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Diversity and Association Analysis of Algal Periphyton community on *Hydrilla verticillata, Vallisneria spiralis*, and *Ceratophyllum demersum.*

Sumit Manna[1], Ria Ghosh[2], Neera Sen Sarkar[3], and Anirban Roy[4]*.

[1]Department of Botany, Moyna College, Affiliated to Vidyasagar University, Moyna, Purba Medinipur -721629, West Bengal, India.
[2]West Bengal Biodiversity Board, (Department of Environment, Govt. of West Bengal), Poura Bhawan (4th floor), FD-415A, Bidhan Nagar, Kolkata- 700106, West Bengal, India.
[3]Department of Botany, University *of* Kalyani*,* Kalyani*,* Nadia, Pin -741235, West Bengal. India.
[4]West Bengal Biodiversity Board, (Department of Environment, Govt. of West Bengal), Poura Bhawan (4th floor), FD-415A, Bidhan Nagar, Kolkata- 700106, West Bengal, India.

ABSTRACT

Various algal periphyton and aquatic macrophyte co-existence determine the foraging and breeding ground of several aquatic animals and thus receiving immense importance in aquaculture worldwide. *Hydrilla verticillata, Vallisneria spiralis, & Ceratophyllum demersum* are the three most dominant aquatic macrophytes of fresh water ecosystems in tropical countries. They vary on their architecture, leaf morphology and surface texture that provide different substratum / habitat conditions for various algal periphyton. Present study was under taken to find out if there is any preference of the periphyton algae to the surface habitat of that the aquatic macrophytes. Algal periphyton association based on the shape / morphology and overall association between them was studied following 2 X 2 and multiple species association index. Crowded and Whorled phyllotaxy of *Hydrilla verticillata* support heighest algal periphyton diversity on its surface through providing a fabulous micro habitat. From the association analysis of periphyton, based on their shape / morphology, the highest significant true positive association was observed between coccoid and discoid - coccoid and discoid form followed by filamentous-single cell form. Schulter Variance Ratio followed by Willcoxon test shows that the highest overall positive association between the algal periphyton genera on the surface of *Ceratophyllum demersum* indicating the strategic sustenance of algal periphyton community rather avoidance on the unfavourable spiny rough surface morphology of the macrophyte.

Keywords: Algal periphyton, association analysis, aquatic macrophytes, diversity

Corresponding author

INTRODUCTION

The algal periphyton community plays a pivotal role in aquatic ecosystem, besides the contribution of phytoplankton and aquatic macrophytes attaining high biomass [1] and primary production, maintaining nutrient cycling [2], energy base for food web and as bio indicators [3]. The structural aspect of such periphyton assemblage is very important to classify water ways in response to environmental condition [4] and [5]. The diversity of the algal periphyton depends on the physio-chemical conditions of the water and suitable availability of the substratum. Aquatic macrophytes especially constitute a vast substrate for the growth of algal periphyton [6]. The difference in architecture of macrophytes, the texture of plant surface including the shape of the leaves give the substrate preference to algal periphyton [7].

The intricate co-existence of various algal periphyton on the aquatic macrophytes also create fabulous conditions for foraging and breeding ground of several aquatic animals and has the potentiality in aquaculture when present in considerable amount. *Hydrilla verticillata, Vallisneria spiralis* and *Ceratophyllum demersum* the most dominant macrophytes of freshwater systems [8], vary in their architecture, leaf morphology and surface texture that may have different substratum/habitat conditions for various algal periphyton assemblage. The analysis of the diversity and association of algal periphyton community on these macrophytes would be helpful in management of aquatic ecosystem and aquaculture as well.

MATERIALS AND METHODS

Study area

The reconnaissance survey was performed in different aquatic bodies of Kolkata to get the habitats of three aquatic macrophytes growing together (co-existence) viz. 1. *Hydrilla verticillata* 2. *Vallisneria spiralis* and 3. *Ceratophyllum demersum*. Several water bodies were surveyed to get these three species growing together. But it was noted that in most of the cases, among these three species, either the growth of a single species or co-existance of two species are there. The only water body Subhas Sorober (22°34' N 88°24' E) where all these species of macrophytes were found to be grown together especially to the northern side of the lake (Fig. 1). This urban lake with a water spread area of 617 X 352 sq. m. is mostly encircled with human habitation. The depth of this water body had a seasonal variation on ranging between 10.30 m (rainy) to 4.8 (summer). The pH of the water ranges between 6 -7.5 depending upon annual precipitation (average annual rainfall 1582 mm. SD. 300 mm.). The temperature of this region varies from 40^0 C (Summer) to 10^0 C (Winter). The monsoon and post-monsoon seasons are the optimum period of growth of different aquatic macrophytes including the present studied species.

