Hydrobiologia **415:** 323–327, 1999.
J. M. Caffrey, P. R. F. Barrett, M. T. Ferreira, I.S. Moreira, K. J. Murphy & P. M. Wade (eds),
Biology, Ecology and Management of Aquatic Plants.

Biomass characterization of *Azolla filiculoides* grown in natural ecosystems and wastewater

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Key words: biofilter, productivity, phosphorus, water quality, *Azolla*

Abstract

The aquatic pteridophyte *Azolla*, a small-leaf floating plant, which lives in symbiosis with a nitrogen fixing cyanobacteria, *Anabaena azollae*, was widespread throughout water channels and hydrographic basins of Portugal. *Azolla* is also the aim of a study for its utilization as a biofilter for wastewater purification, namely for phosphorus removal (± 36%). The goal of this work is to compare the growth characteristics and biomass composition of this water fern in natural ecosystems with those obtained in some wastewaters. Plant growth rate (0.107± 0.037 d^{-1}) and productivity (5.8 g dw m^{-2} d^{-1}) suggest that *Azolla* can grow well in partially treated domestic wastewater, but not in diluted pig wastes. This fact, associated to its biomass composition, namely in phosphorus content (1.38 ±0.20%), increase the possibility of this plant being used to improve wastewater discharge quality. It may also be possible to use the biomass as a biofertiliser or as a feed supplement for aquatic and terrestrial animals due to its protein, crude fiber and mineral content.

Introduction

In Portugal, the aquatic pteridophyte *Azolla*, a small-leaf floating fern that lives in symbiosis with a nitrogen fixing cyanobacteria, is considered as a weed and can be found in many hydrographic basins, namely those of the Tagus, Sado, Mondego, Vouga, Coa and Guadiana rivers. Climatic conditions and eutrophication of many aquatic ecosystem aid its development.

Due to the existence of the symbiosis, *Azolla* can grow in aquatic environments devoid of combined nitrogen, if there is enough phosphorus. In fact, phosphorus is for *Azolla*, as for many other photoautrotrophic aquatic organisms, the limiting nutrient for growth. On the other hand, many eutrophication problems in our country are due to the discharge of domestic or industrial wastewaters only submitted to secondary biological treatment. These effluents contains high levels of nutrients and tertiary chemical treatments for nutrient removal are very expensive.

Macrophyte utilization for wastewater nutrient removal had been already reported and discussed frequently (Fisher, 1988; Kumar & Garde, 1989; Bishop & Eighmy, 1989; DeBusk et al., 1989; Oron, 1990). In fact, the alternative of using macrophytes to treat and polish effluents may present some advantages such as low costs and easy maintenance. The success of this depends on adequate plant growth all over the year and on the rate of nutrient uptake.

Azolla sp. has been intensively studied during the last few years, due to its potential use as a green manure in rice fields of Asia and Africa and as a feed supplement for aquatic and terrestrial animals (Lumpkin & Plucknett, 1980). The plant utilization in wastewater treatment to remove contaminants, particularly phosphorus, was also suggested by some authors (Reddy & DeBusk, 1985; Fisher, 1988). *Azolla* sp. is unique among floating macrophytes, because it can grow in waters devoid of combined nitrogen, due to the symbiosis with a N_2 fixing cyanobacterium, *Anabaena azollae*, that lives in the dorsal lobe cavity of its leaf. So, this plant can grow even after the exaustion of combined nitrogen in secondary effluents, improving an adequate phosphorus removal (Kitoh et al., 1993).

Azolla has not yet been studied for phosphorus removal from wastewater, and as it seemed to be a good phosphorus assimilator, even in the absence of nitrogen, we studied its performance in this field. Besides, the biomass of *Azolla* produced in wastewater treatment plants could be easily harvested and utilized as a fertilizer for agriculture, or as feedstock for animals.

It is known that biomass composition limits its utilization possibilities. So, the goal of this work is to assess and compare the growth rate, productivity, nitrogen and phosphorus uptake and the chemical composition of *Azolla* grown in natural aquatic environments, like Samora Correia irrigation channels (about 3 m width and 1 m depth), Guadiana river (flow rate less than 10 $m^3 s^{-1}$) and Alverca lagoon–Golegã (about 20 m width and 6–7 m depth), with that produced in a maturation pond effluent from a sewage treatment plant, and in a solution with 5% pig wastes.

