

<http://www.pjbs.org>

**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Impact of Soil Environment and Agronomic Practices on Microbial/Dehydrogenase Enzyme Activity in Soil. A Review

Abid Subhani, Huang Changyong, Xie Zhengmiao, Liao Min and A.M. El-ghamry  
Natural Resources Science Department, College of Environmental and Resources Sciences,  
Zhejiang University, Hua Jia Chi Campus, Hangzhou-310029, China

**Abstract:** The present review paper describes the effects of various soil environmental conditions and cultural practices, e.g., soil pH, soil moisture, temperature, nutrient status, insecticide and heavy metal pollutions, on soil microbial activities particularly the dehydrogenase (DNA) or electron transport system (ETS) activity in soil. One of the general criteria used to determine microbial activity and biomass in soil is dehydrogenase/ETS activity. Dehydrogenase activity (DHA) is an indicator for potential non-specific intracellular enzyme activity of the total microbial biomass. It has been used as an indicator for active microbial biomass. Soil pH and temperature can significantly affect microbial activities in soil. It has been reported that dehydrogenase/ETS activity is higher in anaerobically or flooded incubated soils than aerobically incubated soils. Any compound, which alters the number or activity of microorganisms, could affect the soil biochemical properties and ultimately also the soil fertility and plant growth. Dehydrogenase activities increase with increasing microbial populations following amendments of soils with nutrients. The application of organic materials, which contain crop residues, animal feces and their compost, etc., to soil usually, increases the soil biomass and activities. The effects of pesticides and heavy metals addition/presence are also discussed.

**Key words:** Dehydrogenases, soil pH, temperature, moisture, nutrients, organic matter, pesticides, heavy metals

### Introduction

**Basic Concept of Soil Microbial Activity:** The term "soil microbial activity" implies to the overall metabolic activity of all microorganisms inhabiting soil, including bacteria, fungi, actinomycetes, protozoa, algae and microfauna (Nannipieri *et al.*, 1990). The microbial activity plays a vital role in soil productivity sustainability, as it underpins a number of fundamental soil properties such as fertility and structure. The turnover and mineralization of organic substances, nutrient transformations and cycling of organic wastes in and oil are all dependent on the metabolic functions of soil microorganisms (Sparling, 1985; Lee and Pankhurst, 1992). Therefore, the role of microbial activity in the development and functioning of soil ecosystem is inevitable and changes in soil microbial activity may be an indicative of and extremely sensitive to changes in the soil health (Pankhurst *et al.*, 1995). There is considerable interest in study of enzyme activity of soils because such activities may reflect the potential capacity of a soil to perform certain biological transformations of importance to soil fertility. Most studies of soil enzymes have been confined to arable agricultural and forest soils. But a flooded rice soil is predominantly anaerobic and as a result differs from a nonflooded soil in several physical, chemical and biological characteristics. Despite extensive studies on the chemistry (Ponnamperuma, 1972) and microbiology (Yoshida, 1975) of flooded soils. Our knowledge of their enzyme activities is limited. One of the general criteria used to determine microbial activity and biomass in soil is dehydrogenase activity (Ross, 1971; Trevors *et al.*, 1982). Dehydrogenase activity (DHA) is an indicator for potential non-specific intracellular enzyme activity of the total microbial biomass. It has been used as an indicator for active microbial biomass (Ladd, 1978).

Intracellular dehydrogenases belong to the oxidoreductases and catalyze the oxidation of organic compounds by separating two-H atoms. Many specific dehydrogenases 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 redoxsystems and may be considered a good measure of microbial oxidative activities in soils (Tabatabai, 1982). H can be transferred to soluble tetrazolium salts (e.g., TIC, INT) with the formation of red formazans, which can be determined calorimetrically after extraction with a solvent. Trevors *et al.* (1982) reported on the use of 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) for the measurement of dehydrogenase or electron transport system (EIS) activity in soil. The INT is reduced to iodonerotetrazolium formazan (INT-formazan/NTF) by soil enzymes and microorganisms. The INT method offered the advantage of increased sensitivity over the 2, 3, 5-triphenyl tetrazolium chloride (TIC) procedure. At the present time, little information is available on the physical, chemical and biological factors that affect the INT assay. Most research in the past has focused on the factors that affect dehydrogenase activity measurements using TTC. Benefield *et al.* (1977), Trevors (1984), Griffiths (1989) and Von Mersi and Schinner (1991) have also described methods using INT.

Any assay of soil microbial activity may be influenced by numerous conditions, which fluctuate to varying degrees in the same soil. The effects of substrate concentration, O<sub>2</sub> concentrations (moisture), temperature, nutrient availability and pH on microbial dehydrogenase activity in soil are important because they may significantly affect the outcome of ETS activity measurements (Trevors, 1984).

It is demonstrated that soil moisture significantly alters the microbial population, its activity and the relationships between various parameters (Tiwari *et al.*, 1987). Any compound, which alters the number or activity of microorganisms, could affect the soil biochemical properties and ultimately also the soil fertility and plant growth (Wainwright, 1978). Oxygen concentrations and diffusion rates significantly affect the growth and respiration of soil bacteria. They may have pronounced effects on the specific growth rate, maximum growth rate, respiration, numbers and types of bacteria isolated from soil (Nagatsuka and Furusaka, 1980). The

dehydrogenases activity is considered one of the better indicators of microbial activity because dehydrogenases only occur within living cells, unlike other enzymes, which can occur in an extracellular state. However, interpretation of DHA results can be difficult because microbial life is affected by numerous soil factors including organic C, soil pH, N fertilizer, moisture status and soil texture etc. (Cooper and Warman, 1997). It has been reported that dehydrogenase/ETS activity is higher in anaerobically or flooded incubated soils than aerobically incubated soils (Chendrayan *et al.*, 1980; Trevors, 1984).

