Effect of Shifting Cultivation on Soil health and Microbial diversity in JunnarTaluka of Pune District

Background: Shifting cultivation is also referred to as Swidden cultivation, slash and burn cultivation, bush-fallow agriculture. Its name varies in different parts of the world. The physico-chemical characters of the soil are affected by the crop type and agricultural practices. Microbial biomass of soil and its number also influence the soil health.

Aims: The present study has attempted to find out the impact of shifting cultivation on soil bacterial communities and soil quality in the western ghat region of Pune district.

Study design:18 soil samples from 4 villages of the Junnar tehsil were collected after harvesting the cultivated crop.

Place and Duration of Study: The sample was collected within the 2 to 4 acres of the shifting cultivation plots identified in the villages in the western ghat region of Pune district. Methodology: The soil samples were collected after harvesting every crop and from the fallow land. Analysis of Physico-Chemical properties of soil samples was performed. Bacteria were isolated and identified from the soil samples. Statistical analysis was done to interpret the obtained results.

Results: The soil samples included the fallow land and crop (Varai, Khursani, Sawa, Nachani and Hulga) harvested soils. Ten bacterial genuses were isolated from all soils in varying number. It was observed that the Total Viable Count (TVC) of the crop harvest soil significantly decreased as compared to the fallow soil. Different soil parameters were also found to be improved in the fallow period.

Conclusion: The results pointing out the importance of the length of the fallowing on the soil health and thus on the crop yield.

Keywords: Shifting cultivation; Physico-chemical characters, fallow period, TVC, soil health

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1. INTRODUCTION

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The historical background of shifting cultivation is old as the history of agriculture itself [1]. The "Shifting Cultivation", its name varies in different parts of the world and generally known as "Slash and Burn" and "bush fallow" agriculture. It is known as "Jhum" in the hilly states of north-east India, Podu in Orissa, Kumari in Western Ghats, watra in Rajasthan, Penda, Bewar or Dahia in Bastar district of Madhya Pradesh.

22 Shifting cultivation in India is very common in the North Eastern hilly region and Orissa, 23 besides the hilly Eastern Ghats region of Andhra Pradesh, Madhya Pradesh, where it is 24 moderately practiced, and parts of Western Ghats, where it exists in a modified form. 25 Shifting cultivation was widely practiced in the Western Ghats, extending further north along 26

its forested heights till the south west extension of Aravalis in Dungarpur district.

1.1 Changes in soil fertility during shifting cultivation

There are many reports on potential use of microbial properties as measure of soil productivity or microbial activity [2]. Soil enzyme activity which is one of the biochemical properties of soil plays a crucial role in regulating soil nutrient cycling. Soil enzyme activities of the soil are due to the enzymes that are piled up and also from the enzyme activities of proliferating microorganisms. These are very reactive and can give accurate and fast information about the minute changes occurring in soil [3]. Microbial activities directly affect the stability and fertility of the ecosystems and can serve as sensitive indicators of ecology stress suffered by soil. Microorganisms are involved in decomposing soil organic matter nutrient cycling and stabilization of plant ecosystems [4] and thus regulate the soil processes. Moreover, the changes in the microbiota are highly coupled with the soil carbon (C) and Nitrogen (N) processing through their enzyme activities [5]. Unfortunately these parameters and their effects on shifting cultivation with respect to accumulation of forest biomass, nutrient cycling and role of microbes that varies with fallow length are still poorly understood. Therefore the need of hour is to search for sustainable ways of cultivation that can transform shifting cultivation system and have less environmental consequences. Understanding the plant-soil-microbe interactions thus can help finding a solution for transforming shifting cultivation system. The main objective of this research was to study the effect of shifting cultivation system on the microbial diversity and thus in return on the soil

