

Original Research Article

Microbial biomass and enzymatic activity of major cropping systems in soils of Inceptisols and Vertisols at Northern Telangana

ABSTRACT

Under cropping systems, microbial biomass plays a major role in nutrient and energy flow of soil. Similarly, urease and dehydrogenase activities are essential for nitrogen cycle and determining biological index of soils, respectively. However, their information is minimal at major cropping systems of this region. Therefore, surface soil (00-15 cm) samples were collected after 8 years from rice-rice, rice-maize, cotton and turmeric-sesame cropping systems at soils of Inceptisols and Vertisols of Northern Telangana zone during *kharif* 2019. A five replicated soil samples were collected, assessed and statistically analyzed with factorial randomized block design. The results revealed that the forms of microbial biomass carbon (14%) and nitrogen (22%), urease (29%) and dehydrogenase (20%) activity were found to be higher in cropping systems under Vertisols compared to Inceptisols. Among the cropping systems, rice-rice showed significantly higher biological properties than others. The interactions are significant for urease activity. Urease and dehydrogenase activity is positively correlated with soil available nitrogen and organic carbon content of soils, respectively of cropping systems.

INTRODUCTION

The predominant cropping systems grown in Northern Telangana Zone are rice-rice, rice-maize, cotton-fallow and turmeric-sesame. Rice-based cropping systems are the major contributing food production systems and largely cultivated, contributing 84% of total production of the World. Soils of rice-rice system are heavy textured, slow infiltration rate, high water-holding capacity, rich in soil organic matter (SOM) and easy to puddle. In agro-ecosystem, different crops are grown in sequence and they contribute different amounts of crop residues and root exudates (Badole et al., 2020). They may ultimately help to build up different amount of organic carbon in soils. Nature of such organic C also varies because of variation in the nature of root deposition, quality of crop residues and simultaneously there is a build up microorganisms. Mostly the microbial biomass nitrogen, microbial biomass carbon,

urease and dehydrogenase population are found to be higher at root residues when compared to other microbes.

Microbial population is always a sign of healthy soil and they helps in soil quality maintenance. This study helps to know which cropping systems helps in soil health and soil quality maintenance.

Microorganisms play a crucial part in soil nutrient cycling, maintenance of soil structure, degradation of agrochemicals and pollutants, and plant pest control (Stockdale and Brookes, 2006), hence it has often been indicated as an important component of soil fertility (Nogueira et al., 2006). Enzymatic activities in the soil highly affect nutrient cycling and organic matter decomposition (Pavel et al., 2004). Moreover, ureases are in charge of releasing inorganic N in the N-cycle (Bandick and Dick, 1999). A case study indicated that excessive cultivation decreased both microbial biomass and its activities (Gupta and Germida, 1988).

Soil enzyme activities related to SMBC and soil organic carbon (SOC) are often used for comparison of different land use with varying SOM content (Waldrop et al. 2004; Bastida et al. 2007). Measurement of soil enzyme activities in key nutrient cycling (C, N, and P) and oxidation–reduction processes have been used widely as a potential indicator for determining the effect of land use conversions and management practices on soil health (Acosta-Martínez et al. 2007; Pandey et al. 2014; de Medeiros et al. 2015). Therefore, an investigation of soil microbial characters and enzyme activities is critical in studying the land conversions and focusing the soil management.

MATERIAL AND METHODS

An experiment was conducted during *kharif*, 2019 at Agricultural College, Polasa, Jagtial, Professor Jayashankar Telangana State Agricultural University. The experiment was laid out in randomized block design with factorial concept (FRBD) with five replications. Soil samples were collected from four cropping systems viz., rice-rice (CS1), rice-maize (CS2), cotton-fallow (CS3) and turmeric-sesame (CS4) under two soil types viz., inceptisols (S1) and vertisols (S2) from surface soil (0-15cm). Selection of sites was based on continuous cultivation of the same cropping system (at least for 8 year), in Northern Telangana Zone of Telangana State

Microbial biomass carbon

Field-moist soil samples (25.0 g) were exposed to CHCl_3 vapour for 24 h and extracted with 0.5 M K_2SO_4 . Under same conditions the second set of non-fumigated samples were extracted. The difference between C obtained from the fumigated and from the non-fumigated ones was taken to represent the microbial C-flush and converted to MBC using the relationship: $\text{MBC} = 1/0.41 \times \text{C-flush}$ (Voroney and Paul, 1984). All results are expressed on an oven-dry soil basis (105°C, 24 h) and are the mean of three replicate analyses.

