POTENTIAL USES OF *AZOLLA FILICULOIDES'* BIOMASS GROWN IN NATURAL ECOSYSTEMS AND URBAN WASTEWATER

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1 INTRODUCTION

Azolla sp., a small-leaf pteridophyte, is unique among floating macrophytes, because it can grow even after the exhaustion of combined nitrogen in secondary effluents, improving an adequate phosphorus removal. This is due to the symbiosis with a N_2 fixing filamentous cyanobacteria, *Anabaena azollae*, and a variety of bacteria that some have identified as *Arthrobacter sp*. (Carrapiço *et al*., 1996).

According with the results obtained in continuous assays performed earlier (Costa *et al*., 2009; Costa, 2009), the use of *Azolla*, an aquatic fern with high growth rate and productivity, seems to be very promising to improve treated urban wastewater quality, particularly because its phosphorus removal efficiencies (40-65%). The heavy metal biosorption using living *Azolla* (phytoremediation) or dried biomass as a bioadsorvent material are also relatively new technologies for the removal of metals (Rahhshaee *et al*., 2006). These processes success depends on an adequate plant growth all over the year, on nutrients and metals removal efficiencies and on biomass removal and valorisation. *Azolla* presents further potential uses, such as substrate for biogas production or to natural dyes extraction (anthocyanin-E-163, also with anti-oxidative action), as a biological control of submerse weeds, algae growth and insects, but essentially it can be utilized as feed supplements for aquatic and terrestrial animal and as a biofertilizer (Carrapiço *et al*., 1996; Leterne *et al*., 2010). *Azolla* was studied as a biological regenerative life support system (BLSS) integrated in space missions (Soyus- Salyut 6) and Biosphere II project (Arizona) in order to promote food production, gas exchange, water reclamation and nutrient recycling (Carrapiço, 2002; Liu *et al*., 2008[a]; Liu *et al*., 2008[b]; Katayama *et al*., 2008).

It is known that biomass composition can limit the possibilities of its utilization. Therefore, the goal of this work is to assess and compare chemical compositions of *Azolla* grown in natural aquatic environments, in artificial medium and in urban wastewaters. In order to understand its digestibility, protein, crude fat, cellulose, hemicellulose and lignin contents were also analysed.

2 MATERIAL AND METHODS

Azolla from natural aquatic environments was harvested in Samora Correia irrigation channels (about 3m width and 1m depth), River Guadiana (flow rate less than $10m^3s^{-1}$) and Lagoon Alverca -Golegã (about 20m width and 6-7m depth).

Azolla's growth in artificial medium (Hoagland H-40 without nitrogen and 10 mgP L^{-1}) and in urban wastewaters from a facultative pond (CQO-191±89 mgO_2 L^{-1}, N_{Kj}- 40.7±13.8 mg L^{-1}, NH_4^+- 26.27±7.85 mgN L^{-1}, Total P- 9.49±3.40 mg L^{-1}) were carried out in 18.3L capacity (45x32x12.7 cm) PVC reactors (hydraulic detention time equal 5, 7.5 and 10 days), within confining PVC frames ($A=0.11m^2$), inside a Fitoclima 750E culture chamber.

Controlled culture conditions were: 25±0.5°C, during 14h.day light (263 mol photon m^{-2} s^{-1}) and 18±0.5°C during 10h/night period; humidity was between 70 and 75%. These essays were performed under constant initial densities that changed from 6.8 to 68.2 g $_{d.wt.}$ m^{-2}. The excess of the biomass grown during each essay was harvested and further analysed. As biomass composition did not change substantially with the different densities and hydraulic detention time, only media values and standard deviation were found to be present.

Analysis of dry weight, organic matter, ash, crude fibre, fat and nutrients contents were performed according to AOAC methods (1990), regarding different potential uses of *Azolla*. In order to evaluate digestibility and possible toxicity for animals, if it is to be used for animal feedstock, cellulose, hemicelluloses, lignin and heavy metals (Cu, Zn, Pb, Ni, Cd and Cr) were also determined (Goering & Van Soest, 1970; AOAC, 1990). The results of biomass composition are expressed on a dry weight basis.

