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AN ECOLOGICAL STUDY OF *AZOLLA FILICULOIDES* LAM. NEWLY RECORDED IN MISAN WATERBODIES, IRAQ

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Abstract

This study recorded *Azolla filiculoides* Lam. for the first time in Misan aquatic ecosystem in March 2017 after the pre-record for the first time in Iraq by Al-Mayah *et al.* (2016) in Basrah marshland in 2015. Since *A. filiculoides* considered as an invasive species in about 25 country worldwide, a field-ecological study carried out in Huwaizah marshland and Tigris River in Misan province in order to monitor the distribution pattern and to investigate the relationship between *A. filiculoides* and its habitat components in southern Iraqi ecosystem to evaluate its invasion possibility. The study detected that the distribution pattern of *A. filiculoides* associated with environmental conditions (Temperature and water quality), it appeared and developed in early spring and reached its maximum in the mid of summer, then Azolla community begun to deteriorate until it disappeared completely in late fall. *A. filiculoides* interact with many flora and fauna components, it forms a thick mat (4.3-4.6 cm) and affected the underneath submerged plants by reducing its biomass by 16%. Since *A. filiculoides* appeared and disappeared seasonally in southern Iraqi ecosystem, and no economic impact or adverse ecological effects have been recorded, this study suggest no precautions or control programs required since it pose no invasion threat to the ecosystem.

Keywords: Aquatic Plant, Invasive Plant, Huwaizah Marsh, Wetland Ecosystem.

Introduction

Azolla is a fern that floated on water surface can be found in many regions of the world (Mordechi *et al.* 1989; Hussner, 2010). In very rare situations Azolla can inhabit a semi-aquatic habitat (Seckbach, and Grube, 2010). Azolla genera belongs to Azollaceae was a distinct family but now it considered as a synonym to Salviniaceae according to genetic bases (Reid *et al*., 2006), Azolla genera includes 6-7 species (Richard and Keith, 1991; Al-Mayah *et al.* 2016). *Azolla filiculoides* takes a polygonal to triangular shape (Lumpkin and Plucknett, 1980) up to 1 inches. The leaf cavity of *A. filiculoides* inhabited by the Cyanobiont *Anabeana azollae* in form of Azolla-Anabeana system which has ability to provide the total nitrogen requirement of the association (Peters and Meeks, 1989; Plazinski, 1990). Azolla association has higher nitrogen fixation rat compared to the free-living cyanobacteria, therefore, this association system is commonly used as a bio-fertilizer in rice and many other crops (Becking, 1979; Milicia and Favilli, 1992; Pabby *et al.* 2003; Nayak *et al.,* 2004). In addition to Azolla value as an eco-friendly fertilizer (natural nitrogen source) it considered as a higher aquatic macrophytes in terms of protein and amino acid contents especially lysine (Cagauan and Pullin, 1994) therefore, it serves as a stock feed for farm animals, fishes and poultry (Shiomi and Kitoh 2001; Hasan and Chakrabarti 2009; Gowda *et al.,* 2015; Meena *et al.,* 2017; Swain *et al.,* 2018). *A. filiculoides* is a cosmopolitan plant due to its ability to adapt many fresh water environments, it can be found in ponds, marshes, water reservoirs, channels, ditches and slowing rivers, it settles in sunny to partially shady parts of water body (Hussner, 2010). The association with *Anabeana azolla* and the ability of nitrogen fixation enable *A. filiculoides* to thrive in relatively nutrient lack environments. Janes (1998) stated that *A. filiculoides* as the most frost tolerant of Azolla species. It is a fast aquatic fern which floats either individually or forms a mats, it can doubled its surface area in 7-10 days under ideal conditions (Hussner, 2010) where Kitoh *et al.* (1993) recorded a doubling of *A. filiculoides* biomass during 2.2-3.4 days in vitro. *A. filiculoides* can spread and reach new regions via water fowls, in ballast tanks of ships, or by human activities (intentionally or unintentionally). It is one of the fastest alien species that distributed in Egypt among 136 alien species (Shaltout *et al*., 2016). Due to its fast growth, cope with wide range of environmental conditions, adaptation of many dispersal ways and its reported negative impacts on habitats, biodiversity and economy, *A. filiculoides* considered as an invasive species in about 25 country worldwide (McConnachie *et al.,* 2003; McConnachie *et al.,* 2004; Kelly and Maguire 2009; Roxana *et al.,* 2009; Hussner 2010; CABI, 2011; Gratwicke and Marshall, 2011; Sadeghi *et al.,* 2012; DiTomaso *et al*., 2013; Anđelković *et al.,* 2016; Shaltout *et al*., 2016).

