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Study of Dehydrogenase Activity to Select Plant Species for the Perturbed Overburden Soil Environment, Jharia Coalfields, India

Chitralekha Sengupta[1*], Rita Saha[1] and Ram Kumar Bhakat[2]

[1]Central Pollution Control Board, Ministry of Environment and Forest, Eastern Zonal Office, Kolkata, India.
[2]Department of Botany and Forestry, Vidyasgar University, Midnapore, India.
[1]*Central Pollution Control Board, Block 502, Southend Conclave, 1582 Rajdanga Main Road, Kolkata, India.
*Corresponding author email: chitra82ster@gmail.com. Contact No: 9874511954

Abstract

Dehydrogenase activity has been used as an indicator to assess the soil health keeping this in mind, the dehydroganase activity of disturbed overburden dump was observed in the present study to know the status of ecologically perturbed systems such as overburden dumps. These overburden dumps arise due to the open cast mining activities in coal mines. Experiments were also conducted to know the status of this enzyme activity in such overburden dump in Jharia Coalfield, India, when subjected to treatments in order to evaluate how spoil amendments support and boost the biological activities. Maximum DHA was observed in *F. religiosa* followed by *D. strictus*. The plot with cow dung manure depicted the maximum DHA activity than the rest of the plots. Application of VAM proved to be fruitful in enhancing dehydrogenase activity.

Keywords: Dehydrogenase activity; ecological perturbations; overburden spoil; *F. religiosa; D. strictus; A. Indica; E. officinalis; S. indica.*

Introduction

Enzymes are important in catalysing several important reactions necessary for the life processes of micro-organisms and the stabilisation of soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling. The dehydrogenase (DHA) is one such enzyme which reflects the total range of oxidative activity of soil microflora (Liang *et al*., 2014) and imparts good knowledge about the soil fertility status (Woliska and Stepniewska, 2012). This enzyme is considered to be an integral part of intact cells, not accumulating extracellularly in the soil. It is known to oxidise soil organic matter by transferring protons and electrons from substrates to acceptors. These processes form part of respiration pathways of soil microbes and depend on the type of soil and soil air-water conditions (Kandeler *et al*., 1996). Studies on the activities of dehydrogenase enzyme in the soil are very important as it may give indications of the potential of the soil to support biochemical processes. Additionally, dehydrogenase enzyme is often used as a measure of any disruption due to natural as well as anthropogenic disturbances (Dick, 1997). Ecological restoration of overburden dumps involves great challenges. Trees help in improving soil fertility through biological decomposition of organic matter in their rhizosphere. These rhizosphere processes play an important role in plant nutrition and maintenance of soil fertility.

The dehydrogenase activity in unreclaimed overburden dumps of coal mining areas has not been studied. Better understanding of the role of this particular enzyme in environment can serve as a diagnostic tool for better ecosystem assessment and amelioration of perturbed sites, so we decided to (i) evaluate the DHA activity in overburden dumps, seasonally and (ii) to study the effect of some soil amendments on DHA activities to decide reclamation strategies for overburden dumps.

Methodology

2.1 Study area

The study site is located in Bastacola, Jharia coalfield of Jharkhand (23^{o}39’30” and 24°48’ 20’’ N latitude and between 86°11’ 30” and 86° 27’ E longitude). It has an average elevation of 77 m; the climate is tropical monsoon with annual average rainfall is 1169 mm. The mean monthly maximum and minimum temperature during study period (April 2010-July 2011) were, 19.8-45 ^{0}C in summer, 8.5-33.5 ^{0}C in winter and 24-37.8 ^{0}C in monsoon season. The relative humidity varied from 15 – 98 %.

The geological formation belongs to the Gondwana System (Barakar formation), the lower division of which comprises the most important coal measures of India (Chandra *et al*., 2001). The Barakar Formation consists of coarse-grained sand-stones, conglomerates, shales, carbonaceous shales, silt-stones, fireclays and coal seams. The Barakar sandstones show undecomposed feldspars suggestive of unstable source-rock area and rapidly subsiding conditions. The mine-soil is fresh and contains approximately 73% sand, 21% clay and a low concentration of nutrients.

