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Research Article
Effective Stream Health Assessment: Soil Microbes Versus Aquatic Insects

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Abstract

Objective: The present study was examined the reliability of stream soil bacteria and aquatic insect communities as indicators of stream health in three streams located in catchments ranging from pristine to impacted streams. **Methodology:** The 16S rDNA analysis was used to characterize the bacterial communities in stream soil and for comparison aquatic insects were collected. **Results:** Individual rarefaction analysis showed a significant relationship between the richness of bacterial taxa and sampling sites. In contrast, the perplexed result observed in aquatic insects that human impacted site had low rarefaction value. The differences in the evenness of bacterial and aquatic insect communities were detected by similarity indices. Unlike to aquatic insects, seasonality was not influenced the bacterial communities. We could identify the key factors influencing bacterial and aquatic insect assemblages by correspondence analysis. The result of indicator species analysis (IndVal) for each species of soil bacteria and aquatic insects indicates that an unimpacted site had the highest value. **Conclusion:** These findings highlight that the stream soil bacterial and aquatic insect communities respond differently to anthropogenic impacts and the assessment of stream soil bacteria provides an alternative indicators of stream health with less effort.

Key words: Water quality, indicator species, soil microbes, aquatic insects

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Freshwaters can be assessed by physical, chemical and biological attributes. The number and types of organisms that inhabit in water are the significant indicators of water quality and their sensitivity or survival varied with physical and chemical attributes. Water quality assessment using biological measures are fast, efficient and cost effective. The integrity of stream can be assessed by using indicators of algae and other plants[1], macroinvertebrates[2], fishes[3], microbes[4] and litter decomposition[5].

Aquatic macroinvertebrate communities have been frequently used as environmental, ecological and biodiversity indicators[6], since they are relatively easy to sample and various taxa are associated with different levels of water quality[7]. Among macroinvertebrates, the taxa of Ephemeroptera, Plecoptera and Trichoptera are potentially used to assess the streams and rivers[8-11]. Moreover, bacterial growth on stream insects[12] and DNA barcoding of sensitive species[13,14] have been used as bio-indicator for assessing nutrient levels in streams.

Soil bacteria are an essential component of the biotic community in natural forest and they are largely responsible for ecosystem functioning, for example decomposition of organic matter, nitrogen fixing and antibiotic production[15,16]. Bacteria respond quickly to environmental stress compared to higher organisms. The physical and chemical properties of soil are determining the population of bacterial community[17]. Growth of bacteria is affected by the supply of nutrients[18] and very sensitive to human impact in stream[19]. Although several studies were conducted on water quality assessment using physico-chemical parameters, aquatic insects and water microbes, but little attention received on stream health assessment through soil microbes. Hence, the soil bacteria were used for assessing stream health in the present study.

Several studies have been conducted in the assessment of stream health that highlights the potential of diatoms/algae, macroinvertebrates and fishes for bio-indication. Similarly, the application of ciliated protozoa provides the viable assessments of freshwater ecological health[20]. In contrast to macroinvertebrates, relatively few studies have been examined the sensitivity of bacterial communities as indicators of the stream health[7,19,21,22]. A method for assessing stream health using stream soil bacteria requires little sampling effort, easy to characterize the bacterial diversity by 16S rDNA, denaturing gradient gel electrophoresis and automated ribosomal intergenic spacer analysis[23,24] and provides an alternative measure of stream health.

In general, stream health is assessed by the two main tools as aquatic insects/water microbes and physico-chemical parameters. This tool can be flourishing from low to medium flow of water. When the high flow or dryness of stream, this method would be unsuccessful. To overcome this problem, the present study was designed by using soil microbes to assess the stream health during high flow or dryness of stream. Hence, the objective of the present study were to compare the differences in the diversity and population structure of soil bacteria and aquatic insects in streams located in catchments ranging from pristine to impacted streams and the consistency of both of these communities as an indicator of the extent in streams was evaluated.

