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Quantification of soil dehydrogenase enzyme activities in long-term fertilizer experiment under rice-wheat cropping system on *Vertisol*

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Abstract
The present investigation entitled "Quantification of soil dehydrogenase enzyme activities in long-term fertilizer experiment under rice-wheat cropping system on *Vertisol*" during the *kharif* and *rabi* seasons of 2018-19 and 2019-20 at research farm of college of Agriculture, Indira Gandhi Krishi Vishwavidhyalaya, Raipur, Chhattisgarh. The objective of the study were to quantify the microbial respiratory enzyme under inorganic and integrated nutrient management practices. The experiment was designed with Randomized Block Design. The ten treatments of experiment are control, 50% NPK, 100% NPK, 150% NPK, 100% NPK+Zn, 100% NP-K, 100% N-PK, 100% NPK+FYM (5 t ha^{-1}), 50% NPK+BGA (10 kg ha^{-1}), 50% NPK+GM (Sunhemp). The above treatments were randomized with three replications. The soil type was *Vertisol*, also known as *kanhar* in the local language. It can be concluded from the study that the microbial respiratory enzyme under inorganic and integrated nutrient management practices was observed highest in 100% NPK + FYM, as compared to others as well as absolute control.

Keywords: Dehydrogenase enzyme activity, inorganic, integrated nutrient management, *Vertisol*

Introduction
A dehydrogenase is an enzyme belonging to the group of oxidoreductases that oxidizes a substrate by reducing an electron acceptor, usually NAD$^+$ or NADP$^+$ or a flavin coenzyme. According to Zhang *et al*. (2010) [20, 21], dehydrogenases play a part in the biological oxidation of soil's organic matter by transferring hydrogen from the organic substrate to inorganic acceptors. Many long term experiments in India showed increasing yield trends and accumulation of soil organic carbon and biological properties due to the combined application of fertilizer and manures (Saini *et al*. 2005, Bhattacharya *et al*. 2008, Mishra *et al*. 2008, Saha *et al*. 2008 and Bedi *et al*. 2009) [12, 2, 9, 13, 1].

The primary members of the oxidoreductase enzyme class are soil dehydrogenases (Gu *et al*., 2009) [4]. Dehydrogenases are one of the most significant enzymes in the soil environment and are used as a measure of overall soil microbial activity. (Quilchano and Maranon, 2002, Gu *et al*., 2009; Salazar *et al*., 2011) [11, 4, 14], because they occur intracellularly in all living microbial cells (Moeskops *et al*., 2010, Zhao *et al*., 2010, Yuan and Yue, 2012) [10, 21, 19]. Moreover, they are tightly linked with microbial oxidoreduction processes (Moeskops *et al*., 2010) [10].

LTFE carried out in the world have shown, the continuous application of fertilizers NPK alone has caused numerous harms to the physical and biological health of soil, including decreased supply of N, depletion of total soil K, and deficiency of nutrients like sulphur and micro-nutrients due to their continuous removal from soil. Improvement of the soil environment is crucial for the sustainability of such a production system, therefore long-term trials with better balanced nutrition, including maintaining the soil's health, are necessary for managing such a vulnerable ecosystem.

The rice-wheat cropping are very few research on microbial population and enzymatic activity with respect to fertilizer and manure application. Therefore, this study was designed to find out the effect of long term application of fertilizer and manure on crop productivity microbial biomass and enzymatic activities of soil with rice-wheat cropping system on a *Vertisol* in Raipur, Chhattisgarh.

Under rice-based agricultural systems, dehydrogenase activity may have increased due to submergence, which led to an increase in the number of anaerobic bacteria.

When the soil was previously flooded, the soil microflora changed from being predominately aerobic to facultative and obligate anaerobic microbes. Dehydrogenase activity was shown to increase when bacteria switched from aerobic to anaerobic conditions. Several workers reported experiencing comparable outcomes Vandana *et al*. (2012) [17] and Srilatha (2014) [16].