A. filiculoides has been recorded in Iraq for the first time by Al-Mayah *et al.* (2016) in Hartha marshland North of Basrah - Southern Iraq in 2015. After two years (March 2017) we recorded it in Huwaizah marshland east of Misan province - southern Iraq and many other locations in the same city. So far, Al-Mayah *et al.* (2016) is the only one who studied *A. filiculoides* in Iraq in his paper where he described its characteristics, habitat of locations where it found and other ecological and taxonomical points. Because of the possibility of *A. filiculoides* to be an invasive plant in Iraqi waterbodies, and in order to discover its distribution pattern in the environment of South of Iraq and its ecological influences, we conducted a field-ecological study over one year (four seasons) to highlight the relationship between *A. filiculoides* and its habitat components and to assess wither any precautions or programs required to control its spread and impacts.

Materials and Methods

Area of Study

During an aquatic plant survey in Huwaizah marsh we recorded *A. filiculoides* for the first time in Misan (Maysan) province in March 2017, then we conducted many trips for waterbodies (marshes, revers, ditches etc.) in Misan in the same month to discover the availability and distribution of *A. filiculoides* across the area. Azolla samples was collected from study sites and brought to the laboratory, species identified according to the key given by (Al-Mayah *et al*., 2016).

Misan is a governorate located southeastern Iraq in border with Iran between 31.15° and 32.45° latitude, it covers about (16702) km2. The mean temperature of Misan climate varied from 37 °C in July to 11 °C in Jun, therefore the diurnal range of temperature of Misan governorate is 24 °C.

Huwaizah marsh is the biggest southern Iraqi wetlands located to the east of the Tigris river and extended towards the Iraqi-Iranian border (Figure 1). The average size of Huwaizah marsh is 2400 km^2 approximately which extends and shrinks seasonally (Al-Thahaibawi *et al*., 2019). It has been inscribed in the Ramsar Convention on Wetlands in 2009 and then included in the UNESCO's World Heritage List in 2016 due to its unique characteristics and biodiversity.

Five sites have been selected for conducting this study, three at Huwaizah marsh and two at Tigris river (Figure 1), description of study sites is given in Table 1.

