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THE AGE OF SHELTERBELTS AS A FACTOR DETERMINING OF ENZYMES ACTIVITY IN THE AGRICULTURAL LANDSCAPE

WIEK ZADRZEWIENIA JAKO CZYNNIK DETERMINUJĄCY AKTYWNOŚĆ ENZYMÓW W KRAJOBRAZIE ROLNICZYM

Abstract: Shelterbelts belong to the stable elements in the agricultural landscape which reduce very successfully the concentrations of many chemical compounds migrating with ground water, restrain the erosion and regulate water regime in soils. The investigations were conducted in General Dezydery Chlapowski Landscape Park (West Polish Lowland). For purpose of this experiment three shelterbelts and adjoining cultivated fields were selected. Two of them were planted 200 years ago. The dominant species in the first shelterbelts is *Robinia pseudacacia* and the second one includes of *Crataegus monogyna*. The third - new shelterbelt was planted in 1993 and consists of several species of plants. An activity of xanthine, urate, and phenol oxidase was investigated. The obtained results have revealed that annual mean activity of xanthine, urate, and phenol oxidase was statistically significant higher in soils under *Robinia pseudacacia* (6.42, 13.23, 14.22 $\mu mol \cdot h^{-1} \cdot g^{-1}$, respectively), *Crataegus monogyna* (4.21, 7.02, 12.79 $\mu mol \cdot h^{-1} \cdot g^{-1}$, respectively) than in the soil under new shelterbelt (2.14, 2.15, 8.05 $\mu mol \cdot h^{-1} \cdot g^{-1}$, respectively) and adjoining cultivated fields. The study was to investigate the influence of the age of shelterbelts on the activity of xanthine, urate, and phenol oxidase.

Keywords: shelterbelts, xanthine oxidase activity, urate oxidase activity, phenol oxidase activity

Introduction

The tradition of environment protection in Wielkopolska region has its roots in the first half of the previous century before the nature protection movement gained prominence in Poland. General Dezydery Chlapowski the owner of Turew estate was the first to introduce mid-field shelterbelts in order to improve the microclimatic conditions of adjoining fields was the pioneer of landscape management. Shelterbelts are multifunctional forms in the landscape, which reduce very successfully the washing out of nutrient compounds, improve the microclimate for agricultural production, regulate of water ratios in soils, limit soil erosion and the spread of chemical non-point pollution in ground water [1].

Enzymes are a good indicator of changes occurring in the soil under the influence of natural and anthropogenic factors. Enzymatic activities provide quantitative information on the functional diversity of microbial activity, soil chemical processes, the rate of mineralization and humification of soil and the accumulation of organic matter. As well as they take an active part in release and made available to plants mineral substances, molecular nitrogen binding, detoxification of xenobiotics, nitrification and denitrification [2, 3].

The aim of our investigations was to investigate the influence of the age of shelterbelts on the activity of xanthine, urate, and phenol oxidase.

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Materials and methods

Soil samples for this experiment were collected in 2012 from three shelterbelts differing in age and species of trees and adjoining cultivated fields in General Dezydery Chlapowski Landscape Park (40 km South-West of Poznan, West Polish Lowland 52° 3′ 32.382″ N, 16° 49′ 10.736″ E). Two of them were created about 200 years ago. The species composition of the first includes *Robinia pseudacacia* with an admixture of *Quercus robur* and *Larix decidua*, the second - *Crataegus monogyna* and the third - 25 years old shelterbelt consists: *Quercus petraea*, *Q. robur*, *Larix decidua*, *Pinus sylvestris*, *Sorbus aucuparia*, *S. intermedia*, and *Tilia cordata*. These soils were classified as Luvisols (FAO classification) consisting of loamy sands and boulder clays. Soil samples were taken from a depth of 0-20 cm with 10 places under shelterbelts and places located 100 meters from shelterbelts occurring on adjacent cultivated fields. Next, the roots and stones were removed and air-dry the aggregated mineral particles were sieved by 1 mm mesh sieve.