Materials and Methods

The experiment was carried out on *Vertisols* in AICRP- Long Term Fertilizer Experiment at Research Farm, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh), during *kharif* and *rabi* seasons of 2018-19 and 2019-20. The experimental soil was *Vertisol* is *Typic Haplustert* locally called as *kanhar*. The experiment was laid in a Randomized Block Design with ten treatments i.e. T1-Control, T2-50% NPK, T3-100% NPK, T4-150% NPK, T5-100% NPK+Zn, T6-100% NP-K, T7-100% N-PK, T8-100% NPK+FYM (5 t ha^{-1}), T9-50% NPK+BGA (10 kg ha^{-1}), T10-50% NPK+GM (Sunhemp).

Soil dehydrogenase enzyme activity

The dehydrogenase enzyme activity in the soil was determined by method given by Casida *et al*. (1964) [3]. The method used by soil microorganisms which, involves colorimetric determination of 2, 3, 5 - triphenyl tetrazolium chloride (TTC). Three-gram of field moist soil in test tubes take another test tube without soil to prepare a control. Simultaneously, place the field moist soil in aluminium box and place, at oven set at 105 ° Celsius for 24-48 hrs to find the soil's dry weight. Add 0.2 g of $CaCO_3$, 1 ml of 3% 2, 3, 5 triphenyl tetrazolium chloride (TTC) and 2.5 ml of distilled water in the test tubes. Swirl the test tubes content and place them in an incubator with stopper at 37 °C for 24 hr. After 24 hr remove the stoppers, add 10 ml of methanol and shake it for few seconds. A red color will appear due to the reduction of 2,3,5- triphenyl tetrazolium chloride into triphenyl formazan (TPF). Filter the suspension in 100-ml volumetric flask, wash test-tube repeatedly with methanol, and quantitatively transfer supernatant, until reddish color disappears. Dilute the filtrate with methanol to 100 ml. Measures the intensity of the red or pink color in a spectrophotometer at 485 nm.

$$\text{DHA } (\mu g \text{ TPF } g^{-1} d^{-1}) = \frac{Concentration \times Dilution}{Incubation\ time \times Actual\ weight\ of\ soil}$$

Results and Discussion

Dehydrogenase enzyme activity in soil at different intervals of rice

The data on dehydrogenase enzyme activity in soil at different intervals (30, 60, 90 DAT and at harvest) of rice are presented in Table 1 and Fig. 1. The findings demonstrated that dehydrogenase activity in soil at various rice-growing intervals was significantly impacted by inorganic fertilizers and integrated nutrient management (INM) strategies throughout the years 2018 and 2019 and on mean based.

At 30 DAT, the dehydrogenase enzyme activity in soil was significantly higher under treatment T8- 100% NPK + FYM as compared to others, but it was comparable to T10- 50% NPK + GM, T4- 150% NPK, T9- 50% NPK + BGA, T5- 100% NPK + Zn, T3 100% NPK and T6- 100% NP. While, the lowest dehydrogenase enzyme activity (30.89, 34.01 and 32.45 µg TPF g^{-1} 24 hr^{-1}) was noted under T1- control during 2018, 2019 and on pooled mean basis, respectively.

At 60 DAT, the dehydrogenase enzyme activity in soil was significantly higher under treatment T8- 100% NPK + FYM as compared to others, but it was comparable to T10- 50% NPK + GM, T4- 150% NPK, T9- 50% NPK + BGA (blue green algae), T5- 100% NPK + Zn (zinc), and T3- 100% NPK, during 2018 and on mean based. Similarly, during 2019, it was comparable to T10- 50% NPK + GM, T4- 150% NPK, T9- 50% NPK + BGA, T5- 100% NPK + Zn, T3 100% NPK and T6- 100% NP. While, the lowest dehydrogenase enzyme activity (36.96, 42.00 and 39.48 µg TPF g^{-1} 24 hr^{-1}) was noted under T1- control during 2018, 2019 and on pooled mean basis, respectively.

