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Contents

THE PLANTS WITH PROTECTION STATUTE, ENDEMITES AND RELICTS OF PROVADIISKO PLATEAU

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Abstract: The study of the species with protection statute, endemites and relicts of Provadiisko Plateau be performed for the first time. The total number of species with conservation status, established by us, is 80 (9.46% of the total number of species on the plateau). One of those species is included in Appendix II of Directive 92/43/EEC, 19 of the species are included in CITES, 34 species are included in the Red List of Bulgarian vascular plants under the following categories: critical threatened – 1, threatened – 14, vulnerable – 8, nearly threatened – 6 and least concern – 5 species. In the Red book of Bulgaria there are 9 endangered species and 19 rare plants. In the Biological Diversity Act, 21 species are included in Appendix 3 and further 27 species in Appendix 4. The collecting of plants from their natural habitats is prohibited for 15 species, 4 species are under a restriction and 12 species (1.42%) are endemites. These are 3 Bulgarian endemites and 9 Balkan endemites. The Balkan subendemites are 20 species (2.36%). The flora of the plateau includes a significant number of relict species – 40 (4.73%). The majority of them – 37 species are Tertiary relicts, 2 are quaternary relicts and one is a postglacial steppe relict.

Keywords: Provadiisko Plateau, plants with protection statute, endemites, relicts.

1. Introduction

Provadiisko Plateau refers to an area in the hills east of the Danube plain. The Northern plateau border is the Provadiiska River; in the East it reaches to the Devnya Valley; in the South, the Provadiisko Plateau is separated from Roiaksko Plateau by Glavnica River; and finally, west of the Provaddisko Plateau is the Shumensko Plateau. The average altitude is 250 m above sea level. The highest point is Sakartepe in the western parts of the plateau with its height of 389 m. The plateau is located in the Transcontinental climate region, district Dobrudjansko Plateau [1]. Winds are coming mostly from the North and Northeast. The average annual temperature is around 12°C. The average monthly temperatures are always positive. The temperature in January is the lowest (1.2°C) and in July – the highest (22.6°C). The minimum temperature rarely fall to 18°C, and the average maximum temperature reaches 27°C. The maximum rainfalls are in May and June and the minimum – in March and September. The annual amount of rainfalls is around 530 mm. Average humidity is around 76 – 77%; lowest in the summer (70%) and highest in the winter (82%) [2]. The soils, according to the FAO classification, are two types. The first type is calcic chernozems located on the slopes and in the areas with low slope. The second type is calvaric fluvisols located in the Provadiiska Valley [3].

In terms of its flora, the plateau belongs to the region of Northeastern Bulgaria. The vegetation includes: forests of *Carpinus betulus* L. and *Quercus cerris* L., partly with *Carpinus orientalis* Mill.; mixed forests of *Carpinus betulus* L. and *Quercus cerris* L., partly with *Quercus dalechampii* Ten., *Acer campestre* L., etc.; mixed forests of *Tilia tomentosa* Moench., with *Carpinus betulus* L. or *Quercus cerris* L., partly also with *Quercus dalechampii* Ten., *Acer campestre* L., etc.; forest and shrubs of Carpineta orientalis; mixed forests of *Quercus cerris* L., *Quercus pubescens* Willd. and *Cotinus coggygria* Scop., partly with a secondary prevalence of *Cotinus coggygria* Scop.; mixed forests of *Fraxinus ornus* L. and *Carpinus orientalis* Mill., partly of secondary origin; shrubs with prevalence of Paliureta spina-christi, combined with

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xerothermal grass communities mostly replacing xerothermal forest communities of *Quercus cerris* L. and *Quercus frainetto* Ten.; shrub and grass steppe and xerothermal communities; xerothermal grass communities with a prevalence of Dichantieta ischaemi, Poaeta bulbosae, Poaeta concinnae, Chrysopogoneta grylli and Ephemereta; mesoxerothermal grass vegetation with a prevalence of *Poa bulbosa* L., *Loium perenne* L., *Cynodon dactylon* (L.) Pers., partly also *Dichantium ischaemum* (L.) Roberty and rarely *Chrysopogon gryllus* (L.) Trin., mostly in the village com monlands; mesophytous grass communities (meadows), replacing forests of *Ulmus minor* Mill., *Fraxinus oxicarpa* Willd., *Quercus robur* L., *Quercus pedunculiflora* C. Koch.; farm areas, replacing forests of *Fagus sylvatica* ssp. *moesiaca* (K. Maly) Hyelmq.; farm areas, replacing forests of *Quercus dalechampii* Ten.; farm areas, replacing forests of *Ulmus minor* Mill., *Fraxinus oxicarpa* Willd., *Quercus pedunculiflora* C. Koch. [4].

Provadiisko Plateau is a part of the protected zone Provadiisko-Roiaksko Plateau of the European network of protected areas Natura 2000. The objectives of conservation in the protected zone are:

1. Keeping the area of natural habitats and habitats of species and their populations, subject to protection within the protected area.

2. Maintaining the natural state of the natural habitats and the habitats of species subject to protection within the protected area, including natural for these native species composition, characteristic species and environmental conditions.

3. Restoration of the area and the natural state of priority habitats and habitats of species and populations of species subject to protection within the protected area if necessary [5].

It was approved as a protected zone by the National Biodiversity Council on 25 October 2007. It was declared a protected area by the Council of Ministers on 04 December 2007, published in State Gazette number 107 from 18 December 2007 [6].

To the territory of Provadiisko Plateau belongs Corine biotope space Madara [7] and 3 protected areas: 1. Madarski rock wreaths, near to Madara village, Shumen Municipality, Shumen District – it was made to protect the rock wreaths, the habitats of rare and protected plant and animal species. It was announced in 2007 with an area of 319 ha [8]. 2. Snejanska koria, near to Gradinarovo village, Provadia Municipality, Varna District – it was made to protect ancient oak forest (150 years). It was announced in 2002 with an area of 81 ha. 3. The Big Rock, near to Petrov Dol village, Provadia Municipality, Varna District – a Karst canyon habitat of *Buteo rufinus* and *Neophron percnopterus*. It was announced in 1979 with an area of 33 ha [9].

So far, data on the plants with protection statute, endemites and relicts in the area of Provadiisko Plateau have been published by authors for the the territory of Municipality Provadia [10] and by Zahariev and Uzunov for Madarski rock wreaths Protected area [11].

2. Methods of Research

This study was conducted on the route method in 2007 – 2009. The names of the taxons are taken from the Flora of PR Bulgaria, Vol. I – X [12].

The endemites are represented by Asiov et all. [13].

The relicts are represented by Gruev and Kuzmanov [14], Peev [15], Boža et all. [16], Peev et all. [17].

The conservation statute is recognized using the following documents: Council Directive 92/43/EEC of the European Community to protect natural habitats and of wild fauna and flora [18], Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) [19], Red Book of PR Bulgaria [20], Red List of Bulgarian vascular plants [21], Biological Diversity Act [5], Order for special arrangements for the conservation and use of medicinal plants [22].

3. Results and Discussion

As a result of the field survey we found a total of 80 species of conservation statute (Figure 1). This is a 9.46% from the total number of species found on the Provadiisko Plateau. We found the following species:

1. *Adonis vernalis* L.
2. *Aegilops geniculata* Roth
3. **Aesculus hippocastanum* L.
4. *Althaea officinalis* L.
5. *Anacamptis pyramidalis* C. Rich.
6. *Anchusa hybrida* Ten.
7. *Anemone sylvestris* L.
8. *Anthemis regis-borisii* Stoj. et Acht.
9. *Artemisia pedemontana* Balb.
10. *Artemisia pontica* L.

11. *Artemisia santonicum* L.
12. *Asarum europaeum* L.
13. *Asparagus officinalis* L.
14. *Asparagus tenuifolius* Lam.
15. *Asparagus verticillatus* L.
16. *Asplenium trichomanes* L.
17. *Berberis vulgaris* L.
18. *Bupleurum affine* Sadl.
19. *Bupleurum praealtum* L.
20. *Bupleurum tenuissimum* L.
21. *Carlina acanthifolia* All.
22. **Celtis glabrata* Steven
23. *Centaurea marshalliana* Spreng.
24. *Cephalanthera damasonium* (Mill.) Druce
25. *Cephalanthera longifolia* (L.) Fritsch
26. *Cercis siliquastrum* L.
27. *Convallaria majalis* L.
28. *Crocus flavus* West.
29. *Crocus pallasii* Bieb.
30. *Dactylorhiza incarnata* (L.) Soó
31. *Dactylorhiza saccifera* (Brongn.) Soó
32. *Dianthus nardiformis* Janka
33. *Echinops microcephalus* Sibth. et Sm.
34. *Echinops sphaerocephalos* L.
35. *Epipactis helleborine* (L.) Crantz
36. *Erodium hoefftianum* C. A. Mey.
37. *Fibigia clypeata* (L.) Medic.
38. **Ficus carica* L.
39. *Fraxinus pallisiae* Wilmott
40. *Fritillaria pontica* Wahl.
41. *Galanthus elwesii* Hook. fil.
42. *Galanthus nivalis* L.
43. *Galium odoratum* (L.) Scop.
44. *Galium rubioides* L.
45. *Glaucium flavum* Crantz
46. *Goniolimon besseranum* (Schult. ex Reichenb.) Kuzn.
47. *Gypsophila glomerata* Pall. ex M. B.
48. *Gypsophila paniculata* L.
49. *Haplophyllum thesioides* G. Don.
50. *Hedysarum tauricum* Pall. ex Willd.
51. *Helichrysum areanrium* (L.) Moench.
52. *Himantoglossum caprinum* (Bieb.) C. Koch
53. **Juniperus sabina* L.
54. *Jurinea ledebourii* Bunge
55. *Lilium martagon* L.
56. *Limodorum abortivum* (L.) Sw.
57. *Neottia nidus-avis* (L.) Rich.
58. *Ononis adenotricha* Boiss.
59. *Ophrys cornuta* Stev.
60. *Opopanax chironium subsp. bulgaricum* (Vel.) N. Andr.
61. *Orchis morio* L.
62. *Orchis purpurea* Huds.
63. *Orchis simia* L.
64. *Orchis tridentata* Scop.
65. *Phyllitis scolopendrium* (L.) Newm.
66. *Platanthera chlorantha* (Cust.) Rchb.
67. *Polygonatum odoratum* (Mill.) Druce
68. *Pyracantha coccinea* M. J. Roemer
69. *Ruscus aculeatus* L.
70. *Salix caprea* L.
71. *Scilla bifolia* L.
72. *Sedum acre* L.
73. *Sternbergia colchiciflora* Waldst. et Kit.
74. *Stipa capillata* L.
75. *Stipa pulcherrima* C. Koch
76. *Taraxacum bessarabicum* (Hornem.) Hand.-Mazz.
77. **Taxus baccata* L.
78. *Tilia rubra* DC.
79. *Valeriana officinalis* L.
80. *Verbascum dieckianum* Borb. et Deg.

The species marked with an asterisk are found within the plateau just as decorative.

One species is included in Application II of Directive 92/43/EEC – *Himantoglossum caprinum* (Bieb.) C. Koch.

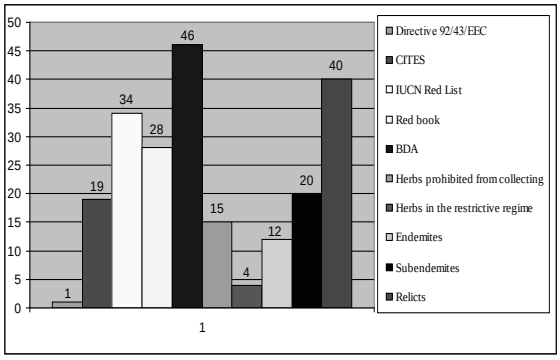

Fig. 1 – A proportion of species with conservation status, endemites and relicts

In the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) are included 19 species: *Adonis vernalis* L., *Anacamptis pyramidalis* C. Rich., *Cephalanthera damasonium* (Mill.) Druce, *Cephalanthera longifolia* (L.) Fritsch, *Dactylorhiza incarnata* (L.) Soó,

Dactylorhiza saccifera (Brongn.) Soó, *Epipactis helleborine* (L.) Crantz, *Galanthus elwesii* Hook. fil., *Galanthus nivalis* L., *Himantoglossum caprinum* (Bieb.) C. Koch, *Limodorum abortivum* (L.) Sw., *Neottia nidus-avis* (L.) Rich., *Ophrys cornuta* Stev., *Orchis morio* L., *Orchis purpurea* Huds., *Orchis simia* L., *Orchis tridentata* Scop., *Platanthera chlorantha* (Cust.) Rchb., *Sternbergia colchiciflora* Waldst. et Kit.

In the IUCN Red List for Bulgaria are included 34 species. One of them is in category „critically threatened" – *Verbascum dieckianum* Borb. et Deg. In category „threatened" are included 14 species: *Aesculus hippocastanum* L., *Anthemis regis-borisii* Stoj. et Acht., *Artemisia pedemontana* Balb., *Celtis glabrata* Steven, *Dactylorhiza incarnata* (L.) Soó, *Dianthus nardiformis* Janka, *Galanthus elwesii* Hook. fil., *Galanthus nivalis* L., *Goniolimon besseranum* (Schult. ex Reichenb.) Kuzn., *Hedysarum tauricum* Pall. ex Willd., *Juniperus sabina* L., *Jurinea ledebourii* Bunge, *Taraxacum bessarabicum* (Hornem.) Hand.-Mazz., *Taxus baccata* L. In category „vulnerable" are included 8 species: *Anacamptis pyramidalis* C. Rich., *Fibigia clypeata* (L.) Medic., *Fraxinus pallisiae* Wilmott, *Haplophyllum thesioides* G. Don., *Himantoglossum caprinum* (Bieb.) C. Koch, *Limodorum abortivum* (L.) Sw., *Ophrys cornuta* Stev., *Opopanax chironium subsp. bulgaricum* (Vel.) N. Andr. In category „nearly threatened" 6 species: *Anemone sylvestris* L., *Artemisia pontica* L., *Cercis siliquastrum* L., *Erodium hoefftianum* C. A. Mey., *Galium rubioides* L., *Ononis adenotricha* Boiss. In category „least concern" are included 5 species: *Aegilops geniculata* Roth, *Ficus carica* L., *Fritillaria pontica* Wahl., *Pyracantha coccinea* M. J. Roemer, *Tilia rubra* DC.

In the Red book for PR Bulgaria are included total of 28 species. In the category „endangered" are included 9 species: *Aesculus hippocastanum* L., *Anemone sylvestris* L., *Artemisia pedemontana* Balb., *Galanthus nivalis* L., *Galium rubioides* L., *Goniolimon besseranum* (Schult. ex Reichenb.) Kuzn., *Pyracantha coccinea* M. J. Roemer, *Taxus baccata* L., *Verbascum dieckianum* Borb. et Deg. In category „rare" are included 19 species: *Anchusa hybrida* Ten., *Anthemis regis-borisii* Stoj. et Acht., *Artemisia pontica* L., *Celtis glabrata* Steven, *Cercis siliquastrum* L., *Dianthus nardiformis* Janka, *Erodium hoefftianum* C. A. Mey., *Fibigia clypeata* (L.) Medic., *Ficus carica* L., *Fritillaria pontica* Wahl., *Haplophyllum thesioides* G. Don., *Hedysarum tauricum* Pall. ex Willd., *Juniperus sabina* L., *Jurinea ledebourii* Bunge, *Limodorum abortivum* (L.) Sw., *Ononis adenotricha* Boiss., *Opopanax chironium subsp. bulgaricum* (Vel.) N. Andr., *Taraxacum bessarabicum* (Hornem.) Hand.-Mazz., *Tilia rubra* DC.

In the Biological Diversity Act are included total of 48 species. In the category „protected" (Application 3) are included 21 species: *Aegilops geniculata* Roth, *Aesculus hippocastanum* L., *Anemone sylvestris* L., *Anthemis regis-borisii* Stoj. et Acht., *Artemisia pedemontana* Balb., *Centaurea marshalliana* Spreng., *Dactylorhiza incarnata* (L.) Soó, *Dianthus nardiformis* Janka, *Galanthus elwesii* Hook. fil., *Galanthus nivalis* L., *Galium rubioides* L., *Goniolimon besseranum* (Schult. ex Reichenb.) Kuzn., *Haplophyllum thesioides* G. Don., *Hedysarum tauricum* Pall. ex Willd., *Himantoglossum caprinum* (Bieb.) C. Koch, *Juniperus sabina* L., *Jurinea ledebourii* Bunge, *Limodorum abortivum* (L.) Sw., *Opopanax chironium subsp. bulgaricum* (Vel.) N. Andr., *Taxus baccata* L., *Verbascum dieckianum* Borb. et Deg.

In the category "under protection and under controlled use" (Application 4) are 27 species: *Asparagus officinalis* L., *Asparagus tenuifolius* Lam., *Asparagus verticillatus* L., *Bupleurum affine* Sadl., *Bupleurum praealtum* L., *Bupleurum tenuissimum* L., *Crocus flavus* West., *Crocus pallasii* Bieb., *Dactylorhiza incarnata* (L.) Soó, *Dactylorhiza saccifera* (Brongn.) Soó, *Echinops microcephalus* Sibth. et Sm., *Echinops sphaerocephalos* L., *Goniolimon besseranum* (Schult. ex Reichenb.) Kuzn., *Gypsophila glomerata* Pall. ex M. B., *Gypsophila paniculata* L., *Helichrysum areanrium* (L.) Moench., *Lilium martagon* L., *Orchis morio* L., *Orchis purpurea* Huds., *Orchis simia* L., *Orchis tridentata* Scop., *Polygonatum odoratum* (Mill.) Druce, *Ruscus aculeatus* L., *Salix caprea* L., *Scilla bifolia* L., *Stipa capillata* L., *Stipa pulcherrima* C. Koch.

Prohibited is the collecting ofherbs from the natural habitats of 15 species: *Adonis vernalis* L., *Althaea officinalis* L., *Artemisia santonicum* L., *Asarum europaeum* L., *Asplenium trichomanes* L., *Convallaria majalis* L., *Glaucium flavum* Crantz, *Helichrysum areanrium* (L.) Moench., *Orchis morio* L., *Orchis purpurea* Huds., *Orchis simia* L., *Orchis tridentata* Scop., *Phyllitis scolopendrium* (L.) Newm., *Ruscus aculeatus* L., *Valeriana officinalis* L.

Under a controlled use are 4 species: *Berberis vulgaris* L., *Carlina acanthifolia* All., *Galium odoratum* (L.) Scop., *Sedum acre* L.

Endemic species (Figure 1) are relatively well represented – 12 species (1.42% of all species on the plateau). 3 species of them are Bulgarian endemites: *Anthemis regis-borisii* Stoj. et Acht., *Campanula euxina* (Vel.) Ančev, *Opopanax chironium* subsp. *bulgaricum* (Vel.) N. Andr. The Balkan endemites are 9 species: *Achillea clypeolata* Sibth. et Sm., **Aesculus hippocastanum* L., *Inula aschersoniana* Janka, *Knautia macedonica* Griseb., *Koeleria penzesii* Ujh., *Koeleria simonkaii* Adam., *Melampyrum scardicum* Wettst., *Salvia ringens* Sibth. et Sm., *Verbascum dieckianum* Borb. et Deg. We found 20 Balkan subendemites (2.36% from the total number of species): *Avenula compressa* (Heuff.) Sauer et Chmelit, *Campanula grossekii* Heuff., *Campanula lingulata* W. et K., *Carduus candicans* Waldst. et Kit., *Carduus thoermeri* Weinm., *Centaurea affinis* Friv., *Centaurea napulifera* Rochel, *Chamaecytisus glaber* (L. fil.) Rothm., *Dianthus petraeus* W. et K., *Doronicum orientale* Hoffm., *Galium paschale* Forsskal, *Galium pseudoaristatum* Schur, *Ophrys cornuta* Stev., *Ornithogalum sibthorpii* Greut., *Senecio papposus* (Reichenb.) Less., *Silene flavescens* Waldst. et Kit., *Symphytum ottomanum* Friv., *Syringa vulgaris* L., *Verbascum banaticum* Schrad., *Verbascum lychnitis* L.

