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Dehydrogenase activity (DHA): Measure of total microbial activity and as indicator of soil quality

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Abstract
Profound agriculture practices may emerge a numerous problem like reduction in soil fertility, erosion, ground water contamination which has direct impact on the soil health. To evaluate soil quality, it is essential to learn all potential changes in soil biological properties. Enzymes are macromolecular biological catalyst in life processes, likewise in soil they are essential to play prospective role in maintaining soil health and to ass's soil quality. There are substantial enzymes in the soil environment, such as oxidoreductases, hydrolyses, isomerases, lyases and ligases. All of these enzymes play crucial role in several soil biological activities and maintaining soil health. In the group of all enzymes in soil environment, dehydrogenase is a most important enzyme belonging to the group of oxidoreductases that oxidizes a substrate by reducing an electron acceptor. Determination of dehydogenase enzyme activity in the soil samples gives us large amount of information about biological characteristic of soil which is essential for maintaining soil fertility as well as soil health. The main intendment of this review paper to define the role of intracellular enzyme dehydrogenase in soil environment as well as different methodology to estimate this enzyme activity in soil. Paper also illustrate that dehydogenases enzyme activity serves as microbial indicator and measure of total microbial activity to asses soil health and is quality.

Keywords: Dehydrogenase enzyme activity, role, microbial indicator, methods.

Introduction
Soil is crucial to life on earth. Soil is a dynamic natural body, non-renewable resource, composed of mineral and organic solids, gases, liquids, and living organism which serve as medium for plant growth and monitoring its fertility is an important objective in the sustainable development of agro-ecosystems The quality of soil depends in part on its natural composition, and also on the changes caused by human use and management (Pierce and Larson, 1993) [10]. There is need to assessment of soil quality for maximize the growth and productivity of crop. Strategies based on biological indicators would be a suitable tool to evaluate the sustainability of the soil ecosystem. Studies of soil enzymatic activity assay are best indicator to measure the ecosystem status and quality of soils.

Soil enzymes are the mediators and catalysts of important soil functions that include: decomposition of organic inputs; transformation of native soil organic matter; release of inorganic nutrients for plant growth; N2 fixation; nitrification; gentrification; and detoxification of xenobiotics (Dick, 1997) [3]. In addition, soil enzymes have a crucial role in C (β-glycosidase and β-galactosidase), N (unease), P (phosphates), and S (sulphatase) cycle (Karaca *et al*., 2011) [7]. There are lots of enzymes in the soil environment, serves as biological indicator of soil. Among all enzymes in soil environment, the dehydogenase enzyme activity is commonly used as an indicator of biological activity in soils (Burns 1978) [1]. Dehydrogenase is an enzyme that oxidizes soil organic matter by transferring protons and electrons from substrates to acceptors. This enzyme is considered to exist as an integral part of intact cells but does not accumulate extra-cellular in the soil (Das and Varma, 2011) [2]. This enzyme occurs only within soil bacteria (e.g. genus Pseudomonas, with Pseudomonas entomophilies as most abundant). They do not act on their own without a bacterial host. Therefore, when dehydrogenase is present in the soil, you can reasonably conclude that bacteria are present (Walls-Thumma, 2000) [14]. Measuring dehydrogenase levels allow to better understand the effect of agricultural practices, such as pesticide use, or other management practices on the health of soil, as well as a direct measure of soil microbial activity.

Estimation of dehydrogenase enzyme activity is generally done by adding alternative electron acceptors to soil samples. Dehydrogenase activity (DHA) in soil was determined, using the reduction of 2, 3, 5-triphenyltetrazolium chloride (3%) method (Klein *et al*., 1971) [8]. The most common laboratory procedure used for soil dehydrogenase activity (DHA) determination is the triphenyltetrazolium chloride (TTC) method described by Klein *et al* (1971) [8], the iodonitrotetrazolium chloride (INT) method described by Spothelfer Magafia and Thalmann (1992) [12]. NADH oxidation by using NADH-tris buffer is another method for measurement of DHA in soil. Thus dehydrogenase enzyme in the soil is very important as it may give indications of the potential of the soil to support biochemical processes, which are essential for maintaining soil fertility as well as soil health.

