Ecotoxicology and Environmental Safety 122 (2015) 377–383

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effects of acute NH_3 air pollution on N-sensitive and N-tolerant lichen species

Luca Paoli [a,*], Ivana Maslaňáková [b], Alice Grassi [a], Martin Bačkor [b], Stefano Loppi [a]

[a] Department of Life Sciences, University of Siena, via P.A. Mattioli 4, I-53100 Siena, Italy
[b] Department of Botany, Institute of Biology and Ecology, P.J. Šafárik University in Košice, Mánesova 23, SK-04001 Košice, Slovakia

ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form
18 August 2015
Accepted 21 August 2015
Available online 20 September 2015

Keywords:
Chlorophyll fluorescence
Dehydrogenase activity
Ergosterol
Industrial composting
Lichens
TBARS

ABSTRACT

Lichens are sensitive to the presence of ammonia (NH_3) in the environment. However, in order to use them as reliable indicators in biomonitoring studies, it is necessary to establish unequivocally the occurrence of certain symptoms following the exposure to NH_3 in the environment. In this paper, we simulated an episode of acute air pollution due to the release of NH_3. The biological effects of acute air pollution by atmospheric NH_3 have been investigated using N-sensitive (*Flavoparmelia caperata*) and N-tolerant (*Xanthoria parietina*) species. Lichen samples were exposed to ecologically relevant NH_3 concentrations for 8 weeks, simulating three areas of impact: a control area (2 $\mu g/m^3$), an area of intermediate impact (2–35 $\mu g/m^3$) and an area of high impact (10–315 $\mu g/m^3$), with a peak of pollution reached between the fourth and fifth week. Ammonia affected both the photobiont and the mycobiont in *F. caperata*, while in *X. parietina* only the photosynthetic performance of the photobiont was altered after exposure to the highest concentration. In the photobiont of *F. caperata* we recorded chlorophyll degradation as indicated by $OD_{435/415}$ ratio, decrease of the photosynthetic performance (as reflected by the maximum quantum yield of primary photochemistry F_V/F_M and the performance index PI_{ABS}); in the mycobiont, ergosterol reduction, membrane lipid peroxidation (as reflected by the increase of thiobarbituric acid reactive substances), alteration (decrease) of the secondary metabolite usnic acid. No effects were detected on caperatic acid and dehydrogenase activity. In *X. parietina*, the only signal determined by NH_3 was the alteration of F_V/F_M and the performance index PI_{ABS}. The results suggest that physiological parameters in N-sensitive lichens well reflect the effects of NH_3 exposure and can be applied as early indicators in monitoring studies.

1. Introduction

Air pollution by ammonia (NH_3) is a notable environmental concern since NH_3 contributes to the deposition of eutrophicating substances which exceeds the critical loads for many ecosystems (Asman et al., 1998) causing impacts ranging from decreased biodiversity, changes in species composition and dominance, and toxicity effects (Fangmeier et al., 1994). Agricultural activities are responsible for over 90% of NH_3 emissions (Galloway et al., 2004), which occur primarily from animal husbandry, manure storage and spreading and application of fertilizers. However, another notable NH_3 source, which is increasingly expanding in many countries, is from decomposition (composting) of organic waste, since the mineralisation of organic N-containing amino acids and urea releases considerable amounts of NH_3, the main cause of N pollution during composting of organic waste (Zeng et al., 2012). In fact, N loss from industrial composting is mainly due to NH_3 emissions, which account for 24–33% and 47–77% of the initial N content of household waste and manure respectively (Beck-Friis et al., 2001; Martins and Dewes, 1992).

Ammonia emission (and hence pollution) is not a uniform and continuous phenomenon, but rather goes through acute episodes. In fact, during aerobic treatment of organic waste, according to the activity of different groups of microorganisms, mineralisation of organic N results in two NH_3 emissions peaks (Zeng et al., 2012) and levels of atmospheric NH_3 up to 700 mg/m^3 have been reported around waste water sludge composting facilities (Haug, 1993). Ammonia is severely irritating to the nose, throat and lungs, and human exposure to excess NH_3 has been shown to be a relevant concern for the health and safety of exposed workers (Rahman et al., 2007).

Once released to the environment, NH_3 is readily converted to NH_4^+ or subject to dry deposition (Fangmeier et al., 1994). Toxicity by NH_4^+/NH_3 has been extensively studied in higher plants (Fangmeier et al., 1994; Britto and Kronzucker, 2002) and detrimental effects can be early detected at physiological level, involving

* Corresponding author. Fax +39 0577 232896.
E-mail address: paoli4@unisi.it (L. Paoli).

http://dx.doi.org/10.1016/j.ecoenv.2015.08.025
0147-6513/

alteration of secondary metabolism and changes due to increased uptake and assimilation of N (Fangmeier et al., 1994). Higher plants, bryophytes, lichens, soil organisms and invertebrates can be profitably used as bioindicators of the effects of N pollution in the environment, integrating non-biological methods of analysis (Sutton et al., 2004).

In particular, lichens are very sensitive to atmospheric reactive N, especially NH_3 (Sutton et al., 2004). Being symbiotic organisms made up by an alga and a fungus, excess N is detrimental to the equilibrium between the two symbiotic partners and hence to the whole lichen, especially if one of the two partners is more able than the other to cope with high N levels (Gries. 1996). The results of previous studies suggested that the photosynthetic apparatus of lichens exposed to ecologically relevant NH_4^+/NH_3 concentration is directly susceptible to these pollutants in the vapour/gas phase (Paoli et al., 2010a; Munzi et al., 2012). In addition, relevant NH_4^+/NH_3 levels may affect membrane lipids and hence alter cell membrane permeability (Fangmeier et al., 1994) and there is evidence that physiological parameters connected to membrane permeability are suitable tools for monitoring biological effects of acute N pollution (Munzi et al., 2009b).