2.2. Experimental design

The seedlings of five plant species grown in the mixed plantation were Neem (*Azardirachta indica:* Meliaceae), Ashok (*Saraca indica:* Fabaceae), Bamboo (*Dendrocalamus strictus:* Poaceae), Peepal (*Ficus religiosa:* Moraceae) and Amla *(Emblica officinalis:* Phyllanthaceae). These species are common in tropical deciduous forests and their two months old seedlings were collected from a nursery of District Forest Department, Dhanbad. The agricultural soils were also collected from Bastacola region used as an amendment. The manure, used in this experiment was cow dung manure and VAM tablets (*Glomus sp*) were obtained from KCP sugar & Industries Corporation, Vuyyuru, Andhra Pradesh. One control and three treatment plots were established. The composition of control and three types of treatment plots used in the experiment are given in Table 1.

Collection of soil samples

Five plant species were planted namely, *Ficus religiosa*, *Azadirachta indica*, *Dendrocalamus strictus*, *Emblica officinalis* and *Saraca indica*. These are the predominant tree species growing in the undisturbed land of Jharia, India. The rhizosphere soil samples were collected from the above plant species of almost uniform age (two months old), growing in the experimental and control plots. The soil strongly adhering to roots and within the space explored by roots was considered as rhizosphere soil (Garcia *et al*., 2005). Samples were taken from three plants (selected randomly) of each species.

Analyses

The soil parameters were estimated as per the methods described by Jackson (1973). The dehydrogenase activity was assayed immediately after collection of soil samples with the modified method of Casida *et al*., (1964). Three determinations per sample were made for dehydrogenase activity. Dehydrogenase activity was determined using 2,3,5-triphenyltetrazolium chloride as the artificial electron acceptor, which is reduced to the red-coloured triphenylformazan (TPF). The red coloured TPF was determined spectrophotometrically at 460 nm (UV-VIS Spectrophotometer, Techcomp). Dehydrogenase activity was expressed as micrograms of TPF formed per gram of soil of the microbial community per hour. The data was subjected to ANOVA analysis and the means were compared using Duncan’s Multiple Range Test (DMRT) at 0.01% level of significance. The software used for this purpose was SPSS-10.

Results

Physico-chemical properties of control and treatment plots

The average value of pH of the mine spoil was slightly acidic (5.67), poor electrical conductivity (0.07mmhos/cm) showed low soluble salts and high bulk density (1.45 g/cc) revealed dominance of sand particles (Table 2), which might be responsible for low moisture content, water holding capacity, organic carbon, available nutrients and microbial activity. Further, these biogeochemical factors may be responsible for week cation exchange capacity (6.89 meq/100g). No heavy metal toxicity was observed in treatment and control plots.

Changes in the physico-chemical properties of coal mine spoil were evaluated seasonally which are given in Table 3. OBS and OBM treatments improved the mine spoil characteristics as seasonal changes in mine spoil characteristics revealed that the spoil amendments (VAM, agricultural soils and organic manure) enhanced the soil fertility by increasing pH. The high cation exchange capacity showed retention of essential nutrients in the rhizosphere after decomposition of organic matter while decreased bulk density lead to reduced compaction, facilitated aeration, better penetration and spreading of roots, thereby making the rhizosphere favourable for massive root development. This gradual increase in favourable physico-chemical properties and nutrient availability in OBC<OBV<OBS<OBM plots facilitated DHA activity to increase as depicted by ANOVA. Correlation of DHA activity with the soil characteristics was not observed.

