Contents of amino acids and osmotic values of epiphytic lichens as indicators for regional atmospheric nitrogen loads

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Zusammenfassung: Die Aminosäuregehalte (Alanin/Arginin, Glutamin, Prolin, Taurin) von fünf verschiedenen Flechtenarten (*Evernia prunastri, Hypogymnia physodes, Parmelia sulcata, Physcia adscendens, Xanthoria parietina*) aus verschiedenen unterschiedlich stickstoffbelasteten Gebieten Westdeutschlands und NW Frankreichs wurden untersucht. Der Gehalt an diesen Aminosäuren ist z.B. in Bonn je nach Lage fünf bis zwölf mal so hoch wie in den Vogesen. In Gebieten mit hohen Stickstoffdepositionen (35 kg/y/ha) ist bei sogenannten Stickstoffzeigern (*Xanthoria parietina, Physcia adscendens*) im Durchschnitt fünf mal so hoch wie in Gebieten mit niedrigeren Belastungen (16 kg/y/ha). Daraus kann geschlossen werden, dass der Stickstoffgehalt von Flechten als Passivsammler die regionale atmosphärische Stickstoffbelastung widerspiegelt und dass der Stickstoffgehalt sogenannter nitrophiler Flechten nicht höher ist als bei anderen Arten.

Hingegen ist der osmotische Wert von sogenannten nitrophilen Flechtenarten höher als bei anderen Arten. Daraus kann man schließen, dass die „Nitrophilie" nicht auf Verträglichkeit hoher Stickstoffaufnahmen beruht, sondern auf eine höhere Toleranz gegen Stickstoffsalze, wie sie in Form von Ammoniumnitrat im Feinstaub enthalten sind. Innerhalb der nitrophilen Arten ist der osmotische Wert von *Phaeophyscia orbicularis* am selben Baum doppelt so hoch wie bei *Physcia adsendens*, was schon bei der höheren Toleranz von *Phaeophyscia* gegen trockene Depositionen aufgefallen war. Die höheren osmotischen Werte bei sog. nitrophilen Flechtenarten dürften eine höhere Trockenresistenz zur Folge haben, weswegen diese nicht nur Stickstoff- sondern auch Trockenzeiger sind und das Vorkommen in unbelasteten aber trockenen Gebieten erklärt. Nitrophytische Flechten sind also Xerophyten, die aufgrund der höheren osmotischen Werte tolerant gegen Stickstoffsalze sind.

Abstract: The amino acid content (alanine/arginine, glutamine, proline, taurine) of five different lichen species (*Evernia prunastri, Hypogymnia physodes, Parmelia sulcata, Physcia adscendens, Xanthoria parietina*) from different parts of Germany and NW France with different atmospheric nitrogen depositions was determined.

The study revealed that the so called nitrophytic lichen species (*Physcia adscendens, Xanthoria parietina*) had no higher amino acid contents as compared with the other species. The amino acid contents of five different lichen species from the same tree varied without regard to the nitrophily of the species. The contents of amino acids of the lichen species studied from Bonn is four to twelve times higher as in the same species in the Vosges Mountains, France. The amount of amino acids in nitrophytic species (*Xanthoria parietina, Physcia adscendens*) from a region with high

load of atmospheric nitrogen (35 kg/y/ha) is in average 5 times higher than in the same species from a region with low nitrogen immission (16 kg/y/ha).
It can be concluded that the amino acid contents of lichens reflects the atmospheric nitrogen load and that the amino acid content of so called nitrophytic lichen species is not higher as in other species, that lichens are passive sampler and take up the available nitrogen but make no use of it but store it as amino acids. On the other hand, the conductivity of the cell liquid (as a measure of the osmotic pressure) of nitrophytic lichen species is higher as compared with non-nitrophytic species. Thus the "nitrophily" of these species is presumably not based upon the facility to higher nitrogen uptake but osmotic tolerance against the salt effects of nitrogen compounds. Within nitrophytic species, the osmotic values of *Phaeophyscia orbicularis* are double as high as those from *Physcia adscendens,* which is explained by the higher tolerance of *Phaeophyscia* against dry deposition. The higher osmotic values of nitrophilous lichen species lead to the conclusion that they are also drought resistant species and occur in regions with low humidity where they are more competitive than other lichen species.