Material and methods

The growth rate and productivity of *Azolla* grown in Samora Correia irrigation channels had been evaluated under confined conditions with 0.16 m^2 circular floating PVC net frames, during seven weeks, from February to April, under the climatic conditions prevailing in the rural area. The natural photoperiod during this period averaged 12 h d^{-1}, with light intensities from less than 25 Wm^{-2} to about 51 Wm^{-2}. The air temperature ranged from 11.5 °C to 20 °C, the corresponding water temperature ranged from 10.5 °C to 15 °C, and the mean relative humidity was about 85%.

Biomass and water samples were collected in Samora Correia irrigation channels during two plant growth cycles, from 1991 to 1993, in the Guadiana river during the 1993 bloom and in Alverca lagoon (Golegã) at 1995. *Azolla*'s productivity in Guadiana river and Alverca lagoon have been estimated from Samora's *Azolla* growth rate, because the growth period and climatic conditions in those sites are similar. Water samples were analysed according to *Standard Methods for Examination of Water and Wastewater Analysis* (APHA, 1992).

The growth of *Azolla* in a solution of 5% pig waste and in a maturation pond effluent, were, carried out in PVC reactors (detention time, tr=10 d), with 18.3l capacity, within confining PVC frames (A=0.11 m^2), inside a Fitoclima 750 E culture chamber. Controlled conditions were: 25±0.5 °C, during 14 h d light (31 Wm^{-2}) and 18±0.5 °C during 10/h night period; humidity was between 70 and 75%.The methodology for growth rate, density and productivity evaluation has been described earlier (Costa et al., 1994, 1996).

The phosphorus and nitrogen uptake (mgP m^{-2} d^{-1} and mgN m^{-2} d^{-1}) were calculated based on growth parameters and on biomass phosphorus or nitrogen content. In order to evaluate phosphorus bioaccumulation, phosphorus yield [mgP (g $dw_{)}{}^{-1}$] had been determined based on phosphorus uptake and productivity.

Analysis of dry and organic matter, ash content, crude fiber, cellulose, hemicellulose, lignin, calcium and magnesium were performed according to AOAC (1990), regarding the potential uses of *Azolla*. Heavy metals (Cu, Zn, Pb, Ni, Cd and Cr) were also determined in order to evaluate possible toxicity for animals when it is used for animal feedstock. The results of biomass composition are expressed on a dry weight basis.

Multiple correlations between different *Azolla*'s growth parameters, biomass composition and medium nutrients concentration, in natural ecosystems and wastewaters, had been calculated.

Results and discussion

Under field conditions, *Azolla filiculoides* grows between November–December and May–June, and its sporulation began in March–April. In these ecosystems, water salinity changed from 0.8 to 1.2 mg L^{-1}. The highest values observed did not affect significantly the fern growth.

Azolla growth rates (Table 1) in maturation pond effluent and in Samora Correia channels were similar, but lower than those observed in Hoagland medium H-40 under the same controlled conditions (0.146±0.044 d^{-1}) (Costa, unpublished data), for densities ranging between 6.82 g dw m^{-2} and 68.2 g dw m^{-2}. Never-

Table 1. Growth parameters, phosphorus and nitrogen uptake of *Azolla*

Variables	Samora	Correia	Guadiana river	Alverca lagoon	Pig waste	Maturation pond effluent
	Confined	Field				
Growth rate (d^{-1})	0.106±0.020 (*n*=2)	–	–	–	0.054±0.012 (*n*=14)	0.107±0.037 (*n*=7)
Density (g dw m^{-2})	66.4	243±10 (*n*=3)	175	258	46.4±11.3 (*n*=14)	54.2±19.7 (*n*=7)
Productivity (g dw $m^{-2}d^{-1}$)	7.04	25.8	18.6	27.4	2.55	5.80
Phosphorus uptake (mgP $m^{-2}d^{-1}$)	23.9	87.7	46.9	119	12.2	80.0
Phosphorus Yield (mgP (g $dw_{)}$ $^{-1}$)	3.40	3.40	2.51	4.34	4.80	13.8
Nitrogen uptake (mgN $m^{-2}d^{-1}$)	227	831	584	718	71.7	242

theless, the maximum value observed in wastewater medium for low densities (13.6 g dw m^{-2}) had been quite high 0.162±0.013 d^{-1}. In diluted pig waste, the growth rate had been much lower, perhaps because in this medium algae grew much more than in domestic effluent.

