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[Review]

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A review of some ecological factors affecting the growth of *Azolla spp.*

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ABSTRACT

The genus *Azolla* forms a group of small-leafed, floating aquatic ferns native to the tropics, subtropics, and warm temperate regions of Africa, Asia, and America. For several decades, these ferns have been utilized for various purposes: e.g. as green manure, feed for animals, but also for the removal of different metals (e.g. Hg, Pb, Cr and Cd) through wastewater treatment or for elimination of nitrogenous compounds from surface water. Notwithstanding, these many advantages of *Azolla*, it has invaded many natural habitats, thus becoming an obnoxious weed. *Azolla* can grow quickly with a doubling time of only 2-5 days and form very dense mats in favourable habitats, causing many difficulties for boat transport, water animals and native plant species and becoming a source of eutrophication. The present paper gives an overview of some important ecological factors affecting *Azolla*'s growth over the past few decades. Moreover, for the most ecological variables discussed in this study, the authors refer to their recent publications for the habitat requirements of *Azolla* in Anzali wetland. Water availability is the key factor for its growth. Growth is further promoted by optimal light intensity (15-18 Klux), temperature (18°- 28°C) and relative humidity (55-83%). Wind and turbulent water can fragment and kill *Azolla*. The importance of both macro (e.g. phosphorus, nitrogen, potassium, calcium and magnesium) and micronutrients (e.g. molybdenum, cobalt and etc.) has also been confirmed from literature. Various types of insects (e.g. caterpillars), bacteria, fungi and viruses can affect *Azolla* growth. As a conclusion, understanding the habitat requirements of *Azolla* is very helpful for managing this aquatic fern, also for decision making in the context of wetland restoration and conservation management.

Keywords: *Alien species, Azolla, physico-chemical variables, structural habitat variables.*

INTRODUCTION

Azolla is a genus of aquatic ferns and small-leafed floating plants, native to the tropics, subtropics, and warm temperate regions of Africa, Asia, and the Americas (Costa *et al.*, 2009). The genus of Azolla spp. is very sensitive to lack of water in aquatic ecosystems such as Stagnant waters, ponds, ditches, canals or paddy fields. These areas may be seasonally covered by a mat of Azolla associated with other free-floating plants species such as Lemna minor L. (duckweed), Pistia stratiotes L. (water lettuce), *Trapa natans* L. (water caltrop), *Wolffia* Horkel ex Schleid (water meal) or *Salvinia molesta* D. S. Mitch and mud-rooting species such as *Ceratophyllum demersum* L. (hornwort), *Ludwigia palustris* (L.) Elliott (water-purslane or water-primrose), *Polygonum arenastrum* Boreau (knotweed) and *Neptunia* Lour (Kannaiyan and Kumar, 2006).

Azolla is one of the world's fastest growing aquatic macrophytes, with a doubling time of only 2-5 days (Taghi-Ganji *et al.*, 2005; Zimmerman, 1985). Though, *Azolla* species have various benefits, they are also considered as annoying weeds, in particular *A. pinnata* (R.Br.) and *A. filiculoides* (Lam.) (e.g. Barreto *et al.*, 2000). Many studies have mentioned *Azolla* as a weed (Bodle, 2008; Delnavaz and Azimi, 2009; Hill, 2003; Kay and Hoyle, 2000). For instance, the North American native *A. filiculoides* has invaded many places in Europe and South Africa (Hill, 2003), where it is now considered as an important exotic weed. *A. pinnata* is another example of an obnoxious weed (Kay and Hoyle, 2000). This fern became naturalized in North Carolina (US) in 1999, where it continues to be present (Bodle, 2008), and also in New Zealand where it displaced the native *A. rubra* in most parts of the country. *A. filiculoides* is also an alien species in Iran (JICA, 2005; Delnavaz and Azimi, 2009). In fact, such invasive aquatic ferns are a major concern for biologists and ecologists dealing with conservation and management of wetland ecosystems due to the threats they may pose to the rich original biological diversity. They can have important harmful and irreversible impacts on wetlands as they may change the local fauna and flora (Sax *et al.,* 2005; Vander Zanden and Olden, 2008). They may reduce the ecological quality through changes in biological, chemical and physical properties of aquatic ecosystems (Boets *et al.,* 2009; Olenin *et al.,* 2007). Some of the biological changes consist of eradication of susceptible or rare species, alteration of native communities and algal blooms. The modification of substrate conditions and the shore zones, alterations of oxygen and nutrient contents, pH and transparency of the water and accumulation of pollutants are also some examples of physical–chemical changes (Olenin *et al.,* 2007). In contrast to native species, invaders can survive and reproduce in a wide range of environmental conditions (Devin and Beisel, 2007; Karatayev *et al.,* 2009). They are often more tolerant to pollution (Wijnhoven *et al.,* 2003; Devin and Beisel, 2007; Normant *et al.,* 2007). *A. filiculoides* is such a particular example that has invaded many aquatic ecosystems in the northern part of Iran (e.g. Anzali wetland). Due to its massive spread in the wetland, some plant species occupying the same ecological niche such as duckweed (*Lemna minor*) completely disappeared and some species such as *T. natans* are facing serious problem (JICA, 2005; Sadeghi *et al*., 2012; Sadeghi *et al*., 2013).