Introduction

After the decrease of SO_2 emissions at the end of the 20. century, a strong increase of epiphytic lichens in Central Europe has been observed. Lichen mappings showed that the increase of species numbers is based in cities and regions with intensive agriculture on a high number of nitrophytic species (Franzen-Reuter 2006). Studies of permanent plots supported this effect (Franzen-Reuter et al. 2006, Janßen et al. 2007).
Studies in cities revealed, that the amount of nitrophytic lichens was correlated with traffic (Stapper et al. 2005, Stapper & Kricke 2004). This effect is not caused by NO_2, since a comparison of the frequency of nitrophytic lichens on trees in Northrhine-Westfalia (Germany) did not correlate with the measurements of NO_2 of monitoring stations nearby (Schumacher et al. 2006). Therefore ammonia was taken into account. Ammonia emissions in Germany count for about one million tons per year. They originate from livestock (86%), fertilizer (9%), traffic (4%) and industry (1%). The main sources of ammonia from traffic are catalysts (Landesumweltamt Nordrhein-Westfalen 1997). In fact, ammonia values measured in the city of Düsseldorf showed a strong correlation with the frequency of nitrophytic lichens as well the N-content of the lichen species *Parmelia sulcata* (Stapper et al. 2005). Measurements of ammonia in the city of Düsseldorf at the places where the lichen samples were taken for analysis of nitrogen gave a strong correlation with the nitrogen contents of the lichen and proved that ammonia is the best nitrogen source (Frahm 2006). Similar results were obtained from fertilizer experiments, during which epiphytic lichens sprayed with fertilizer containing ammonia showed faster growth than those sprayed with fertilizer containing nitrate (Franzen-Reuter & Frahm 2007). This was also the fact for mosses (Solga et al. 2005, Solga & Frahm 2006) and peat mosses (Simola 1975).
Ammonia is a very reactive gas and reacts with NO_2 to ammniumnitrate (Drechsler et al. 2006), which represents between 20 and 70% of particulate matter (Landesumweltamt Baden-Württemberg 2005). By this way gaseous emissions are turned into dry deposition by formation of aerosols. Ammonia nitrate is a nutrient and taken up by lichens as well as bryophytes over the whole surface. It is also a salt. This dry deposition promotes certain species of lichens, which can be used as indicators of dry vs. wet deposition (Janßen et al. 2007). Dry deposition covers the lichens and is dissolved by dew or rain with the result that they may cause osmotic problems for the uptake of water. This leads to the suspicion that nitrophytic lichens in regions with high rates of particulate matter viz. ammoniumnitrate have to be able to tolerate high concentrations of salt.
An indication that nitrophytic lichens do not depend on nitrogen but are in fact drought resistant species is, that the frequency of nitrophytic lichen species on trees is much higher at Bendorf

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(Middle Rhine area) than in industrial suburbs of Düsseldorf, also situated at the Rhine (Frahm et al. 2007) Although the nitrogen emissions are much higher in industrial parts of Düsseldorf than in residential areas in Neuwied, the latter location has more nitrophytic lichens. This paradox was solved by a correlation of the frequency of nitrophytic lichens on trees using a VDI guideline (Verein Deutscher Ingenieure 2004) with the humidity. Neuwied has a humidity of 1,0 (which means that precipitation is as high as the evaporation), but Düsseldorf has 1,4 times more rainfall than evaporation. This proved that nitrophytes are not always indicators for high nitrogen emissions but also for dry climate and lowered the value of the VDI guideline for determing the nitrogen load of a region. This is also supported by the fact that the so called nitrophytic lichens are confined to lower (drier) elevations in North Rhine Westphalia (Frahm & Stapper 2008), where they are apparently more competitive than other species, which dominate at higher elevations.

To test the hypothesis that nitrophytic lichens are salt and drought tolerant, two considerations were made. First of all, halophytic species amongst the flowering plants are characterized by a high contents of proline (Kinzel 1982). In normal plants, proline counts for 2,4-4% of the total amino acids (Stewart & Lee 1974). In contrast, in halophytic plants an average of 54% (30%-70%) of the amino acids is proline. In Triglochin maritimum, proline counts for 20% of its dry weight. In salt marshes, the proline contents of plants raises with the salt concentration in the soil. In non coastal salt vegetation e.g. the sodium lake Neusiedel in Austria, the amount of proline is strongly enlarged in certain plants but not in all. The proline contents is also enhanced in species of dry habitats, where rates of 28-64% are realized (Kinzel 1982). Apparently the water uptake of both, xerophytes and halophytes, is made more difficult. Thus proline seems to be a good marker for salt habitats and drought stress. If a high contents of proline cloud be detected in lichens, salt tolerance and drought tolerance could be attributed to nitrophytic lichens.

