„Babes-Bolyai" University Cluj-Napoca
Faculty of Biology and Geology

Macrolepidoptera communities from Copşa Mică (Sibiu County) and surroundings with emphasis on bio-indicator species (Lepidoptera)

- Summary of the PhD Thesis -

Ph.D. student:
Török Sergiu-Cornel

Scientific coordinator:
Prof. László Rákosy, Ph.D.

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THESIS CONTENTS

Key words: Macrolepidoptera communities, atmospheric pollution, Copșa Mică, biodiversity, industrial melanism

INTRODUCTION

Lepidoptera, together with birds and vascular plants, represents the most frequently monitored taxonomic groups. Several ecological characteristics make Lepidoptera promising biodiversity indicators: easy to survey, intensely studied, worldwide distribution, and more important they represent the dominant fraction of biodiversity and they are sensitive to many environmental changes, natural or atrophic.

Consequently the Macrolepidoptera communities were used as indicators of environmental health in the area of Copșa Mică.

The area of Copșa Mică was regarded, as the most extensively polluted area in Romania and even in Europe. This area was subject of high pollutants concentrations, mainly sulphur compounds in synergistic action with heavy metals (eg. Cu, Pb, Cd, Zn, Mn), pollution effects were felt within a radius of 180 750 hectares, being affected all the ecosystem components.

This paper aims to emphasize on one hand the impact of pollution on Lepidoptera communities from this area, and to discover and analyse the percentages of the melanic forms from certain Lepidoptera species.

The industrial melanism was studied in the period 1987-1988, in Copșa Mică by Rákosy & Rákosy (1997), the results obtained showed a higher percentage of melanic *Biston betularia* forms (carbonaria), in the intensely polluted area these forms reached 100 %. Knowing this data in 2012, the study was repeated, being highlighted some changes in percentages of this species melanic forms.

The aim of our research was to investigate the diversity, similarities and ecological particularities of the Copșa Mică Macrolepidoptera communities from forest sites, aiming to reflect the effects of air pollution in these communities. In order to achieve our aim the following goals were established:

- Identification and establishment of the taxonomic composition and abundance encountered in the analysed area;

- Emphasize rare and endangered species in the area of Copșa Mică;
- Analysis of the Macrolepidoptera communities diversity and structure from the area of Copșa Mică;
- The analysis of the larval trophic spectrum of Macrolepidoptera species from the area of Copșa Mică;
- Identifying and analysing the species with melanic forms and their proportion, to reflect the intensity of pollution from different localities.

Anthropic activities (including pollution) led to a reduction in the habitat of many Lepidoptera species, to the restructuring of faunal complexes found in forest habitats.

These consequently have increased the vulnerability of Macrolepidoptera species, modifying the species composition, structure and abundance.

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THE RESEARCHES HISTORY CONCERNING THE LEPIDOPTERA AS ATMOSFERIC POLLUTION BIOINDICATORS

Environmental degradation has been emphasized using bio-indicator species or communities. Commonly the indicators are species or assemblies which have parameters (biochemical, physiological, ethological or ecological) that describe very precise the state of the environment, indicating their natural or anthropogenic changes.

Butterflies, together with birds and vascular plants, represent the most frequently monitored taxonomic groups (de Heer et al., 2005; Thomas, 2005; Settele et al., 2008).

Several ecological characteristics make butterflies promising biodiversity indicators: due to short life cycles they are more sensitive than other groups to changes in their habitats (Thomas 1994, Van Swaay & Warren 1999, Thomas et al. 2004); due to breeding even in small habitat patches they are likely to reflect changes occurring at a fine scale (Van Swaay et al. 2006); they may be expected to be representative for a wide range of terrestrial habitats (Van Swaay et al. 2006), and more important to be adequate indicators for many groups of terrestrial insects (Thomas & Clarke 2004; Thomas, 2005), which themselves constitute the predominant fraction of biodiversity.

Consequently, monitoring the change in abundance and assessing the distribution of butterflies have been suggested as a potential tool for assessing large-scale biodiversity trends (Grosser, 1989; Măciucă, 2003; Thomas, 2005; van Swaay & van Strien, 2005; van Swaay et al., 2006; van Swaay, 2007; Fleishman & Murphy, 2009).

Butterflies are unusual among insects because they can be studied nearly worldwide (Thomas, 2005).

Assumptions have been made in the literature that the presence of all or selected species in a butterfly assemblies is indicative of general environmental attributes, such as conservation value (Mas & Dietsch, 2004), environmental health (Nel, 1992), and environmental quality (Gordon & Cobblah, 2000; Brown & Freitas, 2002; Mouquet et al., 2005).

Many researchers have examined whether species richness or the occurrence of a subset of a butterfly assemblies typically identified a priori on the basis of taxonomy, ecological similarity, or rarities correlated with species richness of an entire assemblies of butterflies (Beccaloni & Gaston, 1995; Swengel & Swengel, 1997; Swengel & Swengel, 1999; Horner-Devine et al., 2003, Schulze et al., 2004).

In addition, nestedness analyses have generated positive associations between the occurrence of a subset of a butterfly assemblies and an assemblies as a whole (Franzen & Ranius, 2004), numerous other investigations have focused on whether the presence or abundance of some or all species in a given assemblies is correlated with species richness or abundance of other taxonomic groups (Williams & Gaston, 1998; Ricketts et al., 1999; Maes et al., 2005).

Lepidoptera are generally used as indicators of those natural and anthropic factors that affects them.

The natural pressures are: climatic conditions, interspecific relations, from these last pressures most common are parasitism, competition, predators and natural succession.

The anthropic pressured are numerous, but among the main anthropogenic pressures include: pollution, transportation, forestation and deforestation, agriculture intensification, drainage, pasture management, urbanization, overgrazing, tourism, vegetation burning, and oversampling (Rákosy & Stan, 1997; Kovács & Rákosy, 2001; Szekely, 1995).

Lepidoptera species have been used with great success as pollution indicators; among these species some have melanic forms (Ford, 1937, 1940, 1953, 1955; Kettlewell, 1955a, 1955b, 1956, 1957, 1958, 1961a, 1961b, 1973; Clarke şi Shepparde, 1966; Lees, 1968, 1974, 1981; Berry, 1990; etc.). Melanism is a development of dark-colored pigment (melanin) in the epidermal scales.

