Scientific Journal of Impact Factor (SJIF): 5.71

e-ISSN (O): 2348-4470
p-ISSN (P): 2348-6406

International Journal of Advance Engineering and Research Development

Volume 5, Issue 04, April -2018

ASSESSMENT OF SOIL ENZYME ACTIVITIES AND THEIR CORRELATION WITH BIOTIC AND ABIOTIC COMPONENTS IN COASTAL REGION OF GUJARAT

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Abstract - *This study evaluates soil chemical profile, bacterial diversity and a total number of halophytes from three regions, Southern Gulf of Kachchh (SGK) (A, B), South coastline (SC) (C, D) and Northern Gulf of Kachchh (NGK) (E, F). Soil enzymes related to C, N, and P were performed by the chemical and zymological method; while bacterial species were identified through 16s rRNA technique. Results showed that all the sites showed moderate to high alkaline conditions and sodic nature due to high concentration of sodium and chloride ions. The greatest bacterial and halophytes diversity were observed in soil sample of SGK followed by SC and NGK. Soil components and enzymes showed carbon and the associated enzymes positive correlation obtained at all sites ($P < 0.001$). Except the nitrate reductase soil N showed a positive correlation with protease and urease activity in soils ($P < 0.05$). The correlation PCA result observed total variance 74.48 % in biplot which indicates with B and D sites highesr, A and F moderate and E and F least nutrient components obtained from a different region. The correlation between enzymes and related nutrients components disclosed maximum positive correlation for B and D ($P < 0.001$), negative correlation for E and F and both correlation variability at C and A sites ($P < 0.001$, $P < 0.05$). These results indicate a moderate saline condition higher number of halophytes, significant enzymatic and microbial activities were found which essential for nutrient recycling process in the coastal ecosystem.*

Keywords: *Coastal soils, soil enzymes, dehydrogenase, alkaline phosphatase, bacterial diversity, conservation*

I. INTRODUCTION

Coastal habitat is an imperative ecological niche between terrestrial and marine empires, and high productive along with diverse natural conservation sites [1]. The coastal mangroves and associated environment plays a fascinating role in the environment. Mangroves, known as diverse vegetation of halophytes are an essential biotic component of the coastal ecosystem. Earlier in all 66 halophyte species belonging to 57 genera were reported from the Gujarat coast [2]. Soil, a major component of every terrestrial ecosystem, plays a pivotal functional role in carbon sequestration, nutrient recycling process, improving water quality, adjusting the floodwaters, recharging groundwater aquifers, protecting shorelines as well as keep enrich source of flora and fauna diversity [3-7]. India has a 7516.6 km long coastline covered by the variety of marine and coastal ecosystem where, 4660 sq. km is covered by diverse mangrove. Indian mangrove contributes around 2.7 % of the world existing mangrove forest area [8-9]. Indian coast covered by nine states, among them Gujarat covered one-third part around 1600 km of it. The Gujarat coastline is protected by two gulfs, namely Gulf of Kachchh and Gulf of Khambhat and south coast land. Gulf of Kachchh is a part of the western coast of India, facing the Arabian Sea and covers the majority of western coast mangroves [2, 9, 10]. According to DasGupta & Shaw, [2] around 77% of Gujarat mangroves exist in Gulf of Kachchh while the rest are located in Gulf of Khambhat. The coastal wetland functional diversity closely depends on biotic and abiotic components of habitat. Enzyme activities are found under the mangroves and bacterial diversity and it is an important labile fraction of nutrient recycling process and as a sink/source of plant nutrients [11-12].

In the last two decades, the interest in soil biodiversity and ecosystem functioning have become more and more important in ecological science. The prominence of soil enzymes has progressively expanded since the first report on soil enzymes about a century ago [13]. Soil physico-chemical parameters gave information about soil structure while, the halophytes, microbial diversity, and enzyme activity showed ecosystem functions. The microbial community expressed a functional diversity of soil as a result of species variability and gene expression within taxon and environmental effects on ecological interactions [14-15]. Earlier coastal salinity was reported as 4-30 % by the major contribution of sodium and chloride ions with halophytes diversity [12, 16]. The previous study reported adverse effect of salinity and climate condition on microbial biomass carbon and enzyme activities of soil in the coastal region of the Bay of Bengal, Sundarbans, India [11]. Under the plants and microbial action, enzymes showed organic matter degradation, mineralization, and nutrient recycling processes by oxidation, reduction and hydrolysis reaction and released free formed substrate for plants and microbes as a source of nutrients [17-21]. Soil dehydrogenase reflects the total oxidative activity of the microbial biomass and is involved in central

aspects of metabolism by providing glucose as an essential energy source of microbes [22]. Therefore, determination of dehydrogenase and other carbon associated enzymes activity has been suggested as a good indicator of soil quality [23]. Urease is the most prominent enzyme of N cycling, catalyzes the hydrolysis of urea into ammonium ion depending on soil pH [24]. Under the soil acidic or alkaline pH condition phosphatase plays an important role in transforming organic to inorganic phosphorous form for plants [25-26]. Under the controlled condition, influenced of salinity was observed on soil enzyme activities [16] detected that, salinity increase owing to salt water ingression decrease microbial biomass carbon and enzyme activities [27]. However, in India lesser is known about soil microbial and enzyme activities in the coastal region. Although coastal ecosystem associated with numerous environmental functions, its microbial and enzymatic correlation study was not found for coastal region of Gujarat. Coastal diversity is threatened by anthropogenic and natural disasters activities [1, 6].

To known the ecological importance of Gujarat coasl and the status of biotic and abiotic factors, the present study deals with the determination of enzyme activities associated with carbon, nitrogen, phosphorous content along with redox potential at various coastal sites of Gujarat. The study also evaluates high selected fraction of microbial communities and a total number of halophytes species at all three regions. In addition, statistical analysis for intercorrelation between soil nutrients and bacterial distribution with enzymes were performed.

