POLISH JOURNAL OF SOIL SCIENCE
VOL. L/1 2017 PL ISSN 0079-2985

DOI: 10.17951/pjss/2017.50.1.33

KAROLINA FURTAK*, ANNA M. GAJDA*

ACTIVITY OF DEHYDROGENASES AS AN INDICATOR OF SOIL ENVIRONMENT QUALITY

Received: 24.05.2017
Accepted: 04.07.2017

Abstract. Activity of dehydrogenases (DHs) indicates the presence of viable and physiologically active (physiologically active or physiological activity) microorganisms. Their presence (activity) in soil is correlated with the content of organic carbon, microorganisms, nitrifying activity and microbial respiration. Determination of DHs activity allows to control changes in soil microbial population and is considered as an important parameter of soil quality. The aim of the study was to determine the effects of different farming systems on the enzymatic activity in soil under winter wheat. The research was conducted in the years 2014–2016 on long-term field experiment under two different farming systems (ecological and conventional) at the IUNG (PIB) Experimental Station located in Osiny (Lublin Voivodeship), Poland. Each farming system differs in crop rotation system and whole agrotechnics, which have been adapted to its specificity. Determination of DHs activity was performed using Casida *et al*.'s (1964) method with modifications. Measured DHs activity was expressed in milligrams of triphenyl formazan (TPF) per 100 g of soil within 24 hours. The results showed that ecological farming system beneficially influenced soil environment.

Keywords: soil quality, activity of dehydrogenases, farming system

* Department of Agricultural Microbiology Institute of Soil Science and Plant Cultivation (State Research Institute) – IUNG (PIB) Czartoryskich 8, 24-100 Puławy, e-mail: K. Furtak: kfurtak@iung.pulawy.pl; A.M. Gajda: ag@iung.pulawy.pl

INTRODUCTION

Enzymes present in soil environment are involved in many biochemical processes (Błońska 2011). Among them there are dehydrogenases (oxidoreductases), which catalyze disconnection of hydrogen atom from a substrate in process of oxidation.

Dehydrogenases (DHs) activity indicates the presence of physiologically active microorganisms (Kieliszewska-Rokicka 2001). DHs are strongly associated with carbon cycles and soil organic matter (SOM) (Błońska *et al*. 2016). Activity of DHs has also linked with the activity of other soil's enzymes e.g., catalase and β-glucosidase and the presence of nitrogen (Brzezińska and Włodarczyk 2005). DHs play a significant role in the biological oxidation processes in soil (Wolińska and Stępniewska 2012).

Dehydrogenases are very sensitive indicators of changes in soil analysis (Bastida *et al*. 2008, Gajda *et al*. 2013, Gałązka *et al*. 2017). Due to the fact that DHs are active only in living microbial cells, they are highly dependent on the number and structure of microbial communities in soil. It was shown that the activity of that group of enzymes was affected by physical and chemical parameters of the soil, such as: humidity, temperature and pH (Cirilli *et al*. 2012, Levyk *et al*. 2007, von Mersi and Schinner 1991, Wolińska and Stępniewska 2012). Many studies have found that presence of ions of heavy metals (Cu, Pb, Cd) in soil and salinity caused a significant inhibition of DHs activity (Mocek-Płóciniak 2010, Telesiński *et al*. 2015, Xie *et al.* 2009).

Soil dehydrogenases have been widely studied and in relation to other soil parameters became considered as a good, sensitive and useful indicator of changes in soil quality (Salazar *et al*. 2011).

MATERIALS AND METHODS

Experimental site and soil samples collection

The studies conducted in the years 2014–2016 were based on the long-term field experiment at Experimental Station (ES) in Osiny (Lublin Voivodeship) located in Central-Eastern part of Poland (51°27′53″N 22°03′52″E). The ES belongs to the Institute of Soil Science and Plant Cultivation, State Research Institute (IUNG, PIB) in Puławy. Winter wheat (var. *Jantarka*) was grown in monoculture in two different farming systems: conventional farming system (CV) and ecological farming system (EC). The CV was based on the mouldboard plough (up to 20 cm) and traditional soil tillage equipment. The EC was based on soil crushing-loosening equipment a rigid-tine cultivator (up to 10 cm). No mineral fertilizers and plant protection chemicals were used. Weed control was based mainly on mechanical treatments. Each farming system differs in crop rotation system and whole agrotechnics, which have been adapted to its specificity. Winter wheat was grown according to fertilization and weed control recommendations generally used in Poland.