GENERAL CARACTERIZATION OF THE COPȘA MICĂ AREA

The study was conducted in the area of Copșa Mică (46° 6′ N, 24° 13′ E), south-eastern part of the Transylvanian Plain, more exactly in the Târnava and Blaj highlands (northern part of Sibiu county and eastern part of Alba county).

A faunistic study was compiled in 2011, period in which there were analysed a wide range of habitats, being selected and studied twelve sampling points (fig. 01).

In addition to this sampling points, in 2010 were analysed the Daniel Czekelius, Eckbert Schneider, Victor Weindel entomological collections, stored at the Natural History Museum from Sibiu. The same method was used at the Mediaș Municipal Museum, in which we investigated the Szucs Oliveriu Lepidoptera collection. Unfortunately this collection has no labels, the origin of the specimens being ambiguous.

Form these collections and from other scientific papers: Czekelius (1897, 1917, 1935), Burnaz (1993), Ciochia & Barbu, 1980, Popescu-Gorj (1964), Rákosy (1996), Rákosy & Weber (1986), Schneider (1970, 1984), Skolka (1993), Stănescu (1995), König (1986), Vicol (1982), are included species from localities: Șeica Mare (Cb 01), Agârbiciu (Cb 02), Axente Sever (Cb 03), Moșna (Cb 04), Târnăvioara (Cb 05), Bazna (Cb 06), Valea Lungă (Cb 07) și Micăsasa (Cb 08). The day-flying Lepidoptera were collected with an entomological net and the nocturnal Lepidoptera with a portable light trap.

Additionally, 11 points were quantitative investigated; the suitable habitats discovered by the fauna study were the forests. These habitats are rich in species with melanic forms and have high species abundance.

We investigated eleven sites, situated at different distances from the pollution source, all of them situated in forest habitats (fig. 02). We surveyed nocturnal Macrolepidoptera species between 1 May and 30 September 2012.

Moths were collected using a light trap consisting of 8W UV-tubes fixed to the top of black buckets covered by white funnels and powered by a 12 V accumulator (McGregor et al., 1987; Huemer, 2002; Axmacher & Fiedler, 2004; Hilt et al., 2006; Fayle et al., 2007; Hawes et al., 2009; Ramamurthy et al., 2010), once a month in each plot. In every site we have located two such traps. We did not survey on nights with full moon or with low temperature or in the nights with rain. They were placed at minim 200 m from the forest edge, and 200 m from one another.

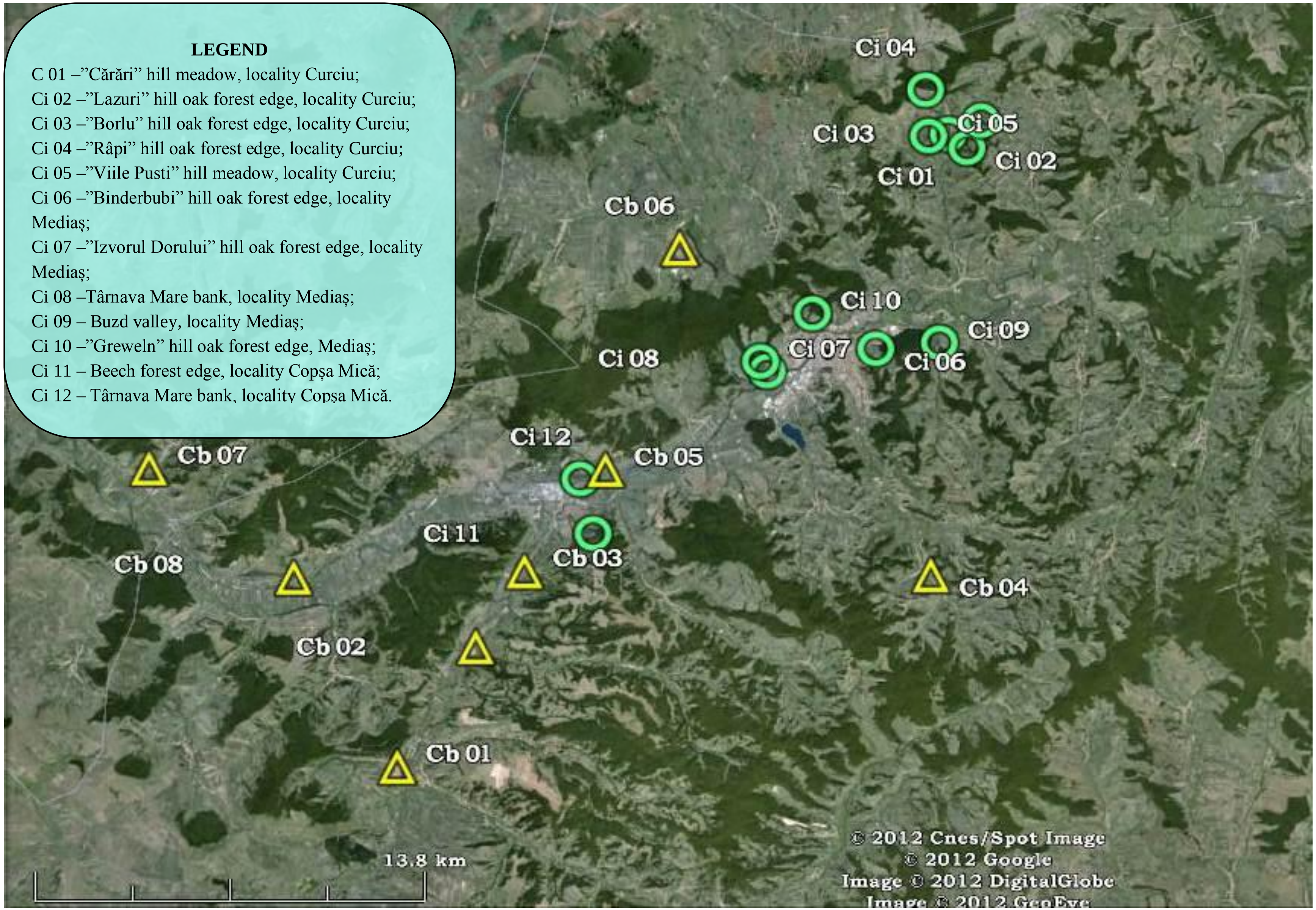

Fig. 01 – The distribution of the qualitative sampling points from the Copșa Mică area

