularly in the year 2010, when the highest concentrations of exchangeable Mg were noted (Table 3). In the organic horizon, both study plots exhibited significant differences in the ratio of Ca to Mg and values of Ca, Mg, K : Al compared with the control sample (Table 3). In the case of the SN treatments, a tendency towards loss of exchangeable potassium from the soil was noted. Effective improvement in the supply of available phosphorus was observed as a result of the use of the superphosphate fertilizer. One year following the fertilization of the Ofh horizon, the phosphorus content increased in the fertilizer variants with P in relation to the control sample. A substantial share of the nickel released in the course of the weathering of serpentinite delivered to the soil is absorbed in the surface horizon, and only small amounts enter the full soil profile (Table 3). A significant difference (<0.005) in soil properties (pH, Y, Al^{3+} , V_e , N, Ni content) was noted in the organic horizon compared with the control sample (Tables 1 and 2).

The activity of dehydrogenase in the organic horizon on the Wisła plot increased in 2009 compared with the control sample (C) in the case of the S and SN fertilization (Table 1). In 2010, the activity of dehydrogenase in the organic horizon increased in the case of all variants of fertilization in relation to the control sample – except for the SNP fertilization (Table 2). The activity of dehydrogenase in the AE horizon on the Wisła plots increased in 2009 in the case of the SP fertilization compared with the control sample (Table 1). The activity of dehydrogenase in the AE horizon increased in 2010 in the case of all the fertilization treatments compared with the control sample (Table 2). The activity of dehydrogenase in the organic horizon increased in 2009 on the Ujsoly plots compared with the control plot in the case of the SN, SNP, and SNPK fertilization (Table 1). The activity of dehydrogenase in the organic horizon decreased in 2010 in the case of all the plot fertilization treatments in relation to the control sample (Table 2). The

Table 1. Mean values of selected properties of the studied soils one year after fertilization

Horizon	Type of fertilization	pH		Y	Al ³⁺	V _c (%)	Total (%)		Cr	Ni	DH	UR
		H ₂ O	KCl	cmol(+) kg ⁻¹	C		N	(mg kg ⁻¹)				
Wisła												
Ofh	C	3.50a	2.71	90.48	21.35	10.46a	29.50	1.30	0.52	2.38a	40.0ab	0.49
	S	3.71ab	2.81	95.12	20.60	19.31ab	29.05	1.33	0.20	6.35ab	62.6b	0.44
	SN	3.74b	2.75	100.68	20.79	17.73ab	31.68	1.41	0.16	5.63ab	42.9ab	0.43
	SP	3.70ab	2.83	95.42	18.20	25.52b	31.56	1.39	0.20	7.12b	30.4 ab	0.55
	SNP	3.75b	2.82	89.42	17.83	24.89b	28.99	1.28	0.10	6.29ab	33.9 ab	0.26
	SNPK	3.55ab	2.71	108.68	21.65	20.76ab	32.88	1.40	0.18	7.60b	10.5 a	0.43
AE	C	3.66	2.71	16.06	6.24	4.11	3.57	0.17	1.29	1.06	23.9	0.19
	S	3.50	2.79	22.59	8.66	4.56	4.02	0.20	0.77	0.28	17.6	0.17
	SN	3.57	2.72	16.54	5.21	5.65	4.20	0.19	0.13	0.15	23.3	0.67
	SP	3.62	2.82	18.68	9.86	3.99	3.97	0.18	0.66	0.54	28.5	0.34
	SNP	3.50	2.79	19.66	7.51	7.80	4.12	0.18	0.48	0.23	20.7	0.15
	SNPK	3.45	2.63	21.82	9.83	5.17	4.53	0.21	0.30	0.29	18.3	0.20
B	C	4.21	3.26	9.23	6.43	2.65	n.d.	n.d.	3.12	2.76	n.d.	n.d.
	S	3.89	3.17	18.85	13.57	1.88	n.d.	n.d.	1.61	0.24	n.d.	n.d.
	SN	3.88	3.05	11.79	6.11	3.45	n.d.	n.d.	1.29	0.71	n.d.	n.d.
	SP	3.71	3.11	16.54	11.19	2.36	n.d.	n.d.	1.36	0.21	n.d.	n.d.
	SNP	3.81	2.95	14.20	8.40	3.37	n.d.	n.d.	0.89	0.10	n.d.	n.d.
	SNPK	3.64	3.18	16.53	11.30	3.19	n.d.	n.d.	1.31	0.73	n.d.	n.d.
Ujsolý												
Oh	C	4.35a	3.41a	58.44b	11.60b	45.99a	22.65	1.01b	2.12	6.38a	111.7a	0.95
	S	5.12c	3.91abc	29.00a	4.01a	75.98bc	12.34	0.62a	1.60	9.44ab	99.8a	0.71
	SN	4.53ab	3.69ab	46.76ab	9.57b	58.69ab	18.69	0.94ab	1.30	7.24a	146.8ab	0.59
	SP	5.07c	4.27c	31.16a	2.94a	85.39c	15.45	0.74ab	2.07	9.26ab	84.5a	0.76
	SNP	5.17c	4.25bc	27.84a	3.90a	78.54c	14.92	0.68ab	2.97	13.66bc	132.0ab	0.62
	SNPK	4.96bc	4.03bc	42.20ab	4.48a	78.13c	20.01	0.92ab	2.02	16.35c	177.7b	0.73

