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AZOLLA FILICULOIDES, A NEW EFFECTIVE DINITROGEN FIXER LAKE EDKU, EGYPT.

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ABSTRACT

Azolla filiculoides was found for the first time in Lake Edku in Autumn 1991. A massive population developed in 1992 to cover more than half of Lake area. In situ nitrogen fixation (C2H2-C2H4 reduction) by Azolla filiculoides in Lake Edku was measured during the year 1992. The highest activity was recorded in Summer (73.1 ug-N_2 plant^{-1} h^{1}), and the lowest activity was in Winter (10.6 ug-N_2 plant^{-1} h^{-1}). The contribution of Azolla to the nitrogen budget of Lake Edku was computed to be 0.7 kg m^{-2} yr^{-1} or 89562 tonnes of nitrogen were fixed annually in the whole lake. This amount represents about 1.36 times of the annual total combined nitrogen (56700 tonnes) entering the lake via drains, which must therefore be considered quite significant and should be taken into consideration for the lake management.

INTRODUCTION

Azolla is a genus of fast-growing, floating aquatic ferns widely distributed throughout tropic and temperate fresh waters. It consists of small vascular water fern of the genus Azolla, a prokaryotic cyanobacteria tentatively identified as Anabaena azollae. and a variety of bacteria (bactobionts) in symbiotic association (Nierzwicki-Bauer and Aulfinger, 1991: Lindblad et al., 1991). In this association, specific functions are performed by each of the partners. The cyano-biont fixes enough atmospheric nitrogen to satisfy both its own requirement for combined nitrogen and that of its host. The fern provides the endosymbiont with carbon source. Both Azolla caroliniana and Anabaena azollae carry out photosynthesis of higher-plant type, thereby enabling each to fix atomospheric CO_2 via Calvin cycle (Ray et al., 1979).

This capacity allows Azolla to grow without being limited by the availability of reduced or oxidized nitrogen ions in its immediate environment. For this reason Azolla is an attractive candidate for photosynthetic production of nitrogen fertilizer. During the last decade, the agronomic potential of Azolla as biofertilizer for increasing rice yields has been confirmed. The nitrogen fixing potential of Azolla and its nitrogen contribution for rice has been well recognized in China (Liu, 1979), Vietnam (Dao and Tran, 1979) USA (Rains and Talley, 1979), Philippines (Watanabe, 1982) and India (Kannaiyan, 1984). A. pinnata, A. caroliniana and A. filiculoides were introduced to Egypt for the first time in 1977-1980 by Hamdy et al., (1980) as a source of biofertilizer for rice fields. It reached Lake Edku via agricultural drains for the first time in Autumn 1991. In 1992 a massive population developed to cover more than half of the lake area.

In this study, nitrogen fixation (nitrogenase activity) was measured using acetylene reduction method to estimate seasonal nitrogen fixation in different parts of Lake Edku as affected by environmental conditions during 1992. Furthermore, the quantitative role of the nitrogen fixed by Azolla in relation to other sources of nitrogen and its significance in Lake Edku was estimated.

Description of the Lake:

Lake Edku which is a coastal lagoon in the eastern Mediterranean, is a shallow brackish water basin extending about 19 km South of Abu-Qir Bay from East to West. It has an average width of 6 km with an average depth of about one meter. The present area of the lake is about 126 km^2. The lake is directly connected with the Mediterranean Sea at its Western extremity through a narrow channel "Boughaz El-Maadiya" (Fig. 1). Lake Edku receives large quantities of drainage water (83-280 x 10^6 m^3 day^{-1} Fig. 2) released from agricultural land of Behera Province via three main drains, Edku, El-Boseily and Barzik, discharging into the eastern part of the lake through the extension of Edku Drain (Shriadah and Tayel, 1992).

MATERIALS AND METHODS

Water samples were collected from five hydrographic stations (Fig. 1) during January and February in winter, April and May in spring, July and August in summer and October and November in autumn, 1992. The physiochemical parameters namely temperature, salinity, pH, dissolved oxygen, oxidizable organic matter, hydrogen sulphide, inorganic introgen compounds (ammonia-N, nitrate and nitrite-N) and phosphate-P were measured. The methods of analysis are summarized in table (1).