Role of dehydrogenase enzyme activity in soil

Among different soil indicators, DHA is one of the most adequate, important and one of the most sensitive bioindicators, relating to soil quality and fertility. Dehydrogenases play a significant role in the biological oxidation of soil organic matter (OM) by transferring hydrogen from organic substrates to inorganic acceptors (Zhang *et al*., 2010) [16]. DHA serves as an indicator of the microbiological redox-systems and could be considered a good and adequate measure of microbial oxidative activities in soil. Additionally, dehydrogenase enzyme is often used as a measure of any disruption caused by pesticides, trace elements or management practices to the soil (Reddy and Faza 1989; Wilke 1991; Frank and Malkomes 1993) [11, 15, 4], as well as a direct measure of soil microbial activity (Trevors 1984; Garcia and Hernandez *et al*., 1997) [13, 6]. Thus, DHA is proposed as the best indicator of the microbiological redox-systems, and could be considered as good and adequate parameter of microbial oxidative activities in soil.

Method of determination of dwhydrogenase activity in soil

a) With TTC acceptor: Lenhard (1956) [9] appears to have been the first to use 2,3,5 triphenyltetrazolium chloride (TTC) in studies of microbial activity in soil The method is based on the assumption that in the absence of O2 TIC acts quantitatively as the terminal H acceptor for dehydrogenase systems, with the formation of red triphenyltetrazoliumformazan (TPF).

TTC + 2 H+ + 2r- = TPF + HCI.

In this method one gram of soil (fresh weigh, sieved <2mm) is placed in 15 ml of screw capped tube and 0.2 ml of 3% TTC solution added. Add 0.5 ml of 1% glucose solution in this tube, sealed with plastic stoppers and incubate at 28^{o}c for 24 hours. Run a blank with addition of 1ml distil water. Add 10 ml methanol after incubation of sample and shake for 1 min and allow standing in dark for six hours. The supernatant from each sample is then filter into 50 ml conical flask and filtrates measured at 485 nm on a spectrophotometer.

b) INT reduction: The INT reduction was determined according to Spothelfer-Magana and Thalmann (1992) [12] as modified by Friedel *et al* [5]. In this method soil samples were placed in test tubes (16x100 mm) and 2.5 ml of INT-TRIS buffer (0.1 M Tris-HCl pH 7.9 containing 2% INT) were added. The tubes were flushed with N2 for 2 min, sealed with plastic stoppers and parafilm and incubated at 46 ºC in the dark for 30 min and 60 min. The formazan formed was extracted by shaking with 10 ml to tetrahydrofuran for 1 h in the dark. After filtration and dilution of the filtrates at a ratio of 1:7 with acetone, the absorbance was measured at 487 nm. Amount of INT reduced was calculated from formazan calibration curve.

c) NADH oxidation: 0.02g and 0.04g of freeze-dried soil or 0.2g and 0.4g of sifted biological culture soil were placed in separate test tubes (16x100 mm) and 2 ml of NADH-TRIS buffer (0.15 M Tris- HCl pH 7.5 containing 20 mM NADH) were added. The tubes were sealed with plastic stoppers and parafilm and incubated at 37ºC for 0 min, 15 min and 30 min with horizontal shaking. NADH oxidation was stopped by adding 5 ml of TRIS-Carbonate buffer (0.1 M sodium carbonate buffered to pH 11.5 with Tris). In the case of incubation for 0 min the TRIS-Carbonate buffer was added previous to the NADH-TRIS buffer. After mixing for a few seconds 0.5 ml of 0.5 M CaCl2 were added and the suspensions were centrifuged at.1500g for 10 min at 4ºC. The supernatants were filtered through a 0.22 mm nylon filter and their absorbance's measured at 340 nm. The differences in the absorbance between the NADH present at the beginning and that remaining after 15 min or 30 min of incubation were converted in mg or mmol of NADH using a calibration curve and dehydrogenase activity was expressed as mg or m mol of NADH oxidized by 1 g of soil during 1h of incubation.

Conclusion

Soil is a dynamic natural body and important part of terrestrial compartment, and supports all terrestrial life forms. Thus, without proper soil management practices, numerous problems may arise, like reduction of soil Fertility, erosion, groundwater contamination, insufficient water holding capacity and loss of Biodiversity. Assessment of soil quality by using soil microbial indicators is very sensitive approach which is responds quickly to environmental alterations.

There are several biological soil properties that can be used as soil quality indicators, alone or in combination with other chemical or physical properties. Among different soil indicators, DHA is one of the most adequate, important and one of the most sensitive bioindicators, relating to soil quality and fertility. DHA is proposed as the best indicator of the microbiological redox-systems, and could be considered as good and adequate parameter of microbial oxidative activities in soil. Furthermore, soil DHA is also used as a measure of any soils disruption posed by pesticides, heavy metals, or other soils contaminates and improper management practices. Exploiting the usefulness of dehydrogenase enzyme activity in assessment of soil quality and health is key to best soil quality indicator in agriculture system. Therefore understanding the role of dehydrogenase activity in soil environment and study of different methodologies for their assessment has large potential to use as soil quality indicator.

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