II. MATERIAL AND METHODS

2.1.Study site

In the present study two major parts of Gujarat coastline (20° 00’ - 24°45' N, 68° 00’ - 73° 30' E) include some of the sites of Gulf of Kachchh (GK), further divided into southern (SGK) and northern (NGK) and Saurashtra coastal (SC) regions were covered. Gulf of Kachchh region is renowned for high tides on a regular basis and Saurashtra coast is less indented and moderately straight. The Gulf of Kachchh is highly productive region covered by the marine national park, coral reef, mudflat, and mangroves diversity [8, 28]. Whereas South coast covered sandy beaches, numerous spits, bars, marshes and small estuaries predominates. Gujarat has maintained different average annual rainfall for Jamnagar 600 to 800 mm, Saurashtra 400 to 800 mm, and in Kachchh less than 400 mm. The alteration in rainfall pattern is responsible for the distinct vegetation in the Gulf of Kachchh (NWAG 2010). The study map Fig. 1 showed the sampling sites of the coastal area of Gulf of Kachchh and Saurashtra coastal region.

2.2 Substrate sample collection

In early pre-monsoon period of 2014, soil samples were collected by random sampling and a total number of halophytes were counted in surrounded one-meter area. Further, two composites were prepared from each region. Samples were encoded with A (Narara, Sikka, Salaya) and B (Dwarka, Okha) for southern Gulf of Kachchh (SGK), C (Veraval, Somnath, Lati) and D (Muldwarka. Tad, Diu) for Saurashtra coastal land (SC), and E (Kashibeach, Mandvi) and F (Mundra, Gangeshwar beach) for northern Gulf of Kachchh (NGK) regions. Then, the collected samples were sieved (2 mm) and some of them were stored in a refrigerator at 4°C for enzyme activities; sub-sample were stored at -20°C for microbial analysis, and remaining samples were air-dried at room temperature for physicochemical analysis.

2.3 Soil physic-chemical properties

Soil pH was measured in triplicate manner using 0.01M calcium chloride solution and the free ion capacity was evaluated using 0.01 M KCl in a 1:2; soil: solution ratio. The gravimetric method was used for the water holding capacity (WHC) [29]. For the chemical examination, soil organic carbon (SOC) was estimated by the colorimetric method and organic matter was

calculated by Van Bemmelen factor [30]. The nitrogen content of the soil was estimated by analysis of nitrate and ammonia in soil by phenoldisulphonic [31] and Nesslerization spectrophotometric method respectively [32]. Soil phosphorus content was determined by Olsen [33] and available sulfur was estimated by turbidometric method [34]. Soil Ca, Mg and chloride ions were determined by the titrimetric method and Fe content was measured by the colorimetric method [35]. Acid digested soil samples were estimated for zinc (Zn), and copper (Cu) micronutrient using atomic absorption spectroscopy (AAS) and available sodium (Na), potassium (K) were analyzed by flame photometry [36].

2.4 Enzyme activities

Various enzyme activities were calculated associated with a different reaction such as redox potential (catalase), cycling of carbon (amylase, invertase, dehydrogenase, phenol oxidase, and CM-cellulase), nitrogen (protease, urease, and nitrate reductase) and phosphorus (alkaline phosphatase) contents. Catalase activity measured by potassium permanganate titration method [17]. Soil amylase, cellulase and invertase activities were measured using starch, sucrose and CM-cellulose as substrates by Guan et al. method [37]. Phenoloxidase activity was analyzed by L-DOPA (L-3, 4-dihydroxy phenylalanine) 10 $mmolL^{-1}$ substrate utilization method [38]. Biological activity in soil sample was identified by dehydrogenase enzyme, and the assay was performed using 0.5% of triphenyl tetrazolium chloride (TTC), which reduced under 24 h incubation at 37 ℃ into a triphenyl tetrazolium formazan and measured at 485 nm [38].

For nitrogen recycling process, protease enzyme activity was identified by catalysis of proteins to polypeptides and amino acids, which further hydrolyzed in ammonia and were estimated by FC reagent at 700 nm [39]. Urease activity was measured by hydrolysis of urea to ammonia under overnight incubation and liberated ammonia was estimated by indophenol method [40]. In nitrate reductase activity liberated nitrate was measured in KCl solution (4 $molL^{-1}$) by the spectrophotometric method at 520 nm [23]. Alkaline phosphatase activity was measured using a *p*-nitrophenyl substrate (*p*NP) with Modified universal buffer (MUB) solution at pH 11.5 and released *p*NP was measured by colorimetrically at 400 nm [20, 25, 26].

2.5 Soil microbial analysis

Soil microbial diversity was assessed using serial dilution technique on Zobel marine agar (HM 2216) media. Further, individual bacterial species were isolated for molecular identification. The bacterial DNA isolation, DNeasy kit (Qiagen) was used for extraction of DNA from the pure culture according to manufacturer's specifications. The 16S rRNA gene fragment from each isolate was amplified by PCR using 8F and 149R primers [41-42]. The PCR process was carried out by taking 50 µl reaction mixture with 2 units of Taq DNA polymerase. Denaturation process was followed at 95 ºC for 3 min; followed by 30 cycles at 95 ºC for 30 s; 50 ºC for 30 s and 72 ºC for 1 min, with a final extension at 72 ºC for 7 min using thermal cycler. Further, amplified sequence was identified by agarose gel electrophoresis and conserved sequence identified [42].

2.5.1 Phylogenetic analysis

The acquired 16S rRNA gene sequence was aligned against representative reference sequence of the most related members, obtained from the National Centre for Biotechnology Information (NCBI) database, by using of BLAST software. The evolutionary distances were computed using the Maximum Composite Likelihood method [43]. Phylogenetic dendrograms were constructed with inferring the Neighbor-Joining method [44]. Evolutionary analyses were conducted in MEGA7 [45] software.

2.6 Statistical analyses

Statistical analysis was performed using OriginPro 8.1 and XLSTAT software. The data were subjected to variance (ANOVA) using Graphpad prism. The means and standard deviations were calculated in a triplicate manner. The relationship between enzyme activities and environmental variables as well as bacterial diversity distribution at various sites were analyzed using correlation analysis and principal component analysis (PCA). Statistical significance was determined at $P \leq 0.05$.