Active dehydrogenases are considered to exist in soils as integral parts of intact cells and dehydrogenase activities are thought to reflect the total range of oxidative activities of the soil microflora. Nevertheless dehydrogenase activities do not consistently correlate with numbers of microorganisms in soils or with rates of oxygen consumption or carbon dioxide evolution by soils (Stevenson, 1962; Casida *et al.*, 1964; Ross, 1973). Dehydrogenase activities increase with increasing microbial populations following amendments of soils with nutrients (Ladd and Paul, 1973) and decrease when soils are dried (Ross, 1970; Pancholy and Rice, 1972). Dehydrogenase activities are related to soil organic matter contents (Lungesen and Mikkelsen, 1974) but vary with the season (Ross and Roberts, 1970; Ross, 1973) and decrease with depth of sample from the soil profile (Musa and Mukhtar, 1969). Activities are best retained by storing undried soil samples in the cold (Ross, 1970) and reduced when soil was stored air-dried at room temperature (Ross, 1970; Ross and McNeilly, 1972), a reduction which tended to increase with storage (Casida *et al.*, 1964; Ross, 1970). Nevertheless, dehydrogenase activity was still detectable in some air-dried soils that had been stored for a few years (Skujins and McLaren, 1968). Activity was also reduced when soil was stored moist at room temperature (Casida *et al.*, 1964; Pancholy and Rice, 1972), at 4°C (Ross, 1970; Pancholy and Rice, 1972); it was generally reduced at -20°C (Ross, 1972) and minimum loss of activity occurred at 4°C (Ross, 1970; Pancholy and Rice, 1972).

Incubation at 37°C increased dehydrogenase activity over that occurring lower temperatures (Casida *et al.*, 1964; Cerna, 1972) and activity was greater in systems incubated anaerobically than in those incubated aerobically (Ross, 1971; Cerna, 1972; Lungesen and Mikkelsen, 1974).

The present review paper describes the effects of different soil ecological conditions and cultural practices, e.g., soil pH, soil moisture, temperature, nutrient status, insecticide and heavy metal pollutions' on soil microbial dehydrogenase (DHA)/electron transport system (ETS) activity in soil.

**Factors affecting soil microbial activities:** There are various soil environmental and cultural practices those influence the soil microbial biomass and their activities. Some of these factors are discussed here with special emphasis on soil dehydrogenase enzyme activity/electron transport system activity:

**Soil pH:** Soil pH can significantly affect microbial activities in soil. Trevors (1984) reported that dehydrogenase activity was decreased when soil samples were adjusted to a lower pH value from the initial soil pH of 7.7. Soil dehydrogenase activity increased in a linear manner when plotted against pH. The correlation coefficient was 0.982 for the relationship. Very little activity was observed below pH 6.6 and above a pH of 9.5 (Trevors, 1984).

Bremner and Zantua (1975) reported on the effect of pH on

enzyme stability in soils. The pH stability of soil enzymes was dependent on the soil being assayed (Frakenberger and Johanson, 1982). Dehydrogenase or ETS activity was reported to be higher in the presence of buffers at pH 7.6 than in soil amended with either calcium carbonate or water (Ross, 1971). The optimum pH range for ETS activity has been reported to be between 7.4 and 8.5 (Nagatsuka and Furusaka, 1980). However, many soil types may not be included in this range.

**Soil temperature:** Baross and Morita (1978) has extensively reviewed the effects of environmental temperatures on microorganisms. Incubation of soil samples at 37°C increased soil dehydrogenase activity above the values normally observed at lower temperatures (Casida *et al.*, 1964; Cerna, 1972). Trevors (1983) reported that ETS activity was 58-fold higher at 20°C than at 4°C in the same sediment sample, whereas an increase in the incubation temperature from 4 to 10°C brought about a 4-fold increase in ETS activity. It also has been reported that urease, phosphatase and sulfatase enzyme activity was detected in soils -10 and -20°C (Bremner and Tabatabai, 1973). However, no data was reported for ETS activity at these subzero temperatures. Soil samples incubated at temperatures ranging from 5-70°C showed a high positive correlation (0.993) between temperature and soil dehydrogenase activity (Trevors, 1984).

Soil samples incubated at temperatures 10, 25 and 40°C showed a high positive correlation (0.820) between soil temperature and soil dehydrogenase/ETS activity, Activity increased in a linear manner over the entire temperature range. For example, the ETS activity at 40°C (839.7 nmol INTF/g soil/min.) was 2.1-fold higher than at 10°C (400.7 nmol INTF/g soil/min.). A significant ( $p = 0.01$ ) increase in ETS activity was noticed when the incubation temperature was increased from 10 to 25 and 40°C under the constant moisture condition (flooded soil), The ETS activity value of 400.7 found at 10°C was improved to 798.6 and 839.7 nmol INTF/g soil/min. at raised temperatures of 25 and 40°C, respectively. The activities at 25 and 40°C were statistically found at par (Subhani *et al.*, 2000).

**Soil moisture:** Microbial populations, their activities and biochemical transformations differ widely in soils of various moisture contents. It is demonstrated that soil moisture significantly alters the microbial populations, its activity and the relationships between various parameters (Tiwari *et al.*, 1987). They found that moisture has a profound influence on the dehydrogenase activity. While in the air dried and  $1/2$  field capacity soils, the activity decreased and stabilized at a very low level, in case of field capacity and waterlogged soils it rose rapidly to a high level. In the case of wetted soils, increased moisture could bring into solution soluble organic matter, which might be responsible for the increased bacterial population. In the case of the air-dried soil, the decrease may be because of the extreme dryness, which became unfavorable for most bacteria and few could survive in the air-dried soil.