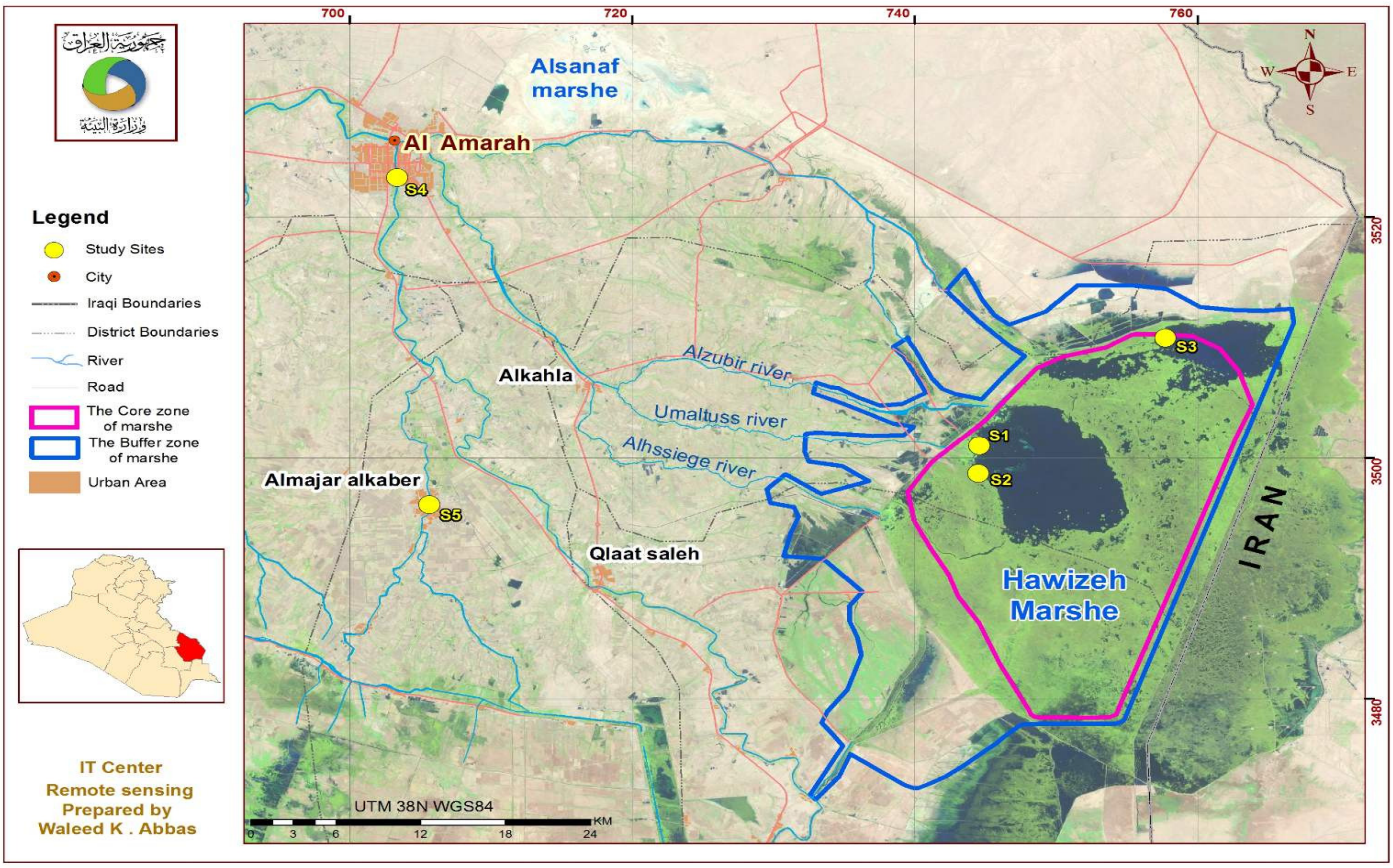

Fig. 1 : Study Area in Misan Governorate, Iraq.

Table 1: Description of study sites

Site No.	Location	Coordinates	Description	Depth*
S1	Huwaizah - Um-Al-Naaj	N 31 37 05.1 E 47 34 42.2	Open water body next to the edge of Chibasha** where the flow water slowed by emergent and submerged plants.	30 – 80 cm
S2	Huwaizah - Um-Al-Naaj	N 313550.4 E 473438.7	Open water body where the flow water slowed by submerged plants.	20 – 76 cm
S3	Huwaizah - Adhaim	N 314145.2 E 474311.3	Open water body	20 – 70 cm
S4	Tigris River - Amara Branch	N 31 49 37.6 E 47 08 55.9	Cliff of the river in city center, where the flow water is slowed by some emergent and submerged plants and some domestic garbage.	Up to 1m
S5	Tigris River - Majar Branch	N 31 34 54.0 E 47 10 05.7	Very slow moving river branch with emergent and submerged plants and some domestic garbage.	Up to 50 cm

* The depth varied over the seasons.

** Chibasha is a local term refers to a small island made by mass of aquatic plants –mainly *Phragmites australis* which make a habitat for many creatures in the open water body of marshland.

Environmental Factors

The study sites were monitored over twelve months from March 2017 till February 2018, mean temperatures were recorded, water pH, EC and depth were measured.

Biomass Production

Three quadrats (0.5 m × 0.5 m) of submerged plants underneath Azolla mat and nearest free Azolla locations were harvested in S1 and S2 to evaluate the biomass production of submerged plants underneath Azolla mat. The collected plants were identified and washed with tap water and left air-

dried, then oven dried at 80 °C and dry weight was determined. The obtained data were analyzed statistically by (ANOVA) test using Statistical Analysis System program (SAS institute, Cary, NC, USA), and means values were compared using (LSD) Fisher's PLSD test at (0.05) level confidence.

The thickness and extending of Azolla mat was measured by measuring tape in S1 and S2 three times (every two weeks) in May 2017.

Interaction with Other Organism:

A. filiculoides association and interaction with other ecosystem biota in the sites was identified and photographed.

Results and Discussion

Presence of *Azolla filiculoides*

The availability of *A. filiculoides* in studied locations varied over different months and seasons as presented in (Table 2, 3, 4, 5 & 6). *A. filiculoides* begun to spread and thrived in Misan aquatic environment with the beginning of spring until it reaches its maximum growth in the mid of summer (Figure 2). Biomass production and Azolla mat extension is increased during April, May and June, it extended its mat more than 200% (double) every two weeks in May 2017 at S1 and S2 (Table 7).