At 90 DAT, the dehydrogenase enzyme activity in soil was significantly higher under treatment T8- 100% NPK + FYM as compared to others, but it was comparable toT10- 50% NPK + GM, T4- 150% NPK, T9- 50% NPK + BGA (blue green algae), T5- 100% NPK + Zn (Zinc), and T3 100% NPK, during 2018 and on mean basis. Similarly, during 2019, it was at par to T10- 50% NPK + GM, T4- 150% NPK, T9- 50% NPK + BGA, T5- 100% NPK + Zn, T3 100% NPK and T6- 100% NP. While, the lowest dehydrogenase enzyme activity (28.97, 31.00 and 29.99 µg TPF g^{-1} 24 hr^{-1}) was noted under T1- control during 2018, 2019 and on pooled mean basis, respectively.

At harvest, the dehydrogenase enzyme activity in soil was significantly higher under treatment T8- 100% NPK + FYM as compared to others, but it was comparable to T10- 50% NPK + GM during 2018. Similarly, during 2019 and on mean basis, but it was comparable to T10- 50% NPK + GM and T4- 150% NPK. While, the lowest dehydrogenase enzyme activity (23.40, 26.46 and 24.93 µg TPF g^{-1} 24 hr^{-1}) was noted under T1- control during 2018, 2019 and on pooled mean basis, respectively.

The above findings revealed that the lowest value of dehydrogenase activity was noted in control and imbalanced fertilizer, whereas balanced dose of fertilizer and INM treatments showed the highest values. The beneficial effects of FYM on dehydrogenase activity might be due to the more easily decomposable components of crop residues on the metabolism of soil microorganisms and due to the increase in microbial growth with addition of carbon substrate. The results are in conformity with the findings of Manna *et al.* (1996 and 2005), Mandal *et al.* (2007) and Verma and Mathur (2009).

Dehydrogenase activity in soil at different intervals of wheat

The data on dehydrogenase enzyme activity in soil at different intervals (30, 60, 90 DAS and at harvest) of wheat are presented in Table 2 and Fig. 2. The results showed that at 30, 60 and 90 DAS, the dehydrogenase enzyme activity in soil was significantly higher under treatment T8- 100% NPK + FYM as compared to others, but it was comparable to T10- 50% NPK + GM, T4- 150% NPK, T9- 50% NPK + BGA (blue green algae), T5- 100% NPK + Zn (zinc) and T3 100% NPK, during 2018-19, 2019-20 and on pooled basis. Therefore, lower value of dehydrogenase enzyme activity noted under T1- control during 2018-19, 2019-20 and on pooled mean basis, respectively.

At harvest, the dehydrogenase enzyme activity in soil was significantly higher under treatment T8- 100% NPK + FYM as compared to others, but it was comparable to T10- 50%

NPK + GM during2018-19, 2019-20 and on mean basis. While, lower value of dehydrogenase enzyme activity (17.29, 21.49 and 19.39 µg TPF g^{-1} 24 hr^{-1}) was noted under T1-control during 2018-19, 2019-20 and on pooled mean basis, respectively.

Kanchikerimath and Singh (2001) studied in semi-arid region of India and Delhi for 26 years on *Cambisol,* the long run application of manure and fertilizers in maize-wheat-cowpea cropping system and reported that DHA significantly enhanced with the application of balanced inorganic nutrients and organic manures. The dehydrogenase assay (DHA) increased with graded levels of mineral fertilizer doses from 50 to 150% NPK. Applications of farmyard manure at 10 t ha^{-1} + 100% NPK recorded significantly higher dehydrogenase activity compared to all other treatments. The increase in DHA due to INM over 100% NPK through mineral fertilizers. The results are in line with the findings reported by Bhattacharya *et al.,* (2008) [2] and they observed 4-5 folds increase in dehydrogenase activity due to farmyard manure application along with NPK. The addition of farmyard manure coupled with mineral fertilization exerted a stimulating influence on the preponderance of bacteria (Selvi *et al*., 2004) [15].

