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ORIGINAL PAPER

EFFECT OF A PREPARATION CONTAINING HUMIC ACIDS ON SELECTED PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES OF REPLANTED SOIL

Zofia Zydlik[1], Piotr Zydlik[2]

[1] Department of Dendrology, Pomology and Nursery
[2] Entomology and Environmental Protection Department
University of Life Science in Poznań, Poland

Abstract

One of the ways to improve the production properties of replanted soil is to use soil fertilisers containing humic acids. The aim of the study was to analyse the impact of a natural soil activator H-850 WG, containing about 60% of humic acids, on some physical and chemical properties of soil and its enzymatic and respiratory activity in replanting conditions. The research was carried out in 2015-2017, on two kinds of soil: replanted soil from an orchard where apple trees had been cultivated since 1949, and the so-called crop rotation soil from an orchard where apple trees had not been cultivated for 10 years. Replanted soil compared to crop rotation soil had a lower enzymatic and respiratory activity, higher salinity and a lower content of some macronutrients. Two variants were set up at both research sites, i.e. control, without any treatment, and a trial, where the soil was sprayed with the preparation H-850 WG, containing humic acids. The results of this three-years experiment indicate a positive effect of humic acids on some physico-chemical properties of soil. The soil pH, soil nutrient content, as well as the level of its respiratory and enzymatic activity increased. The previous soil use had an impact on how efficiently the humic acids were used. Particularly evident effects occurred in replanted soil. An increase in this soil's enzymatic and respiratory activity after the application of humic acids was much higher than in crop rotation one. Both the activity of two soil enzymes, dehydrogenases and proteases, and soil respiratory activity were positively correlated with soil acidity and the soil content of N, P, Mg and Ca. A significant negative relationship was found between soil salinity and its biological activity.

Keywords: soil fatigue, H-850 WG, humic acids, dehydrogenase, protease, soil respiration, macronutrients.

Zofia Zydlik, PhD Eng., Department of Dendrology, Pomology and Nursery, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland, e-mail: zofia.zydlik@up.poznan.pl

INTRODUCTION

Long-term cultivation of one species in the same site results in what is known as soil fatigue, which is conducive to the emergence of replant disease (Pacholak et al. 2009). In such conditions, the biological properties of soil deteriorate, which manifests in a decrease in the number of soil microorganisms, including proteolytic bacteria (Zydlik et al. 2016). The reason may be the accumulation of allelopathic and phenolic compounds in the replanted environment, limiting the development of soil microflora (Politycka, Adamska 2003, Styla 2014). In Poland, there are more and more depleted plots in regions with intensive fruit growing. The situation is aggravated by the limited access to new positions, and therefore the threat of replant disease is becoming more severe.

In practice, various methods are used to alleviate the effects of replant disease. One of them is the use of mineral fertilisers which, however, require a long period of use and are labour- and energy-intensive. In addition, intensive mineral fertilisation, especially with nitrogen fertilisers, can disturb the natural balance in the soil environment, causing a decrease in the number of soil bacteria (Zydlik 2010). Another way to improve the production properties of tired soil is to use so-called soil fertilisers, some of which contain a significant amount of humic acids, which are the basic factor determining soil fertility. The preparations that are a source of humic acids used in practice are most often obtained from a mineral called leonardite, which is an intermediate form between peat and brown coal. It is highly oxidised lignite, which was formed as a result of the brown coal oxidation process during prolonged exposure. It contains a relatively large number of smaller molecular entities (fulvic and humic acids) (Trckova 2018).

Humic substances can provide macro- and microelements to the soil, improve its aggregate structure and water-air relations (Gümüs, Seker 2015), increase the content of organic matter in the soil (El-Desuki 2004), and stimulate the growth and multiplication of useful microorganisms (Tian et al. 2018). By penetrating inside plant cells, such substances can significantly improve the biochemical processes occurring inside them (Matysiak et al. 2010). Humic acids may also contribute to the improvement of soil biological parameters, increasing the activity of soil dehydrogenases (Perez-de-Mora 2006).

The aim of the study was to analyse the impact of a preparation called H-850 WG, containing humic acids, on some soil physical and chemical (pH, salinity) properties, the content of macronutrients in it, as well as enzymatic and respiratory activity of soil in replanting conditions.