Fig. 1: Study area representing the sampling sites

Data collection

It was observed that these three aquatic macrophytes were found to be growing in one side, out of total 617 X 352 sq. m. lake area. A total of 18 sampling units (quadrats of 0.91m. X 0.91m. size) were plotted in the growing region of the lake. Quadrates are plotted randomly in their habitats to avoid biased sampling. All the three studied species were uprooted (pulled from the base) carefully from each of the quadrat so that the surface of these macrophytes remained untouched as much as possible. Each species were transferred to the sample container (filled with 60% formalin solution) separately. The containers were marked separately in the field and transferred to the laboratory. The containers were kept for 7days in undisturbed condition so that the attached periphyton of these macrophytes get separate and suspend in the 70% formaline solution. All containers were placed in a horizontal rotary shaker for 24 hours before microscopic study. From each of the container, 4 sampling units (0.5 ml of solution for each unit) were observed under compound microscope with 630 X magnification. So at present, the sampling unit ultimately rises from 18 to 72. About 0.5 ml of each container solution were placed in a Schaziographic counter and observed under 630 X magnification.

Data processing

Out of the total 37 algal periphyton species growing on the surface of the three aquatic macrophytes, to study the interspecific association between different algal periphyton (if any), a 2 X 2 contingency species association table was prepared from presence-absence data matrix for pair-wise comparison [9]. Large numbers of sampling units (72) were taken throughout all the sampling sites to avoid biased Chi-square value as much as possible (Eq. 1). For further continuity correction to ensure a closure approximation to the theoretical continuous Chi-square distribution, Yate's correction formula was adopted (Eq. 2). Associations between different shapes were measured by computing Dice (*DI*), Ochiai (*OI*), and Jaccard (*JI*) Index (Eq. 3, 4 and 5) [9].

$$\chi_t^2 = \frac{N(ad-bc)^2}{mnrs} \text{........... (Eq. 1)}$$

$$\chi_t^2 = \frac{N\left[\left|(ad)-(bc)\right|-\left(N/_2\right)\right]^2}{mnrs} \text{....... (Eq. 2)}$$

$$DI = \frac{2a}{2a+b+c} \text{........(Eq. 3)}$$

$$OI = \frac{a}{\sqrt{a+b}\,\sqrt{a+c}} \text{.......... ((Eq. 4)}$$

$$JI = \frac{a}{a+b+c} \text{.......... (Eq. 5)}$$

Segregation of the algal periphyton based on their shape/morphology was performed only through selecting the pairs having significant species-species association (0.05% probability) to see if any association exists based on their shape. Index of association between different shapes of algal periphytons were measured through computing the mean of Ochiai, Dice and Jaccard Index that were calculated in the 2 X 2 species association study.

To study the interspecific association (if any) existed between different species/genera of periphyton present in the surface of *Vallisneria spiralis, Hydrilla verticillata* and *Ceratophyllum demersum* multiple species association (MSA) model was adopted here for the large number of co-exsisted periphyton (37 species) (Ludwig and Reynolds, 1988). To show the MSA, at first species association comparison matrix was prepared. Scluters V.R. (variance ratio) test was also performed, based on null association model to test simultaneously for significant association between different between co-existed periphyton species. For this, first we computed the total sample variance (TSV… Eq.5)

$$\sigma_{\mathrm{T}}^2 = \sum_{i=1}^{s} p_i(1-p_i) \text{...... (Eq. 5)}$$

And we had estimated the variance in total sample number (VTSN…Eq. 6). Finally, a variance ratio was calculated using the standard formula (Eq. 7)

$$S_T^2 = \frac{1}{N}\sum_{j=1}^{N}(T_j - t)^2 \text{ .... (Eq. 6)}$$

$$VR = S_T^2 / \sigma_T^2 \text{ .... (Eq. 7)}$$

The expected value under the null hypothesis of independence is 1.VR greater than 1 suggests that, overall the species exhibit a positive association. If VR less than 1, a net negative association is suggested. A statistics 'W' was used to test whether deviations from 1 are significant or not. If the species are not associated, then there is a 90% probability that *W* lies between limits by the Chi-square distribution.

