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Preliminary Ecological Survey of Microflora Inhabitant Different Types of Hydrophytes in Fresh Water Systems at Middle Egypt Belt with Reference to Physico-chemical Parameters and Phytochemistry

A.H. Abo El-lil
Cairo University, Faculty of Science (Beni Suif), Egypt

Abstract: Fungal as well as algal flora inhabiting different types of hydrophytes (floating, submerged and emerged) in case of healthy and unhealthy ones and from different habitats represent different aquatic ecotypes in middle Egypt area (River Nile, channels, drains and pools) were studied. The relations between fungal and algal flora were studied. Some *in vivo* experiments were done to analyze and confirm the *in vitro* observations. In addition to complete analysis of ecological parameters which may affect the abilities of microflora to colonize and inhabitant these hydrophytes, e.g. complete physical and chemical analysis of water of investigated sites, also phytochemical investigations of studied hydrophytes were conducted.

Key words: Ecological, hydrophytes, microflora, freshwater, Egypt

Introduction

Submerged and floating leaves of aquatic plants have different architecture offering different opportunities for epiphytes (Cattaneo, 1998). Epiphytic microalgae are common constituents of aquatic system. These autotrophs are often overlooked due to their minute size (Zimba and Hopson, 1997), however recent work has demonstrated their importance in terms of productivity (Burkholder and Wetsel, 1990) and as a source of food for higher trophic levels (Kitting *et al*., 1984).

Although microorganisms have long been recognized important link between primary and secondary production in detritus-based food webs in aquatic environments (Fenchel and Jorgensen, 1977), little has been published concerning the non aquatic epiphytic fungal flora inhabiting aquatic plants especially in submersed and emmersed ones. Many investigators studied aquatic hyphomycetes condition allochtonoies material, making it more acceptable to stream detritivores (Raviraja *et al*., 1998; Barlocher, 1992a). Others (Gaur *et al*., 1992; Galbraith, 1986 and Butler and Suberkropp, 1986) studied the relative contributions of fungi to water hyacinth decomposition. No enough attentions had been given to fungal flora inhabiting submerged and banking hydrophytes. The studies on microbial colonization of plant detritus in aquatic environments typically indicate that fungi are primary colonizers (Suberkropp and Klug, 1976; Rublee and Roman, 1982).

Burkholder *et al.* (1990) and Cattaneo and Kalff (1978) identified two distinct components of epiphytic algae; the loosely attached and tightly attached or adnate components while the relative importance of these two components has been disputed, efficient removal. Isolation and identification of fungal flora as well as algal flora from different hydrophytes categories must be accomplished to assess the importance of macrophytes and epiphytes (algal and fungal flora) in ecological studies.

The main objective of this study was to identify the fungal as well as algal flora inhabiting different types of macrohydrophytes and to assess the degree of attachment of fungal flora to host tissues, also examine the relationship between epiphytic algal and fungal flora and its aquatic environments, such as chemical components and exudets of hydrophytes, physico-chemical characteristics of water.

Also some *in vivo* experiments were done to emphasize and confirm phenomenon or observation *in vitro* especially for fungal flora patterns and their responsibilities to decay the investigated hydrophytes.

Materials and Methods

Study sites were chosen to cover as far as possible the investigated area (Middle Egypt). Based on different ecological and biological aspects, that represent different habitats supporting the naturally growing flora. The investigated sites are appeared to be populated with dense vegetation and have long history of water plant invasion. Also some sites of the studied area (channel and drain) received loads of untreated sewage and drainage water that come from neighbouring cultivated lands and urban areas. So it is considered as polluted sites, while main streams of Nile are less polluted.

The water resources at the area of study were restricted to River Nile; main stream, irrigation channels, drains and subdrains, which represent different aquatic ecosystems at study sites.

The studied area lies at Middle Egypt belt (lat. 30.25 N) the Pluviothermic quotient of this site is 6.5:5.5.

