LICHENS AS BIOMONITORS OF ATMOSPHERIC AMMONIUM/AMMONIA DEPOSITION IN PORTUGAL

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Abstract

The aim of the present work was to evaluate the potentiality of lichens as biomonitors of NH_4^+/NH_3 (ammonium/ammonia) and NO_3^- (nitrate) atmospheric deposition. For that, we used as a field station a rice plantation which is submitted, once a year, to air spraying fertilization with a misture of nitrogen sources. Samples of an epiphytic lichen, *Ramalina fastigiata*, were collected from an ash-tree bordering the rice-plantation by the Sorraia River Valley (Central Portugal). The study started one month before fertilization and sampling was carried out for five months. The concentration of ammonium in the lichen was highly and significantly correlated with the number of days without precipitation before sampling, and had an inverse correlation with fluorescence values. Under these conditions, the amount of NH_4^+ found in the lichen appers to reflect ammonium/ammonia dry deposition.

1. INTRODUCTION

Since the last century, occurred an increase in atmospheric pollution, being NO_x and NH_x the most common atmospheric sources of nitrogen. In comparison with NH_3, NO_x has a higher relative abundance in industrial and urban areas and also a higher toxicity. The oxidized species are mostly produced by the motor vehicle industry, while the sources of reduced species are essentially of agricultural origin.

In Portugal the monitoring network for atmospheric nitrogen compounds has some flaws mainly related to the small number of sampling stations. Estimated N atmospheric deposition in Portugal through the CORINAIR program showed values similar to those found in the rest of the European Union.

Lichens are extremely sensitive symbiotic organisms consisting of fungi and algae. There are several characteristics which make lichens ideal biological monitors [1, 2]: (1) wide geographical distribuition; (2) no morphological changes with seasons; (3) absence of a well developed cuticle, which results in little control of the uptake of water and solutes from the atmosphere; (4) close dependence on the atmospheric deposition for nutrition; (5) ability to accumulate pollutants througout the year [3]. Sudies of nitrogen metabolism and biomonitoring in lichens were reported by Brown, Bruteig and Crittenden [3–5].

The deposition of contaminants in lichens occurs by dry deposition as well as by wet deposition. The latter results from both precipitation and occult precipitation, mainly fog and dew. The dry deposition includes interception of particles and gas absorption [6].

The chlorophyll fluorescence parameter, Fv/Fm (variable fluorescence to maximal fluorescence) has been studied in different photossintetic organisms, including lichens [7], to access the efficiency of the photosynthetic apparatus and to reflect the efficiency of the primary photochemical reactions in the PSII. Branquinho *et al* [8] have also used this parameter to evaluate the physiological effects of contaminants in lichens.

The objective of this work is to evaluate the ability of lichens as biomonitors of N deposition, their physiological response (Fv/Fm parameter) will be taken into account.