The proline contents of the folious lichen *Parmelia sulcata* was already analyzed by Franzen-Reuter (2004). In this study, the proline contents of the lichen correlated with the nitrogen deposition rate of the habitat. The higher the nitrogen depositions, the higher was the content of arginine, asparagine, glutamine and proline. Reason is that lichens are passive samplers and cannot control the uptake of nutrients. At a deposition rate of 8kg/ha/y, 0,05 µg asparagine viz. arginine per g dry weight were determined. At a deposition rate of 18kg N/ha/y the values raised to 0,45 µg, the ninefold. The amount of proline raised from 0,6 to 2,4 µg/g, the fourfold, glutamine raised from 2,8 to 7,2 µg/g, the threefold. The increase of these amino acids was linear to the increase of nitrogen depositions.
Secondly, xerophytic as well as halophytic species are characterized by high osmotic pressures which are needed for water uptake. If a high osmotic values could be detected in the so called nitrophilous lichens, it could be argued that they are salt and drought tolerant species. Their occurrence could be interpreted as result of high rates of ammonium salts and/or a dry habitat.

Material and methods

To test whether nitrophytic epiphytic lichens are in fact salt tolerant species, the amino acid content as well as the osmotic pressure of certain nitrophytic and non nitrophytic species were determined.

Species selection. Five lichen species with different indicator value for nitrogen (*Hypogymnia physodes, Parmelia sulcata, Physcia adscendens, Evernia prunastri* and *Xanthoria parietina*) were selected. *Physcia adscendens* and *Xanthoria parietina* are regarded as nitrophytes with an indicator value of 6 according (Wirth 2001), *Parmelia sulcata* has a value of 4, *Evernia prunastri* of 3 and *Hypogymnia physodes* of 2.

Collection of samples .

1. To test whether the mode of fixation has an influence upon the results, the species *Physcia adscendens* was collected and (a) frozen in liquid nitrogen, (b) kept in a thermos bottle with ice cubes and (c) packed in a paper bag and transported in silica gel.
2. One set of lichens were collected from the same tree (*Aesculus hippocastanum*) in Bonn-Röttgen on June 5, 2008. The locality is situated in 180 m altitude in a suburb of Bonn with the richest lichen flora in the surrounding of Bonn. Clean parts of the lichens were carefully removed in dry state with a pincer, placed in glass tubes and kept in liquid nitrogen for the transport. Afterwards they were kept in a deep freezer.
3. To see whether a region with lower nitrogen load results in lower contents of amino acids, specimens of the same five lichen species collected in Bonn Röttgen were collected in La Montagne (Vosges Mountains, France) on July 28, 2008, in 645 m elevation in a very extensively used area with forests, meadows and pastures. The nitrogen deposition in Bonn is 19-20 kg/y/ha (Umweltbundesamt 2004) in the Vosges Mountains estimated 10 kg/y/ha.
4. The amino acid contents of the nitrophytic species *Xanthoria parietina* and *Physcia adscendens* were determined in collections from the Lower Rhine area with intensive agriculture (nitrogen deposition 35 kg/y/ha) and a nature preserve in the Ahr valley, about 30 km S of Bonn, with no traffic and no agriculture (nitrogen deposition 16 kg/y/ha), to determine whether higher nitrogen emissions can be correlated with higher amino acid contents. (Lower Rhine: village of Rheurdt ca. 25 m alt., on roadside trees [*Acer pseudoplatanus*], June 15, 2008; Ahr valley, Langfigtal ca. 175 m alt., on *Sambucus nigra*, July 1, 2008).

Determination of conductivity. Because of problems to determine the osmotic pressure of the lichens, the conductivity of the cell liquid was determined instead. For this purpose, the lichens were watered for one minute in VE-water, then kept 48 hours at 100% air humidity to allow full hydration, transferred into liquid nitrogen and squeezed. The conductivity was measured with a conductivity sensor.

To test whether nitrophytic species have the same or higher osmotic pressure as “normal” lichens, the conductivity of 5 different species from the same tree as described under 2. was determined.

To test whether there are differences in the conductivity (= osmotic pressure) of the cell liquid within nitrophytic lichens, samples of *Phaeophyscia orbicularis* and *Physcia adscendens* were collected on roadside trees of the same species (*Acer pseudoplatanus*) along the same tree in Bonn Endenich (part of the city), Bonn Röttgen (a suburb) and the village of Rheurdt (Lower Rhine).