Physico-chemical characteristics of water at all studied sites were evaluated in replicates according to (Golterman *et al.*, 1978), standard methods and recent instruments. Isolation, fixation and identification of epiphytic algal flora and diatoms growing on studied hydrophytes were done according to Prescott (1978) and Hustedt (1959).

Fungal populations were isolated from investigated hydrophytes using washing, impression and maceration techneque and were maintained on Czapeck-Dox agar.

Investigated hosts macrohydrophytes were *Eichhorina crassipes* (floating), *Ceratophyllum demersum* (submerged), *Azolla fern* (floating fern) and *Echinochloa stagninum* (emerged).

Phytochemical evaluation of the investigated hydrophytes as well as elementary analysis were conducted according to Allen *et al.* (1974).

Experimental studies carried out in conditions similar as possible to natural sites, the freshly collected samples of hydrophytes were transfered immediately to laboratory feed tanks that contain steralized water from natural sites and followed for about 80 days. During this period visual observations to investigate hydrophytes were recorded. Statistical analysis of data were done using analysis of variance (ANOVA), Microstat programme (IBM).

Results and Discussion

The results of some physico-chemical characteristics of collected water from study sites evaluated the possible effects of their parameters on epiphytic flora.

The temperature of water varied between 20 and 31°C, while pH value lied generally at alkaline side ranging from 7.5 to 8.4. T.S.S. varied between 307-685 mg l^{-1}. While D.O. ranged from 3.90 mg l^{-1} at drain and about 7.80 mg l^{-1} at River Nile.

Total hardness average was between 1.5 and 4.2 meq/l. The chloride content from different sites was relatively low varing between 40.5 and 103 mg l^{-1} being recorded at drain with an average of 58.6 mg l^{-1}. Also data revealed that sulphate ion contents varied between 38.1 mg l^{-1} at River Nile and 108.5 mg l^{-1} at drain. (Table 5).

Carbonate amounts of water samples seemed not to correlate with bicarbonate, the former was completely depleted at all study sites, while the bicarbonate levels appear of reasonable amounts varied between 152 to 214 mg l^{-1}.

The nitrate and nitrite contents of water samples throughout the study sites may be regarded as being low, actually ranging from 0.004 to 0.1 mg l^{-1}.

Ammonia-N contents fluctuated within narrow range of average 0.1 to 1.22 mg l^{-1}. Phosphate contents of water showed an average varing between 0.02 and 0.1 mg l^{-1}.

The amount of Na and Ca cations appeared to be relatively chigh as compared with other investigated cations. The first one (Na) varied between 24 to 80.5 mg l^{-1}, while Ca ranged from 21.6 to 60.8 mg l^{-1}. Mg come next and varied between 15 to 39 mg l^{-1} and then K was limited with an average of maximum reading 13.2 mg l^{-1} while the minimum one was 5.1 mg l^{-1}.

Microelements such as Fe, Mn, Zn and Cu were investigated and generally showed little amounts less than 0.05 mg l^{-1} except at drain where maximum reading was 0.1 mg l^{-1} for Mn. (Table 5)

So, we can concluded that Nile water was of the best qualities in comparison with other studied sites. Then comes channel with relatively high contents of anions and cations in relation to those recorded in case of River Nile. While drain waters were of the lowest qualities and recorded the highest values of studied parameters and this may regarded to pollutants and remains of human activities and agricultural water excess.

Phytochemical investigation of studied hydrophytes: Hydrophytes used in this study, were collected from its natural habitats, handly cleaned, left to dry in shade and ground to fine powder.

Ash content of investigated plants had great differences, the lowest value 14.5% was recorded for *Echinochloa*, while the highest value (34.1%) was estimated for *Eichhornia*.

The mean values of carbohydrate contents were 19.7, 10.8 and 4.1% while the total nitrogen were 0.91, 0.82 and 1.78 g% for *Echinochloa*, *Eichhornia* and *Ceratophyllum*, respectively.