MATERIALS AND METHODS

Study area: Three third order streams were selected which were all situated within Tamil Nadu Province of South India (Fig. 1). The first sampling site of Kumbakkarai waterfalls (KKWF) is the familiar tourist spot, located at Periakulam, Theni district and tourists take bath in this falls throughout the year. The second site, Kutladampatti waterfalls (KPWF) is the seasonal tourist spot, located at Kutladampatti village, Madurai district, where travelers take bath seasonally (September-February). Karanthamalai waterfalls (KMWF) is the third sampling site, located at Malaiyur village in Natham Taluk, Dindigul district and this waterfalls is pristine and no tourist or anthropogenic impact. The common riparian species are *Pongamia pinnata*, *Syzygium cumini*, *Bambusa* sp. and *Terminalia* sp. The details of site characteristics are presented in Table 1. In the first site of KKWF, stream habitat

Table 1: Physico-chemical characters of three streams in the study area

Parameters	KKWF	KPWF	KMWF
Latitude	10°10'54.7"N	10°08'03.5"N	10°17'39/1"N
Longitude	77°31'47.6"E	78°01'07.1"E	78°14'02.7"E
Elevation (m)	440	453	550
Stream order	3	3	3
Water temperature (°C)	28±3	24±4	29±3
pH	6.7±0.4	6.6±0.3	6.6±0.2
Conductivity (μmhos)	0.33±0.02	0.24±0.01	0.11±0.01
Dissolved oxygen (mg L^{-1})	12.4±1.8	9.7±1.1	13.3±2.2
Total dissolved solids (mg L^{-1})	210±10	50±15	70±30
Stream width (m)	3.3±1.0	3.2±0.5	2.8±0.4
Stream depth (cm)	16±5	25±10	8±2
Surface water current (sec cm^{-1})	0.008±0.002	0.003±0.001	0.008±0.002
Bed rock (%)	20±5	40±10	20±5
Boulders (%)	40±10	30±10	50±20
Pebbles (%)	30±10	20±5	20±10
Sand/silt (%)	10±5	10±5	10±5
Total No. of riparian species (m^2)	6	6	6
Canopy cover (%)	50	80	80