Table 1. Continuation

Horizon	Type of fertilization	pH		Y	Al ³⁺	V _e (%)	Total (%)		Cr	Ni	DH	UR
		H ₂ O	KCl	cmol(+) kg ⁻¹	C		N	(mg kg ⁻¹)				
AB	C	4.46ab	3.46	25.86ab	12.79	22.67	5.67	0.35	3.43	5.42	43.5	0.42
	S	4.75ab	3.64	18.50ab	8.27	37.82	5.06	0.32	2.66	4.91	44.1	0.64
	SN	4.30a	3.50	28.09ab	11.90	25.16	6.59	0.43	2.27	4.17	33.2	0.48
	SP	4.91b	3.88	16.02a	5.22	57.59	4.99	0.31	2.10	4.38	44.1	0.59
	SNP	4.83ab	3.90	16.47a	5.23	58.14	5.18	0.31	2.99	5.55	44.7	0.50
	SNPK	4.45ab	3.59	32.57b	8.01	43.93	4.99	0.33	1.70	3.12	42.6	0.43
B	C	4.72	3.67	14.80	11.61	14.05	n.d.	n.d.	4.12	4.32	n.d.	n.d.
	S	5.04	3.81	10.14	6.92	32.86	n.d.	n.d.	3.47	3.70	n.d.	n.d.
	SN	4.65	3.69	13.99	10.85	13.68	n.d.	n.d.	3.30	3.37	n.d.	n.d.
	SP	5.16	3.84	9.89	5.64	44.17	n.d.	n.d.	2.57	3.18	n.d.	n.d.
	SNP	4.94	3.78	11.73	7.25	31.64	n.d.	n.d.	3.28	3.26	n.d.	n.d.
	SNPK	5.42	4.37	12.76	9.90	20.45	n.d.	n.d.	2.23	2.40	n.d.	n.d.

C – control plot – no fertilizer used, S – ground serpentinite, SN – serpentinite + nitrogen, SP – ground serpentinite + P, SNP – ground serpentinite + NP, SNPK – ground serpentinite + NP + K, Y – hydrolytic acidity, Al³⁺ – exchangeable aluminum, V – saturation of base cations, DH – dehydrogenase activity ($\mu\text{M TPF kg}^{-1} \text{h}^{-1}$), UR – urease activity ($\text{mM N-NH}_4 \text{ kg}^{-1} \text{h}^{-1}$), different lower case letters in the upper index of mean values indicate significant differences.

activity of dehydrogenase in the AB horizon decreased in 2009 on the Ujsoły plots compared with the control sample in the case of the SN and SNPK fertilization (Table 1). The activity of dehydrogenase in the AB horizon increased in 2010 in the case of all the fertilization treatments in comparison with the control sample. The largest increase was observed in the case of the SNP fertilization (Table 2).

Urease activity in the Ofh horizon decreased in 2009 on the Wisła plots compared with the control plot in the case of all the fertilization treatments except the SP fertilization (Table 1). In 2010, urease activity did not increase in the organic horizon for any variant of fertilization. In 2009, urease activity decreased in the AE horizon on the Wisła plots, in comparison with the control sample, in the case of the S and SNP fertilization treatments, and increased for the SN, SP, and SNPK fertilization treatments (Table 1). In 2010, urease activity in the AE horizon increased only for the SNPK variant of fertilization. In 2009, urease activity in the Oh horizon on the Ujsoły plots decreased, compared with the control plot, for all of the fertilization treatments employed (Table 1). In 2009 and 2010, urease activity in the AB horizon on the Ujsoły plots increased, compared with the control sample, in the case of all the fertilization treatments (Tables 1 and 2). Significant differences were

noted in the organic horizon on the Wisła plot as well as in the organic horizon and AB horizon on the Ujsoły plot in terms of dehydrogenase activity (Tables 1 and 2).

The effects of fertilization on the tested surfaces are presented via principal component analysis (PCA) (Figs 1 and 2). The uniqueness of the plots analysed in the experiment in Wisła and Ujsoły in terms of chemical properties was confirmed by the analysis. The soil in the Ujsoły study area is characterized by a higher pH, abundance of alkaline cations, a broad range of Ca:Mg ratios, and a smaller supply of organic matter. The control plot results, compared with the fertilized plots, confirm the effects of the fertilizer use. The variants with the phosphorus fertilization (SP, SNP, SNPK) resulted in a noticeable increase in the content of magnesium and phosphorus in the organic horizon in the first year following the fertilization. The increase in dehydrogenase and urease activity was readily observable for the plots in the Ujsoły area and was also visible in the case of all the fertilization treatments used. A strong correlation between dehydrogenase activity and pH as well as exchangeable calcium content (in particular) was detected (Figs 1 and 2). A positive correlation between dehydrogenase activity and nickel content in the organic horizon of fertilized soils was also noted (Table 4).