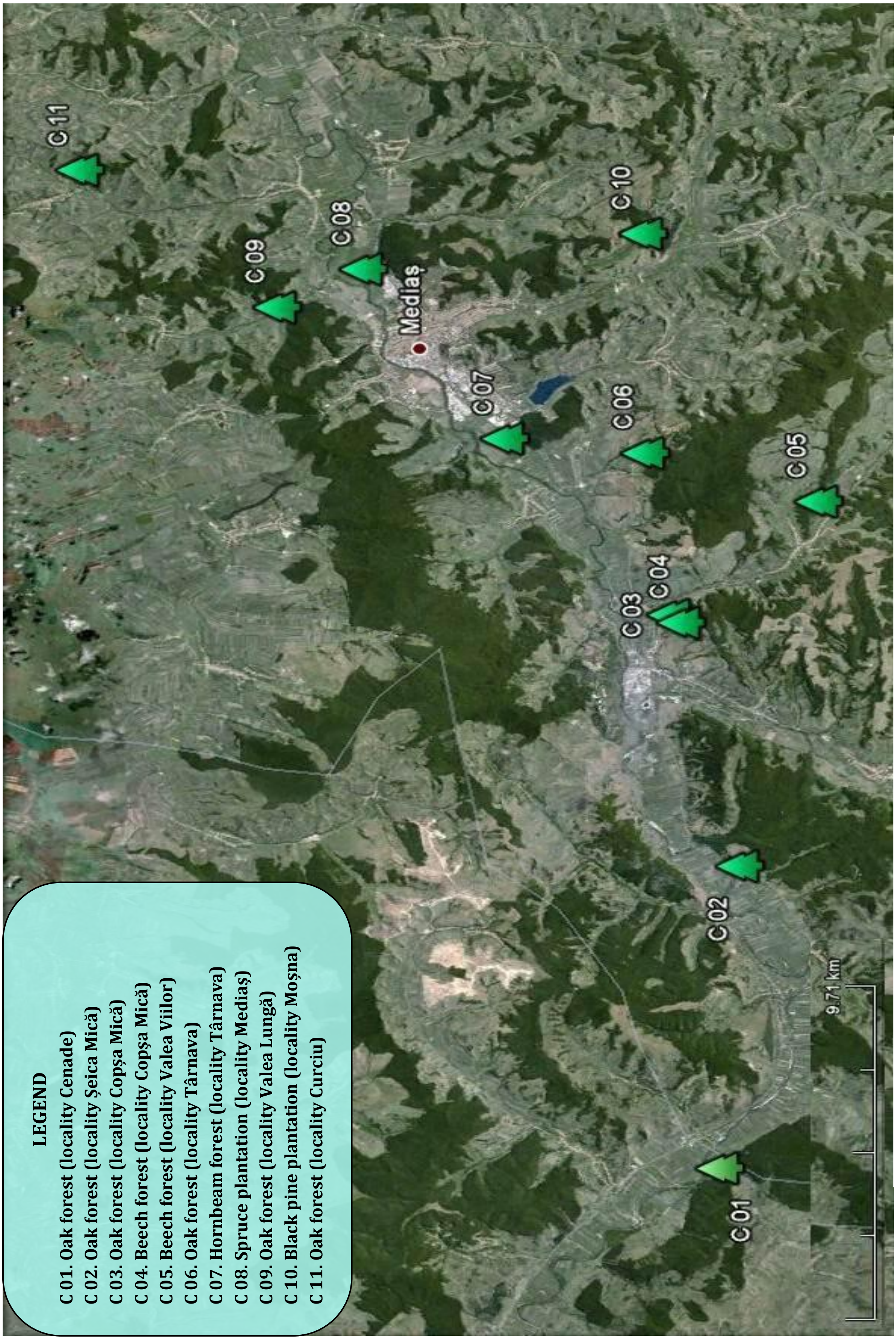

Fig. 02 – The distribution of the quantitative sampling points from the Copșa Mică area

FAUNISTIC CHARATERIZATION OF THE MACROLEPIDOPTERA FROM THE AREA OF COPȘA MICĂ

Species abundance of the Macrolepidoptera from Copșa Mică area

The analysis of the authors and references data, we can now state that in this area is a high taxonomic abundance, given the fact the current pollution level.

A total of 546 species were identified, belonging to 9 superfamilies, 9 families, and 49 subfamilies.

Species abundance in the researched habitats

The analysed data from the quantitative sites indicate a taxonomic diversity dependent on two variables, pollution level and vegetation type.

In these sites we encounter 319 species, the estimated value, using Chao2 index, being 374 species (s.d. +/- 16 species).

