PRELIMINARY STUDY USING LICHEN SPECIES DIVERSITY AS AN INDICATOR OF LOCAL ENVIRONMENTAL QUALITY WITHIN TWO NATURE RESERVES FROM ROMANIA

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Abstract. Within this study was emphasized the difference between the high degree of conservation of Glodeasa Forest Reserve and the impact of eutrophication as a result of human activities from Măluşteni Nature Reserve using lichen species diversity. The major human activities with a strongly impact on environment quality in Măluşteni Nature Reserve area are following: the livestock operations and the crop farming. A comparative assessment on the two protected areas pointed out a significantly differences regarding the preferences of inventoried lichen species to nitrophily. Thus, it was recorded a highest percentage (67%) of nitrophilous lichen species within Măluşteni Nature Reserve in contrast with a highest percentage (93%) of anitrophilous lichen species inventoried from Glodeasa Forest Reserve. The prevailing of some rare and threatened lichen species within Glodeasa Nature Reserve represent a sensitive indicator of highest environmental quality.

Keywords: lichen, environmental quality, nitrophily, anitrophily, Glodeasa, Măluşteni.

INTRODUCTION

The use of living organisms in the study of environmental quality is now widely accepted in many countries [16]. Lichens are extremely sensitive to environmental stress, especially concerning atmospheric pollution, eutrophication, etc. [1, 6].

It is well known that the distribution of epiphytic lichens in forests is restructured by degradation of the environmental conditions. The changes within epiphytic lichen composition in areas with intensive agriculture are caused by increasing of NO_X (nitrogen oxides) in atmosphere therefore take a place a spectacular increasing of nitrophilous species from genera, such as: *Physcia* (Schreb.) Michaux (1803), *Phaeophyscia* Moberg. (1977), and *Xanthoria* (Fr.) Th. Fr. (1860) [20]. Other important source of eutrophication is represented by the density of cattle on farms, this being negatively correlated to restructuring of the epiphytic lichen communities [17].

Protected areas have an important roll to conserve relatively intact habitats and threatened species, but not all protected areas are able to meet their conservation goals because of economic and social constraints [13].

Changes in frequencies of nitrophobe and nitrophilous lichen species can act as an early warning system for long term changes associated with nitrogen or acidifying deposition [25].

This preliminary study aimed to assess the local environmental quality using as indicators lichen diversity. The main objective of this study consists in identification of forest ecosystem with a high environmental quality using as an indicator lichen species. Based on lichen indicator species will be carried out a mapping of environmental quality at local and regional level. The data regarding the researches carried out within Glodeasa Forest Reserve and Măluşteni Nature Reserve are cited for the first time in Romanian lichenological literature.

MATERIALS AND METHODS

Studied areas

The Glodeasa Forest Reserve is a geographical part of ecoregion of the Curvature Carpathians (Fig. 1). This forested area cover 528 hectares from which 347 hectares are protected area belonging from territorial point of view to Câmpina Forest Range, the unit of production Orjogoaia [18]. The basin of Glodeasa stream that include the Glodeasa Forest Reserve belong to Baiului Mountains situated between Prahova and Doftana Valleys. The Baiului Mountains are build up by marl schists as a part of Sinaia layer [4]. The climate is strongly influenced by relief with an temperate – continental character [2]. The annual mean of temperature range from 6°C at medium altitudes to under -2°C on the Carpathian summits. The annual precipitation range between 800-1200 mm [4]. The natural vegetation, an old stand of secular trees, still well preserved, is represented mainly by *Fagus sylvatica* L. (beech) associated with resinous species, such as *Abies alba* Mill. (fir).

The Măluşteni Nature Reserve is situated in The Moldavian Plateau (in the north-east part of Romania) (Fig. 1). This hill-like unit, with a general north-east and south-west inclination, consists of monocline Neozoic sedimentary deposits (clays and sands with intercalations of limestone and sandstone) [4]. The Moldavian Plateau is characterized by its high elevation (400-500 m) and excessive-continental climatic influences with an annual mean temperature between 7.5°C and 9.5°C. The atmospheric precipitation range between 450-600 mm [2].

The Măluşteni Nature Reserve is an important fossiliferous paleontological reserve from Romania. The fossilifer placement of Măluşteni stretches on a surface of 10 hectares and it is situated in the north-western from Măluşteni village at the origin of Românească Valley. The Românească Valley stretches along the western part of the Măluşteni village [18].