3 RESULTS AND DISCUSSION

Dry weight and organic matter values (Table 1) were similar to referred by Van Hove (1989) and Kaplan & Peters, (1998) for *Azolla*, however they were lower than in forage plants (CEIP, 1980). Carbon, admitting that it 50% of organic matter, was about 45%, the same order of magnitude of those reported by Van Hove (1989) (43%) e Kaplan & Peters (1998) (*A. caroliniana*- 41.2%), but higher than the observed by Dinesh & Dubey (1998) (*A. pinnata*- 21.4%).

TABLE 1 ***Azolla* biomass composition grown in different growth media.**

Parameter	**Guadiana river** (n=3)	**Irrigation channels** (n=8)	**Alverca lagoon** (n=1)	**Hoagland H-40** (n=12)	**Urban wastewater** (n=16)
Dry weight (%)	7.4±1.3	6.8±1.5	4.7	5.1±0.2	5.7±0.6
Ash (%)	12.2±0.5	10.0±1.7	8.7	17.0±1.0	14.2±0.5
Phosphorus (%)	0.25±0.01	0.34±0.09	0.43	1.63±0.08	1.32±0.17
Nitrogen Kjeldahl (%)	3.14±0.20	3.22±0.55	2.62	4.05±0.39	3.68±0.36
Protein (%)	19.6±1.2	20.5±3.5	16.4	25.3±2.5	23.0±2.3
Crude fat (%)	5.3±0.8	3.6±1.6	3.2	4.1±0.4	3.6±0.5
Crude fibre (%)	-	14.0±1.4	16.6	14.0±3.5	13.2±2.1
Cellulose (%)	22.5±1.8	19.9±3.9	22.7	13.6±1.4	11.8±2.3
Hemicellulose (%)	10.8±0.6	15.0±4.7	14.8	16.6±3.7	19.1±5.8
Lignin (%)	39.1±1.7	35.5±4.4	41.0	21.8±4.4	20.2±3.5
Lignin/N ratio	12.5	11.0	15.7	5.4±1.3	5.5±0.8

Biomasses from Hoagland H-40 medium and urban wastewater were richer in nutrients (N and P) than those collected in natural aquatic ecosystems (Table 1), because natural waters phosphorus concentrations were lower (0.93±0.72 mgP-PO_4^{3-} L^{-1}) than facultative pond effluent contents (6.85±2.35 mgP-PO_4^{3-} L^{-1}) what can limit nitrogen fixation by symbionts (Costa, 2009). Indeed, phosphorus content in biomass grown in treated wastewater was about three times greater than the highest value observed in *Azolla* grown in natural water bodies, as a result of phosphorus bioaccumulation due to luxury uptake (Costa, 2009). Besides, *Azolla*'s phosphorus utilization efficiencies (EUP- gN. gP^{-1}) observed in biomass collected from natural waters (6.1-12.6) were much higher than those found in biomass from wastewater (2.8±0.2). Furthermore, due to the lower nitrogen content in biomasses grown in aquatic ecosystems, the C/N ratios were higher in these biomasses when compared with those from Hoagland H-40 media and wastewater. So, the biodegradation rate of the biomass grown in wastewater, when incorporated in the soil as biofertilizer, will be faster, more easily releasing nutrients for plants.

When biomasses composition are evaluated in order to its possible use as complement for animal feedstocks, it can be seen that protein, crude fibre and fat contents weren't very different, regardless the plants growth media (Table 1). In all of them, crude protein values were even higher than those observed in some other fodder plants (CEIP, 1980), like maize (Martins, 1996). Nevertheless it will be important to determine lysine, methionine, cysteine and histamine contents. Fibre percentages were high, but less than in some animal food-stuffs (unpublished data). The structural compounds contents, such as cellulose and lignin, in *Azolla* developed in wastewater pond effluent were lower than those observed in biomass grown in natural environments, which could favour its digestibility. Simultaneously, the lignin/N ratio was much lower in biomass grown in wastewater, what helps its biodegradation. However, if the biomass is intended to be used for this purpose it is necessary to evaluate other parameters, namely metals concentrations, which are presented in Table 2.