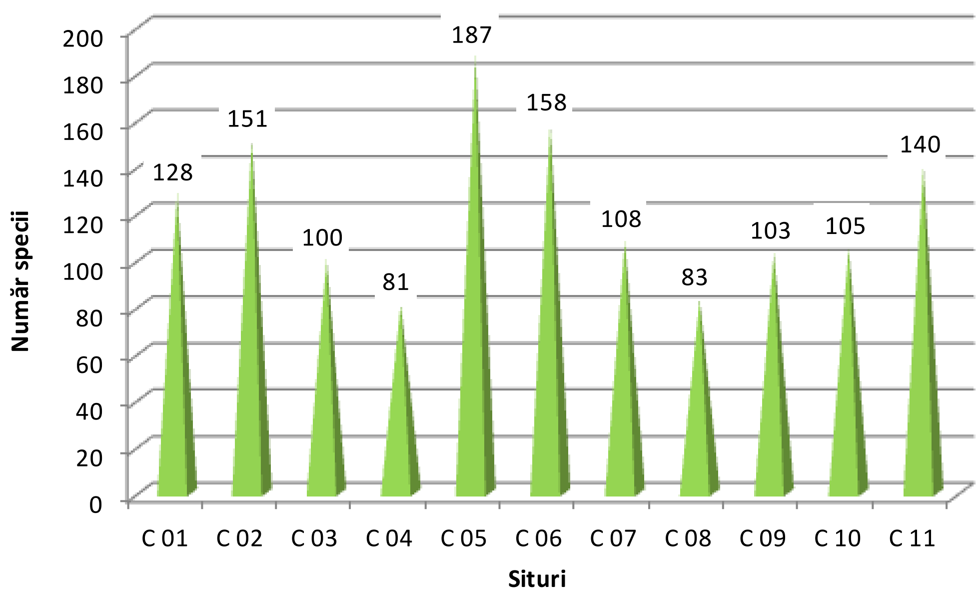

Fig. 03 – Species abundance in the quantitative sites

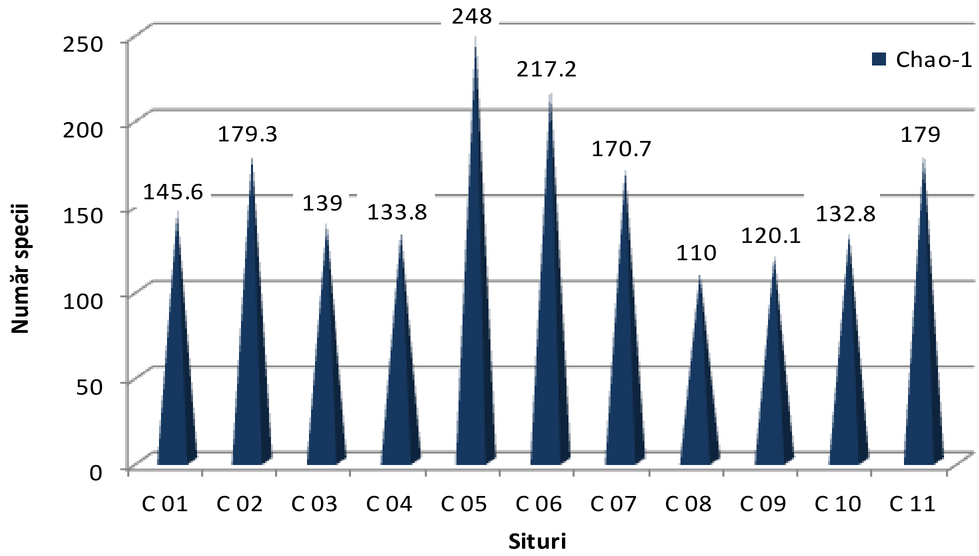

Fig. 04 – Estimated species abundance in the quantitative sites (Chao-1 index)

We've encountered strong dissimilarity in species abundance between different sites (fig. 03). The sites nearest to the pollution source have fewer Macrolepidoptera species (fig. 04). Copșa Mică site situated in a sessile oak forest had only 99 species compared to the furthest site that had 139 species (C11, Curciu site). A similar situation was encountered in the common beech forests (C4, S = 80 and C5, S = 143).

Analysis of the Red list species from Copșa Mică area

From a total of 546 species, 174 species (26, 9 %) are included in Romanian Red List. Here we find the critically endangered species *Euthrix potatoria* (Linnaeus, 1758), identified in six forest sites (C 11; C 06; C 09; C 04; C 05; C 08), also in this area is present the oak forest specialist species *Cyclophora quercimontaria* (Bastelberger, 1897) (C 02; C 05), another critically endangered species is *Ourapteryx sambucaria* (Linnaeus, 1758) identified in the *Pinus nigra* plantation near Moșna locality (C 10).

Unfortunately the critically endangered species *Orgyia recens* (Hübner, 1819) was not found during this study, even dough this species was sampled by Daniel Czékelius in Mediaș late 19th century (25.07.1892).

Also in this area were encountered 8 endangered species (EN), 33 vulnerable species and 126 near threatened species (NT).

The Red list species are very important for insect conservation, in many cases when a habitat is rich in red list species, means that it is an undisturbed habitat. The highest number of Red list species has been recorded from a common beech forest (C05), this site is located not on the Târnava Mare valley, but on a south tributary valley. This valley was presumably protected from the Târnava Mare valley air flow; therefore the pollutants could not affect this site, or they reached this site in a smaller proportion. Another site with high diversity and rich in Red list species is C06, this site is situated in the Târnava Mare valley though the North-East exposure had protected this forest from the main pollutants.

Species of Macrolepidoptera protected by law in the Copșa Mică area

Regarding legislation protecting the Lepidoptera species, part of the species are included in The Habitats Directive annexes, law nr. 49/2011.

Annexe 3A – Animal and plant species whose conservation requires the designation of special areas of conservation and special avifaunistic conservation areas, in this annex are included the species: *Eriogaster catax* (Linnaeus, 1758), *Euphydryas maturna* (Linnaeus, 1758), *Lycaena dispar* (Haworth, 1802) encountered in the area of Copșa Mică.

Annexe 4A – Animal and plant species of community interest in need of strict protection, from this annex we discovered: *Eriogaster catax* (Linnaeus, 1758), *Euphydryas maturna* (Linnaeus, 1758), *Lycaena dispar* (Haworth, 1802), *Maculinea arion* (Linnaeus, 1758), *Maculinea teleius* (Bergstrasser, 1779), *Parnassius mnemosyne transsylvanica* (Schmidt, 1930) and *Proserpinus proserpina* (Pallas, 1772).

Annexe 4A – Animal and plant species of national interest in need of strict protection, in this annex are included the taxa: *Argynnis laodice* (Pallas, 1771), *Colias chrysotheme* (Esper, 1781), *Cupido osiris* (Meigen, 1829), *Cupido (Everes) alcetas* (Hoffmannsegg, 1804), *Heteropterus morpheus* (Pallas, 1771), *Neptis sappho* (Pallas, 1771), *Plebeius sephirus* (Frivaldszky, 1835) și *Pyrgus sidae* (Esper, 1784).

In total in the area of Copșa Mică we have encountered 15 protected species.

ECOLOGICAL ANALISYS OF THE FOREST MACROLEPIDOPTERA COMMUNITIES FROM THE COPȘA MICĂ AREA

Analysis and dynamics of dominant taxa between researched habitats

The separate analysis of similar habitats types such as the oak forests, beech forests or those from the conifers plantations provides us information about the differences between the specie structure from unpolluted sites and polluted sites.