Table 2. Mean values of selected properties of the studied soils two years after fertilization

Horizon	Type of fertilization	pH		Y cmol(+)-kg ⁻¹	Al ³⁺	V _c (%)	Total (%)		Cr (mg kg ⁻¹)	Ni	DH	UR
		H ₂ O	KCl				C	N				
Wisła												
Ofh	C	3.60a	2.83a	88.59	15.68b	22.83a	32.67	1.40	1.00b	3.93a	72.3a	0.44b
	S	3.97b	3.07b	80.61	13.67ab	41.54b	32.41	1.38	0.95ab	18.43b	113.4b	0.37ab
	SN	4.08b	3.13b	77.72	10.17a	51.93b	32.96	1.32	0.82ab	21.88b	85.23ab	0.44b
	SP	3.99b	3.08b	78.38	12.74ab	43.83b	32.55	1.36	0.68ab	18.53b	107.4b	0.36ab
	SNP	4.12b	3.18b	77.05	9.87a	55.24b	35.84	1.43	0.79ab	19.22b	64.3a	0.36ab
	SNPK	3.97b	3.06b	86.12	11.50ab	49.11b	35.11	1.44	0.41a	17.68b	79.3a	0.27a
AE	C	4.00	3.09	12.15	5.95	5.07	2.23a	0.11a	6.15	5.48	15.7	0.15
	S	3.85	3.00	17.76	10.85	6.82	3.69ab	0.16ab	4.50	3.63	16.7	0.13
	SN	3.85	3.02	14.36	5.61	7.43	4.28b	0.19ab	8.08	6.56	16.4	0.12
	SP	4.27	3.28	13.41	7.10	5.82	3.02ab	0.14ab	19.75	15.88	16.0	0.13
	SNP	3.79	2.92	17.83	7.49	7.97	4.21b	0.20b	1.29	1.58	21.1	0.14
	SNPK	3.79	2.90	17.98	11.13	4.31	3.92ab	0.21b	3.82	3.83	19.8	0.17
B	C	4.06	3.22	12.55	7.77	3.27	n.d.	n.d.	4.79	4.05	n.d.	n.d.
	S	3.97	3.11	16.02	11.65	4.14	n.d.	n.d.	3.72	2.85	n.d.	n.d.
	SN	4.01	3.21	10.09	5.75	4.55	n.d.	n.d.	3.00	3.00	n.d.	n.d.
	SP	4.08	3.19	16.95	12.12	2.52	n.d.	n.d.	2.94	2.42	n.d.	n.d.
	SNP	4.01	3.13	11.37	9.41	3.65	n.d.	n.d.	1.44	1.37	n.d.	n.d.
	SNPK	3.96	3.12	13.25	13.53	2.53	n.d.	n.d.	1.57	1.30	n.d.	n.d.
Ujszóló												
Oh	C	4.35a	3.53a	71.24c	5.18b	74.47a	32.04b	1.33b	1.74	5.11a	241.4b	0.53a
	S	5.00b	4.24b	42.50ab	2.29ab	86.52ab	23.66a	0.94a	3.41	23.50b	190.5ab	0.77ab
	SN	4.81ab	4.07ab	51.44b	1.44a	86.28ab	28.48ab	1.16b	1.83	29.48b	146.8a	0.61ab
	SP	5.16b	4.44b	34.58a	1.40a	92.76b	26.16a	1.01a	2.37	26.40b	214.3ab	0.89b
	SNP	4.97b	4.18b	46.57ab	2.92ab	80.13ab	23.96a	0.97a	2.67	24.79b	183.5ab	0.71ab
	SNPK	4.96b	4.14b	44.53ab	3.50ab	82.06ab	25.73a	0.99a	1.94	22.83b	216.9ab	0.67ab

Explanation as in Table 1.

Table 2. Continuation

Horizon	Type of fertilization	pH		Y	Al ³⁺	V _e (%)	Total (%)		Cr	Ni	DH	UR
		H ₂ O	KCl	cmol(+) kg ⁻¹	C		N	(mg kg ⁻¹)				
AB	C	4.47a	3.49a	26.80c	10.71b	26.48a	6.84	0.40	2.50	2.36a	43.7a	0.17a
	S	4.85bc	3.75ab	19.15ab	5.10a	53.80ab	5.98	0.37	2.10	15.54b	62.6ab	0.35b
	SN	4.65ab	3.62a	21.43abc	7.70ab	45.79ab	6.84	0.41	2.83	5.33ab	44.8a	0.24ab
	SP	5.08c	3.89b	15.17a	2.89a	74.38b	5.98	0.36	2.39	5.34ab	63.7ab	0.33ab
	SNP	4.73abc	3.61a	22.46bc	4.44a	64.70b	7.29	0.43	3.00	4.70ab	74.0b	0.26ab
	SNPK	4.67ab	3.61a	23.24bc	6.86ab	45.14ab	6.87	0.40	2.14	2.50a	51.9ab	0.18a
B	C	4.88	3.75	15.5	0.43	22.00	n.d.	n.d.	2.67	1.27	n.d.	n.d.
	S	5.21	3.87	11.89	0.10	43.15	n.d.	n.d.	3.75	3.74	n.d.	n.d.
	SN	4.89	3.76	4.62	0.16	20.97	n.d.	n.d.	3.00	3.00	n.d.	n.d.
	SP	5.03	3.82	12.02	0.30	31.76	n.d.	n.d.	3.53	2.68	n.d.	n.d.
	SNP	4.81	3.74	14.74	0.13	16.40	n.d.	n.d.	3.47	2.62	n.d.	n.d.
	SNPK	4.72	3.76	14.23	0.07	19.97	n.d.	n.d.	2.80	0.98	n.d.	n.d.