In the soil samples were analyzed: pH (in 1M KCl), bulk density [4], contents of total phenolic - colorimetrically by Folin-Ciocalteu reagent [5], total organic carbon (TOC) and dissolved organic carbon (DOC) - Total Organic Carbon Analyzer 5050A [6], total nitrogen (*TN*) - Kjeldahl method, ammonium ions ($N\text{-}NH_4^+$) - ion chromatograph Waters 1515, and nitrate ions ($N\text{-}NO_3^-$) - ion chromatograph HIC-6A [7]. In investigation soils, moisture was under shelterbelts from 12.13 to 27.32 %, and in adjoining cultivated field from 7.25 to 10.20 % (Table 1).

Xanthine oxidase [EC 1.17.3.2] activity was measured by Krawczynski method [8]. Urate oxidase [EC 1.7.3.3.] was evaluated by Martin-Smith method [9]. Phenol oxidase [EC 1.14.18.1] activity was determined by Perucci method [8, 10].

All chemical and biochemical analyses were performed in triplicate and the results were averaged. The confidence intervals were calculated using the following formula: $\bar{x} \pm t_{\alpha(n-1)}\ SE$, where: $\bar{x}$ - mean; $t_{\alpha(n-1)}$ - the value of the Student test for $\alpha = 0.05$, $n - 1$ - degree of freedom, *SE* - standard error.

Results and discussion

The influence of pH the environment on the physical, chemical and biochemical properties of soil are widely known. The change in pH is related to the smaller or greater assimilation of nutrients by the plants and the development of soil microorganisms. In the soil, the stability of enzymes is associated with their structure, stabilization or changes in the activities associated with adsorption in the soil system as well as changes in pH of the soil solution [11]. Soil pH under the new shelterbelt (5.43) was higher compared with an adjoining cultivated field (4.92) (Table 1). Whereas, the soils under *Robinia pseudacacia* and *Crataegus monogyna* were more acidic (3.89 and 3.12, respectively), than in the corresponding cultivated field (5.51 and 4.05, respectively) (Table 1). Our results revealed that the bulk density was significantly lower in the soils under *Robinia pseudacacia* ($1.08\ g \cdot cm^{-3}$), *Crataegus monogyna* ($0.92\ g \cdot cm^{-3}$), and 25 years old shelterbelt ($1.29\ g \cdot cm^{-3}$), than in adjoining cultivated fields (1.34, 1.53, 1.48 $g \cdot cm^{-3}$, respectively) (Table 1).

Table 1

Annual mean data of physical and chemical parameters in soil under shelterbelts and adjoining cultivated field

Parameters	***Robinia pseudacacia* shelterbelt**	**Adjoining cultivated field**	***Crataegus monogyna* shelterbelt**	**Adjoining cultivated field**	**25 years old shelterbelt**	**Adjoining cultivated field**
pH (1M KCl)	3.89	5.51	3.12	4.05	5.43	4.92
Moisture [%]	27.32	10.05	20.10	7.25	12.13	10.20
Bulk density [$g \cdot cm^{-3}$]	1.08 ±0.08	1.34 ±0.09	0.92 ±0.04	1.53 ±0.11	1.29 ±0.12	1.48 ±0.09
Total phenolic [$mg \cdot g^{-1}$]	0.34 ±0.03	0.15 ±0.01	0.17 ±0.02	0.09 ±0.01	0.14 ±0.02	0.04 ±0.01
TOC [$g \cdot kg^{-1}$]	58.2 ±3.2	8.06 ±0.14	42.1 ±3.1	7.44 ±0.89	13.18 ±0.96	5.88 ±0.12
DOC [$g \cdot kg^{-1}$]	4.61 ±0.18	0.76 ±0.08	3.70 ±0.98	0.44 ±0.05	0.80 ±0.07	0.38 ±0.05
NH_4^+ [$mg \cdot kg^{-1}$]	10.28 ±0.63	4.55 ±0.52	6.35 ±0.63	4.32 ±0.43	3.99 ±0.18	5.02 ±0.25
NO_3^- [$mg \cdot kg^{-1}$]	17.3 ±1.2	18.0 ±1.1	10.6 ±1.2	19.0 ±2.1	4.84 ±0.19	7.05 ±0.22
TN [$g \cdot kg^{-1}$]	6.31 ±0.51	1.19 ±0.06	3.97 ±0.19	1.98 ±0.07	2.35 ±0.11	1.56 ±0.07
C/N ratio	9.22	7.22	10.61	3.76	5.61	3.77