RESULT

A total of 37 genera of algal periphyton belonging to 25 families were identified to be attached on the plant body of 3 aquatic macrophytes (*Hydrilla verticillata, Vallisneria spiralis*, *Ceratophyllum demersum*) selecting their surface as their habitat (Table 1). Among 37 genera, the species diversity was maximum in *H. verticillata*, followed by *V. spiralis, C. demersum* having 33, 30, and 23 genera respectively (Table 1). Genera like *Quadrigula, Scenedesmus, Roya, Chaetomorpha, Oedocladium, Kirchneriella* exclusively found to be growing on the surface of *H. verticillata*. It is very interesting to note that 3 genera under filamentous form of algal periphyton were only found on the surface of *Vallisneria spiralis*. No such specific pattern of habitat preference of algal periphyton was observed on *C. demersum*. The present study showed that highest frequency of algal periphyton was found to be supported by *H. verticillata* (Table 1). Members of the family Selanastraceae and Mesotaeniaceae were found to be restricted on the surface of *H. verticillata*. Whereas, the members of two filamentous family - Ulotrichaceae and Spirulinaceae were exclusively grown on the surface of *V. spiralis* (Table 1).

Table 1: Diversity of Periphyton algae and their Frequency Percentage (F%) on different Aquatic Macrophytes

Name of the species	Families	Frequency on macrophyte surface/ substratum		
		V. spiralis	***H. verticillata***	***C. mdemersum***
1. *Gloeocapsa* sp.	Microcystaceae	33.33	44.44	0.00
2. *Chlorococcum* sp.	Chlorococcaceae	22.22	11.11	0.00
3. *Quadrigula quaternata*	Selanastraceae	0.00	11.11	0.00
4. *Coelastrum microporum*	Scenedesmaceae	11.11	44.44	22.22
5. *Chlorella* sp.	Chlorellaceae	44.44	77.78	44.44
6. *Scenedesmus hystrix*	Scenedesmaceae	11.11	55.56	44.44
7. *Oocystis borgei*	Oocystaceae	88.89	77.78	100.00
8. Chlorococcales member	Chlorococcaceae	22.22	11.11	0.00
9. *Scenedesmus* sp.	Scenedesmaceae	0.00	55.56	0.00
10. *Melosira* sp.	Melosiraceae	66.67	88.89	55.55
11. *Navicula* sp.(1)	Naviculaceae	22.22	55.56	77.78
12. *Navicula* sp.(2)	Naviculaceae	44.44	77.78	50.00
13. *Amphora* sp.	Catenulaceae	11.11	22.22	22.22
14. *Characium* sp.	Characiaceae.	11.11	22.22	22.22
15. *Pinnularia* sp.	Pinnulariaceae	0.00	22.22	22.22
16. *Gomphonema* sp.(1)	Gomphonemataceae	33.33	55.56	22.22
17. *Gomphonema* sp.(2)	Gomphonemataceae	11.11	55.56	33.33
18. *Nitzschia* sp.	Bacillariaceae	11.11	33.33	50.00

19. *Netrium* sp.	Mesotaeniaceae	22.22	44.44	50.00
20. *Fragilaria* sp.	Fragilariaceae	55.56	77.78	100.00
21. *Roya* sp.	Mesotaeniaceae	0.00	11.11	0.00
22. *Oscillatoria* sp.	Oscillatoriaceae	22.22	55.56	66.67
23. *Lyngbya* sp.	Oscillatoriaceae	33.33	11.11	33.33
24. *Oedogonium* sp.	Oedogoniaceae	11.11	0.00	0.00
25. *Phormidium* sp.	Oscillatoriaceae	66.67	55.56	22.22
26. *Rhizoclonium* sp.	Cladophoraceae.	11.11	22.22	0.00
27. *Chaetomorpha* sp.	Cladophoraceae	0.00	22.22	0.00
28. *Cylindrocapsa* sp.	Cylindrocapsaceae	22.22	22.22	33.33
39. *Uronema* sp.	Uronemataceae	22.22	44.44	22.22
30. *Ulothrix* sp.	Ulotrichaceae.	11.11	0.00	0.00
31. *Oedocladium* sp.	Oedogoniaceae	0.00	11.11	0.00
32. *Coleochaete* sp.	Coleochaetaceae.	22.22	66.67	33.33
33. *Spirulina* sp.	Spirulinaceae	11.11	0.00	0.00
34. *Crucigenia* sp.	Scenedesmaceae	100.00	22.22	77.78
35. *Closterium* sp.	Closteriaceae	11.11	0.00	22.22
36. *Kirchneriella* sp.	Selenastraceae	0.00	11.11	0.00
37. *Cosmarium* sp.	Desmidiaceae	11.11	22.22	0.00

In respect to the diversity of periphyton algae based on their forms, four major forms, such as coccoid and discoid, boat Shaped, filamentous were identified having different strategy of their mode of attachment to the substratum like Attachment by Mucilage secreted by the algae, remaining as adpressed epiphytes or stalked epiphytes and some time attachment by holdfast/ basal cell *e.g. Oedogonium* sp, *Uronema* sp. etc. (Table 2).

