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Soil Dehydrogenase Enzyme Activity in Natural and Mine Soil - A Review

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Abstract: Soil enzyme activities are very sensitive to both natural and anthropogenic disturbances and show a quick response to the induced changes. Soil dehydrogenase enzymes are one of the main components of soil enzymatic activities participating in and assuring the correct sequence of all the biochemical routes in soil biogeochemical cycles. Dehydrogenase activity is measured by two methods using the TTC and INT substrate; however, various authors reported poor results when TTC is used as substrate. Different biotic and abiotic factors such as incubation time and temperature, pre-incubation, soil aeration and moisture content have significant effect on dehydrogenase activity in soil. Highest dehydrogenase activity is reported from forest soil in autumn seasons while the disturbed soil from coal mines soils containing lowest dehydrogenase activities along the soil erosion gradient of experimental slopes. Least value of enzymes activity is reported from polluted sites than restored and undisturbed sites. Dehydrogenase enzyme is often used as a measure of any disruption caused by pesticides, trace elements or management practices to the soil, as well as a direct measure of soil microbial activity.

Key words: Dehydrogenase Activity · Substrate · Mine Soil · Natural Soil

INTRODUCTION

Soil quality and its degradation depend on a large number of physical, chemical, biological, microbiological and biochemical properties, the last two being the most sensitive since, they respond rapidly to changes. The microbiological activity of a soil directly influence ecosystem stability and fertility and it is widely accepted that a good level of microbiological activity is essential for maintaining soil quality. The soil microbiological activity viz., the enzymatic activities play a key role in soil nutrient cycling, its activity is essential in both the mineralisation and transformation of organic matters and plant nutrients in soil ecosystem [1].

Soil enzyme activities are very sensitive to both natural and anthropogenic disturbances and show a quick response to the induced changes [2]. Therefore, enzyme activities can be considered effective indicators of soil quality changes resulting from environmental stress or management practices. These soil enzymes play a fundamental role in establishing biogeochemical cycles and facilitate the development of plant cover. It is an important aspect of the below-ground processes and give insight into the relative changes in below-ground system functioning as a plant community develops over time. Enzyme activity in soil results from the activity of accumulated enzymes and from enzymatic activity of proliferating microorganisms [3]. They are usually associated with viable proliferating cells, but enzymes can be excreted from a living cell or released into the soil solution from dead cells. Study of soil enzymes gives information about the release of nutrients in soil by means of organic matter degradation and microbial activity as well as indicators of ecological change. Soil enzymes analysis helps to establish correlation with soil fertilization, microbial activity, biochemical cycling of various elements in soil, degree of pollution (heavy metals) and to assess the succession stage of an ecosystem. So, measurements of enzyme activity in degraded soils have useful in examining impacts of environmental change or management on soil enzyme activities. Several works have been reported the potential

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use of enzyme activity as an index of soil productivity or microbial activity [4, 5]. One of the general criteria used to determine microbial activity and biomass in soil is soil dehydrogenase activity (DHA).

Dehydrogenase Enzymatic Activity (DHA): Soil DHA determination in soils was first initiated by [6], since then it has been widely used because of its simplicity as compared to other quantitative methods. The method was later on modified by [7, 8]. Soil dehydrogenase enzymes are one of the main components of soil enzymatic activities participating in and assuring the correct sequence of all the biochemical routes in soil biogeochemical cycles [9]. It has also found that measurement of changes in soil enzyme activities may provide a useful index of changes in soil quality [10]. The soil dehydrogenase activity in soils provides correlative information on the biological activity and microbial populations in soil. The basic idea of using soil enzymes activity as a measure of microbial indicators for soil fertility was introduced and established by Waksman [11]. Soil dehydrogenase activity is considered to exist in soils as integral parts of intact cells. They are not residing extracellular in the soil. Measurement of dehydrogenase activity represents immediate metabolic activities of soil microorganism at the time of the test. Soil dehydrogenase activity is an oxidative degradation process .i.e., dehydrogenation of organic matter by transferring hydrogen and electrons from substrate to acceptors. Dehydrogenase enzymes play a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to accepters. Water-soluble tetrazolium salts are the preferred oxidants because they form water-insoluble colored formazans which can be measured spectrophotometrically.