The lipid content fluctuated generally within narrow range, being varied between 1.11% for *Eichhornia* and 1.31% for *Ceratophyllum*.

The crude fiber content showed relatively higher amounts in *Echinochloa* plants (29.5%) while ranged between 12.5 and 14.8% for rest of the investigated plants.

The analysis showed the fantastic proliferation of these plants and special property of absorbing and accumulating the minerals inside their tissues. Elementary analysis K, Na, Ca and Mg ions showed relatively high concentrations than other investigated elements (Zn, Cu, Mn and Fe), K and Na ions concentration of investigated plants constituted the major compounds of investigated elements, ranging between 2.9 to 3.8% while calcium could be ranked the 3rd important element being varied between 2.2 to 3.4% for *Echinocloa* and *Ceratophyllum*, respectively. Mg showed relatively low values comparing with the above mentioned elements (1.4-1.7%). The other elements Fe, Mn, Zn and Cu showed relatively low contents and varied between 0.02 to 0.1% while Cu recorded as traces being less than 0.01%.

The data showed obviously, the abilities of investigated hydrophytes to absorb and accumulate the macro- and micro-elements, that may help, in addition to other factors, to increase the desire of microflora to inhibitant and colonize these hydrophytes. It may be considered as source of some elements offering suitable microclimate to microflora. Also may discharges some chemical constituents from decaying hydrophytes to external media, which may help some flora to flourish and enhance (Abo Elil, 1995; UNEP, 1986).

Microflora inhabiting investigated hydrophytes: Table 2 showed that the major algal epiphytic community on healthy submerged plant *Ceratophyllum demersum* mainly composed of *Chlorophyta*, *Cyanophyta* and *Bacillariophyta* being amounted to 6.215x10^3 units/g fresh weight. *Ceratophyllum* numerically harboured the maximum number of species being 47. While the other epiphytic algal flora, inhabiting floating hydrophytes *E. crassipes* and *Azolla* fern standing crop yielded 1.85 and 2.31x10^3 units/g fresh weight for *E. crassipes* and *Azolla* fern, respectively.

While *E. stagninum* generated less amount of epiphytic algae being 1.2 x 10^3 units/g fresh weight.

The most common and abundant algae isolated from hydrophytes were diatoms, including *Cyclotella*, *Melosira*, *Comphoneria*, *Navicula*, *Nitzchia*, *Synedra* and *Cocconeis* spp. It is of particular interest to mention that the dominance of diatoms could be attributed to the possible differences in architectural development of communities, which are based on the arrangements of attached algae in relation to the substrate.

On the other hand, the most abundant epiphytic algal species isolated from different hydrophytes belonging to Cyanophyta and Chlorophyta were *Oscillatoria*, *Lyngbya*, *Cladophora*, *Spirogyra*, *Anabaena*, *Microcystis*, *Merismopedia*, *Senedesmus* and *Pediastrum*.

The fungal flora isolated from arial parts of healthy investigated hydrophytes belonged to genus *Aspergillus and Alternaria* (Table 4). Four species of *Aspergillus* identified were *A. niger*, *A. terreus*, *A. carbonareus* and *A. flavus* (Tables 2 and 3). Moubasher *et al.* (1990) has also isolated *Aspergillus* on four baits of cellulose from Nile water.

Table1: Phytochemical parameters of investigated hydrophytes

	Echinochloa	*Eichhornia*	*Ceratophyllum*	*Azolla*
Ash content	14.5	34.1	29.5	22.1
Total carbohydrate	19.7	10.8	4.1	6.3
Total nitrogen	0.91	0.82	1.78	2.12
Crude protein	7.9	5.9	12.1	14.8
Total lipid	1.21	1.11	1.31	0.7
Crude fiber	29.5	14.8	12.5	17.2
K	2.9	3.8	3.6	3.9
Ca	2.2	2.6	3.4	3.1
Na	3.2	2.9	3.5	2.9
Mg	1.4	1.5	1.7	1.8
Fe	0.04	0.1	0.07	0.1
Mn	0.02	0.1	0.09	0.1
Zn	0.05	0.07	0.07	0.07
Cu		Trace		