Soil samples were collected from four soil depths (0–5 cm, 5–10 cm, 15–20 cm, 30–35 cm) at harvest time, and transported to the laboratory and sieved on a sieve 2 mm mesh. The samples were stored at 4°C until analysis.

Dehydrogenases activity

The measurement of dehydrogenases (DHs) activity was performed using Casida *et al*.'s (1964) method with modifications. This method is based on reduction of 2,3,4-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF). From each soil 6 g of subsample (in triplicates) was mixed with $CaCO_3$ (60 mg). After that, 1 cm^3 of 3% (w/v) TTC and 2.5 cm^3 of distilled water were added. Next, samples were shaken and incubated in 37°C for 24 hours in the dark. For TPF extraction an ethyl alcohol was used (25 cm^3). Extracts were filtered and concentration of TPF was measured at 485 nm. DHs activity was expressed in milligrams of triphenyl formazan (TPF) per 100 g of soil within 24 hours.

RESULTS

Figures 1 and 2 show the effects of different farming systems on DHs activity measured in 2015 and 2016. In the year 2015, the measurements of DHs activity in soil under both EC and CV farming systems showed similar trends, but in 2016 major differences were reported (Fig. 1, 2).

In 2015, the highest DHs activity was measured in soil under EC at the layer of 0–5 cm which amounted to 183.6 μg TPF g^{-1} d.w. soil $24h^{-1}$), while at the same layer in CV, the measurement was significantly ($p \leq 0.05$) lower and reached 144.0 μg TPF g^{-1} d.w. soil $24h^{-1}$. Also, in lower layers (5–10, 15–20 cm), higher activities of DHs were measured under EC than CV, but these differences were not statistically significant. However, in the layer of 30–34 cm depth, higher activity of DHs was noted in soil under CV 13.1 μg TPF g^{-1} d.w. soil $24h^{-1}$ than EC 7.2 μg TPF g^{-1} d.w. soil $24h^{-1}$ (Fig. 1).

In 2016, trends in measurements of DHs activity were similar to the observed in 2015 (Fig. 2). The highest activity of DHs was recorded at the layer of 0–5 cm depth under EC (173.3 μg TPF g^{-1} d.w. soil $24h^{-1}$) and the lowest at sub-arable layer of 30–35 cm 9.2 μg TPF g^{-1} d.w. soil $24h^{-1}$. Also, soil taken from layers of 5–10, 15–20 cm depths under EC farming demonstrated 45.6 and 39.5 μg TPF g^{-1} d.w. soil $24h^{-1}$ higher activity of DHs compared with CV, respectively. Similarly to 2015, in 2016, the soil from layer of 30–35 cm under

CV farming system showed higher DHs activity (13.09 μg TPF g^{-1} d.w. soil $24h^{-1}$) in relation to EC (9.1 μg TPF g^{-1} d.w. soil $24h^{-1}$).

Average activity of DHs in all soil layers studied up to 20 cm depth in 2015 under EC was higher and amounted to 129.8 μg TPF g^{-1} d.w. soil $24h^{-1}$ compared to CV 112.7 μg TPF g^{-1} d.w. soil $24h^{-1}$. Also, in 2016, average activity of DHs in all soil layers studied up to 20 cm depth measured under EC was higher (135.2 μg TPF g^{-1} d.w. soil $24h^{-1}$) compared to CV (76.2 μg TPF g^{-1} d.w. soil $24h^{-1}$). Difference in activity of that group of enzymes between systems in soil up to 20 cm depth averaged 17.1 μg TPF g^{-1} d.w. soil $24h^{-1}$ in 2015 (not significant) and 59.0 μg TPF g^{-1} d.w. soil $24h^{-1}$ in 2016 (significant at $p \leq 0.05$). Differences between systems in sub-arable layer 30–35 cm, averaged 6.7 μg TPF g^{-1} d.w. soil $24h^{-1}$ in 2015, and 7.8 μg TPF g^{-1} d.w. soil $24h^{-1}$ in 2016 and were not significant at $p \leq 0.05$ (Fig. 1, 2).