$\bar{x} \pm \Delta x$ - confidence interval of average at confidence level $\alpha = 0.05$ for $n - 1$ degree of freedom

The sorption complex of organic soils opposite to mineral soils contains many organic compounds with free groups such as –COOH, –OH, $–NH_2$, =NH, $–SO_3H$, SO_2H, –SH, as a result of which they act as ion exchangers and have complexing properties. The presence of bridges connecting the aromatic rings of humic acids causes them to have a porous structure. These features determine the absorption of water molecules and sorptive properties [12, 13].

Statistically significantly higher annual mean content of TOC (from 2.2 to 7.2 times) in soil under shelterbelts in comparison to the adjoining cultivated fields was observed (Table 1). For shelterbelt planted in 1993, the process of forming new organic matter is short. In contrary the concentration of TOC in adjoining cultivated field was on lower level. In addition, it was observed that the annual mean content of TOC in soil under *Robinia pseudacacia* was 1.4 times statistically significantly higher than under *Crataegus monogyna* and 4.4 times for new shelterbelt. Moreover, there was statistically significantly higher annual mean content of DOC in soil under *Robinia pseudacacia* (4.61 $g \cdot kg^{-1}$) than under *Crataegus monogyna* (3.70 $g \cdot kg^{-1}$) and 25 years old shelterbelt (0.80 $g \cdot kg^{-1}$) and adjoining cultivated field (0.76, 0.44, 0.38 $g \cdot kg^{-1}$, respectively) (Table 1). DOC is connected to microbial activity and it affects enzymes activity. Therefore, significant higher contents of DOC in soils under 200 years old shelterbelts than young shelterbelt showed higher microbial and enzymatic activity in these soils.

The increase in TOC and DOC content of shelterbelts in comparison with agricultural fields was in line with the increase in the concentration of total nitrogen. *Robinia pseudacacia* soil was characterized by statistically significantly higher annual mean concentrations of total nitrogen (6.31 g · kg^{-1}) in comparison with *Crataegus monogyna* (3.97 g · kg^{-1}), new shelterbelt (2.35 g · kg^{-1}), and adjoining cultivated field (1.19, 1.98, 1.56 g · kg^{-1}, respectively) (Table 1). This increase resulted from the high level of the litter and its degradation impacts on a higher input of nitrogen.

Szajdak and Meysner [14] reported a rapid decrease of nitrogen decrease in the forest floor and suggest that nitrogen sequestering in woody residues may be an important quantity of nitrogen in forest soil. Twenty five years old shelterbelt was characterized by low contents of organic matter. It is caused by shorter time of deposition of organic compounds than in old shelterbelts *Robinia pseudacacia* and *Crataegus monogyna*.

The statistically significant higher annual mean concentration of ammonium ions in soils under 200 year old shelterbelts (10.28 and 6.35 mg · kg^{-1}) than adjoining cultivated fields (4.55 and 4.32 mg · kg^{-1}) revealed that demonstrated differences can be caused by the presence of litter which affects changes in the soil sorptive complex and effectively retain migration of different compounds (Table 1). The adsorptive properties of soils under shelterbelts play an important role in binding cations and cation exchange capacity [1, 15]. The data of nitrate ions ranged from 4.84 to 17.3 mg · kg^{-1} in soil under shelterbelts and from 7.05 to 19.02 mg · kg^{-1} in adjoining cultivated fields (Table 1).

One of the basic factors determining the availability of nitrogen to higher plants which is release during the decomposition of organic matter in the soil is the ratio of carbon to nitrogen [16]. The C/N ratio in soil under *Robinia pseudacacia* was 9.22, *Crataegus monogyna* 10.61 and 25 years old shelterbelt 5.61 (Table 1). These data indicated that there were more favorable conditions for the formation of humus under old shelterbelts than 25 years old shelterbelt.