In respect to the shape/morphology of algal periphyton species, coccoid, filamentous and boat shaped algae, shows their maximum frequencies on the surface of *Hydrilla verticillata* (Fig. 2).Where as four species of other shapes viz. *Crucigenia* sp., *Closterium* sp., *Kirchneriella* sp. and *Cosmerium* sp. (belonging to the families Scenedesmaceae, Closteriacea, Selanastraceae and Desmidaceae respectively) select the surface of *V. spiralis* followed by *C. demersum* as most preferable habitat among the three macrophytes. Surface of *C. demersum* create a fabulous microhabitat for different non-filamentous algae, especially those having boat and coccoid shaped. A similar pattern was also noticed on the surface of *H. verticillata* (Fig. 2)

Out of 666 pairs generated by 37 algal periphyton species, a total of 216 significant inter specific association were resolved out of which there is a strong possibilities of 165 true positive and 51 true negative associations (Table 3). To observe if there is any true significant association exist based on the morphological shape of these algal periphyton, six types of probable single cell- single cell association (coccoid and discoid - coccoid and discoid, coccoid and discoid - boat, coccoid and discoid -others, boat-boat, boat-others, others-others), 3 types of probable single cell- filamentous associatiotn (coccoid and discoid - filamentous, boat-filamentous, others-filamentous) and probable filamentous-filamentous associations were tested using Ochiai, Dice and Jaccard Indices. Out of 6 types of single cell - single cell association, comprised of 108 significant inter specific association, it was observed that coccoid and discoid - coccoid and discoid associations have highest mean chi-square value followed by coccoid and discoid – boat (Table 4). On the other hand, in case of single cell-filamentous and filamentous-filamentous association, comprised of 107 significant species - species association, filamentous – coccoid and discoid association shows highest mean chi-square value followed by filamentous-boat. The coccoid and discoid -coccoid and discoid also shown highest Ochiai, Dice and Jaccard Indices value (Table 3).

Schulter VR (variance ratio) test for multiple species association reflects that algal periphyton grown on the surface of these three aquatic macrophytes shows a true probability of strong overall positive association (Table 4). The highest positive VR value for the overall association of algal periphyton species was obtained on the substratum of *Ceratophyllum demersum* followed by *Vallisneria spiralis* (Table 4).

DISCUSSION

In the present study, out of 37 algal periphyton genera, as identified (Table 1), maximum diversity is found to occur in *Hydrilla verticillata* followed by *Vallisneria spiralis* and *Ceratophyllum demersum*. The whorled phyllotaxy of *H. verticillata* with erect node, distinct internodal gap and crowded leaves, may support habitat\niche of different algal periphyton. Smooth, slightly gelatinous leaf surface of *V. spiralis* also make it a fabulous habitat for different periphyton genera. Out of 37 identified genera, 30 were cited on its surface. In case of *C. demersum*, in spite of the same type of phyllotaxy as in *H. verticillata*, it possess rough, brittle, spine like appearance of the leaves that may repel the periphytons to adhere. This observation is also corroborated by the findings of [10] and [11]. Their Study also indicated that the difference in architecture, the fineness of the leaves and the texture of the plant surface of various macrophytes might affect the structure of the organism inhabiting in the littoral zone of the lakes. Wave action have very significant effect of the detachment of macrophytic organ \ plant which may be disliked by the periphyton as the detached part would be removed to another environmental conditions that cannot be suitable for that particular periphyton. Though *V. spiralis* and *H. verticillta* both have the considerable space, the breaking of the leaves and displacement of the same by the wave action in *V. spiralis* may less favourable to some of the algal periphyton genera in comparison to *H. verticillata* where the more withstanding capability of wave action prevails. Presence of some specific genus like *Quadrigula, Scenedesmus, Roya, Chaetomorpha, Oedocladium, Kirchneriella* only on the surface of *H. verticillata* indicate their high habitat preference. These adpressed epiphytes along with the priphytonic alge having holdfast or basal cell as their attachment machinaries (Table 2) may selecting this aquatic macrophyte of their broad crowded less fragile leaves. This finding also indicating that surface of *H. verticillata* was selected by different algal periphyton with very specific habitat preference having different morphological forms e.g. coccoid and discoid, boat shaped, filamentous and other. It is interesting to note that an adpressed epiphytes *Closterium* sp. having 22.22% and 11.11% frequency percentage (F%) (Table 1) on *C. demersum* and *V. Spiralis* respectively, is totally absent in *H. verticillata*. 2X2 species association indicates there is a strong possibility of true negative association existed between *Kirchneriella* sp. and *Closterium* sp. which repel the co-existance of that 2 species on the surface of *H. verticillata*. It was also noticed that 3 filamentous forms of algal periphyton genera e.g. *Spirulina*, *Oedogonium* and *Ulothrix* were only found on the surface of *V. spiralis*. No such specific pattern of habitat preference of algal periphyton was observed in case of *C. demersum*.