DHA Activity

The DHA activity was maximum in *F.religiosa* followed by *D. strictus* (Table. 3) while *S.indica* showed the least activity in all the plots. However, there was significant difference at 0.01% level of significance in the dehydrogenase activity in various plots in one particular season. Among the plots, OBM depicted the highest DHA activity which varied in monsoon. Control Plot, OBC depicted 5.28 to 14.33 µg/g/hr DHA activity depicting positive effect of organic manure on DHA production. The same was true for agricultural soil addition. Application of VAM increased the DHA activity considerably in OBV plot. ANOVA showed significant difference in the plots and among the plant species. DMRT further ranked the plants and *F. religiosa* performed best.

Seasonal variation in DHA activities

All the plant species had significantly higher DHA activity in their rhizosphere as compared to the control in all the seasons (Table. 3). Significant difference was observed in the DHA activity amongst the three seasons. The initial enzyme activity was low in all the plots. However, an increase was observed in the winter season which was 57 to 211% more than monsoon season, followed by a decrease of about 14 to 38 % in the summer months.

Discussion

Relationships with soil variables

Many workers expressed positive correlation between some soil properties and DHA activity (Leirós *et al*., 2000). However, it must be taken into consideration that these findings hold good only for stable soil like forests and ours is just weathered rocks particles which is far from equilibrium. It is also recognised that statistical analyses act only as a guide for the result interpretation and some of the significant correlation may be of doubtful biochemical significance. Present findings are in accordance with that of Nannipeiri *et al*., (2003) who found no consistent relationship between microbial diversity and soil functions. Due to complex dynamics of soil ecosystem, no single property is satisfactory for the study of microbial activity (Garcia *et al*., 1998). Establishing correlation between parameters in a completely disturbed ecosystem is not a good tool for inferences. Further the relationship between an individual biochemical property and the total microbial activity is not always obvious, especially in the case of complex systems like soils, where the microorganisms and processes involved in the degradation of the organic compounds are highly diverse. Low DHA activity observed throughout the control plot was due to poor fertility status of spoil especially due to lack of soil organic matter (Mummey *et al*., 2002). Presence of many aromatic organic pollutants in the coal mine spoils hamper rhizospheric soil microbial activites (Kumar *et al*., 2010).

However, OBM showed the maximum DHA activity which can be supported by the fact that fresh organic matter contributes to significant increase in microbial metabolism (Joshi *et al*., 2010). Present research also support that of Jarvan *et al*., (2014) who added cattle manure and got increased DHA activity in agro-ecosystem. There was an active increase in DHA activity with the application of organic fertilizers in the experiment of Zhang *et al*., (2009). Poor DHA activity is an indicator of soil degradation (Garcia *et al*., 1997). In general, fine-textured soils have more micropores than sandy soils. Soil micropores protect mineralising microorganisms against grazers (Killham, 1994) and this can be one of the reasons for the higher enzyme activity in OBS plot. Vesicular Arbuscular Mycorrhizal (VAM) fungi also holds promise to increase plant yield and nutrient dynamics as they represent both a conduit for better nutrient uptake and site for accumulation of nutrients in the ecosystem. VAM also affects the enzyme activity by influencing the root exudation and microbial community (Wamberg *et al*., 2003) and thus enzymatic activity. Similar results have been depicted by Acosta-Martinez *et al*., (2008) who reported decline in DHA activity of cultivated land with comparison to undisturbed land. This shows that since mining area are disturbed so the DHA activity has shown decrease. Verma *et al*., (2014) reported very low DHA activity in overburden dumps which was 0.1625 µg/g of soil. However our findings are more than that of Verma et al., (2014) because in our study rhizospheric soil was taken. Most importantly the soil C/N ratio is so little that it cannot provide conducive environment for the microbes to thrive (USDA NRSC, 2011) which is a very significant cause for the low DHA activity.

Heavy metals can reduce enzyme activity by interacting with the enzyme-substrate complex, denaturing the enzyme protein or interacting with the protein-active groups, they could also affect the synthesis of enzyme microbial cells (Pan and Yu, 2011). Xie *et al*., (2009) noted that Cu of 100 mg kg-1 could suppress DHA significantly, while Cd of 5 mg kg-1 had relative greater influence on soil microbial diversity. However, in our study heavy metals were not found in such a high amount. Thus low DHA activity was not because of heavy metal toxicity in the current study.