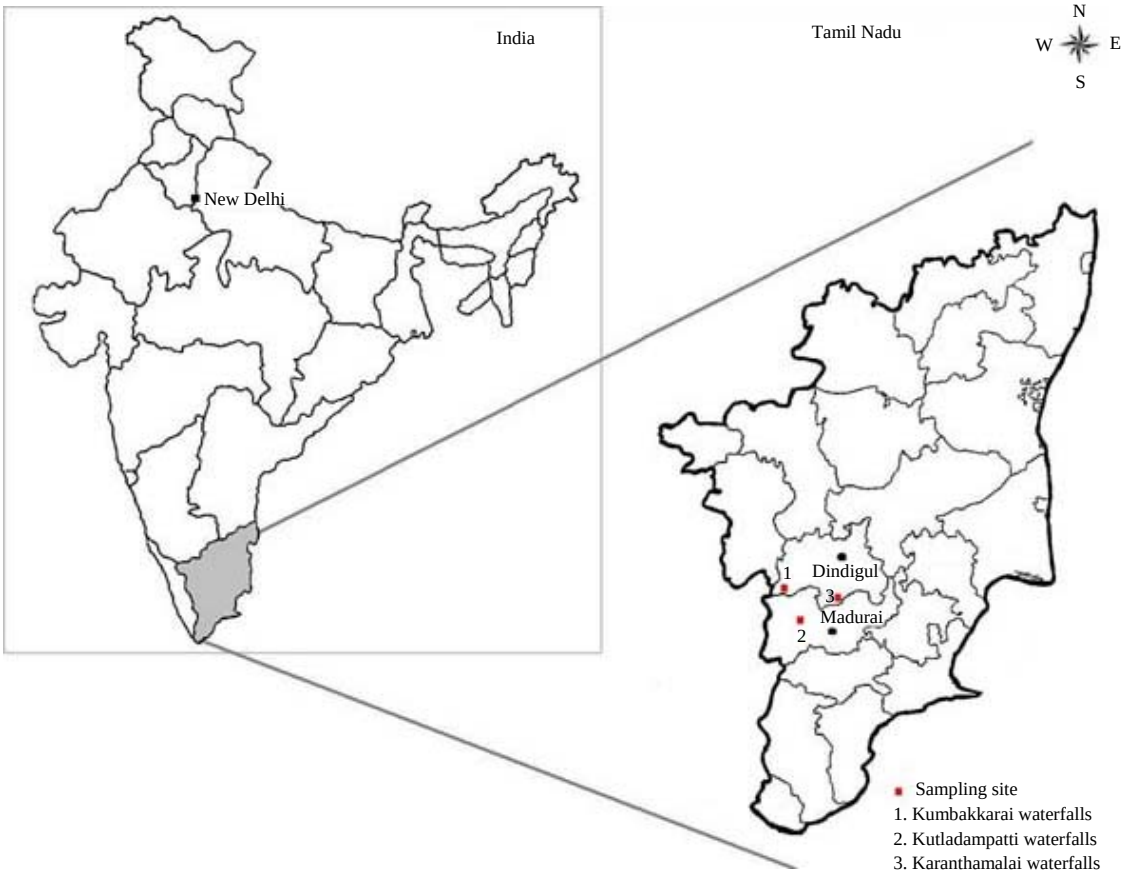

Fig. 1: Map showing sampling sites of Tamil Nadu province, South India

and quality has been degraded by anthropogenic impacts throughout the year due to the introduction of bathing materials (soaps, shampoo, bathing oils, etc.), solid wastes (snacks cover, polythene paper and waste cloths), moreover, liquid waste discharges from hill resorts of Kodaikanal region are directly mixed in headwater of this stream. Since, the habitat structure of the second site of KPWF has been degraded during season with the introduction of bathing materials and solid wastes. The third sampling site of KMWF is natively conserved and less or no anthropogenic impacts found in this region.

Soil collection and processing: The wet soils amidst riffle area of three streams were sampled for 12 months period. Triplicate samples (8-10 cm depth) were collected from each site at intervals of 1 m along transects of 4-5 m in length. The soil samples were taken in the polythene cover (10×10 cm) and also 100 mL water samples were taken in the respective site of collection as control. All samples brought to the laboratory in cooling boxes and then kept at -20°C until analysis. The individual soil and water samples were serially diluted and a 100 µL aliquot of samples was plated onto Nutrient Agar (NA) plates. The NA plates were incubated up to 48 h at 20-24°C and adjacent to stream temperatures. The cultured bacteria were differentiated based on their colony morphological traits and biochemical assays. The cultured bacterial colonies from soil and water samples were compared and identified the soil bacteria. The discriminated soil bacterial colonies were streaked onto the appropriate media for 3 times to ensure pure culture. The analyzed strains were maintained in a 1.5 mL Eppendorf tube containing 500 µL of pure cultures and 500 µL of 30% glycerin with 70% nutrient broth at -80°C. The isolated strains were then subcultured onto Nutrient Broth (NB) for further analyses. Bacterial genera and probable species were obtained by comparing the results with Bergey's Manual[25].

16S rDNA amplification: The extraction of genomic DNA from the isolated bacterial colonies was done by using a protocol described by Moore *et al.*[26]. The extracted DNA were loaded in 1% agarose gel and visualized. The DNA concentration was determined by measuring the absorbance ratio at 260/280 nm

and the DNA suspension was stored at -20°C until 16S rDNA amplification. The 16S rDNA amplification of extracted DNA from each bacterial colony made through the universal eubacterial primers (27 F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-TACGGCTACCTTGTTACGACTT-3'). The final volume of the mix was 50 µL contains 25 µL of PCR master mix, 1 µL of each forward primer, reverse primer and 50 ng of template DNA. Initial denaturation at 94°C for 3 min, cycled for 36 reactions with denaturing the template for 30 sec at 94°C, annealing at 55°C for 1.5 min, the reaction was extended for 2.5 min at 72°C and the reaction was extended finally for 10 min at 72°C. To control for the presence of contaminating nucleic acids, water samples without template DNA were run in parallel. Amplification products were visualized on 1% agarose gels stained with ethidium bromide and then purified using the Hiyield Gel/PCR-DNA extraction kit (Real Biotech Corporation, Taipei, Taiwan). Finally, purified PCR products were sequenced by the automated DNA sequencer model: 3500 (Applied Biosystems, Foster city, CA, USA). All sequences were compared with 16S rDNA gene sequences in the GenBank database using BLASTn search. Isolates were identified when their 16S rDNA sequences shared $\geq$95% homology with complete 16S rDNA sequences found in the GenBank database.

Aquatic insects sampling: In the riffle area of three streams, month-wise sampling was done. Triplicate samplings were taken at intervals of 1 m along transects of 4-5 m in length of stream. The physico-chemical characters of stream were measured using a water analysis kit (Naina Solaris limited, India). The stream profile was estimated according to Dinakaran and Anbalagan[27]. Aquatic insects were collected by using kick-net (mesh size: 200 µm). The collected specimens were preserved in 80% ethyl alcohol in the field. All aquatic insects were identified at the family level and they grouped into functional feeding pattern[28].

Data analysis: The species of soil bacteria collected from three streams were graphically presented to illustrate the site uniqueness. The individual rarefaction analysis was used for comparing species diversity between sampling sites[29]. In the graphical plot of rarefaction analysis, standard errors were converted to 95% confidence intervals. Jaccard similarity index was used and a phylogram was drawn based on the results of Jaccard similarity matrix values with Neigbour Joining (NJ) clustering method[30]. Further, three beta diversity indices for obtained species in sampling sites were calculated according to Koleff *et al.*[31].