2. MATERIAL AND METHODS

2.1 Soil sample collection

The Junnartaluka (19.2048° N, 73.8732° E) which is gently sloping land with moderate erosion has shallow well drained, clayey soil. 18 soil samples from 4 villages of the Junnar tehsil were collected after harvesting the cultivated crop. The sample was collected by laying 5 quadrants of 1m by 1m, within the 2 to 4 acres of the shifting cultivation plots identified in the villages. The soil sample was collected after harvesting every crop and from the fallow land. 200g soil per quadrant was collected from 1cm to 9cm depth, and was pooled together in a 5kg sterile polythene bags and transported at room temperature to the laboratory within 24hours. On arrival at the lab the soil samples were stored at 4°C, the soil samples were processed within 3 to 4 days. To determine all the parameters for each soil sample, portion of soil was air-dried and sieved through a 2 mm sieve and then was diluted in water.

62 2.2 Analysis of Physico-Chemical Properties of Soil

Physico–Chemical Properties of Soil like pH, Electrical Conductivity (EC), organic carbon, available phosphorus and available potash [6], soil CaCO₃[7], total cation (Ca + Mg)available sodium [9], soil moisture [10], soil texture [11], Water Holding Capacity (WHC) [12] were determined as described earlier.

2.3 Isolation and Identification of bacteria

The microbial community analysis was done by total viable count of soil bacteria using serial dilution of the soil. 1gm of soil was mixed in 100ml saline and serially diluted this up to 10^{-10} and then 0.1ml of each dilution was spread on sterile nutrient agar plate in triplicates. These plates were incubated at room temperature for 24 to 72 hours. The subsequent bacterial colonies developing on the plates were counted and the colony forming units (cfu) per ml of soil sample was estimated. It is the number of cfu multiplied by the volume of dilution multiplied by the dilution factor. The isolated bacterial colonies growing on these plates were visually identified and picked up daily, the colony morphology recorded and the bacterial

colonies were subject to Gram staining, Spore staining, capsule staining and biochemical identification was done up to genus level as per the Bergey's manual of Determinative Bacteriology, 9th edition.

2.4 Statistical Analysis

Statistical analyses were performed using SPSS software version 21. The correlation between all the soil parameters tested in this study was generated. Using R software version 3.6.1, Heat maps were developed for this correlation analysis data generated from SPSS. On the basis of the data obtained richness, Simpson's diversity and evenness indexes were calculated. The results were submitted to principal component analysis (PCA) in order to determine the common relationships between parameters.

3. RESULTS AND DISCUSSION

3.1 Physico-chemical analysis

Soil sample from various villages of each tehsil was collected and the physico-chemical analysis was performed for each sample. From 4 villages of the Junnar, 18 soil samples of the shifting cultivation plots were collected. Average and standard deviation of each parameter was statistically calculated and was considered for further analysis.

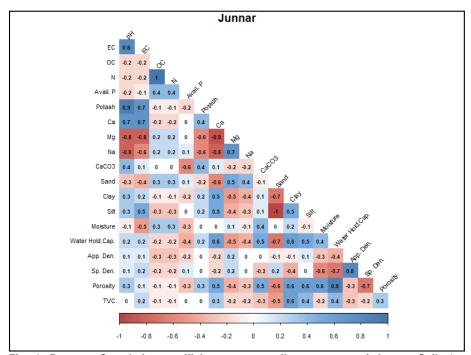


Fig. 1. Pearson Correlation coefficient among soil parameters of Junnar Soil; the values indicated in red are a negative correlation, and those in blue are a positive

correlation; the number of degrees of freedom (df) = 1, Strong positive correlation (above +0.6), strong negative correlation (above -0.6) and neutral relation (zero).