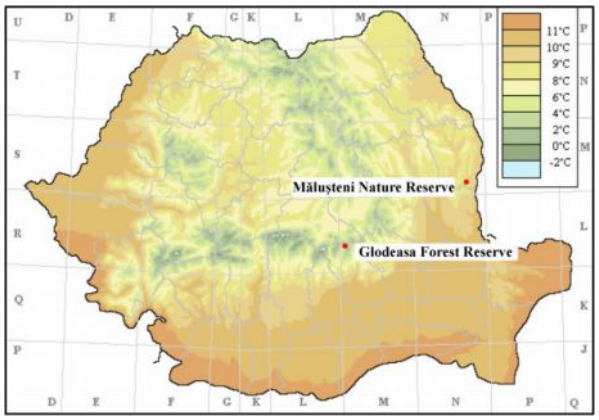

Figure 1. The investigated sites [25].

Within Măluştini Nature Reserve, the vegetation is represented by a second-growth forest in which species such as *Populus nigra* L. and *Robinia pseudacacia* L. are prevailed. The *Salix* L. genus is also well represented along a stream that crosses the researched area. The anthropogenic activities, especially animal waste are responsable to the presence of sinanthropic species both, cormophytes (*Iva xanthifolia* Nutt. (*Cyclachaena xanthiifolia* (Nutt.) Fresen.), *Urtica urens* L., *Cannabis sativa* subsp. *spontanea* (Vavilov) Sereb. (*C. ruderalis* Janish.), *Consolida regalis* S. F. Gray, etc.) and lichen species (*Xanthoria parietina* (L.) Th. Fr., *Physcia adscendens* (Fr.) Oliv., *Phaeophyscia orbicularis* (Nëck.) Moberg., etc.). The adjacent areas of Măluşteni Nature Reserve are intensively used as agricultural lands and pastures. Thus, fertilized crop and concentrated animal waste from livestock enclosures are major point sources of NH_3 in rural areas [14].

Sampling procedure

The researches activities concerning the inventory of lichen species was carried out on May 2009 within Glodeasa Forest Reserve and on June 2009 within Măluşteni Nature Reserve. Within both investigated forests the lichen species were collected from the base of the trunks up to 1.5 m height. The lignicolous lichen species were taken into account. The collected lichen species were carried into laboratory to be determined using the bibliography [8-9, 19].

The high plants (host trees) were determined according to Ciocârlan [7] and Beldie [5]. The nomenclature used is according to Ciurchea [9]. The identification of lichen species was also based on spot test using chemical reagents such as: KOH, $CaCl_2$, and IIK. Indicator values to assessment nitrophily were extracted from bibliography [10, 27]. The anatomical aspects of thalline formations were analyzed on the basis of microscopic slide preparation. The specimens are conserved in the Lichen Collection, BUCM Herbarium within Institute of Biology, Romanian Academy from Bucharest.

RESULTS

A total of 26 lichen species were recorded in the studied areas, of which 46% lichen species were found within Măluşteni Nature Reseve and 54% lichen species were recorded for Glodeasa Forest Reserve, respectively (Fig. 2). The percentage distribution regarding the species richness within both investigated forests is close related to microhabitat conditions offered especially by chemical properties of the corticolous substrata. Thus, the greater number of lichen, especially rare and threatened species inventoried in a great deal on lignicolous substrata (Fig. 4), within Glodeasa Forest Reserve reflects, a highest conservation value. In Măluşteni Nature Reserve, species richness sampled on corticolous substrata is structured by rural anthropogenic activities. It was observed that, the lichen species composition is completly different in the two investigated forests, as a consequence of environmental conditions (Table 1).

From ecological point of view, one of the most important variables taken into account is the lichen species nitrophily. Thus, in the Glodeasa Forest Reserve, 93% of investigated lichen species are anitrophilous and 7% are moderate-nitrophilous, respectively (Fig. 3). Within Glodeasa Forest Reserve the structure of lichen species composition is close related to a high conservation value, especially based on the presence of threatened lichen species, such as: *Cetrelia cetrarioides* (Del. ex Duby) W. Culb. & C. Culb., *Evernia prunastri* (L.) Ach., *Flavoparmelia caperata* (L.) Hale, *Lobaria pulmonaria* (L.) Hoffm., *Pertusaria amara* (Ach.) Nyl., *Phlyctis agelaea* (Ach.) Flot., *Ramalina farinacea* (L.) Ach., *Ramalina pollinaria* (Westr.) Ach., and *Usnea ceratina* Ach.

