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Analysis of Physico Chemical Parameters in Relation to Soil Fungi of Bhadrachalam Forest, Khammam District, Andhra Pradesh, India

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Abstract The study area is located in Bhadrachalam forest located at Khammam district, Andhra Pradesh state, India is enriched with fungal diversity. The present location is empowered by river Godavari which is believed to be a divine river by the local tribes. The study zone extends through $80^0$21' to $81^0$09'E and $17^0$36' to $18^0$38'N. The soil samples were carefully collected from Northern part of Bhadrachalam forest from 2009 to 2010 at regular intervals. The main aim of the current work is to systematically analyze the physico chemical parameters such as Nitrogen, Phosphorus, Potassium, Iron, Copper, Zinc and Manganese which effects the fungal population present in the soil. An attempt has been made to analyze and describe the effect of key physico chemical factors on fungal population existing in the study area. The current work was initiated as the soil fungi are essential ecological entities to support the forest plant species. It is observed that there is a stringent necessity to practice the sustainable practices to save the microflora in order to save the forest ecosystem. The soil microflora is determined by the abiotic factors. This work is focused on the quantification of the abiotic factors on the soil fungi of Bhadrachalam forest.

Keywords *Atomic Absorption Spectrophotometer, Abiotic Factors, Fungal Population Soil Microflora, Statistics*

1. Introduction

The forest area is one of the territorial divisions of Khammam zone of Bhadrachalam forest. The study area is located on the bank of Godavari River. The present study area is a partially surveyed valley during 1885. The area is covered by rifts due to folding and lowering of rocks. The current work was initiated as the soil fungi are essential ecological entities to support the forest plant species. It is observed that there is a stringent necessity to practice the sustainable practices to save the microflora in order to save the forest ecosystem. The soil microflora is determined by the abiotic factors. This work is focused on the quantification of the abiotic factors on the soil fungi of Bhadrachalam forest. The hyphae which were observed in T.subterraneum are slightly wider in nitrogen deficient soils (LK

Abbott and AD Robson, 1976). The current study area extends from $80^0$21' - $81^0$09' E and $17^0$36' - $18^0$38' N and falls in the Survey of India toposheet No. 55 G/2, 5. It is a well know green cover area present in the entire Khammam district with scenic beauty.

2. Methodology

Soil samples were collected on regular basis from 2009 to 2010. Composite samples were collected from a predefined area of 50 x 50 m area. The rhizosphere soil was collected in the polythene bags along with tiny root bits. The microelements such as Iron, Copper, Zinc and Manganese were systematically studied using atomic absorption spectrophotometer. The samples were kept in refrigerator at 5^0C until further tests commenced. Rhizoshpere soil samples were collected from Hardwikia bipinata (Caesalpiniaceae) and Dalbergia Paniculata to study the soil microflora and propagules after isolating the unwanted debris. The root bits were subjected to cytological investigation for observing soil micro flora with a special focus on fungal members.

2.1. Chloride Content

The chloride content of the soil was determined by chromate titration method (Piper, 1944) of Wilcox and Hatcher (1950).20 g of soil was taken in 250 ml Erlemeyer flask and 100 ml of glass distilled water was added. The contents were shaken on mechanical shaker for one hour and then filtered. 50 ml of filtrate was taken in another 250 ml Erlemeyer flask and titrated against N/20 Silver nitrate solution using 1ml Potassium chromate as indicator. The yellow colour was changed to brick red. The chloride content (in ppm) was calculated as follows:

Chlorides (in ppm) = Volume of silver nitrate X 35.457 X f (f=0.45)
N/20 Silver Nitrate: 4.2472 g of $AgNO_3$ was dissolved in 1000 ml of distilled water.
Potassium chromate solution: 10 g of $K_2Cr_2O_4$ was dissolved and diluted in 200 ml of distilled water

2.2. Microelements

The quantity of microelements like Iron, Copper, Zinc and Manganese was determined on atomic absorption spectrophotometer following the method recommended by Isaac and Kerber (1944). 1 g of 0.5 mm sieved soil was taken in a big corning tube and to it added 5 ml of conc.HNo_3. The contents were digested in water bath for 4 hrs. and left overnight. Next day, they were diluted to 20 ml (15 ml of distilled water was added) and filtered through Whatman 41 filter paper. The filtrate was corked and kept as stock solution.

For Iron (in ppm), the stock solution was diluted to 190 (i.e. for 1ml of stock solution 18 ml of HNO_3 acidified distilled water was added and from this solution 1 ml was taken and added 9 ml of HNO_3 acidified distilled water) and fed to atomic absorption spectrophotometer (AS) adjusting the wavelength to 248.3 mm. For Copper content (in ppm) the stock solution is directly fed to the AS with the wavelength adjusted to 324.8 mm, for Zinc content (in ppm) 1/19 dilution of the stock solution was taken and fed to AS adjusting the wavelength to 213.9 mm. For Manganese content (in ppm) 1/19 dilution of the stock solution was taken and fed to AS adjusting the wavelength to 279.5 mm.