The species structure from the oak forests for instance is extremely diverse, even though it has some common features.

In these forests the highest Dzuba values have the *Cyclophora sp.* (fig. 05), consumers of Fagaceae species, *Cyclophora punctaria* prefers only the *Quercus* species and *Cyclophora linearia* prefers a wide range of Fagaceae species.

We also notice that the *Cyclophora* species have a definite dominance in those sites that are affected by human activities (such as pollution or logging).

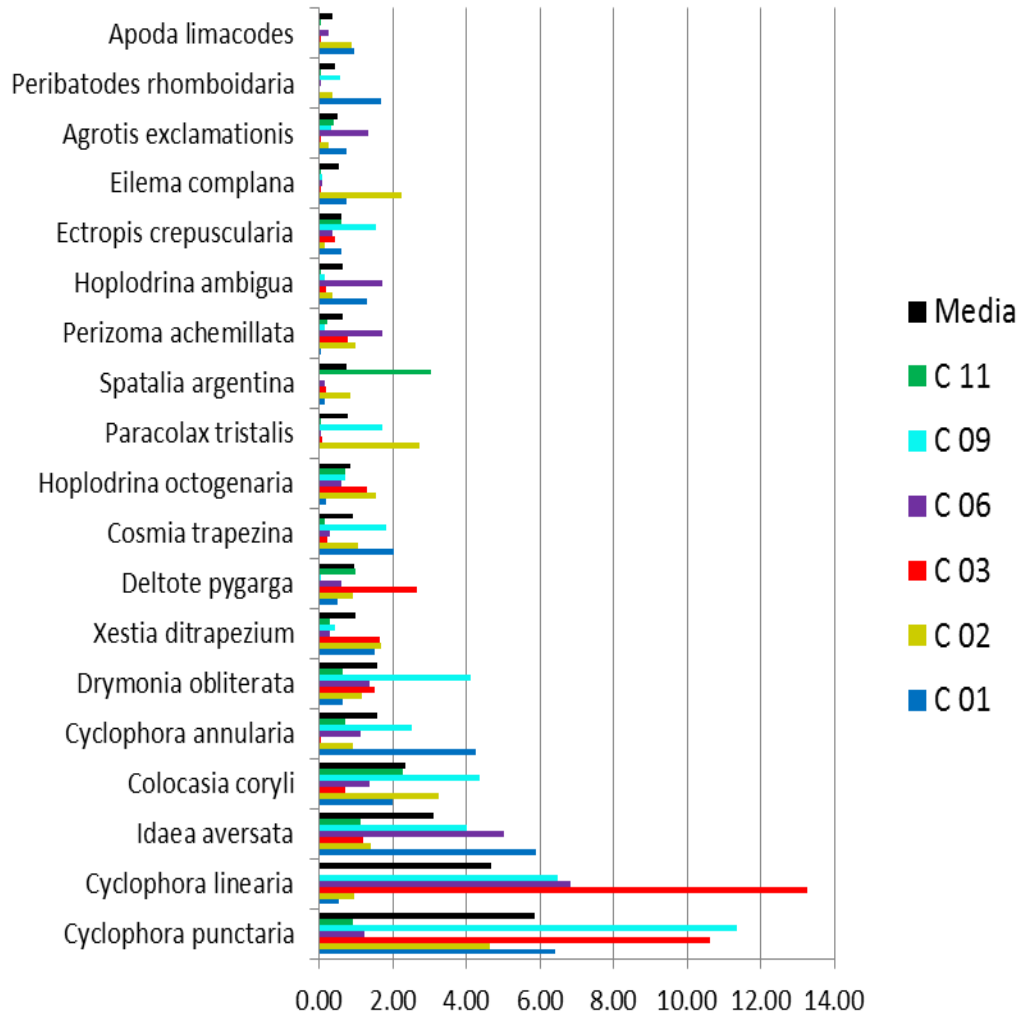

Fig. 05 – The percentages of the species with the highest Dzuba index values for the oak forests

The same trend is found in the beech forests and in the conifers plantations.

Community composition similarity of Macrolepidoptera from Copșa Mică area

The Jaccard index, is a statistic used for comparing the similarity and diversity of the forest Macrolepidoptera communities.

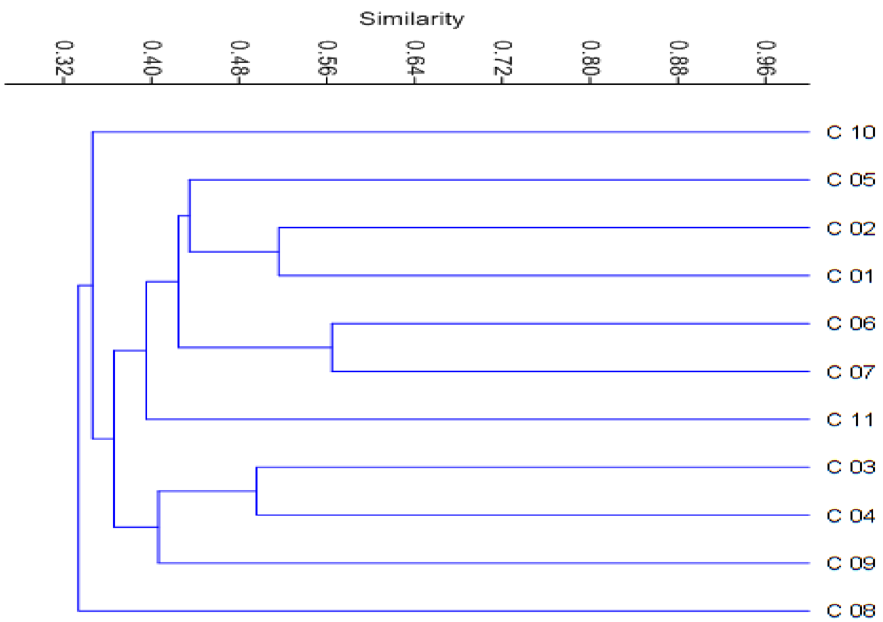

Fig. 06 – Dendrogram illustrating the similarities between the Macrolepidoptera communities from Copșa Mică area

Cluster analysis based on Jaccard similarity index revealed significant compositional differences in moth communities sampled (Fig. 06). Jaccard index recorded high similarity between a sessile oak forest (C06) and a hornbeam forest (C07), two very close sites (J=0, 56). The most different Macrolepidoptera communities are those from a hornbeam forest (C07) and a sessile oak forest (C04) (J=0, 28).