The intracellular dehydrogenase enzymes belong to the oxidoreductases and catalyse the oxidation of organic compounds by separating two–H atoms. Many specific dehydrogenases act as to transfer the separated H to either nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate. Through these co-enzymes the H atoms take part in the reductive processes of biosynthesis. Therefore, the overall dehydrogenase activity of a soil depends on the activities of various dehydrogenases, which are a fundamental part of the enzyme system of all microorganisms (enzymes of the respiratory metabolism, the citrate cycle and N metabolism). Dehydrogenase activity thus serves as an indicator of the microbiological redox systems and may be considered a good measure of microbial oxidative activities in soils [12]. H can be transferred to soluble tetrazolium salts (e.g., TTC, INT) with the formation of red formazans, which can be determined calorimetrically after extraction with a solvent.

Different Method Used for Measurement of Dehydrogenase Activity

Use of Tetrazolium Salts: Dehydrogenase assays based on the reduction of 2, 3, 5- triphenyltetrazolium chloride (TTC) to the creaming red-colored formazan (TPF), have been used to determine microbial activity in soil. Water-soluble tetrazolium salts are the preferred oxidants because they form water-insoluble colored formazans which can be measured spectrophotometrically. Soil was Prepared with $CaCO_3$ and dispensed in three tubes as 6g each. The total volume of fluids added to the soil was 3.5 ml; this included any fluids added during preincubation of the soil. Most substrate solution concentrations were 1% dextrose solutions. All of these were sterilized by autoclaving and 1 ml was added to the soil at the time of TTC addition. However, the substrates were added as 0.5 ml of a double-strength solution if there was danger of exceeding the 3.5-ml fluid volume limit. TTC (Calbiochem, San Diego, Calif.) was prepared as a 3% aqueous solution and was sterilized by passage through a 0.30-, um membrane filter (Millipore Corp., Bedford, Mass.). Each tube received 1 ml of TTC, except for the soil blank, which received water instead. After addition, the water, substrate and TTC were simultaneously mixed through the soil with a sterile glass rod; then rubber stoppers were inserted and the tubes were incubated at 37°C at different incubation period of 6hrs, 12hrs, &24hrs. Upon completion of incubation, the tubes could be extracted immediately. Each soil sample was transferred with methanol to a funnel containing Whatman no. 5 filter paper (W. and R. Balston, Ltd.) placed on a 100-ml graduated cylinder. Additional portions of methanol were passed through the soil until 50 ml of methanol, containing the formazan, had been collected in the graduated cylinder. If the filtrate passing from the funnel still had a red color, additional methanol was passed through the soil until all formazan had been extracted and corrections in calculations were made for the additional methanol. The red methanolic solutions of the formazan were read at 485 nm against the extract from the non-TTC soil blank by using a Spectronic 20 colorimeter

(Bausch and Lomb, Rochester, N.Y.). The values obtained were compared against a formazan (Calbiochem) standard curve prepared with methanol and they are reported as milligrams of formazan per gram of soil.

Use of P-Iodonitrotetrazolium Violet, or INT salts: Various authors have used INT 2-(p-iodophenyl)-3-(p-nitrophenyl)- 5-phenyltetrazolium chloride (p-iodonitrotetrazolium violet, or INT) which act as an artificial electron acceptor as a substrate to determined DHA in soil [13]. Later, Trevors *et al*. [14] used INT for the determination of dehydrogenase activity in soils, finding it to be more suitable than TTC for this purpose. Benefield *et al*. [15], Grifiths [16] and Von Mersi and Schinner [17] have reported that INT affords a more accurate estimation of dehydrogenase activity than TTC, which they attributed to its higher electron affinity compared to TTC. As a consequence of this high electron affinity, INT competes more effectively with oxygen for free electrons and so it is not necessary to carry out the determinations under anaerobic conditions. Furthermore, under both aerobic and anaerobic conditions, more INT than TTC is reduced and so sensitivity is also improved [18]. They observed that the reduction from INT to INTF (iodonitrotetrazolium formazan) gives a more exact measurement of DHA in soil extracts, as O_2 does not interfere with the INT reduction. More formazan is produced from the INT reduction than TTC, both with anaerobic and aerobic incubation [18].