Microbial biomass nitrogen

Microbial biomass nitrogen (MBN) was also estimated using the same principle of microbial biomass carbon (Brooks et al., 1985). The K_2SO_4 extractant of both fumigated and unfumigated soil was digested for 3 hr. by adding H_2SO_4 and digestion mixture. After cooling, distillation was carried out to find the nitrogen content. The difference between fumigated and unfumigated extracted nitrogen of soil divided by a calibration factor (K_{EC}) 0.38 gives the measure of microbial biomass nitrogen in soil and expressed as micro gram of microbial biomass-N per gram of dry soil.

Urease

This method is based on determination of NH_3 released other incubation of soil with urea solution for 2 hours at 30 °C (Tabatabai and Bremner, 1972). Five gram of soil was taken in duplicate in 50 ml volumetric flask. 0.2 ml toluene and 9 ml of THAM buffer (pH- 9; 0.05M) were added to it. The flasks were swirled for few second to mix the content. Then 1 ml of 0.2 M urea solution was added and swirled again for a few second. The flasks were then stoppered and placed in an incubator at 30°C for 2 hours. After 2 hours the stoppers were removed and approximately 35 ml of $\text{KCl} - \text{Ag}_2\text{SO}_4$ solution was added and the flasks were swirled for a few seconds and allowed to stand until the contents have cooled to room temperature. The volume of the flasks was made upto mark (50 ml) by addition of $\text{KCl} - \text{Ag}_2\text{SO}_4$ solution. In order to mix the contents the flakes were inverted several times with the help of a stopper in the flake. To perform control, the above procedure was followed, but 1 ml 0.2M urea solution was added after the addition of 35 ml $\text{KCl}-\text{AgSO}_4$ solution.

A 40 ml aliquot of the suspension was pipetted out into 100 ml distillation flask and 0.2g MgO was added to it for the determination of $\text{NH}_4\text{-N}$ in the resulting soil suspension. The content of the flask was then distilled for 15 minutes and the

distillate was collected in a 50 ml conical flask containing 5 ml of 2 per cent boric acid indicator solution. The distillate was then titrated with 0.005(N) H₂SO₄. The urease activity is expressed as micro gram NH₄ – N per gram dry soil per hour at 30⁰C (µg NH₄⁺ released g soil⁻¹ h⁻¹).

Dehydrogenase

Five grams soil was weigh into glass tubes and mixed with 5 ml TTC solution. The tubes were sealed with rubber stopper and inoculated for 24 hours at 30⁰C. The control contain only 5 ml tris buffer (without TTC). After incubation 40 ml acetone was added to each tube and tubes were shaken thoroughly and further incubated at room temperature for 2 hour in dark (shaking the tubes at intervals). The suspension was then filtered and optical density of clear supernatant was measured against the blank at 546 nm (red colour). The activity of dehydrogenase is expressed in µg TPF formed per gram of dry soil per day (µg TPF produced g⁻¹ soil h⁻¹).

Statistical analysis

The data were analysed using analysis of variance (ANOVA) – two way classification. Two factor factorial ANOVA was used to determine the existence of interaction effect between soil orders and cropping systems. Simple correlation coefficient was also developed to evaluate relationships between the response variables using the same statistical package. The 5% probability level was regarded as statistically significant (Panse and Sukhatme, 1978)

Results and discussion

The higher content of soil organic carbon, the more active the soil microorganisms. Microorganisms accelerate the degradation of organic matter, which is reflected in soil respiration and release of carbon dioxide from the rhizosphere (Zhang *et al.*, 2010). The results on the effect of cropping systems and soil type on microbial biomass and enzyme activity are in the Table.1.