The C 03, C4, C 09 sites influenced by different human activities form different group even though this group contains oak forests (C 03 and C 04) and beech forests (C 04).

High similarity is recorded between C 01 and C 02 sites, these sites have a similar geographic position, vegetation, soil and landscape.

The C 11 sites species composition is very different from other oak forest sites.

The permANOVA performed (5000 permutations) confirms this theory, similarly showing that are significant differences between forest sites (p=0.0053, F=2.39, Df=11). Thus, our results suggest that the local differences in moth communities are mediated by differences in host-plant resources and geographical position than on levels of atmospheric pollution. No significant differences between oak forest sites (permANOVA, 5000 permutations, p=0.4, F=1.05, Df=6) and also no significant differences between the forests in the vicinity of Mediaș locality (permANOVA, 5000 permutations, p=0.1628, F=1.494, Df=5).

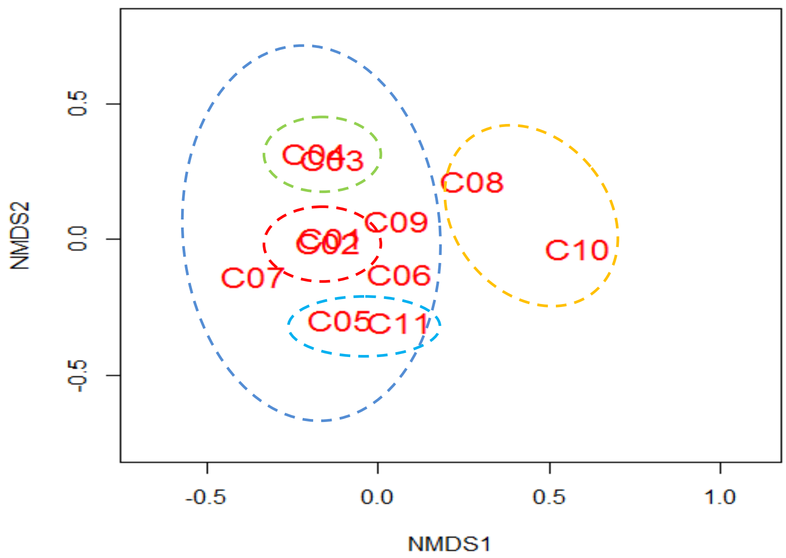

Fig. 07 – NMDS similarity (based on the Bray-Curtis similarity index) for all sampling points (Stress=0.04)

NMDS similarity cluster shows a higher degree of resemblance between the conifers plantations and between the deciduous forests, these types of forests forming two different groups (fig. 07).

In the deciduous forests group there is a great similarity between the C 01 and C 02 sites, these two sites having resembling numeric density, the same situation is encountered between the intensely polluted C 03 and C 04 sites.

Significant similarities in the numeric density are recorded between the unpolluted C 05 and C11 sites, although these two sites have two different vegetation types.

At the opposite pole are the habitats from the coniferous plantations which have low density numeric, although their species composition is rather different.

Diversity and equitability of Macrolepidoptera communities from Copșa Mică area

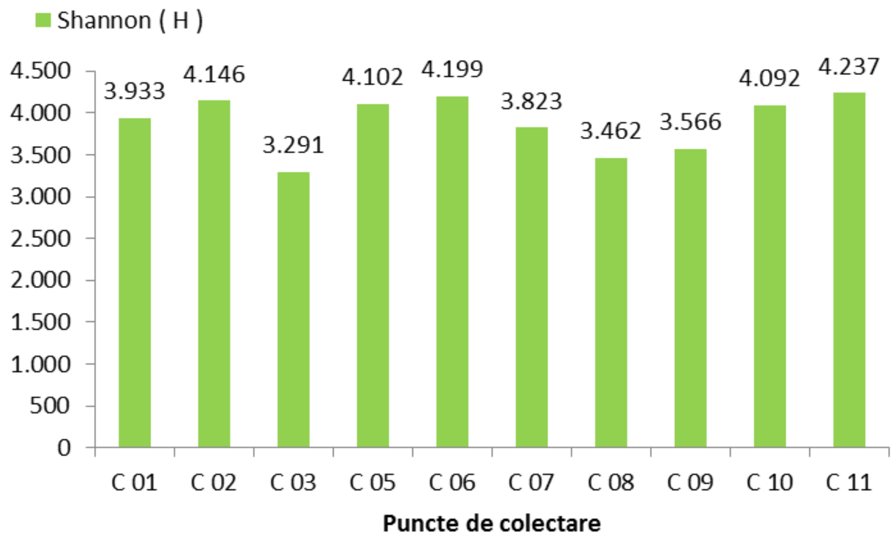

Fig. 08 – Shannon-Wiener index diversity measures for all sampling sites

Regarding the Shannon-Wiener (H`) diversity index the highest diversity is present in the C 11 oak forest site, this site has the highest compositional stability, the site with the minimum structural stability is C 03 oak forest site, existing pollution from this site causing this decrease (fig. 08). C 02, C 05 and C 06 sites also have high diversity level, these sites are unpolluted or they have a small pollution rate.

The diversity rate from C 03 and C04 sites is largely due to degradation and loss of vegetation diversity, that Macrolepidoptera being dependent on this vegetation, the competition for available resources is also low in these habitats, some species taking advantage of the remaining resources, such as the species of *Cyclophora sp.* from the oak forests. In contrast in other types of habitats such as the spruce plantation that provides resources for those species that are capable of adapting to this condition, such as *Idaea aversata* species.

In consequence the spruce plantation (C 08) has a low diversity rate.

According to Simpson's diversity index, each site contained normal diversity level in the range of 0.921 – 0.976. In addition Shannon–Wiener index had shown similar results, ranging from 3.280 – 4.255.

Increase or decrease diversity is the result of interactions among many both abiotic and biotic factors, both natural and anthropogenic.