The marked with an asterisk *Aesculus hippocastanum* L. is an ornamental plant on the plateau, but occurs as wild in different places.

The data for the relicts (Fig. 1) on the plateau are published for the first time. The flora of the plateau included significant number of relict species – 40. They account for 4.73% of the total species. The majority of them, 37 species, are Tertiary relicts: **Abies alba* Mill., *Acer campestre* L., *Acer pseudoplatanus* L., *Acer tataricum* L., **Aesculus hippocastanum* L., **Betula pendula* Roth, *Carpinus betulus* L., *Carpinus orientalis* Mill., **Celtis australis* L., **Celtis glabrata* Steven, *Cercis siliquastrum* L., *Clematis vitalba* L., *Corylus avellana* L., *Cotinus coggygria* Scop., *Fraxinus excelsior* L., *Fraxinus ornus* L., *Hedera helix* L., *Juniperus communis* L., *Lathyrus aureus* (Stev.) Brandza, *Phragmites australis* (Cav.) Steud., *Picea abies* (L.) Karsten, **Pinus nigra* Arm., *Populus alba* L., *Populus nigra* L., *Populus tremula* L., *Pyracantha coccinea* M. J. Roemer, *Quercus cerris* L., *Ruscus aculeatus* L., *Salix alba* L., *Salix caprea* L., *Salix fragilis* L., *Smilax excelsa* L., **Taxus baccata* L., *Ulmus laevis* Pall., *Ulmus minor* Mill., *Viburnum lantana* L., *Viscum album* L. They were widespread during the Tertiary, but their habitats today are much smaller. The species marked with an asterisk are distributed as decorative or plantation.

The second group are quaternary relicts. They have become part of our flora as a result of glaciation during the Quaternary. Therefore, they are considered glacial relicts. On the plateau, there are two such species: *Limodorum abortivum* (L.) Sw. and *Galanthus nivalis* L. From the third group, the postglacial steppe relict, only one species is found: *Sternbergia colchiciflora* Waldst. et Kit.

The species with highest conservation value, i.e. those that fall into the categories of being endangered and vulnerable, are 23 in number.

The species covered simultaneously in 4 different categories, there are five in number: *Dactylorhiza incarnata* (L.) Soó, *Galanthus nivalis* L., *Goniolimon besseranum* (Schult. ex Reichenb.) Kuzn., *Himantoglossum caprinum* (Bieb.) C. Koch, *Limodorum abortivum* (L.) Sw. It is a larger group of species that refer both to the 3 categories – 18 species: *Aesculus hippocastanum* L., *Anemone sylvestris* L., *Anthemis regis-borisii* Stoj. et Acht., *Artemisia pedemontana* Balb., *Dianthus nardiformis* Janka, *Galanthus elwesii* Hook. fil., *Galium rubioides* L., *Haplophyllum thesioides* G. Don., *Hedysarum tauricum* Pall. ex Willd., *Juniperus sabina* L., *Jurinea ledebourii* Bunge, *Opopanax chironium subsp. bulgaricum* (Vel.) N. Andr., *Orchis morio* L., *Orchis purpurea* Huds., *Orchis simia* L., *Orchis tridentata* Scop., *Taxus baccata* L., *Verbascum dieckianum* Borb. et Deg.

The remaining 57 species with conservation statute fall only in one or two categories.

4. Conclusions

The study of the species with protection statute, endemites and relicts of Provadiisko Plateau be performed for the first time.

The total number of species with conservation status, established by us, is 80 (9.46% of the total number of species on the plateau). The species that we described generally appear in 13 lists of endangered species.

The endemic species that we found on the plateau and described are 12 species (1.42% of the

total number of species). These are 3 Bulgarian endemites and 9 Balkan endemites.

The Balkan subendemites are 20 species (2.36%).

The flora of the plateau includes a significant number of relict species – 40 (4.73%). The majority of them, 37 species, are Tertiary relicts, 2 are quaternary relicts and one is a postglacial steppe relict.

The largest number of species of conservation statute confirms the importance of Provadiisko Plateau as a protected site, included in the European network of protected areas Natura 2000.

5. References

[1] VELEV, S., 2002 – Climatic zoning, in Kopralev, I. (main ed.). Geography of Bulgaria. Physical and socio-economic geography, Institute of Geography, BAS, Farkom, Sofia, 760 pp.

[2] KOPRALEV, I. (main ed.), 2002 – Geography of Bulgaria. Physical and socio-economic geography, Institute of Geography, BAS, Farkom, Sofia, 760 pp.

[3] NINOV, N., 2002 – Soils, in Kopralev, I. (main ed.). Geography of Bulgaria. Physical and socio-economic geography, Institute of Geography, BAS, Farkom, Sofia, 760 pp.

[4] BONDEV, I., 1991 – The vegetation of Bulgaria. Map in M 1:600 000 with explanatory text, University Press St. Kliment Ohridski, Sofia, 183 pp.

[5] Biological Diversity Act, State Gazette number 77 from 9 August 2002, pp. 9–42. Amended in State Gazette number 94 from 16 November 2007.

[6] Resolution number 802 of Council of Ministers on 4 December 2007 adopting the List of protected areas for conservation of wild birds and the List of protected areas for conservation of natural habitats and of wild fauna and flora, State Gazette number 107 from 18 December 2007.

[7] NATURA 2000 Standard Data Form for Provadiisko – Roiaksko Plateau Protected Area (BG0002038), Ministry of Environment and Waters of Bulgaria, 12 pp.

[8] http://www.riosv.icon.bg/index.php (Access 23 January 2011).

[9] http://www.riosv-varna.org (Access 23 January 2011).

[10] ZAHARIEV, D., D. Dimitrov. 2011. The medicinal plants of Provadiisko Plateau. Ovidius University Annals – Biology-Ecology Series, Vol. 14.

[11] ZAHARIEV, D., G. Uzunov. 2010. A study of the flora in Madarski rock wreaths Protected area. University of Shumen Annals, Vol. XX B6, Natural sciences, Biology, University publishing, Shumen, pp. 123–135.

[12] Flora of PR Bulgaria, Vol. I–X, 1963–1995, Publishing House of BAS, Sofia.

[13] ASIOV B., Petrova A., Dimitrov D., Vasilev R., 2006 – Conspectus of the Bulgarian vascular flora. Distribution maps and floristic elements, Bulgarian Biodiversity Foundation, Sofia, 452 pp.

[14] GRUEV, B., Kuzmanov B., 1994 – General biogeography, University Press St. Kliment Ohridski, Sofia, 498 pp.

[15] PEEV, D., 2001 – National park Rila. Management plan 2001 – 2010. Adopted by Resolution number 522 of Council of Ministers on 4 Juli 2001, Sofia, 338 pp.

[16] BOŽA, P., G. Anačkov, R. Igić, D. Vukov, D. Polić, 2005, Flora "Rimskog šanca" (Vojvodina, Srbija), 8th Symposium on the flora of Southeastern Serbia and Neighbouring Regions, Niš, 20–24.06.2005, Abstracts, pp. 55.

[17] PEEV, D., St. Kozuharov, M. Anchev, A. Petrova, D. Ivanova, S. Tzoneva, 1998, Biodiversity of Vascular Plants in Bulgaria, In: Curt Meine (Ed.), Bulgaria's Biological Diversity: Conservation Status and Needs Assessment, Volumes I and II, Washington, D.C., Biodiversity Support Program, pp. 55–88.

[18] Council Directive 92/43/EEC of the European Community to protect natural habitats and of wild fauna and flora.

[19] Convention on International Trade in Endangered Species of Wild Fauna and Flora, State Gazette number 6 from 21 Januari 1992.

[20] Red Book of Popular Republic of Bulgaria, Vol. 1, Plants, 1984, Publishing House of BAS, Sofia, 447 pp.

[21] PETROVA A., V. Vladimirov (Eds), 2009, Red List of Bulgarian vascular plants, *Phytologia Balcanica* 15 (1), Sofia, pp. 63–94.

[22] Order number RD–72 from 3 Februari 2006 for special arrangements for the conservation and use of medicinal plants, State Gazette number 16 from 21 Februari 2006.

THE MEDICINAL PLANTS OF PRESLAVSKA MOUNTAIN – A PROTECTED AREA BY NATURE 2000

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Abstract: A considerable taxonomical diversity of the medicinal plants of Preslavska Mountain – a protected area by Nature 2000, is established: 355 species of vascular plants from 244 genera and 78 families. The most of the families and the genera are represented by a small number of inferior taxa. The analysis of their life form indicates that the geophytes dominante, followed by the groups of the phanerophytes and the hemi cryptophytes. The biological types are represented mainly by perennial herbaceous plants (56.62%) and annual herbaceous plants (13.24%). The largest percentage of species are of the circumboreal type (41.69%). Among the medicinal plants, there are 2 Balkan endemites, 3 Balkan subendemites and 26 relicts. 31 species with protection statute are described. The anthropophytes among the medicinal plants are 227 species (63.94%). The deposits of medicinal plants are in relatively stable condition. Only species of the genera *Tilia* and *Rosa* need of protection due to increased interest in collecting herbs from them.

Keywords: Preslavska Mountain, Nature 2000, medicinal plants, analysis of medicinal plants, protected species.

1. Introduction

Preslavska Mountain was declared a protected area as part of the European network of protected areas Natura 2000 with the following objectives of conservation:

1. Keeping the area of natural habitats and habitats of species and their populations, subject to protection within the protected area.

2. Maintaining the natural state of the natural habitats and the habitats of species subject to protection within the protected area, including natural for these native species composition, characteristic species and environmental conditions.

3. Restoration of the area and the natural state of priority habitats and habitats of species and populations of species subject to protection within the protected area if necessary.

Preslavska Mountain was approved as a protected zone by the National Biodiversity Council on 21 November 2006. It was declared a protected area by the Council of Ministers on 02 March 2007, published in State Gazette number 21 from 9 March 2007 [1]. The documentation has been submitted to the European Commission in March 2007.

Preslavska Mountain is part of the eastern part of Stara Planina Mountain. To the east it is separated from Dragoevska Mountain of Goliama Kamchia River. West territory of the Mountain extends to the Vrana River. The north bordering with the valley of the Vrana River and its tributaries, and to the south – with the valley of Goliama Kamchia River and its tributaries. The area of the zone amounted to 14060.01 ha. The minimum altitude is 116 m asl and the highest point – Mt Goliama Vashkadalnitsa is situated 723 m asl. The Mountain enter into Continental climatic zone, Dobrudja plateau region [2]. According to data from Hydrometeorological Observatory in Shumen average annual temperature is 11.0 ºC. The lowest average monthly temperatures are in January (–1.1 ºC) and the highest – in July (22.0 ºC). The annual rainfall is 641 mm. The lowest average monthly rainfall is in September (34 mm), while the highest – in June (73 mm). The soils, according to the FAO classification, are two types. The first type is haplic phaeozems located at the foot of the Mountain. The second type is haplic luvisols on the slopes and

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ridge of the Mountain [3]. In terms of its flora, the Mountain belongs to the region of Northeastern Bulgaria. The vegetation includes: mixed forests of *Fagus sylvatica* L. ssp. *moesiaca* and *Carpinus betulus* L.; forests of *Carpinus betulus* L., partly with *Carpinus orientalis* Mill.; mixed forests of *Carpinus betulus* L. and *Quercus dalechampii* Ten.; mixed forests of *Carpinus betulus* L. and *Quercus cerris* L., partly with *Quercus dalechampii* Ten., *Acer campestre* L. etc.; mixed forests of *Quercus dalechampii* Ten., *Quercus cerris* L. and *Quercus frainetto* Ten.; mixed forests of *Tilia tomentosa* Moench. with *Carpinus betulus* L. or *Quercus cerris* L., partly also with *Quercus dalechampii* Ten., *Acer campestre* L. etc.; forest of *Quercus cerris* L.; mixed forests of *Quercus cerris* L. and *Quercus frainetto* Ten.; mixed forests of *Quercus cerris* L. and *Carpinus orientalis* Mill., partly also with *Fraxinus ornus* L.; mixed forests of *Quercus pubescens* Willd., *Quercus virgiliana* (Ten.) Ten. and *Carpinus orientalis* Mill.; forests of *Fraxinus ornus* L., partly also with *Carpinus orientalis* Mill.; mesoxerothermal grass vegetation with a prevalence of *Poa bulbosa* L., *Loium perenne* L., *Cynodon dactylon* (L.) Pers., partly also *Dichantium ischaemum* (L.) Roberty and rarely *Chrysopogon gryllus* (L.) Trin., mostly in the village com monlands; xeromesothermal and xerothermal grass communities; farm areas, replacing forests of *Quercus cerris* L. [4].

The natural habitats [5] which are the subject of protection within the protected area are:

1. Rupicolous calcareous or basophilic grasslands of the Alysso-Sedion albi (6110*).
2. Semi-natural dry grasslands and shrubland facies on calcareous substrates (Festuco–Brometalia) (6210*, important orchid sites).
3. Hydrophilous tall herb fringe communities of plains and of the montains to alpine levels (6430).
4. Lowland hay meadows (*Alopecurus pratensis, Sanguisorba officinalis*) (6510).
5. Caves not open to the public (8310).
6. Medio-European limestone beech forests of the Cephalanthero – Fagion (9150).
7. Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (Alno-Pandion, Alnion incanae, Salicion albae) (91E0*).
8. Galio-Carpinetum oak-hornbeam forests (9170).
9. Pannonian woods with *Quercus pubescens* (91H0*).
10. Pannonian-Balkanic turkey oak-sessile oak forests (91M0).
11. Moesian silver lime woods (91Z0).

They are are included in Annex I to Directive 92/43/EEC. The habitats, marked with an asterisk, are priority for conservation under Council Directive 92/43/EEC of the European Community to protect natural habitats and of wild fauna and flora [6].

2. Methods of Research

The field studies were conducted on the route method in 2005 – 2010. The names of the taxons are taken from the Flora of Popular Republic of Bulgaria, Vol. I – X [7]. The update of the taxons is consistent with APG III [8]. The life forms are presented by Raunkier [9]. In their determination was used Flora of PR Bulgaria, Vol. I – X [7]. The biological types are presented by Kozuharov [10]. The floristic elements and endemites are presented by Asiov et all. [11]. The relicts are presented by Gruev and Kuzmanov [12], Peev [13], Boža et all. [14], Peev et all. [15]. The protection status is presented using the following documents: Council Directive 92/43/EEC of the European Community to protect natural habitats and of wild fauna and flora [16], Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) [17], Red Book of PR Bulgaria [18], Red List of Bulgarian vascular plants [19], Biological Diversity Act [20], Order for special arrangements for the conservation and use of medicinal plants [21].

The anthropophytes are presented by Stefanov and Kitanov [22].

3. Results and Discussion

As a result of the research of the medicinal plants of the Preslavska Mountain protected area 355 species of vascular plants from 244 genera and 78 families have been indetified. They represent 8.88% from all species, 26.93% from all genera and 50.98% from all plant families in Bulgaria.

The most of the families and the genera are represented by a small number of inferior taxa.

Almost all families – 67 (85.90%) are represented with 1 – 4 genera. Only 11 (14.10%) from the families included 5 or more genera (Table 1). Most genera are found in the families Asteraceae (29) and Lamiaceae (24).

Table 1 – Families with greatest number of genera

Families	**Genera**
Asteraceae	29
Lamiaceae	24
Apiaceae	17
Fabaceae	15
Rosaceae	12
Brassicaceae	10
Scrophulariaceae	8
Ranunculaceae	8
Boraginaceae	7
Poaceae	6
Solanaceae	5

Most families – 59 (75.64%) have 1 – 4 species. Only 19 (24.36%) of the families are represented by 5 or more species (Table 2). Most species belong to the following families: Lamiaceae (43), Asteraceae (39), Fabaceae (25) and Rosaceae (23).

Table 2 – Families with greatest number of species

Families	**Species**
Lamiaceae	43
Asteraceae	39
Fabaceae	25
Rosaceae	23
Apiaceae	18
Ranunculaceae	14
Scrophulariaceae	14
Brassicaceae	11
Boraginaceae	8
Malvaceae	7
Orchidaceae	7
Oleaceae	6
Poaceae	6
Polygonaceae	6
Solanaceae	6
Amaryllidaceae	5
Aspleniaceae	5
Betulaceae	5
Geraniaceae	5

This relationship is very strong in genera – almost all (95.49%) are represented by 1 – 3 species. Only 11 (4.51%) of the genera have more species – 4 or 5 species (Table 3).

In the analysis of the life forms were obtained the following results (Table 4): The phanerophytes (Ph) are represented by 70 species (19.72%). The megaphanerophytes are represented by 8 species, the most common of which are: *Acer pseudoplatanus* L., *Fraxinus excelsior* L., *Fraxinus oxycarpa* Wilid. and *Quercus frainetto* Ten.

Table 3 – Genera with greatest number of species

Families	**Genera**	**Species**
Lamiaceae	*Salvia*	5
Asteraceae	*Achillea*	4
Fabaceae	*Lathyrus*	4
Geraniaceae	*Geranium*	4
Lamiaceae	*Salvia*	4
	Stachys	4
Orchidaceae	*Orchis*	4
Ranunculaceae	*Ranunculus*	4
Rosaceae	*Prunus*	4
Sapindaceae	*Acer*	4
Scrophulariaceae	*Veronica*	4

The mezophanerophytes are 32 species, of which essential are: *Carpinus betulus* L., *Fagus sylvatica* L., *Fagus orientalis* Lipsky, *Fraxinus ornus* L., *Tilia tomentosa* Moench and *Ulmus minor* Mill.

The microphanerophytes are 21 species, the most common of which are: *Cornus mas* L., *Corylus avellana* L., *Crataegus monogyna* Jacq., *Ligustrum vulgare* L., *Paliurus spina-christi* Mill., *Prunus spinosa* L., *Rosa canina* L., *Rubus discolor* Weiche et Nees, *Sambucus nigra* L. and *Syringa vulgaris* L.

The nanophanerophytes are 6 species, which are essential: *Genista tinctoria* L., *Jasminum fruticans* L. and *Rubus caesius* L.

The succulents are represented by 3 species: *Sedum acre* L., *Sedum album* L. and *Sedum maximum* (L.) Suter.

The group of Chamephytes (Ch) includes 6 species (1.69%): *Dictamnus albus* L., *Ruscus aculeatus* L., *Satureja montana* L., *Teucrium chamaedrys* L., *Teucrium polium* L. and *Thymus pulegioides* L.

The hemicryptophytes (H) are 58 species (16.34%), of which most common are: *Agrimonia eupatoria* L., *Carlina vulgaris* L., *Cichorium intybus* L., *Clinopodium vulgare* L., *Echium vulgare* L., *Eryngium campestre* L., *Lotus corniculatus* L., *Marrubium peregrinum* L., *Plantago lanceolata* L., *Plantago media* L., *Polygala major* Jacq., *Ranunculus ficaria* L., *Salvia nemorosa* L., *Silene vulgaris* (Moench) Garcke, *Taraxacum officinale* Web., *Trifolium pratense* L., *Trifolium repens* L., *Verbena officinalis* L. and *Viola odorata* L.

Table 4 – Life forms

Group	Subgroup	Species
Phanerophytes (Ph)	Megaphanerophytes	8
	Mezophanerophytes	32
	Microphanerophytes	21
	Nanophanerophytes	6
	Epiphytes	–
	Succulents	3
Chamephytes (Ch)		6
Hemicryptophytes (H)		58
Therophyte – hemicryptophytes (Th-H)		41
Cryptophytes (Cr)	Geophytes	132
	Helophytes	1
	Hydrophytes	–
Therophytes (Th)		47

The transition group therophytes – hemi cryptophytes (Th-H) comprises 41 species (11.55%), of which essential are: *Alliaria petiolata* (Bieb.) Cavara et Grande, *Arctium lappa* L., *Capsella bursa-pastoris* Moench., *Daucus carota* L., *Erodium cicutarium* (L.) ĽHer., *Heracleum sibiricum* L., *Malva sylvestris* L., *Plantago major* L., *Stellaria media* (L.) Vill., *Tordylium maximum* L., *Verbascum densiflorum* Bertol. and *Viola tricolor* L.