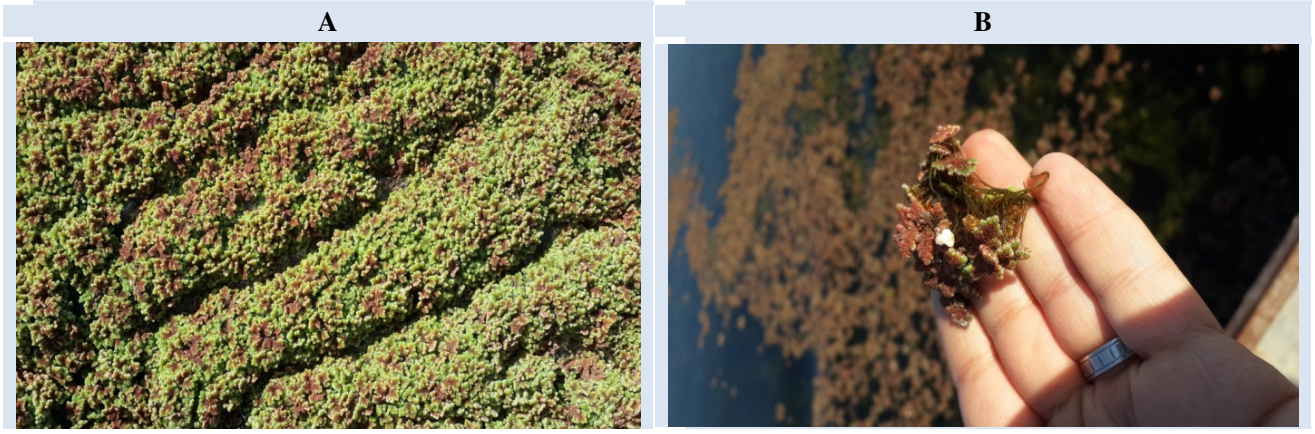

Fig. 2 : (A) *Azolla filiculoides* mat (S2). (B) *Azolla filiculoides* individuals (S1).

Table 2: Presence of Azolla from Mar. 2017 to Feb. 2018 in Site 1**.**

Month & year	Non present	Present as individuals	Present as discontinuous community	Present as continuous community (mat)
Mar. 2017		●		
Apr. 2017			●	
May 2017				●
Jun. 2017				●
Jul. 2017				●
Aug. 2017			●	
Sep. 2017		●		
Oct. 2017		●		
Nov. 2017		●		
Dec. 2017	●			
Jan. 2018	●			
Feb. 2018	●			

Table 3: Presence of Azolla from Mar. 2017 to Feb. 2018 in Site 2.

Month & year	Non present	Present as individuals	Present as discontinuous community	Present as continuous community (mat)
Mar. 2017		●		
Apr. 2017			●	
May 2017				●
Jun. 2017				●
Jul. 2017				●
Aug. 2017		●		
Sep. 2017	●			
Oct. 2017	●			
Nov. 2017	●			
Dec. 2017	●			
Jan. 2018	●			
Feb. 2018	●			

Table 4: Presence of Azolla from Mar. 2017 to Feb. 2018 in Site 3

Month & year	Non present	Present as individuals	Present as discontinuous community	Present as continuous community (mat)
Mar. 2017		●		
Apr. 2017			●	
May 2017				●
Jun. 2017				●
Jul. 2017			●	
Aug. 2017		●		
Sep. 2017	●			
Oct. 2017	●			
Nov. 2017	●			
Dec. 2017	●			
Jan. 2018	●			
Feb. 2018	●			

Table 5: Presence of Azolla from Mar. 2017 to Feb. 2018 in Site 4

Month & year	Non present	Present as individuals	Present as discontinuous community	Present as continuous community (mat)
Mar. 2017		●		
Apr. 2017			●	
May 2017				●
Jun. 2017				●
Jul. 2017				●
Aug. 2017	●			
Sep. 2017	●			
Oct. 2017	●			
Nov. 2017	●			
Dec. 2017	●			
Jan. 2018	●			
Feb. 2018	●			

Table 6: Presence of Azolla from Mar. 2017 to Feb. 2018 in Site 5

Month & year	Non present	Present as individuals	Present as discontinuous community	Present as continuous community (mat)
Mar. 2017			●	
Apr. 2017				●
May 2017				●
Jun. 2017				●
Jul. 2017				●
Aug. 2017			●	
Sep. 2017			●	
Oct. 2017	●			
Nov. 2017	●			
Dec. 2017	●			
Jan. 2018	●			

The thriving of Azolla community in Iraqi southern marshes and other water bodies during spring and early summer months can be explained by the improvement of environmental conditions in terms of temperature and water quality, Cheng and his colleagues indicated that 29/21°C was more suitable for *A. filiculoides* growth (Cheng, W, *et al*. 2010), the expansion of Azolla during late winter and early spring and its response to local warming has been discussed by (José, *et. al*. 2014). Water level is increased, therefore, water quality is improved relatively during spring and early summer in the water bodies southern of Iraq due to the prior rainy season and increasing water releases from Turkish and Iran.

Table 7: Azolla Mat Extension during May 2017.

Site 1		
Measuring Date	**Length (cm)**	**Extension rate (%)**
02.05.2017	145	-
15.05.2015	360	248.27
30.05.2017	830	230.55
Site 2		
Measuring Date	**Length (cm)**	**Extension rate (%)**
02.05.2017	215	-
15.05.2015	523	243.25
30.05.2017	1212	231.74

In S1, S2, S3 and S5 Azolla community begun to deteriorate in August (Figure 3) from continuous community to discontinuous community, individual plants until it disappeared in November.