One group of compounds that appears to be a component of the “unknown” soil nitrogen is nucleic acid bases, that is, purines and pyrimidines [17]. In purine metabolism, a key role plays xanthine oxidase. This enzyme from the oxidoreductase class catalyzes the conversion of hypoxanthine into xanthine and xanthine into uric acid [18]. Our investigations have shown significant differences in xanthine oxidase activity in soil under shelterbelts and adjoining cultivated fields. The activity of this enzyme estimated in adjoining cultivated fields ranged from 1.15 to 3.02 µmol · h^{-1} · g^{-1}, and in soil under shelterbelts was from 2.14 to 6.42 µmol · h^{-1} · g^{-1} (Fig. 1). Investigations showed that xanthine oxidase activity was statistically significantly higher in soil under *Robinia pseudacacia* than *Crataegus monogyna* (1.6 times), new shelterbelt (3.0 times) and agriculture fields (2.1 times).

Also, urate oxidase is an important enzyme in the ureide pathway. This enzyme participates in the cycle of nitrogen transformation in soil because it catalyzes the oxidation of uric acid of the final product of purine metabolism to allantoin, hydrogen peroxide and carbon dioxide. In soils, urate oxidase and its substrate uric acid are adsorbed on clay minerals and under these conditions enzymatic degradation of uric acid to allantoin takes place [19]. Statistically significant the highest urate oxidase activity in soil under *Robinia pseudacacia* (13.23 µmol · h^{-1} · g^{-1}), and the lowest in adjoining cultivated field (0.63 µmol · h^{-1} · g^{-1}) was found (Fig. 2). Our results revealed significant lower urate oxidase activity in soil under *Crataegus monogyna* (1.9 times) and new shelterbelt (6.2 times) in comparison with *Robinia pseudacacia*. Research carried out by Zhou et al. [20] indicated no effect of carbon and higher oxygen concentration on the increase of urate oxidase activity. On the other hand, authors proved an increase in the activity of this enzyme after using organic nitrogen.

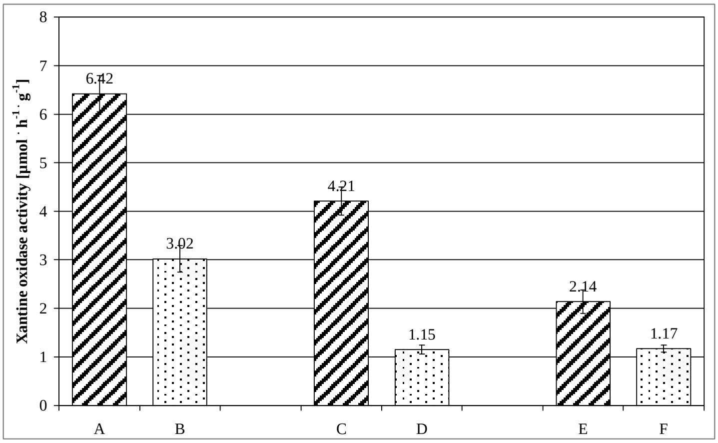

Fig. 1. Annual mean xanthine oxidase activity in soil under shelterbelts and adjoining cultivated fields: A - 200 years old shelterbelt including *Robinia pseudacacia*, B - adjoining cultivated field to *Robinia pseudacacia* shelterbelt, C - 200 years old shelterbelt including *Crataegus monogyna*, D - adjoining cultivated field to *Crataegus monogyna* shelterbelt, E - 25 years old shelterbelt including several species of plant, F - adjoining cultivated field to 25 years old shelterbelt