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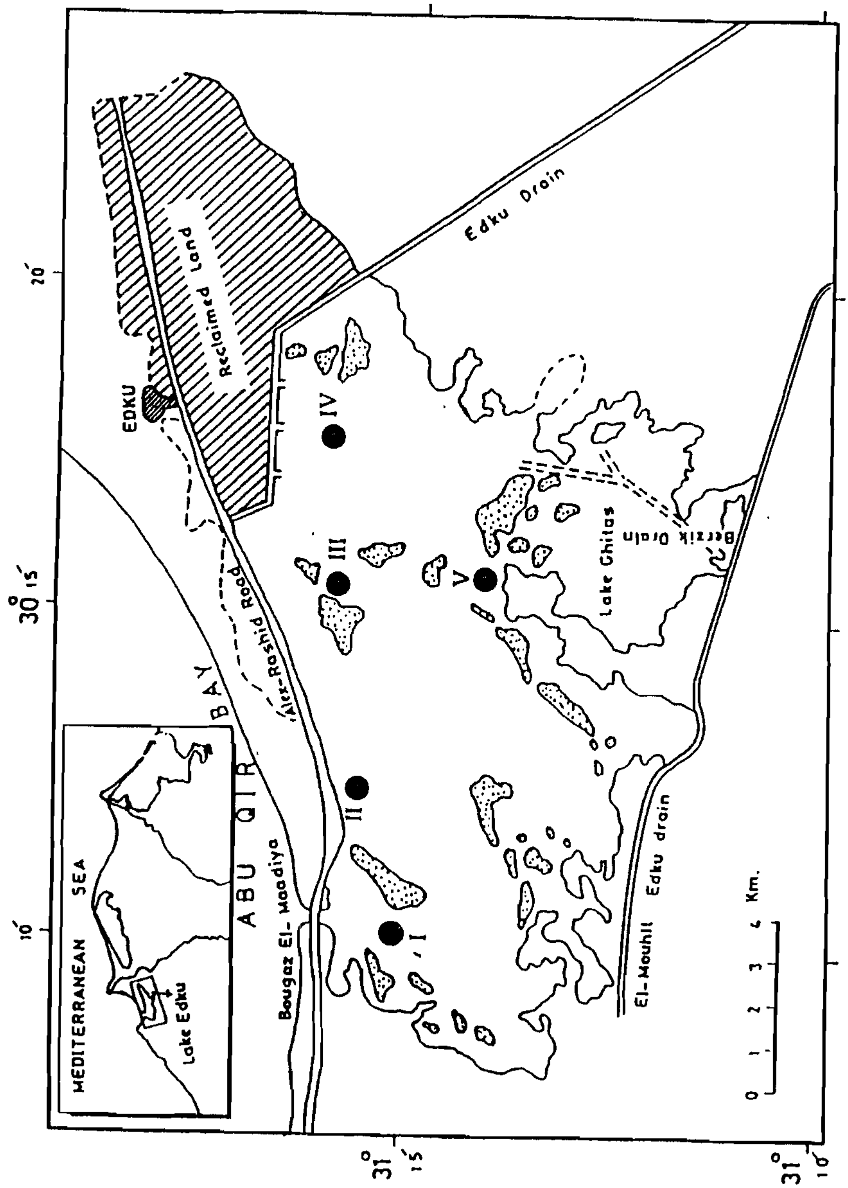

Figure (1): Lake Edku and sites of sampling stations.

Table (1): Methods of analysis of different hydrochemical parameters.

Parameter	Methods of Analysis	References
Temperature (oC)	Ordinary thermometer graduated to 0.1 oC	
Salinity (%o)	Beckmen induction Salinometer model R-70	
Hydrogen ion concentration (pH)	Potentiometrically	Strikland Parsons; 1965
Dissolved oxygen (mg/l)	Winkler method	Grassoff; 1976
Hydrogen Sulphide (mg/l)	Thiosulphate titration method	APHA; 1985
Oxidizable organic matter	Permanganate titration method	FAO,; 1975
Nutrient salts	Spectrophotometerically	Grasshoff; 1976.

Table (2): Rate of nitrogen fixation of Azolla filiculoides during 1992. (ng N2 fixed plant-1 h-1).

Station	Winter	Spring	Summer	Autumn
II	6.8	14.9	78.1	69.9
III	13.7	28.8	101.2	50.8
IV	8.8	16.7	66.5	42.1
V	13.4	20.3	46.6	38.4
Average	10.6	20.2	73.1	50.3

Azolla collected from Lake Edku was identified as Azolla filiculoides by Dr. S.N. Shalan, Soil and water Research Institute, Agricultural Research Center, Giza, Egypt.