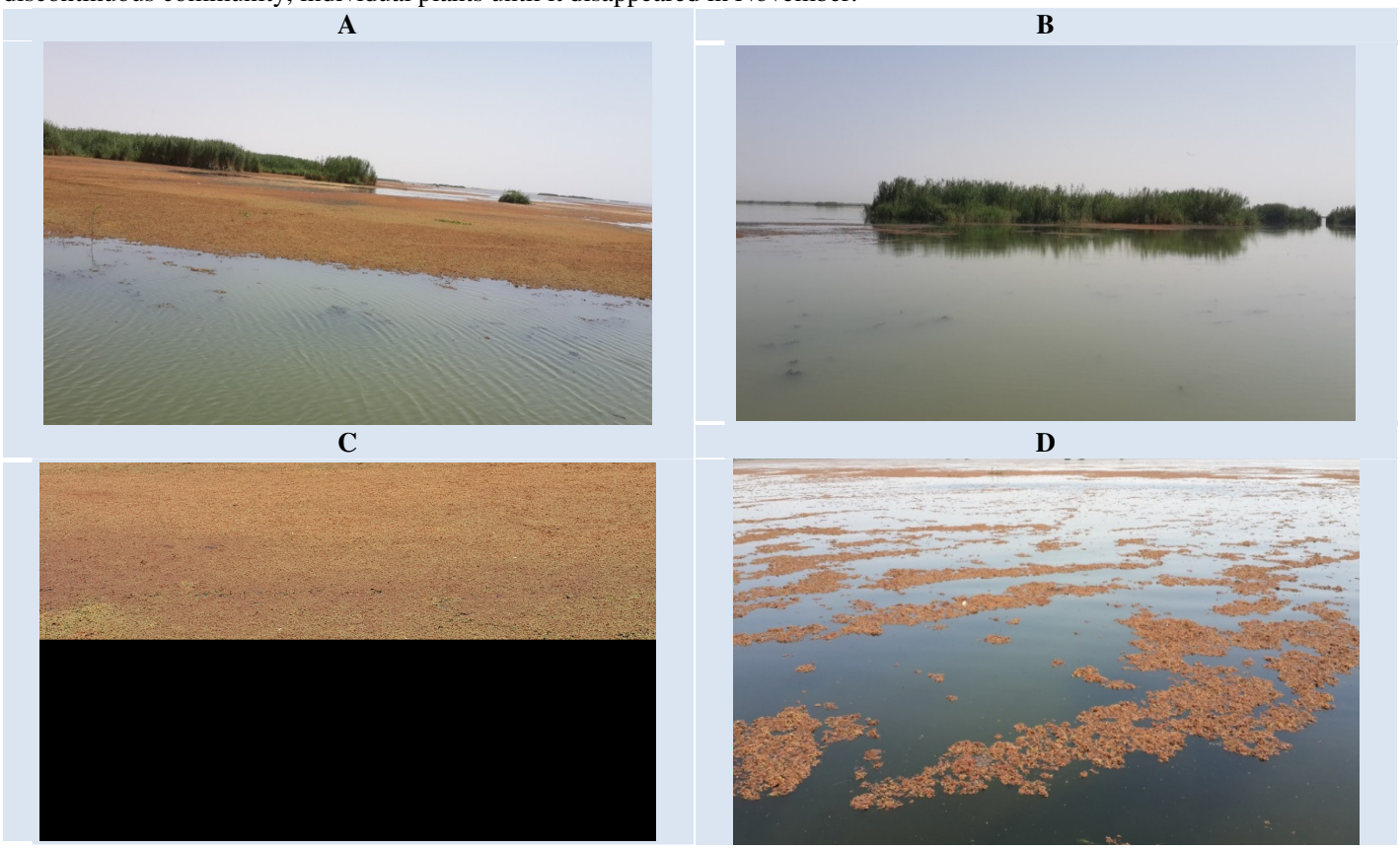

Fig. 3. (A) Azolla community thrives at Jun 2017 (S1). (B) Azolla community deteriorated at August 2017 (S1). (C) Azolla community thrives at Jun 2017 (S2). (D) Azolla community deteriorated at August 2017 (S2).

This deterioration of *A. filiculoides* community owing to environmental stress, the temperature degree raised to its maximum in July and August where it reaches up to 49 °C (with max. temp. mean 46°C in August) as shown in (Table 8). There have been many studied regarding the variation in temperature responses of *A. filiculoides*, optimum temperature ranges for *A. filiculoides* between 25 - 30°C (Ashton and Walmsely, 1984), however, temperature up to 35°C can be inhibit and harmful for *A. filiculoides* growth (R. Sadeghi, *et al*. 2013).