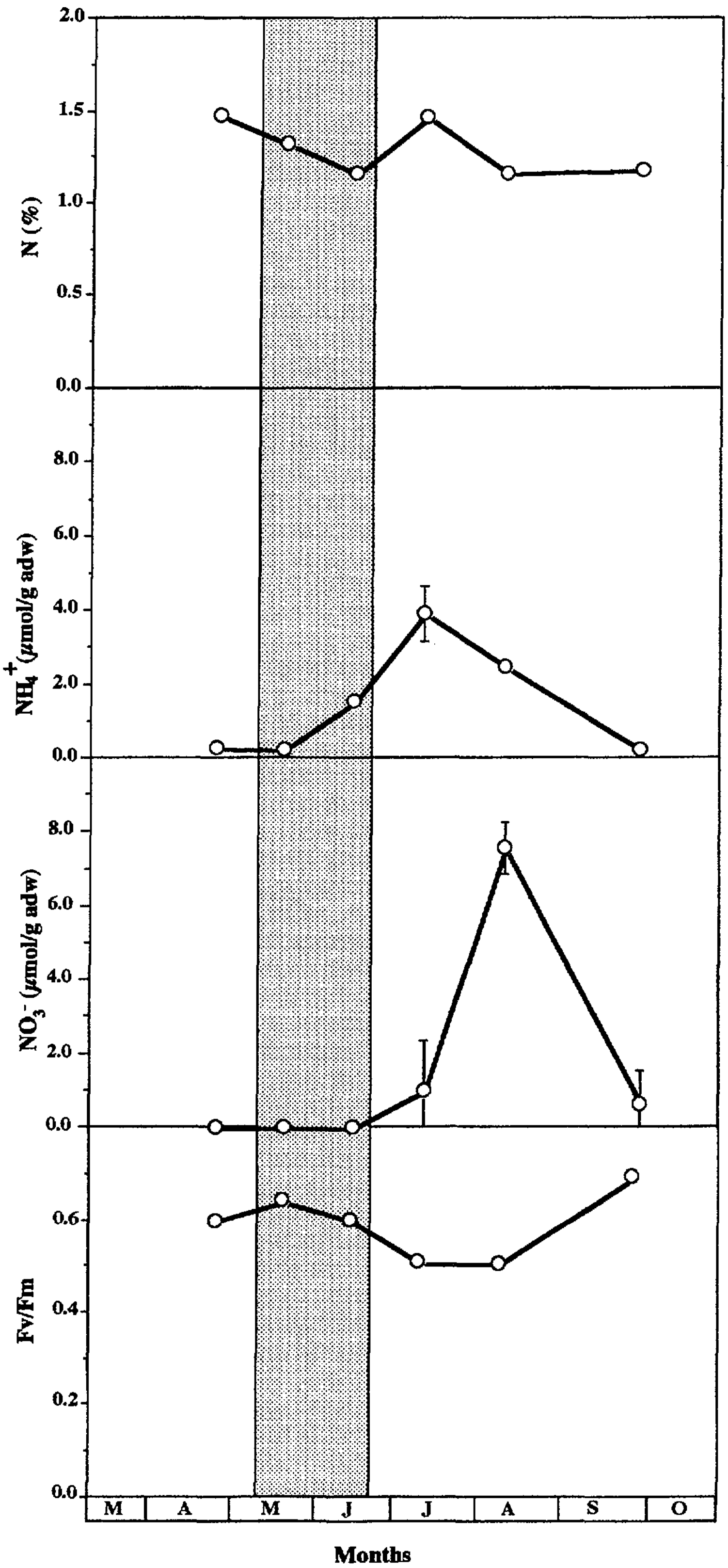

FIG. 1. Monthly variation of the total N (A), NH_4^- (B) and NO_3^- (C) concentrations in Ramalina fastigiata in 1996. Each symbol and the bars represent the mean and standard deviation (respectively) of 3 samples (A, B and C). In D each point corresponds to the measurement of one sample of the fluorescence ratio of variable fluorescence to maximal fluorescence (Fv/Fm). The fertilization period is indicated by shaded area.

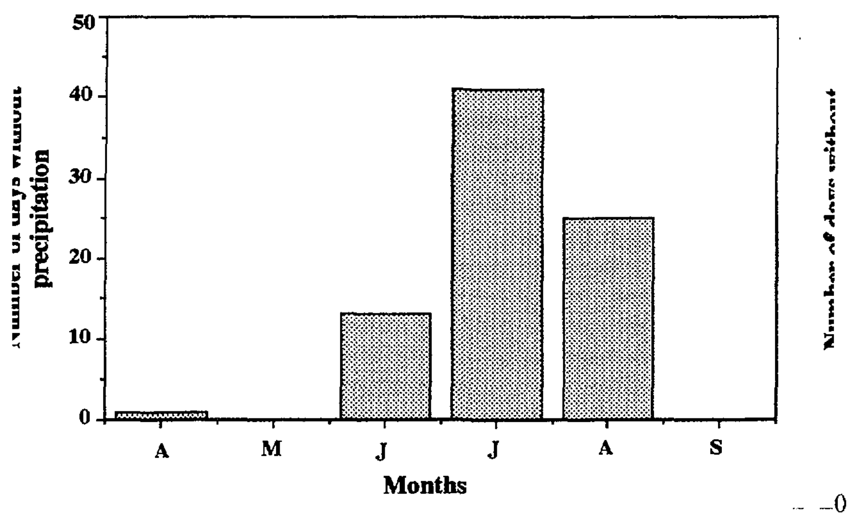

FIG. 2. Cumulative number of days without precipitation before each sampling.