Objectives And Methodology

Until now, many publications have focused on the *Azolla*-*Anabaena* association and nitrogen fixation in *Azolla* (e.g. Van Hove and Lejeune, 2002; Arora and Singh, 2003; Fernández-Zamudio *et al*., 2010), while very little is known about its habitat needs particularly as an invasive species in a new environment. Recently, Sadeghi *et al.* (2012a, b) have conducted a project in the Anzali wetland about the habitat requirements of *A. filiculoides*.

Knowing the habitat requirements of this fern can be very helpful for developing decision making in the context of wetland restoration and conservation management.

The literature search of the present paper is based on different *Azolla* species. The authors used all available information as published on web of science (WOS). These sources consist of different databases containing information gathered from scholarly journals, books and book series. On the basis of this, this paper aims to give a thorough review of the most important abiotic and biotic factors affecting *Azolla* growth since the last few decades till now (without time limitation). Moreover, for the most structural habitat and water quality variables reviewed in the present paper, the authors cite their recent publications for the habitat requirements of *Azolla* in the Anzali wetland. The relationship between abiotic factors (physical-chemical and structural habitat factors) and biotic characteristics and *Azolla* can give more insight into how to manage this mosquito fern and hence aquatic ecosystems. Since date of publications is unlimited, some conflicting ideas of information might be expected. Therefore, there might be inconsistencies in some parts of abiotic and biotic data presented in this review. It is worth mentioning that each laboratory or research group might use specific analytical tools. Therefore, we strongly recommend readers to find the original articles for more detailed analytical methods and interpretation of results; all resources used for this review are duly cited.

Origin and distribution of *Azolla* species

It is believed that *Azolla* domestication dates back to the 11th century and was first done in Vietnam. The genus *Azolla* was botanically established by Lamarck in 1783 (Kannaiyan and Kumar, 2006). The species of *Azolla* are divided into two subgenera: *Euazolla* and *Rhizosperma* (Mey.) Strasb based on the sporocarp characters (e.g. Sood *et al*., 2007). Some other authors use the taxonomic "section" level instead of subgenus (e.g. Saunders and Fowler, 1992). Subgenus (section) *Azolla* has 5 species, namely *A. caroliniana* Willd., *A. filiculoides* Lam., *A. mexicana* Presl., *A. microphylla* Kaulf. and *A. rubra* R.Br., while section *Rhizosperma* has only 2 species called *A. nilotica* Decne. ex Mett. (NI) and *A. pinnata* R. Br. (Raja *et al*., 2012).

According to Kannaiyan and Kumar (2006), *Azolla* species distribution corresponds to fresh water ecosystems of temperate and tropical regions all over the world (Fig. 1). A study of the distribution of *Azolla* has indicated that species of section *Euazolla* have originated from North and South America: 1) *A. filiculoides*, which occurs in southern South America, and western North America to Alaska; 2) *A. caroliniana*, eastern North America, central America, north South America, the Caribbean, Mexico and West Indies; 3) *A. mexicana*, northern South America to British Columbia, western North America and eastward to Illinois; and 4) *A. microphylla*, western and northern South America to southern North America and the West Indies.

Section *Rhizosperma* covers only two species and is distributed as follows: 1) *A. pinnata*, tropical Africa and southern Africa, South East Asia, Japan and Australia; and 2) *A. nilotica*, central Africa, upper Nile Sudan, Uganda, Tanzania, Congo and Namibia (Carrapiço *et al.*, 2000; Kannaiyan and Kumar, 2006).