Oxygen concentrations and diffusion rates significantly affect the growth and respiration of soil bacteria (Nagatsuka and Furusaka, 1980). They may have pronounced effects on the specific growth rate, maximum growth rate, respiration, numbers and types of bacteria isolated from soil (Nagatsuka and Furusaka, 1980). It has been reported that dehydrogenase activity is higher in anaerobically incubated soil than aerobically incubated soil (Ross, 1971; Cerna, 1972). The dehydrogenase and invertase activities of three soils were

studied under flooded and nonflooded (60% WHO) conditions. Flooding increased ( $\times 1.25$ - $2.5$ ) the dehydrogenase activity (Chendrayan *et al.*, 1980). Initial  $O_2$  concentrations ranging from 0 to 20% displayed a high negative correlation of  $-0.868$  when plotted against soil ETS activity (Trevors, 1984). One of the important consequences of flooding soil is a marked shift in favor of anaerobic microorganisms. Presumably, the increased dehydrogenase activity was related to the increase in the population of anaerobic microorganisms in flooded soils. Most dehydrogenases are of anaerobic origin (Ross and Roberts, 1970). Iron reduction is probably the most dominant of all redox reactions in a flooded soil (Ponnampertuma, 1972). Tate (1979) observed a significant increase in the dehydrogenase activity of soil under anaerobic conditions though no change in the microbial numbers was detected. This, he suggested, might be due to an increased role for the facultative and anaerobic bacteria. Brzezinska *et al.* (1998) studied the effect of soil oxygen status on dehydrogenase activity. The soils were subjected to soil water tensions of 0, 5 or 15.9 kPa and temperatures of 10, 20 or 30°C. Dehydrogenases activity in these treated soils increased with increased soil water content and temperature: 10°C rise increased dehydrogenase activity by 2.6-4.6 times. The combined effect of flooding and temperature increase to 30°C increased dehydrogenase activity by an average 129-times as compared with the 15.9 kPa at 10°C treatment. Flooding and temperature increases caused a decrease in soil redox potential and  $O_2$  diffusion rates. Soil dehydrogenase activity was highly correlated with redox potential and it is suggested that soil water content and temperature influence the dehydrogenase activity indirectly by affecting the soil oxidation-reduction status.

Subhani *et al.* (2000) noticed a high positive correlation (0.867) between soil moisture contents and ETS activity. The ETS activity was increased significantly ( $p = 0.01$ ) as the soil moisture contents were increased and the maximum (800.5 nmol (NTF/g soil/min.) activity was found under flooded soil condition (anaerobic soil) and the minimum (38.7 nmol INTF/g soil/min.) in air-dried soil. The ETS activity of 38.7, observed in air-dried soil, was enhanced to 60.4, 689.3 and 800.5 nmol INTF/g soil/min., in 50% WHC, 100% WHC and flooded soil, respectively.

**Nutrient availability:** Nutrient availability also influences the enzyme activities, as various microorganisms control their enzyme production in response to nutrient availability (Chrost, 1891). There are contradictory reports concerning the relationship between the enzymes and the inorganic nutrient content in the soil (Speir and Ross, 1978; Benckiser *et al.*, 1984). Dehydrogenase activities increase with increasing microbial populations following amendments of soils with nutrients (Ladd and Paul, 1973). Ammonium sulfate at concentrations ranging from 40-120  $\mu\text{g/g}$  had no inhibitory effect on ETS activity, however, at 160 and 200  $\mu\text{g/g}$ , activity was reduced 13.8 and 10.3%, respectively (Trevors, 1984). Subhani *et al.* (2000) applied N at various levels ranging from 0 to 400  $\mu\text{g/g}$  soil and observed different response on ETS activity. The lower rates of N i.e., 25, 50, 100  $\mu\text{g/g}$  soil increased the activity by 2.3, 5.4 and 17.5%, respectively, as compared with the control. The lowest level (25  $\mu\text{g/g}$  soil/min) failed to produce any significant increase, while  $N_{50}$  and  $N_{100}$  resulted in significant increase in ETS activity. However, higher N concentrations of 200 and 400  $\mu\text{g/g}$  soil, caused significant reduction of 4.5 and 8.7, respectively. The maximum activity (942.1 nmol INTF/g soil/min.) was observed with  $N_{100}$ , while minimum (731.6 nmol INTF/g soil/min.)

activity was recorded at N application rate of 400  $\mu\text{g/g}$  soil. There appears to be no linear relationship between N concentrations and ETS activity, since higher rates reduced activity and lower concentrations did not cause an inhibition.

**Organic matter:** Microbial biomass acts as a labile sink and source of plant nutrients in soil. The biomass is generally increased by applications of organic matter, which may have same advantages over chemical fertilizers both in terms of sustainability and from an environmental view point (Jenkinson and Ladd, 1981).

It has been known that the application of organic materials, which contain crop residues, animal feces and their compost, etc., to soil usually increases the soil biomass and activities (Jenkinson and Powlson, 1976; Powlson *et al.*, 1987; Fraser *et al.*, 1988). Kazunori and Yutaka (1991) measured the relationship between the amount of organic material applied and soil biomass content in soils amended with various organic materials. They found that the values of the soil biomass in the soil amended with organic material were greater than those in the soil amended with inorganic fertilizer.