One-way ANOVA was calculated for homogeneity of environmental variables (Water temperature, pH, conductivity, dissolved oxygen, total dissolved solids, water current, stream width and depth and stream substrates) in sampling sites and it was tested with unequal variance (Welch). The percentage of dissimilarity for soil bacteria and aquatic insects were measured by multi-group SIMPER (Similarity percentage) method[32]. Correspondence Analysis (CA) was calculated, measuring the relationship between 13 environmental variables species richness of soil bacterial and aquatic insects among sampling sites[33]. All the above statistics were calculated by using PAST version 2.08. Indicator species analysis (IndVal) was used to examine the fidelity and specificity of individual taxa to the impacted and unimpacted sites[34] with the help of indicspecies package[35] version 1.6.7.

RESULTS

Soil samples from three sampling sites in all sampling occasion contained 12 bacterial species (KKWF: 3 species, KPWF: 4 species, KMWF: 9 species, Fig. 2). Two species, *Bacillus megaterium* and *Bacillus* sp1., accounting for 89% in KKWF, *Bacillus* sp1., comprising 63% in KPWF and *Paenibacillus* sp., occupying 24% in KMWF of the overall soil sample. In aquatic insect sampling from three streams, 22,710 individuals belonging to 28 species, 26 families and 8 orders were recorded. The genus *Simulium*, comprising the greatest percentage (47%) rather than other insect taxa in KKWF and KPWF, while it was lowest (27%) in KMWF.

Species richness showed that soil bacteria had the higher species richness in unimpacted site of KMWF (No. of species: 9), in reciprocal KMWF (No. of species: 15) site had the lower species richness of aquatic insects than KPWF (24) and KKWF (25). Individual rarefaction curves and richness estimators of soil bacteria showed significant difference between sampling sites (mean richness estimators: KMWF-8.46, KPWF-3.47, KKWF-2.25, Fig. 3). The rarefaction analysis for aquatic insects showed the uncertain outcome that although year round anthropogenic impacted site of KKWF had lower value (9.5) than a moderately impacted site of KPWF (10.7, Fig. 3). Species richness and abundance of aquatic insects was high at monsoons (South-West and North-East monsoons). In contrast, soil bacteria were not influenced by the monsoonal effects in anthropogenic impacted sites and changing of species richness observed in low impacted sites.

The total aquatic insects sampled 28 species of which 5 species were unshared, 10 species shared between two sites and 13 species shared between three sites (Fig. 4). Neigbour-joining tree (NJ) based on the Jaccard index showed that the 46% constitutes 13 shared aquatic insect species between three sampling sites (Fig. 4). Abundance based

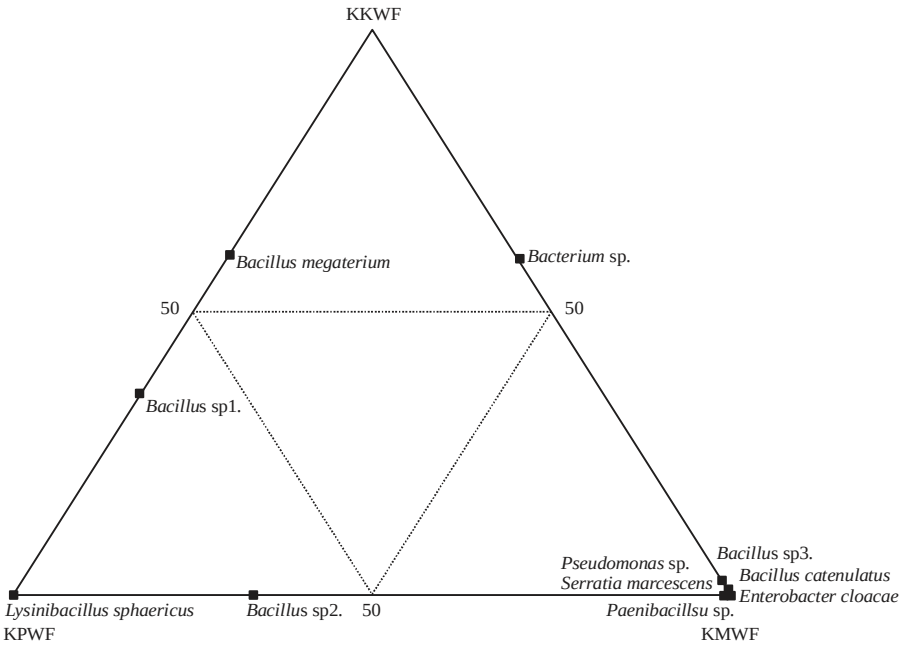

Fig. 2: Ternary plot showing the distribution of soil microbes between three sampling sites