Among the *Azolla* species, *A. filiculoides* is the only fern which is found in Anzali wetland, northern Iran (Sadeghi *et al.*, 2012a, b; Sadeghi *et al*., 2013). This wetland is an international aquatic ecosystem based on Ramsar convention (1971) which is located in northern Iran, at 37°28'N, 49°25'W (Imanpour Namin *et al*., 2011) and south west of the Caspian Sea. It is an important ecosystem for various aquatic plants and animals. This ecosystem provides valuable habitat to 43 fish species (Dadras and Kardovani, 2010) in particular for spawning grounds of anadromous fish species such as *Sander lucioperca, Abramis brama* (Moradinasab *et al.*, 2012) and *Rutilus frisii kutum* which is a commercial species (Ahmadnezhad *et al*., 2012; Khara *et al*., 2012; Moradinasab *et al*., 2012;

Pourkazemi and Razikazemi, 2012). Moreover, this wetland is a breeding and wintering area for a wide variety of waterfowl such as wintering ducks, geese and swans (Mansoori, 1995). The overgrowth of *Azolla* is now considered as a big problem in this unique ecosystem as this exotic fern competes with these indigenous fauna and flora for vital resources such as light and nutrients (Fig.2).

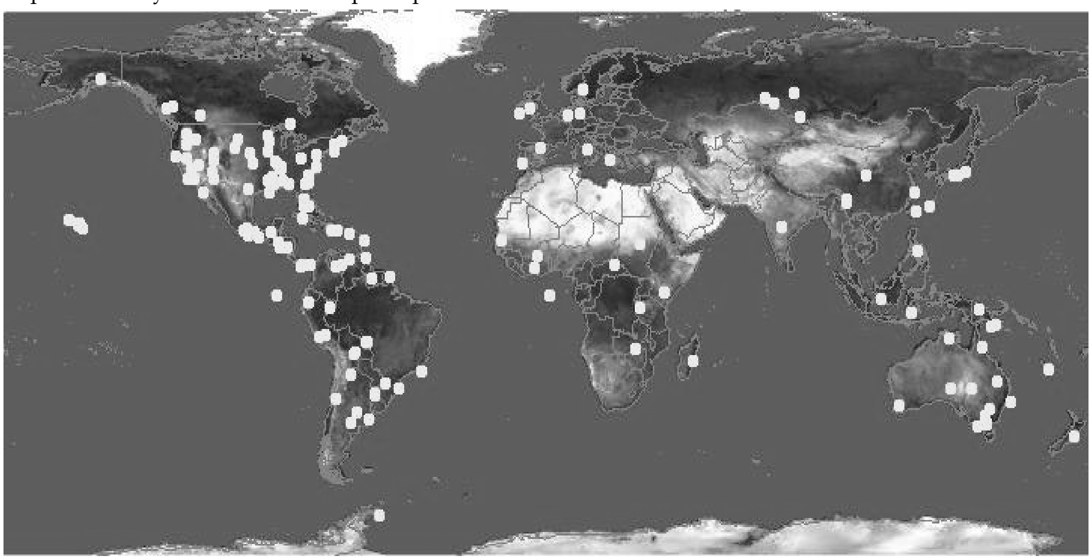

Fig.1. Occurrence and distribution of *Azolla* species represented with white marks in the world (Source: http://www.discoverlife.org).

Fig. 2. *Azolla filiculoides* (photo taken by the authors from Selkeh wildlife refuge, Anzali wetland, in the northern part of Iran).

Main abiotic and biotic factors affecting *Azolla*'s growth

The abiotic characteristics influencing the growth of *Azolla* can be classified into two main categories: structural habitat and physical-chemical factors. Sadeghi *et al.* (2012a, b) reported that for the excessive growth of *Azolla* in Anzali wetland, the structural habitat variables are more important than physical-chemical ones. Moreover, the growth of *Azolla* can also be affected by biological factors. In what follows, we will very briefly describe some of the most important structural habitat and physical-chemical factors to meet the habitat requirements of *Azolla* under laboratory and field conditions

Structural habitat factors

Water

As it is clear from the etymology of *Azolla* (derived from a Greek origin, namely *azo* and *ollyo* which means "killed by drought") (Carrapiço *et al.*, 2000), this fern cannot survive without water. In other words, water is a vital and important factor for the survival of *Azolla*. This small aquatic fern should float on the water surface to stay alive. When enough water is available, it can form a layer with a height of around 2-3 cm that needs less space compared to other aquatic plants (Liu *et al.*, 2008). Though, it is able to grow on a wet mud surface or wetted peat litter, this fern prefers to grow in free-floating conditions (Serag *et al.*, 2000) on calm water surfaces, and may thus be found on the surface of ponds, canals, and lakes as well as on some slow-moving rivers (Ghorbanzadeh and Tajer Mohammad, 2009). It was reported that the optimal growth and biomass production of *Azolla* in the Anzali wetland might have a close relation to water depth (Sadeghi *et al.*, 2012a, b) since very low water depths might slow down the growth and hence reduce its biomass production (Biswas *et al.*, 2005).