Seasonal variation

The observed increase in DHA from monsoon to winter can be due to litter decomposition which decay of litter in the plots through the litter bag experiment which was conducted for different study. The litter addition favoured the overall soil oxidative activity; as the litter underwent decay, smaller and simpler organic molecules are leached down from the litter layer to the surface soil horizon in the form of water-soluble organic matter, thus providing a labile organic substrate for soil microorganisms (Görres *et al*., 1998).The decrease summer season, may be due to decrease in moisture content which would have led to the decrease in microbiological activity. Garcia *et al*., (1994) found that the rainy season enhanced the enzyme activity (DHA) of soils in the south-east arid region of Spain. Other authors also found increase in microbial activity in forest and grassland soils due to higher soil moisture contents (Banerjee *et al*., 2000).

Soil with low moisture level have shown to DHA close to zero (Marzadori *et al*., 1996). As soils dry, the water potential increases, and as well microbial activity as intracellular enzyme activity slows down (Mukhopadhyay and Maiti, 2010).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 in a study by Kumar *et al*., (2010) on Jharia coalfields.

Plant species wise DHA occurrence

Sinha *et al*., (2009) did a remarkable study on Jharia Coalmines and reported 93.3 µg TPF/g/hr in the rhizosphere soil of *Aegle mermalos*. Their research highlighted that the microbial activity of the tree root zone is important for screening plant species for reclaiming coal mines spoils. The maximum DHA activity has been found in the rhizosphere of *F. religiosa* which may be due to higher root growth and exudates. *F. religiosa* has been found to have the highest root biomass and a potent grower in poor spoils. *D. strictus* is also a fast grower and its association with VAM has been found to be about 32% in the control sites. There is evidence that mycorrhiza affect the growth, composition and activity of microbial communities by altering root exudation. VAM have been found to considerably increase the dehydrogenase activity (Alguacil *et al*., 2005).

In this study a new observation was marked. Higher DHA activity does not always refer to good health of the plant. Such an observation was highlighted in the case of *S. indica* which showed death and very poor growth performance. Its root exudation stopped and dead root material was available for decay to the soil microbes as some *S. Indica* plants wilted. This shift in carbon availability probably resulted in growth of saprotrophic fungi and bacteria that replaced micro-organisms living on watersoluble carbohydrates from the living roots and hence increased the DHA activity (Wamberg *et al*., 2003). The initial DHA activity was more or less similar amongst the plants because they were not very well acclimatise to the adverse condition of the ump spoil. Variation was seen after that when the plants stared growing and as per there growth response root exudation was also different.

The complexity of the interaction between different tree species and micro flora is too great to be generalized (Pellisier and Souto, 1999). Soil chemical changes related to the release of organic and inorganic compounds, and the respective products of their microbial metabolism are important factors affecting microbial populations, availability of nutrients, solubility of toxic elements in the rhizosphere, and thereby the ability of plants to cope with adverse soil-chemical conditions (Pandey and Palni, 2007).

Conclusion

This is a short term study to propose plant species fit for restoration. Initial lower DHA activities depicted poor microbial activity in the spoil as a consequence of ecological perturbations. Slowly the microbes got accustomed to the spoil environment and promoted the DHA activity. VAM application has showed increased DHA activity which highlights its importance in restoring disturbed soil. Application of soil amendments are suggested to decrease ecological perturbations by promoting faster and beneficial microbiological processes. Though it is too early to comment but tree species like *F. religiosa* and *D. strictus* can be proposed for quick revive of the spoils through better enzymatic activity.