PCA analysis performed on the basis of data acquired via experimental means over a period of two years after the fertilization suggests a stronger effect of the fertilization on the studied soil properties. This was confirmed by a clear distinction between the fertilization treatment data compared with data for the control plot. Soil samples obtained from the organic horizon two years following the fertilization differ mainly in the content of magnesium, nickel, and to a lesser degree phosphorus. The effect on pH and increases in the concentration of calcium as well as increases in the level of activity of dehydrogenase and urease are more strongly reflected in the Ujsoly plots (Fig. 1).

DISCUSSION

It seems that the selection of serpentinite to fertilize the studied soils improves their conditions. Higher concentrations of magnesium as well as a larger percentage of magnesium in the sorption complex were detected for each of the fertilizer treatments, compared with the control plot. The selected dose for serpentinite (2 000 kg ha⁻¹) proved to be correct in the case of the Ujsoly plot. No adverse effects were noted for the fertilized soils of Ujsoly. A negative effect was noted following the application of a much larger dose on the Wisła plot (4 000 kg ha⁻¹): acidifying fertilizers (potassium sulphate: 226 kg ha⁻¹) and ammonium nitrate (440 kg ha⁻¹). The soil pH increased in all soil horizons on the Ujsoly plot compared with the control plot in the case

of all the fertilization treatments used. The highest pH was observed in soil in the case of the S and SP fertilization treatments. A decrease in pH and an increase in the concentration of exchangeable aluminium were noted in the AE and B horizons following the fertilization on the Wisła plot. The maximum decrease in pH in H₂O recorded in the B horizon in 2009 (0.57 pH units from a pH of 4.21 for the control variant to a pH of 3.64 for SNPK) corresponds to the 3.7 times larger concentration of the hydrogen ion in one litre of solution in the B horizon of the SNPK variant compared with the control variant. In the AE horizon, this difference was less pronounced (0.21 units, from pH = 3.66 to 3.45) and corresponded only to the 1.6 times larger concentration of the hydrogen ion in one litre of solution in the AE horizon of SNPK compared with the soil in the AE horizon of the control sample used. The decrease in pH and the increase in the concentration of aluminium in the upper mineral horizons (AE and B) in 2009 and 2010 were the result of illuviation by rainwater-displaced acid cations from the sorption complex, hydrogen, and aluminium by magnesium, potassium, and ammonium released from the fertilizers introduced. The acidity of the upper mineral horizons induced by the use of dolomite was previously described in the literature (Kreutzer, 1995; Lundström *et al.*, 2003), but it was not described for the use of a silicate fertilizer rich in alkaline cations. Therefore, lower doses of serpentinite should be used, especially when the serpentinite

Table 3. Mean trace element content and molar ratios for the studied soils one year and two years after fertilization

Type of fertilization	Ca	K	Mg	Na	P	$\frac{(Ca+Mg+K)}{Al}$	Ca:Mg	Ca	K	Mg	Na	P	$\frac{(Ca+Mg+K)}{Al}$	Ca:Mg	
	(mg 100 g ⁻¹)							(mg 100 g ⁻¹)							(mg kg ⁻¹)
2009								2010							
Wisła															
Ofh															
C	31.28a	37.67	7.19a	2.22a	18.40a	0.15a	2.64a	62.03a	41.18	9.97a	2.47	18.43a	0.32a	3.78b	
S	34.12a	37.05	34.84b	2.38ab	21.54a	0.27b	0.59a	70.57a	34.59	79.66b	2.12	16.10a	0.86b	0.54a	
SN	39.36ab	40.25	32.82b	2.02a	20.44a	0.27b	0.73a	77.63a	35.68	88.95b	2.95	15.87a	1.44b	0.53a	
SP	68.08c	46.57	40.95b	3.07b	40.41b	0.44b	1.01a	87.10a	38.67	67.21b	2.44	35.70b	0.87b	0.79a	
SNP	83.34c	33.99	25.71ab	2.09a	42.16b	0.40b	1.97ab	145.17b	39.89	65.04b	3.56	34.30b	1.49b	1.35ab	
SNPK	65.08bc	46.40	33.74b	2.19a	36.40ab	0.33b	1.17a	99.03ab	43.93	74.16b	3.19	28.23b	1.06b	0.81a	
AE															
C	1.91a	4.22	1.03a	0.36	4.51	0.05	1.13	2.57a	3.32	0.90a	0.38	5.95	0.06	1.76	
S	2.25a	4.67	2.07ab	0.51	2.54	0.05	0.66	3.56a	5.04	1.81b	0.62	4.08	0.08	1.18	
SN	1.97a	4.43	1.65ab	0.40	4.17	0.07	0.73	2.77a	3.98	2.45bc	0.62	7.00	0.08	0.69	
SP	3.02a	4.72	1.72ab	0.48	8.05	0.04	1.07	2.53a	4.00	1.85b	0.35	10.15	0.04	0.85	
SNP	5.52b	4.24	2.17b	0.37	8.01	0.07	1.54	6.17b	4.59	2.85c	0.54	9.92	0.09	1.30	
SNPK	3.10ab	6.02	2.06ab	0.67	7.53	0.05	0.91	3.28a	4.54	1.85b	0.66	11.32	0.04	1.07	
B															
C	1.56	2.41	0.53	0.28	6.65	0.03	1.78	1.92	2.94	0.69	0.31	7.23	0.03	1.69	
S	1.42	3.10	0.76	0.40	5.28	0.02	1.14	2.66	4.23	1.11	0.45	28.82	0.03	1.36	
SN	1.62	1.89	0.70	0.23	6.82	0.03	1.40	2.06	2.71	1.04	0.37	13.30	0.04	1.21	
SP	2.01	2.55	0.94	0.38	7.46	0.02	1.29	2.20	3.50	1.30	0.06	13.53	0.03	1.03	
SNP	3.12	1.87	0.96	0.35	10.43	0.03	1.98	3.39	2.82	1.54	0.17	10.38	0.04	1.34	
SNPK	2.42	5.61	1.22	0.34	16.44	0.03	1.20	2.39	3.62	1.28	0.16	18.43	0.02	1.13	
Ujsoty															
Oh															
C	187.45a	26.70ab	15.91a	1.78	19.11a	0.96a	7.02b	330.17	42.99	24.09a	1.79	14.16ab	3.78a	8.32b	
S	228.87ab	22.45a	36.35b	1.91	7.98a	3.68b	3.75a	299.65	41.09	74.44bc	1.06	8.28a	9.66b	2.44a	
SN	257.75ab	30.50ab	34.16b	1.71	9.26a	1.70ab	4.50ab	314.09	38.53	104.40c	1.01	9.96a	17.53c	1.83a	
SP	356.97b	33.80ab	39.82b	9.86	31.38ab	7.36c	5.34ab	426.00	46.23	98.35bc	0.77	18.64abc	20.53c	3.07a	
SNP	316.49ab	26.31ab	37.74b	1.25	64.67b	4.95b	5.00ab	288.03	28.30	54.52a	1.06	26.65c	6.71c	3.21a	
SNPK	299.54ab	34.95b	47.52b	1.56	38.51ab	4.37b	3.78a	301.09	38.35	62.47ab	0.93	24.94bc	6.04c	2.92a	