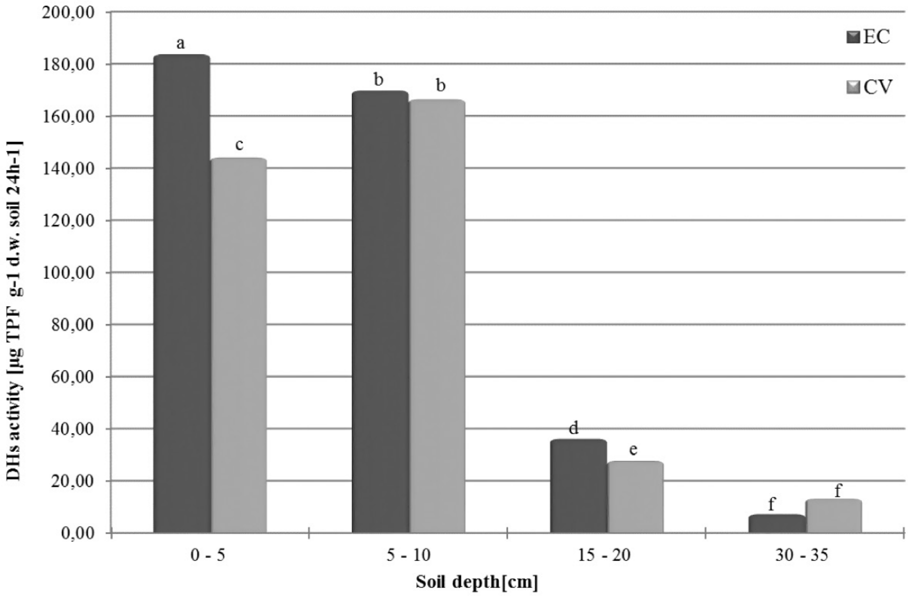

Fig. 1. Activity of dehydrogenases [μg TPF g^{-1} d.w. soil 24 h^{-1}] in 2015 in soil under both tillage systems: CV and EC. a, b, c, d, e, f – values marked with different letters are statistically significant at $p \leq 0.05$

Analysis of changes in DHs activity within two years showed insignificant changes in the top layer (0–5 cm) of soil under EC farming system (Fig. 3). At the layer of 5–10 cm depth the significant ($p \leq 0.05$) decrease was noticed by about 51.5 μg TPF g^{-1} d.w. soil $24h^{-1}$, on average. For the deeper soil layers of 15–20 and 30–35 cm depths, the DHs activity increased in time by 78.1 (significant at $p \leq 0.05$) and 1.9 (non significant at $p \leq 0.05$) μg TPF g^{-1} d.w. soil $24h^{-1}$, respectively.

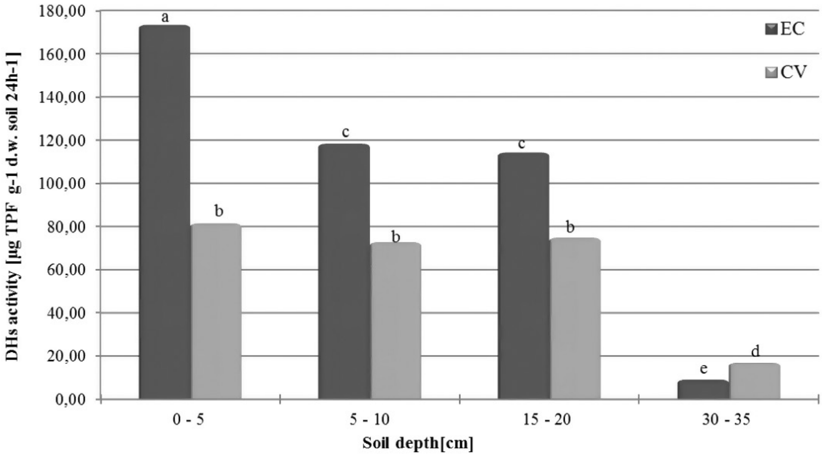

Fig. 2. Activity of dehydrogenases [µg TPF g^{-1} d.w. soil 24 h^{-1}] in 2016 in soil under both tillage systems: CV and EC. Explanations as in Fig. 1

The observed trends in DHs activity for soil under CV farming system appeared to be contrary to these under EC (Fig. 3). In the soil layers of 0–5 and 5–10 cm depths the average DHs activity decreased significantly ($p \leq 0.05$) by 62.6 and 93.7 µg TPF g^{-1} d. w. soil $24h^{-1}$, respectively. At the deeper layer of soil profile 15–20 cm depth the significant ($p \leq 0.05$) increase of DHs activity by 53,8 TPF g^{-1}d.w. soil $24h^{-1}$ was measured. Slight increase by about 3.8 µg TPF g^{-1} d. w. soil $24h^{-1}$ in DHs activity was also observed in the 30–35 cm layer but this change in time was no significant. The least dynamic changes occurred in the lowest depth of the soil profile (30–35 cm) under both EC and CV farming systems (Fig. 3).