The group of cryptophytes (Cr) is the largest and includes 133 species (37.46%). Their significant proportion can be explained by the dominance of forest habitats within the protected area. Geophytes dominate with total of 132 species; the most widespread of them are: *Achillea millefolium* L., *Anemone ranunculoides* L., *Artemisia absinthium* L., *Artemisia vulgaris* L., *Chelidonium majus* L., *Convolvulus arvensis* L., *Coronilla varia* L., *Filipendula vulgaris* Moench, *Fragaria vesca* L., *Galanthus elwesii* Hook. fil., *Galanthus nivalis* L., *Galium verum* L., *Geum urbanum* L., *Hypericum perforatum* L., *Isopyrum thalictroides* L., *Mercurialis perennis* L., *Origanum vulgare* L., *Polygonatum odoratum* (Mill.) Druce, *Potentilla argentea* L., *Sanguisorba minor* Scop., *Scilla bifolia* L., *Urtica dioica* L. The helophytes is represented by one species only: *Typha latifolia* L.

The therophytes (Th) are 47 species (13.24%). The most widespread are: *Galium aparine* L., *Lactuca serriola* L., *Lamium purpureum* L., *Lolium temulentum* L., *Melilotus officinalis* (L.) Pall., *Sideritis montana* L. and *Xeranthemum annuum* L.

The largest group species in terms of biological types (Figure 1) are perennial plants (p) – 201 species (56.62%). Their dominance can be explained with the wide variety of communities and habitats within the protected area.

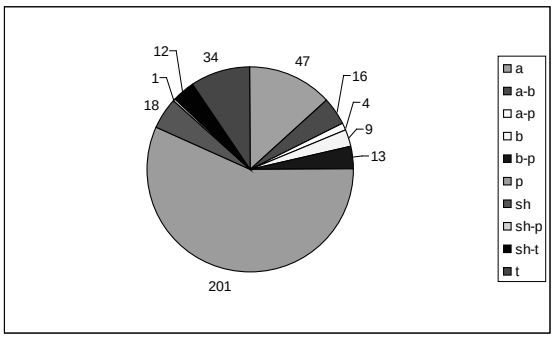

Fig. 1 – Biological types

The annual plants (a) are 47 species (13.24%), which can be explained by the presence of dry rocky terrain.

The tree species (t) are 34 (9.58%). The shrubs (sh) are 18 species (5.07%). The next group includes transition groups from annual to biennial plants (a-b) includes 16 species (4.51%), from biennial to perennial plants (b-p) – 13 species (3.66%) and from shrubs to tree (sh-t) with 12 species (3.38%).

The biennial plants (b) are 9 species (2.54%). The smallest are transition groups from annual to perennial plants (a-p), which is represented by 4 species (1.13%) and transition groups from shrubs to perennial plants (sh-p) – with 1 species (0.28%).

The specific physiographic conditions on the Preslavska Mountain determined considerable diversity of floristic elements. 7 different types of

floristic elements and the other 2 groups are established (Table 5). The dominant elements are elements from circumboreal type – 148 species (41.69%), followed by European elements – 91 species (25.63%) and Mediterranean elements – 60 species (16.90%). The smallest are Euxinian elements and Hybridogenous elements. Each of them has 1 species (0.28%) only. The endemic component is represented by 5 species (1.06%). It includes 2 Balkan endemites – *Achillea clypeolata* Sibth. et Sm. and *Acanthus balcanicus* Heyw. et Richards. and 3 Balkan subendemites – *Achillea grandifolia* Friv., *Ophrys cornuta* Stev. and *Syringa vulgaris* L.

Table 5 – Floristic elements

Floristic elements	**Species**
Circumboreal type	148
European type	91
Mediterranean type	60
Pontic type	20
Cosmopolitan type	19
Adventive type	10
Balkan endemic and subendemic type	5
Euxinian elements	1
Hybridogenous elements	1

This distribution can be explained by the location of the Mountain in the Transcontinental climate region. The proximity of the Mountain to the border of a temperate region is the reason for the prevalence of Circumboreal and European floristic elements. At the same time, the impact of the Continental-mediterranean region in terms of the Black Sea create conditions for the development of a large number of Mediterranean species.

Among the identified medicinal plants contribute a significant number of relict species – 26. They account for 7.32% of the total number of species. The majority of the relict species are Tertiary relicts. They are 25 species: *Abies alba* Mil., *Acer campestre* L., *Acer pseudoplatanus* L., *Acer tataricum* L., *Alnus glutinosa* (L.) Gaertn. *Betula pendula* Roth, *Carpinus betulus* L., *Carpinus orientalis* Mill., *Cercis siliquastrum* L., *Clematis vitalba* L., *Corylus avellana* L., *Cotinus coggygria* Scop., *Fagus orientalis* Lipsky, *Fraxinus excelsior* L., *Fraxinus ornus* L., *Hedera helix* L., *Picea abies* (L.) Karsten, *Populus alba* L., *Populus nigra* L., *Populus tremula* L., *Ruscus aculeatus* L., *Salix alba* L., *Salix fragilis* L., *Ulmus minor* Mill. and *Viscum album* L. One of the relict species is quaternary – *Galanthus nivalis* L.

31 species with protection statute are described. One of them – *Himantoglossum caprinum* (Bieb.) C. Koch., is included in the list of species, protected by the Berne Convention and Natura 2000. 9 species are included in CITES: *Anacamptis pyramidalis* C. Rich., *Galanthus elwesii* Hook. fil., *Galanthus nivalis* L., *Himantoglossum caprinum* (Bieb.) C. Koch., *Ophrys cornuta* Stev., *Orchis morio* L., *Orchis purpurea* Huds., *Orchis simia* L. and *Orchis tridentata* Scop. In the Red List of Bulgarian vascular plants, 2 species are included under the category „Threatened": *Galanthus elwesii* Hook. fil. and *Galanthus nivalis* L., 2 species are included under the category „Vulnerable": *Anacamptis pyramidalis* C. Rich. and *Himantoglossum caprinum* (Bieb.) C. Koch, and 2 species are in the category „Nearly threatened": *Anemone sylvestris* L. and *Cercis siliquastrum* L. In the Red book for Bulgaria 2 species are included in the category „Endangered": *Anemone sylvestris* L. and *Galanthus nivalis* L., and 1 species is included in the category „Rare" – *Cercis siliquastrum* L. In the Biological Diversity Act 6 species are included in the category „Protected": *Anacamptis pyramidalis* C. Rich., *Anemone sylvestris* L., *Galanthus elwesii* Hook. fil., *Galanthus nivalis* L., *Himantoglossum caprinum* (Bieb.) C. Koch. and *Ophrys cornuta* Stev. In the category "Under the protection and regulated use of nature" are 14 species: *Asparagus officinalis* L., *Bupleurum rotundifolium* L., *Dryopteris filix-mas* (L.) Schott, *Echinops ritro* L., *Lilium martagon* L., *Orchis morio* L., *Orchis purpurea* Huds., *Orchis simia* L., *Orchis tridentata* Scop., *Paeonia peregrina* Mill., *Polygonatum odoratum* (Mill.) Druce, *Primula veris* L., *Ruscus aculeatus* L. and *Scilla bifolia* L. Collecting herbs is prohibited from the natural habitats of 10 species: *Asarum europaeum* L., *Asplenium trichomanes* L., *Inula helenium* L., *Orchis morio* L., *Orchis purpurea* Huds., *Orchis simia* L., *Orchis tridentata* Scop., *Phyllitis scolopendrium* (L.) Newm., *Ruscus aculeatus* L. and *Valeriana officinalis* L. Under a restrictive regime are 7 species: *Atropa belladonna* L., *Berberis vulgaris* L., *Betonica officinalis* L., *Galium odoratum* (L.) Scop., *Paeonia peregrina* Mill., *Primula veris* L. and *Sedum acre* L.

The anthropophytes among the medicinal plants are 227 species (63.94%). Many of them are considered ruderal plants. The most common as ruderal plants are: *Capsella bursa-pastoris* Moench., *Cardaria draba* (L.) Desv., *Chamomilla recutita* (L.) Rausch., *Chelidonium majus* L., *Conium maculatum* L., *Conyza canadensis* (L.) Cronq., *Heracleum sibiricum* L., *Lactuca serriola* L., *Sambucus ebulus* L. and *Urtica dioica* L.

During the period of study we established an annual gathering of flowers of *Tilia species diversa* in industrial quantities. The methods of collection include the cutting of branches, which must be controlled so as not to lead to destruction of their fields. In a much lesser extent, used stocks of fruits of *Rosa species diversa* and *Cornus mas* L. This is a traditional occupation of part of the Roma population. Of the remaining species of medicinal plants used in small quantities for personal use only.

4. Conclusions

A considerable taxonomical diversity of the medicinal plants of the protected area is established: 355 species of vascular plants from 244 genera and 78 families.

The most of the families and the genera are represented by a small number of inferior taxa.

The analysis of their life form indicates that the geophytes dominante, followed by the groups of the phanerophytes and the hemicryptophytes.

The biological types are represented mainly by perennial herbaceous plants (56.62%) and annual herbaceous plants (13.24%).

The identified medicinal plants can be categorized into 7 types of floristic elements. The highest percentage species are of the circumboreal type (41.69%).

Among the medicinal plants, there are 2 Balkan endemites, 3 Balkan subendemites and 26 relicts.

31 species with protection statute are described: the use of 1 species is restricted by the Berne Convention and Natura 2000; 9 species are included in CITES; 6 species are included in IUCN Red List for Bulgaria; 3 species appear in the Red book for Bulgaria; 20 species are included in the Biological Diversity Act; the collecting of herbs from their natural habitats is prohibited for 10 species, and 7 species are under a restrictive regime.

The anthropophytes among the medicinal plants are 227 species (63.94%).

The deposits of medicinal plants are in relatively stable condition. Only species of the genera *Tilia* and *Rosa* need of protection due to increased interest in collecting herbs from them.

5. References

[1] Resolution number 122 of Council of Ministers on 2 March 2007 adopting the List of protected areas for conservation of wild birds and the List of protected areas for conservation of natural habitats and of wild fauna and flora, State Gazette number 21 from 9 March 2007.

[2] VELEV, S., 2002 – Climatic zoning, in Kopralev, I. (main ed.). Geography of Bulgaria. Physical and socio-economic geography, Institute of Geography, BAS, Farkom, Sofia, 760 pp.

[3] NINOV, N., 2002 – Soils, in Kopralev, I. (main ed.). Geography of Bulgaria. Physical and socio-economic geography, Institute of Geography, BAS, Farkom, Sofia, 760 pp.

[4] BONDEV, I., 1991 – The vegetation of Bulgaria. Map in M 1:600 000 with explanatory text, University Press St. Kliment Ohridski, Sofia, 183 pp.

[5] NATURA 2000 Standard Data Form for Preslavska planina Protected Area (BG0000421), Ministry of Environment and Waters of Bulgaria, 19 pp.

[6] KAVRAKOVA, V., Dimova, D., Dimitrov, M., Tzonev, R., Belev, T. (editors), 2005, A guidance for identifying the habitats of European importance in Bulgaria, Geosoft, Sofia, 128.

[7] Flora of PR Bulgaria, Vol. I-X, 1963-1995, Publishing House of BAS, Sofia.

[8] Angiosperm Phylogeny Group, 2009, An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III, *Botanical Journal of the Linnean Society*, 161 (2): 105–121.

[9] PAVLOV, D., 2006 – Phytocoenology, Publishing House of University of Forestry, Sofia, 251 pp.

[10] KOZUHAROV, S. (ed.), 1992 – Identifier of the vascular plants in Bulgatia, Nauka i izkustvo, Sofia, 788 pp.
[11] ASIOV B., Petrova A., Dimitrov D., Vasilev R., 2006 – Conspectus of the Bulgarian vascular flora. Distribution maps and floristic elements, Bulgarian Biodiversity Foundation, Sofia, 452 pp.
[12] GRUEV, B., Kuzmanov B., 1994 – General biogeography, University Press St. Kliment Ohridski, Sofia, 498 pp.
[13] PEEV, D., 2001 – National park Rila. Management plan 2001 – 2010. Adopted by Resolution number 522 of Council of Ministers on 4 Juli 2001, Sofia, 338 pp.
[14] BOŽA, P., G. Anačkov, R. Igić, D. Vukov, D. Polić, 2005, Flora "Rimskog šanca" (Vojvodina, Srbija), 8th Symposium on the flora of Southeastern Serbia and Neighbouring Regions, Niš, 20–24.06.2005, Abstracts, pp. 55.
[15] PEEV, D., St. Kozuharov, M. Anchev, A. Petrova, D. Ivanova, S. Tzoneva, 1998, Biodiversity of Vascular Plants in Bulgaria, In: Curt Meine (Ed.), Bulgaria's Biological Diversity: Conservation Status and Needs Assessment, Volumes I and II, Washington, D.C., Biodiversity Support Program, pp. 55–88.
[16] Council Directive 92/43/EEC of the European Community to protect natural habitats and of wild fauna and flora.
[17] Convention on International Trade in Endangered Species of Wild Fauna and Flora, State Gazette number 6 from 21 Januari 1992.
[18] Red Book of Popular Republic of Bulgaria, Vol. 1, Plants, 1984, Publishing House of BAS, Sofia, 447 pp.
[19] PETROVA A., V. Vladimirov (Eds), 2009, Red List of Bulgarian vascular plants, *Phytologia Balcanica* 15 (1), Sofia, pp. 63–94.
[20] Biological Diversity Act, State Gazette number 77 from 9 August 2002, pp. 9–42. Amended in State Gazette number 94 from 16 November 2007.
[21] Order number RD–72 from 3 Februari 2006 for special arrangements for the conservation and use of medicinal plants, State Gazette number 16 from 21 Februari 2006.
[22] STEFANOV, B., Kitanov B., 1962 – Kultigenen plants and kultigenen vegetation in Bulgaria, Publishing House of BAS, Sofia, 275 pp

THE TROPHIC SPECTRUM OF FIVE GOBIES SPECIES (PISCES, GOBIIDAE) FROM DOBROUDJA'S COASTAL WATERS

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Abstract: Five species of gobies (*Apollonia melanostomus, Ponticola cephalargoides, Neogobius fluviatilis, Gobius jozo* and *Mesogobius batrachocephalus*) were studied for determine food composition and calculate the mean filling index. Except for *M. batrachocephalus* which eats almost only benthonic fishes, all other species of gobies show a preference for mollusks and crustaceans. The highest values of mean filling index are noticed during the fall after the matting period and for *A. melanostomus,* probably because of his wide ecological valence and of its feeding behavior, more aggressive than other gobies species with it coexists. The lowest values characterize *Gobius jozo*, because it is represented in our samples only by juveniles.

Keywords: gobies, food, digestive tract content, mean filling index

1. Introduction

The study of fish trophic spectrum permits the estimation of its place, role and relation with other species in their living biocenosis.

As a general rule, gobies are carnivorous species, eating most invertebrates and fewer vertebrate or invertebrate eggs and other small fish species, including cannibalism. The feeding and trophic spectrum depends on external or internal factors (light, temperature, oxygen, food type and richness, age, sex, parasites, matting period). Thus, we've been notice that during summer the fishes eat early in the morning, when water temperature is low. At noon, when waters get warmer, the fishes go down to dormancy, and feeding is temporarily interrupted. Svetovidov (1964) said that round goby doesn't feed at 27 degrees ad up.

The warming of water causes a different behavior response. Thus, *Mesogobius batrachocephalus* migrates to depths in the summer for feeding and come back to near shore in fall. Contrary, *Apollonia melanostomus* migrates to depths in the cold season when the water temperature is lower than 5-6 degrees, the migration amplitude being from 0.2-0.5m in matting season up to 10-20 or even 50-60 in the winter.

The period before matting season is always characterized by an intensive feeding. In matting season, the entire energy is focused to build the nest, finding the partner, spawning and caring the offspring. This is why the most specimens don't eat, and some of them are dying (example: *A. melanostomus* males), so they can reproduce once in lifetime. The females have a long life to perpetuate the species.

The trophic spectrum is different from juveniles to adults. As a general rule, the body length determines the size of the prey. The young eat small organisms as crustaceans and polychaetes, and only sometimes little mollusks, while adults choose adults mollusks and other fishes. This is the reason why sometimes they have been noticed segregation on body length range, for a better usage of different ecosystem niches' trophic capacity. This is probably the reason of why in the summer of 2000 we find near the shore only juveniles specimens.

Nevertheless, analyzes of digestive tract content show a similitude between the foods of this two age cluster, because of poverty of their living biocenosis in food.

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Important data about some Romanian gobies species (*Apollonia melanostomus, Ponticola. cephalargoides* and *Mesogobius batrachocephalus*) food were given by Porumb (1961). This author referred only on marine populations of round goby probably because the entire quantity of fishing capture economically important has marine provenience.

Till now there are no information about Romanian populations gobies, even if they have been noticed a lot of changes which affected directly or indirectly gobies fauna.

This is the reason why this study aimed to determine food composition for estimate de capacity of using trophic base from their biocenosis and calculate the mean filling index.

2. Material and Methods

We studied five specie of gobies (*Apollonia melanostomus, Neogobius fluviatilis, Ponticola cephalargoides, Gobius jozo* and *Mesogobius batrachocephalus*), the most frequent fishes in Romanian coastal waters.

The samples have been collected from 1998 to 2003, by purse-seining, angling, longlining or other fishing tools with bait hooks, using nets with a mesh diameter around 4 mm or by diving up to 5m depth from Romanian coastal waters of Black Sea and from paramarine lakes (Danube Delta complex, including Sinoe and Razim lakes, Siutghiol, Tasaul, Neptune lakes) as we figured below (fig. 1).

The samples have been preserved in formaldehyde 4%, then transferred in alcohol. Then the fishes have been disecated for prelevance of digestive tract.

The digestive tube full and empty of food, was weight and the composition of food has been determined by analysing at microscop and binocular lamp.

The mean filling index was calculated using the formula:

Filling Index (FI) = the weigth of digestive tract content x 10000/total body wegth

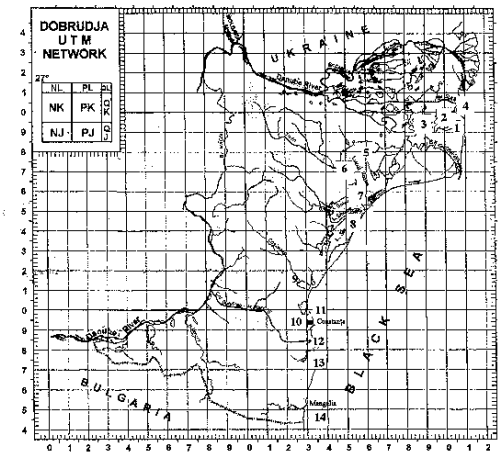

Fig. 1 – Map of station. 1. Roşuleţ; 2. Puiuleţ; 3. Puiu; 4. Sulina; 5. Razelm; 6. Sarichioi; 7. Cap Doloşman; 8. Sinoe; 9. Taşaul; 10. Siutghiol; 11. Perla; 12. Port Tomis; 13. Eforie Sud; 14. Mangalia; 15. Vama Veche

3. Results and Discussions

A. melanostomus is almost an exclusive molluscivorous species. Due to its basal pharyngeal plates and a very strong musculature of jaws and esophagus, the round goby is able to rive shells with 1.6 mm length and wrest the content. Little mollusks can be eaten completely, but different authors (in Miller, 2004) affirm that there are always broken valve in gobies stomach and intestine. The digestive tract is longer than the tract from other gobies species, represented 90 percent of body length. A real stomach and gastric digestion don't exist, so the digestion can take even 72 hour.