Table 2: Showed standing crop of algal epiphytic communities isolated from different hydrophytes

Name of hydrophytes	Standing crops of epiphytic algaex10^3 (units/g)	No. of species
Ceratophyllum demersum	6.215	47
Eichhornia crassipes	1.85	36
Azolla fern	2.31	38
Echinochloa stagninum	1.2	31

Table 3: The density of fungi isolated from water hyacinth

Fungi genera	Old plant	Mature plant	Young plant
Aspergilli	+	+++	++
Alternaria	+	++	+++
Fusarium	-	+	+++
Paecilomyces	-	+	+
Cercospora	-	-	+

Table 4: Showed the fungal flora isolated from different investigated hydrophytes according to age and its densities

Old plant	Mature plant	Young plant
Aspergilli (++)	*Aspergilli* (+++)	*Aspergilli* (+)
A. niger	A. niger	A. niger
	A. terreus	A. terreus
	A. carbonareus	A. carbonareus
	A. flavus	A. flavus
*Alternaria (+++)	**Alternaria* (++)	**Alternaria* (+)
**Fusarium* (+++)	**Fusarium* (+)	
	Paecilomyces (++)	
**Cercospora*		

*, Isolated from infected hydrophytes, +++, frequent; ++, abundant; +, isolated

On the other hand, *Fusarium* sp. completely not isolated from healthy hydrophytes (*E. crassipes*, *Azolla* ferns and *E. stagninum*). While fungus *Alternaria* rarely isolated. So we can consider *Aspergillus* species as the natural

Table 5: Physico-chemical parameters of water at studied sites

Water temperature °C	20	31	25.5
T.S.S. mgl	307	658	496.0
DO mgl	3.9	7.8	5.9
PH	7.5	8.4	7.9
Total hardness meql	1.5	4.2	2.9
Chloride mgl	40.5	103	72.0
Sulphates mgl	38.1	108.5	73.3
Bicarbonates mgl	152	214	183.0
Nitrate&nitrites mgl	0.004	0.1	0.05
Ammonia mgl	0.1	1.22	0.66
Phosphates mgl	0.02	0.1	0.06
Na mgl	24.0	80.5	52.3
Ca mgl	21.6	60.8	41.2
Mg mgl	15.0	39.0	27.0
K mgl	5.1	13.2	9.1
Mn mgl	0.05	0.1	0.08

flora inhabiting the arial parts of above mentioned hydrophytes and do not cause any damage or diseases to these hydrophytes. Also we found that, *Alternaria* sp. appeared to be dominant and isolated frequently from old hydrophytes, while *Fusarium* sp. was isolated frequently from geatiatric hydrophytes which were able to be infected by fungal diseases especially at parts in contact with water level. These species have been reported as cellulose decomposers (Stewart and Walsh, 1972); this may explained the abilities of above mentioned fungi to invase the old hydrophtes,charactarized by weak cortex in opposite to yong ones has strong tissues resist enzymatic activities of fungi.

Reddish brown patches especially at old leaves of *E. crassipes* due to presence of *Alternaria* sp., while *Fusarium* sp. was isolated from yellowish old parts. Also *Cercospora* fungus was isolated from infected parts characterized by crimson or gray colour in the middle of old leaves of *E. crassipes*.

In conclusion *Alternaria*, *Fusarium* and *Cercospora* cause a fungal diseases to some hydrophytes due to their abilities to penetrate the wall of hydrophytes mechanically and/or enzymatically and then they excrete their toxins which leading to visual and marked changes in the colour of areal parts of investigated hydrophytes. Park (1974a,b and 1980) also reported cellulose degradation in fresh water ecosystem by fungi.