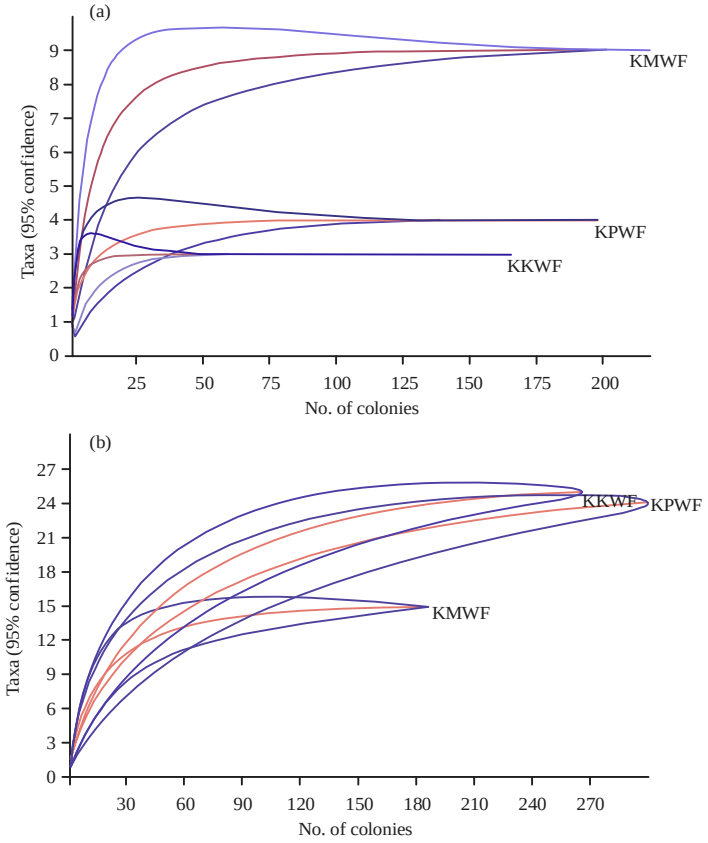

Fig. 3(a-b): Individual-based rarefaction curves for (a) Soil microbes and (b) Aquatic insects of three streams

similarity indices for the total aquatic insects were 0.3125 (Whittaker index), 0.0116 (Harrison index) and 0.0821 (Routledge index). In contrast, there are no shared species of soil bacteria between sampling sites revealed by NJ tree