MATERIALS AND METHODS

A field experiment was carried out in 2015-2017, at the area of the Agricultural-Pomological Experimental Farm (52°31′ N, 16°38′ E) in Przybroda, Poznań University of Life Science, Poland. The farm is situated in Wysoczyzna Wielkopolska. The object of the experiment was soil from an apple orchard planted in spring 2009 at a spacing of 3.33 x 1.5 m. It was *Endocalcaric Cambisol* – sandy loam soil (WRB 2014) with the floatable fraction of 17-20% (sand with high loam content). The soil came from two different types of sites. First was a site of grubbed-up apple tress cultivated in this place since 1949 (replanted soil). During that time, trees had been replaced (i.e. replanted) three times without any treatments connected with the preparation of the field for an orchard. The fact of soil fatigue in that site was confirmed by our previous research results (Zydlik et al. 2011, Zydlik 2012). The second site was an orchard in which apple trees had grown since 1949, but there was a break in the cultivation of trees from 1998 to 2009. During that period, cereal crops green manure for ploughing were grown (so-called crop-rotation soil).

The experiment used a natural soil activator called H-850 WG, based on leonardite. This preparation contains humic acids (65%), fulvic acids (10%) and water-soluble K_2O. It was applied in the form of soil spraying under the tree crowns three times during the growing season: in mid-April, at the beginning of May and at the beginning of September, supplying a total dose of 3 kg ha^{-1}.

The experiment included the following combinations: 1 – replanted soil; 2 – replanted soil using the H-850 WG preparation; 3 – crop rotation soil; 4 – crop rotation soil with the H-850 WG preparation. All combinations in the experiment were carried out in two blocks of 20 trees each.

During the three-year research period, the following soil measurements and analyses were carried out: pH, volumetric weight, salinity and content of total form of mineral elements ($N\text{-}NO_3$, P, K, Ca, Mg). The assessment of the soil biological properties included the determination of the activity of two soil enzymes, dehydrogenases and proteases, and its respiratory activity. All the analyses were performed in four replications.

During the growing season, soil samples for the analysis of physical and chemical properties were taken twice: in the early spring (early March) and after the fruit harvest (end of September). Soil samples for analysis of its biological activity were collected three times: in spring – two weeks after the end of flowering of the apple trees, in summer – after the end of the intensive vegetative growth of the trees, and in autumn – two weeks after the fruit harvest. In each combination, soil samples were taken within the root system from a layer of 0 to 40 cm in depth, under the tree crowns; afterwards, the samples were mixed and a composite soil sample of a weight of 1 kg was taken.

The physical and chemical analyses of soil properties, including the assessment of $N\text{-}NO_3$, P, K, Ca and Mg content, were performed at the Chemical and Agricultural Station, using a universal method in accordance with the research procedures adopted at the station.

Dehydrogenase activity in the soil (cm^3 H_2 24 h^{-1} kg^{-1} s.m.) was determined by the colorimetric method in accordance with Thalmann (1968), and proteases (mg tyrosine h^{-1} kg^{-1} s.m) – by the spectrophotometric method according to Ladd, Butler (1972). Soil respiratory activity (CO_2 mg kg^{-1} 48 h^{-1}) was determined based on the amount of CO_2 released by the absorption method according to Gołębiowska, Pędziwilk (1984).

The results of the analyses were subjected to one or two-factor statistical analysis. The method of variance analysis was used applying the Duncan's test at a significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

The physico-chemical properties of the soil affect its productivity. A consequence of replant disease may be a change in some of these properties. The replanted soil used in the experiment compared to the crop rotation one was more acidic and more saline (Table 1). After applying the preparation containing humic acids at both sites, the pH of the soil increased significantly, and in the replanted soil its salinity decreased from 0.24 to 0.10 g NaCl dm^{-3} (the average for the three-year study period) – Table 1. Humic acids could neutralise soil pH, so that many nutrient elements become available to the plant (Yilmaz 2007). The soil acidity reduction after the application of humic acids is also indicated by Akinci et. al. (2009) and Katkat et. al. (2009). An increase in soil pH is beneficial as high concentrations of hydrogen ions in the soil reduce its biological activity, including enzymatic activity.