Air and water temperature

Temperature is one of the most important factors determining growth rates of free-floating macrophytes in the field (Van Der Heide *et al.*, 2006). Some free-floating plants such as red water fern (*A. filiculoides*), water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) can grow very quickly and thus cause severe problems mainly in tropical and subtropical regions (Mehra *et al.*, 1999; Hill, 2003; Van Der Heide *et al.*, 2006; Sadeghi *et al.*, 2012a, b). There have been numerous studies about the differences in temperature responses of *Azolla* species and their eco-physiological strains indicating that a very high (above 30°C) or very low temperature (below -4°C) could play an inhibitory role in the growth of *Azolla* (e.g. Fernández-Zamudio *et al.*, 2010; Liu *et al.*, 2008; Serag *et al.*, 2000; Sadeghi *et al*., 2012a).

The optimum temperature range for *Azolla* growth has been shown to be between 18 and 28°C (e.g. Tuan and Thuyet, 1979). In fact, higher temperatures (e.g. 35°C) can inhibit or even be harmful for its growth. However, different *Azolla* species, strains or varieties have different temperature sensitivities (Uheda *et al.*, 1999). Optimum temperature ranges for *A. caroliniana*, *A. filiculoides* (Ashton and Walmsely, 1984) and *A. pinnata* are between 25 and 30°C. *A. mexicana* will not tolerate frost, while *A. filiculoides* will survive it (Talley and Rains, 1980).

Debusk and Reddy (1987) studied growth and nutrient uptake of *A. caroliniana* Willd, and *Salvinia rotundifolia* Willd under controlled conditions at constant temperatures (from 10 to 30°C). Maximum plant density was obtained at a temperature range of 15-20°C. Net primary productivity was highest within the 20-30°C range.

Light intensity

Photosynthetic activity, growth and nitrogen fixation of *Azolla* and its symbiont are all affected by light intensity (Pabby *et al.*, 2003). Sporulation is regulated by the interacting effects of light intensity, photoperiod, temperature and other factors such as pH, nitrogen and phosphate supply (Pabby *et al.*, 2003). When light intensity is high and amount of nutrients in water is low, *Azolla* turns red. During hot summer or cold winter, it also turns red or brownish-red when under shaded conditions, whereas in nutrient-rich conditions, it becomes green. Irradiance interacts with temperature in influencing growth of *Azolla* species (Janes, 1998).

Growth rate and nitrogen fixation activity of *Azolla* change with increasing light. Pabby *et al.* (2003) demonstrated that growth rate and nitrogen fixation activity of *Azolla* change with increasing light. Tuan and Thuyet (1979) pointed out that at pH 5, high light intensity increased *Azolla* growth, whereas at pH 6 and 7, it inhibited growth and high light intensities (above 90 Klux) inhibited N_2 fixation, whereas low light intensities or shading had a good effect on *Azolla* growth and multiplication. Sadeghi *et al.* (2012a, b) reported that the availability of sufficient light intensity together with enough air temperature during most of time in the Anzali wetland can give *A. filicoluoides* the opportunity to have optimum growth under field conditions. Bar *et al.* (1991) and Costa *et al.* (2009) stated that light intensity lower than 10-13 Klux can decrease nitrogen fixation in *Azolla*. In fact, this species only requires 25-50% full sunlight for regular growth. In field conditions, *Azolla* benefits from shade for its growth. However, when light intensity is lower than 1.5 Klux, biomass production of *Azolla* significantly decreases (Liu *et al.*, 2008). In conclusion, optimal light intensity for *Azolla* growth is 15-18 Klux, and its growth and photosynthesis are inhibited at higher intensities.

Humidity

Increase in the biomass of *Azolla* relies to some extent on air humidity. At relative humidity of less than 60%, *Azolla* becomes dry and fragile (Bocchi and Malgioglio, 2010). Many authors (e.g. Forni *et al.*, 2001; Costa *et al.*, 2009) found that relative humidity should be between 70 and 75% for optimal growth. Sadeghi *et al.* (2012a) demonstrated that when the air humidity in the Anzali wetland exceeds 80%, the prevalence of *Azolla* would be low. Mean relative humidity for allowing *Azolla* growth was estimated at 55-83% (Lumpkin and Bartholomew, 1986) and it was in the range of 65-75% based on the study of Biswas *et al.* (2005). However, according to the latter authors, for optimum *Azolla* growth and biomass production, high temperature, high humidity, and low water depth may not be good conditions in particular during the dry season. When the weather is completely dry, the fern dies (Biswas *et al.*, 2005). Therefore, *Azolla* needs enough humidity in order to have successful growth and multiplication.