Results

1. The mode of fixation (deep freezing, cooling, drying) had almost no influence upon the contents of amino acids. For example, the values of glutamine varied between 1.1 and 1.3 mg/g dry weight, proline between 0.2 and 0.35 mg/g dry weight (Fig. 1). The values varied independently of the mode of fixation; no method had significantly higher or lower values.

2. The amino acid contents of the five lichens species collected on the same tree in Bonn Röttgen varied without regard to the nitrophily of the species. The highest values were found in *Evernia prunastri*, which is a fruticose lichen; the others are thallose species (fig. 2). *Physcia adscendens*

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and *Xanthoria parietina*, both nitrophytic, had lower values. The lowest values were found in *Hypogymnia physodes* (acidophyte) and *Parmelia sulcata* (neutrophilous, moderately nitrogen tolerant).

3. The contents of amino acids in *Evernia prunastri, Hypogymnia physodes* and *Parmelia sulcata* is much higher in Bonn as compared to the Vosges mountains (tab. 1). In the Vosges Mountains, the proline contents of *Hypogymnia physodes* is 0,1 mg/g dry weight, in Bonn 0,8 mg/g. The glutamine contents of *Evernia prunastri* is 0,8 mg/g in the Vosges Mountains but 7.4 mg/g in Bonn. In general the amino acid contents in Bonn is three to twelve times as high as in the Vosges.

Tab. 1: Amino acid contents of different lichen species in regions with low (Vosges) and moderate (Bonn) loads of atmospheric nitrogen.

	Evernia Vosges	Evernia Bonn	Factor	Hypogymnia Vosges	Hypogymnia Bonn	Factor	Parmelia sulcata Voges	Parmelia sulcata Bonn	Factor
Taurine	0,19	2,9	15	0.1	1.5	15	0.04	0.5	12,5
Glutamine	0,82	7,4	9	0.62	1.1	2	0.26	1.1	4
Glutamat	0,38	4,6	12	0.8	4	5	0.32	4.3	15
Alanine	0,4	1,3	3.2	0.4	0.2	0,5	0.4	0.3	0,75
Proline	0,3	2,3	7.6	0.1	0.8	8	0.1	0.4	4

4. The amino acid content of the nitrophytic species *Physcia adscendens* and *Xanthoria parietina* in regions with low nitrogen loads (Ahrtal) are much lower than in regions with high nitrogen loads (Lower Rhine). The glutamine contents in the Ahr valley was 0.9 – 1 mg/g dry weight as compared with 4,8-5,1 mg/g dry weight in the lower Rhine Region, thus five times higher (fig. 3). Proline is 2-5 times higher. Alanine and arginine shows hardly any differences and glutamate shows no correlation with the nitrogen load of the habitat.

5. The content of proline in the nitrophytic lichens studied is not significantly higher as compared with the other species. These lichen species are either no halophytes or halophytic species of lichens have no higher proline contents like flowering plants.

6. The conductivity of the cell liquid of lichens collected from the same tree varied between 0,03 and 0,7 mS (tab. 2).

Tab. 2: Conductivity of cell liquids of lichen species from the same tree.

Species	Weight (mg)	Conductivity (µS/cm)
Physcia adscendens	15.13	0.7
Xanthoria parietina	15.08	0.15
Parmelia sulcata	15.71	0.09
Evernia prunastri	15.71	----
Hypogymnia physodes	15.03	0.03

7. The conductivity of the cell liquid of the nitrophytic *Phaeophyscia orbicularis* is much higher than the conductivity of the nitrophytic *Physcia adscendens* (tab. 3). The values of *Physcia adscendens* are conspicuously uniform in the three different study areas, which might depend on

the fact that the samples were taken on roadside trees of roads with high traffic. The values of *Phaeophyscia orbicularis* are double or three times as high.

Tab. 3: The conductivity (µS/cm) of the cell liquid of the nitrophytic lichen species *Phaeophyscia orbicularis* and *Physcia adscendens* growing on the same tree trunk in different regions.