Reference

Acosta-Martinez V, Acosta-Mercado D, Sotomayor-Ramirez D and CruzRodriguez L 2008 Microbial communities and enzymatic activities under different management in semiarid soils. Applied Soil Ecology. 38: 249-260

Alguacil MM, Caravaca F and Rolda´n A 2005 Changes in rhizosphere microbial activity mediated by native or allochthonous AM fungi in the reafforestation of a Mediterranean degraded environment. Biology & Fertility of Soils. 41: 59-68

Banerjee MR, Burton DL, McCaughery WPP and Grant CA 2000 Influence of pasture management on soil biological quality. J. Range Management. 53:127-133

Chandra D, Singh RM and Singh MP 2000 Text book of the coal (Indian context). Ist edition. Tara Book Agency, Varanasi

Casida LE, Klein DA and Santoro T 1964 Soil dehydrogenase activity. Soil Sci. 98: 371-376

Dick RP 1997 Soil enzyme activities as integrative indicators of soil health. In: Pankhurst CE, Doube BM, Gupta VVSR (eds) Biological indicators of soil health. CAB International, New York, 121–156

Garcia C, Hernandez T and Costa F 1994 Microbial activity in soils under Mediterranean environmental conditions. Soil Biology & Biochemistry. 26: 1185-1191

Garcia C and Hernández T 1997 Biological and biochemical indicators in derelict soils subject to erosion. Soil Biology & Biochemistry. 29: 171-177

Garcia C, Hernandez T, Albaladejo J, Castillo V and Roldán A 1998 Revegetation in semiarid zones: influence of terracing and organic refuse on microbial activity. Soil Science Society of America J. 62: 670-676

Garcia C, Roldan A and Hernandez, T 2005 Ability of different plant species to promote microbiological processes in semiarid soil. Geoderma 124: 193-202

Jackson, ML 1973 Soil Chemical Analysis. Prentice Hall of India private Limited, New Delhi

Järvan M, Edesi L, Adamson A and Võsa T 2014 Soil microbial communities and dehydrogenase activity depending on farming systems. Plant Soil Environment. 60(10): 459-463

Joshi N, Ngwenya BT and French CE 2012 Enhanced resistance to nanoparticle toxicity is conferred by overproduction of extracellular polymeric substances. J. Hazard. Mater., 241–242: 363-370

Kandeler E 1996 Nitrate. In: Schinner F, Öhlinger R, Kandeler E, Margesin R (eds). Methods in soil biology. Springer, Berlin Heidelberg New York: 408-410

Killham K 1994 Soil ecology. Cambridge University Press, Cambridge

Kumar S, Chaudhuri S and Maiti SK 2010 Dehydrogenase activity in natural and mine soil - A review. Environment and Ecology Research

Leirós MC, Trasar-Cepeda C, Seoane S and Gil-Sotres F 2000 Biochemical properties of acid soils under climax vegetation (Atlantic oakwood) in an area of the European temperatehumid zone (Galicia, NW Spain): general parameters. Soil Biology & Biochemistry. 32: 733-745

Liang Q., Chen H., Gong Y., Yang H., Fan M and Kuzyakov Y 2014 Effects of 15 years of manure and mineral fertilizers on enzyme activities in particle-size fractions in a North China Plain soil. European J. Soil Biology, 60: 112-119

Marzadori C 1998 Effect of lead pollution on different soil enzyme activity. Bio. Fert. Soils. 22: 53-58

Mukhopadhyay S and Maiti SK 2010 Dehydrogenase activity in natural and mine soil-a review. IJEP. 30(11): 921-933

Mummey DL, Stahl PD and Buyer JS 2002 Microbial biomarkers as an indicator of ecosystem recovery following surface mine reclamation. Applied Soil Ecology. 21: 251-259

Nannipieri P, Ascher J, Ceccherini MT Landi L, Pietramellara G and Renella G 2003 Microbial diversity and soil functions. Eur. J. Soil Sci. 54: 655-670

Pan J, Yu L 2011 Effects of Cd or/and Pb on Soil Enzyme Activities and Microbial Community Sructure. Ecological Engineering. 37:1889-1894