III. RESULTS

3.1 Soil chemical profile

There was no statistically significant ($P < 0.05$) variation in average soil pH of all sites. The all physic-chemical parameters were mentioned in Table 1. Soil collected from different sites exhibited a range of pH from 7.75 to 8.42. The average EC of soils varied widely from 2.17 to 7.43 mS/cm and the mean WHC varied from 23.01 to 51.04 %. SOC and OM showed significant ($P < 0.05$, $r = 0.8309$) variability at all sites . Soil N in the form of nitrate and ammonia showed non significant variation ($P < 0.05$) and higher conent of NH_4-N was detected at major sites. All sites observed nonsignificant variation for P and S varied from 26.19 to 48.79 mg/kg; P, and 150 to 582.6 mg/kg S. Higher content of the soil S was observed in compared

to other components. All sites showed a high concentration of sodium and chloride ions and low concentration of K, Ca and Mg ions (Table 1). For the cations and anions all sites nonsignificant variation observed. Statistically analyzed correlation results showed that sodium ion maximum positive correlation and highest significanct variation obtained with all analyzed parameters ($P < 0.001$), whereas sulfur showed positive correlation at minimum significant level with carbon, organic matter, Ca, and Mg mineral ions ($P < 0.05$). No significant variation was observed in remaining parameters. ANOVA result for chemical profile exhibited P-value for sites variation is 0.6698, which indicate least significant variation.

Table 1. The chemical properties of the soil samples (mean ± sem) from the southern and northern Gulf of Kachchh and Saurashtra coastal land.

			MACRO NUTRIENT					
	pH $CaCl_2$	**EC mS/cm**	**OC %**	**OM %**	**Nitrogen**		**Phosphorous mg/kg**	**Sulphur mg/kg**
					Nitrate (NO_3^-N) mg/kg	**Ammonia (NH_4^+N) mg/kg**		
A	7.75 ± 0.12	7.43 ± 2.47	3.29 ± 0.92	4.89 ± 0.47	31.79 ± 3.24	71.23 ± 3.84	27.53 ± 3.14	447.5 ± 3.56
B	7.91 ± 0.25	2.68 ± 0.89	6.71 ± 0.76	11.32 ± 0.95	47.78 ± 2.54	62.2 ± 2.63	53.74 ± 2.96	582.6 ± 2.12
C	7.82 ± 0.18	2.17 ± 0.39	1.08 ± 0.54	1.03 ± 0.26	56.23 ± 7.41	48.96 ± 1.42	39.37 ± 1.89	150.00 ± 0.07
D	7.88 ± 0.09	7.37 ± 2.45	7.02 ± 0.18	13.16 ± 1.18	39.75 ± 4.44	75.78 ± 4.0	45.97 ± 1.03	487.52 ± 2.4
E	8.42 ± 0.32	7.24 ± 2.41	0.59 ± 0.26	0.83 ± 0.19	35.04 ± 1.89	39.52 ± 3.71	44.55 ± 2.14	265.11 ± 1.56
F	8.20 ± 0.14	5.03 ± 1.67	4.34 ± 1.37	5.35 ± 0.65	49.17 ± 3.27	41.4 ± 2.22	50.47 ± 1.46	152.5 ± 2.74

MICRONUTITRIENT				
Na mg/kg soil	**K mg/kg soil**	**Cl meq/100 g soil**	**Ca meq/100 g soil**	**Mg meq/100 g soil**
1052.80 ± 12.24	9.92 ± 2.314	22.46 ± 1.06	6.00 ± 0.19	0.85 ± 0.04
1473.40 ± 13.56	11.04 ± 1.867	57.46 ± 1.66	22.303 ± 0.90	3.91 ± 0.95
479.40 ± 8.422	7.20 ± 2.004	2.75 ± 0.65	1	0.27 ± 0.05
1409.80 ± 10.03	12.61 ± 1.967	24.60 ± 3.5	7.55 ± 0.55	0.80 ± 0.4
415.6 ± 9.54	5.22 ± 1.008	11.26 ± 0.73	1.35 ± 0.15	0.56 ± 0.23
492 ± 6.33	5.61 ± 2.158	8.23 ± 0.73	1.55 ± 0.05	0.25 ± 0.15

3.2 Halophytes diversity

The study sites showed marshy, rocky, muddy, and sandy habitat variability in soil structure. More vegetation was found on marshy and muddy sites in compared to sandy and rocky sites. Different halophytes species include *Suaeda maritime* (L.) Dumort., *Avicennia marina* (Forrssk.) Vierh., *Prosopis chilensis* (Mollina.) Stunz, *Heliotropium curassavicum* L., *Salicornia brachiata* Roxb., *Sesuvium portulacastrum*, (L.) Linn., *Suaeda vermiculata* Forssk.ex J, F.Gmel., *Salvadora persica* Linn., *Limonium stocksii*, (Boiss.) and *Convolvulus microphyllous* Siber ex Spreng were identified from the study area. The regions showed total number of 33, 21, and 14 halophytes from SGK, SC and NGK regions respectively.

3.2 Enzyme activities

That average activity of catalase enzyme is presented in Fig. 2, which indicate nonsignificant variation at all sites. The soil C was positively correlated with amylase ($r = 0.600$, $P < 0.01$), cellulase ($r = 0.829$, $P < 0.01$), invertase ($r = 0.629$, $P < 0.01$), dehydrogenase ($r = 0.687$, $P < 0.01$) and negatively correlated with phenoloxidase ($r = -0.481$) Fig.3. The soil nitrogen was positively correlated with urease ($r = 0.600$, $P < 0.01$) and protease ($r = 0.95$, $P < 0.01$) but negatively correlated with nitrate reductase (-0.431) Fig. 4. Correlation data of phosphorous with alkaline phosphatease exposed very less positive relation ($r = 0.123$, $P < 0.05$) (Fig.5, Table 2).

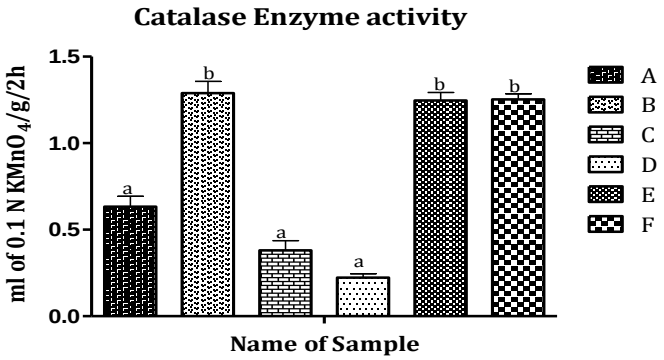


Figure. 2. Catalase enzyme activity among all three regions sites. Small alphabet letter shows no significance difference between all sites.