Shifts in microbial numbers and activity in soil are often related to changes in C inputs to soil as a result of management-related changes in crop type or residue addition. Soil microbial populations are increased by addition of animal manures or by synthetic fertilizers where crop residue production and soil organic matter levels are simultaneously increased (Martyniuk and Wagner, 1978). Fraser *et al.* (1988) studied the effects of 7 organic herbicide and insecticide, singly or in combination, on soil microbial population and found that soil microbial biomass, bacterial and fungal contents and  $CO_2$  evolution were greater in soils receiving manure. Microbial biomass levels in manure-amended soils were 10 to 26% greater than in non-manured soils receiving commercial fertilizer or pesticides. Shinjiro *et al.* (1988) investigated the effect of fertilizer and manure application microbial numbers, biomass and enzyme activities in volcanic ash soils. They observed that microbial numbers and soil microbial biomass C was the largest in the farmyard manure plot, followed by the chemical fertilizer plot (N-fused magnesium phosphate 12-300  $\text{kg ha}^{-1}$  annually) and smallest in the no fertilizer plot. Jenkinson and Powlson (1976) reported that the microbial biomass-C of a wheat field soil was 20 mg C/100g dry soil in the plot without fertilizer application, 22 in the NPK Mg (Na) plot and 47 in the FYM plot.

Schnurer *et al.* (1985) studied the change in soil fertility caused by various organic (1800  $\text{kg C ha}^{-1} \text{yr}^{-1}$  as straw or FYM) and N-fertilizer (as  $Ca(NO_3)_2$  at 80  $\text{kg N ha}^{-1} \text{yr}^{-1}$ ) amendments in a long-term field trial and observed that both biomass estimates and activity measurements have a highly significant correlation with soil organic matter. Lovell and Jarvis (1996) and Fujiyoshi and Kazuyuki (1997) reported similar results.

**Pesticides addition:** A different opinion exists about the use and effect of pesticide on soil microbial biomass and their activities. For examples: the use of pesticides is thought to be harmful to microorganisms and their activities contributing to soil fertility and is therefore avoided (Wolf, 1977). Junnila (1994) reported that metsulfuron-methyl had a little effect on microbial dehydrogenase and nitrification activities. Pesticides generally appear to have no adverse effects on the population of total bacteria in soil except at concentrations exceeding recommended rates (Anderson, 1981) and stated that usually the adverse effects of pesticides are manifested only at higher concentrations, while at recommended field levels they are merely of an ephemeral

nature. The application to a clay loam soil 11.0% organic carbon, pH of 7.2) of different pesticides including diazinon, leptophos, malathion, parathion, triazophos, dieldrin, captan, etc., all at 5 and 10 mg kg<sup>-1</sup> and of dichloropropanedichloropropene and nitrapyrin, at usual field rates, resulted in no inhibition of dehydrogenase activity within 7 days, although several of the pesticides stimulated the activity, probably as a result of an increase in resistant genotypes that catabolically utilized the affected microorganisms (Tu, 1981).

In a sandy soil, with 3.0% organic matter and a pH of 7.5, 5 mg kg<sup>-1</sup> atrazine reduced the dehydrogenase activity by about 4.0%, but after 30 days the activity was similar to that of the control. However, no complete recovery of activity within this period was observed at concentrations above 50 mg kg<sup>-1</sup>. A less pronounced effect was observed with fluometuron, i.e., a reversible 30% reduction at 50 mg kg<sup>-1</sup> and triflurain, which inhibited the activity by less than 30% at 500 mg kg<sup>-1</sup>. All pesticides tested were formulated products. The formulated herbicides pendimethalin and difenzoquat, both at 0.5 mg kg<sup>-1</sup> and thiobencarb at 2.5 mg kg<sup>-1</sup> had no adverse effects on dehydrogenases in different soils. The fungicide folpet and captafol, at 1.0 mg kg<sup>-1</sup>, inhibited dehydrogenases slightly. However, a 10-fold increase in the dosage of folpet and captafol reduced the activity significantly, but it recovered to the control level after 21 days (Atlas *et al.*, 1978). Ready recovery of the affected microorganisms as a result of fast degradation or immobilization of the pesticides under the experimental conditions might explain the reversible effects of the pesticides on the oxidoreductive and cellulolytic activities. On the other hand, it is possible that the impairment of one segment of the microbial community was compensated by increased activity of other genotypes with a resulting unchanged overall microbial activity. Whatever mechanism is responsible for the observed effects in this study, both reflect the high degree of soil homeostasis of the microbiological activity (Atlas *et al.*, 1978).

The effects on dehydrogenase activity of formulated hexachloro-cyclohexane (HCH), carbaryl, benomyl and atrazine in an alluvial soil (0.7% organic matter, pH 6.4) were tested under flooding conditions: i.e., after pesticides application the soil was flooded with standing water. At 1 mg/kg, no inhibition occurred with any of the pesticides, but at a 10-fold rate with HCH, dehydrogenase activity dropped temporarily to about 30% but recovered after 20 days. An even more drastic reduction was observed with benomyl at 10 mg/kg, with only 10% residual activity detected initially and still less than 50% after 20 days of incubation. Carbaryl at the same concentrations showed no effect. At a dose of 100 mg/kg, HCH and benomyl essentially abolished dehydrogenase activity with no recovery after 20 days, whereas carbaryl reduced the activity by only a factor of 2 throughout the testing period. Only atrazine induced no depression at all concentrations. In a different experiment, the same soil was flooded with standing water for 15 days before treatment with pesticides. Under such conditions HCH and benomyl at 100 mg kg<sup>-1</sup> induced only negligible loss in activity (Chendrayan and Sethunathan, 1980), which shows that the experimental conditions have a major influence on the activities of soil enzymes. Absence of any information on the persistence of the chemicals under the experimental conditions is an imperfection of many reports on side effect testing of pesticides. Clearly, 20 days of monitoring microbial activities will be considerably shorter than the half-lives of some of the pesticides tested in this study.