Table 1: Effect of organic and inorganic fertilizers on activity of soil dehydrogenase enzyme DAT: Days after transplanting

DHA (µg TPF g^{-1}24 hr^{-1})												
Treatment	**30 DAT**			**60 DAT**			**90 DAT**			**At harvest**		
	2018	**2019**	**Pooled mean**	**2018**	**2019**	**Pooled mean**	**2018**	**2019**	**Pooled mean**	**2018**	**2019**	**Pooled mean**
Control	30.89^{d}	34.01^{d}	32.45^{d}	36.96^{e}	42.00^{d}	39.48^{e}	28.97^{e}	31.00^{d}	29.99^{e}	23.40^{e}	26.46^{de}	24.93^{ef}
50% NPK	39.47^{bc}	42.59^{bc}	41.03^{bc}	45.54^{cd}	50.58^{bc}	48.06^{cd}	37.55^{cd}	39.58^{bc}	38.56^{cd}	27.60^{d}	30.66^{cd}	29.13^{de}
100% NPK	43.80^{abc}	46.92^{abc}	45.36^{abc}	49.87^{abc}	54.91^{abc}	52.39^{abc}	41.88^{abc}	43.91^{abc}	42.90^{abc}	28.50^{cd}	31.56^{c}	30.03^{cd}
150% NPK	46.42^{a}	49.54^{a}	47.98^{a}	52.49^{ab}	57.53^{a}	55.01^{ab}	44.50^{ab}	46.53^{a}	45.51^{ab}	32.40^{bc}	35.46^{abc}	33.93^{abc}
100% NPK+Zn	45.47^{ab}	48.59^{ab}	47.03^{ab}	51.54^{ab}	56.58^{ab}	54.06^{abc}	43.55^{ab}	45.58^{ab}	44.56^{ab}	30.00^{cd}	33.06^{c}	31.53^{cd}
100% NP-K	42.01^{abc}	45.13^{abc}	43.57^{abc}	48.08^{bcd}	53.12^{abc}	50.60^{bcd}	40.09^{bcd}	42.12^{abc}	41.11^{bcd}	21.70^{e}	24.76^{e}	23.23^{f}
100% N-PK	37.71^{c}	40.83^{c}	39.27^{c}	43.78^{d}	48.82^{cd}	46.30^{d}	35.79^{d}	37.82^{c}	36.80^{d}	32.13^{bc}	35.19^{abc}	33.66^{bc}
100% NPK+FYM	48.13^{a}	51.25^{a}	49.69^{a}	54.20^{a}	59.24^{a}	56.72^{a}	46.21^{a}	48.24^{a}	47.23^{a}	36.55^{a}	39.61^{a}	38.08^{a}
50% NPK+BGA	45.55^{ab}	48.67^{ab}	47.11^{ab}	51.62^{ab}	56.66^{ab}	54.14^{ab}	43.63^{ab}	45.66^{ab}	44.65^{ab}	31.13^{cd}	34.19^{bc}	32.66^{bcd}
50% NPK+GM	47.13^{a}	50.25^{a}	48.69^{a}	53.20^{ab}	58.24^{a}	55.72^{ab}	45.21^{a}	47.24^{a}	46.23^{ab}	35.13^{ab}	38.19^{ab}	36.66^{ab}
S.Em ±	2.08	2.24	2.16	1.78	2.30	2.04	1.49	2.09	1.79	1.31	1.65	1.48
CD (5%)	6.19	6.64	6.42	5.30	6.82	6.06	4.44	6.21	5.32	3.90	4.89	4.40

Table 2: Effect of organic and inorganic fertilizers on activity of soil dehydrogenase enzyme