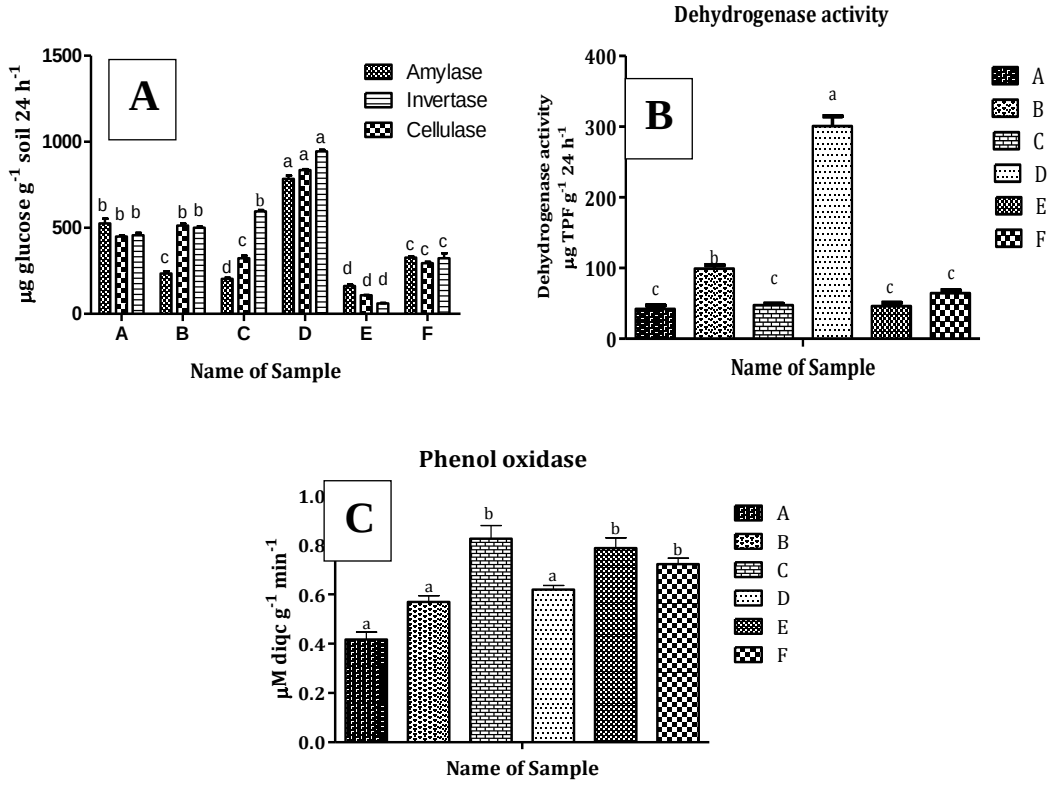


Figure 3. Carbon cycling enzymes amylase, invertase, CM-cellulase (A), dehydrogenase (B), and phenol oxidase (C) activity among all sites. G-glucose concentration, TPF: triphenyl formazan released from 2,3,5 triphenyl tetrazolium chloride, diqc:3, dihydroindole 5-6, quinone-2-carboxylate liberated substrate from L-DOPA. The lower case indicates the non-significant difference.

Table 2. Correlation between carbon, nitrogen, and phosphorus with associated enzymes.

	Amylase	Invertase	Cellulase	Dehydrogenase	Phenol oxidase	Protease	Urease	Nitrate reductase	Alkaline phosphatase
Carbon	0.600**	0.629**	0.829**	0.687**	-0.481	0.474*	0.685**	-0.567	0.878**
Nitrogen	0.807**	0.764**	0.886**	0.599**	-0.746	0.950**	0.6**	-0.431	0.862**
Phosphorus	-0.240	-0.021	0.061	0.284	0.365*	-0.443	0.338*	-0.105	0.123*

* P < 0.05, **P < 0.01 showed significant variance and ns: not significant.