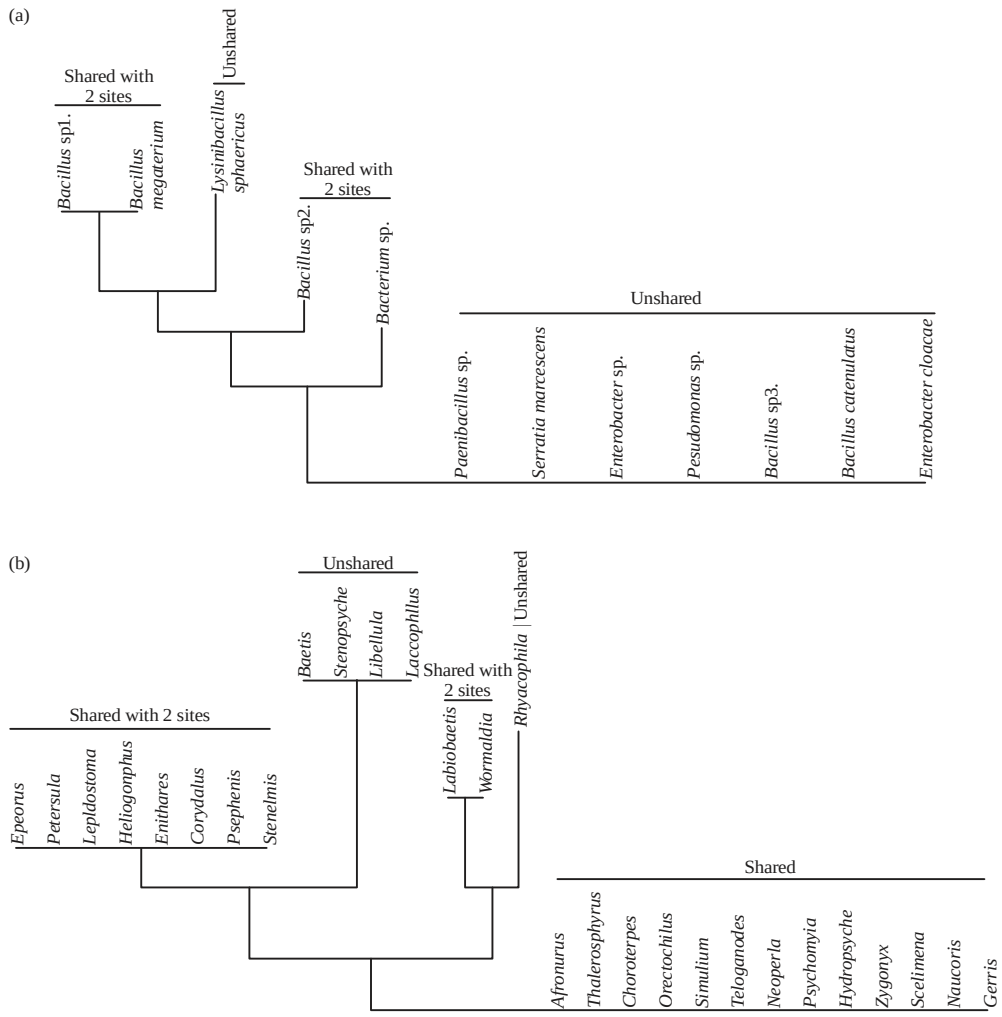

Fig. 4(a-b): Neighbour-joining tree based Jaccard index for (a) Soil microbes and (b) Aquatic insects

based Jaccard index (Fig. 4) and had lower similarity between sites (Whittaker: 1.0526, Harrison: 0.0877 and Routledge: 0.2397) than aquatic insects.

Differences in the environmental variables between three sites were not statistically significant (F = 0.082, p = 0.922) revealed by Welch F-test. The SIMPER model with the highest dissimilarity of soil bacteria (61.69%) was observed between sampling sites rather than aquatic insects (51.15%). Among 13 environmental variables tested for correspondence analysis (Fig. 5), total dissolved solids and conductivity identified as the key factors influencing soil microbial species assemblages, while pH, water temperature, stream width and pebbles as the main factors influencing aquatic insect assemblages.

IndVal analysis for soil microbes between three sites revealed that 8 species (66%) out of 12 out of species showed a significant indicator value considering site specificity. *Paenibacillus* sp., *Bacillus* sp3. and *Enterobacter cloacae* had significant indicator value and unique site-specific, whereas *Bacillus* sp1. and *Bacillus megaterium* had considerable indicators in human impacted sites and they were associated with the combination of two sites (Table 2). Among the 28 species of aquatic insects, 5 species (18%) showed a significant association in the indicator value analysis considering unique site-specific and remaining 23 species associated with the combination of two and three sites (Table 2). The result of IndVal analysis for each species of soil bacteria and aquatic insects indicates that an unimpacted site of KMWF had the highest value (3.1 and 4.1) (Fig. 6).

DISCUSSION

The present study admirably highlights the importance of soil microbes in streams. This finding is discussed in detail of this section that ecologists frequently using aquatic

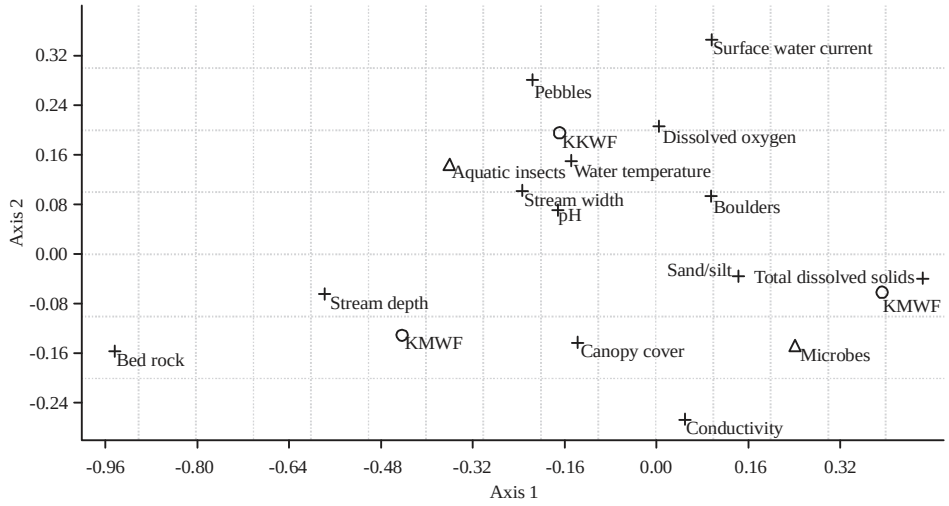

Fig. 5: Scatter diagram of correspondence analysis showing the relationships between environmental variables and soil microbes and aquatic insects of three sampling sites