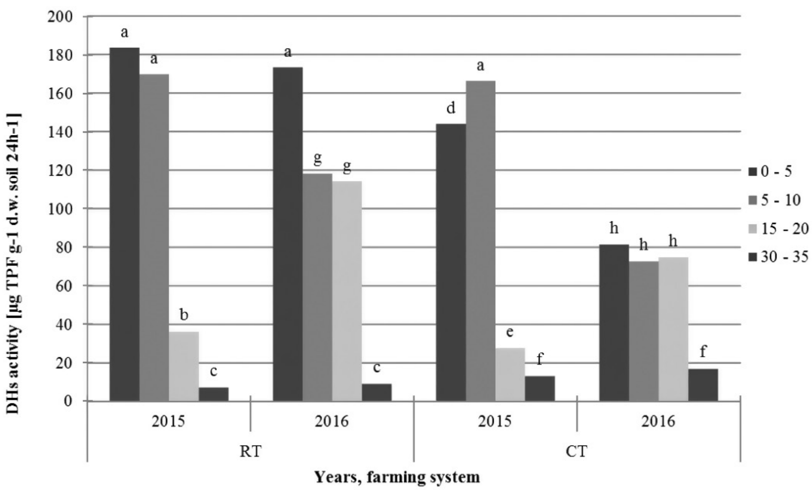

Fig. 3. Activity of dehydrogenases [µg TPF g^{-1} d.w. soil $24h^{-1}$] in soil under different farming systems EC and CV in 2015 and 2016. Explanations as in Fig. 1

DISCUSSION

The estimation of enzymatic activity is recommended as a sensitive indicator of abundance and activity of microorganisms inhabiting in soil. It provides information about the quality of the soil environment (Swędrzyńska and Grześ 2015; Wolińska *et al*. 2013; Wolińska *et al*. 2014; Wolińska *et al*. 2015).

Results indicating differences in the DHs activity from the different systems of cultivation were obtained in many studies e.g. Alvear *et al*. (2005), Daif *et al*. (2013), Frąc *et al*. (2011), Marinari *et al*. (2006). They showed that the soil under ecological farming system, and/or simplified tillage were characterized by a higher DHs activity than soil under farming systems based on conventional tillage. The study of Caporali *et al*. (2003) conducted in Italy suggested that the ecological farming and tillage systems contribute to the maintenance of biodiversity. Similar study conducted in 2006 and 2007 in Rogów and Grabów (Poland) on long-term experimental fields also showed the highest DHs activity in soil under reduced tillage (Gajda 2008). Higher DHs activity in the soil under barley in direct sowing compared to plough cultivation was also showed in studies of Majchrzak *et al*. (2014). However, the authors conclude that the farming system has a significant effect on the DHs activity in soil only before sowing and at the tillering phase. Pociejowska *et al*. (2013) also reported that the use of direct sowing increases the enzymatic activity of the soil. Research conducted on agricultural soils in the Lublin Voivodeship by Wolińska *et al*. (2015) showed that in arable soils the DHs activity was lower than in control soils – uncultivated.

Many studies have shown the decrease of the DHs activity with increasing depth in the soil profile, e.g. Gajda (2008), Swędrzyńska and Grześ (2015), Swędrzyńska et al. (2013), Wolińska *et al*. (2013), Wolińska *et al*. (2015) and support the results presented in this paper.

CONCLUSIONS

1. Significantly higher activity of dehydrogenases was measured in soil under ecological farming system (EC) versus conventional (CV).
2. The highest activity of dehydrogenases has been recorded at the soil layer of 0–5 cm depth in both farming systems with growing trend noted.
3. In both farming systems studied the activity of dehydrogenases decreased with an increasing depth in the soil profile.
4. During the year, the activity of dehydrogenases in the surface layers of the soil has increased in both cultivation systems.
5. At the soil layer of 30–35 cm the higher dehydrogenases activity showed soil under conventional farming system (CV) as compared with ecological (EC).