DHA (µg TPF g^{-1}24 hr^{-1})												
Treatment	**30 DAS**			**60 DAS**			**90 DAS**			**At harvest**		
	2018	**2019**	**Pooled mean**	**2018**	**2019**	**Pooled mean**	**2018**	**2019**	**Pooled mean**	**2018**	**2019**	**Pooled mean**
Control	23.78^{e}	27.92^{e}	25.85^{e}	29.83^{e}	34.98^{e}	32.41^{e}	22.89^{e}	28.01^{e}	25.45^{e}	17.29^{f}	21.49^{f}	19.39^{f}
50% NPK (50% NPK)	32.36^{cd}	36.50^{cd}	34.43^{cd}	38.41^{cd}	43.56^{cd}	40.98^{cd}	31.47^{cd}	36.59^{cd}	34.03^{cd}	21.49^{e}	25.69^{e}	23.59^{e}
100% NPK (100% NPK)	36.69^{abc}	40.83^{abc}	38.76^{abc}	42.74^{abc}	47.89^{abc}	45.32^{abc}	35.80^{abc}	40.92^{abc}	38.36^{abc}	22.39^{de}	26.59^{de}	24.49^{de}
150% NPK (150% NPK)	39.31^{ab}	43.45^{ab}	41.38^{ab}	45.36^{ab}	50.51^{ab}	47.93^{ab}	38.42^{ab}	43.54^{a}	40.98^{a}	26.29^{bc}	30.49^{bc}	28.39^{bc}
100% (100% NPK+Zn)	38.36^{ab}	42.50^{ab}	40.43^{ab}	44.41^{ab}	49.56^{ab}	46.98^{ab}	37.47^{ab}	42.59^{ab}	40.03^{ab}	23.89^{cde}	28.09^{cde}	25.99^{cde}
100% NP (100% NP)	34.90^{bcd}	39.04^{bcd}	36.97^{bcd}	40.95^{bcd}	46.10^{bcd}	43.53^{bcd}	34.01^{bcd}	39.13^{bcd}	36.57^{bcd}	15.59^{f}	19.79^{f}	17.69^{f}
100% N (100% N)	30.60^{d}	34.74^{d}	32.67^{d}	36.65^{d}	41.80^{d}	39.22^{d}	29.71^{d}	34.83^{d}	32.27^{d}	26.02^{bc}	30.22^{bcd}	28.12^{bc}
100% NPK (100% NPK + FYM)	41.02^{a}	45.16^{a}	43.09^{a}	47.07^{a}	52.22^{a}	49.65^{a}	40.13^{a}	45.25^{a}	42.69^{a}	30.44^{a}	34.64^{a}	32.54^{a}
50% NPK (50% NPK+BGA)	38.44^{ab}	42.58^{ab}	40.51^{ab}	44.49^{ab}	49.64^{ab}	47.07^{ab}	37.55^{ab}	42.67^{ab}	40.11^{ab}	25.02^{cd}	29.22^{cde}	27.12^{cd}
50% NPK (50% NPK+GM)	40.02^{ab}	44.16^{a}	42.09^{a}	46.07^{ab}	51.22^{a}	48.65^{ab}	39.13^{a}	44.25^{a}	41.69^{a}	29.02^{ab}	33.22^{ab}	31.12^{ab}
S.Em ±	1.74	1.70	1.72	1.78	1.71	1.75	1.49	1.46	1.47	1.05	1.23	1.14
CD (5%)	5.17	5.05	5.11	5.29	5.09	5.19	4.41	4.33	4.37	3.13	3.66	3.39

DAS: Days after sowing, Figures in subscript and parenthesis showed *kharif* experiment treatments