Explanations as in Table 1.

Table 3. Continuation

Type of fertilization	Ca	K	Mg	Na	P	$\frac{Ca+Mg+K}{Al}$	Ca:Mg	Ca	K	Mg	Na	P	$\frac{Ca+Mg+K}{Al}$	Ca:Mg
	(mg 100 g ⁻¹)							(mg 100 g ⁻¹)						
2009								2010						
AB														
C	58.70	10.82	5.61a	0.65	2.74a	0.28	6.23	56.60a	16.16	7.68a	1.04	1.21a	0.36	4.47
S	75.75	12.22	9.80ab	0.63	2.41a	0.58	4.61	88.46ab	18.61	17.30ab	0.95	1.06a	1.24	3.10
SN	62.37	13.19	9.08ab	0.74	3.73ab	0.35	4.10	90.81ab	15.85	17.69ab	0.88	1.29a	0.83	3.12
SP	123.43	14.38	14.48b	1.02	5.42ab	1.46	5.08	134.26ab	18.43	19.61ab	1.07	3.30ab	3.04	4.15
SNP	126.96	12.01	12.18ab	0.75	11.52b	1.44	6.21	227.53b	33.93	32.52b	2.66	5.35b	3.35	4.25
SNPK	68.24	11.30	8.59ab	0.73	3.00a	0.54	4.77	85.31ab	19.74	11.86ab	0.72	3.21ab	0.84	4.37
B														
C	27.60	8.59	4.02	0.76	1.30	0.16	4.09	43.40	11.52	5.78	0.81	0.34	0.30	4.55
S	50.30	9.85	6.45	0.78	1.47	0.47	4.65	68.50	12.16	7.57	0.76	0.32	0.66	5.49
SN	24.52	7.10	3.80	0.68	1.39	0.16	3.85	38.67	8.04	4.41	0.49	0.28	0.29	5.31
SP	71.43	10.96	9.46	0.77	1.70	0.81	4.50	60.87	9.82	7.07	1.13	0.26	0.45	5.22
SNP	53.13	8.99	6.95	0.74	1.67	0.47	4.55	32.50	6.90	4.17	0.65	0.54	0.20	4.72
SNPK	37.94	9.40	5.74	0.93	1.63	0.26	3.97	30.09	9.57	4.57	0.37	0.45	0.26	4.00

is applied in a mixture with fertilizers causing acidification. Fertilization with serpentinite in combination with nitrogen, phosphorus, and potassium affected the activity of enzymes such as dehydrogenase and urease. In the case of dehydrogenase activity in the O horizon of soils from the Wisła and Ujsoły plots, a marked increase was observed two years after the fertilization. The highest increase in soil dehydrogenase activity was noted after the serpentinite fertilization on the Wisła plots and after the application of the SNPK fertilizer on the Ujsoły plots. The increase in dehydrogenase activity may be due to an increase in the number of microorganisms and microbial activity. According to Xiaoguang *et al.* (2011), an increase in the level of soil enzyme activity in fertilizer-treated soils may be the consequence of both microbial growth and stimulation of microbial activity by enhanced resource availability, as well as due to changes in the makeup of the microbial community. While chemical N fertilizers inhibited soil enzyme activity, P and K fertilizers enhanced it (Yang *et al.*, 2008; Yao *et al.*, 2009). The research results obtained in this study confirmed the above findings. Fertilization in the SN variant poorly stimulated dehydrogenase activity compared with the variant with P and K.