Microbial Biomass Carbon

Microbial biomass carbon (MBC) content in surface soils was significantly higher in vertisols (132 µg C g⁻¹ soil) than inceptisols (116 µg C g⁻¹ soil) the results were in agreement with Prasad *et al* 2013. The high clay and organic matter contents in vertisols might have contributed for higher MBC values compared to inceptisols. Different cropping systems were found to have significant effect on MBC. MBC was in the range of 89 to 152 µg C g⁻¹ soil MBC values was found highest in CS₁ (141 µg

g^{-1}) followed by CS_4 ($134 \mu\text{g g}^{-1}$), CS_2 ($123 \mu\text{g g}^{-1}$) and CS_3 ($99 \mu\text{g g}^{-1}$). Higher MBC values are commonly found in cropping systems that include high residue-producing crops (Omay *et al.* 1997), crops with intensive root growth and root density (Stone and Buttery 1989; Perfect *et al.* 1990). Thus CS_1 has found to maintain significantly higher MBC values than other cropping systems this may be due to the amount of crop residues, the proportion of easily decomposable organic compounds returned to the soil, root density and microclimate in rice-rice cropping systems (Anantha et al., 2020; Moore *et al.* 2000). Interactional effect of soil types and cropping system were found to be non significant.

Microbial Biomass Nitrogen

Microbial biomass nitrogen (MBN) content in surface soils was significantly higher in vertisols ($11.21 \mu\text{g NH}_4^+ \text{g}^{-1} \text{soil}$) than inceptisols ($9.16 \mu\text{g NH}_4^+ \text{g}^{-1} \text{soil}$). The result is also in accordance with Prasad *et al.*, 2013. The high clay and organic matter contents in vertisols might have contributed for higher MBN values compared to inceptisols. Different cropping systems were found to have significant effect on MBN. The values of MBN ranged from 8.85 to $11.64 \mu\text{g NH}_4^+ \text{g}^{-1} \text{soil}$. MBN values were found highest in CS_1 ($11.64 \mu\text{g NH}_4^+ \text{g}^{-1} \text{soil}$) followed by CS_2 ($10.63 \mu\text{g g}^{-1}$), CS_4 ($9.6 \mu\text{g g}^{-1}$) and CS_3 ($8.86 \mu\text{g g}^{-1}$). Thus MBN count was higher in CS_1 as this system has more substrate production when compared to other systems above. The microbial biomass C and N pools will increase in one cropping system relative to another only if microbes have access to sufficient substrates and non-growth requirements have been satisfied. Therefore, greater microbial biomass in diversified cropping systems may be a consequence of increased substrate availability, where greater retention and recycling of C and N enhance available substrate to support microbial growth and biosynthesis (Geyer *et al.*, 2016). Interactional effect of soil types and cropping system were found to be non significant.

Urease

Soil types had significant impact on the activity of urease. Greater urease activity was recorded in vertisols ($3.32 \mu\text{g NH}_4\text{-N g}^{-1} \text{hr}^{-1}$) than inceptisols (2.58) as biological activities are more in verisols than inceptisols Prasad *et al.*, 2013. In cropping systems, rice-rice (CS_1) has maintained higher amount of urease activity followed by $\text{CS}_2 > \text{CS}_4 > \text{CS}_3$ with the activity of 3.84, 3.62, 2.41 and $1.92 \mu\text{g NH}_4\text{-N g}^{-1} \text{hr}^{-1}$ respectively. Biogenic elements were more in rice-rice cropping system compared to other systems (Strachel, 2016) which helps in increase of microbial

properties, such as urease (Strachel, 2016, Saiya-Cork *et al.* 2002, and Wang *et al.* 2008). Urease participate in ammonification, during which ammonia is released from urea, amino acids, and purine bases. Soil fertility and productivity depend on soil organic matter, which is a reserve of nutrients and is very important in nutrient cycling (Bai *et al.*, 2018) as well as improves soil physical, chemical, and biological properties (Bhattacharya *et al.*, 2010). Processes associated with organic matter transformations in soil occur with the participation of soil microorganisms and their enzymes (Schimel *et al.*, 2004)