The Ca and Mg concentrations in biomasses grown in wastewater were lower than in biomass grown in natural aquatic ecosystems, but K content was much higher and overcame the earlier reported for different *Azolla* species grown in rice fields (Rother & Whitton, 1988; Lumpkin & Plucknett, 1980). Because of that, the Mg/K ratio in biomasses from wastewater treatment effluent (0.13-0.16) was about ten times lower than the ratio found in biomass from natural water bodies (1.0-1.4). However, it is not foreseen that Mg deficiencies might occur if *Azolla* is used as feed complement for herbivorous animals, because the values obtained were in the same order of magnitude of those reported for leguminous (0.10-0.12), cereals and forage plants (0.10-0.59) (SAPEC, 1989; CEIP, 1980).

TABLE 2 **Metals concentrations in different biomasses**.

Parameter* **(mg kg$_{d.w.}^{-1}$)**	**Guadiana river** (n=3)	**Samora Correia Irrigation channels** (n=8)	**Alverca lagoon** (n=1)	**Hoagland H-40** (n=12)	**Urban wastewater** (n=16)
K	13800	19801±2985	12500	57106	31528±4759
Ca	-	17708±2664	-	14438±1397	7306±4371
Mg	20000	28161±10812	12500	9293±819	4605±2366
Fe	1300.1	20863.1±3059.4	430.0	-	-
Mn	200.0	1075.9±771.2	170.0	-	-
Cu	34.0	29.0±11.5	21.0	38.5±0.8	14.9±7.2
Zn	20.0	37.0±23.0	81.0	-	61.4±37.5
Pb	11.0	14.7±6.8	n.d.	-	5.6±3.0

* Cd, Cr and Ni were not detected; n.d.- not detected.

Zinc was the heavy metal present in higher concentrations, but heavy metals concentrations in wastewater biomasses weren't higher than in natural aquatic ecosystems biomasses, which were in the same order of magnitude of the referred for different *Azolla* species grown in rice fields (Rother & Whitton, 1988; Lumpkin & Plucknett, 1980). Zn and Cu concentrations in biomasses from wastewater were lower or similar to those found in cereals and leguminous plants (SAPEC, 1989; CEIP, 1980). However, these values were higher than those calculated based on the one reported by Katayama *et al.* (2008) for *Azolla* cultivated in IRRI medium to be incorporated in the diet of spatial mission crews. In what concerns lead's concentration, they were higher than the maximum reported by Fleurence (1991) for algae in human diet (5 mg kg $_{m.s.}^{-1}$), which could limit its future use as feed supplement for animals.

Despite the presence of heavy metals in *Azolla* grown in wastewater, its macronutrients contents (N- 3.52 a 3.76%, P- 1.28 a 1.37%, K- 3.06 a 3.32%) were higher than the observed in biomass from natural aquatic ecosystems what suggest that it be more valuable as biofertilizer (Table 1 and 2). Indeed, the level of heavy metals in *Azolla*'s biomass was found to be lower than in others substrates incorporated in soil, such as compost from vegetal residues (Guigi *et al.*, 1990). However, the values obtained were higher than the reported for plants (spinach) cultivated in soil incorporated with compost from urban solid wastes and sludge (Guigi *et al.*, 1990). Thus, the use of *Azolla's* biomass as animal feedstock complement should be done with great precaution.

4 CONCLUSIONS

Azolla's biomass grown in urban wastewater can be incorporated in the soil and the high macronutrients (N, P, K) contents will be more available for plant growth, due to C/N and lignin/N ratios that are lower than those that occur in biomass from natural waters bodies.

The high levels of protein and crude fat, and the lower cellulose and lignin contents of *Azolla*'*s* biomass grown in wastewater can favour its digestibility when it is used as feed supplement for animals. However, the presence of heavy metals in wastewaters, due to industrial discharges, could limit its use.

The biomass reuse could improve wastewater treatment sustainability reducing their costs. In fact, the use of macrophytes as an alternative to treat and polish effluents, in rural areas, may present some advantages such as low costs, easy maintenance and lower CO_2 emissions.

If *Azolla* is used, namely as biofertilizer, its harvesting could have less economical constraints which could decrease the impact of this plant's bloom on aquatic ecosystems (water channels and hydrographic basins of Portugal, namely those of Tagus, Sado, Mondego, Vouga, Coa and Guadiana rivers), where sometimes it is largely widespread. Besides, *Azolla*'s growth in wastewaters, namely due to its phosphorus removal capacity, could improve treated effluents quality, helping to control the eutrophication of natural waters.

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