In respect to the morphological shape of algal periphyton, filamentous, boat and coccoid and discoid forms (Table 2 & Fig. 2) were found to be abundant on the surface of *H. verticillata* for its reliable unique habitat. Strong negative association of *Kirchneriella sp.* with *Closterium sp.* may be one of the reason of decreasing cumulative F% of the other forms of algal periphyton on the surface of *H. verticillata.*

Fig. 2: Comparison of Mean F% of algal periphyton genera (on the basis of their Morphological shape) for Different Habitat

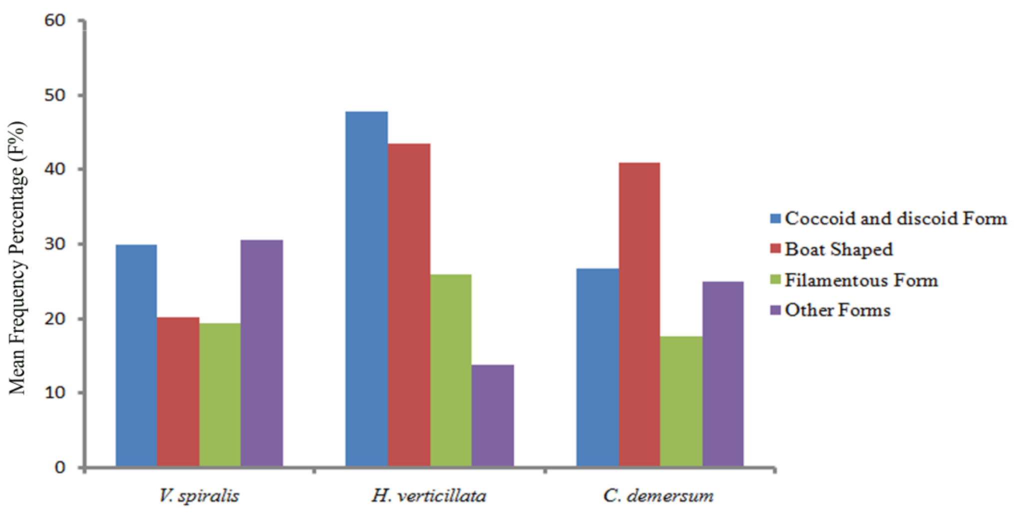

Table 2: Diversity of Periphyton algae based on their forms and their mode of attachment to the substratum