Interactional effect of cropping system and soil types were found to have a profound influence on urease activity. CS₁ cropping system maintained higher urease activity followed by CS₂, CS₄ and CS₃ in inceptisol. Similar trend also recorded with vertisols. Soil available nitrogen content in soil had positive influence on urease activity. With higher the content of nitrogen in soil more will be the urease activity, such results were also found with (Strachel, 2016, Saiya-Cork *et al.* 2002; Wang *et al.* 2008). Relationship between urease with available nitrogen content was positively correlated ($Y=26.80 X + 128.2$; $R^2=0.499$), is shown in the Figure. 1. Nitrogen stimulates soil microorganisms which produce more soil enzymes when biogenic elements become more available (Strachel *et al.*, 2016). As urease is the enzyme that catalyzes hydrolysis of urea to CO₂ and NH₃, which is a vital process in the regulation of N supply to plants after urea fertilization (Balota *et al.*, 2010).

Dehydrogenase

Activity of dehydrogenase reflects oxidative activity of soil microflora and is a good indicator of microbial activity (Nannipieri *et.al.*, 1990; Velmourougane *et al.*, 2013). Activity of dehydrogenase enzyme was significantly higher in vertisols (2.71 µg TPF g⁻¹ hr⁻¹) over inceptisols (2.25 µg TPF g⁻¹ hr⁻¹). The results were in the same line with Prasad *et al.*, 2013. The greater amount of organic carbon, nutrients and stimulated microbial activity (Velmourougane *et al.*, 2013) in Vertisols might have contributed for the increase in dehydrogenase activity compared to inceptisol. Different cropping systems were found to have significant effect on dehydrogenase activity. Irrespective of soil order, dehydrogenase activity was found in the decreasing order of CS₁ > CS₂ > CS₄ > CS₃ with the activity of 3.52, 2.65, 2.27 and 1.48 µg TPF g⁻¹ hr⁻¹, in respectively in surface soils. It was found that dehydrogenase activity was highest in CS₁, as biological activity was found highest in rice ecology. Under CS₁ very labile pool of SOC was higher which might have used as feed for micro

organism (Casida, 1977 and Zaman *et al.*, 2002) and enhances soil enzymatic activity. Relationship between organic carbon and dehydrogenase activity was positively correlated, is shown in the Figure 1. Similar correlation was also observed by Bergstrom *et al.*, 1998 and Roldan *et al.*, 2005. Soil organic carbon has been considered as an indicator of soil quality, because of its character of nutrient sink and source that can enhance soil physical and chemical properties, and also promote biological activity (Salazar *et al.*, 2011) and highest carbon and biological activity were found in rice ecology when compared to other cropping systems. Interactional effect of soil type and cropping system were found to be non significant.