	Endenich (city of Bonn)	Röttgen (suburb of Bonn)	Rheurdt (Lower Rhine)
Physcia adscendens	108	113	105
Phaeophyscia orbicularis	207	180	359

Janßen et al. (2007) could show that *Phaeophyscia orbicularis* is an indicator for dry nitrogen deposition. The species has much increased over the years 2004 to 2006 in a 20 x 20 cm permanent plot situated nearby a control station for nitrogen depositions. The plot was covered mainly by *Physcia adscendens* before. In the same time, the deposition of total nitrogen had decreased, however, the amount of dry deposition had increased. Since the increase of *Phaeophyscia* was correlated with the increase of dry deposition, this species was regarded as indicator for dry deposition, however, the reasons were not clear. Our results indicate the higher osmotic value of *Phaeophyscia* as compared with *Physcia* enables the species to tolerate dry deposition with a higher salt concentration. Dry depositions can accumulate on the surface of the lichen thallus. If they are wetted by dew or slight rainfall, the predominant compound ammoniumnitrate is dissolved into a high concentrated salt solution. The concentration is much higher than the salt concentration of wet deposition in rainfall.

Discussion

The content of analyzed amino acids are higher in regions with higher nitrogen load and seem to correlate with the nitrogen load of the habitat. They were highest in the Lower Rhine area and lowest in the Vosges Mountains but intermediate n Bonn.

The results prove the fact that lichens are passive sampler. The higher the atmospheric nitrogen, the higher are the values of amino acids. The species apparently have no mechanism to control the uptake of nitrogen but store the surplus, which is not used for metabolism, as amino acids.

The results support studies in the city of Düsseldorf, where the content of total nitrogen in the lichen *Parmelia sulcata* was strongly correlated with the ammonia measured by samplers on the same tree (Frahm 2006) as well studies in Northrhine-Westphalia, where the contents of certain amino acids in lichens correlated with the local nitrogen deposition (Franzen-Reuter 2004).

The amount of amino acids in the lichen thalli are not higher in nitrophytes (*Xanthoria parietina*, *Physcia asendens*) as compared with non-nitrophytes on the same tree. It is possibly depending on the growth form. So all lichen species studied have the same facility for absorption of nitrogen. Nitrophytes have no higher absorption and possibly no special adaptation to nitrogen in their metabolism.

The determination of the conductivity showed that the nitrophyte *Physcia adscendens* has by far the highest value. *Xanthori*a with the same indicator value has a lower conductivity of the cell liquid and *Parmelia sulcata* as well as *Hypogymnia physodes* the lowest.

If the conductivity is taken as a measure for the osmotic pressure, *Physcia adscendens* is able to tolerate the highest salt concentrations and can therefore tolerate the highest amounts of particulate matter in form of ammonium salts. This correlates with the observation that this is the most common species in rural and urban areas with the highest nitrogen load. In contrast, *Hypogymnia*

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physodes has the lowest conductivity and probably the least facility for water uptake from salt solutions. It correlates with its distribution in regions with lowest nitrogen loads. The conductivity of *Parmelia sulcata* as a moderate nitrogen tolerant species is in between that of the nitrophytic and non-nitrophytic species. In general the conductivity of nitrophytic lichen species is higher than that of non-nitrophytic species. This supports the theory that nitrophytic lichens are drought tolerant species. The higher osmotic pressure allows the uptake of water vapour at lower rates of relative humidity. This is an advantage as compared to other lichen species with lower values of osmotic pressure, which cannot survive in dry regions. This is supported by the dominance of nitrophytic lichen species in a part of Germany with low humidity but no apparently higher nitrogen emissions (Frahm et al. 2007).

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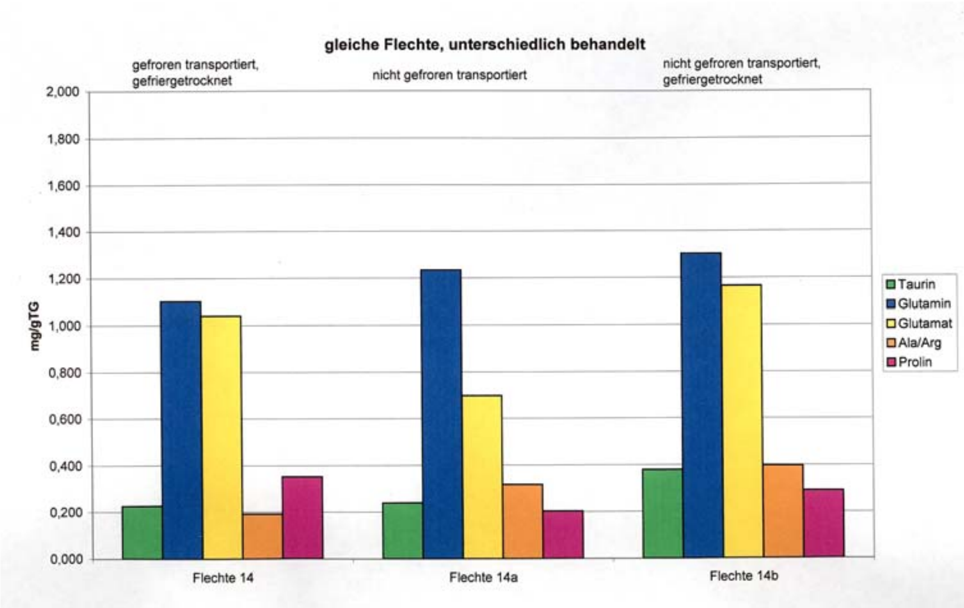

Fig. 1: Amino acid contents of *Physcia adscendens* after different modes of fixation.