Water quality also declined with continuation of summer forward fall season due to high evaporation rate and decreasing of water releases to Tigris river and its associated marshes which leads to increasing salts concentrations and reached its maximum levels in fall (Sep., Oct. & Nov.) (Table 9), therefore *A. filiculoides* community was affected. Masood, *et al*. (2006) ranked *A. filiculoides* as a salt sensitive, where the growth of *A. filiculoides* was greatly reduced by 38.4% at 10mM NaCl and it failed to stand with 20mM NaCl. In same context, Al-Mayah *et al*. (2012) also stated the disappearance of most of hydrophytes (except salt tolerance species) from Huwaizah region during September, October and November due to reduction of water inflows from the rivers that feeding the marshland.

Table 8: Maximum Temperatures in Misan during Mar.2017 – Feb.2018.

Month	Temperature °C		
	Least Max.	Highest Max.	Max. Mean
Mar. 2017	19	26	22.5
Apr. 2017	21	35	28
May 2017	23	42	32.5
Jun. 2017	40	45	42.5
Jul. 2017	44	47	45.5
Aug. 2017	44	49	46.5
Sep. 2017	40	44	42
Oct. 2017	32	38	35
Nov. 2017	26	35	30.5
Dec. 2017	20	25	22.5
Jan. 2018	19	23	21
Feb. 2018	19	24	21.5

Table 9: pH and EC values of study sites from Mar.2017 to Feb.2018.

Month	S1		S2		S3		S4		S5	
	EC (ms/cm)	pH	EC (ms/cm)	pH	EC (ms/cm)	pH	EC (ms/cm)	pH	EC (ms/cm)	pH
Mar. 2017	3120	7.4	2920	7.4	3615	7.35	2275	7.3	2496	7.4
Apr. 2017	2066	7.1	2566	7.2	3980	7.7	1780	7.1	2120	7.7
May 2017	1032	7.8	1132	7.8	1661	7.2	1175	7.4	1105	7.6
Jun. 2017	2675	7.9	2375	7.7	5030	8.2	1312	7.5	1285	7.5
Jul. 2017	3320	8.1	3422	7.9	7140	8.2	1980	7.7	1874	7.7
Aug. 2017	3531	8.2	3611	8.0	3571	7.9	2371	7.5	2180	7.5
Sep. 2017	4600	8.2	3730	8.3	4310	8.1	2765	7.6	2414	7.2
Oct. 2017	3920	7.8	3803	7.6	14160	8.3	2580	7.6	2523	7.5
Nov. 2017	4213	7.8	4142	7.7	13250	8.3	2622	7.5	2516	7.4
Dec. 2017	5422	7.4	5212	7.7	13870	8.2	2137	7.4	2246	7.5
Jan. 2018	2571	7.8	3220	8.1	14221	8.3	2200	7.5	2276	7.4
Mar. 2017	2950	8.2	2836	8.5	12530	7.8	2270	7.4	2337	7.4

The disappearance of Azolla from S4 was earlier compared to other studied sites which was completely vanished during late July and early August 2017 (Table 5). That is because of dissimilarity of sites habitat, S4 is a relatively wide river (approximate 100 m) with fast water flow, the *A. filiculoides* settled in the cliff of the river where the flow water is slowed down by some emergent and submerged plants (mainly *Phragmites australis* and *Ceratophyllum demersum*) and some domestic garbage. However, in July the water level is decreased and water regression from the cliff puling Azolla toward river center which is moved freely and no more suitable for free floating plants to be established.

Plant Associations and Accompanying

A. filiculoides associated and interacted with many flora and fauna species in the studied habitats. *Phragmites australis, Typha australis* (emergent plants) and *Ceratophyllum demersum* (submerged) provided a slow moved water and allow Azolla to settled its community in S4 and S5. However, in S1 (Figure 4) along with *P. australis* and *C. demersum* some other submerged plants provide the same service for Azolla like *Hydrilla verticillata* and *Najas armata*. Whereas, in S2 and S3 only submerged plants were found to slow the water flow down and enable Azolla to grow.