The larval trophic spectrum of Macrolepidoptera species

from Copșa Mică area

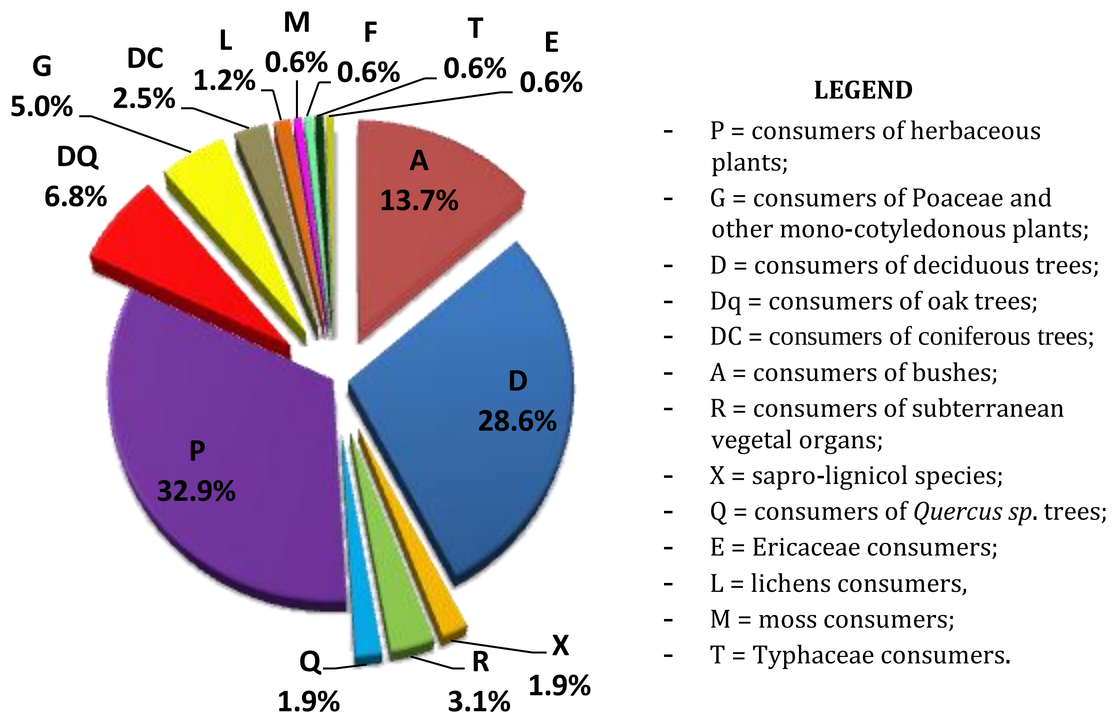

Fig. 09 – Larval trophic spectrum of the Macrolepidoptera species identified in the forest habitats from the area of Copșa Mică

The analysis of the larval trophic source from the forest habitats reveals the dominance of the herbaceous plants consumers (32,9 %) and of the deciduous trees consumers (28,6 %). Followed by the bushes consumers 13,7 % (*Crataegus monogyna, Clematis vitalba, Sambucus nigra, Prunus spinosa, Berberis vulgaris*, etc.), and of Fagaceae consumers (6,8 %).

5 % prefers the Poaceae and other mono-cotyledonous plants as main trophic source, followed by the subterranean vegetal organs consumers, coniferous trees consumers (3,1 %) and of *Quercus sp*. trees consumers (1,9 %) (fig. 09).

Regarding the larval trophic source from the investigated sampling points, the species consume preponderantly herbaceous plants and deciduous trees, in many sites the proportion of this two categories being approximately equal.

The oak forest sites have a similar larval trophic spectrum, even though there are some minor differences.

ANALYSIS OF THE *BISTON BETULARIA* (Linnaeus, 1758) MELANIC FORMS ENCOUNTERED IN ZONA COPȘA MICĂ AREA

Knowing the high level of air pollution from Copșa Mică, through synergistic action of heavy metals and sulphur compounds, this area has the right conditions for the occurrence of melanic forms of the *Biston betularia* (Linnaeus, 1758) species.

Rákosy & Rákosy (1997) have cited that the melanic forms (carbonaria form) percentage decreases with the distance increase from the main pollutant source.

The analysis of the pollution levels reveals the existence of three pollution levels: light, medium and highly polluted sites. In 1989 in the light polluted sites the dominant form was the insularia (48, 8%) followed by the carbonaria form (39%), in 2012 the insularia form is also dominant, the carbonaria form percentage declines.

In the medium polluted sites in 1989 the carbonaria form is dominant (56 %), being followed by the insularia form, in 2012 the carbonaria is just half (25 %), the insularia form being dominant. We can observe that in 2012 the *Biston betularia* forms from the medium polluted sited have similar proportions to those from the unpolluted sites, and correlation with the reduction in pollution can be made.

The intensely polluted sites near Copșa Mică locality, in 1989 were dominated by the carbonaria form (96 %), the insularia form percentage is very low (4 %) and the typical form is absent.

In 2012 the melanic carbonaria form decreases with 36 %, here being present individuals with the typical form. We can conclude that the pollution level had decreased even though the remaining pollution is very high.

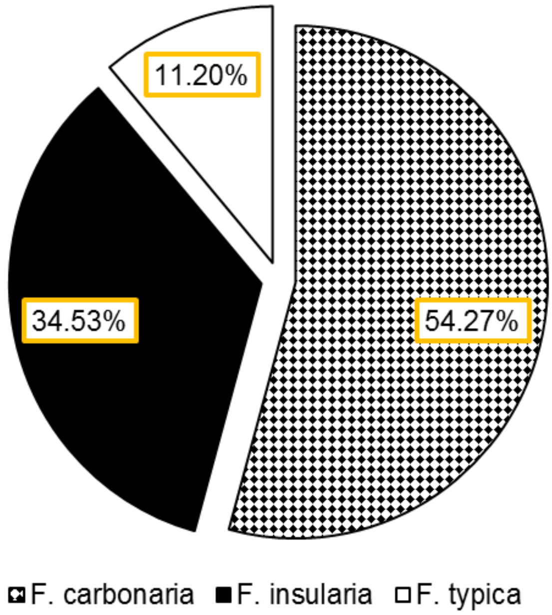

Fig. 10 – The percentages of *Biston betularia* melanic forms in 1989 from Copșa Mică area