Growing seasons and day length

The length of the growing season and day length are other climatic factors which regulate production of aquatic plants (Serag *et al.*, 2000). Biomass production and growth of *Azolla* are also dependent on the specific growing season. The growing seasons in *Azolla*, however, are linked with other factors such as nutrients, pH, salinity and wind. A water body with rich phosphorus and neutral pH is better than one with poor phosphorus and acidic conditions. Overall, production in summer is higher than in other seasons (Speelman *et al*., 2009).
Water temperature has a close relationship with air temperature. Therefore, any changes in weather conditions over different seasons have an important effect on *Azolla* growth (Ferdoushi *et al.*, 2008). Janes (1998) studied growth and survival of *A. filiculoides* in Britain where it is considered a serious weed. According to the author, long-term survival of *Azolla* might be limited by winter death

Wind And Waves

Wind and waves are other factors affecting the production of aquatic plants, in particular for *Azolla* which is a free-floating fern. In shallow lakes, wind-induced waves can have either direct effects on plant growth (e.g. mechanical damage) or indirect effects such as increased siltation (Vermaat *et al*., 2000; Santamaria, 2002). Since *Azolla* is a small free floating plant, wind and waves are not favourable for its growth. Water agitation can break up the fronds. This fragmentation as such can have a negative effect on N_2 fixation capacity. Therefore, wind and turbulent water can fragment and kill *Azolla*. In addition, big waves (or typhoons) are very harmful for its growth. Bunds, debris, wooden floats and big macrophtes such as *Phragmites* spp and *Typha* spp can protect *Azolla* against winds and waves.

Physical-Chemical Factors

Nitrogen

In contrast to other minerals, availability of nitrogen and to some extent dissolved oxygen are not considered as limiting factors for *Azolla* growth, but growth of algae-free plants relies on combined nitrogen. According to a study (Sadeghi *et al.*, 2012a), an increase in nitrate concentration might result in a low coverage of *Azolla* in the Anzali wetland.
The reason is that through its symbiosis with *Anabaena*, *Azolla* can have free access to atmospheric nitrogen (N_2) for fulfilling all of its N requirements (Costa *et al.*, 2009), while algae-free plants are deprived of such opportunities. The upper lobes of *Azolla* have free contact with air, which is important for nitrogen fixation.
Based on a study by Costa *et al.* (2009), high ammonium concentrations in wastewater had no effect on nitrogen fixation dynamics. Fang *et al.* (2007) surveyed nutrient fluxes along the roots of 4 wetland plants (*Azolla* spp., *Vallisneria natans*, *Bacopa monnieri* and *Ludwigia repens*) in order to see the dynamics of nutrient removal from eutrophicated water systems. When different ions (e.g. NH_4^+, NO_3^-) were tested, *Azolla* spp. showed a preference for NO_3^- uptake.

Growth and nitrogen fixation was estimated at about 3.1 to 4.6 kg N/ha/day in *A. pinnata* under field conditions (Kulasooriya and Hirimburegama, 1982). A seven to eightfold increase in biomass was observed in 15 days resulting in a doubling time of 4.8 days. Most reports indicate a N_2-fixation rate ranging from 0.4 to 3.6 kg N/ha/day (Watanabe, 1982; Roger and Ladha, 1992) depending on the absence/presence of N fertilizers. Based on many studies (Cary and Weerts, 1992; Biswas *et al.*, 2005; Costa *et al.*, 2009), when field conditions are optimal for this species, its N accumulation rate is estimated to be 5.0-9.0 mg N per/g dry weight per day. This can result in a dry matter increase of 0.135-0.290 g per g *Azolla* dry weight per day. This value is consistent with a doubling time of 2.5-5.5 days.