The productivity values obtained in confined systems (Samora channels and wastewaters) were similar to those reported by Vicenzini et al. (1985) and DeBusk &Ryther (1987). Higher values have been observed under field conditions (Samora Correia field, Guadiana river and Alverca lagoon) due to the higher densities found in these aquatic ecosystems, where wind and hydraulic patterns could promote local biomass accumulation. Nevertheless, these values are similar to those repported by Moorhead & Reddy (1987) for other macrophytes. Phosphorus uptake in the Guadiana river and in Samora Correia confined assays were similar to that reported by Reddy et al. (1985), but other field values (Samora Correia and Alverca lagoon) were much higher because of high levels of productivity found there.

The nitrogen uptake values under field conditions were higher than those reported by Reddy et al. (1985) and Kumarasinghe & Eskew (1993), but more consistent with those obtained by Vicenzini et al. (1985), what could be a result of the high productivities calculated under these conditions. The value calculated for Samora Correia assays performed under confined conditions, must be more realistic due to the densities observed which led to lower productivity. Even considering the lowest values of nitrogen uptake obtained with *Azolla*, these were much greater than those reported for nitrogen fixation by leguminous plants (15mg m^{-2} d^{-1} to 138 mg m^{-2} d^{-1}) (Barber, 1995; MADRP, 1997). Indeed, the nitrogen uptake was found to be positively correlated with density (r = 0.972; p = 0.01) and productivity (r = 0.985; p = 0.01). Nevertheless, the high nitrogen and phosphorus uptake observed under field conditions were in the same order of magnitude to those repported by DeBusk (1989), in wastewater tertiary treatment using water hyacinth.

The analysis of growth characteristics indicates that *Azolla* can grow quite well in treated urban wastewater, having growth rates and productivities great enough to encourage its utilisation as a biofilter for nutrient removal and biomass production. Indeed, its productivity is even greater than those of some conventional cultures in Portugal, like rice and alfalfa (1.3 g m^{-2} d^{-1} to 8.2 g m^{-2} d^{-1}, SAPEC, 1989).

The macronutrient content of *Azolla* (Table 2) is high enough to allow it to be used as a fertiliser. When *Azolla* grew in domestic effluent, its phosphorus content was about five times higher than when it grew in natural waters. This value is also greater than that reported by Lumpkin & Plucknett (1980). This has happened because *Azolla's* phosphorus content depends on growth medium phosphate concentration (Reddy & DeBusk, 1985; Mian &Azmal, 1989; Costa et al., 1994). In fact, the phosphate level in natural waters were less (0.93±0.72 mg P–PO_4^{3-} L^{-1}) than the observed in maturation pond effluent (8.26

Table 2. Composition of *Azolla* biomass

Variables	Samora Correia channels (n=8)	Guadiana river (n= 3)	Alverca lagoon	Diluted pig waste (n=10)	Maturation pond effluent (n=7)
Dry matter (%)	6.83±1.50	7.41±1.31	4.69	6.80±1.50	5.43±0.24
Organic matter(%)	90.01±1.5	87.97±0.51	91.29	84.15	85.65
Ash (%)	9.99±1.67	12.21±0.51	8.71	16.85±0.07	14.35
Phosphorus(%)	0.34±0.09	0.25±0.01	0.434	0.48±0.03	1.38±0.20
Nitrogen (%)	3.22±0.55	3.14	2.62	2.81±0.02	4.31±0.65
K (%)	1.97±2.97	1.38	1.25	1.91±0.53	3.17±0.79
Protein (%)	20.49±3.45	19.36±1.31	16.38	17.56±2.00	26.96±4.04
Crude fat (%)	3.60±1.64	5.34±0.82	3.25	5.82±0.10	4.04±0.81
Crude fiber (%)	13.99±1.40	–	16.55	13.35±4.30	12.53±0.83
Cellulose (%)	19.87±3.92	22.48±1.79	22.72	–	11.03±2.05
Hemicellulose (%)	15.05±4.66	10.75±0.65	14.82	–	18.05±7.90
Lignin (%)	35.51±4.39	39.12±1.73	40.99	–	19.52±1.74

– Not determined.