The effect of the herbicide dalapon at 2.6, 26.6, 266 and 2660 mg/kg on the activity of the soil microflora was tested in a loamy sand 12.4% organic carbon pH 6.4) and a sandy loam (1.7% organic carbon, pH 7.6). The pesticide was

readily degraded in both soils: no residues were detected with the two lowest concentrations after 2 and 4 weeks, respectively. However, with application at 2660 mg kg<sup>-1</sup>, degradation was retarded and 1215 mg kg<sup>-1</sup> of the parent compound was analyzed in the loamy sand and 190 mg kg<sup>-1</sup> in the sandy loam after 32 weeks. Dehydrogenase activity was inhibited at the two lowest concentrations, but the effects were statistically not significant. However, the activity was inhibited for 32 weeks at 266 and 2660 mg kg<sup>-1</sup>, i.e., at much higher concentrations than those used for normal agricultural management practices. The conclusion of this study is that dalapon used at the recommended concentration will not impair soil fertility, as all effects were reversible due to the ready microbial degradation of the pesticide and of its major metabolites (Greaves *et al.*, 1981).

In a loamy soil with a sand content of 6.2% (1.4% organic carbon, pH 6.8) simazine, atrazine, propazine and pronetryne at conventional field rates stimulated dehydrogenase activity: e.g., with simazine at 1 kg ha<sup>-1</sup> the activity was about 140% of the control activity. On the other hand, aminotriazole, fenuron, defenuron, lenacil, chlorpropharn and propham inhibited the activity, the last by almost 50%. These effects paralleled the influence of the chemicals on populations of soil fungi but not of bacteria. However, in a soil with 64.6% sand content (0.7% organic carbon pH 5.9) all pesticides had an inhibitory effect on dehydrogenase activity, with proximpham, propham and atrazine at 10, 10 and 2 kg ha<sup>-1</sup>, respectively, reducing the activity by more than 50% 10 days after application. The more pronounced inhibitions in the sandy soil with its limited nutrient supply may be explained by the induction of significantly higher numbers of bacteria by the pesticides and concurrent reduction of other metabolic activities (Hickisch *et al.*, 1984).

The effect of cycloate (2.9 kg ha<sup>-1</sup>) alone and in combination with the phospholipid phosphatidylcholine (1.6 kg/ha) was tested in a sandy loam (0.9% organic carbon, pH 7.11) and in silt loam soil (2.6% organic carbon, pH 7.31). Dehydrogenase activity was decreased by both treatments during the first week and then returned to that of the control soils, but the effects were more pronounced in the soil with a lower organic carbon content. The inhibitory effect was still observed after 20 weeks of incubation when a 10-fold concentration of the chemicals was applied (Malkomes and Halstrick, 1985).

**Heavy metals:** A large number of studies have measured the enzyme activities in relation to heavy metal pollution in soil (Baath, 1989; Schnurer, 1989; Bardgett *et al.*, 1994). These include the measurements of some very unspecific enzymes, like dehydrogenase, to those that are involved in more specific reactions, like urease, protease etc. Generally, the enzyme activities in soils have been shown to decrease drastically in response to increasing metal pollution. Wilke (1987) found, a significant inhibition of the dehydrogenase and proteinase activities even eight and ten years after the last additions of zinc and copper, respectively. Zn reduced the enzyme activities in the tested soil although its total content (222 mg Zn kg<sup>-1</sup>) did not exceed the recommended guidelines (300 mg kg<sup>-1</sup>). In another study, addition of Br, F, iii and V as inorganic salts reduced the activities of protease and dehydrogenase enzymes in a sandy cambisol, though the total concentrations of these elements were not above the maximum recommended levels (Wilke, 1988). Br had an inhibitory effect even below the concentration of 10 mg kg<sup>-1</sup> soil. Belitsyna *et al.* (1989) found, the greatest suppression of invertase and dehydrogenase activity in soil at 10, 500 and 1000 mg kg<sup>-1</sup> of Cd, Pb and Zn, respectively. Kandler *et al.* (1992) reported, a decrease of dehydrogenase, protease, alkaline phosphatase and arylsulfatase activities in soil samples at 5.10 cm depth

close to a smelter. The significant declines of dehydrogenase activity in all the above studies indicate it to be the most sensitive parameter for the detection of harmful effects of inorganic pollutants on the soil microbial activity (Wilke, 1988).

Addition of low metal sludges, composts and other organic materials has been found to increase the activities of dehydrogenase, phosphatase and beta-Glucosidase (Chander and Brookes, 1991; Giusquiani *et al.*, 1994). Increases in enzyme activities (urease, phosphatase) were also observed in some other cases, where the organic substrates like glucose or ryegrass were applied as energy sources (Nannipieri *et al.*, 1983), or organic lead compounds were added to soils (Naplekova and Bulavko, 1983). The increased enzyme activities, in the above studies, were attributed to the enhanced microbial activity in soils, stimulated by the addition of nutrients and organic matter contained in the sludges and composts (Eivazi and Zakriya, 1993). However, the addition of sludges or composts at the higher rates reversed this effect, causing a decrease in the activities of dehydrogenase, phosphatase, arylsulfatase and L-asparaginase (Reddy *et al.*, 1987). This inhibition of enzyme activities was related to the heavy metal (Cu, Zn, Ni, Cd, Fe and Mn) concentrations in the sludge-amended soils. A significant decline in the dehydrogenase activity was observed in soils amended with Cu-enriched sludges (Chander and Brookes, 1991; Chander *et al.*, 1995).