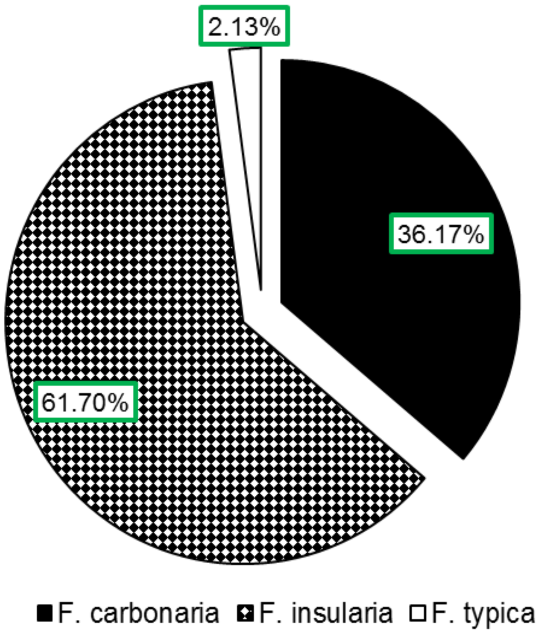

Fig. 11 – The percentages of *Biston betularia* melanic forms in 2012 from Copșa Mică area

The percentages from all three pollution levels from the 1988-1989 reveals a strong dominance of the melanic forms (carbonaria and insularia), reaching 88, 8 %, indicating the pollution level from this area (Rákosy & Rákosy, 1997) (fig. 10).

In 2012 the insularia form is dominant this form percentage has increased, the carbonaria has also increased slightly, the increase in insularia is the result of pollutants decrease and regeneration of the investigated forest habitats (fig. 11)

In the same year have been encountered melanic forms in other Macrolepidoptera species: *Agrotis exclamationis* (Linnaeus, 1758) (12,4 %), *Lymantria monacha* (Linnaeus, 1758)(6,3 %), *Oligia strigilis* (Linnaeus, 1758), *Calliteara pudibunda* (Linnaeus, 1758)(9,2 %) and *Ectropis crepuscularia* (Denis & Schiffermüller, 1775)(10,2 %).

The decline of the melanic forms is due to the change in bark coloration, on which the melanic forms rest, this consequently had led to an increased in bird predation (Cook et al., 2012).

The changes in bark coloration are effect of the decommissioning of the principal atmospheric pollutants source, Carbosim and Sometra.

CONCLUSIONS

- In the area of Copșa Mică we identified 546 species, belonging to 9 superfamilies, 9 families, and 49 subfamilies; day-flying Macrolepidoptera numbered 99 species and 447 nocturnal species. We can state that this area is characterized by high taxonomic abundance, given the fact the current pollution level.

- The analysed data from the quantitative sites indicate a taxonomic diversity dependent on two variables, pollution level and vegetation type. In these sites we encountered 319 species, the estimated value, using Chao2 index, being 374 species (s.d. +/- 16 species).

- 174 species (26, 9 %) are included in Romanian Red List. Here we find the critically endangered species *Euthrix potatoria* (Linnaeus, 1758), *Cyclophora quercimontaria* (Bastelberger, 1897) (C 02; C 05), *Ourapteryx sambucaria* (Linnaeus, 1758), and *Orgyia recens* (Hübner, 1819) species that wasn't found during this study, even though this species was sampled by Daniel Czékelius in 19th century from Mediaș (25.07.1892). Moreover, were encountered 8 endangered species (EN), 33 vulnerable species and 126 near threatened species (NT).

- In polluted sites the Red list diversity is smaller than in unpolluted sites.

– *Cyclophora* species have a definite dominance in those sites that are affected by human activities (such as pollution or logging), in these sites the composition is heterogeneous.

– Jaccard index recorded medium similarity (J=0, 56). between a sessile oak forest (C06) and a hornbeam forest (C07), two very close sites

– In the C11 unpolluted site species composition was very different from other oak forest sites.

– PermANOVA analysis (5000 permutations) showed significant differences between forest sites (p=0.0053, F=2.39, Df=11). However, no significant differences were recorded within oak forest sites (permANOVA, 5000 permutations, p=0.4, F=1.05, Df=6) and also within forests in the vicinity of Mediaș (permANOVA, 5000 permutations, p=0.1628, F=1.494, Df=5).

– NMDS similarity analysis showed a higher degree of resemblance between the conifers plantations and between the deciduous forests, these types of forests forming two different groups. In the deciduous forests group, there is a great similarity between the C 01 and C 02 sites, in terms of numeric density, the same situation being encountered between the intensely polluted C 03 and C 04 sites. Significant similarities in the numeric density are recorded between the unpolluted C 05 and C11 sites, although these two sites have two different vegetation types.

– Regarding Shannon-Wiener (H`) diversity index, the highest value was recorded in the C 11 oak forest site; this site has the highest compositional stability. In contrast, the site with the minimum structural stability is C 03, where, the existing pollution caused a decrease of diversity. C 02, C 05 and C 06 sites also have high diversity levels, these sites are unpolluted or they have a small pollution rate.

– The analysis of the larval trophic source from the forest habitats reveals the dominance of the herbaceous plants and of the deciduous trees consumers. However, bushes and Fagaceae consumers were highly represented. Other consumers types have were low percentages.

– The melanic forms (carbonaria form) percentage decreases with increase of distance from the main pollutant source.

– The percentages from all three pollution levels from the 1988-1989 reveals a strong dominance of the melanic forms (carbonaria and insularia), reaching 88, 8 %, indicating the pollution level from this area. In 2012, the insularia form is dominant (it has an increased percentage). Moreover, the carbonaria has slightly increased. The increase of insularia is the result of pollutants decrease and regeneration of the investigated forest habitats. The decline of the melanic forms is due to the change in bark coloration, on which the melanic forms rest; this consequently had led to an increased bird predation. The changes in bark coloration are effect of the decommissioning of the principal atmospheric pollutants source, Carbosim and Sometra.

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