Porumb said that during the spring, the round goby can eat 36.02% young shells, 7.84% polychaetes, 5.47% *Idothea baltica* , 4,61% *Pachigrapsus marmoratus*, 4,41% gobies sapling, 4.2% *Xantho* and 2,27% *Chiton* which is picked from the stones.

During the fall, the same author identified that the most important components of food, in order of their frequency and abundance, are *Xantho*, polychaetes, *Upogebia*, *Mytilus*, *Carcinus*, *Cardium*

etc. In this period he calculated the higher value for filling index (155.6).

Our studies reveal that round goby has a preference for shells as *Mytilus, Mytilaster, Corbula, Mya* and *Cardium* (for marine populations) or *Dreissena* (for freshwaters populations). The shells quantities vary from 69.7% (Constanţa) to 40.2 % (Siutghiol) from total weight of stomach content.

In the same time, it can be noticed that fishes and crustaceans are permanent presence in round goby food (Fig. 2).

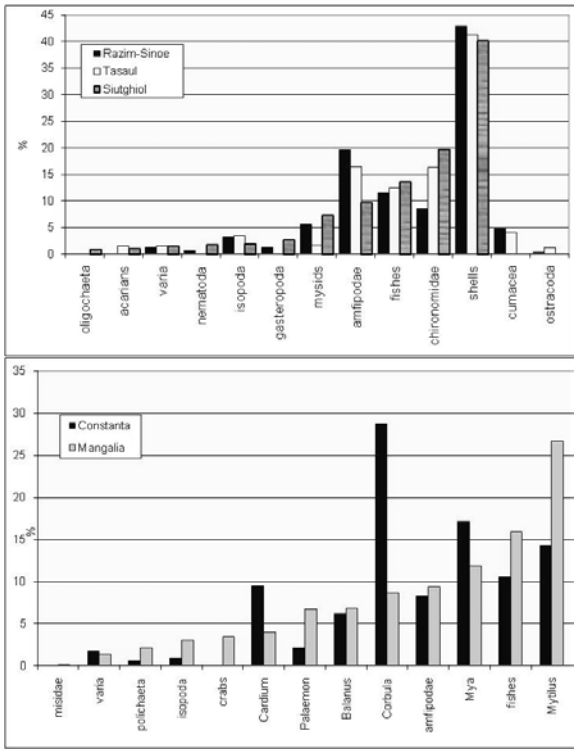

Fig. 2 – The trophic spectrum of *Apollonia melanostomus* freshwater and marine populations (1998-2002**)**

Analyzes of digestive tube content show some differences between sexes and different period of the year. As a general rule, the females prefer small food (crustaceans, worms, small shells), probably because of their small length and their behavior less aggressive than males' (Fig. 6.4.1.10). In the same time, during the warm season of year we noticed an increase of crustaceans' quantities (fig. 3).

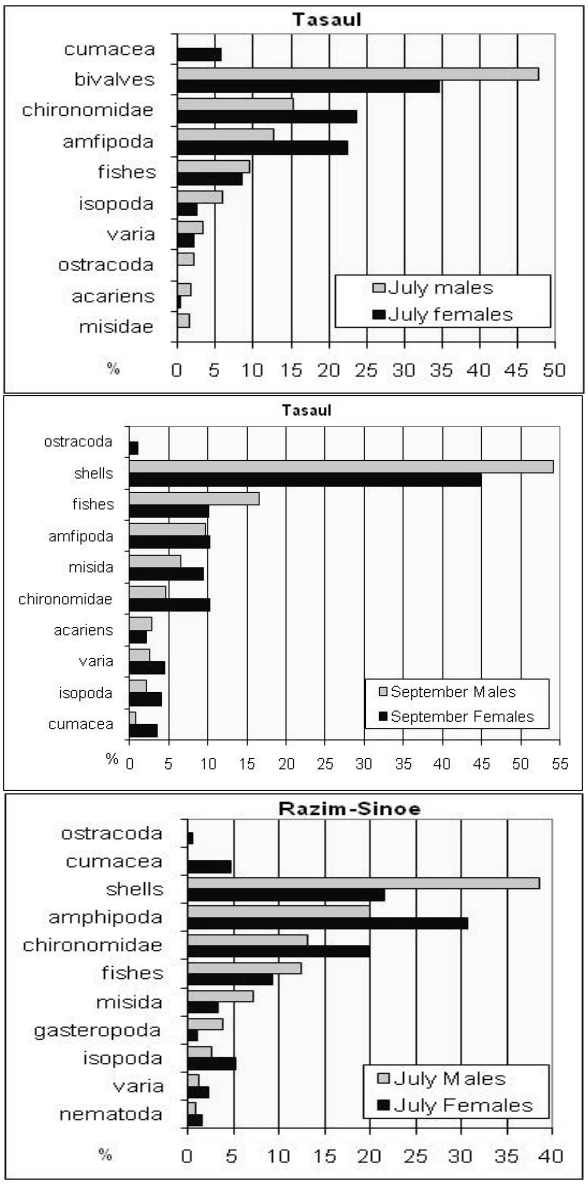

Fig. 3 – Qualitative and quantitative food compound of *A. mellanostomus* in Taşaul and Razim-Sinoe lakes (1998-2002)

For example, in Tasaul, in July, we identified 30.7% bivalves and 34.5% crustaceans from entire digestive tract weight content, while in September we

found 28.3% bivalves and 44.8% crustaceans. Male eat a proportion of 22.2% crustaceans and 47.8% bivalves in July, but 19.1% crustaceans and 53.2% bivalves in September (Fig. 3).

Marine populations of *A. melanostomus* have similar feeding behavior. Speaking about females, crustaceans represent the most important part of food content in warm season (fig. 4). With water cooling, the quantity of eaten shells grows, probably because of their reduced mobility against crustaceans which bury in sand or gravel.

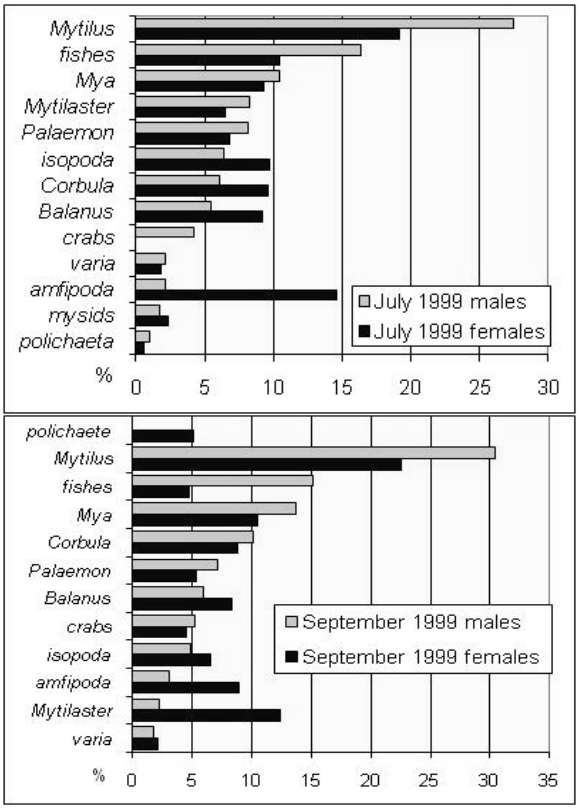

Fig. 4 – Quantitative and qualitative food compound of *A. melanostomus* (sea waters, 1999)

The digestive filling index (FI) depends on season, age and aquatorium characteristics (Table 1). Even some specialists consider that in mate period the nourishing is interrupted, the digestive content of collected gobies has shown only a reducing intensity of feeding.

Also, based on our observations, we can say that the FI of marine populations are higher than of freshwater population. This is can be explained by the changes induced by the adaptation of this species belonging to a primary marine family to a freshwater environment.

Table 1 – Filling index values at *A. melanostomus* from Romanian coastal waters

Basin	**min**	**max**	**average**
Mare	9.27	236.46	98.98
Siutghiol	10.37	233.83	73.77
Taşaul	9.65	212.42	72.91
Razim-Sinoe	13.11	309.56	98.42
2002	**Taşaul**		**Constanţa**
June	71.92		78.31
July	67.31		82.97
August	94.67		85.63
September	112.73		142.5

Anyway, the FI values for marine gobies are higher than those from literature data: 113.62 in summer and 155.6 in fall (Porumb, 1961). It is possible that the changes from latest decades in coastal biocenoses to affect the feeding behavior of ichtyofauna. Thus, gobies spent a lot of effort to adapt to the new conditions of life, including food resources changes.

Having the same dentition with round goby, *Neogobius fluviatilis* prefers the mollusks, as *A. melanostomus* does. The variations depend on the trophic base available in biocenoses. Thus, the comparative study of populations belonging to three different aquatories (Razim-Sinoe, Taşaul, Siutghiol) shows that in 2001, even if the majority of digestive tract content is represented by shells (*Dreissena*, *Monodacna*) and by fishes, all the other components are represented in different percentage (Fig 5).

In Taşaul and Siutghiol lakes, because of the poverty of crustaceans and bivalves fauna, gobies orientate to atypical food elements, as insects larva and spiders. Apparently, the presence of acarians seem to be an accident, but the constancy of their presents, even in small quantities, reveal the gobies reorientation thru new nourishing supplement. Also, the higher values of body length of gobies from Razim populations make possible the access to big elements of food in this lake.

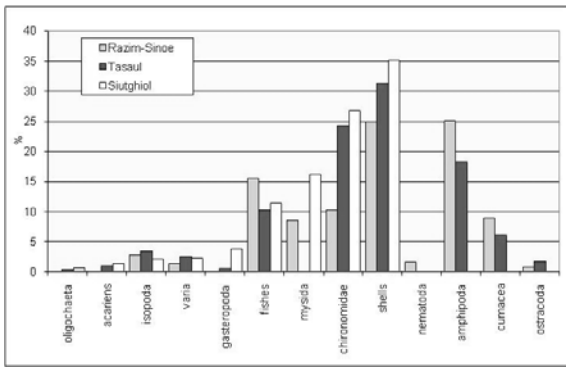

Fig. 5 – The trophic spectrum of *Neogobius fluviatilis* populations (2001)

There are differences in food composition function of extreme conditions (temperature). Thus we noticed an increase of chironomides and crustaceans quantity during summer. For example, in Tasaul Lake (1999) in July, crustaceans represented 35.5%, and chironomides 26.9% from entire digestive tract content, while in September the percentage decrease to 18.6% for insects and 16.7% for crustaceans (Fig. 6).

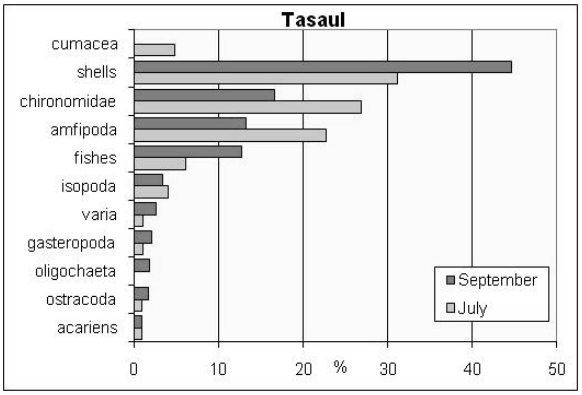

Fig. 6 – The trophic spectrum of *N. fluviatilis* (Taşaul, 1999) in July-September

The phenomenon can be explained by water cooling at the beginning of fall, when the larval development of insect ends, and crustaceans bury in the bottom.

The various trophic bases from all studied basins show different FI values (Table 2), with maximum for Razim-Sinoe complex (90.75) and minimum for Siutghiol Lake (73.32). In the same time, as we have already seen at *Apollonia melanostomus*, the spawning period is followed by o stronger nourishment. So, the average values of digestive tract content weight are higher in September than are in July (Table 2).

Table 2 – Filling index values at *N. fluviatilis* from paramarine waters

	min	**max**	**average**
Siutghiol	9.16	197.53	73.22
Taşaul	13.65	241.69	82.64
Razim-Sinoe	12.21	261.14	90.75
	Taşaul		
July	22.54	206.99	74.51
September	23.65	241.69	108.73

When *N. fluviatilis* and *A. melanostomus* coexist, the study shown that monkey goby prefers crustaceans instead of mollusks. The explanation could be the aggressive food behavior of round goby.

For *P. cephalargoides* is no evidence of preference for any kind of food, this species being an omnivorous one. With its lateral teeth stronger than those from dentary bone, it eats both small or big crustaceans (*Palaemon*, *Crangon*) or polychaetes, and mollusks.

The digestive tract content study revealed differences in species predominance during the year (**Porumb**, 1961). Thus, in the winter the nourishing doesn't completely stop and the mean value of FI is 53.09. The author noticed an increasing in feeding in the beginning of spring, when water temperature is minimum 6^0C. The values of FI are 41.67 in January and 55 in February. In this time of year, benthonic crustaceans dominate in gobies food (*Porcellana longimana* – 31.03% *Xantho* – 13.70%), followed by *Palaemon* (2.19%), Blenniidae (1.62%), *Upogebia* (1.34%).

In the summer, the feeding coefficient has a mean value of about 108.38, and polychaetes (17.23%) are dominant in food, being followed by shells and crustaceans. Because in summer and fall the fishes are very active, they can catch pelagic and good swimmer species which go down to the bottom during the day. Polychaetes are replaced by *Xantho* and *Palaemon*, followed by small fishes, mussels and *Idothea*.

In our samples from Mangalia, starting from June till September, we identified specimens from 5 great taxonomic groups of invertebrates (crustaceans, shells, polychaetes, fishes and varia).

This segregation of food type depends on the length of consumer which determines a certain length of prey. For example, we make a comparison between gobies with body length of 46-65 mm and 116-135 mm. For both of these body length ranges the crustaceans are predominant (60.4% for 46-65 mm and 65.1% for 116-135 mm, following by bivalves (Fig. 7). Nevertheless, the small fishes (46-65 mm) prefer small crustaceans (amphipods, mysids, isopods) instead of decapods (6.2%), beside polychaetes (14.6%). The fishes and crabs are missing completely.

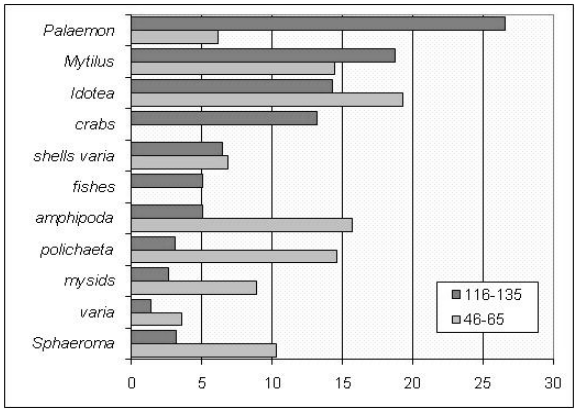

Fig. 7 – The trophic spectrum at different body length ranges of *Ponticola cephalargoides* (Mangalia, 2003)

For length range of 116-135 mm, the food consists in *Palaemon* (26.6%), bivalves (25.3%), isopods (15.5%), and crabs (13.2%) and different species of fishes (5.1%).

The values of FI are lower than literature's data (Porumb 1961). There are no significant differences between sexes and body length ranges. However, FI values depend on season, with maximum in autumn (76.6) and minimum in summer (68.8).

The fourth species, *Gobius jozo* is a species which lives at higher depths, and it is very rare in Romanian waters.

For black goby, the literature's data are very pauper, especially for Romanian coastal water of Black Sea. Bănărescu (1964), in his effort to describe the ecology of this species, said that "fishes and other small animals (for ex. *Corbulomya*)" are its favorite foods. Our observations are more closely of Miller's (1986) data, the trophic spectrum consisting almost exclusively in amphipods, isopods and shells (Fig. 8). FI values vary between 14.32 and 197.53 with an average of 68.89. It is possible that *Gobius jozo* eats important quantities of other fishes, but our captures was represented only by juveniles which eat only small animals.

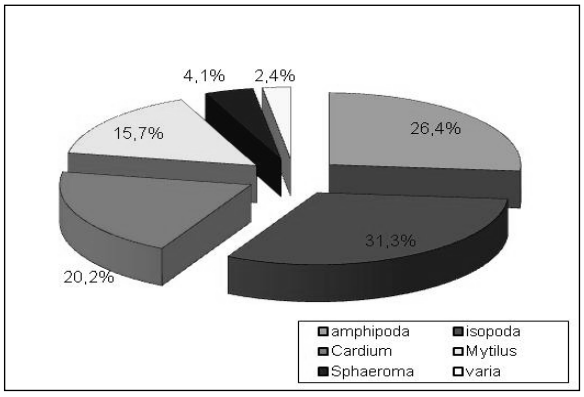

Fig. 8 – The trophic spectrum of *Gobius jozo* (Constanţa, 2000)

Mesogobius batrachocephalus proves to be a real predator. It has been noticed a differentiation of dentition with canines on dental bone, real canines and smaller teeth on pharyngeal upper bone and curved teeth on pharyngeal lower bone, in concordance with this feeding behavior. In its' diet prevail other species of gobies and big crustaceans. As a general rule, **Porumb** (1961) didn't remark any difference between season in food composition of knout goby, probably because of the same body length and feeding behavior.

We faced difficulties in *Mesogobius* trophic spectrum estimation, because of the time passed between the proper nesting and pulling to the water surface. In this time digestion continued and there are more chances that fishes to have an empty digestive tract. On the other hand, in mixed capture, some big knout gobies eat other small fishes.

In Romanian coastal waters, we haven't noticed any significant differences of *M. batrachocephalus* food composition during June-September period. Fishes bones and scales prevail, followed by

crustaceans and mollusks (Fig. 9). Comparative study based on sex composition of this goby population showed a light preference for small food for female, while males ate more fishes.

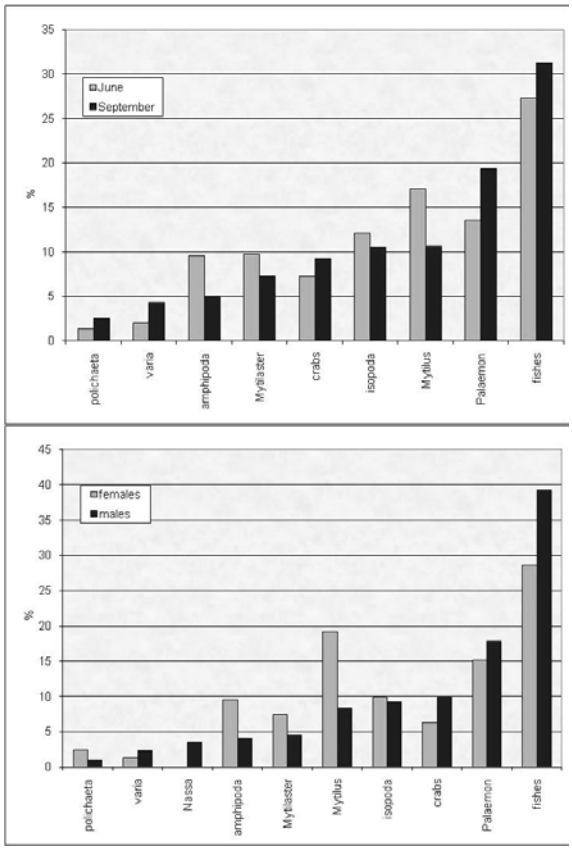

Fig. 9 – Qualitative and quantitative compound of *Mesogobius batrachocephalus* food (Mangalia, 1999)

Although there is a trophic spectrum differentiation between males and females, the values of FI for June and September overlap, with a mean value of 73.54 in June and 88.79 in September, instead of 22.17 during the winter and 106.89 in the fall, as Porumb (1961) said.

4. Conclusions

The *Apollonia melanostomus* trophic spectrum analyze prevails its preference for mollusks, both marine (*Mytilus, Mytilaster, Corbula, Mya, Cardium*) and freshwaters (*Dreissena*) populations. The quantities of bivalves vary from 69.7% (Constanta) to 40.2% (Siutghiol) from the total weight of digestive tract content. In the same time, fishes and crustaceans represent important part of round goby trophic base, being permanent in its food.