Nitrogenase activity was measured by the acetylene reduction technique (Stewart et al, 1971). Then plants (= 2g fresh weight) were put onto the surface of 100 ml lake water in a 270 ml glass bottle sailed with rubber teats to eliminate any possible over pressure or vacuum in reaction space (El-Samra and Olah, 1979). The acetylene concentration was kept at 10 % (v/v). Reactions were run for 4 hours (in situ incubation) starting at 10 am. After the incubation time, gas samples were withdrawn using glass syringes and stored in 7 ml sterilized-evacuated blood tubes for gas chromatographic analysis. One ml of gas sample was injected into Varian-Aerograph series 1400 gas chromatograph, fitted with a Porapak R column and a FID detector. The produced ethylene concentrations were calculated from the chromatogram peaks height. Triplicates were assayed for each sample. Two types of controls were made, the first was the lake water without Azolla, while the second was the Azolla added to lake water with no addition of acetylene. The number of plants per area was calculated from 12 random samples per station using quadrat each measuring 20 cm x 20 cm. Samples of fresh Azolla were dried in an oven at 60 °C for 48 h before recording dry weights.

RESULTS AND DISCUSSION

Seasonal Variations :

The net value of nitrogen fixation activity by Azolla is the result of the combined effect of environmental factors, as temperature, light and mineral nutrients specially phosphorus, in addition to Azolla species itself. For example salinity has its considerable effect as there is an exchange of water masses between the lake and the sea. Therefore it is important to discuss how the interaction of the environmental factors do affect nitrogen fixation by Azolla in Lake Edku in different seasons.

The maximum activity of nitrogen fixation (73.1 ug N_2 $plant^{-1}$ h^{-1}) was recorded in summer (July and August) while the minimum (10.6 ug N_2 $plant^{-1}$ h^{-1}) in winter (January and February). Winter value, thus represented 14 % of the summer value, while spring and autumn represented 28 % and 66 % respectively (Table 2 & Fig. 3).

A positive correlation was found between water temperature and Azolla nitrogenase activity in Lake Edku. Talley and Rains (1980) recorded maximum growth and nitrogen fixation when morning and afternoon temperatures were between 25 and 30°C which permitted maximum sustained nitrogenase activity. This is in agreement with the results of Vu Van Vu et al ., (1986); Lumpkin, (1987); Kannaiyan and Somporn, (1989) and Laurinavichene et al., (1990).

The most remarkable characteristic of Azolla is its complete lack of dependence on nitrogen source (Kannaiyan, 1992). The concentrations of combined nitrogen, in lake water during 1992 was not higher than 1.5 mg l^{-1} (Table 3). The correlation between N_2- fixation activity and nutrient salts (ammonia, nitrate and nitrite) was not significant. This indicates that combined nitrogen has no effect on Azolla nitrogenase activity in the lake.

The manifestation of nitrogen fixing ability was reported to be adversely affected by combined nitrogen beyond certain levels (Satapathy and Singh, 1985; Kitoh and Shiomi, 1991), while the extent of inhibition depended on type of nitrogen source. Okoronkwo et al., (1989), reported that in presence of ammonia (40 mg l^{-1}), the percentage of nitrogen derived from air varied from 30 to 80 % of Azolla nitrogen depending on its species.

The use of Azolla as a source of nitrogen is severely limited by its high phosphorus requirement (around 0.3 mg l^{-1}) for replication and nitrogen fixation (Watanabe et al., 1989; Singh and Singh 1990; Nierzwicki-Baue, 1990). In Lake Edku the phosphorus concentration ranged between 6.2 and 26.2 ug l^{-1} (Table 3) which might limit Azolla nitrogen fixation. It was noted that Azolla in many areas of the lake had a reddish brown discoloration that spread from the center of the frond to the tip of the body as a symptom of phosphorus deficiency (Kannaiyan 1992). Subudhi and Watanabe (1980) and Kannaiyan 1985 reported that (5-10 mg-P l^{-1}) were adequate for growth and multiplication of Azolla filiculoides. However, Subudhi and Watanabe (1979) found that it can grow normally at 0.06 mg-P l^{-1} but the biomass, chlorophyll content and acetylene reducing activity were less at 0.03 mg-P l^{-1}.