A stimulation of enzyme activities was also observed in the rhizosphere soils. Reddy *et al.* (1987) found that enzyme activities in the rhizosphere soils (R) were higher than in before planting (BP) and non-rhizosphere (NR) sludge-amended soils. The root effect (enzyme activity in rhizosphere: enzyme activity in non-rhizosphere soil) on the dehydrogenase, urease and phosphatase ranged from 1.05 to 1.42, 1.2 to 3.06 and 0.94 to 1.23 mg g<sup>-1</sup> soil, respectively. Increasing concentrations of sludge reduced the urease activity by 20-73% in the BP and 38-40% in the NR soils, but increased by 16-43 % in the R soils. Marzadori *et al.* (1996) observed that the soil dehydrogenase activity was influenced by variations in the soil moisture content in a lead spiked soil, but the effects of variation in soil moisture were less evident on the phosphatase activity.

**Summary:** The microbial activity plays a vital role in soil productivity sustainability, as it underpins a number of fundamental soil properties such as fertility and structure. One of the general criteria used to determine microbial activity and biomass in soil is dehydrogenase activity. Dehydrogenase activity (DI-IA) is an indicator for potential non-specific intracellular enzyme activity of the total microbial biomass. Dehydrogenase activity thus serves as an indicator of the microbiological redoxsystems and may be considered a good measure of microbial oxidative activities in soils. It has been used as an indicator for active microbial biomass. It is effected by various soil conditions and management practices, e.g., O<sub>2</sub> concentrations (moisture), temperature, nutrient availability, soil pH, organic matter Contents of the soil and presence or addition of heavy metals, pesticides, manure etc. etc.

## References

Anderson, J.P.E., 1981. Methods to evaluate pesticide damage to the biomass of the soil microflora. *Soil Biol. Biochem.*, 13: 149-153.

Atlas, R.M., D. Pramer and R. Bartha, 1978. Assessment of pesticide effects on non-target soil microorganisms. *Soil Biol. Biochem.*, 10: 231-239.

Baath, E., 1989. Effects of heavy metals in soil on microbial processes and populations: A review. *Water Air Soil Pollut.*, 47: 335-379.

Bardgett, R.D., T.W. Speir, D.J. Ross, G.W. Yeates and H.A. Kettles, 1994. Impact of pasture contamination by copper, chromium and arsenic timber preservative on soil microbial properties and nematodes. *J. Biol. Fertil. Soils*, 18: 71-79.

Baross, J.A. and R.Y. Morita, 1978. *Microbial Life at Low Temperature: Ecological Aspects*. In: *Microbial life in extreme environments*, Kushner, D.J. (Ed.). Academic Press, New York.

Belitsyna, G.D., N.Y. Dronova, I.N. Skovortsova and L.N. Tomilina, 1989. Alteration of certain indices of soil biological activity under anthropogenic stress. *Soviet Soil Sci.*, 21: 40-44.

Benckiser, G., S. Santiago, H.U. Neue, I. Watanabe and J.C.G. Ottow, 1984. Effect of fertilization on exudation, dehydrogenase activity, iron-reducing populations and Fe<sup>++</sup> formation in the rhizosphere of rice (*Oryza sativa* L.) in relation to iron toxicity. *Plant Soil*, 79: 305-316.

Benefield, C.B., P.J.A. Howard and D.M. Howard, 1977. The estimation of dehydrogenase activity in soil. *Soil Biol. Biochem.*, 9: 67-70.

Bremner, J.M. and M.A. Tabatabai, 1973. Effects of some inorganic substances on TTC assay of dehydrogenase activity in soils. *Soil Biol. Biochem.*, 5: 385-386.

Bremner, J.M. and M.I. Zantua, 1975. Enzyme activity in soils at subzero temperatures. *Soil Biol. Biochem.*, 7: 383-387.

Brzezinska, M., Z. Stępniewska and W. Stępniewski, 1998. Soil oxygen status and dehydrogenase activity. *Soil Biol. Biochem.*, 30: 1783-1790.

Casida, L.E., D.A. Klein and T. Santro, 1964. Soil dehydrogenase activity. *Soil Sci.*, 98: 371-376.

Cerna, S., 1972. Effect of some ecological factors on dehydrogenase activity in soil. *Rostl. Vyroba*, 18: 101-106.

Chander, K. and P.C. Brookes, 1991. Is the dehydrogenase assay invalid as a method to estimate microbial activity in copper-contaminated soils? *Soil Biol. Biochem.*, 23: 909-915.

Chander, K., P.C. Brookes and S.A. Harding, 1995. Microbial biomass dynamics following addition of metal-enriched sewage sludges to a sandy loam. *Soil Biol. Biochem.*, 27: 1409-1421.

Chendrayan, K. and N. Sethunathan, 1980. Effects of HCH, carbaryl, benomyl and atrazine on the dehydrogenase activity in a flooded soil. *J. Bull. Environ. Contam. Toxicol.*, 24: 379-382.

Chendrayan, K., T.K. Adhya and N. Sethunathan, 1980. Dehydrogenase and invertase activities of flooded soils. *Soil Biol. Biochem.*, 12: 271-273.

Chrost, R.J., 1991. Environmental Control of the Synthesis and Activity of Aquatic Microbial Extracellular Enzymes. In: *Microbial Enzymes in Aquatic Environments*, Chrost, R.J. (Ed.). Springer-Verlag, New York, pp: 29-53.

Cooper, J.M. and P.R. Warman, 1997. Effects of three fertility amendments on soil dehydrogenase activity, organic C and pH. *Can. J. Soil Sci.*, 77: 281-283.

Eivazi, F. and A. Zakariya, 1993. Beta-glucosidase activity in soils amended with sewage sludge. *Agric. Ecosyst. Environ.*, 43: 155-161.

Frakenberger, Jr. W.T. and J.B. Johanson, 1982. Effect of pH on enzyme stability in soils. *Soil Biol. Biochem.*, 14: 433-437.

Fraser, D.G., J.W. Doran, W.W. Sahs and G.W. Lesoing, 1988. Soil microbial populations and activities under conventional and organic management. *J. Environ. Qual.*, 17: 585-590.