Although *N. fluviatilis* is a molluscivorous species, in Tasaul and Siutghiol lake, because of a pauper fauna of mollusks and crustaceans, it was noticed a reorientation toward atypical elements, as insects and hydracariens.

Where *A. melanostomus* and *N.* fluviatilis coexist, *Neogobius* eat predominant crustaceans, maybe because of his food behavior less stronger than *Appolonia*.

Ponticola cephalargoides prooved to be an omnivorous species, eating crustaceans, bivalves, polychaetes, fishes etc, with a crustaceans dominancy. The food segregation function of fishes body length, shows a predominance of crustaceans and polychaetes for small fishes (46-65 mm) and of *Palaemon* (26.6%) an bivalves (25.3%) for big fishes (116-135 mm)

For *M. batrachocephalus* there was a constancy of food type ans composition from June till September, with fishes (27.3% in June, 31.3% in September), crustaceans (30.4% in June, 44% in September) and mollusks (26.8% in June, 17.9% in September).

Gobius jozo eats almost exclusively crustaceans (amphipods, isopods) and bivalves, the mean filling index values being 68.89. It is possible that *Gobius jozo* to eat important quantities of fishes, but our captures was represented only by juveniles which eat only small animals.

The digestive tract filling with food depends on season, with an intensification of eating outside of matting period. The mean filling index values are: for *A. melanostomus* FI=98.98 in sea and FI=81.7 in freshwaters, for *N. fluviatilis* FI=82.02, for *P. cephalargoides* FI=73.4, for *M. batrachocephalus* FI=83.16 and for *G. jozo* FI=68.89. The maximum values characteristic to *A. melanostomus* probably due to its wide ecological valence and to aggressive feeding behavior as against the other gobies species with which cohabitates (*N. fluviatilis* in freshwaters or *P. cephalargoides* in marine waters. The lowest

values have been noticed for *Gobius jozo* represented in our samples only by juveniles.

5. References

[1] BĂNĂRESCU, P., 1964 - Pisces. Osteichthyes în Fauna R.S.R, Acad. R.S.R, Bucureşti, Vol. XIII: 815-865

[2] MILLER, P.J., 1986 - Gobiidae in Fishes of the North-eastern Atlantic and Mediterranean, P.J.P. Whitehead, M-L. Bauchot, J-C Hureau, J.Nielsen&Tortonese E. (ed.), UNESCO, Paris: 1019-1085

[3] MILLER, P.J., 2004 – Fam Gobiidae in "The Freshwater Fishes of Europe", Vol. 8, I Aula-Verlag: 157-404

[4] MILLER, P.J., 2004 – Fam Gobiidae in "The Freshwater Fishes of Europe", Vol. 8, II Aula-Verlag, 478 pp

[5] PORUMB, I.I., 1961 – Contributii la cunoasterea biologiei guvizilor (*Gobius batrachocephalus*, *Gobius cephalarges* si *Gobius melanostomus*) din dreptul litoralului romanesc al Marii Negre (date preliminare), *Hidrobiol*, Buc: 271-282

[6] SVETOVIDOV, A.N., 1964 – Black Sea Fishes (in russian), Nauka, Moscova, 512 pp

BIOMETRICS, SEX STRUCTURE AND LENGTH-WEIGHT CORRELATION ANALYZES ON SOME GOBIES SPECIES POPULATIONS (PISCES GOBIIDAE) FROM ROMANIAN COASTAL WATERS

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Abstract: Biometrical population structure and length-weight relationship were determined to five species of gobies (*Apollonia melanostomus, Ponticola cephalargoides, Neogobius fluviatilis, Gobius jozo* and *Mesogobius batrachocephalus*). Some differences were recorded in gobies populations, depending on species, sex, life cycle or environmental conditions (water salinity, trophic base, competition). The analyze of length body distribution to both sexes of species reveals a decreasing value in postmatting period, when females, smaller than males, predominate. The average value of Fulton index is maximum for *P. cephalargoides* and *M. batrachocephalus* and minimum for *N. fluviatilis*.

Keywords: gobies, biometric population structure, sex ratio, Fulton index

1. Introduction

Forming the largest family of percomorph teleosts, gobies are mainly tropical and temperate benthic fishes, inhabiting inshore marine, estuarine and freshwaters. 24 species of gobies live in Black Sea basin, and 19 can be found in Romanian waters. Among them, only three have a small and local economical importance *(Apollonia melanostomus, Mesogobius batrachocephalus, and Ponticola cephalargoides).*

The length-weight relationship is a very useful tool in fisheries assessment (Anderson & Neumann (1996)), in estimating crop biomass (Morey *et al*., 2003) or weigh based on knowing length (Beyer,1987), in tracking the seasonal variation in fish growth (Ritcher *et al*., 2000), in predicting the condition, reproductive history, and life history of fish species (Nikolsky, 1963; Bolger & Connolly, 1989, Wootton, 1992), and in morphological comparison of species and populations (King, 1996; Gonçalves *et al*., 1997).

When making comparisons between populations, it is essential to use standard measures for all populations so that the results will be more reliable.

The correlation between length and weight expressed by Fulton index, indicate the nutrition degree of population, and thus the health statement of it.

2. Material and Methods

We studied five specie of gobies (*Apollonia melanostomus, Neogobius fluviatilis, Ponticola cephalargoides, Mesogobius batrachocephalus* and *Gobius jozo*) which are the most frequent fishes in Romanian coastal waters.

The study developed between 1998 and 2003 when the samples have been collected by purse-seining, angling, long lining or other fishing tools with bait hooks, using nets with a mesh diameter around 4 mm or by diving up to 5m depth from Romanian coastal waters of Black Sea and from paramarine lakes (Danube Delta complex, including Sinoe and Razim lakes, Siutghiol, Tasaul, Neptune lakes) as we figured below (fig. 1).

The samples have been preserved in formaldehyde 4% and then transferred in alcohol.

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For establish the length ranges of gobies was measured the standard length of body. After that the fishes were weight to determine the length-weight correlation and Fulton index.

$$\text{Fulton index} = \frac{\text{total weight} \times 100}{\text{Standard length}^3}$$

The sexes were determined by dissection and exposing the gonads.

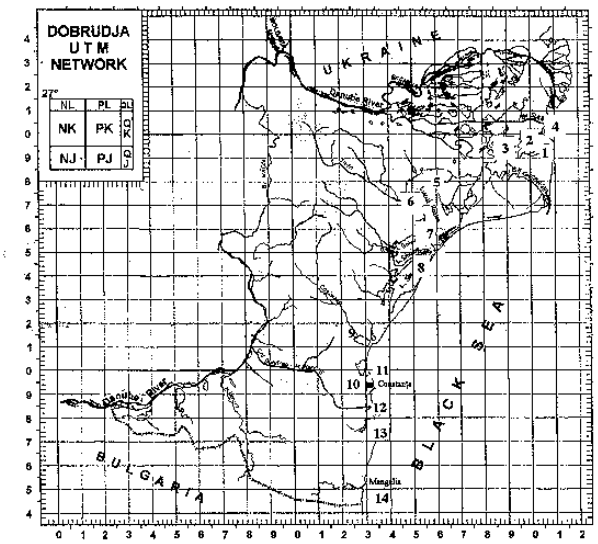

Fig. 1 – Map of station: 1. Roşuleţ; 2. Puiuleţ, 3. Puiu, 4. Sulina; 5. Razelm; 6. Sarichioi; 7. Cap Doloşman; 8. Sinoe; 9. Taşaul; 10. Siutghiol; 11. Perla; 12. Port Tomis; 13. Eforie Sud; 14. Mangalia; 15. Vama Veche

3. Results and Discussions

Biometric and sex structure of some gobies species populations

Apollonia melanostomus is a euryhaline species which lives almost in all Romanian coastal waters. It was made a comparative study referring to two populations collected in the same period of year (July 1999) from Tasaul Lake and marine waters from Constanţa. The marine specimens are larger (an average of 106-115 mm) than freshwater ones (an average of 76-95 mm) (fig. 2). Because gobiids are primary marine species, we can presume that the accommodation to freshwater environment supposes a redirection of energetic consumption to adaptation to different life condition, simultaneous with decrease of size.

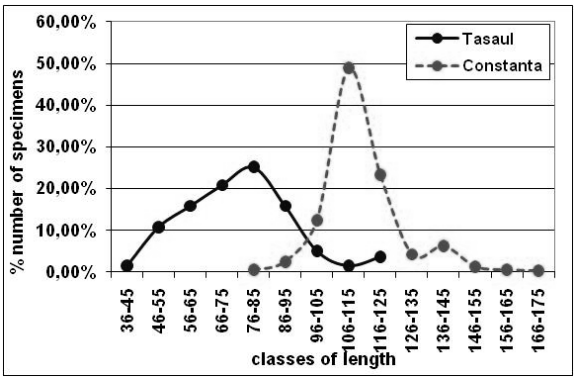

Fig. 2 – The biometric structure of *Apollonia melanostomus* populations (July, 1999)

This phenomenon is not unique among fishes. Thus, Kessler described a similar situation for a lot of species from Black Sea and Mediterranean Sea, showing that pontic fishes are smaller than Mediterranean ones. The author considers that temperature and salinity are the main factors which determine the nanism or gigantism phenomenon.

The dynamic of body size for individuals from the same basin shows a variation of average value during the year (fig. 3**)**. Thus in summer-fall period of 1998, in Constanţa, the higher values of length of body have been recorded in July (116-125 mm) against August-September (106-115 mm).

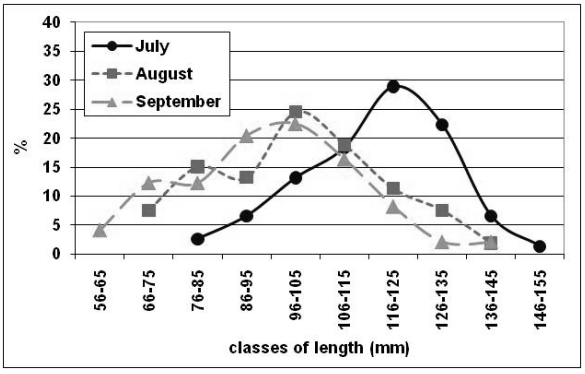

Fig. 3 – The cummulative curves based on biometrical population structure and months for *Apollonia melanostomus* (Constanţa, 1998)

It can be possible that this variation to been determined by the life cycle of *A. melanostomus*. This hypothesis is sustained by sex structure of population. It is well known that round goby mates in warm season, with females that reach sexual maturity younger than males. It is why the body size differs a lot, males having during two first years of life a double growing rate than females. After the matting period, the most males die, probably because they don't feeding, suffering weight loss, cankers of tegument (Kostyucenko, in Miller 2004). In this case, the average value of gobies body length should decrease, being determined by a higher preponderance of females and immature young males, which are shorter than their predecessors.

Besides this, we have to take in consideration that summer fishing, which hints the large individuals, can also determine the decrease of average length of body in the marine population.

The comparative analyses of body length of round goby males in July and September confirm the former statement (Fig. 4). Thus, at the beginning of summer, the most individuals are 76-115 mm length, while in September, the most specimens are 76-85 mm length.

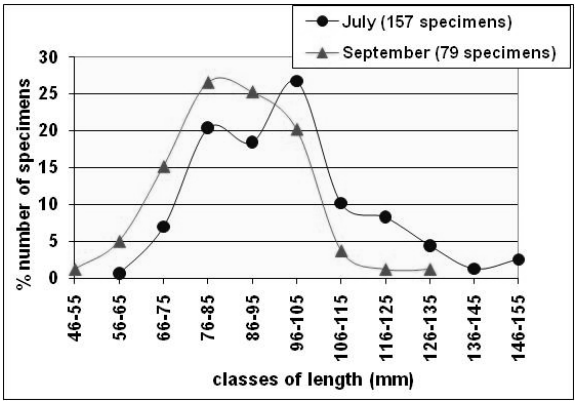

Fig. 4 – The percent biometric structure of an *Apollonia melanostomus* population (July and September, Constanţa, 1998)

We can presume as the smaller the sex ratio (females/males) value is, the smaller is the difference between the average body lengths values throughout the summer-autumn period.

The sex-ratio values (females/males) vary between 0.802 in July and 1.02 in September (fig. 5). The sub unitary values can indicate a low survival percent in future generations, because a small number of female means a small number of eggs.

In Siutghiol Lake the situation is different from Black Sea water (fig. 6). In July-September period, the average values of body length were 66-85 mm. We have to notice that during all these months, in Siutghiol Lake the water temperature was constantly high (an average of 26^0C). This determined a prolongation of matting period till September, which can explain the situation describe above.

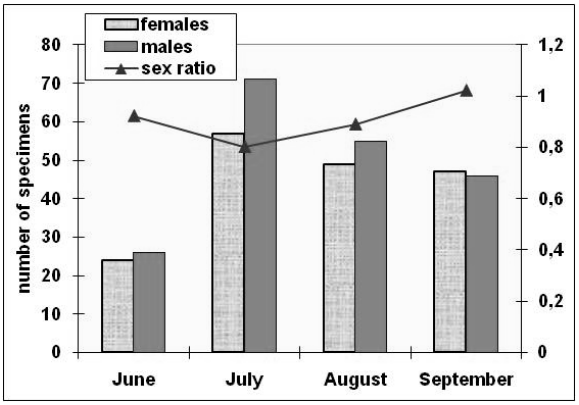

Fig. 5 – The sex structure to a population of *Apollonia melanostomus* (June-September, Constanţa, 1998)

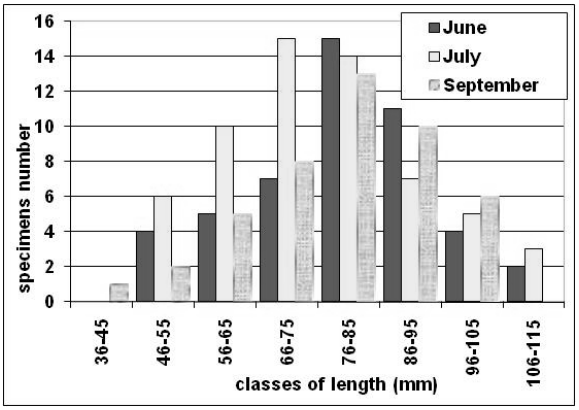

Fig. 6 – The percent biometric structure of an *Apollonia melanostomus* population from Siutghiol Lake (June-September 1998)

During the automn months, when the water temperature decrease and the matting period end, the average values body length became lower (fig. 7).

The body length analyse of each sex for a population from Tasaul Lake (1999) show that in July, during the period of matting, males were larger than females (fig. 8) which confirm that males length determine a high average values of population length.

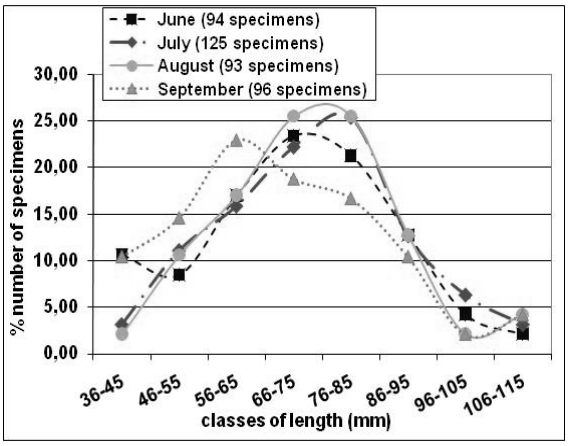

Fig. 7 – The percent biometric structure of a round goby population from Taşaul Lake (June-September, 1999)

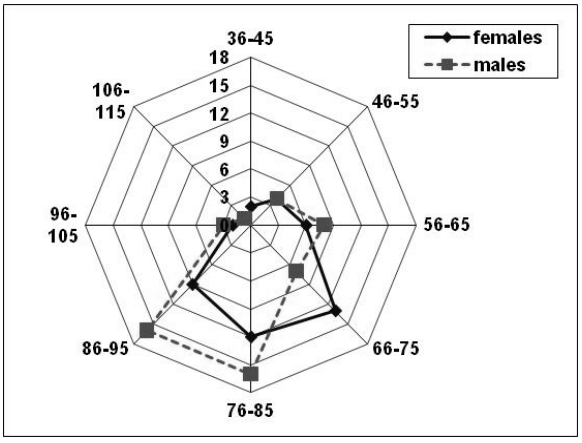

Fig. 8 – The length and sex structure of an round goby population from Taşaul Lake (July, 1999**)**

The sex ratio values for freshwater populations varies between 0.704 in Siutghiol (in July) and 1.5 in Taşaul (in August), probably because of the environmental conditions and because of life cycle (Table 1).

Table 1 – Sex structure and sex ratio values of two *Apollonia melanostomus* populations from Taşaul and Siutghiol lakes (1998)

	Siutghiol				**Taşaul**			
	VI	**VII**	**VIII**	**IX**	**VI**	**VII**	**VIII**	**IX**
♀	54	50	49	43	45	40	54	51
♂	68	71	38	38	51	43	36	37
Sex ratio	0.79	0.7	1.29	1.13	0.88	0.93	1.5	1.38

For *Neogobius fluviatilis*, the comparative analyses of populations from Taşaul, Siutghiol and Razim Lakes show differences between the average body length values: 86-95 mm for Siutghiol and Taşaul Lakes, instead of 96-105 mm for Razim Lake (fig. 9), probably due to the richness of trophic base from the last aquatorium. For example, in Razim fauna there can be found 4 species of bivalves and 20 species of peracarids instead of one species of bivalve and 7 species of peracarids in Taşaul Lake (Serban, personal data).

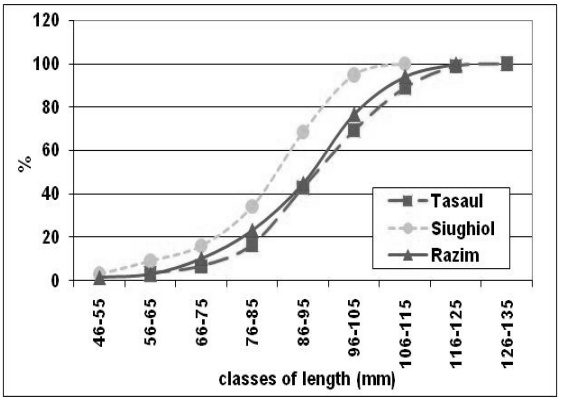

Fig. 9 – The percent cumulative diagram of biometric structure to *Neogobius fluviatilis* populations from Siutghiol, Taşaul and Razim lakes

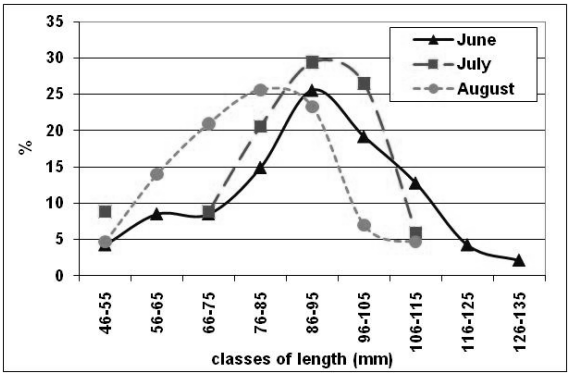

Fig. 10 – The percent cumulative diagram of the biometric structure of *Neogobius fluviatilis* populations from Taşaul (June-August 1999)

As in round goby case, an analyze of body biometric structure population of *N. fluviatilis* from Tasaul Lake during the summer showed an average values lower in August (76-85mm) than June (86-95 mm) (fig. 10) probably because the death of males, which are larger than females, after the matting period. Thus, males have usually a body length of 96-105 mm (25%) while females are usually 86-95 mm (31.58%) in body length (fig. 11).