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The soil analysis of Junnar tehsil shows that the pH of this soil is strongly positively related to EC and strongly negatively related to Mg and Na. Electric conductivity was shown to be positively related to potash and Ca and negatively related to Mg. Carbon was strongly positively related to the nitrogen and clay and neutral to; similarly nitrogen was neutral to CaCO₃. Available phosphorus was strongly negatively related to CaCO₃ and neutral to Ca and Mg. Also, potash was neutral to moisture and both densities and negatively related to Mg and Na. In the same way, calcium was strongly negatively related to Mg and Na and neutral to moisture. Sodium showed neutral relation with both densities. Magnesium was positively related to Na whereas CaCO₃ was also neutral to both densities. Sand had a neutral relation with moisture and negatively related to specific density. WH was positively related to porosity and negatively related to specific density. Lastly moisture showed positive relation to porosity while negative to both densities. TVC of this soil showed a strong positive correlation with clay and a neutral relation with pH, phosphorus and potash. This correlation can be visualized in figure 1.

116 3.2 Microbial analysis

All samples, which were analysed, contained various bacteria in varying population number
 and thus are dominant in the soil. In the soil samples studied here, they seem to be unevenly

spread. Any type of soil shows presence of bacteria but their population number varies and

120 is dependent on the soil texture and organic substrate in the soil. Some of the bacteria can 121 survive in unfavourable ecosystem due to their endospore forming ability. Few are also

known to withstand extreme conditions. Bacterial population is highly influenced by pH,

123 temperature, humidity, agriculture practice, fertilizers, pesticide, and the addition of organic

124 matter (Rao, 1994).

TVC of 634 X 10⁶cfu/ml was determined for the 18 samples collected from 4 villages of Junnar Tehsil. Total ten different bacterial genuses isolated from all the 18 soil samples were

127 Brocothrix, Bacteroidetes, Staphylococcus, Frankia, Kurthia, Streptomyces, Nocardia,

Actinobacter, Amphibacillusand Bacillus in the increasing order of the number of each genus

129 isolated (Fig. 2).

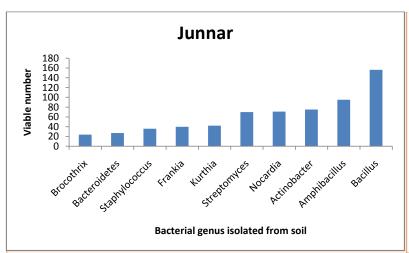


Fig. 2. Number of each bacterial isolate from all the samples

3.2.1Simpson's diversity index (SDI)

It was observed that the SDI was close to 1 which states that the diversity is high in all the soil studied here. The range of biogeochemical processes, the complexity of interactions, multifunctionality, and sustainability of the soil ecosystem has been impacted by the diversity of soil microbial communities and thus the soil quality [13]. Our results for soil bacterial composition coincide with those of a study[14], who gave a Global Atlas of the Dominant Bacteria Found in Soil.

Table 1: Simpson's diversity index

Bacterial Isolates	Total Number	Bacterial Isolates	
Brocothrix	24	Brocothrix	
Bacteroidetes	27	Bacteroidetes	
Staphylococcus	36	Staphylococcus	
Frankia	40	Frankia	
Kurthia	42	Kurthia	

143 3.2.2Richness

The number of species per sample is a measure of richness. The more species present in a sample, the 'richer' the sample. Accordingly the soil Junnar was lower in richness with only 10 different isolates (Fig. 2).

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3.2.3Evenness

- Evenness is a measure of the relative abundance of the different species making up the richness of an area. If a community dominated by one or two species, it is considered to be less diverse than the one in which several different species have a similar abundance. Thus, all samples showed unevenness because the total numbers of individuals in the sample are quite unevenly distributed between their respective isolates, dominated by Bacillus isolate.
- As species richness and evenness increase, diversity also increases. Simpson's Diversity Index takes into account both richness and evenness to measure the diversity. This compliment (1-D) represents the probability that two individuals randomly selected from a sample will belong to different species. Thus high diversity is observed in all the soils.