The salinity variation in Lake Edku is affected by the invasion of marine saline water (salinity > 20 ‰) from the Mediterranean Sea (Abu-Qir Bay) specially in winter (Table 3), which is the main reason for Azolla disappearance near El-Boughaz and in the affected west areas of the lake.

Dual variations in nitrogen fixation rates by Azolla:

Diurnal variations of nitrogen fixation were measured seasonally at four hours intervals. Three types of diurnal patterns were recorded (Fig. 4). In spring and winter, significant amount of nitrogen fixation occured in the morning, increased

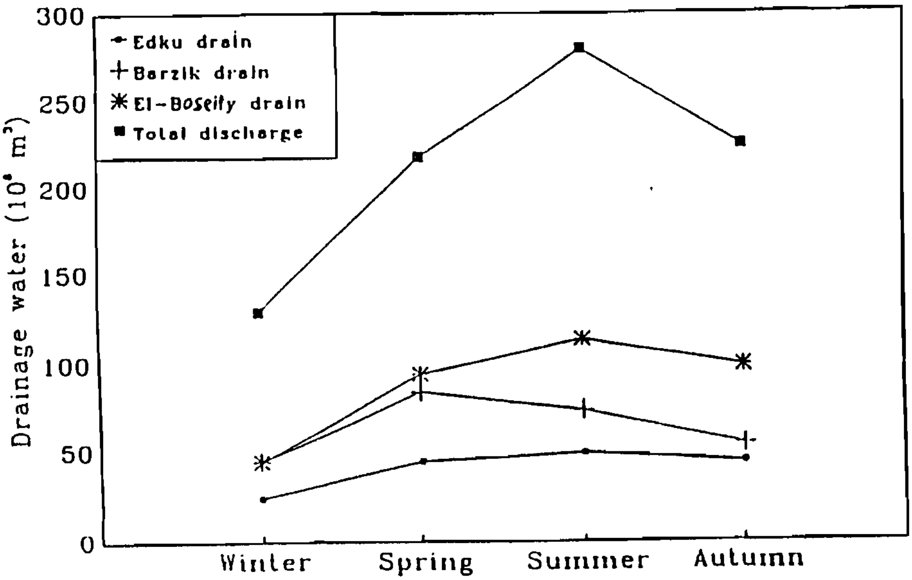

Figure (2): Seasonal variations of drainage waters discharged into lake Edku during 1992.

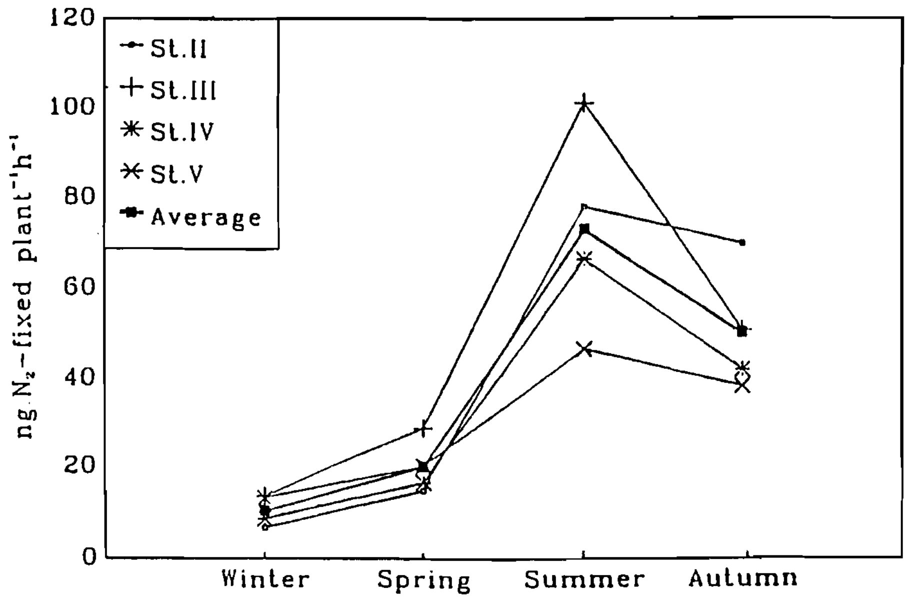

Figure (3): Seasonal variations of nitrogen fixation by <u>Azolla</u> <u>filiculoides</u> during 1992.

Table (3): Physio-chemical characteristics of Lake Edku during 1992.