The research was supported partly by the IUNG (PIB) Research Statute Program 2.26 and IUNG (PIB) Research Programmes 1.3 and 1.4.

REFERENCES

[1] Alvear, M., Rosas, A., Rouanet, J.L., Borie, F., 2005. *Effects of Three Soil Tillage Systems on Some Biological Activities in an Ultisol from Southern Chile.* Soil Till Res., 82: 195–202.

[2] Bastida, F., Kandeler, E., Moreno, J.L., Ros, M., García, C., Hernández, T., 2008. *Application of Fresh and Composted Organic Wastes Modifies Structure, Size and Activity of Soil Microbial Community under Semiarid Climate*. Applied Soil Ecology, 40, 2: 318–329.

[3] Błońska, E., Lasota, J., Gruba, P., 2016. *Effect of Temperate Forest Tree Species on Soil Dehydrogenase and Urease Activities in Relation to Other Properties of Soil Derived from Loess and Glaciofluvial Sand.* Ecol Res., 31, 5: 655–664, **http://dx.doi.org/10.1007/s11284-016-1375-6**

[4] Błońska, E., 2011. *Soil Enzymes and Their Importance in Assessing the Biological Activity of Forest Soils on the Example of Nature Reserves in the Polish Lowlands and Uplands* (in Polish). Rocz. Glebozn., 62, 4: 163–172.

[5] Brzezińska, M., Włodarczyk, T., 2005. *Enzymes of Intracellular Redox Transformations (Oxidoreductases)* (in Polish). Acta Agrophys. Rozpr. Monogr., 3: 11–26.

[6] Caporali, F., Mancinelli, R., Campiglia, E., 2003. *Indicators of Cropping System Diversity in Organic and Conventional Farms in Central Italy.* International Journal of Agricultural Sustainability, 1, 1: 6772.

[7] Casida, L., Klein, D., Santoro, T., 1964. *Soil Dehydrogenase Activity*. Soil Science, 98: 371–376.

[8] Cirilli, F., Bellincontro, A., De Santis, D., Botondi, R., Colao, M., Muleo, R., Mencarelli, F., 2012. *Temperature and Water Loss Affect ADH Activity and Gene Expression in Grape Berry During Postharvest Dehydration.* Food Chem., 132: 447–454.

[9] Daif, M.A., Khater, H.A., Abdul Aleem, M., Rehan, M.M., 2013. *Chemical and Biological Indicators of Soil Quality in Organic and Conventional Farming Systems.* J. Soil Sci. And Agric. Eng., Mansoura Univ., 4, 11: 1273–1288.

[10] Frąc, M., Lipiec, J., Rutkowska, A., Oszust, K., Półtorak, M. 2011. *Microbiological Properties of Soil under Winter Wheat in the Ecological and Conventional Cropping Systems* (in Polish). Acta Agrophys., 18, 2: 245–254.

[11] Gajda, A.M., 2008. *Effect of Different Tillage Systems on Some Microbiological Properties of Soils under Winter Wheat.* Int. Agrophysics, 22: 201–208.

[12] Gajda, A.M., Przewłoka, B., Gawryjołek, K., 2013. *Changes in Soil Quality Associated with Tillage System Applied*. Int. Agrophysics, 27: 133–141.

[13] Gałązka, A., Gawryjołek, K., Perzyński, A., Gałązka, R., Księżak, J., 2017. *Changes in Enzymatic Activities and Microbial Communities in Soil under Long-Term Maize Monoculture and Crop Rotation.* Pol. J. Environ. Stud., 26, 1: 39–46.

[14] Kieliszewska-Rokicka, B., 2001. *Soil Enzymes and Their Importance in Investigate of Microbiological Activity of Soil.* In: Dahm, H., Pokojska-Burdzej, A. (eds.), *Microorganisms of the Soil Environment. Physiological, Biochemical and Genetic Aspects* (in Polish). Toruń, Wydaw. Adam Marszałek, pp. 37–47.

[15] Levyk, V., Maryskevych, O., Brzezińska, M., Włodarczyk, T., 2007. *Dehydrogenase Activity of Technogenic Soils of Former Sulphur Mines (Yvaoriv and Nemyriv, Ukraine).* Int. Agrophysics, 21: 255–260.