In a previous work we investigated whether NH_3 emissions released during composting of organic waste influenced during short-term exposures the lichens in the surrounding environment (Paoli et al., 2014a). It was shown that exposing lichens around a composting plant allowed detecting early physiological indications of potential biological changes before these consequences were apparent at the community level. In particular, N-tolerant species were not affected by the proximity to the facility and some parameters even suggested a better performance, while N-sensitive species showed reduced performances approaching the source. In addition, it was hypothesised that the concentrations of NH_3 were highly fluctuating, with peaks during outdoor handling and maturation of the compost, suggesting that acute episodes of pollution could be the reason for the observed effects (Paoli et al., 2014a). It was concluded that lichens can provide useful data for decision-makers to establish correct science-based environmentally sustainable waste management policies. However, since the interpretation of the results of field studies is often complicated by the interactions among many environmental factors, experiments under controlled conditions are necessary to separate the effects of specific environmental variables. The present experiment was thus carried out to investigate the biological effects of a simulated acute air pollution by atmospheric NH_3 on N-sensitive (*Flavoparmelia caperata*) and N-tolerant (*Xanthoria parietina*) lichens.

2. Materials and methods

2.1. Lichen species

Samples of the lichens *Flavoparmelia caperata* (L.) Hale and *Xanthoria parietina* (L.) Th.Fr. were collected at the beginning of May 2013 from a remote area of central Italy far from pollution sources (Murlo, Tuscany 43°11′60″ N, 11°21′33″ E, 310 m a.s.l.) and transferred to the Botanical Garden of the University of Siena.

Both lichen species have a similar foliose habitus and a green-algal photobiont (*Trebouxia*). They are however characterized by a different sensitivity to the presence of N compounds in the environment (Nimis and Martellos, 2008): *F. caperata* grows in sites with no or weak eutrophication (non-nitrophilous) and is sensitive to excess N in the environment, whereas *X. parietina* is a nitrophilous lichen, which may grow in sites with high eutrophication. In addition, *F. caperata* is a mesophytic species chiefly growing in sites with diffuse light but scarce direct solar irradiation, up to sun-exposed sites, but avoiding extreme solar irradiation (Nimis and Martellos 2008), while *X. parietina* is rather xerophytic and can tolerate extreme radiations. Both species are widely spread in lichen communities of the eu-mediterranean belt (i.e., in areas with a humid-warm climate, such as Tyrrhenian Italy): *F. caperata* is one of the most common species in *Quercus* stands and *X. parietina* is diffused in open stands, also in dry environments.

2.2. Experimental design and sample treatment

Samples of *F. caperata* and *X. parietina* were divided in 3 batches and placed inside 3 experimental fumigation chambers of $60 \times 40 \times 25$ cm^3, located within one of the greenhouses of the Botanical Garden of the University of Siena. Based on previous field studies (Paoli et al., 2014a), each experimental chamber simulated a different situation of impact according to a gradient of NH_3 pollution: no impact (control), intermediate impact, high impact. In order to work with a similar lichen biomass, each experimental chamber contained about 50 thalli of *F. caperata* and about 100 thalli of *X. parietina* (whose thalli are generally more little than those of *F. caperata*).

Samples were treated for 8 weeks as shown in Table 1: during the first 3 weeks samples were acclimated to low atmospheric NH_3; then an episode of acute pollution from atmospheric NH_3 was simulated for 2 weeks and during the last 3 weeks a moderate impact was simulated. Control samples were constantly treated at the concentration of 2 μg/m^3, roughly corresponding to background values in Tuscany (Frati et al., 2007). Intermediate samples were treated at concentrations of 2 μg/m^3 during the first 3 weeks, at a peak of 100 μg/m^3 during the 4th and 5th weeks of exposure and at 10 μg/m^3 during last 3 weeks. High impact samples were treated at concentrations of 10 μg/m^3 during the first 3 weeks, at peaks of 300 μg/m^3 during the episode of acute pollution (4th and 5th weeks) and at 100 μg/m^3 during last three weeks (Table 1).

Ammonia was applied as follows: water solutions containing liquid NH_3 were prepared and placed into open Petri dishes within the experimental chambers, then let evaporate within each chamber, which remained closed. In the control chamber only water was applied. Relative humidity increased during water evaporation: every two days, after water evaporated, a further solution containing NH_3 (or only water in controls) was applied opening the chamber only for the time necessary and closing it after the treatment. Therefore, each chamber represented a sort of closed environment. The level of atmospheric NH_3 (Table 1) was measured with passive air samplers (Radiello® diffusion tubes, Aquaria). For each treatment two samplers were placed in each chamber for 7 days during the 3rd, 5th and 8th week. Samplers contained a filter impregnated with phosphoric acid that adsorbs gas-phase NH_3 as NH_4^+, which can be measured spectrophotometrically by the indophenol blue method (Allen, 1989). The detection limit was 0.7 μg/m^3, uncertainty was 6.5%.

The experiment was run between May and June 2013. Microclimatic parameters under the experimental conditions were regularly recorded between 12:00 and 1:00 p.m. and values were in the following range: solar radiation (1000–1550 μmol s^{-1} m^{-2}),

Table 1
Atmospheric NH_3 concentrations (μg/m^3) measured with passive air samplers.

Weeks	Ammonia concentrations (μg/m^3)		
	Control	Intermediate impact	High impact
1–3 Acclimation	2.1 ± 0.8	1.9 ± 0.7	10.3 ± 0.6
4–5 Acute pollution	2.2 ± 0.8	35 ± 4	315 ± 4
6–8 Pollution	1.9 ± 0.5	10 ± 1	101 ± 2