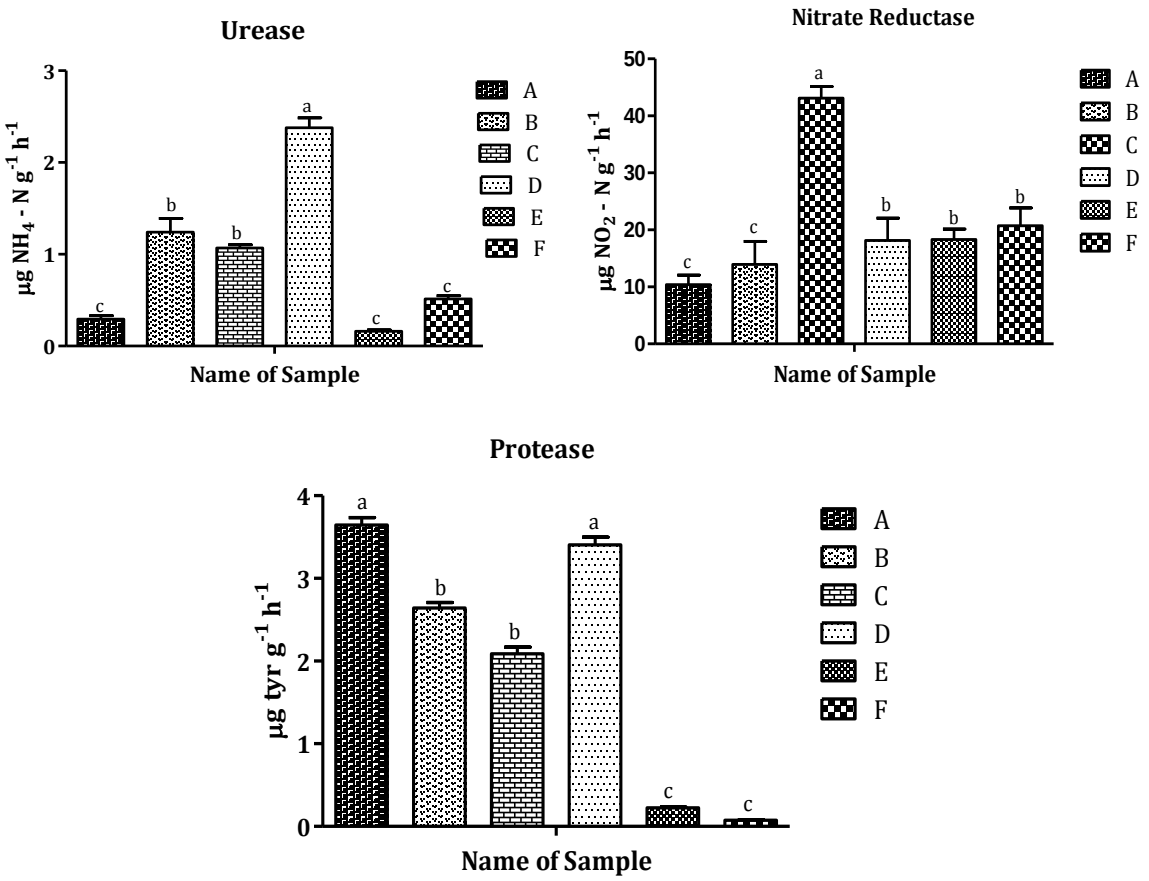


Figure 4. Nitrogen cycle associated enzymes urease, nitrate reductase and protease showed non-significant variation between sites which mentioned in alphabet small lower case. Tyr: tyrosine, ammonia-nitrogen, and nitrate estimated concentration reported in the graph.

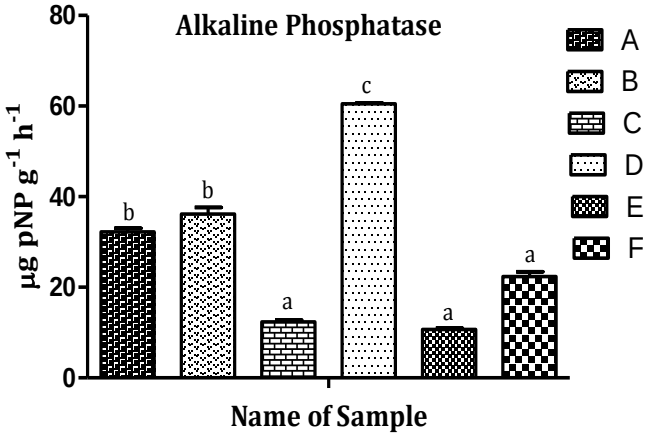


Figure 5. Alkaline phosphatase activities between all sites showed non-significant variations by lower case. pNP: p-nitrophenol liberated concentration measured.

3.3 Soil microbial diversity

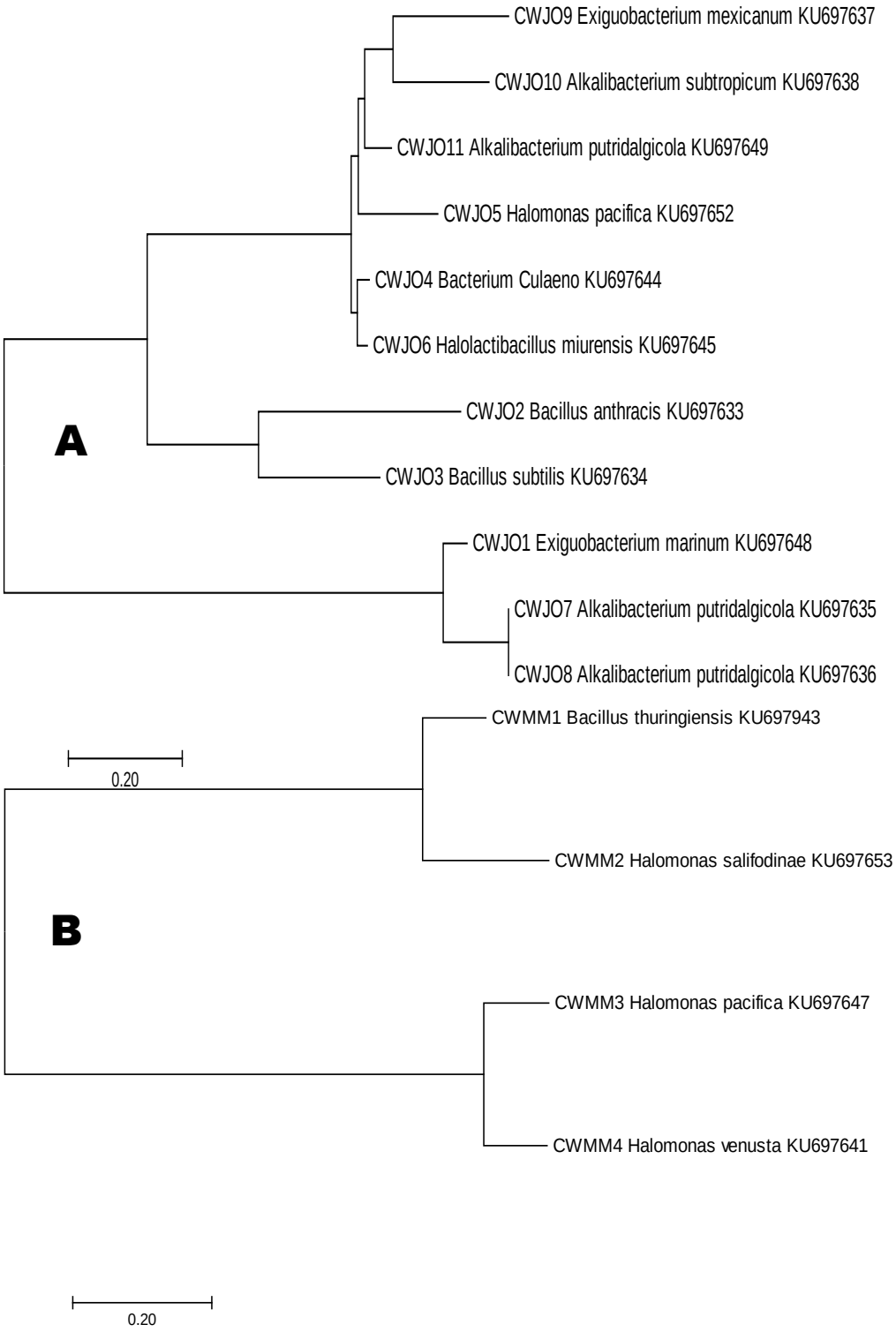
The bacterial species observed were Halophiles, Alkaliphiles, Bacillus, Extremophiles, Exigobacterium, and Salinicoccus group of diversity. Identified bacterial strains using 16S rRNA partial sequence displayed percentage similarity using BLAST software with those of environmental clones or known species in the NCBI database Fig 6 (A, B, & C); Table 3. The branch length value of optimal trees obtained for SGK, NGK and SC regions were 2.977, 2.193 and 1.748 respectively. The tree was drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. For the evolutionary distance, a major number of the sequence involved in 11, 6, and 4 nucleotides for SGK, SC, and NGK regions. For whole process position 1st+2nd+3rd+Noncoding involved for nucleotide database analysis. Final data sets positions obtained for SGK, SC, and NGK were 269, 501, and 427 respectively; missing data and positions were eliminated throughout the analysis.