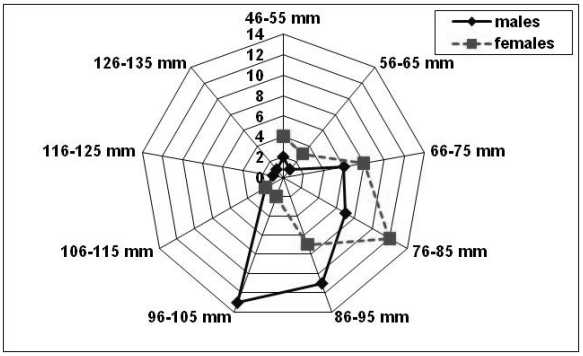

Fig. 11 – The length and sex structure of a *N. fluviatilis* population from Taşaul Lake (1998)

The sex-ratio values of *N. fluviatilis* populations from Razim, Taşaul and Siutghiol (1998) show males predominance in the matting period (June), while females are more numerous in feed period (August). The minimum value of sex ratio was recorded in August in Siutghiol Lake where the matting period extends till September because of high constant water temperature (Table 2).

Table 2 – Sex structure and sex ratio of *N. fluviatilis* populations from Razim, Taşaul and Siutghiol lakes (1998)

	Razim		**Taşaul**		**Siutghiol**	
	VI	**VIII**	**VI**	**VIII**	**VI**	**VIII**
♀	28	14	38	36	41	25
♂	29	11	44	22	48	23
Sex ratio	0.97	1.27	0.86	1.64	0.85	1.09

Ponticola cephalargoides is a very common species in Romanian coastal marine water. The body length of fishes from a Constanţa coastal waters population varies between 96-145 mm, with an average of 96-115 mm (fig. 12), smaller than the values mentioned in literature (an average of 15 cm and a maximum of 24 cm (Borcea, 1934)).

The males are larger than females (106-115 mm for males and 96-105 for females) (Fig. 13).

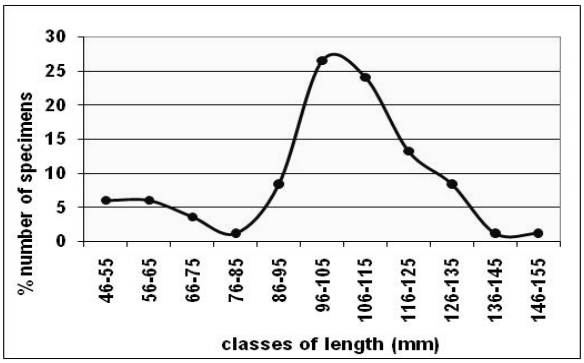

Fig. 12 – Percent biometric structure of *Ponticola cephalargoides* populations (Mangalia)

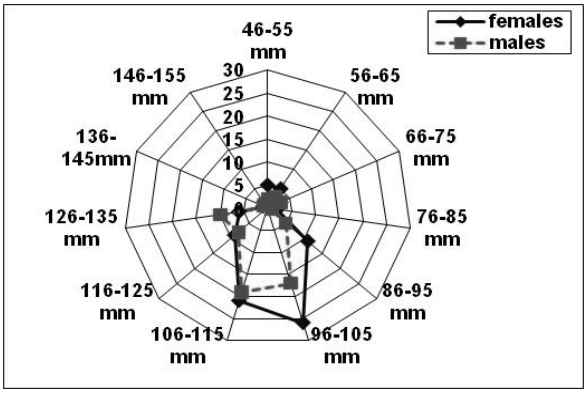

Fig. 13 – Sex and length *Ponticola cephalargoides* population structure (Mangalia, 2002)

We have to notice that, because of a very early matting period (April for N-W of Black Sea and March-May for Bulgarian coast of Black Sea), the average values of body length is determined a lot by the length of females which are more numerous. The sex ratio values sustain this presumption (table 3).

Table 3 – Sex structure and sex-ratio of *Ponticola cephalargoides* populations from Mangalia

	Mangalia		
	August 2002	**August 2003**	**September 2003**
Femele	64	89	53
Masculi	51	70	47
Sex ratio	1.255	1.271	1.127

Porumb (1961) described a completely different situation: males predominate during all the year, including matting period, when females represented only 41% from entire population.

Gobius jozo, the fifth species which we proposed to analyze, lives in depths, closing to seacoast only exceptionally. In July 2000, a population of young fishes, with 41-77 mm body length, was noticed nearby Mamaia station. The most individuals had 46-60 mm length (fig. 14).

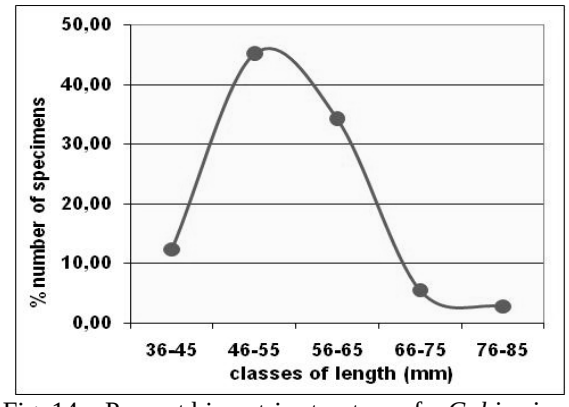

Fig. 14 – Percent biometric structure of a *Gobius jozo* population from Mamaia (2000)

The migration of black goby was stimulated by the circulation of cold water currents which determined the decrease of water temperature till 14-15^{0}C. The matting period which implies the nest and eggs defense can explain the lack of adult larger than 100 mm from this population. On the other hand, we can suppose that younger individuals migrated to find the characteristic food or higher oxygenate water, because they are more susceptible to water oxygen fluctuation than adults.

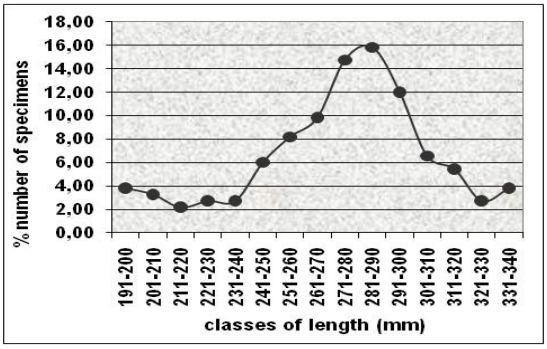

Fig. 15 – Percent biometric structure of Mesogobius *batrachocephalus* populations from Mangalia

In *Mesogobius batrachocephalus* population there have been recorded specimens with body length varying between 192,7 mm and 362 mm (fig. 15), larger than values quoted in literature (345 mm in Bănărescu, 1964). Only Gheorghiev (1966, in Miller 2004) reported specimens of 370 mm length for Bulgarian seacoast.

As *P. cephalargoides*, the knout goby was fishing out of matting period. The predominance of females (sex ratio=1.46) wasn't determined by the males mortality but because the migration of both sexes in horizontally water masses in order to find food.

Length-weight relationship. Fulton index

As a general rule, there is no difference in length-weight correlation between males and females from the same or different populations, between freshwater or marine populations or between specimens collected in different period of the year.

In *Apollonia melanostomus* case the value of length-weight correlation is high (0.925) both in marine or freshwater basins (fig. 16).

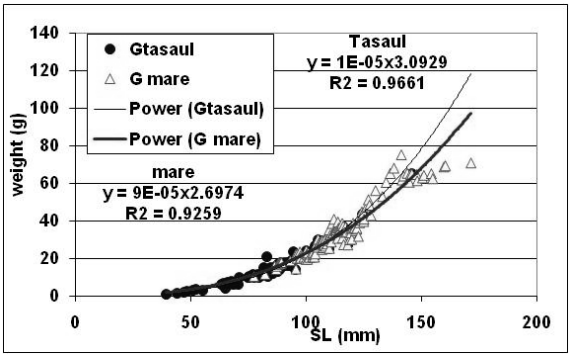

Fig. 16 – Growth equation of *Apollonia melanostomus* populations from Taşaul Lake and marine waters

Instead, the Fulton index values are different for this two type of aquatories, varying between 1,55 and 3,61 for freshwaters and 1,41 and 2,9 for marine waters, but with the same average value (2,21 and 2,20).

Because the Fulton index varies with physiological status, it is possible that in the matting period the weight of gonads to have a major contribution to total body weight. After this period, the survivors are emptied of sexual organs, and thus, the Fulton index values are lower than the matting period ones. (Example: in a Constanţa population K_{mediu}=2.99 in July and 1.74 in September; in a population from Taşaul Lake K_{mediu}=3.09 in June and 1.94 in September).

For *N. fluviatilis*, the index of length-weight correlation is 0,9083 (fig. 17), and Fulton index varies between 1,14 and 2.67, with an average value of 1,62. As in round goby case, the Fulton index value varies with seasons and physiological status, with an average of 1,83 in June-July (matting period) till 1,58 after that.

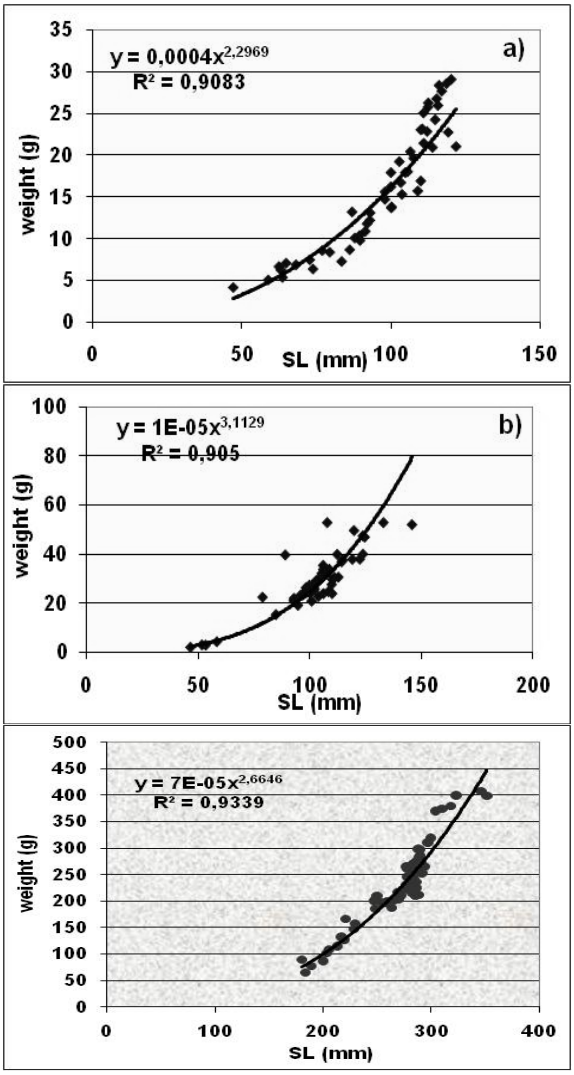

Fig. 17 – Growth equation on *Neogobiuas fluviatilis* (Razim) (a) *Ponticola cephalargoides* (b) and *Mesogobius batrachocephalus* (c) populations

P. cephalargoides şi *M. batrachocephalus* show the same pattern of length-weight correlation, with R^2=0.905 for *Ponticola* and R^2=0.934 for *Mesogobius* (Figure 6.4.2.3). Instead, because these two species mate early in the spring, K values are determined much more by the efficiency and capacity of every specimen's food acquirement. Thus, K=1,67-5,57 with an average value of 2,51 for *P. cephalargoides* and K=0,83-4,94 with an average value of 2,38 for *M. batrachocephalus.*

Reporting to *G. jozo*, the Fulton index wasn't determined by gonads weight, because the fishes weren't sexually matured. K values varied between 1,62 and 2,99 (average of 2.01), and R value was 0.955 (fig. 18).

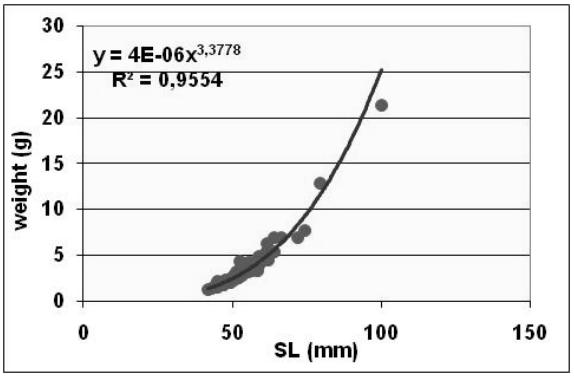

Fig. 18 – Growth equation on *Gobius jozo* population

In conclusion, the maximum values of Fulton index were noticed for *P. cephalargoides* and *M. batrachocephalus*. The specimens of these species, which were collected after the matting period, were mainly looking for feeding in order to assure the energetically needs for growing and recreate of gametes stock.

The minimum values of Fulton index were recorded for *Neogobius fluviatilis*. This species have the same food preferences as round goby, a more aggressive fish, with which cohabitated.

4. Conclusions

Marine specimens of *Apollonia melanostomus* are larger than freshwater ones, with an average body lenght value of 106-115 mm for Black Sea populations instead of 76-95 mm for paramarine lakes populations. Because gobiids are primary marine species, we can assume that the accommodation to freshwater environment supposes the redirection of energetic consumption to adaptation to different life condition, simultaneous with decrease of body length.

Reffering to *Neogobius fluviatilis*, there are some differences between Razim population on one hand, and other southern romanian paramarine lakes populations, on the other hand. The specimens from Razim basin were larger than other lakes specimens (an average body lenght value of 96-105 mm instead of 86-95 mm), probably because the richness of Razim trophic base.

For *Ponticola cephalargoides* and *Gobius jozo* the average body lenght values are lower comparative with those quoted in literature. For the second species, the result can be explained by the early age of collected specimens, which migrated to low waters looking for proper life conditions (food, oxygen).

Mesogobius batrachocephalus maximum standard body lenght values are higher than those from quoted literature (362 mm instead of 345 mm).

The analyze of length body distribution to both sexes of all species reveals a decreasing values in postmatting period, when females, smaller than males, predominate.

The average value of Fulton index are maximum for *P. cephalargoides* and *M. batrachocephalus* cases (2.51 and 2.38), because this species, after the matting period, were mainly looking for feeding in order to assure the energetically needs for growing and reestablishing of gametes stock.

The minimum value of Fulton index was recorded in *N. fluviatilis* case (1.62), because of the aggresive behavior of *A. melanostomus*, a species with which it coexists in all studied aquatories.

5. References

[1] ANDERSON, O.R., NEUMANN, R.M., 1996 - Length, weight and associated structural indices, p. 447-482 *In:* L.A. Nielsen & D.L. Johnson (Eds). Fisheries Techniques**.** Bethesda, American Fisheries Society, 732p

[2] MOREY, G., MORANTA, J., MASSUTI, E., GRAU, A., LINDE, M., RIERA, F. and MORALES-NIN, B., 2003 -Weight-length relationship ships of littoral to lower slope fishes from the western Mediterranean. *Fish. Res*., 62: 89-96.

[3] BEYER, J.E., 1987 - On lenght-weigth relationships. Part I: Computing the mean weight of the fish of a given lenght class. Fishbyte, Manila, **5**: 11-13

[4] RICHTER, H.C., LUCKSTADT, C., FOCKEN, U. AND BECKER, K., 2000 - An improved procedure to assess fish condition on the basis of length-weight relationships. *Arch. Fish. Mar. Res*. 48:255-264.

[5] NIKOLSKI, G.V., 1962 – Ecologia pestilor, Academia R.P.R., 362 pp

[6] BOLGER, T., CONNOLLY, P.L., 1989 - Selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology*, Dumfries, **34**: 171-182

[7] WOOTTON, R.S,. 1992 - Fish Ecology, Thomson Litho Ltd., Scotland.

[8] **King, R.P.**, 1996 - Length-weigth relationshipships of Nigerian freshwater fishes. *Fishbyte*, 19(4): 53-58.

[9] GONÇALVES, J.M.S., BENTES, L., LINO, P.G., RIBEIRO, J., CANARIO, A.V.M. AND ERZINI, K., 1997 - Weight-length relationshipships for selected fish species of the mallscale demersal fisheries of the south and southwest coasts of Portugal.

[10] MILLER, P.J., 2004 – Fam Gobiidae in "The Freshwater Fishes of Europe", Vol. 8, I-II Aula-Verlag: 157-404; 478 pp

[11] SERBAN, VERGINICA – Ovidius University, unpublished personal data.

[12] BORCEA, I., 1934 - Revision systematique et distribution geographique des Gobiides de la Mer Noire et particulierement des eaux roumaines, *Ann. Scient. Univ. Jassy*: 1-231

[13] PORUMB, I.I., 1961 – Contributii la cunoasterea biologiei guvizilor (*Gobius batrachocephalus, Gobius cephalarges* si *Gobius melanostomus*) din dreptul litoralului romanesc al Marii Negre (date preliminare), *Hidrobiol*, Buc: 271-282

[14] BĂNĂRESCU, P., 1964 - Pisces. Osteichthyes în Fauna R.S.R, Acad. R.S.R, Bucureşti, Vol. XIII: 815-865

PRELIMINARY DATA ON CARAPACE HEALTH OF *TESTUDO GRAECA* FROM CETATEA HISTRIA, DOBRUDJA COUNTY

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Abstract: *Testudo graeca* is one of Dobrudja's endangered species and highly impacted by habitat destruction and fragmentation caused by human activities. The area near Histria fortress holds a large population of this land tortoise. In this preliminary study, a total of 54 tortoises where evaluated for estimating overall carapace health and anthropic impact considering the degree of erosion, injuries, human made scars and malformations. The results showed that this tortoise population is healthy and with seldom anthropic impact.

Keywords: *Testudo graeca*, carapace health, Histria fortress

1. Introduction

Testudo graeca is an attractive species for ecological studies because of its long life, easiness of capture, the presence of the carapace that allows for permanent marking for individual identification. Their importance as flagship species, status of conservation (priority species under the Habitats Directive 92/43/EEC) and public attractiveness makes them important for conservation.

T. graeca is unevenly distributed in Dobrudja, with large populations located in the north, Măcin Mountains National Park [1] and forested areas in south, and isolated ones in the central area. The studied population in this article is located in the enclosed area of Histria fortress Museum Complex, situated in Danube Delta Biosphere Reserve, which is relatively well separated from other similar populations because of habitat discontinuity, water bodies or fenced areas. This area has a low human impact, considerable vegetation and complex landscape, pits and hillocks, remnants of past archeological studies

The present paper describes the preliminary results of the monitoring done in 2010.

2. Material and Methods

The preliminary study on the *Testudo graeca* population from the enclosed are of Histria fortress Museum Complex was done between May-October 2010 on an area of 32 ha. The chosen area has no current archeological activity and combined with the fencing around the perimeter, which limits both the dispersal of tortoise and entrance of domestic animals, and lack of touristic activity makes it ideal for evaluating the health of this tortoise.

The study was done during day time, between 9:00 and 14:00 hrs. [2], covering the area with 24 transects, with 400 m average length with a gap distance of 20 m [3]. The location of each tortoise along transect was recorded with a handheld GPS Garmin eTrex. All tortoise captured were sexed, weighed with a portable electronic balance maxim weight 3kg, 1g precision, measured, photographed and temporary marked with a marker [4]. Age estimation was done for juvenile and subadults [5] by counting the number of rings on at least three carapace scutes [6].

Estimation of carapace health was done by recording, on site or by photography analyses, the erosion class of carapace according to the model of Berry and Woodman [7], injuries from anthropic

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activity, fire, and malformation consisting in abnormal number of scutes or abnormal growth.

The erosion class analyses imply grouping the tortoise by the degree of erosion on carapace in seven classes. The first class corresponds to newly born tortoise and the last class to tortoise with large carapace and old age. The degree of erosion of each tortoise depends mainly on dimension of carapace, age, habitat and diseases affecting carapace.

3. Results and Discussions

During the study period were inventoried 54 individuals of *T. graeca*, 27 females, 21 males and six juveniles with a density of 1.68 individuals/ ha, a lower density then in other studies on near similar zone in respect to area [8].

Overall the carapace and plastron of Cetatea Histria tortoises are in good shape, fact that correlates with the reduced human activities. Nevertheless were inventoried a number of tortoise which showed man made scars and malformations, doubled humeral scute on plastron or abnormal scutes growth and numbers on carapace (Fig. 1). Those anomalies in scutes number or abnormal growth can be attributed to isolation, limited population exchange and genetic diversity.