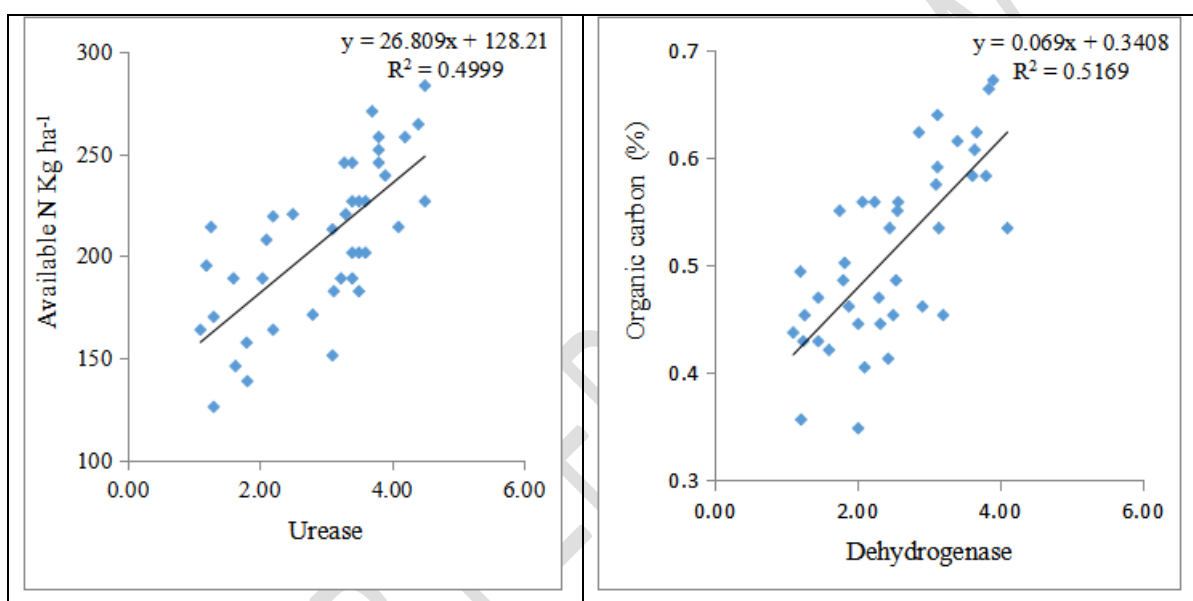


Figure. 1 Correlation studies a) Soil available nitrogen Vs Urease b) Organic carbon Vs Dehydrogenase.

Table 1. Influence of soil types and cropping systems on soil microbial biomass carbon ($\mu\text{g C g}^{-1}$ soil), microbial biomass nitrogen ($\mu\text{g NH}_4^+ \text{g}^{-1}$ soil), urease ($\mu\text{g NH}_4\text{-N g}^{-1} \text{hr}^{-1}$) and dehydrogenase ($\mu\text{g TPF g}^{-1} \text{hr}^{-1}$)

Soil order	MBC	MBN	Urease	Dehydrogenase
S ₁	116.15	9.16	2.58	2.25
S ₂	132.41	11.21	3.32	2.71
S.Em	4.73	0.35	0.09	0.09
CD@5%	13.71	1.00	0.27	0.27
Cropping System				
CS ₁	141.14	11.64	3.84	3.52
CS ₂	123.24	10.63	3.62	2.65
CS ₃	98.73	8.86	1.92	1.48
CS ₄	134.02	9.60	2.41	2.27

S.Em	9.00	0.49	0.13	0.13
CD@5%	26.07	1.42	0.39	0.38
Interactions				
S₁CS₁	130.74	10.78	3.65	3.32
S₁CS₂	119.15	9.82	3.44	2.25
S₁CS₃	88.58	7.91	1.52	1.32
S₁CS₄	126.12	8.11	1.71	2.12
S₂CS₁	151.55	12.49	4.04	3.72
S₂CS₂	127.31	11.45	3.80	3.05
S₂CS₃	108.85	9.80	2.32	1.64
S₂CS₄	141.94	11.08	3.12	2.43
S. Em	9.47	0.69	0.19	0.18
CD@5%	NS	NS	0.55	NS
CV	17.03	15.20	14.28	16.50

S₁- Inceptisols, S₂- Vertisols, CS₁- Rice-Rice, CS₂- Rice-Maize, CS₃- Cotton –Fallow, CS₄- Turmeric-Sesame, SE m: Standard error of mean, CD: Critical difference, CV: Critical Variance

CONCLUSION

Biological properties *viz.*, microbial biomass carbon, microbial biomass nitrogen, dehydrogenase and urease enzyme activities of soils showed higher values under vertisols over inceptisols. Under cropping systems compared rice- rice cropping system showed significantly higher biological activities in soil. Lowest activities were recorded in cotton-fallow cropping system. Urease was positively correlated with available nitrogen and dehydrogenase was positively correlated with SOC content. Microbial activities in soil were found higher under vertisols and in rice-rice cropping system.