Soil enzymes are involved in biochemical processes and are closely linked with the circulation of nutrients, energy conversion, and environmental quality. The observed change in enzyme activity was caused by the use of the fertilizer, but this was not the only major factor. Fertilization directly affects the pH of the soil, with which dehydrogenase and urease activity is closely correlated. The increase in pH in the surface horizon is a result of fertilization. This affects the recovery of microbial activity and, consequently, leads to an increase in the activity of urease and dehydrogenase – mainly in the O horizon. Dehydrogenase and urease activity remained positively correlated with pH two years after the application of the fertilizer to the soil. The observed increase in dehydrogenase and urease activity with increasing pH confirmed the stimulatory effect of pH on enzyme activity. Rusek (2006) found that dehydrogenase activity decreases with decreasing soil pH. The composition of microbial communities depends on soil pH (Januszek *et al.*, 2015; Lauber *et al.*, 2008). According to Wolińska and Stępniewska (2012), pH affects soil enzyme activity by changing the ionic form of the active site of the enzyme as well as by affecting the substrate of the enzyme. Brzezińska *et al.* (2001) observed maximum

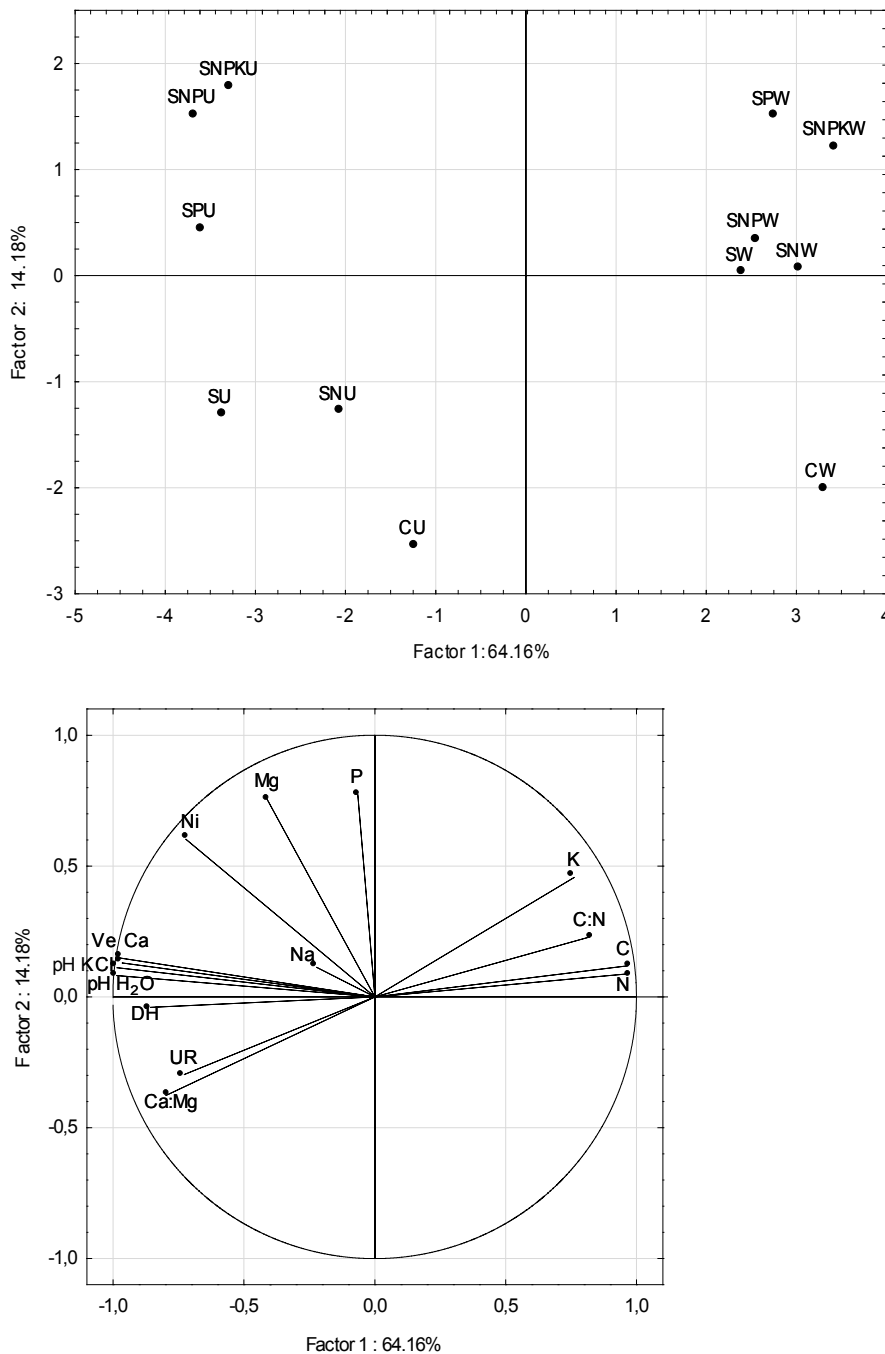


Fig. 1. Factorial plan and projection of variables on the factor-plane 1x2 (effect of fertilization in 2009, organic horizon); CU, SU, SNU, SPU, SNPU, SNP KU – variants of fertilization on the Ujsoły plots; CW, SW, SNW, SPW, SNP W, SNP KW – variants of fertilization on the Wisła plots.

dehydrogenase activity at a pH of 6.6 to 7.2 and found that organic matter was the most important factor significantly affecting the mobility of enzymes (Štursowá and Baldrian, 2011; Xiao-guang *et al.*, 2011). In addition, Nazarkiewicz and Kaniuczak (2008) described a positive effect of the interaction between liming and mineral fertilization on the enzyme activity of dehydrogenase, phosphatase, and protease, including an increase in pH.