Table 2: Physico-chemical analysis of soil samples

Sam ple No.	Villag e	рН	EC	0	N	Ava il. P	Pota sh	Ca	Mg	Na	CaC O3	San d	Cla y	Silt	Moi stur e	Wate r Hold. Cap.	App Den	Sp. Den	Por osit y	TVC
			(M c/c m)	(%	(kg /ha)	(kg/ hc)	(kg/h c)	(%)	(%)	(%	(%)	(%)	(%)	(%)	(%)	(%)	(gm/ cc)	(gm/ cc)	(%)	(x10 ⁶ cf u/ml)
JK 1 Hulga (1st yr)	Khire shwar	5.9	0.1 5	0.7 6	53 2	13.5 7	188	55.4 8	35.5 6	8.2 1	2	55.2 5	21.3 2	23.3 3	12.2	56.49	2.14	1.15	57.6 7	23
JK 2 Varai(2nd yr) JK 3	Khire shwar	6.3 2	0.1 7	2.2	15 47	14.0 1	872.3 9	60.7 4	31.9 4	3.8	2.5	50.4 1	25.6 2	23.9	17.7 7	53.9	2.14	1.13	62.5 5	7
Khura sni 3rd yr)	Khire shwar	6.1 9	0.0	0.7 3	51 1	13.7 9	653.7 6	64.3 6	31.8 4	0.9 6	2.13	56.9	23.3 3	19.6 3	15.2 2	57.47	2.09	1.09	58.3 6	4
JK 4 Nach ani(4t h yr)	Khire shwar	5.8 2	0.0	1.0 6	74 2	14.2 4	356.9 8	54.1 6	39.2 7	5.0 7	1.88	67.0 1	18.3 4	14.5	11.1 7	52.86	2.1	1.16	55.5 2	3
JK 5 Fallo w)	Khire shwar	5.5 9	0.1 3	1.8 2	12 74	14.2 4	126.7 4	48.8 1	41.7 1	8.8 2	1.5	67.1 9	18.2 4	14.4 7	8.9	46.69	2	1.17	54.9 2	35
JA 1 Varai	Ambe	6.5 4	0.5 1	0.3 8	26 6	14.0 1	1158. 61	70.2 1	20	2.5 9	1.8	37.8 9	23.1 9	38.7 8	9.11	52	2.18	1.2	57.5	4
JA 2 Nach ani	Ambe	6.4 8	0.4 4	0.9 1	63 7	14.2 4	653.7 6	64.2 7	27.9 5	2.1	1.38	70.7 4	12.5 1	16.5 4	6.43	43.46	2.42	1.48	50.3 3	4

