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Methodological approaches to evaluate dehydrogenase activity as a good indicator of soil functionality.

Juan Antonio Campos, Carmen Moreno, Jaime Villena, Jesús D. Peco, Eva M. García-Noguero, and Marta M. Moreno
ETSIA. University of Castilla La Mancha. Ronda de Calatrava 7. Ciudad real 13071 Spain

Dehydrogenase activity (DHA) has been widely used as a good indicator to assess the oxidative status in soils. The common method determination relies on the reduction of an artificial electron receptor by the soil microorganisms, namely, a soluble tetrazolium salt that acquires a red color in its reduced form (formazan), being this way easily measured by colorimetry, after extraction by a proper solvent. This activity is very sensitive to all the factors that can reach the upper layer of soils, especially temperature and moisture, and its use has become very useful to determine the degree of xenobiotics toxicity or the goodness, or not, of agricultural procedures and management. To establish an appropriate methodology for the measurement and monitoring of this activity, in our work we evaluate the most relevant aspects that must be taken into account so that the determination of this activity is as consistent as possible.

Incubation time and pre-incubation: The incubation time appears as the main source of trouble in the interpretation of results. Most of the time, an incubation time of 24 hours is used, but some authors recommend shorter incubation periods to make the measurements at an initial rate and that way use a linear function. For this reason, some authors advise shorter periods of incubation after having a pre-incubation time with glucose or yeast extract. This way the reducing potential of the soil will be better represented.

Soil moisture: For the DHA results of a certain area to be comparable, the degree of soil humidity has to be necessarily standardized since any change in soil moisture will lead to changes in DHA. Dry soils give figures of DHA close to zero. Precise readings of DHA can only be obtained minimizing the moisture interactions. A pre-incubation of 10 days with the soil hydrated with 50% of the water holding capacity, not only ensures equal moisture for all the samples but also serves to reactivate the soil microorganism population. After that, samples should be immediately incubated with the electron receptor and analyzed.

Formazan extraction: Special physicochemical properties of soils can lead to better or worse extraction of formazan. Some authors advise carrying out a simple trial to establish the degree of extraction of the formazan according to the slope of a linear function between the added formazan and that extracted.

Optimal TTC concentration: Some toxicity of TTC has been raised recently. Although the

concentration of the substrate must be sufficient to saturate all the enzymatic capacity, it is advisable not to add an excess of TTC. The quantity and quality of organic matter may be behind the degree of severity of the toxic effect of TTC.