The examined organic horizons exhibited the highest level of enzyme activity, which decreased with the depth of the studied soil profiles. The high activity of enzymes in the upper horizons of the soil is associated with the presence of microorganisms and their activity and the content of the organic matter constituting the feeding base. This pattern is linked mainly with the location of humus in the soil and the amount of available carbon substrates for the

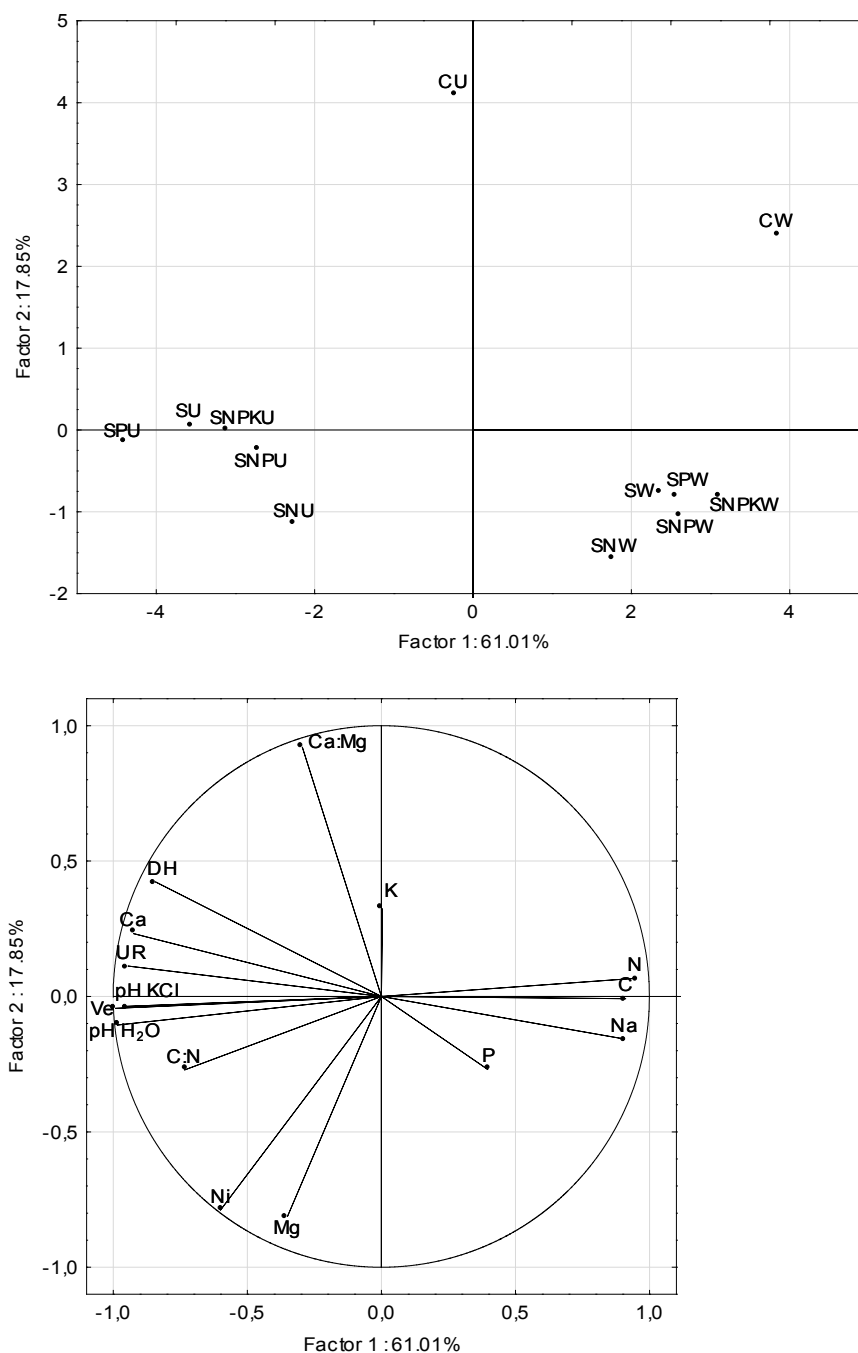


Fig. 2. Factorial plan and projection of variables on the factor-plane 1x2 (effect of fertilization in 2010, organic horizon). Explanations as in Fig. 1.

microorganisms present in the soil as well as the decreasing quantity of enzymes with the increasing depth of the soil profile. The observed decline in enzyme activity in the soil profile is fully consistent with the observations of Eivazi and Tabatabai (1990), Trasar-Cepeda *et al.* (1998), Kandeler *et al.* (1999), and Wang and Lu Qin (2006). Wolińska *et al.* (2015) as well as Veres *et al.* (2013) showed that soil enzyme activity acts as a 'sensor' of soil organic

matter (SOM) decomposition. The SOM decomposition rate depends on substrate properties as well as availability of microorganisms and their enzymes.