Different Factors Affecting the Dehydrogenase Enzymatic Activities: Dehydrogenase activity can be considered to be a good measure of microbial oxidative activity in soils. It is usually determined by measuring the amount of an artificial electron acceptor reduced by microbial activity, such as a soluble tetrazolium salt with a red colored reduced form (a formazan) that can be determined colorimetrically following extraction with a suitable solvent. Studies of dehydrogenase enzymatic activities in soil are important as they indicate the potential of the soil to support biochemical processes which are essential for the maintenance of soil fertility. Soil dehydrogenase activity is often used as a measure of any disruption caused by pesticides, trace elements or management.

Incubation Time and Temperature: The most used incubation periods is 24 hrs or longer at 37°C to study DHA. Klein *et al*, [19] incubated soil for 96 hr with glucose at 27°C and Skujins [20] incubated soil for 24 hrs at 30°C without added substrate. The 24 hrs incubation periods seems to be the part of the dehydrogenase technique most likely to cause problems in the interpretation of results. The dehydrogenase technique for measuring the metabolic activity of microorganism in soil was modified to use a 6 hr, 37°C incubation with either glucose or yeast extract as the electron donating substrate by Casida [21]. He reported that the rate of formazan production remained constant during this time interval and cellular multiplication did not occur. The technique was used to follow changes in the overall metabolic activities of microorganism in soil undergoing incubation with a limiting concentration of added nutrients. Ross [22] studied soil dehydrogenase determinations conducted at 30°C. He too concluded that 6 hr incubation was more valid than longer incubations; however, he recommended a 1 hr anaerobic incubation.

Pre-Incubation of Soil: Pre-incubation of soil with yeast extract or glucose increase the DHA of the soil. Pre-incubation of a dry soil with moisture, however, also caused an increase in DHA. A phenomenon somewhat similar to the latter was studied by Stevenson [23], who pointed out that nutrients become more readily available when dry soil is remoistened. In addition, however, there probably is some breaking of dormancy for cells and depending on the duration of incubation of the remoistened soil, there can be cellular multiplication. Substrate added to the soil for incubation trails usually are added as an aqueous response is a component of the response is a component of the response to the substrate. Perhaps dry soils remoistened and incubated before substrates are added or dehydrogenase determinations are made. Regardless of whether a soil has been pre-incubated, the dehydrogenase curve (formazan production) does not remain linear during 24 hr of incubation but, instead, show a change to a decreased rate at about 9 to 12 hr.

Soil Aeration: Soil aeration parameters such as oxygen diffusion rate (ODR), redox potential (Eh), concentration of (Fe^{2+}), water content and bulk density have been significantly effects on dehydrogenase activity in soil (Brzezinska *et al*., [24]. They have found positive correlation with Fe^{2+}, water content and bulk density, while negative correlation in case of ODR and Eh. Maximum DHA was observed at soil pH of 6.6-7.2. The soil physical properties and the content of decomposable organic matter are the main factors determining the soil aeration status.

Soil Moisture Content: The soil moisture influences both the microbial activity and DHA in the soil. Since moisture found to be an important factor in microbial activity, after the initial addition of water to the soils, 10 day were necessary to reactivate the microbial population [25]. In succeeding weeks, the reduction in the soil moisture content caused a corresponding reduction in the active biomass and thus also in DHA, which continued until after the 6th week of incubation, when water was again added to the soil, once again allowing an increase in enzyme activity. In soil having low moisture level the DHA was reported close to zero [26]. The highest DHA was reported in rainy season and lowest in winter. Soil DHA was positively and significantly correlated with soil pH, Ca, Mg, K and water content. Mersi and Schinner [27] also suggested that samples should be analysed immediately if possible; where an immediate analysis is not possible a maximum of 1 week and all analyses be carried out with field moist soils.