Species based on forms	Based on Mode of Attachment to the substratum
Coccoid and discoid Form	**Attachment by Mucilage secreted by the algae**
1. *Gloeocapsa* sp.	1. *Gloeocapsa* sp.
2. *Chlorococcum* sp.	2. *Chlorococcum* sp.
3. *Quadrigula quaternata*	3. *Quadrigula quaternata*
4. *Coelastrum microporum*	4. *Coelastrum microporum*
5. *Chlorella* sp.	5. *Chlorella* sp.
6. *Scenedesmus hystrix*	6. *Oocystis borgei*
7. *Oocystis borgei*	7. *Kirchneriella* sp.
8. Chlorococcoles member	8. *Lyngbya* sp.
9. *Scenedesmus* sp.	9. *Phormidium* sp.
10. *Melosira* sp.	10. *Spirulina* sp.
Boat Shaped	11. *Melosira* sp.
11. *Navicula* sp.(1)	12. *Navicula* sp. (1)
12. *Navicula* sp.(2)	13. *Navicula* sp. (2)
13. *Amphora* sp.	14. *Pinnularia* sp.
14. *Characium* sp.	**Adpressed epiphytes**
15. *Pinnularia* sp.	1. *Amphora* sp.
16. *Gomphonema* sp.(1)	2. *Coleochaete* sp.
17. *Gomphonema* sp.(2)	3. *Scenedesmus hystrix*
18. *Nitzschia* sp.	4. *Scenedesmus* sp.
29. *Netrium* sp.	5. *Crucigenia* sp.
20. *Fragilaria* sp.	6. *Closterium* sp.
21. *Roya* sp.	7. *Cosmarium* sp.
Filamentous Form	8. *Netrium* sp.
22. *Oscillatoria* sp.	9. *Roya* sp.
23. *Lyngbya* sp.	10. *Nitzschia* sp.
24. *Oedogonium* sp.	25. *Fragilaria sp.*
25. *Phormidium* sp.	26. *Oscillatoria* sp.
26. *Rhizoclonium* sp.	27. Chlorococcoles member
27. *Chaetomorpha* sp.	**Stalked epiphytes**
28. *Cylindrocapsa* sp.	28. *Gomphonema* sp. (1)
29. *Uronema* sp.	29. *Gomphonema* sp. (2)
30. *Ulothrix* sp.	30. *Characium* sp.
31. *Oedocladium* sp.	**Attachment by holdfast/ basal cell**
32. *Coleochaete* sp.	31. *Oedogonium* sp.
33. *Spirulina* sp.	32. *Chaetomorpha* sp.
Other Forms	33. *Rhizoclonium* sp.
34. *Crucigenia* sp.	34. *Oedocladium* sp.
35. *Closterium* sp.	35. *Uronema* sp.
36. *Kirchneriella* sp.	36. *Ulothrix* sp.
37. *Cosmarium* sp.	37. *Cylindrocapsa* sp.

Association of periphyton, based on their shape / morphology, the highest significant true positive association was observed between coccoid and discoid - coccoid and discoid form followed by filamentous-single cell form. It may be due to probability of their coherence to a multicellular nature of a single individual or rather a single colony that can move easily during unfavourable condition for their survival. In addition, the number of periphyton genera also may be higher in number while forming colony, within a small space in respect to any other periphyton-pairs.

Whereas in case of filamentous-filamentous form, the minimum Chi-square value (Table 3) indicates less positive association between this form of shape. It may be due to anastomos nature of the filament that inhibits their photosynthetic ability. But in single cell-filamentous type of association, the anastomose nature is not prevailing, thereby single cells algal periphyton get away to attach the filamentous surface.

Table 3: Association analysis on the basis of morphological shape of the algal periphyton

Basic Structure	Periphyton pair	No. of species pair	Student's chi square (mean)	Yate's Chi square (mean)	Ochiai index (mean)	Dice index (mean)	Jaccard index (mean)
Single cell - single cell	CD. – CD.	17	13.42	10.26	0.60	0.55	0.41
	CD. – B.	42	8.20	5.96	0.46	0.41	0.30
	CD. – O.	15	6.18	3.80	0.33	0.26	0.15
	B. – B.	22	7.73	5.29	0.41	0.36	0.27
	B. – O.	10	6.68	4.45	0.23	0.18	0.14
	O. – O.	2	5.94	4.3	0.35	0.34	0.23
Single cell - filamentous	F. – CD.	32	6.76	4.18	0.39	0.34	0.22
	F. – B.	42	6.01	3.88	0.34	0.29	0.19
	F. – O.	11	4.72	2.59	0.28	0.22	0.13
Filaments only	F. – F.	23	4.95	2.75	0.33	0.26	0.16

Coccoid and discoid: CD; Boat: B; Others: O; Filamentous: F;

Schulter VR ratio (Table 4) followed by Wilcoxon test (W test) indicates, the highest overall positive association between the algal periphyton genera exists on the surface of *C. demersum*. Unfavourable surface morphology of *C. demersum,* for most of the algal periphyton, may be strategically overcome by these periphyton genera through creating this strong overall positive association between them.

Table 4: Overall associations among algal periphyton genera on three different aquatic macrophytes surface habitats

Name of the different Habitats	Total no. of Algal Periphyton Genera	Total Sampling Variance (TSV)	Variance in Total Species Number (VTSN)	Variance Ratio (VR)	Wilcoxon Test (W test)
Hydrilla verticillata	33	6.17	13.47	2.18	156.96
Vallisneria spiralis	31	4.69	11.55	2.46	177.12
Ceratophyllum demersum	24	4.09	15.06	3.67	264.24

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