According to the field observation, rural anthropogenic activities are the main drivers responsible for restructuring lichen species composition within Măluşteni investigated area. Regarding the preference of lichen species to nitrophily from Măluşteni Nature Reserve, a highest percentage of nitrophilous lichen species (67%), a lower percentage of anitrophilous lichen species (25%), and 8% for anitrophilous-moderate nitrophilous lichen species, were recorded (Fig. 3).

Within Glodeasa Forest Reserve, both euryhygrophilous and xeromesophilous-mesophilous lichen species have recorded equal percentage (29%). The xeromesophilous and mesohygrophilous lichen species have recorded 14% each. The mesophilous-mesophilous-hygrophilous and mesophilous lichen species are less represented (7% each). The preferences of lichen species to temperature indicate that the micro-mesothermal lichen species are prevailed (43%), followed by eurythermal (29%), microthermal (14%), and moderately thermophilous (14%), lichen species. Regarding the preferences towards substrata characteristics a great majority of lichen species are acidophilous-moderately acidophilous (36%) these are followed by moderately acidophilous (29%), acidophilous (21%), euryionic and moderately acidophilous-subneutrophilous with 7% each. The photosciaphilous and moderately photophilous lichen species have recorded 29% each, accompanied by photosciaphilous-moderately photophilous (21%), pho-

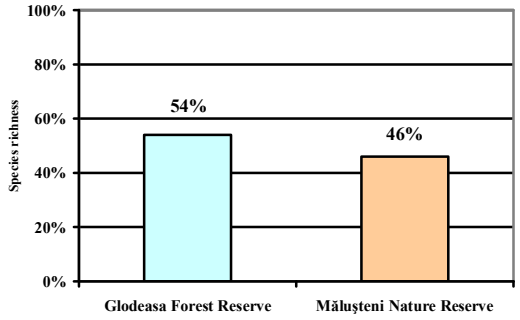

Figure 2. The lichen species richness in relation with investigated forests.

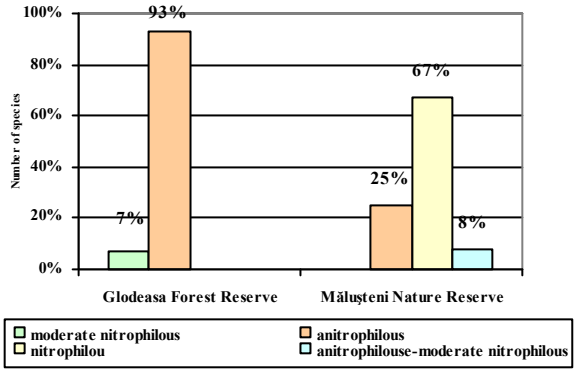

Figure 3. The percentage distribution of lichen species in relation with preference to nutrients within Glodeasa Forest Reserve and Măluşteni Nature Reserve.

Table 1. The listed lichens species identified within the two nature reserve in relation to substrata and values of the ecological indices.