2.3. Nitrogen

The nitrogen content was estimated by Kjeldahl's method (Piper, 1944). 10 g of soil was taken in a Kjeldahl flask and to that 30ml of conc. H_2SO_4, 8 g of potassium sulphate and 0.5 g of copper sulphate were added. The digestion was started on a low flame and the heat was gradually increased. The digestion was continued for one to one and half hours till the contents become colourless. After

cooling the flask, the contents were diluted to 100 ml and the fluid transferred to a distillation flask leaving the soil behind. The soil residue was washed with four lots of 60ml of distilled water, decanting the washings into the flask. To this sufficient saturated NaOH solution was added to make the contents of flask alkaline. NaOH solution was poured along the side of the flask so that it forms a heavy layer at the bottom. The flask was kept for distillation. The distillate was collected into N/10 H_2SO_4 and was titrated against N/10 sodium hydroxide using methyl red as indicator. A blank titration was also carried out earlier.

The percentage of total nitrogen in the soil corresponds to:
% of Nitrogen = (Blank titre Value – distillate titre Value) X Normality of acid X 0.14

2.4. Phosphates

For estimating available phosphorus Olsen's method (Jackson, 1973) was followed. 5 g of soil was weighed into a 250 ml Erlenmeyer flask, 100 ml of 0.5 M Sodium bicarbonate and teaspoon full of activated charcoal were added, Stoppard and shaken for 30 min. on electric shaker. The contents were filtered through Whatman 41 filter paper. 5 ml of the filtrate was pipetted out into 25 ml volumetric flask and was neutralized with 1:4 H_2SO_4. paranitrophenol as indicator. The volume was made up by adding distilled water and 1 ml of molybdic acid reagent, avoiding excess effervescence. When few crystals of stannous oxalate were added, blue colour will develop. The solution was shaken well and read in Elico calorimeter within 10 min after adding stannous oxalate using red filter.

Available phosphorous (ppm) corresponds to:

$$\frac{\text{Reading of calorimeter} \times 2.5}{0.05} \times \frac{100}{5} \times \frac{1}{5}$$

2.5. Potassium

Ammonium acetate method (Muhr et.al., 1965) was followed for estimating the available potassium. 5 g of soil was taken into a 250 ml conical flask and 25 ml of 1 N neutral ammonium acetate was added. It was allowed to shake for 5 min on an electric shaker and filtered through Whatman – 40 filter paper. The filtrate was read in a standardized Elico flame photometer using potassium interference filter. The readings of the test sample were recorded and it should be between the readings of the standards.

The percentage of potassium then corresponds to:

$$C_1 + \frac{R - r1\ X\ C2 - C1}{r2 - r1} \times 25 \times \frac{100}{5} \times \frac{1}{10^3}$$

Where C1= Lower ppm taken,
C2= Higher ppm taken,
r_1= Flame photometer reading of C1,
r_2= Flame photometer reading of C2,
R= Reading of the test sample,
25= Volume of ammonium acetate used,
100/5= for getting percentage,
$1/10^3$ = to convert microgram into mg/100 g

2.6. Quantitative and Qualitative Estimation of Soil Fungi

For quantitative estimation of soil fungi, the dilution plate method (Waksman, 1922) as modified by Brierley et.al, (1927) was employed. Sterile distilled water was used to make the dilutions. 25 g of soil was weighed into an Erlenmeyer flask containing 250 ml sterile distilled water. The liquid was thoroughly shaken with hand to break up the soil particles. Further dilutions were made immediately. Potato- Dextrose - Agar (PDA) medium was employed for isolation and estimation of soil fungal numbers. The pH of the medium was adjusted to 4.5 by adding 25% lactic acid. Dilutions of 1: 10,000 of sample were used. The sterilized plates were inoculated with 1 ml portion of the ultimate dilution. Six plates were inoculated with each sample every month and were incubated at 25^0C. The number of colonies developing was counted from the 4^{th} to the 7^{th} day after inoculation and the number of fungi per gram dry soil was calculated (Waks man, 1931).

Table 1: *Result of Chemical and Statistical Analysis of Chloride Concentrations (ppm) of Soil Samples*

Month	Soil-1	Soil-2
April	12.7645	27.1246
May	12.7645	12.7645
June	7.9778	9.5733
July	7.9778	11.1689
August	11.1689	19.1467
October	7.9778	11.1689
November	11.1689	7.9778
December	9.5733	7.9778
Min	7.9778	7.9778
Max	12.7645	27.1246
Median	10.3711	11.1689
Std Dev	2.078176	6.602832
Skewness	0.10509	1.595797
Kurtosis	-1.92229	2.120627

Soil-1: *Dalbergia Paniculata;* ***Soil-2:*** *Hardwickia bipinata*

Table 2: *Result of Chemical and Statistical Analysis of Iron Concentration (ppm) in Soil of the Study Area*

Month	Soil-1	Soil-2
April,2009	274.55	478.04
June	458.09	461.89
August	785.27	403.37
October	315.78	296.97
December	341.81	335.16
February, 2010	381.14	241.88
Min	274.55	241.88
Max	785.27	478.04
Median	361.475	369.265
Std Dev	186.7033	94.01139
Skewness	1.88304	-0.14487
Kurtosis	3.757802	-1.70319