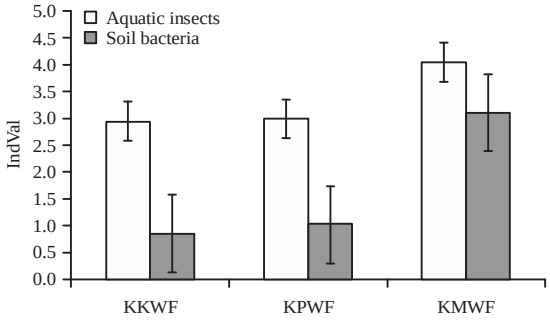

Fig. 6: IndVal analysis (Mean±SE) for soil bacteria and aquatic insects in three sampling sites

macroinvertebrates for measuring the anthropogenic impact and they developed various biometric indices for assessing stream health. Aquatic insect community mainly influenced by chemical properties of water and stream microhabitats. When assessing the stream integrity using aquatic insects, concern chemical properties or organic load or microhabitats are fixing the distribution of species diversity. For example, high organic load and less microhabitat in stream may cause loss of diversity and high organic load and good microhabitat possible to retain few sensitive and intermediate (neither sensitive nor tolerant) and tolerant species. It is evidenced by the present study that the year round anthropogenic impacted site of KKWF had higher aquatic insect species richness than the moderate (KPWF) and unimpacted site (KMWF). This may be due to either diversified microhabitats support insect diversity or more tolerant taxa present in stream. Hence, this finding may give a perplex effect of biomonitoring assessment. To overcome this problem may seek the help of microorganisms. In promising, soil bacterial analysis in streams of the present study afforded precise result that unimpacted site (KMWF) holding higher species richness and species richness decreased from moderate to high anthropogenic impacted sites.

The anthropogenic factors of pesticides, chemical pollutants, heavy metals and habitat degradation can potentially affect soil microbial diversity[36,37]. Likely anthropogenic impacted sites of KKWF and KPWF had the lower bacterial species richness than low impacted site. It may be due to chemical discharges (soaps, shampoo, oils, etc.) from tourist people layered with topsoil of the stream[38]. The high percentage of *Bacillus megaterium* and *Bacillus* sp., found in anthropogenic impacted sites and they are absent in low impacted sites, where largely occupied with *Paenibacillus* sp., *B. megaterium* and *Paenibacillus* are common soil bacteria, found in a variety of environments, including antarctic geothermal lake and they produce industrial important enzymes and antimicrobial substances[39,40].

In aquatic insects sampling, *Simulium* comprising greater percentage than the other insect taxa in impacted sites, while it was lower in unimpacted site. This is similar to findings by Buss *et al.*[41] and Anbalagan *et al.*[42] that waste discharges from tourist people highly influenced by larval abundance in streams. *Simulium* constitutes a crucial component and is employed as bioindicators of quality of aquatic habitats due to high sensitivity to environmental degradation[43]. The diversity of soil bacteria was low in degraded land, although highest rainfall area of the world[37]. A similar pattern was observed in the present study that effect of the monsoons was not influenced by the diversity of soil bacteria in impacted sites.

Table 2: Results of indicator species analysis on three streams (K-KKWF, P-KPWF and M-KMWF)