Fujiyoshi, S. and I. Kazuyuki, 1997. Effects of organic matter application on microbial biomass and available nutrients in various types of paddy soils. *Soil Sci. Plant Nutr.*, 43: 191-203.

Giusquiani, P.L., D. Businelli and G. Gigliotti, 1994. Long-term effects of heavy metals from composted municipal waste on some enzyme activities in a cultivated soil. *J. Biol. Fertil. Soils*, 17: 257-262.

Greaves, M.P., H.A. Davies, J.A.P. Marsh and G.I. Wingfield, 1981. Effects of pesticides on soil microflora using dalapon as an example. *J. Arch. Environ. Contam. Toxicol.*, 10: 437-449.

Griffiths, B.S., 1989. Improved extraction of idonitrotetrazolium-foramazan from soil with dimethylformamide. *Soil Biol. Biochem.*, 21: 179-180.

Hickisch, B., G. Machulla and G. Mueller, 1984. Side effects of herbicides on soil organisms after one and several applications thereof. Second communication. Laboratory experiments: Results obtained for dehydrogenase activity and soil respiration after one application. *Microbiol. Res.*, 139: 13-20.

Jenkinson, D.S. and D.S. Powlson, 1976. The effects of biocidal treatments on metabolism in soil-V: A method for measuring soil biomass. *Soil Biol. Biochem.*, 8: 209-213.

**Subhani *et al.*: Dehydrogenases, soil pH, temperature, moisture, nutrients, organic matter, pesticides, heavy metals**