Pandey S and Palni LMS 2007 The rhizosphere effect in trees of the Indian Central Himalaya with special reference to altitude. Applied Ecology & Environmental Res. 5: 93-102

Pellisier F and Souto XC 1999 Allelopathy in northern temperate and boreal seminatural woodland. Critical Reviews in Plant Sciences. 18: 637-652

Prasad R and Mertia RS 2005 Dehydrogenase activity and VAM fungi in tree rhizospher of agroforestry systems in Indian arid zone. Agoforestry Forum. 63: 219-223

Sinha S, Masto RE, Ram LC, Selvi VA, Srivastava NK, Tripathi RC and George J 2009 Rhizosphere soil microbial index of tree species in a coal mining ecosystem. Soil Biology & Biochemistry. 41: 1824-1832

USDA NRCS 2011 Carbon to Nitrogen Ratios in Cropping Systems. soils.usda.gov/sqi

Verma S, Singh A P, Devi S, Mewaram R R, Sharma S and Dubey K 2014 Assessment of Microbial Community and Soil EnzymeActivity of Coal Mine Dumps of Sonbhadra UttarPradesh, India. Proc. of the Int. Conference on Advances In Bio-Informatics, Bio-Technology And Environmental Engineering-ABBE 2014. doi: 10.15224/ 978-1-63248-009-5-90

Wamberg C, Christensen S, Jakobsen I, Müller AK and Sørensen SJ 2003 The mycorrhizal fungus (*Glomus intraradices*) affects microbial activity in the rhizosphere of pea plants (Pisum sativum). Soil Biology & Biochemitry 35:1349-1357

Wolinska A and Stepniewska Z 2012 Dehydrogenase activity in the soil environment. In: Canuto R.A. (ed.): Dehydrogenases. Intech, Rijeka. Available at http://www.ebook3000.com/

Xie W, Zhou J, Wang H, Chen X, Lu Z, Yu J and Chen X 2009 Short-Term Effects of Copper, Cadmium and Cypermethrin on Dehydrogenase Activity and Microbial Functional

Diversity In Soils After Long-Term Mineral or Organic Fertilization. Agriculture, Ecosystems & Environment. 129: 450-456

Zhang L, Zhijie WU, Chen Lijun, Jiang Yong and Dongpo LI 2009 Kinetics of catlase and dehydrogenese in main soils of northeast China under different soil moisture conditions. Agricultural J. 4(2):113-120

Table: 1 Summary about control and treatment plots.

Plot Name	Abbreviation	Remarks
Over Burden spoil as Control	OBC	Mine spoil, seedlings of five plant species, no amendments
Over Burden Spoil + VAM	OBV	Mine spoil, seedlings of five plant species, treatment with vesicular arbuscular mycorrhizae (VAM)
Over Burden Spoil + VAM + Agricultural Soil	OBS	Mine spoil, seedlings of five plant species, treatment with vesicular arbuscular mycorrhizae (VAM) and agricultural soil to mine spoil ratio was 1:4
Over Burden Spoil+ VAM + Manure	OBM	Mine spoil, seedlings of five plant species, treatment with vesicular arbuscular mycorrhizae (VAM) and manure to mine spoil ratio was 1:4

Table: 2 Physico-chemical parameters of the control and treatment plots in three years of study. DTPA extractable metals are depicted.