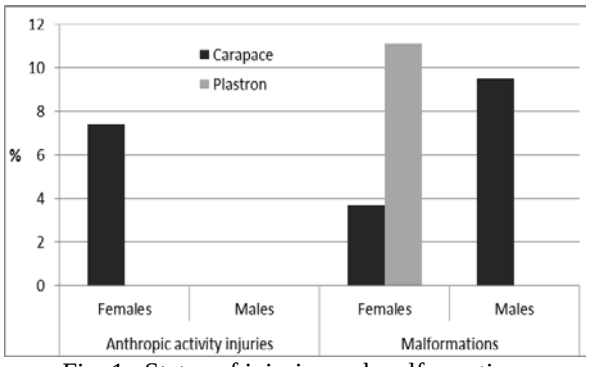

Fig. 1– Status of injuries and malformation

None of the tortoise showed visible marks of fire on both carapace and plastron. The erosion class analyses showed that all juvenile, of age three to seven were comprised in the first three classes of erosion and the adult females and males in the upper classes which corresponds to the model of erosion classes. Because of unreliable age estimation for captured tortoise over the 10-12 years old a correlation between age and erosion class was not suitable because a large number of tortoise were older than 12 years. Instead, a correlation between length of carapace and erosion class was done which showed that the carapace erosion increase with the length of carapace (Fig. 2, 3, 4). Considering that carapace increase in length with age we may assume that in general an older tortoise will have a more eroded carapace.

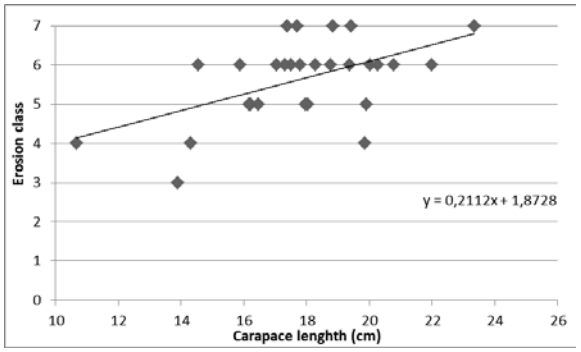

Fig. 2 – Correlation between carapace length and erosion class of carapace in females *T. graeca* from Histria fortress (n=27, R= 0,53, P= 0,95)

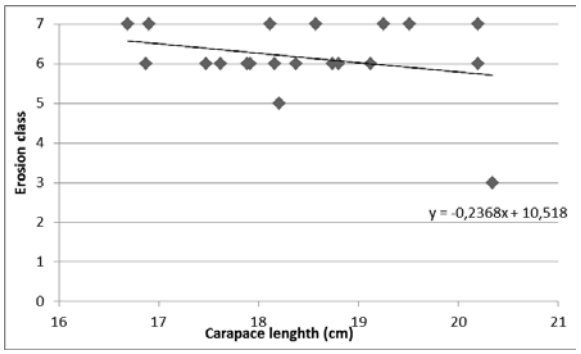

Fig. 3 – Correlation between carapace length and erosion class of carapace in males *T. graeca* from Histria fortress (n= 21, R= -0,27, P= 0,46)

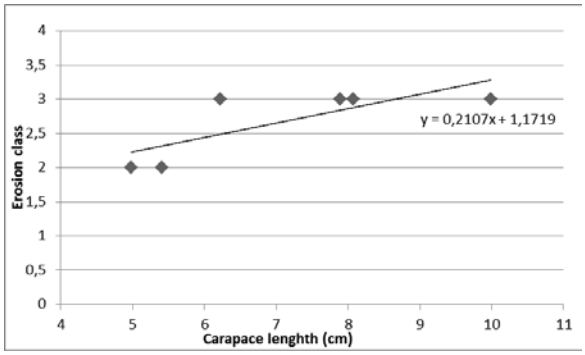

Fig. 4 – Correlation between carapace length and erosion class of carapace in juveniles *T. graeca* from Histria fortress (n= 6, R= 0,77, P= 0,77)

4. Conclusions

This preliminary study showed that the health carapace of *T. graeca* from the enclosed area of Histria fortress Museum Complex is overall good, with few injuries which could be attributed to human activity and reduced number of malformations. The erosion of carapace is consistent with the applied model of study showing a clear correlation between length of carapace and scutes erosion although this area has only abundant vegetation.

5. References

[1] COGĂLNICEANU, D., TUDOR, M., BĂNCILĂ, R., PLĂIAȘU, R., 2007 – Amphibians and reptiles in Macin Mountains National Park. Inventory of flora and fauna, description and mapping of habitats in Macin Mountains National Park and population assessment of conservation interest, Project UNDP/GEF No. 4711, www.parcmacin.ro (in Romanian).

[2] HILL, D., FASHAM, M., TUCKER, G., SHEWRY, M., SHAW, P., 2007 – Handbook of Biodiversity Methods, Cambridge University Press, New York.

[3] MAZEROLLE, M., BAILEY, L., KENDALL, W., ROYLE, A., CONVERSE, S., NICHOL, J., 2007 – Making Great Leaps Forward: Accounting for Detectability in Herpetological Field Studies. *Journal of Herpetology*, 41 (4): 672-689.

[4] STUBBS, D., HAILEY, A., PULFORD, E., TYLER, W., 1984 – Population Ecology of European Tortoise: Review of Field Tehniques. *Amphibia-Reptilia*, 5 (1): 57-68.

[5] GERMANO, D., BURY, R. B., 1998 – Age determination in turtles: evidence of annual deposition of scute rings. *International Journal of Turtle and Tortoise Research*, 3 (1): 123.

[6] BERTOLERO, A.; CARRETERO, M., A.; LLORENTE, G., A., 2005 – An assessment of the reliability of growth rings count for age determination in the Hermann's Tortoise *Testudo hermanni*. *Amphibia-Reptilia*, 26 (1): 17-23.

[7] BERRY, K. H., and A. P. WOODMAN. 1984 – Preliminary investigations of shell wear in determining adult age groups in desert tortoises. Appendix IV VII in K. H . Berry (ed.), The Status of the Desert Tortoise *Gopherus agassizii*) in the United States, A4-1 – A4-54.

[8] KADDOUR, K. B., SLIMANI, T., EL MOUNDEN, E. H., LAGARDE, F., BONNET, X., 2006 – Population structure, population density and individual catchability of *Testudo graeca* in the central Jbilets (Morocco), *Vie et Milieu*, 56 (1): 49-54.

DIVERSITY OF TESTATE AMOEBAE (PROTOZOA: ARCELLINIDA AND EUGLYPHIDA) IN OVCHARITSA RESERVOIR (SOUTHEASTERN BULGARIA)

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Abstract: In eighteen samples collected from the Ovcharitsa Reservoir, 75 taxa (including species and varieties) of testate amoebae of 18 genera were established. The fauna was composed mainly of aquatic species of the genera *Difflugia* (34 species), *Centropyxis* (12) and *Difflugiella* (5). The genera *Centropyxis* (28.0%), *Difflugia* (21.5%), *Trinema* (14.5%), *Corythionella* (12.2%) and *Phryganella* (9%) predominated considering the number of specimens. Frequently dominating species were *Centropyxis aculeata* (88.9%), *C. aerophila* (77.8%), *Phryganella hemisphaerica* (77.8%), *Centropyxis hirsuta* (50.0%), *C. ecornis* (44.4%), *Cyclopyxis eurystoma* (44.4%), *Pseudodifflugia gracilis* (38.9%) and *Centropyxis marsupiformis* (27.8%).

Keywords: Testate amoebae, Ovcharitsa Reservoir, dominance frequency, similarity.

1. Introduction

Testate amoebae are abundant and diverse in natural lakes, pools and rivers, as well as in artificial water basins such as reservoirs [1], [2]. They are found with high diversity and frequency in the biggest reservoirs in Bulgaria - Beli Iskar, Ticha and Batak, built mainly to supply the inhabitants of major cities with drinking water [3], [4], [5]. Relatively rich and varied is the testate amoebae fauna in Rabisha Reservoir, whose main function is to irrigate the adjacent farmland [6].

In the middle of last century a number of reservoirs in Bulgaria are built with a purpose the using of its waters to provide electric power or cooling of different systems, but their testacean fauna has not been the subject of research until now. One of them is Ovcharitsa Reservoir. It is especially build to cool the circulating water in the electric plant Maritza Iztok-2 and to protect of flood located in the vicinity mine Troyanovo.

The aim of this article is to present the results of our investigations on the taxonomic composition and diversity of the testate amoebae in Ovcharitsa Reservoir. This paper is the one of two describing the testate amoebae communities in the reservoir.

2. Methods of Research

The material for the present study (18 samples) was collected from different biotopes of the Ovcharitsa Reservoir, in June 2010. Eckmann's grab was used for the collection of the benthic samples. The plankton samples were collected with a plankton net.

Test morphology and measurements of the species were made using an light microscope "Amplival", at 400x magnification. For species identification was used taxonomic monographs [7], [8], [9], [10], [11], [12], [13].

The relative significance $D=n_i/N \times 100$, where n_i is the number of the specimens of each species and N – the total number of all specimens was used to established the dominant structure. The dominance frequency was calculated by the formula: $DF=d/n \times 100$, where d is the number of samples in which one species dominates and n is the total number of samples.

The comparison among testacean fauna of the Ovcharitsa Reservoir and other studied reservoirs in Bulgaria was made by the Jaccard's similarity factor (k): $k = C / A+B-C \times 100$, where A is the number of species (genera) in the studied reservoir; B – the number of found species (genera) in the compared reservoir; C – the number of the common species.

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3. Results and Discussion

Seventy-five taxa (including species and varieties) of testate amoebae, belonging to 18 genera of the orders Arcellinida and Euglyphida were found in the Ovcharitsa Rezervoir. A total of 768 testate amoeba shells were counted. The list of the observed taxa and their dominance freguency in the reservoir are presented in Table 1.

Table 1 – List of taxa and their dominance freguency (DF) in the Ovcharitsa Reservoir.

Taxa	DF
Arcella *discoides* Ehrenberg, 1843	0.0
A. hemisphaerica Perty, 1852	0.0
Centropyxis *aculeata* (Ehrenberg, 1830) Stein, 1857	88.9
C. aerophila Deflandre, 1929	77.8
C. aerophila v. sphagnicola Deflandre, 1929	11.1
C. cassis (Wallich, 1864) Deflandre, 1929	11.1
C. constricta (Ehrenberg, 1841) Deflandre, 1929	0.0
C. deflandriana Bonnet, 1959	0.0
C. delicatula Penard, 1902	11.1
C. ecornis (Ehrenberg, 1841) Leidy, 1879	44.4
C. hirsuta Deflandre, 1929	50.0
C. marsupiformis (Wallich, 1864)	27.8
C. platystoma (Penard, 1890) Deflandre, 1929	0.0
C. sylvatica (Deflandre, 1929) Bonnet & Thomas, 1955	5.6
Corythionella *georgiana* Nicholls, 2005	22.2
Cyclopyxis *aplanata* Deflandre, 1929	0.0
C. eurystoma Deflandre, 1929	44.4
C. kahli Deflandre, 1929	5.6
Difflugia *ampullula* Playfair, 1918	5.6
D. angulostoma G. - Lievre et Thomas, 1958	0.0
D. balcanica Ogden & Zivkovic, 1983	5.6
D. bicornis Penard, 1890	0.0
D. brevicolla Cash, 1909	0.0
D. bryophila (Penard, 1902) Jung, 1942	5.6
D. capreolata Penard, 1902	0.0
D. corona Wallich, 1864	5.6
D. decloitrei Godeanu, 1972	5.6
D. dificilis ecornis Chardez, 1956	0.0
D. distenda Ogden, 1983	0.0
D. dragana Ogden & Zivkovic, 1983	0.0
D. elegans Penard, 1890	22.2
D. glans Penard, 1902	11.1
D. globularis (Wallich, 1864) Leidy, 1877	5.6
D. globulosa Dujardin, 1837	0.0
D. gramen Penard, 1902	11.1
D. lacustris (Penard, 1899) Ogden, 1983	0.0
D. lanceolata Penard, 1890	0.0
D. levanderi Playfair, 1918	5.6
D. lobostoma Leidy, 1879	0.0
D. lob. f. multilobata (Leidy, 1879) G.-Lievre & Thomas, 1958	0.0
D. lucida Penard, 1890	5.6
D. manicata Penard, 1902	22.2
D. mica Frenzel, 1892	5.6
D. minuta Rampi, 1950	5.6
D. penardi Hopkinson, 1909	0.0
D. pristis Penard, 1902	11.1
D. pulex Penard, 1902	16.7
D. sarissa Li Sun Taï, 1931	16.7
D. serbica Ogden & Zivkovic, 1983	0.0
D. tenuis (Penard, 1890) Ogden, 1983	0.0
D. tricornis Ogden, 1983	0.0
D. viscidula Penard, 1902	0.0
Difflugiella *angusta* Schönborn, 1965	5.6
D. oviformis Bonnet &Thomas, 1955	11.1
D. sacculus (Penard, 1902) Deflandre, 1953	0.0
D. pusilla Playfair, 1918	0.0
Taxa	**DF**
D. vulgaris (France, 1913) Grospietsch, 1964	0.0

Euglypha *acanthophora* (Ehrenberg, 1841) Perty, 1849	0.0
E. rotunda Wailes, Penard, 1911	11.1
E. tuberculata Dujardin, 1841	5.6
Hyalosphaenia *subflava* Cash, 1909	0.0
Microchlamys *patella* (Claparede & Lachmann, 1885) Cockerell, 1911	11.1
Pentagonia *shablensis* Todorov & Golemansky, 1998	0.0
Phryganella *hemisphaerica* Penard, 1902	77.8
Plagiopyxis *declivis* Thomas, 1955	22.2
Pl. minuta Bonnet, 1959	0.0
Psammonobiotus *linearis* Golemansky, 1970	11.1
Pseudodifflugia *compressa* Schulze, 1874	16.7
P. gracilis Schlumberger, 1845	38.9
Schaudinnula *arcelloides* Awerintzew, 1907	5.6
Tracheleuglypha *acolla* Bonnet & Thomas, 1955	5.6
T. dentata Deflandre, 1938	0.0
Trinema *enchelys* (Ehrenberg, 1838) Leidy, 1878	22.2
T. lineare Penard, 1890	0.0
Zivkovicia *compressa* (Carter, 1864) Ogden, 1987	5.6
Total species/genera	**75/18**

The obtained results show that the genera *Difflugia* (34 taxa), *Centropyxis* (12) and *Difflugiella* (5) had the highest number of species and they belong 68.0% of all established species in the reservoir (Fig. 1). *Two genera (*Cyclopyxis *and* Euglypha*) were represented by 3 species, five genera – by 2 species and 8 or 44.4% of a*ll genera were represented by 1 species only. Considering the number of specimens of each species, the genera *Centropyxis* (28.0%), *Difflugia* (21.5%), *Trinema* (14.5%), *Corythionella* (12.2%) and *Phryganella* (9%) predominated in the testacean communities of the reservoir (Fig. 2). Together they made up 85.2% of the total count. Although the genus *Difflugia* is established with the greatest number of species, in terms of the number of specimens, its role decreases and the genus *Centropyxis* had the highest relative abundance. The dominance of the genus *Trinema* is due to the presence of the species *Trinema enchelys* mainly, and that of the genera *Corythionella* and *Phryganella* - of only one species too, respectively – *Corythionella georgiana* and *Phryganella. hemisphaerica*.

Only 8 or 10,7% of all found in the reservoir 75 taxa were frequently dominating species (with dominance freguency equal to or higher than 25%): *Centropyxis aculeata* (88.9%), *C. aerophila* (77.8%), *Phryganella hemisphaerica* (77.8%), *Centropyxis hirsuta* (50.0%), *C. ecornis* (44.4%), *Cyclopyxis eurystoma* (44.4%), *Pseudodifflugia gracilis* (38.9%) and *Centropyxis marsupiformis* (27.8%).

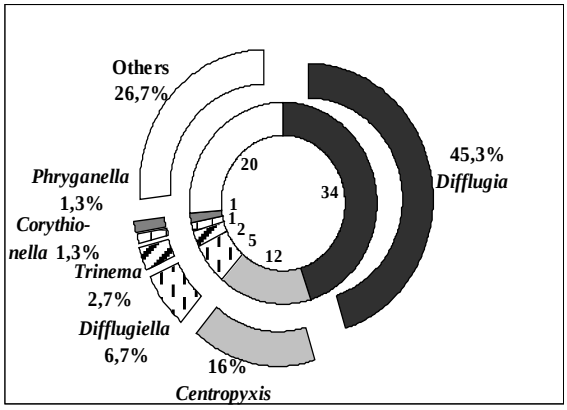

Fig. 1 – Pie chart indicating the number of species (inner circle) and relative significance, according to the number of species of dominating genera

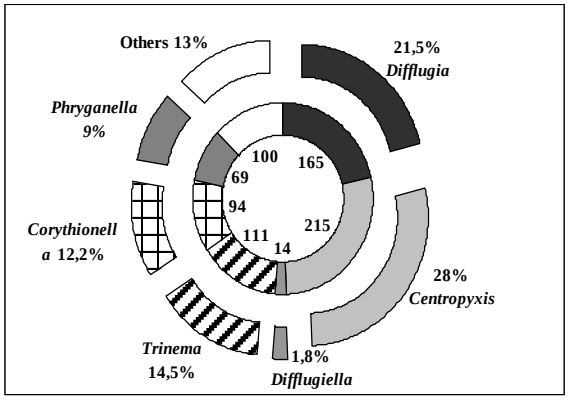

Fig. 2 – Pie chart indicating the number of specimens (inner circle) and relative significance, according to the number of specimens of dominating genera

One of the important features of the fauna appears to be its degree of similarity in different reservoirs. Data on the comparison of the testacean fauna of the Ovcharitsa Reservoir with that of four other studied reservoirs in Bulgaria are given in Table 2. Analysis of results showed that the established species richness in the Ovcharitsa Reservoir is not great and corresponds with the diversity found in other studied reservoirs – Rabisha and Beli Iskar. Most testate amoeba species observed in this study are commonly found in the many natural lakes and reservoirs in the world [2], [5] [14], [15], [16], [17], [18], [19], [20], [21]. In qualitatively regard, more significant (but not a big) similarity exists between testate amoebae of investigated by us reservoir with these of the reservoirs Rabisha, Ticha and Batak - the coefficients of faunal similarity on a species level is between 33.3 and 51.5, and on a genus level - between 39.3 and 60.8. The observed differences and established values of the coefficient of similarity between the testate amoebae fauna of compared water basins are not surprising and are due to some differences in their characteristics – the different age, varied settlement with a littoral vegetation, differences in chemical characteristics of the water and other.

Analyzing the structure of testate amoebae communities in the five reservoirs were established significantly greater similarity. In all studied reservoirs dominated representatives of the genera *Difflugia* and *Centropyxis*. For reservoirs Ovcharitsa, Rabisha, Ticha and Batak the values of the ratio of the number of species of the genera *Difflugia* and *Centropyxis* to the total number of species are very similar, which again proves the more similarity between testate amoebae fauna of these water basins. And in the five reservoirs studied are found relatively low diversity of the genus *Arcella* which everywhere presented with several species and low population density. Such distribution of the testate amoebae fauna is a characteristic for the continental aquatic ecosystems.

Relatively low similarity on a species level with the testate amoebae fauna of Beli Iskar Reservoir, and lower value of the ratio of the number of species of the genera *Difflugia* to the total number of species is probably due (besides of the indicated above reasons) to the fact that the dam has been studied only in terms of the watercatchment area and the littoral zone. No data on testate amoebae living in the deep zone of the reservoir, where it is expected to be dominated by representatives of the genus *Difflugia* - species reported as characteristic for the sediments benthos from the deep–water of the lakes.