Season	SPRING					SUMMER					AUTUMN					WINTER				
Station	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Temperature	21	22	22	22	22	28.5	29	29.5	29	29.5	24	25	25	25	25	17.8	18	18	18	18
pH	8.1	8	7.6	7.8	7.7	8.20	8.1	8.2	8.2	8.3	8.2	8.1	8.6	8.5	8.5	8.4	8.3	8.5	8.4	8.3
Salinity S ‰	12.8	11.8	13.3	13.2	12.8	14.8	17.7	10.6	7.7	15.8	16.6	10.2	11.1	8.2	16.1	27.8	2.4	2.7	2.3	1.7
Dissolved O_2 (mg/l)	1.4	1.1	0.7	0.8	0.7	3.8	4.1	4.1	3.2	1.7	4.2	3.5	3.5	3.2	2.8	1.1	3.7	2.0	2.6	3.7
Organic matter (mg/l)	3.3	2.0	3.3	3.5	4.0	3.7	11.6	6.3	9.5	11.6	9.0	12.0	6.0	8.0	7.5	6.1	1.2	1.5	1.5	0.4
Hydrogen sulphide (mg/l)	0.5	0.3	0.4	0.4	0.3	1.1	0.9	0.6	0.8	0.9	0.6	0.7	0.8	0.9	0.8	0.7	0.5	0.6	0.6	0.4
Ammonia-N (mg/l)	0.2	0.6	0.7	0.5	0.5	0.4	0.8	0.8	0.7	0.6	0.3	0.9	0.7	0.8	0.6	0.1	0.3	0.3	0.6	0.2
(NO_3 - NO_2)-N (mg/l)	0.5	0.8	0.7	0.6	0.6	0.2	0.7	0.5	0.4	0.5	0.2	0.4	0.6	0.8	0.6	0.2	0.3	0.2	0.4	0.3
Phosphate-P (ug/l)	6.2	14.3	15.4	15.3	14.1	6.5	25.1	26.0	26.2	25.6	9.1	20.1	23.9	22.7	22.2	7.8	12.3	13.6	10.1	11.2