[16] Majchrzak, L., Niewiadomska, A., Natywa, M., 2014. *Evaluation of Dehydrogenase Activity of Spring Barley Depending on the Tillage System, Previous Crop and Type of Crop Residua* (in Polish). Annales Universitatis Mariae Curie-Skłodowska Lublin-Polonia, 69, 4: 103–111.

[17] Marinari, S., Mancinelli, R., Campiglia, E., Grego, S., 2006. *Chemical and Biological Indicators of Soil Quality in Organic and Conventional Farming Systems in Italy.* Ecol. Indicators, 6: 701–711.

[18] Mocek-Płóciniak, A., 2010. *Utilisation of Enzymatic Activity for the Evaluation of the Impact of Anthropogenic Changes Caused by Heavy Metals in Soil Environment* (in Polish). Nauka Przyr. Technol., 4, 6: 86

[19] Pociejowska, M., Natywa, M., Majchrzak, L., Cłapa, T., Selwet, M., 2013. *Effect of Site Preparation for Spring Wheat on the Number of Microorganisms and Biochemical Activity of Soil* (in Polish). Polish Journal of Agronomy, 15: 21–26.

[20] Salazar, S., Sanchez, L., Alvarez, J., Valverde, A., Galindo, P., Igual, J., Peix, A., Santa-Regina, I., 2011. *Correlation Among Soil Enzyme Activities Under Different Forest System Management Practices*. Ecological Engineering, 37: 1123–1131.

[21] Swędrzyńska, D., Grześ, S., 2015. *Microbiological Parameters of Soil Under Sugar Beet as a Response to the Long-Term Application of Different Tillage Systems*. Pol. J. Environ. Stud., 24, 1: 285–294.

[22] Swędrzyńska, D., Małecka, I., Blecharczyk, A., Swędrzyński, A., Starzyk, J., 2013. *Effects of Various Long-Term Tillage Systems on Some Chemical and Biological Properties of Soil.* Pol. J. Environ. Stud., 22, 6: 1835–1844.

[23] Telesiński, A., Dobrowolska, A., Stręk, M., Płatkowski, M., Onyszko, M., 2015. *Comparison of Some Heavy Metal and Salinity Effects on Enzymatic Activity in Horticultural Growing Media Used in Pot Technology of Lavender (Lavandula angustifolia Mill)* (in Polish). Woda–Środowisko–ObszaryWiejskie, 15, 1, 49: 123–132.

[24] von Mersi, W., Schinner, F., 1991. *An Improved and Accurate Method for Determining the Dehydrogenase Activity of Soils with Iodonitrotetrazolium Chloride*. Biol. Fertil. Soils, 11: 216–220.

[25] Wolińska, A, Stępniewska, Z., 2012. *Dehydrogenase Activity in the Soil Environment.* In: Canuto, R.A. (ed.) *Dehydrogenases*, InTech, Chapter 8; pp. 183–210, **DOI: 10.5772/48294**

[26] Wolińska, A., Rekosz-Burlagar, H., Goryluk-Salmonowicz, A., Błaszczyk, M., Stępniewska, Z., 2015 *Bacterial Abundance and Dehydrogenase Activity in Selected Agricultural Soils from Lublin Region.* Pol. J. Environ. Stud., 24, 6: 2677–2682.

[27] Wolińska, A., Stępniewska, Z., Szymańska, E., 2013. *Dehydrogenase Activity of Soil Microorganisms and Total DNA Level in Soil of Different Use.* J. Agric. Sci. Tech., B., 3, 9: 613–621.

[28] Wolińska, A., Szafranek-Nakonieczna, A., Banach, A., Rekosz-Burlaga, H., Goryluk-Salmonowicz, A., Błaszczyk, M., Stępniewska, Z., Gorski, A., 2014. *Biological Degradation of Agricultural Soils from Lublin Region (SE Poland).* Int. J. Curr. Microbiol. App. Sci., 3, 11: 558–571.

[29] Xie, W., Zhou, J., Wang, H., Chen, X., Lu, Z., Yu, J., Chen, X., 2009. *Short-Term Effects of Copper, Cadmium and Cypermethrin on Dehydrogenase Activity and Microbial Functional Diversity in Soils after Long-Term Mineral or Organic Fertilization.* Agriculture, Ecosystems & Environment, 129: 450–456.