The study also showed that at the next stage of infections by above mentioned fungi, there are 2ry infection phenomena, some saprophytic fungi such as *Rhizopus*, *Mucor* and *Absidia* which feed on decomposed polysaccharides resulted from enzymatic activities of *Alternaria* and *Fusarium* considered as parasitic fungi, were also isolated frequently. This may explain the phenomenon of complete decay of infected parts of hydrophytes at latest stage. Many species of fungi, including those we have isolated are now known to produce high activity of cellulase capable of degrading insoluble cellulose to soluble sugars (Bisaria and Ghose, 1981).

On the other hand, fungal flora isolated from submerged parts of investigated hydrophytes showed some new genera differing from those isolated from aerial parts, for example *Glomus*, *Jeleroelie*, *Aspergilli* and *Alternaria* were isolated. The fungal flora differed according to the parts from which isolated. Despite the hydrophyte is the same, like *E. crassipes* it revealed marked change in fungal flora isolated from areal parts and those from roots and rhizome (emmersed parts).

To confirm the result of study *in vitro* and under the condition related as far as possible to natural condition in field, we observe and follow young and healthy *E. crassipes* hydrophyte for about 80 days till complete decay and isolated fungal flora at different stages, the results showed that, after 3-4 days of decay, some visual fungal colonies start growing. While after 10 days, fast growing fungi like *Trichodermai* and *Absida*, characterized by high ability to enzymatic activities were isolated.

After 3 weeks, we could isolate marked amounts of common fungi (*Rhizopus* and *Mucor*) from phycomycetes and (*Myrothecium* and *Phoma*) from ascomycetes.

About 2 months latter *Acremonium*, *Gliocladium* in addition to *Penicillium* were isolated and at the end of the experiment (after 80 days) the hydrophytes completely decayed and phycomycetes like *Absida*, *Rhizopus* and *Mucor* became abundant and dominant ones.

Relation between epiphytic algal and fungal flora: The results *in vivo* and *in vitro* observations revealed that, in case of healthy hydrophytes the algal flora specially green algae and blue green non filamentous algae were dominant while at start of infection by fungi the green algae decreased markedly and fungal flora from deuteromycetes became dominant. While at late stage of infection and start of plant decay, the filamentous blue green algae like *Lyngbya* and *Oscillatoria* and diatoms like *Nitzchia* and *Comphanema* were isolated but only scarcley. While some fungal phycomycetes as mentioned before were dominant and abundant.

Such observation may regard to dramatic and sharp changes in nature and structure of hydrophytes after colonization by fungal flora due to their highly enzymatic activities of fungal flora and also may regards to aflatoxins secreted by these fungi which affected the algal flora specially green ones as epiphytes on hydrophytes. The above mentioned data come in harmony with several authors (Galbraith, 1986; Moubasher *et al.*, 1990; Gaur, 1992; Zimba and Hopson, 1997; Raviraja *et al.*, 1998; Cattenea and Kalft, 1978 and 1998).

Such results may indicate that *Lyngbya* and *Oscillatoria* from filamentous algae and *Nitzchia* and *Gomphorema* from diatoms has great ability to resist the pollution and can survive with fungal population, both as colonizers or epiphytes on investigated hydrophytes. On the contrary green algae can fail to do that, disappeared and substituted by above mentioned fungal flora.

Statistical analysis: Statistical analysis of variance (ANOVA) revealed that Statistically significant difference between fungal flora isolated from healthy hydrophytes and infected ones at 5% level.

Highly significant difference between epiphytic algal flora isolated from healthy ones and infected decayed hydrophytes at 1% level.

The analysis also showed significant difference at 5% level between fungal and algal flora isolated from new healthy hydrophytes and old infected ones.

Significant differences at 5% level were appeared depending the types of water bodies which samples collected from it (mainly river Nile and drains).

Non significant difference was detected by changing the host from which microflora were isolated.

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