2. MATERIALS AND METHODS

2.1. Study area

The Sorraia River Valley (38°56'N, 8°35'W) is a rice plantation area. It receives an annual input of nitrogenous compounds as fertilisers sprayed by aeroplane, from April until mid-June. Besides this agricultural activity, grazing may also represent an important contribution to the rise of N levels in the atmosphere.

2.2. Sampling

The experiment was carried out for 6 months (April to September) in 1996. Once a month, samples of the fruticose lichen *Ramalina fastigiata* were collected from a *Fraxinus angustifolia* tree bordering the cultivated area. During transport to the laboratory the lichen material was kept in plastic bags. The samples were kept at room temperature until analysis.

2.3. Total Nitrogen Analyses

After being air dried, the samples were ground to powder in a mill (Glen Creston Lt. MM 200). Subsequently they were analysed in an EA1108 CHNS-O Fisson Instruments Analyser.

2.4. Inorganic Nitrogen Analyses

Prior to analysis, 50 mg of air dried samples were extracted in 5 ml of destilled water at 100°C. The extracts were filled up to 10 ml, filtered and used for determination of the concentration of NO_3^- and NH_4^+ and kept cool (4°C).

For the determination of NH_4^+, the extracts were analysed by the Indophenol-Blue method [9]. This method measures all the ammonia N (NH_4^+ and NH_3).

For the determination of NO_3^-, the extracts were analysed according to a colorimetric method in which the NAS reagent (Diphenylamine Sulfonic Acid Cromogene) is added to the solution to be analyzed.

The absorbance of the samples was read at 630 nm (NH_4^+)[9] and 570 nm (NO_3^-) in a Philips PU8620 Spectrophotometer. All the results were expressed in μmol/g adw (air dried weight).

2.5. Chlorophyll fluorescence

A PAM 101 Chlorophyll Fluorometer (Walz, Effeltrich, Germany) was used to measure chlorophyll fluorescence of *Ramalina fastigiata*. Lichen samples were dark adapted for 10 min before the measurements. The minimum and maximum fluorescence (Fo and Fm, respectively) were determined. Variable fluorescence, Fv, is the difference between Fm and Fo, and it was calculated in order to obtain the parameter Fv/Fm.

3. RESULTS AND DISCUSSION

A small variation in the total N concentration (Fig.1A) of the lichen samples after the fertilization period was observed (1 - 1.5%). These values are within the range of those obtained by Crittenden [10] for other lichen species (0.51 - 4.01 %). Considering that 90% of a lichen mass is fungi, lichen N concentration should reflect fungal N concentration rather than algal N concentration. Concentrations of N in fungi under optimal nitrogen supply range between 0.23 and 5.3 % [11]. Therefore the concentrations found in *Ramalina fastigiata* seem to be whitin the normal values.

The total N concentration showed no substancial increase, NH_4^+ (Fig.1B) and NO_3^- (Fig.1C) concentrations increased 4 and 7 times respectively, after the fertilization period. This observation agrees with the results of Boonpragob *et al* [12] who obtained the maximum NH_4^+ and NO_3^- concentration during Summer, also in lichen samples.

The concentration of ammonium in the lichen was highly and significantly correlated ($p \leq 0.01$) with the number days without precipitation (Fig.2). The agreement between the variation patterns in Figs.1B and 2 suggests that the accumulation of NH_4^+ in lichen talli is determined by the dry deposition. We suggest that within the precipitation events the lichen surface is washed, leading to lower NH_4^+ concentration values.

The Fv/Fm values in *Ramalina fastigiata* (Fig.1D), used to indicate the physiological state of the lichen, decreased after the fertilization period. The lowest values were obtained in July-August, followed by a recovery in September. A significant negative correlation was observed between NH_4^+ concentration and Fv/Fm values ($p \leq 0.01$). Similar results were obtained by Boonpragob *et al* [13] using the chlorophyll and net photossyntesis parameters. Our results might indicate a physiological effect of the fertilizers in the lichen talli or just a seasonal variation of fluorescence, with lower Fv/Fm values during Summer (drought season).

This work shows that lichens can be used as biomonitors of N dry deposition.

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