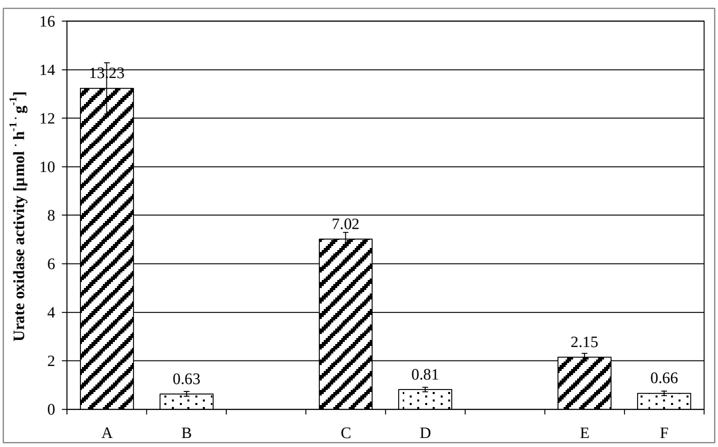

Fig. 2. Annual mean urate oxidase activity in soil under shelterbelts and adjoining cultivated fields: A - 200 years old shelterbelt including *Robinia pseudacacia*, B - adjoining cultivated field to *Robinia pseudacacia* shelterbelt, C - 200 years old shelterbelt including *Crataegus monogyna*, D - adjoining cultivated field to *Crataegus monogyna* shelterbelt, E - 25 years old shelterbelt including several species of plant, F - adjoining cultivated field to 25 years old shelterbelt

The significant higher activity of xanthine oxidase and urate oxidase activity in soil under *Robinia pseudacacia* and *Crataegus monogyna* contribute to the increasing degradation of peptides and purine basis which may indicate the dominance of catabolic processes. Peptides, purine basis, and aldehydes can be completely decomposed and formed compounds undergo heteropolycondensation giving macromolecules of humic substances [21].

Phenolic compounds include organic acids derived from dihydroxybenzoic acid and dihydroxycinnamic acid, flavone derivatives as well as flavonoids, catechins, coumarins, anthocyanins, lignins and many other substances. The varied structure of phenolics determines the specific directions of their action. Phenolics in plants occur as free aglycones or combined with sugar as glycosides. In soils, they are decomposed by fungi e.g., *Basidiomycetes* and *Ascomycetes* and bacteria e.g., *Pseudomonas* [22]. Phenol oxidase catalyzes the oxidation of phenolic compounds to quinones in the presence of oxygen. The reaction of quinones with amino compounds may result in the formation of nitrogen-containing heterocyclic compounds, or melanin [23].

Shelterbelts showed the statistically significant higher annual mean content of total phenolic in *Robinia pseudacacia* (0.34 mg $\cdot$ g^{-1}) and *Crataegus monogyna* (0.17 mg $\cdot$ g^{-1}) than in new shelterbelt (0.14 mg $\cdot$ g^{-1}) and agricultural fields (from 0.04 to 0.15 mg $\cdot$ g^{-1}) (Table 1). There was a trend of increasing relative phenol oxidase activity with phenolic compounds for both shelterbelts compared to agricultural fields. The highest phenol oxidase activity in *Robinia pseudacacia* 14.22 µmol $\cdot$ h^{-1} $\cdot$ g^{-1}, and *Crataegus monogyna* 12.79 µmol $\cdot$ h^{-1} $\cdot$ g^{-1}, the lowest in agricultural fields in range from 4.17 to 5.38 µmol$\cdot$ h^{-1}$\cdot$ g^{-1} was observed (Fig. 3). These differences can be attributed to the processes of soil decomposition under shelterbelts owing to the loosening action of the roots of the trees and higher activity of microorganism in comparison with the soil of agricultural fields.

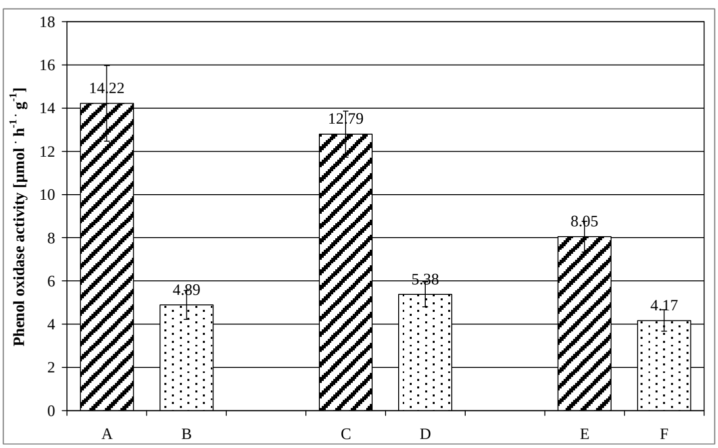

Fig. 3. Annual mean phenol oxidase activity in soil under shelterbelts and adjoining cultivated fields: A - 200 years old shelterbelt including *Robinia pseudacacia*, B - adjoining cultivated field to *Robinia pseudacacia* shelterbelt, C - 200 years old shelterbelt including *Crataegus monogyna*, D - adjoining cultivated field to *Crataegus monogyna* shelterbelt, E - 25 years old shelterbelt including several species of plant, F - adjoining cultivated field to 25 years old shelterbelt