LITERATURE CITED

- Acosta-Martínez V, Mikha MM, Vigil MF (2007) Microbial communities and enzyme activities in soils under alternative crop rotations compared to wheat-fallow for the Central Great Plains. *Appl Soil Ecol* 37(1-2):41–52
- Anantha KC, Majumder SP, Badole S, Padhan D, Datta A, Mandal B, Srinivas, Ch. 2020. Pools of organic carbon in soils under a long-term rice–rice system with different organic amendments in hot, sub-humid India. *Carbon Management*, 11 (4): 331-339.
- Badole S, Datta A, **Krishna Chaitanya A**, Majumder SP, Mandal B. 2020. Soil Carbon Dynamics Under Different Land-Use and Management Systems. In book: *Carbon Management in Tropical and Sub- Tropical Terrestrial Systems*.

Editors: Ghosh, P.K. Mahanta, S.K. Mandal, D. Mandal, B. Ramakrishnan, S.
Springer Nature Singapore Pte Ltd. 2020.

- Bai Z, Casparia T, Gonzaleza MR, Batjesa NH, Mäderb P, Bünemannb EK, de Goedec R, Brussaardc L, Xud M and Ferreirae CS. 2018. Effects of agricultural management practices on soil quality: A review of long-term experiments for Europe and China. *Agriculture, Ecosystem & Environment*. 265: 1-7.
- Balota EL, Chaves JC. 2010. Enzymatic activity and mineralization of carbon and nitrogen in soil cultivated with coffee and green manures. *Revista Brasileira de Ciência do Solo*. 34: 1573-1583.
- Bandick AK, and Dick PP. 1999. Field management effects on soil enzyme activities. *Soil Biol. Biochem.* 31, 1471–1479. doi: 10.1016/S0038-0717(99)00051-6
- Bastida F, Moreno JL, Hernández T, García C (2007) The long-term effects of the management of a forest soil on its carbon content, microbial biomass and activity under a semi-arid climate. *Appl Soil Ecol* 37(1-2):53–62
- Bergstrom DW, Monreal CM, King DJ. 1998. Sensitivity of soil enzyme activity to conservation practices. *Soil Science Society of America Journal*. 62, 1286-1295.
- Bhattacharyya T, Pal DK, Chandran P, Mandal C, Ray SK, Gupta RK, Gajbhiye KS. 2004. Managing Soil Carbon Stocks in the Indo-Gangetic Plains, India, RWC-CIMMYT, New Delhi. 44.
- Brookes PC, Kragt JF, Powlson DS, Jenkinson DS. 1985. Chloroform fumigation and the release of soil nitrogen: the effects of fumigation time and temperature. *Soil Biology and Biochemistry*. 17 (6), pp. 831-835. [https://doi.org/10.1016/0038-0717\(85\)90143-9](https://doi.org/10.1016/0038-0717(85)90143-9)
- Casida LE. 1977. Microbial metabolic activity in soil as measured by dehydrogenase determinations. *Applied and Environmental Microbiology*. 34: 630-636.
- de Medeiros EV, Notaro KA, de Barros JA, Moraes WS, Silva AO, Moreira KA (2015) Absolute and specific enzymatic activities of sandy entisol from tropical dry forest, monoculture and intercropping areas. *Soil Tillage Res* 145:208–215
- Geyer KM, Kyker-Snowman E, Grandy AS, Frey SD, 2016. Microbial carbon use efficiency: accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. *Biogeochemistry*. 127:173–188.
- Gupta VVSR, Germida JJ. (1988). Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. *Soil Biol. Biochem.* 20, 777–786. doi: 10.1016/0038-0717(88)90082-X
- Moore JM, Klose S, Tabatabai MA. 2000. Soil microbial biomass carbon and nitrogen as affected by cropping systems. *Biology and Fertility of Soils*. 31:200-210.