Phosphorus
Similar to many other photoautotrophic aquatic organisms, some nutrients such as phosphorus (in the form of phosphate) are major limiting nutrients for *Azolla* growth. Phosphorus is an important nutrient to yield a successful and rapid growth of these species (El Katony *et al.*, 1996). The effect of this important nutrient on the overgrowth of *Azolla* has also been confirmed in the Anzali wetland (Sadeghi *et al.*, 2012a, b).
In laboratory experiments, Janes (1998) found that increasing phosphorus supply and/or plant density led to increased sporulation. If there is enough phosphorus in the aquatic environment, *Azolla* will be able to grow without the need to provide combined nitrogen such as NH_4NO_3 (Costa *et al.*, 1999).
There have been different reports about the levels of phosphorus needed for sustained *Azolla* spp. growth. In laboratory experiments, a concentration of around 0.06 ppm (2µM) is reported to be adequate to sustain *Azolla* growth. A range between 0.3 and 1 ppm (10 to 33µM) is suggested from field surveys. Optimum growth of different *Azolla* species responds to different concentrations of phosphorus (Kushari and Watanabe, 1992).

Salinity
Various studies have shown the negative effect of salinity on growth of different *Azolla* species. There are still no in depth studies about variation in salt tolerance of different species of *Azolla*. Overall, however, this genus is considered to be extremely sensitive to NaCl (Rai and Rai, 2000; Fernández-Zamudio *et al.*, 2010). Masood *et al.* (2006) stated that salinity inhibits growth of *A. pinnata* and *A. filiculoides* leading to a significant decrease in dry weight. Therefore, the growth of *A. pinnata* and *A. filiculoides* plants gradually decreased when NaCl concentration increased. *A. filiculoides* was more sensitive to high salt concentration than *A. pinnata.* Salt concentrations above 10mM NaCl inhibited growth of *A. filiculoides,* but growth of *A. pinnata* was only stopped at 40mM NaCl.
The presence of nutrients like nitrate in the saline habitat plays a key role in conveying salt tolerance to *Azolla* (Mishra and Singh, 2006; Singh *et al.*, 2008). Fernández-Zamudio *et al.* (2010) studied germination characteristics and spore germination success of *A. filiculoides* (as an invasive fern, in a Mediterranean temporary wetland). Germination rate showed a significant decrease when salinity increased.
Arora and Singh (2003) conducted a study on six different *Azolla* species (*filiculoides*, *mexicana*, *microphylla*, *pinnata*, *rubra* and *caroliniana*) in New Delhi. Based on the observations, salinity drastically decreased biomass production in all 6 species. *A. microphylla* showed high tolerance to salinity than other species.
Khosravi *et al.* (2005) studied the toxic effect of heavy metals (Pb, Cd, Ni and Zn) on growth of *A. filiculoides* in Anzali wetland (Iran). It was stated that increasing salt concentration in water (in terms of NaCl) can reduce the removal of heavy metals by *Azolla*. In other words, high salinity level can inhibit *Azolla* growth (Arora and Singh 2003; Fernández-Zamudio *et al.,* 2010) leading to a decrease in removal of heavy metals by the species. Sadeghi *et al.* (2012b) found that salinity can play an intermediately role on the growth of *A. filiculoides* in the Anzali wetland.

pH
The response of *Azolla* to pH depends on many factors such as temperature, light intensity, nutrients (nitrogen and phosphorus), and the presence of soil and iron (Wagner, 1997). Usually, *Azolla* prefers a medium near to neutrality or to some extent, acidic conditions. The optimal growth pH varies from 4.5 to 7.5 (e.g. Cary and Weerts, 1992). *A. filiculoides* and *A. pinnata* can, however, grow well at pH values between 5 and 7, while *A. pinnata* can grow relatively well in a pH range between 5 and 8, while *A. filiculoides* only grows well in the 5 and 7 range (Cary and Weerts, 1992). The range of the given pH was also very close to the growth of *A. filiculoides* in the Anzali wetland (Sadeghi *et al.*, 2012b).
Serag *et al.* (2000) demonstrated that optimal growth is at pH of the culture solution between 4.5 and 7, but *Azolla* can survive even at pH values ranging from 3.5 to 10 (provided all important elements are available). Mousa (1994) demonstrated that under Egyptian conditions, *Azolla* grows well with a pH ranging from 7.1 to 9.0. As a conclusion, optimum growth in *Azolla* is dependent not only on pH but also on other environmental conditions.

Other Nutrients
In addition to the nutrients described above, other macronutrients (e.g. potassium, calcium and magnesium) are also needed for *Azolla* growth. Moreover, *Azolla* requires micronutrients (e.g. Mo^{6+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+} and Co^{2+}) in order to have an optimal growth. Some of these macro and micronutrients are briefly described below.