- Jenkinson, D.S. and J.N. Ladd, 1981. Microbial Biomass in Soil: Measurement and Turnover. In: Soil Biochemistry, Paul, E.A. and J.N. Ladd (Eds.). Marcel Dekker, New York, USA., pp: 415-471.
- Junnila, S., H. Heinonen-Tanski, L.R. Ervio and P. Laitinen, 1994. Phytotoxic persistence and microbiological effects of chlorsulfuron and metsulfuron in Finnish soils. *Weed Res.*, 34: 413-423.
- Kandeler, E., G. Luftenegger and S. Schwarz, 1992. Soil microbial processes and Testacea (Protozoa) as indicators of heavy metal pollution. *Zeitschrift Pflanzenernahrung Bodenkunde*, 155: 319-322.
- Kazunori, S. and O. Yutaka, 1991. Relationship between the amount of organic material applied and soil biomass content. *Soil Sci. Plant Nutr.*, 37: 387-397.
- Ladd, J.N. and E.A. Paul, 1973. Changes in enzymic activity and distribution of acid-soluble, amino acid-nitrogen in soil during nitrogen immobilization and mineralization. *Soil Biol. Biochem.*, 5: 825-840.
- Ladd, J.N., 1978. Origin and Range of Enzymes in Soil. In: Soil Enzymes, Burns, R.G. (Ed.). Marcel Dekker, New York, USA., pp: 51-96.
- Lee, K.E. and C.E. Pankhurst, 1992. Soil organisms and sustainable productivity. *Aust. J. Soil Res.*, 30: 855-892.
- Lovell, R.D. and S.C. Jarvis, 1996. Effect of cattle dung on soil microbial biomass C and N in a permanent pasture soil. *Soil Biol. Biochem.*, 28: 291-299.
- Lungesen, K. and J.P. Mikkelsen, 1974. Dehydrogenase activity in danish soils. *Tids. Planteavl.*, 177: 516-520.
- Malkomes, H.P. and S. Halstrick, 1985. Effect of the herbicide Ro-Neet and its combination with a phospholipid on biological activities in soil under laboratory conditions. *Zentralblatt fur Mikrobiologie*, 140: 381-391.
- Martyniuk, S., and G.H. Wagner, 1978. Quantitative and qualitative examination of soil microflora associated with different management systems. *Soil Sci.*, 125: 337-395.
- Marzadori, C., C. Ciavatta, D. Montecchio and C. Gessa, 1996. Effects of lead pollution on different soil enzyme activities. *Biol. Fertil. Soils*, 22: 53-58.
- Musa, M.M. and N.O. Mukhtar, 1969. Enzymatic activity of a soil profile in the Sudan Gezira. *Plant Soil*, 30: 153-156.
- Nagatsuka, T. and C. Furusaka, 1980. Effects of oxygen tension on growth, respiration and types of bacteria isolated from soil suspensions. *Soil Biol. Biochem.*, 12: 397-403.
- Nannipieri, P., L. Muccini and C. Ciardi, 1983. Microbial biomass and enzyme activities: Production and persistence. *Soil Biol. Biochem.*, 15: 679-685.
- Nannipieri, P., S. Grego and B. Ceccanti, 1990. Ecological Significance of Biological Activity. In: Soil Biochemistry, Bollang, J.M. and G. Stotzky (Eds.). Marcel Dekker, Inc., New York, pp: 293-355.
- Napleikova, N.N. and G.I. Bulavko, 1983. Enzyme activity of soils polluted by lead compounds. *Soviet Soil Sci.*, 15: 33-38.
- Pancholy, S.K. and E.L. Rice, 1972. Effect of storage conditions on activities of urease, invertase, amylase and dehydrogenase in soil. *Soil Sci. Soc. Am. J.*, 36: 536-537.
- Pankhurst, C.E., B.A. Hawke, H.J. McDonald, C.A. Kirby and J.C. Buckerfield *et al.*, 1995. Evaluation of soil biological properties as potential bioindicators of soil health. *Aust. J. Exp. Agric.*, 35: 1015-1028.
- Ponnamperuma, F.N., 1972. The chemistry of submerged soils. *Adv. Agron.*, 24: 29-96.
- Powlson, D.S., P.C. Brooks and B.T. Christensen, 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. *Soil Biol. Biochem.*, 19: 159-164.
- Reddy, G.B., A. Faza and R. Bennett Jr., 1987. Activity of enzymes in rhizosphere and non-rhizosphere soils amended with sludge. *Soil Biol. Biochem.*, 19: 203-205.
- Ross, D.J. and B.A. McNeilly, 1972. Effects of storgae on oxygen uptakes and dehydrogenase activities of beech forest litter and soil. *N. Z. J. Sci.*, 15: 453-462.
- Ross, D.J. and H.S. Roberts, 1970. Enzyme activities and oxygen uptakes of soils under pasture in temperature and rainfall sequences. *Eur. J. Soil Sci.*, 21: 368-381.
- Ross, D.J., 1970. Effects of storage on dehydrogenase activities of soils. *Soil Biol. Biochem.*, 2: 55-61.
- Ross, D.J., 1971. Some factors influencing the estimation of dehydrogenase activities of some soils under pasture. *Soil Biol. Biochem.*, 3: 97-110.
- Ross, D.J., 1972. Effects of freezing and thawing of some grassland topsoils on oxygen uptakes and dehydrogenase activities. *Soil Biol. Biochem.*, 4: 115-117.
- Ross, D.J., 1973. Biochemical activities in a soil profile under hard beech forest, 2. some factors influencing oxygen uptake and dehydrogenase activities. *N. Z. J. Sci.*, 16: 225-240.
- Schnurer, E., 1989. Enzyme activities and microbial biomass in old landfill soils with long-term metal pollution. *Verhandlun. Gesellsch. Okol.*, 18: 339-348.
- Schnurer, J., M. Clarholm and T. Rosswall, 1985. Microbial biomass and activity in an agricultural soil with different organic matter contents. *Soil Biol. Biochem.*, 17: 611-618.
- Shinjiro, K., A. Susumu and T. Yasuo, 1988. Effect of fertilizer and manure application on microbial numbers, biomass, and enzyme activities in volcanic ash soils: I. Microbial numbers and biomass carbon. *Soil Sci. Plant Nutr.*, 34: 429-439.
- Skujins, J.J. and A.D. McLaren, 1968. P Persistence of enzymatic activities in stored and geologically preserved soils. *Enzymologia*, 34: 213-225.
- Sparling, G.P., 1985. The Soil Biomass. In: Soil Organic Matter and Biological Activity, Vaughan, D. and R.E. Malcolm (Eds.). Martinus Nijhoff Dr. W. Junk, Boston, Lanchester, pp: 223-239.
- Speir, T.W. and D.J. Ross, 1978. Soil Phosphatase and Sulphatase. In: Soil Enzymes, Burns, R.G. (Ed.). Academic Press, London, pp: 197-250.
- Stevenson, I.L., 1962. The effect of decomposition of various crop plants on the metabolic activity of the soil microflora Can. *J. Micorbiol.*, 8: 501-509.
- Subhani, A., M. Liao, C.Y. Huang and Z.M. Xie, 2000. Effects of some management practices on electron transport system (ETS) activity in paddy soil. *Pedosphere*, 10: 257-264.
- Tabatabai, M.A., 1982. Soil Enzymes. In: Methods of Soil Analysis, Part 2, Agronomy 9, American Society of Agronomy, Page, A.L., R.H. Miller and D.R. Keeney (Eds.). Madison, Wis., USA., pp: 903-947.
- Tate, L.R.III., 1979. Effect of flooding on microbial activities in organic soils: Carbon metabolism. *Soil Sci.*, 128: 267-273.
- Tiwari, S.C., B.K. Tiwari and R.R. Mishra, 1987. The influence of moisture regimes on the population and activity of soil microorganisms. *Plant Soil*, 101: 133-136.
- Trevors, J.T., C.I. Mayfield and W.E. Inniss, 1982. Measurement of electron transport system (ETS) activity in soil. *Microb. Ecol.*, 8: 163-168.
- Trevors, J.T., 1983. Effect of mercuric chloride on electron transport system (ETS) activity in sediment. *J. Water Air Soil Pollut.*, 20: 265-271.
- Trevors, J.T., 1984. Effect of substrate concentration, inorganic nitrogen, O<sub>2</sub> concentration, temperature and pH on dehydrogenase activity in soil. *Plant Soil*, 77: 285-293.
- Tu, C.M., 1981. Effects of pesticides on activities of enzymes and microorganisms in a clay soil. *J. Environ. Sci. Health Part B*, 16: 179-191.
- Von Mersi, W. and F. Schinner, 1991. An improved and accurate method for determining the dehydrogenase activity of soils with idonitrotetrazolium chloride. *Biol. Fertil. Soils*, 11: 216-220.
- Wainwright, M., 1978. A review of the effects of pesticides on microbial activity in soils. *Eur. J. Soil Sci.*, 29: 287-298.
- Wilke, B.M., 1987. Long-term effects of copper and zinc on microbial activity in a humic, loamy sand. *Landwirtschaftliche Forschung*, 40: 336-343.
- Wilke, B.M., 1988. Long-term effects of inorganic pollutants on microbial activity of a sandy Cambisol. *Zeitschrift Pflanzenernaehrung Bodenkunde*, 151: 131-136.
- Wolf, R., 1977. Book Reviews of Organic Farming: Yesterdays and Tomorrow Agriculture. Rodale Press, Emmaus, PA., ISBN-13: 9780878571758, Pages: 344.
- Yoshida, T., 1975. Microbial Metabolism of Flooded Soils. In: Soil Biochemistry, Paul, E.A. and A.D. McLaren (Eds.). Vol. 3, Marcel Dekker, New York, pp: 83-122.