Soil enzymatic activity is seen as a reliable indicator of its biological activity (Arriagada et. al. 2012). Its intensity depends to a large extent on the proper course of several soil processes, e.g. the mineralisation of organic compounds, and consequently the supply of nutrients to the

Table 1

The impact of the examined factors on selected physico-chemical properties of soil in 2015-2017

Type of soil	Humic acids	Volumetric weight (g dm^{-3})	pH (H_2O)	Salinity (g NaCl dm^{-3})
Replanted	-	1 690	5.1	0.24
	+	1 536	5.6	0.10
Crop rotation	-	1 616	5.7	0.07
	+	1 670	6.8	0.06

plants. The most important role in soil is played by enzymes belonging to oxidoreductases (dehydrogenases) and hydrolases (proteases and ureases). The experiment showed significant differentiation in the activity of both tested soil enzymes depending on the previous soil use. On average, in 2015-2017, the activity of both dehydrogenases (0.58 cm^3 H_2 24 h^{-1} kg^{-1} s.m.) and proteases (1.64 mg tyrozysine h^{-1} kg^{-1}s.m.) in the replanted soil was more than twice lower compared to the crop rotation ones (values 1.19 and 3.81, respectively) – Tables 2, 3. Thus, confirmation of earlier results

Table 2

Dehydrogenase activity in soil (cm^3 H_2 24 h^{-1} kg^{-1} s.m.) in 2015-2017

Type of soil	Humic acids	2015	2016	2017	Average
Replanted	-	0.44*a*	0.75*a*	0.54*a*	0.58*A*
	+	1.83*cd*	1.32*b*	1.63*bc*	1.59*C*
Crop rotation	-	0.74*a*	1.36*b*	1.46*b*	1.19*B*
	+	1.43*b*	1.29*b*	2.11*d*	1.62*C*
Average for the year		1.11*a*	1.18*a*	1.44*b*	-

Note: Means marked with the same small letter do not differ significantly at $\alpha = 0.05$; Means marked with the same capital letter in columns do not differ significantly at $\alpha = 0.05$.

Table 3

Activity of proteases in soil (mg tyrosine h^{-1} kg^{-1} s.m.) in 2015-2017

Type of soil	Humic acids	2015	2016	2017	Average
Replanted	-	2.25*b*	1.73*b*	0.95*a*	1.64*A*
	+	4.16*d*	4.76*d*	3.33*c*	4.08*B*
Crop rotation	-	2.23*b*	4.72*d*	4.49*d*	3.81*B*
	+	5.94*e*	4.95*d*	6.64*e*	5.84*C*
Average for the year		3.64*a*	4.03*a*	3.86*a*	-

Explanation under Table 2

of the authors indicating low biological activity of replanted soil was obtained (Zydlik 2010).

Soil obtains production properties as a result of the activity of soil microorganisms that btransform organic matter. One of the indicators of soil microbial activity is its respiratory activity, measured by the amount of CO_2 released as a result of decomposition of organic matter. It is estimated that about 90% of CO_2 in soil is generated by the action of soil microorganisms. According to the authors of the study, the average three-year transpiration activity of replanted soil found in the experiment was significantly lower (47.93 mg CO_2 kg^{-1} 48 h^{-1}) than on the crop rotation one (69.44 mg CO_2 kg^{-1} 48 h^{-1}) – Table 4.

Soil spraying with the activator containing humic acids caused a significant increase in both dehydrogenase and protease activity in the soil at both

Table 4

The soil respiratory activity (mg CO_2 kg^{-1} 48 h^{-1}) in 2015-2017

Type of soil	Humic acids	2015	2016	2017	Average
Replanted	-	42.71*a*	60.96*bc*	40.12*a*	47.93*A*
	+	71.06*de*	99.28*g*	67.13*cde*	79.16*C*
Crop rotation	-	59.26*b*	88.74*f*	60.31*dc*	69.44*B*
	+	65.94*bcd*	98.09*g*	73.37*e*	79.14*C*
Average for the year		59.74*a*	86.77*b*	60.23*a*	-

Explanation under Table 2

sites (Tables 2 and 3). Bielińska et al. (2013) came to a similar conclusion, indicating an increase in dehydrogenase, protease and urease activity in soil treated with soil conditioners. The effectiveness of the use of humic acids in the experiment varied depending on previous soil use. The increase in enzymatic activity in the replanted soil was clearly higher than in the crop rotation one. On average for 2015-2017, after the application of humic acids, in the first site, the activity of dehydrogenases increased almost threefold (Table 2), and that of proteases – by approx. 2.5-fold compared to the combination without this treatment (Table 3). For the crop rotation soil, these differences were much smaller. Higher enzymatic activity of the replanted soil in the variant with the use of the soil activator could have resulted from a decrease in soil acidity at this site (Table 1). Low soil pH limits the activity of soil microorganisms, which in turn results in a reduction of soil enzymatic activity (Zhan, Sun 2014).