JP 4 Fallo w	Pimp arwa di	6.4 1	0.3 6	0.1 3	91	14.0 1	861.8 3	74.8 7	17.9 1	2.8 7	2.13	27.7 6	22.4 1	49.6 3	12.0 6	60.83	2.14	1.16	63.6 7	
JP 3 Sawa	Pimp arwa di	6.3 9	0.3 9	0.7 5	52 5	14.2 4	847.0 4	74.5 8	20.0 6	1.5 9	1.63	43.1 7	25.4	31.2 4	10.4 2	60.6	1.66	1.05	61.1 5	
JP 2 Khura sani	Pimp arwa di	6.7 7	0.4 5	1.3 8	96 6	14.0 1	1396. 24	76.0 6	7.9	1.1 4	2.63	54.2 7	28.3	17.2 5	10.9 8	57.18	1.96	1.07	59.5 2	
JP 1 Varai	Pimp arwa di	6.3	0.3 5	0.1 3	91	13.1 2	766.7 7	70.9 4	23.0 9	1.7 4	2.88	43.8 8	26.7	29.3	13.6	61.41	2.12	1.12	61.2 4	
JH 4 Fallo w	Hatw eez	6.0 2	0.3 2	1.2	84 0	14.2 4	321.0 7	73.7 8	22.4 6	2.1 3	2	26.2 6	25.1 8	48.4 6	11.9 3	60.6	2.11	1.12	60.4 4	
JH 3 Sawa	Hatw eez	6.1 8	0.3 6	1.3 4	93 8	13.7 9	203.8 4	74.9 6	22.2 3	1.8	2	37.5 6	32.1 1	30.2	11.6 8	59.69	2.13	1.13	59.9 7	
JH 2 Khura sani	Hatw eez	6.7 4	0.4 8	1.6 6	11 62	13.5 7	1417. 37	67.7 3	24.7 3	1.8 1	3.88	42.6 2	21.2 1	36.0 5	11.6 6	59.56	2.07	1.14	60.2 3	
JH 1 Varai	Hatw eez	6.3 3	0.3 6	2.5 9	18 13	14.2 4	739.3 1	72.8 9	20.7	2.0 9	1.5	59.7 2	20.2	19.9 7	13.8 6	55.33	1.82	1.07	57.9 5	
JA 5 Fallo w	Ambe	6.5 6	0.4 3	0.9 1	63 7	14.0 1	1168. 11	67.4 3	24.1 4	1.6 9	1.38	22.6 2	33.1 8	44.1	8.78	55.79	2.26	1.27	58.2	
JA 4 Khura sani	Ambe	6.3 2	0.4	0.7 5	52 5	14.2 4	650.5 9	74.5 7	18.4 8	2.8	1.8	38.0 7	27.1	34.7 3	8.38	57.85	2.14	1.15	59.7 6	
JA 3 Sawa	Ambe	6.5 5	0.4 2	0.1 3	91	13.5 7	1302. 25	70.1 2	22.9 1	1.5 2	2	56.3 7	22.3 1	21.2 6	10.1 9	56.64	2.17	1.2	59.6 9	

Sample	VIII	Bacill	Amphiba	Sta	Actinoba	Nocar	Streptom	Fran	Kurt	Broncho	Bacterio	Viable count(in triplicat	TVC (x10 6 cfu/
No UKA (Ukala)	Village	us	cillus	ph.	cter	dia	yces	kia	hia	thrix	des	e)	ml)
JK1(Hulg a) JK2(Varai	Khiresh war Khiresh	6	3	1	1	3	3	2	2	1	1	30+15+2 4	23
) JK3(Khur	war Khiresh	2	1	1	1	1	0	0	1	0	0	5+8+7	7
sani) JK4(Nach	war Khiresh	1	1	0	1	0	1	0	0	0	0	1+6+4	4
ani)	war)	1	1	0	0	0	1	0	0	0	0	3+1+5	3
JK5(Fallo w)	Khiresh war)	11	6	3	3	3	2	2	2	2	1	42+40+2 3	35
JA1(Varai) JA2(Nach	Ambe	3	1	0	0	0	1	0	0	0	0	7+1+4	4
ani)	Ambe	1	0	1	0	1	1	0	0	0	0	10+1+1	4
JA3Sawa JA4Khura	Ambe	1	0	0	0	1	1	0	0	0	0	5+3+0 78+75+3	3
sani	Ambe	22	5	5	4	6	6	3	4	4	4	6	63
JA5Fallo w	Ambe Hatwee	44	16	3	12	16	9	6	8	1	4	145+75+ 137 58+32+3	119
JH1Varai	Z	3	11	4	6	4	4	4	3	1	1	3	41
JH2Khura sani	Hatwee z Hatwee	2	1	0	1	2	1	0	0	0	0	9+8+4 49+21+4	7
JH3Sawa	Z	5	8	3	2	6	5	4	2	2	1	4	38
JH4Fallo w	Hatwee z	3	6	1	6	9	8	4	1	2	2	26+31+6 6	41

	Pimpar											59+82+1	1
JP1Varai JP2Khura	wadi Pimpar	19	9	6	12	10	9	8	4	3	4	12 12+23+2	84 <u>2</u>
sani	wadi Pimpar	4	4	1	3	3	2	1	1	0	0	2 109+75-	19 +
JP3Sawa	wadi	22	19	6	16	6	14	4	13	6	8	160	114
JP4Fallo	Pimpar											32+12+3	3
W	wadi	6	3	1	7	0	2	2	1	2	1	1	25