Soil-1: *Dalbergia Paniculata;* ***Soil-2:*** *Hardwickia bipinata*

Table 3: *Result of Chemical and Statistical Analysis of Copper Concentration (ppm) of Soil from the Study Area*

Month	Soil-1	Soil-2
April,2009	0.365	0.559
June	0.901	0.481
August	0.638	0.647
October	0.389	0.344
December	0.657	0.590
February, 2010	0.490	0.340
Min	0.365	0.34
Max	0.901	0.647
Median	0.564	0.52
Std Dev	0.201432	0.129022
Skewness	0.739695	-0.30222
Kurtosis	-0.00796	-1.91772

Soil-1: *Dalbergia Paniculata;* ***Soil-2:*** *Hardwickiabipinata*

Table 4: *Result of Chemical and Statistical Analysis of Zinc Concentration (ppm) of Soil from the Study Area*

Month	Soil-1	Soil-2
April,2009	2.736	2.242
June	2.185	2.242
August	2.660	3.477
October	1.862	3.002
December	1.558	4.465
February,2010	0.855	1.273
Min	0.855	1.273
Max	2.736	4.465
Median	2.0235	2.622
Std Dev	0.711828	1.115722
Skewness	-0.59478	0.288225
Kurtosis	-0.34925	-0.10397

Soil-1: *Dalbergia Paniculata;* ***Soil-2:*** *Hardwickia bipinata*

Table 5: *Result of Chemical and Statistical Analysis of Manganese (ppm) Content of Soil from the Study Area*

Month	Soil-1	Soil-2
April, 2009	14.801	5.795
June	19.532	14.516
August	13.015	15.770
October	4.826	5.567
December	13.737	12.445
February, 2010	13.680	4.769
Min	4.826	4.769
Max	19.532	15.77
Median	13.7085	9.12
Std Dev	4.760437	4.982895
Skewness	-0.97645	0.157348
Kurtosis	2.773713	-2.80945

Soil-1: *Dalbergia Paniculata;* ***Soil-2:*** *Hardwickia bipinata*

Table 6: *Result of Chemical and Statistical Analysis of Nitrogen Concentration (ppm) of Soil from the Study Area*

Month	Soil-1	Soil-2
April, 2009	297	220
June	1830	163
August	220	4282
October	109	5099
December	757	889
February, 2010	263	230
Min	109	163
Max	1830	5099
Median	280	559.5
Std Dev	652.0499	2258.919
Skewness	1.901579	0.967632
Kurtosis	3.54546	-1.55901

Soil-1: *Dalbergia Paniculata;* ***Soil-2:*** *Hardwickia bipinata*

Table 7: *Result of Chemical and Statistical Analysis of Phosphorus Content (ppm) of Soil from the Study Area*

Month	Soil-1	Soil-2
April, 2009	3.9	4.0
June	4.0	3.8
August	0.3	0.2
October	0.2	0.4
December	1.9	1.8
February, 2010	2.1	3.0
Min	0.2	0.2
Max	4	4
Median	2	2.4
Std Dev	1.657307	1.663731
Skewness	0.089996	-0.22514
Kurtosis	-1.8749	-2.23153

Soil-1: *Dalbergia Paniculata; Soil-2: Hardwickia bipinata*

Table 8: *Result of Chemical and Statistical Analysis of Potassium Concentration (ppm) of Soil from the Study Area*

Month	Soil-1	Soil-2
April,2009	295	140
June	250	225
August	205	150
October	210	230
December	175	200
February,2010	185	200
Min	175	140
Max	295	230
Median	207.5	200
Std Dev	44.94441	37.73813
Skewness	1.02602	-0.54664
Kurtosis	0.288268	-1.6987

Soil-1: *Dalbergia Paniculata;* ***Soil-2:*** *Hardwickia bipinata*

3. Results and Discussion

The results obtained through this study emphasize that the Chloride, Nitrogen, Zinc concentrations are predominant in soils harboring Hardwickia bipinata. The Iron, Potassium, Manganese concentrations are considerably more in soils harboring Dalbergia Paniculata. This reflects that there is a relation between the physic chemical factors and the fungal components of the forest soil microcosm. Further in situ investigations via novel physico chemical aids can give a fool proof scenario of the abiotic factors and fungal interplay conditions existing in this area.

4. Conclusion

The physico chemical factors were statistically analyzed and represented in Table1 through Table 8. The statistical parameters including minimum, maximum, median, standard deviation, skewness and kurtosis were calculated. The present analyses were carried in order to explain the importance of key elements present in the soil. The key parameters such as Chloride, Iron, Copper, Zinc, Manganese, Nitrogen, Phosphorus, and Potassium were carefully studied and the values are given in tabulated format. The primary objective of the current study was to understand the physico chemical parameters present in the rhizosphere soils of the forest area in Bhadrachalam forest of Khammam district, Andhra Pradesh. The current study was formulated and programmed to statistically analyze and represent key physico chemical parameters which effects fungal population of the study area. The results of the present study indicate that the soils in this forest area need immediate attention so as to sustain the ecological balance of the area.

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