Soil respiratory activity was another parameter that responded positively to humic acid applications in the experiment. Same as before, the increase in the respiratory activity in the replanted soil was several times higher than in the crop rotation one. On average for the years 2015-2017, the amount of CO_2 secreted in the replanted soil treated with the H-850 WG preparation increased from 47.93 to 79.16 mg kg^{-1} 48 h^{-1}**,** and in the crop rotation soil it rose from 69.44 to 79.14 mg kg^{-1} 48 h^{-1} (Table 4). As noted by Gajda et al. (2012), high rates of soil respiratory activity testify to an equally high level of soil microorganisms responsible for humus formation and providing nutrients to plants, as a result of which the soil's production value increases.

In addition to soil pH, mineral and organic colloid content, abundance and species composition of microorganisms, soil biological activity may also be significantly affected by climatic conditions. In this experiment, both the enzymatic activity of the soil and the intensity of its respiration showed large variation at different plant growing periods. In the three-year study period, the highest soil dehydrogenase activity was recorded in the crop rotation soil in autumn (2.14 cm^3 H_2 24 h^{-1} kg^{-1}s.m.), while the lowest one was in spring (0.44 cm^3 H_2 24 h^{-1} kg^{-1}s.m.) – Table 5. In autumn, soil moisture is relatively high and, as Brzezińska et al. (2001) pointed out, an increase in the soil water content increases dehydrogenase activity. This is because these enzymes are produced mainly by anaerobic bacteria under anaerobic conditions (for example due to flooding). The high activity of soil dehydrogenases in the autumn period was also determined by STYLA (2014) and by ZYDLIK et al. (2016).

Soil moisture conditions could also affect the activity of soil proteases. As stated by SARDANS et. al. (2005), a decrease in soil moisture by 10% causes a decrease in the activity of these soil enzymes by 20 to 60%. In the current experiment, the soil protease activity was more varied, depending on the type of site and on the growing season. On the replanted soil, the high activity of these soil enzymes was generally noted in spring and summer, whereas on the crop rotation soil, it peaked at the end of the growing season.

The soil respiratory activity, apart from the availability of oxygen, organic matter content and salinity, is significantly affected by soil temperature (SZAFRANEK-NAKONIECZNA, STĘPNIEWSKA 2014). The analysis of soil respiratory activity carried out in the experiment during various plant growing periods indicates that summer was the time of the highest respiratory intensity (Table 5).

Lower enzymatic and respiratory activity of the replanted soil compared to the crop rotation one, as demonstrated in the experiment (Tables 2-4), may result from insufficient effective activity of soil microorganisms responsible for the decomposition of organic matter in this environment and, as a result, of a lower content of nutrients. Table 6 presents the total content of some macronutrients in the replanted and in the crop rotation soil. It was

Table 5

The impact of the examined factors on enzymatic and respiratory activity of the soil during different periods of plan growth in 2015-2017

Type of soil	Humic acids	Season	Dehydrogenases	Proteases	Respiratory activity
Replanted	-	spring	0.44*a*	2.24*b*	42.71*bc*
		summer	0.75*a*	1.73*b*	60.96*de*
		autumn	0.54*a*	0.96*a*	40.12*a*
	+	spring	1.83*cd*	4.17*d*	71.06*de*
		summer	1.32*b*	4.75*d*	99.28*g*
		autumn	1.63*bc*	3.32*c*	67.13*cde*
Crop rotation	-	spring	0.74*a*	2.23*b*	59.23*b*
		summer	1.36*b*	4.72*d*	88.74*f*
		autumn	1.47*b*	4.49*d*	60.31*bc*
	+	spring	1.44*b*	5.59*e*	65.94*bcd*
		summer	1.29*b*	4.94*d*	98.09*g*
		autumn	2.14*d*	6.63*e*	73.37*e*

Note: statistical analysis was made separately for each parameter

Table 6

The impact of the examined factors on the total content of some macroelements (mg kg^{-1}) in soil in 2015-2017