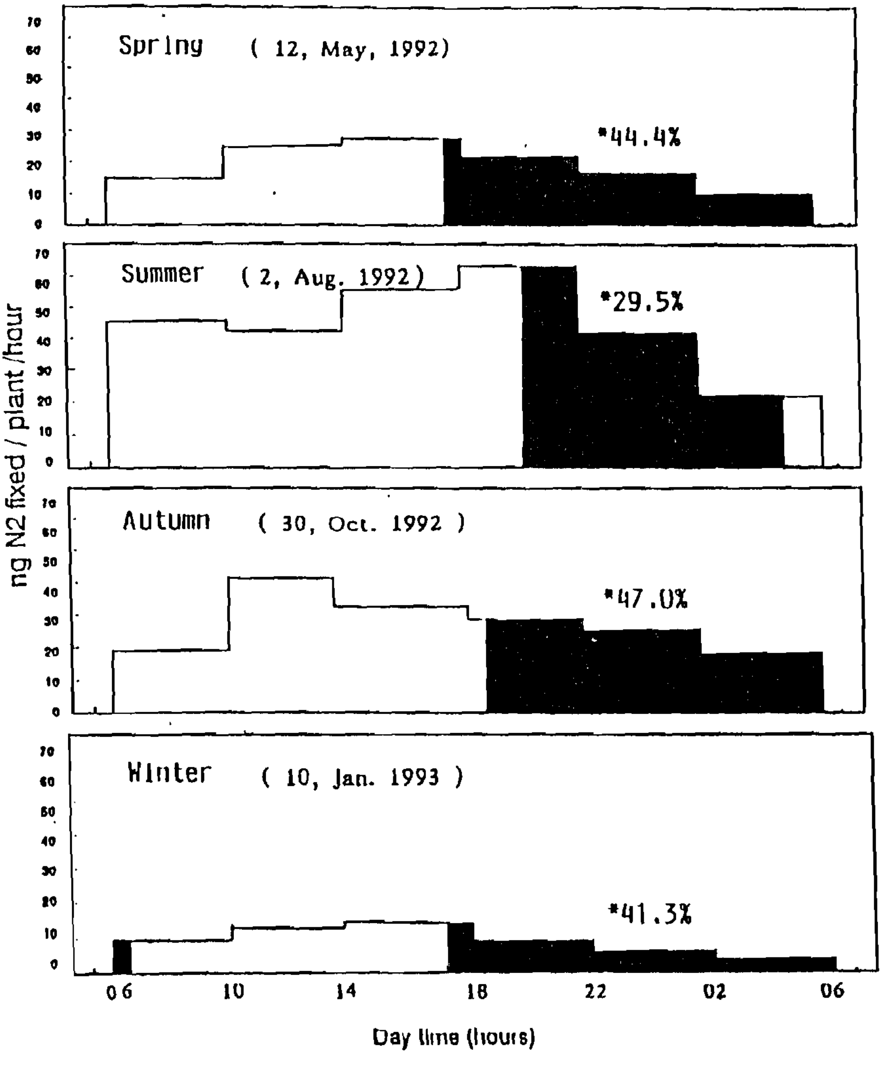

Figure (4): Diel variations in nitrogen fixation by *Azolla filiculoides* during different seasons.

* % of night fixation per day.

gradually to reach maximum in the afternoon, then decreased gradually during the night to early morning. The same patterns were observed with phytoplankton (Lundgren, 1978; Pearl 1979; Kellar and Pearl; 1980).

In summer, the pattern was characterized by two peaks, the first before midday and the second just before night which was similar to those of El-Samra and Olah (1980) and El-Shenawy (1989) using epiphytes associated with Elodea and Ceratophyllum. In autumn, the maximum activity was recorded around noon, followed by gradual decrease to early morning. This was typically like those of Fink and Seeley (1978) and El-Shenawy (1989) by the epiphytes of some aquatic plants.

It has been generally established that there is a correlation between the light and nitrogen fixation because of the close dependence of N_2-fixation on the photosynthetic products (Lewis and Levine 1984). The two peaks characterizing the summer pattern may be due to inhibition of high irradiance in midday (Talley and Rains, 1980; Dey and Kushari, 1986).

Night fixation:

Night fixation activity was recorded in all seasons (Fig. 4). It represented 44.4 %, 29.5 %, 47.0 % and 41.3 % of the Diel fixation in spring, summer, autumn and winter respectively. The night fixation recorded was so high that it must be taken into consideration in calculating the nitrogen budget. These results are higher than that of Tally and Rains (1980). They reported that the night fixation by Azolla filiculoides in California agricultural drainage was 26 % of the Diel ones. According to Horne (1979), the night activity was attributed to the potential accumulation of photosynthetic products in the night period which powered the night fixation process.

Significance of Azolla nitrogen fixed in Lake Edku:

Azolla can accumulate 30 to 60 Kg N hectare^{-1} in 30 days in rice fields (Watanabe, 1987). Recent work (Kumarasinghe et al 1985; Okoronkowo et al, 1989) showed that 80-95 % of the nitrogen accumulated by Azolla represents a major input of nitrogen into the ecosystem.

To calculate the biological nitrogen input by Azolla in Lake Edku, we have measured the areas covered by Azolla and its density (plant m^{-2}) through the year 1992 (Table 4) and multiplied that by the nitrogen fixation activity. The amounts of nitrogen fixed in each season were presented in table (5) (The Diel rhythm per season was taken for calculation of total nitrogen fixed). The input of inorganic nitrogen via drains was computed from the volume and the concentration values of waters discharged annually into the lake. The annual amounts were calculated to be about 56700 tonnes of inorganic nitrogen added to Lake Edku via drains. Table (5) shows that the biological nitrogen input by Azolla to the lake through fixation was 89562 tonnes N $year^{-1}$. This amount was about 1.36 times of the total combined nitrogen input via drains.

Table (4): Density of Azolla filiculoides in Lake Edku during 1992.

	Spring	Summer	Autumn	winter
Azolla density (plant m-2)	2000	3000	5000	4000
Percentage of areas covered by Azolla	5 %	30 %	60 %	45 %

Table (5): Amounts of nitrogen fixed by Azolla filiculoides in Lake Edku during 1992.

	Winter	Spring	Summer	Autumn	Annual N2 fixed
g-N m-2	2.3	39.3	473.7	195.5	710.8 g m-2
Tonnes-N	288.58	4947.8	59684.6	24641.1	89562 tonnes

It is well known that in the natural aquatic ecosystems, nitrogen and phosphorus are the principal limiting nutrients to primary production on one hand and they are the main nutrients responsible for increased eutrophication on the other. However, this estimation is an approximate figure, Azolla as a new vegetation in Lake Edku provides a new source of nitrogen which can be a significant item among the sources of eutrophication and must be taken into consideration for the lake management.

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