Macronutrients
Macronutrients like potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) (Serag *et al.,* 2000) are also very important to yield a successful and rapid growth of these species (Biswas *et al.*, 2005). Among the given nutrients, the magnesium was recognized as an important factor in order to meet the habitat requirements

of *Azolla* in the ecosystem of Anzali wetland (Sadeghi *et al.,* 2012b). Potassium and organic compound of nitrogen and phosphorus in the biomass of *Azolla* can be good indicators for its use as a biofertilizer in domestic wastewaters or natural environments (Costa *et al*., 1999).

Azolla is able to take up potassium from the soil and make it available to the rice plants. A good source of macro and micronutrients could be cow manure which is suitable for *Azolla* growth. Compared to the other nutrients sources, biomass production of *Azolla* can be increased by providing cow manure to the growth medium (Biswas *et al*., 2005).

Micronutrients

The importance of some micronutrients (Biswas *et al*., 2005) such as iron and trace elements like Mo has long been recognized for a successful and quick growth of *Azolla,* particularly in relation with its nitrogen fixation metabolism. Nitrogen fixation by *Azolla-Anabaena* requires cobalt and molybdenum (Zahran *et al*., 2007). Singh *et al.* (2010) studied the effect of micronutrients (e.g. Mo^{6+}, Mn^{2+}, Zn^{2+}, Cu^{2+} and Fe^{2+}) on cellular and extracellular activities of two *Azolla* species (*A. microphylla* and *A. filiculoides*) exposed to a P-deficient, saline (20mM NaCl) medium. At lower concentrations (0-0.01mM), the micronutrients showed a significant enhancement in the given activity, whereas higher concentrations (e.g. at 10 mM) played an inhibitory role. Sadeghi *et al.* (2012b) reported a moderate effect of Fe on the growth of *A. filiculoides* in the Anzali wetland. Wagner (1997), on the other hand, examined that the threshold levels of the micronutrients such as Fe, Mn, Mo, and B for *Azolla* growth were 50, 20, 0.3, and 30 μgL^{-1}, respectively. The availability of Fe to be accessed by *Azolla* itself is influenced by pH value. When pH value is high, iron is precipitated as hydroxide so that it becomes less available to *Azolla*. When *Azolla* has no access to iron, it turns yellow.

Jain *et al*. (1992) studied the toxic effect of some elements on *Azolla*. Their study showed that iron and manganese did not have any toxic effect on the anaerobic fermentation of *Azolla*, while copper, cobalt, lead and zinc showed toxicity.

Biological Factors

The presence of some macrophytes communities such as (e.g. *Phragmites* spp and *Typha* spp) can play an important role for the overgrowth of *Azolla* in wetlands. In the shallow part of wetlands, macrophytes occupy the wetland (JICA, 2005, Sadeghi *et al*., 2012a, b). This provides a good opportunity for the distribution of *Azolla* because *Phragmites* create a windbreak and shelter to this species.

In contrast, some biological factors negatively affect *Azolla* growth. For example, insects such as Lepidoptera (caterpillars) and Diptera, as well as Cephalapoda, Crustaceae and snails affect growth of *Azolla* by "grazing" on its biomass. Use of chemical pesticides or herbicides is considered to be harmful for its growth (McConnachie *et al*., 2003). Hence, these biological factors can be used for controlling *Azolla*. Mechanical removal for some *Azolla* species (e.g. *A. filiculoides*) will not be practical because of the rapid rate of surface area doubling time (McConnachie *et al*., 2003). Biological control of weeds like *Azolla* could be a viable option provided the target plant population can be significantly reduced and other extra control methods are not needed (Ghosheh, 2005). This indicates that biological weed control methods would need to be examined in depth.

The effect of microbiological pathogens like bacteria, fungi and viruses has been studied for biocontrol of *A. philoxeroides* and *A. filiculoides* and other aquatic plants in tropical regions. These pathogens may affect growth and reproduction of species by reducing growth and nitrogen fixation of the association (Barreto *et al*., 2000).

Among *Azolla* species, *A. filiculoides* has been the target of biocontrol programs. The frond-feeding weevil *Stenopelmus rufinasus* (Gyll.) can occur on *A. caroliniana* Willd, and *A. filiculoides* (Gassmann *et al*., 2006). In South Africa, for instance, *S. rufinasus* was imported from Florida and used as a biological control agent in 1997. The weevil was able to contain the *Azolla* population for 3 years. After this period, *Azolla* was no longer considered a problem in South Africa (Ghosheh, 2005).