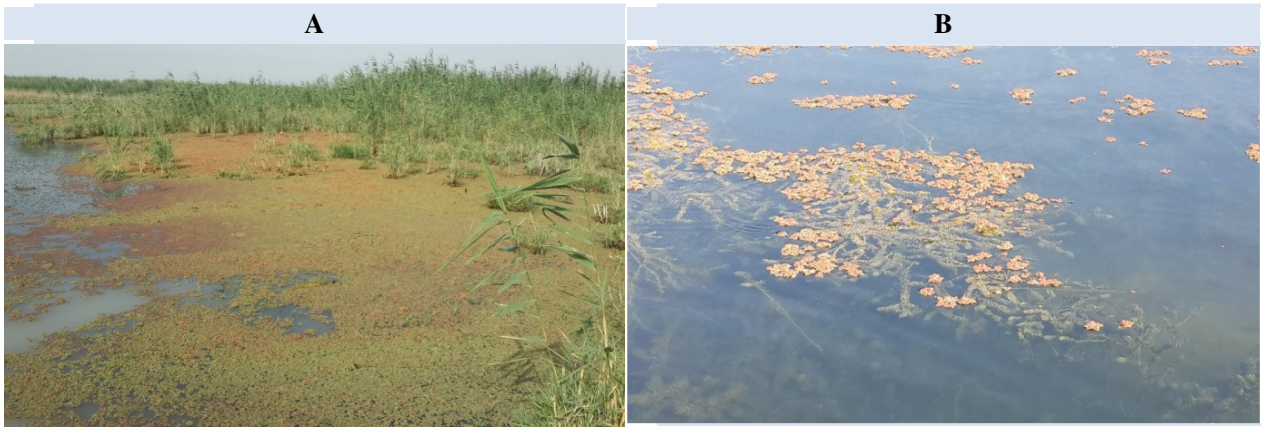

Fig. 4. (A) Emergent aquatic plants (*Phragmites australis* and *Typha australis*) slowing moved water down and helping *Azolla filiculoides* to be settled. (B) Submerged aquatic plants (*Ceratophyllum demersum*, *Hydrilla verticillata* and *Najas armata*) served *Azolla filiculoides* to be settled in moving water.

On the other hand, the submerged plants affected by Azolla mat as indicated by the reduction of biomass production by 16% compared with non-Azolla areas (Table 10). The negative impact of Azolla mat on submerged plants and algae also reported by (Janes *et al.* 1996) by affecting their photosynthesis and blocking O_2 diffusion.

Table 10: Effect of Azolla Mat on Submerged Plants Biomass.

Quadrat No.	Site 1		Site 2	
	Dry weight gm/m^2		Dry weight gm/m^2	
	Under Azolla Mat *	No Azolla Coverage	Under Azolla Mat *	No Azolla Coverage
1	572.5	462.5	562.3	438.5
2	553	463.2	581.4	425
3	565.7	485.7	557.7	472.6
Mean	563.73 A	470.467 B	567.13 A	472.033 B
LSD	26.443		22.881	
Biomass Reduction Percentage	16.45 %		16.77 %	

* *Azolla filiculoides* mat thickness: 4.3 – 4.6 cm.

Lemna minor, Salvinia natans, *Persicaria lapathifolia, Bacopa monnieri* and *Jussiaea repens* recorder in many sites as associated plants with *A. filiculoides* (Figure 5).