± 1.07 mgP–PO_4^{3-} L^{-1}). Indeed, phosphorus biomass content was highly significantly correlated with nutrients present in the growth medium (N–NH_4^+- r=0.916, p=0.01; P–PO_4^{3-} - r=0.979, p=0.01). We had observed earlier that *Azolla* can accumulate phosphorus by luxury uptake (Costa, 1994). Results from Phosphorus Yield [mgP (g $dw_)$$^{-1}$] confirm that observation.

Biomass content of protein and fiber weren't very different regardless of where the plants were grown (Table 2). Crude protein values were even greater than those observed in some other fodder plants (CEIP, 1980) like maize (Martins, 1996). Nevertheless, it will be important to determine lysine, methionine and histamine content. Fiber was high, but less than in some animal feed (Costa, unpublished data). Cellulose and lignin from *Azolla* developed in maturation pond effluent were lower than that observed in natural conditions, which could favor its digestability.

In order to use *Azolla* as feedstock, it is important to analyse its mineral content. Heavy metals content of *Azolla* harvested from natural environments (Table 3) were in the same order of magnitude of those reported by Lumpkin & Plucknett (1980). The copper and zinc levels were similar to those of oligoelements concentrations in some cereals and leguminous plants (CEIP, 1980). Zinc and lead contents of biomass grown in treated sewage and pig wastes were quite high, especially zinc. Other heavy metals, like Ni, Cd and Cr, were not detected, but special attention should always be paid when this plant is grown in wastewater, because of its potential bioaccumulation of some metals.

Table 3. Metals in *Azolla*

Metal content (mg (kg $dw_)$$^{-1}$)	Samora Correia channels (n = 5)	Guadiana river	Alverca lagoon	Diluted pig waste	Maturation pond effluent (n=6)
Ca	17708±2664	–	–	–	5355±1084
Mg	28161±10812	20000	12500	–	3383±491
Cu	29.00±11.54	34	21	26	15.34±11.01
Zn	37.00±23.00	20	81	115	52.31±31.30
Pb		n.d.	n.d.	–	6.07±3.07

– Not determined.
n.d. – Not detected.

The mineral content of all biomass samples (K, Ca and Mg – Tables 2 and 3) was normal when compared with what has been found in reported earlier studies (Lumpkin & Plucknett, 1980), but comparatively higher than other traditionally used forage plants, especially magnesium in *Azolla* grown under field conditions.

Conclusions

Azolla can grow quite well in treated urban wastewater, having growth rates and productivities great enough to encourage its utilisation. Indeed, its productivity is even greater than those of some conventional crops like rice and alfalfa (SAPEC, 1989). The maximum plant growth rate in secondary treated wastewater was 0.161 d^{-1} and the phosphorus removal efficiency was about 36% (Costa et al., 1996). So, the *Azolla* utilisation as a biofilter, namely for phosphorus removal, can be improved for wastewater purification.

The biomass analysis shows that the great nitrogen, phosphorus, potassium and organic content could favor the use of *Azolla* as a biofertiliser, especially when it is growing in domestic wastewaters, but also when it is harvested from natural environments. The nitrogen content was even greater than that found in some other nitrogen fixing plants, and because it can bioaccumulate phosphorus, the concentration of this element in the plant was as higher as the growth medium was rich on phosphate.

Azolla's biomass can be used in feedstock, but further research is needed to determine protein and fat quality, and also digestability and toxicological aspects. With *Azolla* grown in wastewaters, it would be necessary to pay special attention to heavy metals content, especially copper, zinc and lead.

As *Azolla* presents some interest for its utilization as biofertilizer or feed supplement for animals, harvesting could have less economical constraints what could decrease this plant bloom impacts on aquatic ecosystems. In the other hand, *Azolla*'s growth in wastewaters, namely due to its phosphorus removal capacity, could improve discharged water quality helping to control natural waters eutrophication.

Acknowledgements

This research was supported by JNICT/FCT Projects (PEAM/P/APR/121/91 and PEAM/C/GRH/581/95) and INAG.

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