3.4 Bacterial community distribution in relation to environmental variables

In the PCA study, the first two axes (principle components) explained 77.78 % (54.75% + 22.99%) of the variability in community data (Fig. 7). The PCA ordination of the alkaliphiles, exigobacterium, bacterial community, halophytes and environmental variables OM, OC, NH_4-N, and S demonstrated that strongly correlated with first PCA axis, whereas extremophiles, salinococcus, halophiles, and bacillus were the most correlated with the second axis. The P and NO_3-N had weak correlation with second axis and very weak correlation with first axis. The distribution was mainly related to first axis variables.

Table 3. Bacterial 16S r RNA gene sequence data obtained from the coastal regions.

CWJO	Accession ID	% identification	CWMM	Accession ID	% identification	CWVV	Accession ID	% identification
CWJO-1	KU697648	99%	CWMM-1	KU697643	99%	CWVV-1	KU697642	99%
CWJO-2	KU697633	86%	CWMM-2	KU697653	99%	CWVV-2	KU697639	99%
CWJO-3	KU697634	85%	CWMM-3	KU697647	100 %	CWVV-3	KU697650	97%
CWJO-4	KU697644	100%	CWMM-4	KU697641	86%	CWVV-4	KU697651	96%
CWJO-5	KU697652	99%				CWVV-5	KU697640	86%
CWJO-6	KU697645	99%				CWVV-6	KU697646	99%
CWJO-7	KU697635	99%						
CWJO-8	KU697636	99%						
CWJO-9	KU697637	88%						
CWJO-10	KU697638	89%						
CWJO-11	KU697649	99%						



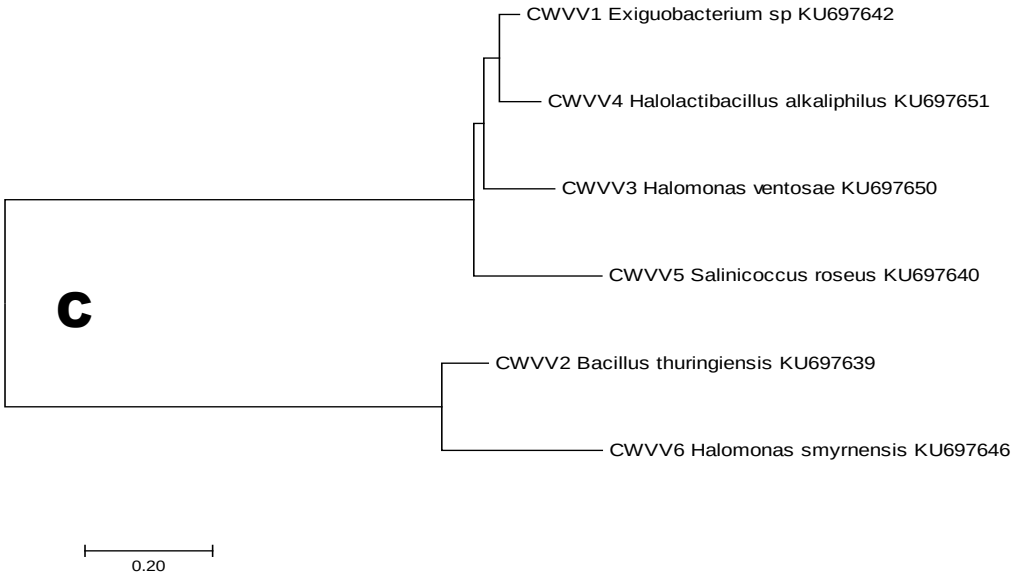


Figure 6. A, B, C represent the phylogenetic relationship of the 16S rRNA gene sequence of SGK, NGK, and SC regions. The evolutionary history was inferred from the Neighbor-Joining method. Partial sequence was analyzed by 16S rRNA technique and evolutionary analysis conducted by MEGA 7 software.

3.5 Principle components analysis (PCA) of nutrient and enzyme data

The PCA showed that nutrients profile and enzyme activities of various sites were different to each other (Fig.9). The first two PCs (PC1 and PC2) explained 55.21% and 19.27% of the variance in the nutrients and enzymes datasets. The largest loadings on the PC1 were SOC (0.851), SOM (0.915), NH_4-N (0.924), available S (0.883), total S (0.832) chemical components, and amylase (0.829), cellulase (0.960), invertase (0.784), dehydrogenase (0.804), protease (0.786), urease (0.762), alkaline phosphatase (0.982) enzymes whereas on the PC2 axis were nitrate (0.581), inorganic phosphorous (0.979), total phosphorous (0.985) chemical components, and catalase (0.459), and phenol oxidase (0.485) enzymes.