Dehydrogenase Activity in Natural Soil: Estimates of the dehydrogenase activity of soil are simple to carry out and may be used more frequently for studying various aspects of the oxidation of organic compounds in soil or for indicating possible levels of biological activity in soil. Quilchano *et al*. [28] studied the seasonal effect on soil dehydrogenase activity in upper 10 cm of Mediterranean forest soils in Los Alcornocales Natural Park (Southern Spain). They have found that the DHA of forest soil in autumn 527±165 nmol p-iodonitrotetrazolium formazan (INTF) g^{-1} h^{-1} was almost double that in summer 289±95 nmol INTF g^{-1} h^{-1}, for one of the studied plots. During the dry season, DHA of forest control soils 324±85 nmol INTF g^{-1} h^{-1} was higher than in the thinned and shrub-cleared forest 253±93 nmol INTF g^{-1} h^{-1}. The values of DHA obtained are almost double those reported by Leirós *et al*. (2000) in an Atlantic forest of *Q. robur*, exposed to a temperate climate in north-west Spain, in soils containing a higher amount of soil organic matter than those of the Los Alcornocales forest. Other authors also attributed the increase in microbial activity in forest [29] and in grassland soils [30] to higher soil moisture contents.

The soil microbial activity act as a biomarker of degradation and remediation processes of natural soil and abandoned agricultural soils were studied by Pascual *et al*. [31] at the department of Soil and Water Conservation and Organic Waste Management, Spain. They have observed that natural soil contains the 61.1±4.6 mg INTF g^{-1} of soil h^{-1} compared to agricultural soil of aged less than 10 years, which containing about 50.1±6.2 mg INTF g^{-1} of soil h^{-1}, another 10-20 years old agricultural soil contains about 16.2±5.2 mg INTF g^{-1} of soil h^{-1} and greater than 20 years abandoned agricultural soil containing the same dehydrogenase activity as 10-20 years old abandoned agricultural soil. The decrease in activity they have reported may be with the passing time may be due to progressive erosion of the abandoned soils as a consequence of the low levels of plant cover and the low levels of organic matter.

An experiment was conducted by Simek *et al*. [32] to study the evidence of rich microbial communities in the subsoil of a boreal acid sulphat soil conducive to greenhouse gas emissions from research farm at the University of Helsinki, Finland. They have found that dehydrogenase activity (DHA) was the highest in the Ap horizon of both pedons 12.3±0.1µg TPF g^{-1} h^{-1} in the non-acid sulphate soil and 14.3±0.1µg TPF g^{-1} h^{-1} in the acid sulphate (AS) soil. In the B horizons, the DHA decreased to about 3% of the values in the Ap horizons. In the C horizons of both pedons, it increased again to relatively high values. High microbial activity in these horizons of the AS soil are due to presence of large carbon and nitrogen stocks as well as high substrate induced respiration**.** The potential impact of environmental change on ecosystem structure and function and to understand the relationships between enzyme activity and biotic and abiotic factors was studied by Li, *et al*. [33] at the Department of Geography, Israel. They observed that with increasing aridity, the soil dehydrogenase activities decreased significantly in both 0–2- and 5–10-cm soils. They have also observed that when the field-moist soil which was air-dried, dehydrogenase activity was increased significantly. Dehydrogenase activities at two sites Giv'at Ye'arim (GIV) and MIS were least in winter, with no significant difference between spring and summer samples at both soil depths. At Kalia (KAL), the value of soil dehydrogenase activity at 0–2 cm was reported least in the summer samples.

Bonanomi *et al*. [34] studied the soil quality under intensive cultivation and tree orchards in Southern Italy. They have selected 20 agricultural farms in five geographical areas of Southern Italy with different soil types. In each farm, with different management regime which are classified as high-input (HIMR, intensive cultivation under plastic tunnels) or low-input (LIMR, tree orchards). Results showed that there is effect of management regimes on enzymatic activities. Dehydrogenase activities were reduced by 84%

(0.89 µg TPF g^{-1} h^{-1} vs 5.41µg TPF g^{-1} h^{-1}) in the HIMR compared to the LIMR, respectively. This result is consistent with the findings of many studies that reported a decline of enzymatic activities in cultivated soils when compared to the corresponding uncultivated or less-disturbed soils [35]. They have also showed that enzymatic activities were positively correlated only with total soil organic C content and with fungal mycelium.

The drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface of California grassland soils (USA) were studied by Xiang *et al*. [36]. They have reported that microbial activity, as measured by dehydrogenase activity, was roughly 4-fold higher in surface than in subsurface soil. In both soils, dehydrogenase decreased slightly with the initial equilibration and decreased further with extended dry incubation. In both soils, dehydrogenase increased with initial wetting, but decreased with increasing dry/wet cycles. In surface soils, dehydrogenase levels in the 12-cycle treatment fell to levels below those of the continuously moist treatment. In subsurface soils, all the cycling treatments increased total dehydrogenase activity over the course of the incubation; in the 4- and 6-cycle treatments dehydrogenase activities at the end of the incubation were higher than in the continuously moist treatment. The increases in dehydrogenase activity in cycling soils, however, were much was the dominant mechanism driving overall C and biomass dynamics through multiple dry/wet cycles in these soils, dramatically so in the subsurface soil.

The effect of moisture and disaggregation on the microbial activity of soil was studied by Paradelo *et al*. [37] at Departamento de Edafoloxıae Quımica Agrıcola, Spain. The dehydrogenase activity (DHA) of a vineyard soil was measured at different moisture conditions, in different aggregate size fractions and after breaking the existing aggregates. The highest values of DHA (144 mg TPF kg^{-1} day^{-1}) were found for the undisturbed soil at a suction pressure of 0.1 MPa and decreasing values were obtained at pressures above and below that water potential. The disruption of the aggregates strongly reduced the biological activity at suction pressures under 0.1 MPa, but had no effect in drier conditions. Both in disturbed and undisturbed soil, an important part of the biological activity remained at high suction pressures (over 0.1 MPa), with DHA values around 50% of the maximum values.

Dehydrogenase Activity in Degraded Soil: Moreno-de las Heras [38] studied the development of soil physical structure and biological functionality in mining spoils affected by soil erosion in a Mediterranean-Continental environment of Spain. The dehydrogenase activity reported considerably low in the most eroded slopes which are ranged from 0.34–0.56 µg INTF g^{-1}soil h^{-1}. The maximum dehydrogenase activities were reported from least eroded slopes which ranged from 0.87-0.98 µg INTF g^{-1}soil h^{-1}. Exponential decreases in enzymatic hydrolytic activities were found along the soil erosion gradient represented by the five experimental slopes. These decreasing trends are in accordance with the differences found in soil organic matter and vegetation development between the slopes. Sinha *et al*. [39] studied the microbial characteristic of rhizospheric tree species used for re-vegetation of degraded land in Jharia Coalfield (India). They have selected three groups of different plants species used for revegetation. In the first group, containing the highest DHA activity and were reported as >60 mg TPF g^{-1} h^{-1} in *A. marmelos, M. alba, A. indica, D. sissoo* of different plant species; intermediate value of DHA were reported as 20–40 mg TPF g^{-1} h^{-1} under *T. indica, M oleifera, F. religiosa, E. jambolana, B. bauhinia* plantation; and low values <20 mg TPF $g^{-1}h^{-1}$ were recorded under *Eucalyptus, T. grandis, B. monosperma* plantation. The lowest dehydrogenase activity, observed under *Eucalyptus* could be due to the allelopathic effect of root exudates. The presence of many aromatic organic pollutants in the coal mine spoil could also be one of the limiting factors for the survival of the plant species and also the rhizospheric microbiological properties.

The influence of mycorrhizal fungi on the growth of different tree species and their nutrient uptake in gypsum mine spoil in India were studied by Rao *et al*. [40]. They have observed that dehydrogenase activities in the rhizosphere soils were significantly enhanced to varying degrees upon inoculation with *G. fasciculatum.* The dehydrogenase soil enzymatic activities reported under the *A.lebbeck* plantation at the control site is 6.1 pkatal and the DHA value reported under *A.lebbeck* plantation inoculated with *G. fasciculatum* is 8.2 pkatal. The DHA activities recorded under the *A.indica,* 4.4 pkatal in control site and the DHA activities found in inoculated site is 6.1 pkatal. These observations are in conformity with many reports of higher microbial activity in the rhizosphere of plants inoculated with AMF [41, 42].