Species	Locality		Values of ecological index
	Glodeasa Forest Reserve	Măluşteni Nature Reserve	
Caloplaca placidia (Mass.) Stein	-	+	$H_6T_5R_7L_{.7}$
Cetrelia cetrarioides (Del. ex Duby) W. Culb. & C. Culb.	+	-	$H_6T_4R_5L_5$
Cladonia pyxidata (L.) Fr.	+	-	$H_0T_0R_0L_7$
Cladonia coniocraea (Flk. Spreng. em Sandst.	+	-	$H_0T_0R_4L_5$
Cladonia fimbriata (L.) Fr. em Sandst.	+	-	$H_0T_5R_4L_7$
Evernia prunastri (L.) Ach.	-	+	$H_3T_5R_3L_7$
Flavoparmelia caperata (L.) Hale	+	-	$H_4T_6R_4L_6$
Fuscidea cyathoides (Ach.) V. Wirth & Vèzda	-	+	$H_7T_4R_4L_5$
Hypogymnia physodes (L.) Nyl.	+	-	$H_3T_0R_3L_9$
Lecanora hagenii (Ach.) Ach.	-	+	$H_3T_0R_8L_6$
Lobaria pulmonaria (L.) Hoffm.	+	-	$H_7T_5R_5L_5$
Melanelia olivacea (L.) Essl.	+	-	$H_7T_5R_3L_8$
Parmelia saxatilis (L.) Ach.	-	+	$H_5T_4R_3L_6$
Parmelia sulcata Taylor.	+	-	$H_3T_0R_5L_7$
Pertusaria amara (Ach.) Nyl.	+	-	$H_4T_5R_3L_6$
Phaeophyscia nigricans (Flk.) Moberg.	-	+	$H_0T_0R_8L_8$
Phaeophyscia orbicularis (Nëck) Moberg.	-	+	$H_0T_0R_7L_7$
Phaeophyscia sciastra (Ach.) Moberg.	-	+	$H_0T_0R_8L_8$
Phlyctis agelaea (Ach.) Flot.	+	-	$H_4T_6R_6L_5$
Physcia adscendens (Fr.) Oliv.	-	+	$H_3T_5R_7L_7$
Physcia stellaris (L.) Nyl. Em. Harm.	-	+	$H_3T_5R_6L_7$
Physconia distorta (With.) J. R. Laudon	-	+	$H_3T_5R_6L_7$
Ramalina farinacea (L.) Ach.	+	-	$H_4T_5R_5L_6$
Ramalina pollinaria (Westr.) Ach.	+	-	$H_5T_3R_4L_7$
Usnea ceratina Ach.	+	-	$H_0T_5R_4L_8$
Xanthoria parietina (L.) Th. Fr.	-	+	$H_3T_5R_7L_7$

Legend of the table: + the presence of the lichen species; - there is no available data; H-humidity; T-temperature; R-value of pH; L-light

tophilous (14%), and strongly photophilous (7%) (Table 1).

Regarding the preferences of the inventoried lichen species within Măluşteni Nature Reserve towards humudity a great majority of these are xeromesophilous (50%), followed by euryhygrophilous (25%). The mesophilous, mesohygrophilous, and mesophilous-mesohygrophilous lichen species have recorded lower percentage (8% each). The preferences to temperature indicate a high percentage of micro-mesothermal lichen species (50%), followed by eurythermal and microthermal lichen species (33% and 17%, respectively). From chemical properties of substrata point of view, the subneutrophilous lichen species are prevailed (33%), accompanied by neutrophilous (25%), acidophilous-moderately acidophilous (17%), moderately acidophilous-subneutrophilous (17%), and acidophilous (8%) lichen species. The prevailing of moderately-photophilous (58%) is close related to weakly closed canopies of poplar trees within Măluşteni Nature Reserve. The moderately-photophilous lichen species are accompanied by photosciaphilous-moderately photophilous (17%), photophilous (17%), and photosciaphilous (8%) (Table 1).

Within Glodeasa Forest Reserve, a great majority of inventoried lichen species were identified on lignicolous substrata (50%), followed by species found on rhytidoma of *Abies alba* (29%) and *Fagus sylvatica* (14%). A lower percentage (7%) was attributed to tericolous lichen species (Fig. 4). In Măluşteni Nature Reserve, all investigated lichen species are corticolous,

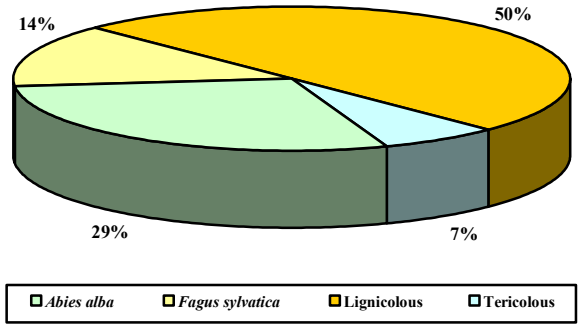

Figure 4. The spectrum of lichen species as a function of substrata from Glodeasa Forest Reserve.

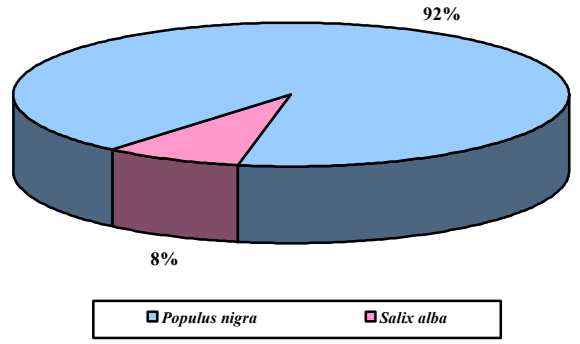

Figure 5. The spectrum of lichen species as a function of substrata from Măluşteni Nature Reserve.

of which a highest percentage (92%) was recorded for species found on rhytidoma of *Populus nigra*, followed by a lower percentage (8%) recorded for species identified on *Salix alba* bark (Fig. 5).