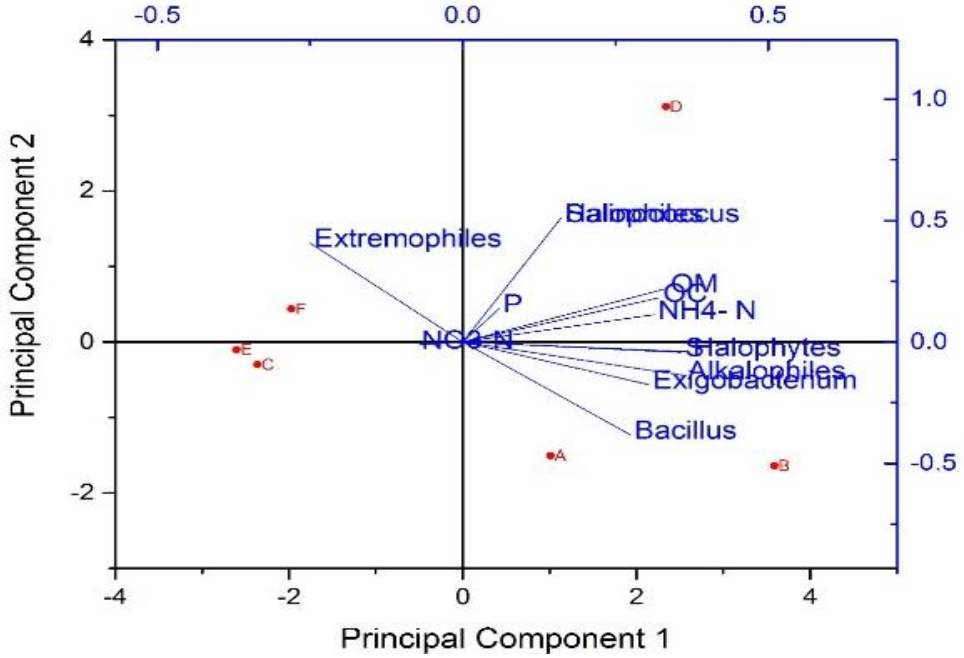


Figure 7. PCA of microbial group diversity data from all coastal samples. PC1 and PC2 accounted 54.75 % and 22.99 % of the total variance (77.78 %), respectively. Bacterial communities and nutrients distribution were analyzed at all sites.

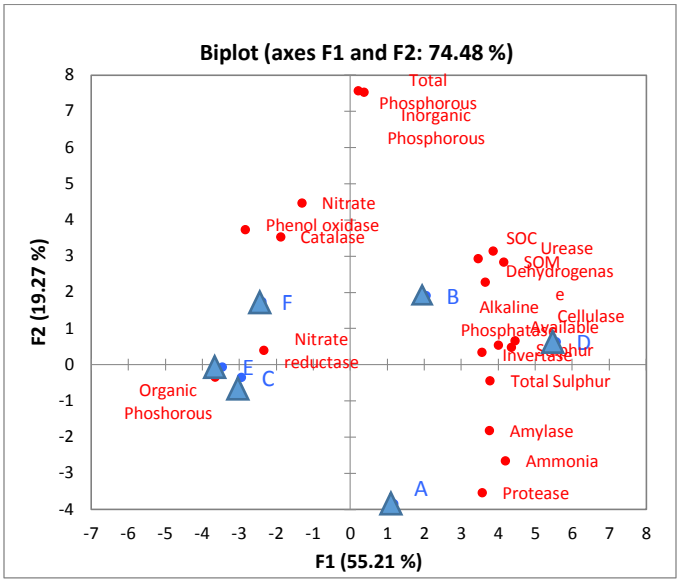


Figure 8. Sites-environment biplot from the PCA analysis summarize differences in nutrients and associated enzymes. F1 and F2 accounted for 55.21 % and 19.27 % of the variance, respectively. Soil samples were tested from different sites composites, which covered three regions of Gujarat coastland.

IV DISCUSSION

4.1 Variability in soil physico-chemical profile

All the sites followed moderate to high alkaline conditions. The soils displayed provisionally flexible salinity and the dominant cation and anion were sodium and chloride respectively. Less amount of other ions were observed at all sites. These results are in alignment with the Indian coastal soil results [27]. According to Satyanarayana D. et al. [46], organic carbon and organic matter percentage of coastal habitat depends upon the type of vegetation prevalent in the study area. Here we found lower percentage of OC and OM in NGK region due to the higher salinity effect. High salt concentration caused an adverse effect on soil and responsible for low vegetation production [46]. The nitrogen content showed varied in the concentration of nitrate and ammonical nitrogen from each site which indicates significant denitrification process at all sites. Different concentration of ammonical nitrogen indicates atmospheric nitrogen fixation by the bacterial and vegetation of sites [47-48]. In comparison to other components, weak mobility and lower utilization of soil phosphorous was found due to alkaline pH. Thus, soil phosphorous which is directly linked with poor degradation of organic matter is obtained by microbes in the coastal environment. This result was consistent with the observations reported by Satyanarayana et al., [46]. The effect of river vehicular pollution and low oxidation rate of sulfur ion in this area may be considered the reason for high sulfur concentration in coastal soil [49-50].

4.2 Effects of bacteria and halophytes

Our data indicates a great cultivable bacterial diversity and variable halpophytes species from a coastal region of Gujarat. Fig 6 indicates major proportion diversity covered by halophiles and alkaliphiles, while Bacillus, Exigobacterium, and Extremophiles covered less portion. Soil enzyme activities detected at all sites could be those immobilized in the soil colloids and originating from the surviving salt-tolerant plants and microbial species [12]. Based on correlation study between the substrate and associated enzyme activities significant result of carbon and associated enzymes persisted due to the active participation of halophytes and soil microbial diversity [51]. Our findings support this statement and showed a large number of mangrove species and bacterial diversity showed significant enzyme activity at site A, B and D in comparison to C, E, and F sites. Microbes utilize carbon substrate as a source of energy; while halophytes involved in degradation process of polyaromatic hydrocarbon made the recycling process in a continuouse manner [18, 53-55]. Although the atmosphere is having higher nitrogen content, it could not be uptake directly. The soil is indispensable medium to support the nitrogen mineralization process and plant roots and associated microbes fix it from atmosphere. Here we obtained urease activity mainly origined from plants as well as microbes found as both intra and extra cellular and degrade urea into ammonia and CO_2 [24]. Protease, plays a vital role in a mineralization process and regulating the amount of plant available N and plant growth. In a soil it associated with inorganic and organic soil colloids [14, 39]. Nitrate reductase activity is mainly performed by soil microbes and they have ability to convert nitrate in to ammonia in a soluble form for plants and other living components. The activity support our results, where urease activity found due to the plants and microbes extra and

intracellular enzymes; while higher tyrosine concentration showed supportive action of plant and bacterial diversity at sites and for nitrate reductase nitrogen fixers may be responsible. Results showed the bilogical capacity of soil for enzyme action as well as have an important role in the microbial ecology in the coastal ecosystem [55-56]. In addition, nonsignificant activities of all enzymes could be associated with the osmotic potential of soils due to high salt concentrations and salt out effects of soluble salts on enzyme protein. In unfavorable environmental condition, microbes and plants could not perform a substantial metabolic activity [12, 58].