Table 1: Status of Dehydrogenase Activity (DHA) in different types of land use

Site description	Types of soil	DHA activity	References
Mediterranean forest soils in Los Alcornocales Natural Park (Southern Spain)	Forest soil in autumn. Forest soil in summer Forest control soils. Shrub-cleared forest.	527±165 nmol (INTF) g^{-1} h^{-1} 289±95 nmol INTF g^{-1} h^{-1} 324±85 nmol INTF g^{-1} h^{-1} 253±93 nmol INTF g^{-1} h^{-1}	Quilchano *et al*. (2002).
Natural soil and abandoned agricultural soils, Spain.	Natural soil Agricultural soil 10-20 years old agricultural soil.	61.1±4.6 mg INTF g^{-1} of soil h^{-1} 50.1±6.2 mg INTF g^{-1} of soil h^{-1} 16.2±5.2 mg INTF g^{-1} of soil h^{-1}	Pascual *et al*. (2000).
Research farm at the University of Helsinki, Finland.	Ap horizon in non-acid sulphate soil. Ap horizon in acid sulphate (AS) soil.	12.3±0.1µg TPF g^{-1} h^{-1} 14.3±0.1µg TPF g^{-1} h^{-1}	Simek *et al*. (2011)
Cultivation and tree orchards in Southern Italy.	Intensive cultivation under plastic tunnels. Low-input (LIMR, tree orchards).	0.89 µg TPF g^{-1} h^{-1} 5.41µg TPF g^{-1} h^{-1}	Bonanomi *et al*. (2011).
Vineyard soil, Spain	Undisturbed soil at a suction pressure of 0.1 MPa.	144 mg TPF kg^{-1} day^{-1}	Paradelo *et al*. (2009).
Mining spoils affected by soil erosion in a Mediterranean-Continental, Spian	Eroded slopes Least eroded slopes	0.34-0.56 µg INTF g^{-1}soil h^{-1} 0.87-0.98 µg INTF g^{-1}soil h^{-1}	Moreno-de las Heras (2009)
Re-vegetation of degraded land in Jharia Coalfield (India).	Rhizospheric soils of *A. marmelos, M. alba, A. indica, D. sissoo* plantation. Under under *T. indica, M oleifera, F. religiosa, E. jambolana, B. bauhinia* plantation. Under *Eucalyptus, T. grandis, B. monosperma* plantation	>60 mg TPF g^{-1} h^{-1} 20-40 mg TPF g^{-1} h^{-1} <20 mg TPF $g^{-1}h^{-1}$	Sinha *et al*. (2009).
Gypsum mine spoil, (India)	Rhizosphere soils of *A.lebbeck* plantation at the control site. Rhizosphere soils of *A.lebbeck* plantation inoculated with *G. fasciculatum.*	6.1 pkatal 8.2 pkatal	Rao *et al*. (2001)
Reclamation of post-mining barrens lands, Poland	Loam sandy texture soil Sandy soil Under Birch plantation Mixed plantation Pine plantation.	72.7±26.9 µg INTF g^{-1} h^{-1} 52.2±11.2 µg INTF g^{-1} h^{-1} 77.3±26.8 µg INTF g^{-1} h^{-1} 61.4±26.4 µg INTF g^{-1} h^{-1} 48.7±14.8 µg INTF g^{-1} h^{-1}	Chodak *et al*. (2010).
Heavy metal-contaminated and reclaimed soils, Spain.	Non-polluted area Reclaimed area Polluted area	71.4±5.2 µg TPF g^{-1} dw h^{-1} 53.0±0.5 µg TPF g^{-1} dw h^{-1} 2.9±12.1 µg TPF g^{-1} dw h^{-1}	Hinojosa *et al*. (2004).