According to field observations, lichen species composition, as sensitive indicators of environmental conservation value are structured by environmental drivers both within natural and anthropogenic conditions, respectively.

DISCUSSIONS

There is a major difference regarding the local environmental quality between the two studied woodlands.

The affinity of certain epiphytic lichen species for NH_3 (ammonia) is well documented in the lichenological literature [12, 15, 20, 22]. These indicator species, known as, ''nitrophytes'' are common, conspicuous components of the lichen flora in urban and agricultural landscapes. In rural areas the relative abundance and diversity of nitrophilous species is caused by NH_3 volatilization from both fertilized fields and concentrated animal wastes at livestock enclosures such as dairies farm and pasture [14]. However the lichen diversity depends on many factors such as: climate, substratum ecology and different type of anthropogenic interference [11, 21].

It was observed that the species richness depends on environmental conditions in both studied sites. Thus, the most lichen species from Măluşteni Nature

Figure 6. The concentrated animal wastes disposal on eastern side of Măluşteni Nature Reserve (photo: Vicol Ioana, 2009).

Figure 7. The abundance of *Xanthoria parietina* on a trunk of *Populus nigra* from Măluşteni Nature Reserve (photo: Vicol Ioana, 2009).

Reserve are nitrophilous, for instant: *Lecanora hagenii*, *Phaeophyscia nigricans*, *Phaeophyscia orbicularis*, *Phaeophyscia sciastra*, *Physcia adscendens*, *Physcia stellaris*, *Physconia distorta*, and *Xanthoria parietina*. The concentrated animal wastes represent a major sources of NH_3 within this protected area (Fig. 6).

On the other hand, it was observed a positive correlation between the highest number of nitrophilous lichens species and their host trees (Fig. 7).

Thus, the sampled host trees, such as: *Populus nigra* and *Salix alba* are eutrophic species. In a strongly contrast to environmental conditions from Măluşteni Nature Reserve, within other ecosystems from Romania, for instant in Bucharest Municipality, Colentina District, along the Colentina River was observed that in the absence of the agriculture practices and concentrated animal waste the species of *Populus* genus do not represents a host to nitrophilous lichen species (Vicol, Bucharest, pers. comm. 2010). The richness of nitrophilous lichen species is one of the most important indicator to assessment local eutrophication conditions from Măluşteni Nature Reserve. The nitrophilous lichen species are growing on subacid to basic substrata, normally exposed to direct sunlight, in conditions of relatively high aridity, rather tolerant of air pollution [11]. Agriculture and concentrated animal wastes may induce nitrophytic

lichen vegetation because of nutrient enrichment of the habitat and the synergistically effect of dust and light which induce the drying of the bark [16]. In a similar study performed in the Eastern Sayan (South Siberia, Russia) it was revealed the impact of alkaline dust and ammonia (NH_3) on lichen species. Thus, a rise of the bark pH, it was accompanied by an increase in the proportion of nitrophilous lichen species, whereas the proportion of the acidiphilous lichen species, gradually decreasing [20]. The presence of two acidophilous lichen species *Evernia prunastri* and *Parmelia saxatilis* within Măluşteni Nature Resrve, might be explained by the fact that lichens which growing on tree trunks do not respond earlier to changes in atmospheric conditions. Thus, lichens are often relatively long-lived and nitrophobes may continue to survive on an older substratum despite moderately increasing ammonia concentrations [26]. On the Swiss Plateau (Central Switzerland), in environmental conditions similar to those from Măluşteni, 70% of lichen taxa (*Xanthoria, Physcia, Phaeophyscia*), common to this area were nitrophilous, whereas 30% were anitrophilous. The nitrophilous lichen species increased in regions with intensive agriculture and with the intensity of farming [22]. Thus, as indicators of environmental quality, lichens provide an excellent and inexpensive alternative for estimating at a fine-scale, the distribution of ammonia [15].