4.3 Effects of soil enzymes in relation to carbon, nitrogen and phosphorous substrate

Soil components are closely associated with soil enzymes in a biochemical transformation process of nutrient cycling. However, their distribution and soil enzymes activities in the coast land environment have been lesser described. In our study catalase enzyme showed non significant activity at all sites. This intracellular activity found due to the presence of anaerobic and aerobic bacteria mainly halophiles, and alkaliphiles showed redox potential activity [58]. It involved in cell protecting action from damage caused by reactive oxygen species. This study displayed that catalase activities did not significantly impact at all sites. The catalase activities of each site represented negative correlation with carbon and nitrogen content. This activity was identified based on soil samples ability to break down hydrogen peroxide in the presence of potassium permanganate [59]. Phosphorous content showed slightly positive correlation with the catalase activities at all sites. We suspected that the catalase enzyme activities were not dependent on other abiotic components of the coastal environment and soil higher concentration of sodium and chloride ions may also be responsible for the non-significant activity [16, 57]. Carbon associated enzymes showed significant activities in the soil-plant environment. Amylase activity is widely distributed in soil and plays a significant role in the breakdown of starch and its conversion to oligosaccharides. The enzymes productive activity by soil indicates directly supplying enzymes from their residues or indirectly providing substrates for the synthetic activities of microorganisms [60]. Cellulase activities in the soil samples is mainly a result of catalysed degradation of cellulose and polysaccharides to release reduced sugars as an end product by the microorganisms. Invertase enzyme showed similar activities like amylase and cellulase in the soil samples. It is mainly involved in breakdown reaction in which the catalysis of the hydrolysis of sucrose to glucose and fructose has been broadly studied because of its widespread and distributed in soil microorganisms. In our study, biplot analyzed data showed a positive correlation of carbon with amylase, cellulase, and invertase enzymes activities (Fig. 8). The soil environment microbial activities and vegetation may responsible for it and they utilized soil carbon as a major source of nutrient for energy [61].

Soil dehydrogenase activity is mainly associated with C–cycling in electron transformation process. This enzyme activity occurs as an integral part of all viable microbial cells but does not occur extracellularly in soil [17]. This enzyme oxidizes soil organic matter by transferring protons and electrons from substrate to acceptor. It is involved in respiration process of soil microorganisms and are closely related to the type of soil and moisture content of soil [51]. However, in this study dehydrogenase activity showed a positive correlation with the carbon content of soil. This was probably related to the higher amount of oxygen molecules uptake by the sand soil for respiration process and oxidized carbon source for energy through soil microbial activity. It is directly linked with the biological activity of soil [22]. In a soil phenol oxidase enzymes, activity shows catalysis and oxidation of lignin, phenolic, and other aromatic compounds. This enzyme is excreted by soil microorganisms, and the catalytic process was carried out in a soil environment. The potential activity was measured by the rate of oxidation of a L-DOPA substrate into red coloured compound 2-carboxy-2,3-dihydroinole-5,6-quinone using oxygen as the final electron acceptor [53, 62]. Oxidation rate for phenoloxidase substrates is strongly dependent on pH and structure of the soil. Here, the negative correlation of phenol oxidase and carbon content indicate the concentration of hydrogen ions had a prominent effect on the rate of oxidation, assay neutral and slightly acidic pH, as well as an unfavorable condition for degradation of phenolic compounds [51, 53]. This result hypothesizes that the enzyme activities of cellulase, invertase, amylase, dehydrogenase and phenol oxidase (Table 2) were found due to the presence of soil carbon source, organic matters and microbial diversity metabolic action [51].

Nitrate reductase enzyme plays a key role in nutrient recycling process of nitrogen content. It is involved in denitrification process in which dissimilatory nitrate reductase catalyzes the first step of denitrification by reducing nitrate to nitrite [63]. In our study results predicted that nitrogen content of the soil was not showing the significant activity of nitrate reductase. It depends upon soil microbial diversity, the moisture content of soil, and vegetation biomass of particular environment. Lacking nitrifying bacterial presence as well as unfavorable abiotic components are responsible for it [63-64]. In urease activity, microorganisms catalysed urea into ammonia and carbon dioxide and are widely distributed in the soil environment. It was interesting that in a present study urease enzyme showed a positive correlation with soil nitrogen content which indicates the presence of nitrogen-fixing bacteria in a coastal soil environment. According to Houtl and McGarity (1986), on-site vegetation also may help in a urease activity of soil [24]. Protease, the nitrogen cycling enzyme is mainly involved in the catalysis of protein molecules. Here, casein molecules catalyzed by soil heterotrophic bacteria, fungi, and actinomycetes into tyrosine. In our investigation protease activity, positive correlation with nitrogen content indicates anaerobic and

heterotrophic microbial diversity presented significant activity at all sites. The vegetative plant roots and associated microflora also contribute active participation in catalysis process [39, 52]. Soil alkaline phosphatase activity, as measured here with an artificial substrate (*p*-nitrophenyl Phosphate), in which inorganic phosphate released from organic matter. Soil pH plays an essential role for soil phosphatase activity. This activity derived from the higher plants root, fungi, phosphate solubiliser bacteria, and some soil micro fauna diversity [55](Olander and Vitousek 2000). Soil phosphorous content and alkaline phosphatase significant negative correlation indicate lower microbial biomass and reducing activity of phosphatase activity [26, 52, 54, 63].

V. CONCLUSION

The study gave insights on close relationship of biotic and abiotic components with soil enzymes activity describing the coastal ecological importance. We employed soil enzyme activities as good indicators of soil quality for coastal region. Results showed that under environmentally favourable conditions plant and bacteria secrete extra and intracellular enzyme and significant activity is seed at various sites. Our findings showed that high alkaline condition, low moisture content and low plant and bacterial diversity did not support sufficient enzymes activity. It is essential to conserve coastal ecosystem because halophytes species and associated microbial diversity are the potent driving factors for organic matter decomposition and nutrients transformation in a coastal ecosystem. Overall, our findings and recommendations suggest suitable sources (biotic and abiotic) of coastal habitat are necessary to be evaluated for conservation and sustainable development. The study provides the first insight into the bacterial communities and soil enzyme activities on Gujarat coastalecosystem.

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