CONCLUSIONS

In order to have a successful wetland restoration and conservation management program one has to get acquainted with the habitat requirements of invasive aquatic fern species such as *Azolla.* However, this mosquito fern has many benefits (e.g. nitrogen fixation, phosphorus removal from wastewater, or use as green fertilizer), until now, little is known about the negative impacts of *Azolla* (as an invader or alien species) on a new environment. This paper reviewed the most important structural habitat variables in order to meet the habitat requirements of *Azolla* including water, light intensity, air and water temperature, relative humidity, wind velocity and waves. Moreover, the importance of physical-chemical variables for *Azolla* has been confirmed from the cited literature. Phosphorus, nitrogen, potassium, calcium and magnesium are considered to be the most important macronutrients to induce the growth of *Azolla*.

On the other hand, some micronutrients (e.g. molybdenum, cobalt and vanadium) are well-known to stimulate the growth of *Azolla*. The structural habitat variables probably have a more important effect on growth of *Azolla* compared to the physical–chemical ones. Among the biological factors covered, insects, bacteria, fungi and viruses have been shown to affect growth and development of *Azolla*. As a final conclusion, getting more insight into abiotic and biotic factors affecting growth of *Azolla* will help future research and management of this aquatic fern.

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پایشگری مروری بر بعضی از فاکتورهای اکولوژیکی و تاثیر آنها روی رشد گونه‌های آزولا

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چکیده

جنس آزولا متعلق به گروهی از سرخس‌های آبزی با برگ کوچک و شناور می‌باشد.آزولا بومی مناطق گرمسیری، نیمه گرمسیری و گرم معتدله آفریقا، آسیا و آمریکا است. برای چندین دهه است که از جنس‌های مختلف آزولا برای اهدافی مختلفی استفاده شده است مثلا استفاده به عنوان کود سبز، غذا برای حیوانات، و همچنین جذب فلزات مختلف از فاضلاب‌ها (مثل جیوه، سرب، کروم و کادمیوم) یا حذف ترکیبات نیتروژنی از آب‌های سطحی. علیرغم سودمندی‌های بسیار زیادی که می‌توان برای آزولا در نظر گرفت، این سرخس آبزی بسیاری از زیستگاه‌های طبیعی در مناطق گرمسیری را مورد هجوم قرار داده است و در واقع به عنوان یک گیاه مزاحم محسوب می‌شود. آزولا قادر است در مدت زمان کوتاهی به حد اکثر رشد خود برسد. به طوری که مدت زمان ۲ برابر شدن آن ۲- ۵ روز است. این سرخس آبزی می‌تواند در این مدت زمان کوتاه پوشش بسیار ضخیمی در زیستگاه‌های که برای رویشش مناسب است تشکیل دهد. این کار می‌تواند مشکلات زیادی را برای تردد قایق ها و دیگر گونه های بومی گیاهی و جانوری ایجاد کند. از طرفی دیگر افزایش بیش از حد این سرخس‌های آبزی می‌تواند باعث ایجاد پر غذایی در تالاب‌ها شود. مقاله فعلی که یک کار تحقیقی مروری است به نقش بعضی از فاکتورهای مهم بوم شناختی بر روی رشد آزولا در طول چند دهه اخیر می‌پردازد. مضافاً در بسیاری از قسمت‌های این مقاله یک مطالعه موردی نیز روی تالاب انزلی انجام گرفته تا نقش این عوامل بوم شناختی روی رشد و پراکنش آزولا در این اکوسیستم با ارزش بین المللی مقایسه شود. یکی از مهمترین این فاکتورها موجودیت آب است که می‌تواند برای رشد آزولا بسیار حیاتی باشد. فاکتورهای نظیر میزان شدت نور (۱۸-۱۵ کیلو لوکس)، درجه حرارت (۲۸-۱۸ درجه سانتی گراد) و رطوبت نسبی (۸۳-۵۵ درصد) نیز باعث افزایش شدت رشد آزولا می‌شود. در حالیکه باد و تلاطم آب می‌تواند باعث تلاشی و مرگ آزولا شود. اهمیت مواد مغذی درشت مصرف‌ها (مثل فسفر، نیتروژن، پتاسیم، کلسیم و منیزیم) و ریز مصرف‌ها (مولیبدن، کبالت، و غیره) نیز برای رشد آزولا در مقالات علمی تأیید شده است. تیپ‌های مختلفی از حشرات (کاترپیلارها)، باکتری ها، قارچ ها و ویروس شناخته شده اند که می‌تواند باعث محدودیت رشد آزولا شوند. شناخت نیازهای زیستگاهی آزولا می‌تواند برای مدیریت این سرخس آبزی بسیار سودمند باشد و این کار به نوبه خود می‌تواند نقش مهمی در احیا و حفاظت تالاب ایفا نماید.

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