^{162 *}Fallow samples are highlighted yellow 163

Soils can be naturally acidic due to processes such as removal of base cations, microbial respiration, and production of organic acids. pH range of 5.5 to 7 is suitable for almost all crops. Fallowing of the land tends to reduce the soil pH and make it suitable for good yield. Same is observed from the table 2. In contrast, increasing soil pH with fallow period was reported by study[15]. In another study it was shown that the pH of the rhizosphere soil decrease with increase fallow length which may be due large root exudates of annual plants in different fallow land [16]. The cultivation of khursani and varai makes the soil more alkaline as compared to sawa, nachani and hulga. Thus fallowing such lands for more than 7 to 8 years would bring back the soil pH to normal.

Almost similar pattern was observed for all the crop harvested soil parameters when compared with the fallow soil. In case of Pimparwadi village, the fallow soil parameters and the TVC showed some deviation in its results. The TVC of fallow soil was less than crop harvested soil. The reason could be the fallowing period of the land which might be less than two years.

Pimparwadi village showed maximum presence of all the isolates in the soils collected after harvesting Varai and Sawa, while Ambe village soil collected after harvesting Khursani exhibited maximum presence of all isolates as seen in table 3. This shows that the crop cultivation did not influence the number and diversity of the bacteria in that soil.

3.3. Correlation Analysis

 A Pearson correlation coefficient was computed to assess the relationship between the various soil parameters and bacterial genus isolated from these soils.

In Junnar tehsil, there is a significant negative correlation between sand, soil apparent density and the bacterial viable count, whereas, a positive correlation exists between clay and the total bacterial count, along with dominance of Bacillus, Actinobacter, Amphibacillus, Nocardia and Streptomyces, during this period the crops cultivated are Hulga (Macrotyloma uniflorum, or horse gram) in the first year, followed by Varai (Panicummiliaceum, or proso millet) in the second year, Khurasani (Hyocyamusniger, Niger seed) in the third year, Nachani (Eleusinecorocana or African millet) in the fourth year, followed by fallow period.

Bacterial isolates Soil parameters	Bacillus	Amphibacillus	Staph.	Actinobacter	Nocardia	Streptomyces	Frankia	Kurthia	Bronchothrix	Bacteriodes
pH	0.104	0.016	0.079	0.112	0.047	0.001	0.083	0.07	0.169	0.015
EC	0.221	0.197	0.155	0.23	0.252	0.234	0.169	0.181	0.107	0.197
OC	0.201	0.089	0.021	-0.17	-0.035	0.184	0.099	0.072	-0.2	0.235
N	0.201	0.089	0.021	-0.17	-0.035	0.184	0.099	0.072	-0.2	0.235
Avail. P	0.021	0.157	0.021	0.006	-0.088	0.037	0.202	0.153	0.139	0.085
Potash	0.11	0.042	0.183	0.084	-0.025	0.096	0.186	0.034	0.265	0.042
Ca	0.139	0.315	0.294	0.396	0.276	0.416	0.343	0.252	0.337	0.33
Mg	0.143	0.247	0.216	0.329	-0.217	0.298	0.241	0.201	0.216	0.237