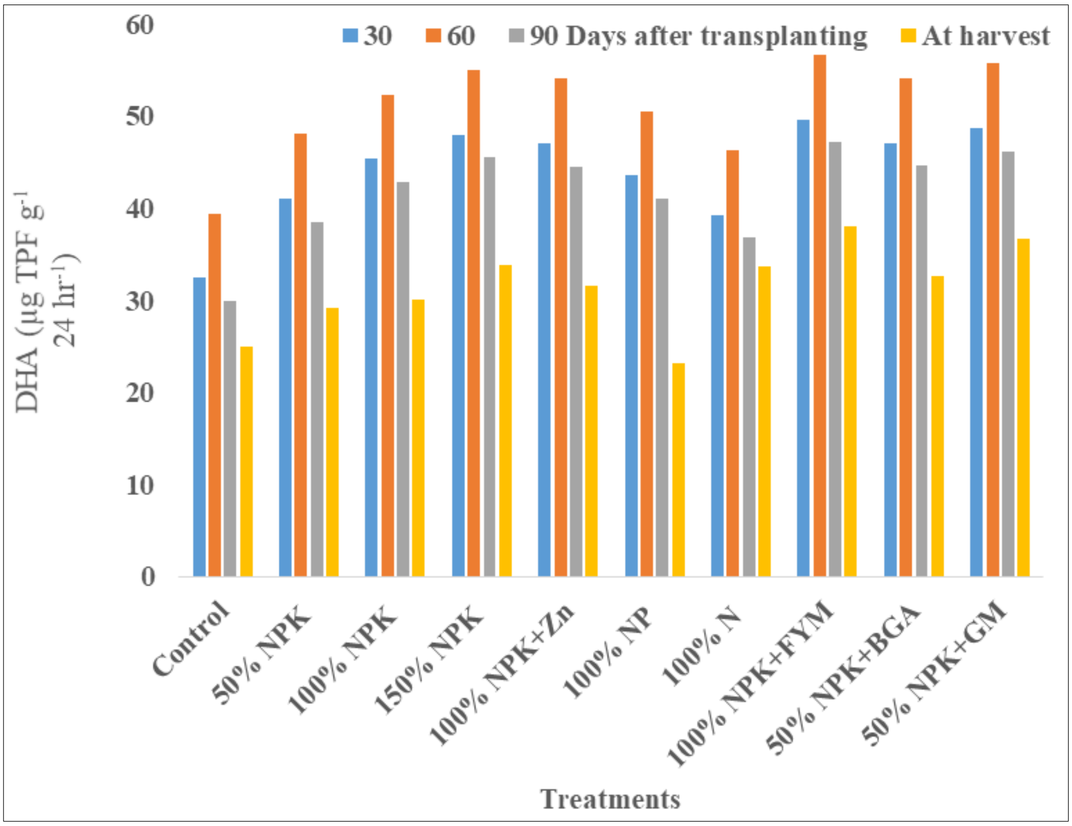

Fig 1: Effect of organic and inorganic fertilizers on activity of soil dehydrogenase enzyme (Pooled mean of 2018 and 2019)

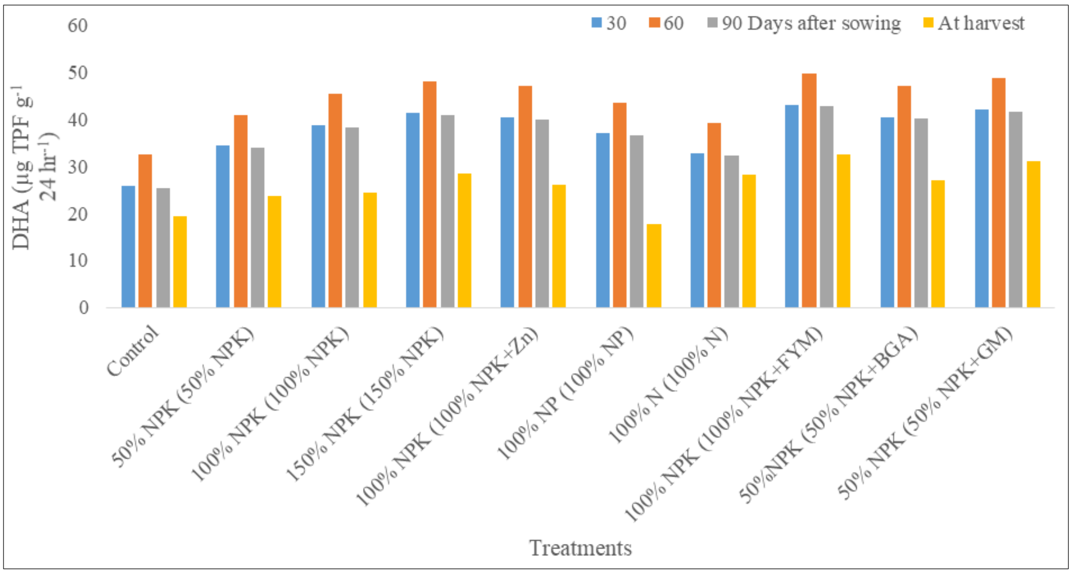

Fig 2: Effect of organic and inorganic fertilizers on activity of soil dehydrogenase enzyme (Pooled mean of 2018-19 and 2019-20)

Conclusions

From the experiment, it can be concluded that the effect of 20 years of different treatments on application of inorganic fertilizer, integrated nutrient management (INM) practices on microbial respiratory enzyme under inorganic and integrated nutrient management practices was recorded highest in 100% NPK + FYM, as compared to others as well as absolute control.

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