Chodak *et al*. [43] studied the re-establishment of soil microbial communities is a prerequisite for successful reclamation of post-mining barrens to assess the effect of texture of soil substrate and the planted tree species on microbial properties of mine soils reclaimed for forestry in Poland. The dehydrogenase enzyme activity reported in loam sandy texture soil is 72.7±26.9 µg INTF g^{-1} h^{-1} and in sandy soil it is 52.2±11.2 µg INTF g^{-1} h^{-1}. The effect of different plantation on DHA activities reported are under Birch plantation is 77.3±26.8 µg INTF g^{-1} h^{-1}, in mixed plantation the activities is 61.4±26.4 µg INTF g^{-1} h^{-1} and in Pine plantation it is 48.7±14.8 µg INTF g^{-1} h^{-1}. The afforested loamy sands contained signidficantly more organic matter and total nitrogen and had higher pH than the sands. Consequently, they maintained larger and more active microbial biomass and higher DHA activities.

The effect of soil moisture pre-treatment on status and health of soil microbiological enzymatic activities act as indicators of heavy metal-contaminated and reclaimed soils were studied by Hinojosa *et al*. [44], in Spain. The different soil samples were collected from polluted plot just underneath the pyrite mud layer, which was removed prior to sampling. The field moisture content of soil samples reported was 4.0% (±2.8). It has been observed that DHA activities were significantly greater for pre-treated rewetted and incubated soils. The greater amount of DHA activities were found in non-polluted area is 71.4±5.2 µg TPF g^{-1} dw h^{-1}, average activities were reported in reclaimed area as 53.0±0.5 µg TPF g^{-1} dw h^{-1} and least value reported in polluted area as 2.9±12.1 µg TPF g^{-1} dw h^{-1}. Enzyme activities decreased significantly with increasing degree of pollution: non-polluted soil

showed the highest enzyme activity values, polluted the lowest and restored soil intermediate values. However, rewetting generally increased enzyme activities of non-polluted and reclaimed soils which improved discrimination from polluted treatments. Polluted soils had much smaller increases or even decreases in enzyme activities with rewetting.

Importance of Dehydrogenase Enzymes Activity in Soil: Dehydrogenase enzyme is often used as a measure of any disruption caused by pesticides, trace elements or management practices to the soil, as well as a direct measure of soil microbial activity [45, 46]. It can also indicate the type and significance of pollution in soils. It has been found that dehydrogenase enzyme is high in soils polluted with pulp and paper mill effluents [47] but low in soils polluted with fly ash [48]. Similarly, higher activities of dehydrogenases have been reported at low doses of pesticides and, lower activities of the enzyme at higher doses of pesticides [49]. As most areas of the world are often polluted by different industrial bio-chemical products, better understanding of the role of this enzyme in environmental science will open greater possibilities of using it as a diagnostic tool for better ecosystem assessment and amelioration.

CONCLUSIONS

The following are the significant findings emerging from the present study.

- Soil dehydrogenase enzymes are one of the main components of soil enzymatic activities participating in and assuring the correct sequence of all the biochemical routes in soil biogeochemical cycles. It has also found that measurement of changes in soil enzyme activities may provide a useful index of changes in soil quality.
- Dehydrogenase enzymatic activities is measured by two methods, namely use TTC and INT as substrate, however, various reported the poor results of DHA when TTC is used as substrate.
- Several biotic and abiotic factors affected soil dehydrogenase activity such as the incubation time and temperature, soil aeration status and soil moisture content and is also often used as a measure of any disruption caused by pesticides, trace elements or management.
- Several workers have used incubation periods of 24 hrs or longer at 37°C to study DHA. Some researchers modified with either glucose or yeast extract as the electron donating substrate. Pre-incubation of dry soil with moisture, also caused an increase in DHA because nutrients become more readily available when dry soil is remoistened.
- The DHA activity was greater in surface than sub-surface soil which is due to accumulation of greater organic matter. It has been also found that the DHA activity was greater in natural soil than the degraded soil.
- The dehydrogenase activities in the rhizosphere soils were significantly enhanced to varying degrees upon inoculation with *G. fasciculatum* as reported from the growth of different tree species and their nutrient uptake in gypsum mine spoil.
- The DHA activity was found greater in forest soils than grassland area. The DHA activity was also found maximum in loamy sandy soil than sandy soil. The afforested loamy sands contained significantly more organic matter and total nitrogen and had higher pH than the sands.

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