Differences in enzyme activity may be related to the morphology of the studied soil profiles. The research plots at the Wisła and Ujsóly sites differ in the type of humus, which may produce a direct effect on enzyme activity. In the soil profile at the Wisła site, there is a larger organic

Table 4. Correlation between types of enzyme activity and soil characteristics

Activity	pH _{H₂O}	pH _{KCl}	Ni
	2009		
DH	0.21	0.20	0.36*
UR	0.26	0.20	0.16
	2010		
DH	0.65*	0.65*	0.35*
UR	0.78*	0.82*	0.09

DH – dehydrogenase activity, UR – urease activity, * $p < 0.05$.

Ofh horizon, while the Oh horizon at the Ujsoły site is shallower. These horizons differ in the rate of decomposition of organic matter and in the rates of various biochemical processes. In addition, the thickness and the degree of decomposition of these horizons are determined by the temperature and moisture in the soil profile. According to Brzezińska *et al.* (2001), Brzostek and Finzi (2012), and Wallenstein *et al.* (2012), temperature and moisture are both important factors influencing enzyme activity in the soil. Adequately high levels of soil moisture determine the level of soil enzyme activity. According to Kubista (1982), the activity of dehydrogenase is closely related to the energy of the metabolism of microorganisms. Kubista (1982) suggests that microbial activity increases in the soil due to an increase in moisture. According to Brzezińska *et al.* (2001), an increase in water content in the soil significantly affects the level of activity of dehydrogenase. Steinweg *et al.* (2012) demonstrated that low soil moisture can strongly limit in-situ enzyme activity in soils.

Urease and dehydrogenase activities react well to the changes caused by fertilization. Mohammadi *et al.* (2012) suggested that urease activity could be used as an indicator of changes in the soil. In the organic and mineral horizons of the Wisła and Ujsoły research plots, urease activity decreased following fertilization. In addition, urease activity became reduced in the years that followed in the case of most fertilization treatments. According to Bhattacharya and Sahu (2013) as well as Liao and Raines (1984), urease activity in the soil may be inhibited by phosphorus fertilization. Hence, a reduction in the rate of the use of nitrogen obtained from urea may occur. Such a pattern was observed in this study in the second year following the fertilization in the A and AE horizons, where the fertilizer treatments with phosphorus inhibited the previously mentioned effect on urease activity. Many studies have shown that soil contamination with heavy metals may lead to a negative impact on ambient soil organisms and, consequently, on the biochemical activity of the soil (Friedlová, 2010; Kandeler *et al.*, 1996). Bielińska (2006) found a negative correlation between the soil content of zinc, lead, and copper and the activity of enzymes – dehydrogenase,

phosphatase, urease, and protease. High levels of heavy metal contamination (Cd and Pb) reduce the number of soil microorganisms and affect nutrient cycling (Chen *et al.*, 2005; Kucharski and Wyszowska, 1998; Sardar *et al.*, 2010). Dehydrogenase activity can be inhibited in the range from 10 to 90%, which depends on the degree of soil contamination (Kucharski and Wyszowska, 1998). According to the work by Wyszowska *et al.* (2008), the activity of dehydrogenase and urease may decrease as a result of the introduction of nickel into the soil – already at a dosage of 100 mg kg⁻¹. In this study, 6.69 kg of nickel were introduced into the soil with four tons per hectare introduced with serpentinite. However, stimulating effects of heavy metals have also been shown in the literature (Bååth, 1989). Metals detected in small amounts in the soil are not harmful and can even stimulate certain enzyme processes. It may be the case that a metal could be one of the components of the catalytic centre and may activate selected enzymes by creating a metal-substrate complex (Balicka and Varanka, 1978). No decrease in the level of urease activity following the fertilization with serpentinite in 2010 was observed and there was a lack of correlation with urease activity; the concentration of nickel in the soil did not inhibit the urease activity of the studied soils. Dehydrogenase activity in the studied O horizon increased within two years of the time of plot fertilization. This may suggest an increase in microbial activity after fertilization using all the treatments provided in the study and an absence of negative effects of the nickel used.

CONCLUSIONS

1. As a result of the use of the fertilizer (ground serpentinite, ground serpentinite + N, ground serpentinite + P, ground serpentinite + NP, ground serpentinite +NPK) the studied soils became significantly enriched in magnesium, and serpentinite contributed to the reduction in the molar ratio of exchangeable calcium to magnesium ions, which should facilitate the uptake of magnesium by the roots of trees due to the competition between calcium ions and magnesium ions. Lower doses of serpentinite (<4 000 kg ha⁻¹) should be used to fertilize light soils with a magnesium deficiency and weakened tree stands. Higher doses of serpentinite increase the level of acidification of the upper mineral horizons.
2. An increase in the supply of phosphorus was observed after the fertilization of all the soil horizons of both experimental plots with superphosphate.
3. Fertilization of the soil using serpentinite in combination with nitrogen, phosphorus, and potassium fertilizer stimulates the activity of dehydrogenase in soil under spruce stands.
4. The positive impact of soil fertilization on dehydrogenase activity is reflected in the organic horizon. Reduced activity of urease was noted in the studied organic horizon fertilized in combination with phosphorus in the years after

the fertilization period, which confirmed that it can reduce urease activity and contribute to inhibition of nitrification in this way.

5. The absence of a negative impact of the serpentinite fertilizer used in the study on the enzyme activity of the studied soils under spruce stands suggests an absence of toxic concentrations of heavy metals in the soil, and further suggests the feasibility of using this type of fertilizer in forestry.

Conflict of interest: The Authors do not declare conflict of interest.

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