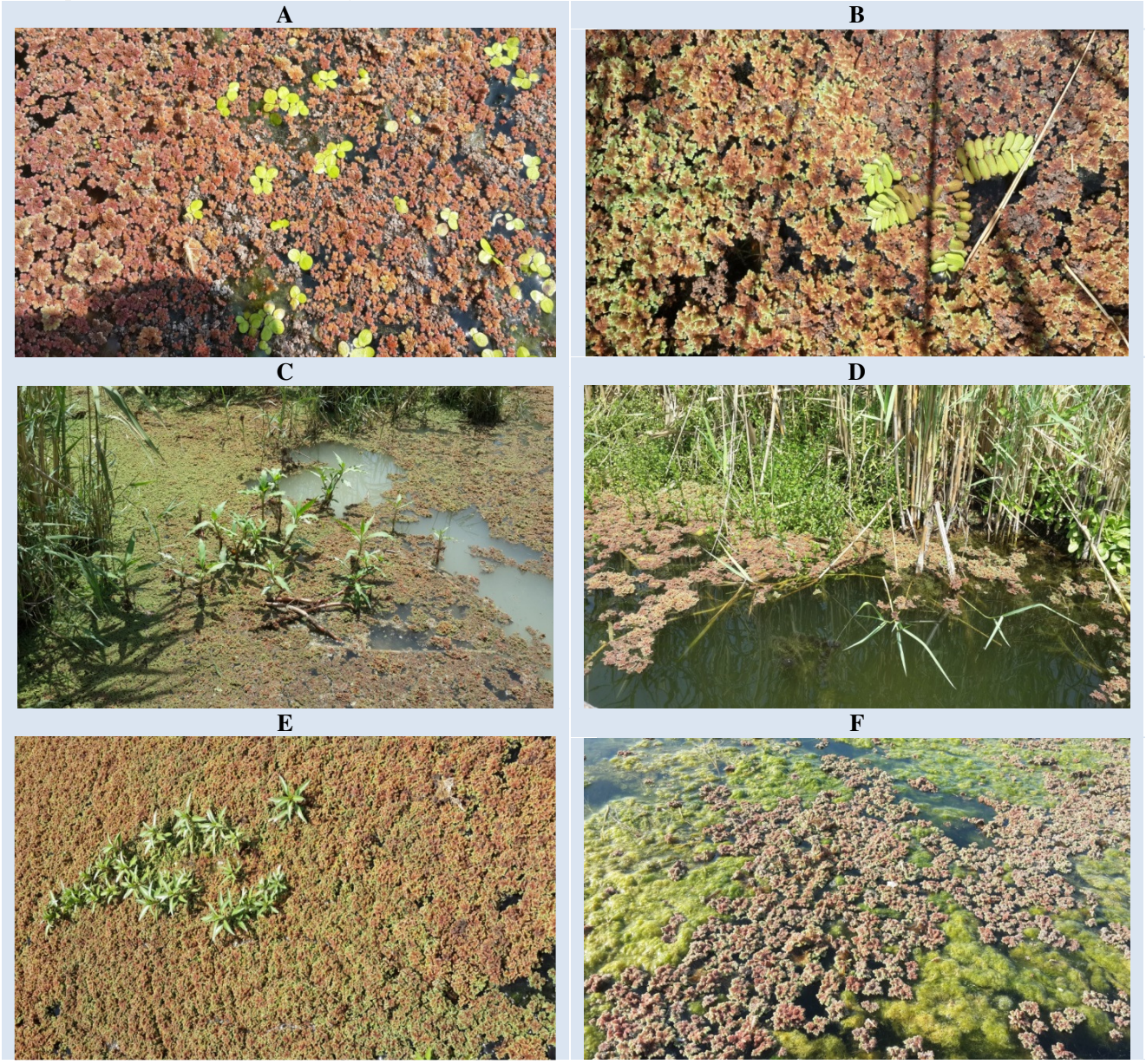

Fig. 5 : Azolla Association with Other Aquatic Plants: (A) Azolla and *Lemna minor* L. (B) Azolla and *Salvinia natans* L. (S1). (C) Azolla and *Persicaria lapathifolia* (D) Azolla and *Bacopa monnieri* (E) Azolla and *Jussiaea repens* L. (F) Azolla and Algae (*Cladophora crispata L.*), All Photos are Taken in Huwaizah Marsh.

The emergent plants (*Phragmites australis* and *Typha australis*) provided a shelter from direct sun during summer, this effect was indicated in variation of Azolla mat color located next to *P. australis* community where it varied from green plants under partially shade to red color under direct sun (Figure 6). *A. filiculoides* terns to red when it under environmental stress whether extreme hot or cold (Kelly and Maguire, 2009; Hussner, 2010).

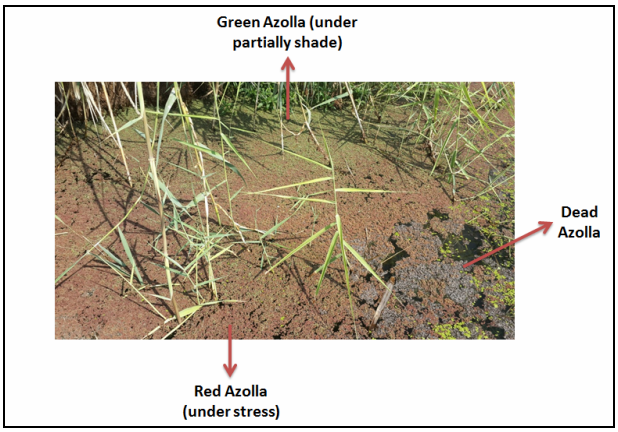

Fig. 6 : Different Azolla Color: from Green (Azolla under Partially Shade of Emergent Plants) to Radish (Azolla under Heat Stress and Direct Sun Light), Photo Taken in S1 (10.Aug. 2017).

Interaction between Azolla and some fauna also recorded during sites monitoring, Azolla mate provides a platform for the activities of many insects, arthropods and worms as shown in (Figure 7).

Fig. 7: Azolla Interaction with Worms and Arthropods: (A) Azolla and Worms. (B) Azolla and Butterfly. (C) Azolla and Anisoptera. (D) & (E) Azolla and Different Arthropods. (F) Arthropods Pupa on Azolla Mat, All Photos are Taken in Huwaizah Marsh.

Conclusion

This study indicated that the distribution pattern of *A. filiculoides* in Misan revers and Huwaizah marshland do not represent an invasion threats to the aquatic ecology of southern Iraq within environmental conditions that recorded along with four seasons (March 2017 - February 2018). The Azolla individuals appeared in early spring in some stand and slow moved water and reproduced gradually until it forms a dens mats during early and mid of summer, then Azolla community deteriorated until it disappeared during late summer and fall season due to environmental stress (temperature and water quality). No adverse impacts of *A. filiculoides* observed on other flora, fauna or economic impacts as it found in other countries where *A. filiculoides* considered as an invasive species (CABI 2011).

Recommendations

A comprehensive investigation of distribution pattern and spread of *A. filiculoides* involved other regions of Iraq over different years and thorough study of interaction with submerged fauna (like fishes) will be useful to understand the ecological effect of *A. filiculoides* as a new species to the aquatic pteridoflora of Iraq.

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