Type of soil	Humuc acids	$N\text{-}NO_3$	P	K	Ca	Mg
Replanted	-	5.36	26.6	60.3	108.7	31.0
	+	6.60	33.3	66.7	159.0	57.7
Crop rotation	-	6.57	33.3	74.3	240.7	63.0
	+	9.30	88.3	109.3	1040.7	199.7

found that the content of each of the five studied macroelements in the crop rotation soil was notably higher than in the replanted one. Particularly visible differences occurred in the case of Ca and Mg, the content of which in the replanted soil was more than two-fold lower than the crop rotation one (Table 6). These results confirming a decrease in the content of some nutrients in the replanted soil are confirmed in earlier reports by other authors (Wyszkowska et al. 2009, Li et al. 2010, Zydlik et al. 2016).

One way to increase the soil nutrient content can be by applying humic acids (Aknici et al. 2009, Gümüs, Seker 2015). The results of this three-year research justify this claim. After the application of humic acids tn both types of sites, the content of $N\text{-}NO_3$, P, K, Ca and Mg in soil increased. The content of Ca (from 240 to 1 040 mg kg^{-1}) and Mg (from 63 to 199.7 mg kg^{-1}) increased the most, while that of K increased the least (Table 6).

The statistical analysis indicates presence of some relationships, expressed with correlation coefficients, between soil enzymatic and respiratory activity, and some of its physical and chemical properties. A significant positive relationship was found between dehydrogenase and soil protease activity, as well as soil respiratory activity and soil acidity and the content of such components as $N\text{-}NO_3$, P, Ca and Mg (Table 7). Other authors also point to similar relationships between enzyme activity and the level of soil acidity (Jiang et al. 2003) and the content of nutrients in soil (Li et.al. 2001, Mandal et al. 2007, Xu et al. 2008, Kucharski et al. 2009, Wyszkowska et al. 2009).

Soil salinity is a degrading factor. A high salt content alkalises soil, reduces the bioavailability of macro- and microelements, and limits its enzymatic activity by interfering with the ionic balance in cells. The experiment showed the existence of significant negative correlations between soil salinity and the other examined properties (Table 7).

In summary, it is stated that spraying soil with a preparation containing humic acids contributed to the improvement of its biological parameters, expressed by the activity of two soil enzymes, and to the increase of its respiratory intensity, in addition to which it increased the amount of nutrients in the soil. Higher effectiveness in this respect was noted in the replanted

Table 7

Relationships expressed by correlation coefficients between physico-chemical and biological properties of soil

Specification	Respiratory activity	Dehydrogenases	Proteases	pH	Salinity	$N\text{-}NO_3$	P	Ca	Mg
Respiratory activity	1	0.122	0.502**	0.130	-0.191	0.364**	0,158	0.218*	0.245*
Dehydrogenases		1	0.449**	0.324**	-0.419**	0.419**	0.386**	0.394**	0.471**
Proteases			1	0.238*	-0.395**	0.443**	0.421**	0.508**	0.493**
pH				1	-0.790*	0.341**	0.854**	0.733**	0.865**
Salinity					1	-0.376**	-0.616**	-0.579**	- 0.755**
$N\text{-}NO_3$						1	0.351**	0.435**	0.603**
P							1	0.914**	0.926**
Ca								1	0.930**
Mg									1

* significance at the level $\alpha = 0.05$, ** significance at the level $\alpha = 0.01$

soil, which may indicate the desirability of using humic acids under stress conditions for plants, which certainly should also include the consequences of replant disease.

CONCLUSIONS

1. There was a lower activity of dehydrogenases and soil proteases, a lower level of soil respiratory activity, higher salinity and a lower content of some macronutrients in the replanted soil compared to the coil rotation one.

2. The use of a preparation with humic acids in apple orchard caused an increase in soil pH, a decrease in its salinity, a significant increase in the activity of two soil enzymes, dehydrogenases and proteases, and an increase in its respiratory activity.

3. Earlier soil use had an impact on the effectiveness of using humic acids. The increase in soil enzymatic and respiratory activity after their application was much higher in the replanted soil than in the crop rotation one.

4. The influence of some physico-chemical properties of soil on its enzymatic activity was demonstrated. Significant positive relationships were found between the enzymatic activity of soil and its acidity, as well as the content of $N\text{-}NO_3$, P, Ca and Mg in soil.

5. A significant negative relationship was found between soil salinity and its biological activity and the soil content of macronutrients.

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