Parameters	Values ± Standard Deviation			
	OBC	**OBV**	**OBS**	**OBM**
pH	6.21±0.39	6.02±0.07	6.55±0.73	6.69±0.20
EC(mmhos/cm)	0.17±0.05	0.51±0.09	0.70±0.10	1.46±0.42
BD(g/cc)	1.45±0.02	1.45±0.01	1.33±0.06	1.30±0.07
MC (%)	1.60±0.10	1.89±0.08	2.17±0.13	3.07±0.06
WHC (%)	18.20±0.43	18.61±0.47	25.63±0.345	29.67±0.22
OC (µg/g)	0.97±0.10	1.03±0.12	2.16±0.10	3.89±0.13
OM (µg/g)	1.68±0.17	1.79±0.20	3.74±0.17	6.74±0.23
Avai- N(µg/g)	23.23±1.50	23.29±2.34	34.98±0.93	60.10±1.37
Avai-P(µg/g)	1.95±0.80	2.38±0.62	8.23±0.79	15.64±0.54
C/N	0.04	0.04	0.061	0.24
Cu (ppm)	0.43±0.19	0.70±0.20	0.40±0.14	1.17±0.67
Mn (ppm)	3.24±0.74	3.75±0.81	9.76±0.13	59.70±1.67
Zn (ppm)	11.44±1.4	11.76±1.18	1.98±0.20	4.50±0.29
Ni (ppm)	0.13±0.06	0.16±0.04	0.53±0.14	0.44±0.07
Cd (ppm)	0.19±0.06	0.12±0.06	0.02±0.00	0.12±0.04
Pb (ppm)	0.82±0.08	0.84±0.03	0.66±0.07	1.34±0.41
Fe (ppm)	1.43±0.67	1.32±0.48	12.17±0.46	14.98±0.28

Table: 3 Seasonal variation in DHA activity (µg/g/hr) in the rhizosphere of five plant species along with their F values and outcomes of DMRT.

Plant Species	Plots			
	OBC	**OBV**	**OBS**	**OBM**
A. indica				
M (F=52.24)	1.34 (c) ± 0.10	2.03 (c) ± 0.38	3.40 (b) ± 0.73	8.00 (a) ± 0.59
W (F=28.29)	3.60 (c) ± 0.95	5.88 (bc) ± 0.80	8.03 (ab) ± 1.56	13.28 (a) ± 1.72
S (F=174.20)	2.94 (c) ± 0.57	4.07 (bc) ± 0.93	6.21 (b) ±1.02	10.58 (a) ± 1.61
E.officinalis				
M (F=740.53)	1.17 (d) ± 0.80	1.99 (c) ± 0.63	2.82 (b) ± 0.63	5.41 (a) ± 0.69
W (F=28.07)	2.14 (c) ± 0.57	3.73 (bc) ± 0.92	5.47 (b) ± 0.92	8.74 (a) ± 1.38
S (F=56.48)	2.52 (c) ± 0.4	3.20 (c)± 0.91	5.54 (b) ±1.21	9.56 (a) ± 1.19
D. strictus				
M (F=837.34)	1.34 (d) ± 0.51	2.83 (c) ± 1.51	4.39 (b) ± 1.22	12.74 (a) ± 2.19
W (F=214.29)	4.42 (c) ± 1.19	6.37 (c) ± 1.73	9.99 (b) ± 2.17	19.77 (a) ± 2.18
S (F=200.09)	4.01 (c) ± 1.04	5.66 (c) ± 1.29	8.35 (b) ± 1.92	16.55 (a) ± 1.87
F. religiosa				
M (F=424.08)	1.26 (d) ± 0.19	2.80 (c) ± 1.77	2.80 (b) ± 1.92	13.90 (a) ± 3.17
W (F=117.61)	5.77 (c) ± 1.16	8.59 (c) ± 2.18	8.59 (b)± 1.94	22.98 (a) ± 4.11
S (F=146.40)	4.75 (c) ± 1.97	6.03 (c) ± 1.39	6.03 (b) ± 2.18	19.58 (a) ± 2.72
S. indica				
M (F=102.38)	1.17 (c) ± 0.72	1.99 (bc) ± 1.42	2.82 (ab) ± 2.18	5.41 (a) ± 1.59
W (F=36.53)	2.14 (c) ± 0.95	3.73 (bc) ± 1.33	5.47 (b) ± 1.18	8.74 (a) ± 3.28
S (F=15.79)	2.52 (b) ± 0.38	3.20 (b) ± 1.91	5.54 (b) ± 1.55	9.56 (a) ± 3.17

Note: M= monsoon, W= winter and S= summer.