Na	-0.08	0.172	0.075	0.262	-0.202	0.246	0.146	0.131	0.018	0.161
CaCO3	0.271	0.326	0.215	-0.14	-0.145	0.208	0.126	0.304	0.205	0.225
Sand	- .492*	0.385	-0.19	- .489*	570*	- .498*	- .484*	0.332	0.353	0.418
Clay	.559*	.498*	0.348	0.441	.648**	.499*	.541*	0.424	0.299	0.411
Silt	0.377	0.268	0.088	0.425	0.438	0.411	0.375	0.234	0.315	0.348
Moisture	-0.31	0.092	0.082	0.016	-0.152	0.134	0.013	0.152	0.152	0.177
Water Hold.Cap.	0.186	0.329	0.26	0.466	0.334	.482*	0.449	0.297	0.417	0.403
App. Den.	0.041	- .541*	0.444	0.455	-0.011	0.418	0.201	- .517*	- .504*	0.444
Sp. Den.	0.058	0.279	-0.25	0.265	-0.023	0.254	0.209	0.218	-0.33	0.228
Porosity	0.13	0.213	0.194	0.39	0.17	0.291	0.288	0.226	0.342	0.292

Fig, 3. Pearson Correlation coefficient among soil parameters and the bacterial isolates; the values indicated in red are a negative correlation, and those in green are a positive correlation; the number of degrees of freedom (df) = 1, Strong positive correlation (above +0.5), strong negative correlation (above -0.5) and neutral relation (near zero).

All isolates showed a positive correlation with EC, Ca, clay, water holding capacity and porosity. They all exhibited a negative correlation with Mg, Na, apparent density, CaCO₃ and sand. Frankia was only bacterial isolate that had a positive relation with moisture and in the same way Bacillus solely showed a positive relation with specific density of soil.

The development of nutrients in the soil is mainly due to the biological transformations which are caused by soil microorganisms [17]. They have a significant influence on the soil function and thus can be used as soil quality indicators. The current research revealed that the bacterial populations in study area were significantly influenced by fallow age. Another study mentioned that the bacteria are less vulnerable to changes in soil and environmental conditions unlike fungi which are easily controlled by changes in soil pH, nutrient and harsh environmental conditions [18].

Similar study [19] was carried out recently to compare chemical soil quality parameters (soil pH, available P and K, organic carbon, and total nitrogen content) and soil enzymatic activity (dehydrogenase, acid phosphatase, alkaline phosphatase, urease, protease) in organic and conventional farming systems and determined that organic system was positively correlated (statistically significantly) with favorable soil pH, a higher content of organic C, and total N, and C/N ratio.

3.4 Principal Component Analysis

Agricultural data from different villages were subjected to principal component analysis (PCA) to find having similar overall patterns in the reduced multivariate data space without loss of information due to dimensionality reduction. All the statistical analysis was done in SPSS version 21.

From the Factor matrix of Varimax rotation from the PA extraction method shows that Na and Mg has the similar pattern with factor component of -0.496 and -0.505 in component 2 as shown in 2 dimensional figure of Junnar; Whereas, N and OC also showing similar Varimax rotation component of 0.287 and PH (0.815) and Potash 0.907 (Fig 4).

Component Plot in Rotated Space

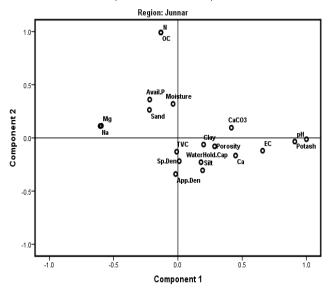


Fig. 4: PCA for JunnarTaluka

4. CONCLUSION

The results demonstrate significant differences between the soils studied, both regarding physico—chemical and biological parameters. Based on both these parameters it can be concluded that the length of the fallow period influences the soil health. The TVC obtained in the fallow soils was significantly higher than the crop harvested soils. Thus the land management systems should take to satisfy the needs of the cultivators by providing various alternatives for their sustainability. Few remedial measures suggested at the end can be considered to improve and protect the environment.

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COMPETING INTERESTS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

P. Vasudevan and R. G. Jaybhaye designed the study. P. Vasudevan conducted data gathering. P. Vasudevan and S. Panicker wrote the article.All authors read and approved the final manuscript.

CONSENT (WHEREEVER APPLICABLE)

Not Applicable

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

Not Applicable

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