Species	S.Comb.	r_{pb}	p-value
Soil bacteria			
8 species associated to one site			
Paenibacillus sp.	M	0.826	0.032
Bacillus sp3.	M	0.723	0.021
Enterobacter cloacae	M	0.299	0.019
Bacillus catenulatus	M	0.279	0.024
Serratia marcescens	M	0.259	0.001
Lysinibacillus sphaericus	P	0.223	0.001
Pseudomonas sp.	M	0.209	0.001
Enterobacter sp.	M	0.188	0.001
4 species associated to two sites			
Bacillus sp1.	K+P	0.883	0.132
Bacillus megaterium	K+P	0.461	0.202
Bacillus sp2.	P+M	0.271	0.021
Bacterium sp.	K+M	0.369	0.012
Aquatic insects			
5 species associated to one site			
Labiobaetis sp.	M	0.984	0.221
Wormaldia sp.	M	0.962	0.187
Stenopsyche kodaikanalensis	K	0.072	0.011
Libellula quadrimaculata	K	0.011	0.001
Rhyacophila sp.	P	0.125	0.018
10 species associated to two sites			
Lepidostoma nuburagangai	K+P	0.531	0.154
Heliogomphus kalarensis	K+P	0.293	0.019
Psephenis sp.	K+P	0.012	0.003
Epeorus sp.	K+P	0.267	0.031
Petersula sp.	K+P	0.193	0.001
Corydalus	K+P	0.015	0.002
Enithares sp.	K+P	0.041	0.015
Stenelmis sp.	K+P	0.013	0.007
Baetis sp.	K+P	0.091	0.013
Laccophilus anticatus	K+P	0.083	0.012
13 species associate to three sites			
Simulium gurneyae	K+P+M	0.191	0.001
Scelimena sp.	K+P+M	0.142	0.001
Hydropsyche sp.	K+P+M	0.198	0.011
Teloganodes kodai	K+P+M	0.931	0.283
Choroterpes alagarensis	K+P+M	0.915	0.231
Orectochilus sp.	K+P+M	0.228	0.021
Neoperla biseriata	K+P+M	0.925	0.232
Thalerosphyrus flowersi	K+P+M	0.961	0.275
Psychomyia sp.	K+P+M	0.315	0.042
Gerris sp.	K+P+M	0.292	0.031
Afronurus kumbakkaraiensis	K+P+M	0.798	0.171
Zygonyx sp.	K+P+M	0.191	0.012
Naucoris sp.	K+P+M	0.219	0.001

The percentage of shares of aquatic insects is greater and no sharing of soil bacterial species between sampling sites revealed by beta diversity indices in the present study. This may be due to site specificity, physical and chemical properties of water and substrate availability. Bacterial species are constantly exposed to physical, chemical and trophic gradients, as well as intra- and inter-specific interactions that may take part in a supplementary role in determining bacterial biodiversity in natural environments and more susceptible to environmental stress[44,45]. They usually have increased generation times when compared to multi-cellular organisms and are genetically more diverse and these aspects would help to retain the bacterial population in natural environments[46].

In this study, Total Dissolved Solids (TDS) and conductivity were reflected by changes in soil bacteria assemblage composition, demonstrating that the taxonomic group is sensitive to human impacted sites. Changes in TDS concentrations in natural waters often result from industrial or human activities, changes in the water balance and they affect aquatic organisms[47]. While, multiple environmental parameters (pH, water temperature, stream width and pebbles) were influencing aquatic insect assemblages in the present study, revealed by statistical analysis. Changes in the aquatic insect community by water temperature and stream width are natural factors[48,49] and velocity[50]. This result builds on the findings of a meta-analysis examining aquatic insect assemblage related to natural and anthropic environmental variables[51] and provide evidence for their richness with environmental variables.

Indicator species are used to determine the relationship between the observed species presence-absence or abundance values in a set of sampled sites[34]. Previous assessment of the streams using aquatic insects demonstrated the reliability of indicator species for determining or identifying the habitat modification[52-55], but it may not be carried out all streams due to habitat types, stream inputs, riparian vegetation, land use and anthropogenic variables. It is evidenced in the present study that high anthropogenic impacted stream (KKWF) had the higher species richness and IndVal compared to moderate impacted and low impacted streams. However, IndVal analysis for soil bacteria analysis of the present study provides a valid result that unimpacted site (KMWF) had the highest indicator value than impacted sites. In addition, 8 species appeared to be sensitive to anthropogenic impact and unique site specificity was given by IndVal analysis.

CONCLUSION

Although bio-monitoring studies using aquatic insects provide valid results in stream assessment, sometimes it may not a success in applying all streams due to faunal endemism, adaptability and microhabitats and also during summer and monsoon time. The present study showed that soil bacteria of stream are highly sensitive indicators of low to high anthropogenic habitat disturbance rather aquatic insects. Our findings demonstrate that stream soil bacteria are more

sensitive to rapid changes in water quality and are an ideal component to analyze stream integrity in all time and even dry season rather aquatic insects.

SIGNIFICANT STATEMENTS

- Water quality assessment can be done by using soil microbes through this study
- It is inexpensive and easily assesses the polluted streams and rivers with less time
- Even dry season, river/stream assessment can be done by using soil microbes

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