- Nannipieri, P., Gregos, S. and Ceccanti, B. 1990. "Ecological significance of the biological activity in soil". Smith, J. L. and Paul (eds) - *Soil Biochemistry*. 6: 293-354.
- Nogueira MA, Albino UB, Brandao-Junior O, Braun G, Cruz M F, Dias BA. (2006). Promising indicators for assessment of agroecosystems alteration among natural, reforested and agricultural land use in southern Brazil. *Agr. Ecosyst. Environ.* 115, 237–247. doi: 10.1016/j.agee.2006.01.008
- Omay AB, Rice CW, Maddux LD, Gordon WB, 1997. Changes in soil microbial and chemical properties under long-term crop rotation and fertilization. *Soil Science Society of American Journal*. 61: 1672-1678.
- Pandey D, Agrawal M, Bohra JS. (2014) Effects of conventional tillage and no tillage permutations on extracellular soil enzyme activities and microbial biomass under rice cultivation. *Soil Tillage Res* 136:51–60
- Panse VC, Sukhatme, P. V. 1978. Statistical methods for agricultural workers. Indian Council of Agricultural Research, New Delhi. 87-89.
- Pavel R, Doyle J, Steinberger Y. (2004). Seasonal pattern of cellulase concentration in desert soil. *Soil Biol. Biochem.* 36, 549–554. doi: 10.1016/j.soilbio.2003.10.024
- Perfect E, Kay BD, Van Loon WKP, Sheard RW, Pojasok T. 1990. Factors influencing soil structural stability within a growing season. *Soil Science Society of American Journal*. 54: 173-179.
- Prasad P, George J Mastro RE, Rout TK, Ram LC, Selvi VA. 2013. Evaluation of Microbial Biomass and Activity in Different Soils Exposed to Increasing Level of Arsenic Pollution: A Laboratory Study. *Soil and Sediment Contamination*. 22(5): 483-497.
- Saiya-Cork KR, Sinsabaugh RL, Zak DR. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biology and Biochemistry*. 34(9):1309–1315.
- Salazar S, Sanchez L, Alvarez J, Valverde A, Galindo P, Igual J, Peix A, Santa Regina I. 2011. Correlation Among Soil Enzyme Activities Under Different Forest System Management Practices. *Ecological Engineering*. 37: 1123-1131.
- Schimel JP, Bennett J, 2004. Nitrogen mineralization: Challenges of a changing paradigm. *Ecology*. 85, 591–602.
- Stockdale EA, Brookes PC. (2006). Detection and quantification of the soil microbial biomass impacts on the management of agricultural soils. *J. Agric. Sci.* 144, 285–302. doi: 10.1017/S0021859606006228
- Stone JA, BATTERY BR. 1989. Nine forages and the aggregation of a clay loam soil. *Canadian Journal of Soil Science*. 69: 165-169.

- Strachel. R., Wyszowska, J and Baćmaga. M. 2016. The Influence of Nitrogen on the Biological Properties of Soil Contaminated with Zinc. *Bull Environ Contam Toxicol.* 2017; 98(3): 426–432.
- Tabatabai MA, Bremner JM. 1972. Assay of urease activity in soils. *Soil Biology and Biochemistry.* 4: 479-487.
- Velmourougane K, Venugopalan MV, Bhattacharyya T, Sarkar D, Pal DK, Sahu A, Ray SK, Nair KM, Prasad J, Singh RS. 2013. Soil dehydrogenase activity in agro-ecological sub regions of black soil regions in India. *Geoderma.* 197-198: 186-192.
- Waldrop MP, Zak DR, Sinsabaugh RL (2004) Microbial community response to nitrogen deposition in northern forest ecosystems. *Soil Biol Biochem* 36(9):1443–1451
- Wang QK, Wang SL, Liu YX (2008) Responses to N and P fertilization in a young *Eucalyptus dunnii* plantation: microbial properties, enzyme activities and dissolved organic matter. *Applied Soil Ecology.* 40(3): 484-490.
- Zaman M, Cameron KC, Di HJ, Inubushi K, 2002. Changes in mineral N, microbial and enzyme activities in different soil depths after applications of dairy shed effluent and chemical fertilizer. *Nutrient Cycling in Agroecosystems.* 63: 275-290.