Within the Glodeasa Forest Reserve, the richness of anitrophilous lichen species such as: *Cetrelia cetrariodes*, *Flovoparmelia caperata*, *Hypogymnia physodes*, *Lobaria pulmonaria*, *Parmelia sulcata*, *Pertusaria amara*, *Ramalina farinacea*, etc. represents an important indicator to assessment the highest conservation value of this habitat. There is a positive correlation between the dominant host trees (*Fagus sylvatica* and *Abies alba*) and acidophilous lichen species which growing on their bark. Thus, 29% of acidophilous lichen species (*Hypogymnia physodes, Pertusaria amara, Ramalina farinacea, and Ramalina pollinaria*) were identified on *Abies alba* rhytidoma. A lower of acidophilous lichen species (14%) were were found on *Fagus sylvatica* bark (*Lobaria pulmonaria* and *Usnea ceratina*). In an other similar study performed within a native Caledonian pinewood (Aberdeenshire, Scotland), the acidophilous lichen species (*Calicium parvum, Chaenotheca chrysocephala, Hypocenomyce friesii, Imshaugia aleurites,* and *Lecidea hypopta*), were used as indicators of ecological continuity [27]. As in the Glodeasa Forest Reserve, in other studies, *Lobaria pulmonaria*, is cited as a species specifically to habitats with a high conservation degree [3], highly sensitive to increase of nitrogen compounds in atmosphere [26], and an associated species to old-growth forests [23]. The lichen species such as: *Flavoparmelia caperata, Pertusaria amara,* and *Ramalina farinacea* are sensitive to air pollution and they are growing in microclimates with higher atmospheric humidity and lower bark eutrophication [16].

Several studies carried out in central and northern Europe recognized NH_3 air pollution as an important factor affecting the epiphytic lichen vegetation. In The Netherlands, during of 10 years, take a place an increasing of nitrophilous lichen species, paralleled by a decrease in acidophytic ones, has occurred in areas with high cattle density, and this phenomenon was especially apparent on acid-barked trees, on which nitrophytes were previously scarce or absent [12]. A similar shift in species composition was also observed in the UK [27], Switzerland [22], and rural area of Central Italy [16].

Studies performed in the western part of Denmark (Kås Forest) and in the West of Great Britain (Tycanol National Nature Reserve) have shown major differences between the twig lichen communities at the two sites. Thus, within Tycanol National Nature Reserve, was recorded a high frequency of nitrophobes (nitrogen sensitive species) and a low frequency of nitrophiles, while at Kås there is a low frequency of nitrophobes and a high frequency of nitrophiles [26].

In the Protected Landscape Area Bohemian Karst (Czech Republic) a high environmental alterations was detected using lichens as indicator of environment quality in areas where intensive agriculture are practiced. Within this area the most common are nitrophilous species (*Physcia adscendens*, *Physcia tenella*, *Phaeophyscia orbicularis*, *Xanthoria parietina*, etc.) and the rarest are acidophilous species (*Flavoparmelia caperata*, *Pleurosticta acetabulum*, *Ramalina pollinaria*, etc.) [24].

The ecological preferences of inventoried lichen species are correlated to environmental conditions specifically to both investigated forests. Thus, within Glodeasa Forest Reserve prevailing anitrophilous lichen species accompanied in a great deal by euryhigrophilous and xeromesophilous-mesophilous, micro-thermal, acidophilous-moderately acidophilous and photosciaphilous lichen species. Within Măluşteni Nature Reserve, prevailing nitrophilous lichen species with preferences to substrata that are enrichment in organic nutrients in xeric and better illuminated stations.

Assessment of local environmental conditions both within Glodeasa Forest Reseve and Măluşteni Nature Reserve reveal significantly differences based on indicator lichen species. Thus, within the Măluşteni Nature Reserve the intensive livestock operations and intensive agriculturally practices have led to the eutrophication of local environmental conditions, reflected by richness of nitrophilous lichens species. On the contrary, the highest conservation value within Glodeasa Forest Reserve is due to the lowest impact of human activities, the proof being the richness of threatened and anitrophlious lichens species.

Acknowleggements. The author is gratefully to Mr. Vicol Ioan for helpful regarding field activity, also thankful to PhD Ştefănuţ Sorin, Phd Ion Mihaela, and Phd Fiera Cristina for their help in field work. The field activity took place under a project funded by Romanian Academy.

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Received: 31 January 2011
Accepted: 8 April 2011
Published Online: 16 April 2011
Analele Universităţii din Oradea – Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433