However, it was documented that enzymes activity significantly increased with an annual mean concentration of TOC, DOC, total phenolic, bulk density total nitrogen, ammonium, and nitrate ions in soil under 200 years old shelterbelts in compared to the new shelterbelt and adjoining cultivated fields. According to Dhillon et al. [13], the location of the litter and its decomposition rate had a significant impact on the composition of organic matter under shelterbelts. The higher content of processed forms of organic matter such as ketones showed that organic matter in soils under shelterbelts was at a more advanced stage of decomposition compared to agricultural fields.

Conclusions

It was shown that the age of shelterbelts was the main factor causing the differences in enzymes activity. On the base of obtained results, it was assumed that statistically significantly higher xanthine, urate and phenol oxidase activities in soil of the 200 years old *Robinia pseudacacia* and *Crataegus monogyna* in comparison with new shelterbelt and adjoining arable fields.

The annual mean enzymes activity in the soil under old shelterbelts corresponded with higher average concentrations of total nitrogen, TOC, and DOC.

These results of xanthine and urate oxidase activity indicated that the soils under old shelterbelts were found to be more effective for the degradation of purine basis and peptides. Moreover, the differences in concentration of total phenolic componds in soil under old shelterbelts and agricultural fields are possible to be caused by differences in phenol oxidase activity. The activity of these enzymes could affect the accumulation of nitrogen, carbon and phenolic compounds in the litter and soil under old shelterbelts and lead a change in organic matter.

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WIEK ZADRZEWIENIA JAKO CZYNNIK DETERMINUJĄCY AKTYWNOŚĆ ENZYMÓW W KRAJOBRAZIE ROLNICZYM

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Abstrakt: Zadrzewienia należą do stabilnych elementów krajobrazu rolniczego, które skutecznie redukują stężenia wielu biogenów migrujących z wodą gruntową, ograniczają erozję i regulują reżim wodny w glebie. Funkcje, jakie spełniają zadrzewienia w krajobrazie rolniczym, są ważne ze względu na zrównoważony rozwój obszarów wiejskich. Badania prowadzono w Parku Krajobrazowym im. gen. Dezyderego Chłapowskiego w Turwi (województwo wielkopolskie). Próbki gleb pobierano pod trzema zadrzewieniami różniącymi się wiekiem i składem gatunkowym drzew oraz na przyległych polach uprawnych. Dwa z nich powstały około 200 lat temu. W skład gatunkowy pierwszego wchodzi *Robinia pseudacacia*, natomiast drugiego *Crataegus monogyna*. Trzecie, nowe wielogatunkowe zadrzewienie powstało w 1993 roku. W glebach oznaczono aktywność oksydazy ksantynowej, moczanowej i fenolowej. Badania wykazały, że średnia roczna aktywność oznaczonych enzymów była statystycznie istotnie wyższa i wynosiła odpowiednio w glebach pod zdrzewieniem robiniowym 6,42, 13,23, 14,22 μmol · h^{-1} · g^{-1}, pod zadrzewieniem głogowym 4,21, 7,02, 12,79 μmol · h^{-1} · g^{-1} niż pod nowym zadrzewieniem 2,14, 2,15, 8,05 μmol · h^{-1} · g^{-1} i na przyległych polach uprawnych. Stwierdzono wpływ wieku zadrzewień na aktywność oksydazy ksantynowej, moczanowej i fenolowej.

Słowa kluczowe: zadrzewienia, aktywność oksydazy ksantynowej, aktywność oksydazy moczanowej, aktywność oksydazy fenolowej