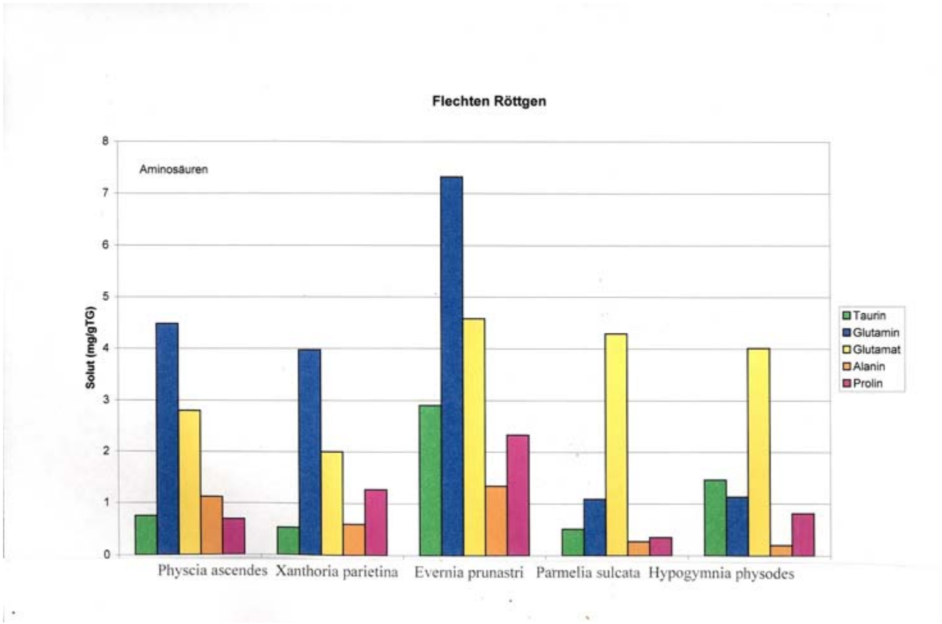

Fig. 2: Amino acid content of five different lichen species from the same tree.

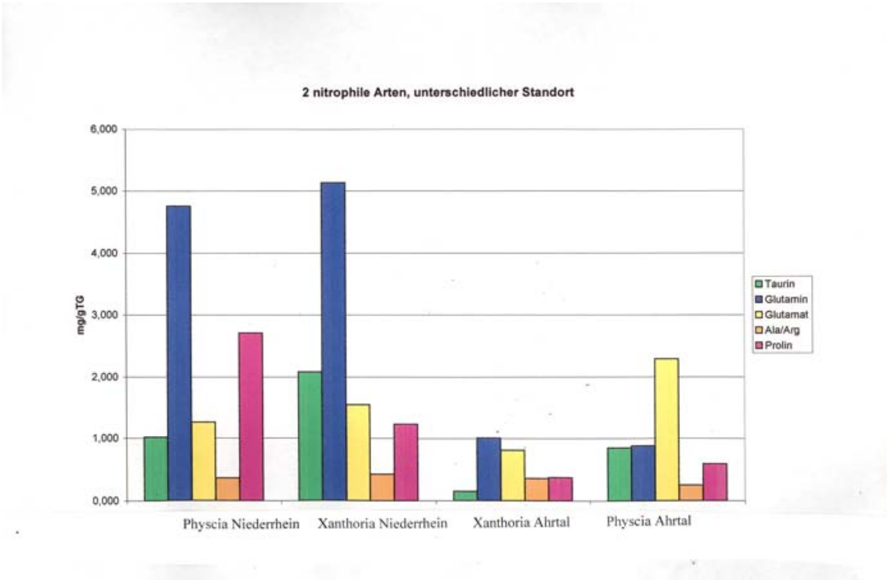

Fig. 3: Amino acid contents of two nitrophytic lichen species from different regions.