4. Conclusions

This study showed that testate amoebae fauna in the Ovcharitsa Reservoir is not much abundant. The established diversity correspond with that found in some other similar reservoirs. Most testate amoebae species observed in this study belong to the genera *Difflugia*, *Centropyxis* and *Difflugiella* and are commonly found in studies of lacustrine biotopes. Comparison with other studied dams in Bulgaria showed that the similarity expressed not so in composition, much in community structure. The assemblage structure and especially the proportion of dominant taxa observed in it correspond well with the result, established in other lakes and reservoirs.

Acknowledgements. This study was carried out under a project № RD-07-336 / 2011 funded by the University of Shumen.

5. References

[1] LANDA G., R. L. MOURGUES-SCHRUTER R., 2000 – Caracterização da comunidade zooplanctônica de um sistema artificial (represa Zootecnia) nos campos da Universidade Federal de Lavras – MG, *Acta Limnologica. Bras*., vol. 12, p. 63-68.

[2] LANSAC-TÔHA F., ALVES G., VELHO L., ROBERTSON B., JOKO C., 2008 – Composition and occurrence of testate amoebae in the Curuá-Una Reservoir (State of Pará, Brasil), *Acta Limnologica Bras*., **20** (3): 177-195.

[3] GOLEMANSKY V., TODOROV M., 1993 – Testate Amoebae (Protozoa, Rhizopoda) in the watercatchment area and littoral of the 'Beli Iskar' dam. – *Acta zoologica bulgarica*, **46:** 3-8.

[4] DAVIDOVA R., GOLEMANSKY V., TODOROV M., 2008 – Diversity and Biotopic Distribution of Testate amoebae (Arcellinida and Euglyphida) in Ticha Dam (Northeastern Bulgaria), *Acta zoologica bulgarica*, 60, Suplementum **2:** 7-18.

[5] TODOROV M., GOLEMANSKY V., TEMELKOV B., 2008 – Diversity and Biotopic Distribution of Testate amoebae (Protozoa: Arcellinida and Euglyphida) in the Batak

Reservoir (Soutern Bulgaria), *Acta zoologica bulgarica*, 60, (2): 115-124.

[6] DAVIDOVA R., 2010 – Testate Amoebae Communities (Protozoa: Arcellinida and Euglyphida) in Rabisha Reservoir (Northwestern Bulgaria), *Acta zoologica bulgarica*, 62 (3): 259-269.

[7] DEFLANDRE G., 1928 – Le genre *Arcella* Ehrenberg, *Arch. Protistenk*., 64: 152-287.

[8] DEFLANDRE G., 1929 - Le genre *Centropyxis* Stein, *Arch. Protistenk*., 67: 323-374.

[9] GAUTHIER-LIEVRE L., THOMAS R., 1958 – Les genres *Difflugia, Pentagonia, Maghrebia et Hoogenraadia* (Rhizopodes testaces) en Afrique, *Arch. Protistenk*.103: 241-370.

[10] DECLOITRE L., 1962 – Le genre *Euglypha* Dujardin, *Arch. Protistenk*., 106: 51-100.

[11] OGDEN C., HEDLEY R., 1980 – An Atlas of Freshwater Testate Amoebae, Bull. Br. Mus. (Natural History), Oxford University press, 222 pp.

[12] OGDEN C., 1983 – Observations on the systematics of the genus *Difflugia* in Britain (Rhizopoda, Protozoa), *Bull Br Mus (Natural History)*, Zoology series 44, 73 pp.

[13] OGDEN C., ŽIVCOVIC A., 1983 – Morphological studies on some Difflugiidae from Yugoslavia (Rhizopoda, Protozoa), *Bull. Br. Mus. Nat. Hist. (Zool.)*, 44 (6): 341-370.

[14] MORACZEWSKI J., 1967 – Formation des taxocenoses des Testacea dans le lac de Zegrze. – *Acta Protozoologica*., IV, **30:** 327-341.

[15] GOLEMANSKY V., 1967 – Materiaux sur la systematique et l`ecologie des thecamoebiens (Protozoa, Rhizopoda) du lac D`Ohrid, *Fac. Nat. Sci and Mathem*., Univ. in Scopje, Ann. XIV, **4,** 76: 3-21.

[16] BERECZKY M., 1973 – Beiträge zur Kenntnis der im Eprofundal des Balaton lebenden testaceen, *Annles Univ. Scient. bpest. Rolando Eötvös* (*Sect. Biol.*), **15:** 117-127.

[17] GROSPIETSCH T., 1975 – Beitrag zur Kenntnis der Testaceen-Fauna des Lago Valencia (Venezuela), *Verh. Internat. Verein. Limnol.*, **19:** 2778-2784.

[18] KULIKOVSKAYA I., 1983 – About fauna of the Testaceous Amoebae (Rhizopoda, Testacea) of the Glubokoye lake, in: Biocenoses of the mesotrophic lake Glubokoye. M., 1983, 149-181.

[19] TODOROV M., GOLEMANSKY V., 1998 – Testate amoebae (Protozoa: Rhizopoda) of the coastal lakes Shabla and Ezerets (Northeastern Bulgaria), a description of *Pentagonia shablensis* sp. nov., in: Biodiversity of Shabla Lake System. 'Prof. M. Drinov' Academic Publishing House, 69-90.

[20] TODOROV M., GOLEMANSKY V., 2000 – Testate Amoebae (Protozoa: Testacea) of the Glacial Lakes in the Rila National Park (Southwestern Bulgaria), in: Biodiversity and evolution of glacial water ecosystems in the Rila Mountains. (Eds.): Golemansky V., W. Naidenow, Instute of Zoology, 15-26.

[21] LORENCOVÁ M., 2009 – Thecamoebians from recent lake sediments from the Šumava Mts, Czech Republic. *Bulletin of Geosciences* 84(2), 359–376.

Table 2 – Comparison among testacean fauna of the Ovcharitsa Reservoir and other studied reservoirs in Bulgaria

Reservoirs	Number of species/ genera	Number of the common with Ovcha-ritsa Reservoir species/ genera	Similarity (k, %) on the species/ genera level	A ratio of the number of species of the genus *Difflugia* to the total number of species	A ratio of the number of species of the genus *Centro-pyxis* to the total number of species	A ratio of the number of species of the genus *Arcella* to the total number of species
Ticha	104 / 24*	55 / 15	44.4 / 55.6	55.8%	13.5%	2.0%
Batak	93 / 21**	42 / 11	33.3 / 39.3	50.5%	11.8%	6.5%
Beli Iskar	78 / 30***	22 / 14	16.8 / 41.2	7.7%	7.7%	3.8%
Rabisha	78 / 19****	52 / 14	51.5 / 60.8	41.0%	15.4%	3.8%
Ovcharitsa	75 / 18	-	-	45.3%	16.0%	2.7%

* According to Davidova at al. [4]; ** According to Todorov at al. [5]; *** According to Golemansky, Todorov [3]; **** According to Davidova [6];

STUDY OF THE IDENTIFICATION AND BIOCHEMICAL CHANGES PRODUCED BY *APRICOT CHLOROTIC LEAFROLL PHYTOPLASMA* (ACLR)

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Abstract: The ecological conditions in the South-Eastern part of Romania are very favorable for the apricot culture (*Prunus armeniaca*). Unfortunately, the same conditions are favorable also for some pathogens (viruses, phytoplasma, bacteria or fungi), the causal agents for many damaging diseases. The final syndrome of this diseases complex is known as apoplexy or premature dye back of the apricots. Recognition of this diseases complex is very important for the study and cure of the phenomena. If for the majority of the apricot diseases the symptoms are easy to be evidenced, the ones produced by *Apricot chlorotic leafroll phytoplasma*, a key damaging agent in premature dye back of the apricots, are more difficult to be revealed.
This paper present the researches carried out in order to realize the symptomatic diagnosis of these phytoplasma attacks and the quantification of the biochemical changes in the leaves of some apricot cultivars with different degrees of sensibility infected with A.C.L.R., compared with the leaf biochemical pattern of the same but healthy cultivars. The research was carried out at the Research Station for Fruit Growing Constanta, Romania, in our national apricot genetic resource collection with 550 cultivars. The biochemical changes referred to the leaf cell plasma content in: polyphenol-oxidase, total phenols and tannins, free amino-acids, soluble dry mater, water, carbohydrates and assimilation pigments.

Keywords: polyphenol-oxidase, phenols, tannins, free amino-acids, photoasimilate pigments.

1. Introduction

Time every year, in the South-Eastern part of Romania, an important number of apricots dies from the causes unknown to the growers. Researches caused in many European countries mention that on this fruit species, 80% of the dead trees or the suffering ones, are attacked by the A.C.L.R (Cornaggia, et al., 1994; Jarausch et al., 1998; Labonne et al., 2000).

A.C.L.R. is a quarantine disease (OEPP/EPPO, 1986) belonging to the mycoplasama group (Morvan et al., 1973). The disease was also evidenced on many fruit species, although some of them (plum) are not showing symptoms (Németh, 1986).

The researches carried out during 2006-2010 were focused mainly on revealing of the disease symptoms under the concrete eco-pedo-climatic conditions from Research Station for Fruit Growing Constanţa, for early and right diagnosis of this malady. More over, during the study period were assessed the biochemical changes occurred in the cellular juice of the leaves sampled from the diseased trees compared to the biochemical changes occurred in the cellular juice of the leaves sampled from healthy trees.

2. Materials and methods

In order to describe the symptoms produced by natural infections with A.C.L.R, periodical observation were done during vegetation and tree dormancy period. Trees with symptoms were marked and details of external and internal macroscopic display of pathogen and their evolution in time were observed for an easier recognition of the attack in the commercial apricot orchards.

For the quantification of the biochemical changes in the leaves of the apricot cultivars the biological material used in this study was represented by some apricot cultivars extended in production

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(Goldrich, Harcot, Dacia, Earliril, Royale and Olimp) integrated in four different classes of resistance (function of the F% and I value faced to the attack of the A.C.L.R.) trought natural infection. Biochemical analyses were performed of the cellular juice from leaves, in the course of two vegetation periods: in fully activity (June) at the end of the period (October). The determinations were mode using the known methodologies and they refer to the content in:

- Plyphenol-oxidase - titrimetical method
- Total phenols, spectrophotometrical method
- Tannis, Lowenthol, Neubeur method
- Free amino-acids, spectrophotometrical method
- Dry matter, gravimetrical method
- Assimilate pigments, Hagarsi Bertensath method
- Carbohydrates-titrimetrie by Bertland, Jijin method
- Crude protein , spectrophotometrical method

3. Results and discussion

In the spring, the A.C.L.R. symptoms are mainly the early leafing, preceding or at the same time with the blooming (Fig.1), the phenomena being spotted firs on one or two branches.

When the summer starts, the leaves from the attacked branches are smaller in size with green-yellow color(Fig. 2). The typical symptoms occurs at the end of the summer, when the apricots leaves rolls along the main stalk and vein, taking a cone (or spoon) shape, and falls prematurely (Fig. 3). The fruits, numberless, are smaller, slight asymmetric, and rips later or they remain green and fall to the ground prematurely (Fig. 4). The apricot branches growth slows down, the internodes are shorter and after a variable tie period the internodes are drying (Fig. 5). A section trough these branches evidence the phloem brunification and necrosis (Fig. 6), the structure where the pathogen develops (Morvan, 1973). In the orchard, the disease occurs on isolated apricots, or affect the trees in groups. As regard the transmission, the researches did not identified *Cacopsylla pruni* species mentioned as vector (Carrol, et al., 1998), but revealed the disease transmission with the shoots used for grafting or budding and detached from diseased apricot trees.

Fig. 1 – ACLR Symptoms in spring

Fig. 2 – ACLR Symptoms at start of the summer

Fig. 3 – ACLR Symptoms at the end of the summer

Fig. 4 – ACLR Symptoms on fruits

Fig. 5 – ACLR Symptoms on the branches

Fig. 6 – Oxidation and necrosis in phloem

As regard the biochemical changes the content in proteins ferments-polyphenol oxidase was found in higher quantities at the cultivars showing a stronger resistance to the attack of A.C.L.R., but in smaller quantities in the susceptible ones (Fig.7). This can be explained by the fact that the polyphenol-oxidase plays a role in the catalise synthesis of the phytoalexins and other components as indicators of the infection process.

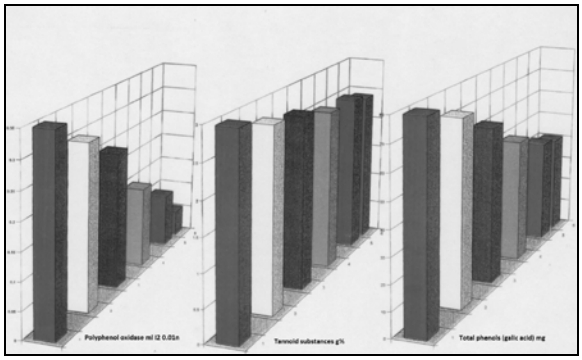

Fig.7 – The content of poliphenols, total phenols and tannoid substances of some apricot cultivars

The content in tannoid substances, playing a role in the inhibition of the infection process is higher of the resistance cultivar. The phenols substances are the first ones which are opposing to reproduction of the pathogen in the plant organism. Through their oxidation the chinons obtained, are very toxic substances also for the pathogenic agents.

Regarding the free amino-acids content, our results have evinced that the more susceptible varieties have a greater content (Fig. 8). This fact may be explained both by the reduction in the protein synthesis as by the increase in the protein decomposition due to the disturbance induced by the pathogenic agent.

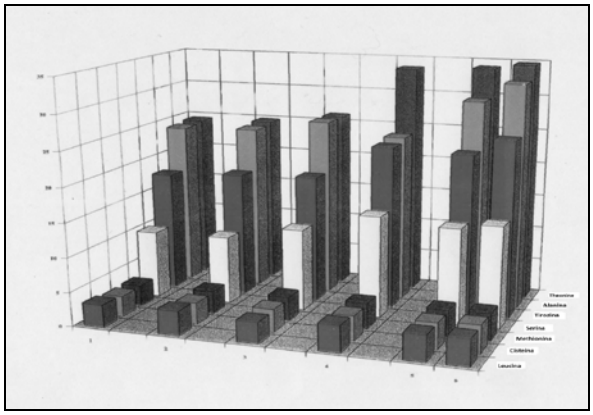

Fig. 8 – The content in free amino-acids (mg alanina)

As for the dry substance content the higher resistant cultivars have presented an increased content in terms of mean annual value (Fig. 9).

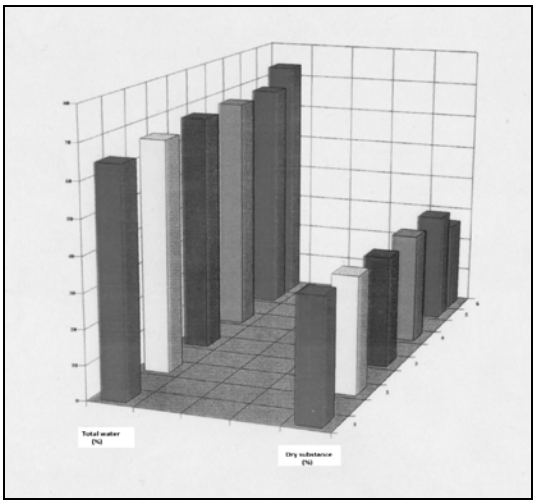

Fig. 9 – The content in dry substance and total water of some apricot cultivars

There is a direct correlation between the water content of the leaves and the different behaviour of the apricoth cultivars under the A.C.L.R. attack.

The quantity of **a; b; c** forms of assimilation pigments, especially of the **(a+b)/c** ratio are an obvious indicator viewing the appearance of some factors which disturb the normal methabolic processes. Thus, the **(a+b)/c** ratio was much smaller in the susceptible cultivars than in the resistant cultivars (Fig. 10).

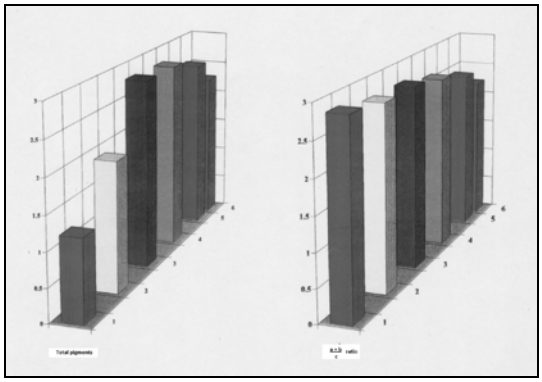

Fig. 10 – The concentration in assimilation pigments (mg / g wet substance)

The percentage variation of the glucids the resistant cultivars presented an elevated quantity of direct reducting and total forms (Fig. 11). The mean value of crude protein (%) is decreasing once with the increasing of the sensitivity to the attack by A.C.L.R. This could be explained both through the reduction in the capacity of the proteins synthesis also through the intensification of the proteins decomposition in the case of some cultivars whith a higher susceptibility to the attack.

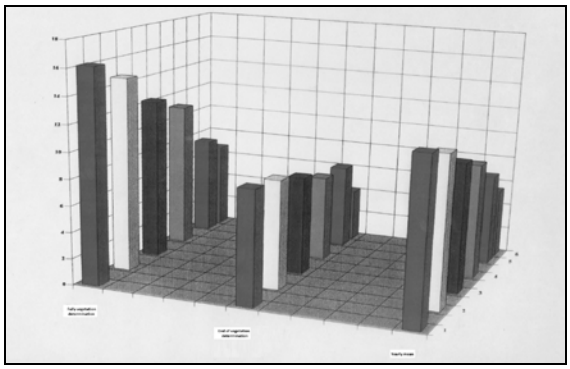

Fig. 11 – Percentage variation of crude proteine (%)

4. References

[1] CARRARO L, OSLER R, LOI N, ERMACORA P, REFATTI E, 1998 – Transmission of European stone fruit yellows phytoplasma by Cacopsylla pruni. *Journal of Plant Pathology*, 80: 233-242.

[2] CORNAGGIA D, AUDUBERT A, DESVIGNES JC, 1994 – Enroulement chlorotique de l'abricotier en Roussillon. *Phytoma* 460: 20-23.

[3] JARAUSCH W, DANET JL, BROQUAIRE JM, SAILLARD C, GARNIER M, DOSBA F, 1999 – Apricot chlorotic leaf roll in Roussillon: specific detection of the pathogen, search for alternative host plants, attempts to determine infection periods and attempts to identify the insect vector. *Acta: Horticulturae*, 488: 739-783.

[4] LABONNE G, BROQUAIRE JM, JARAUSCH W, FREYDIER M, QUIOT J-B, 2000 –Base d'une strateagie de lutte contre l'enroulement chlorotique de l'abricotier en vergers d'abricotier. *Phytoma*, 530: 32-37.

[5] MORVAN, G., 1977 – Apricot chlorotic leafroll. *Bulletin OEPP/EPPO Bulletin* 7 : 37-55.

[6] NÉMETH, M., 1986 – Apricot chlorotic leaf roll. In: *Virus, mycoplasma and rickettsia diseases of fruit trees*. Akadémiai Kiado, Budapest, Hungary.

[7] OEPP/EPPO, 1986 – Data sheets on quarantine organisms No. 146, Apricot chlorotic leafroll MLO. *Bulletin OEPP/EPPO Bulletin* 16: 43-45.

DENDROFLORA OF THE STUDENTS' PALACE PARK (CONSTANTA CITY)

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Abstract: In the paper an inventory of the woody species of the Students' Palace Park of Constanta (Soveja area), one of the largest green area of Constanta city is presented. Some considerations regarding biological forms and phytogeographic origin of the trees, shrubs and lianas of the Student Park are also given in the paper. The field studies achieved during the years 2008 and 2009 led to the identification of 71 plant taxa, of which 37 trees species, 30 shrubs and 4 lianas. The inventoried woody plant taxa belong to 30 families and 22 orders. In terms of biological forms, the woody taxa of Student Park belong to phanerophytes and epiphytes. Analyses of phytogeographic origin of studied woody species emphasize the prevalence of North American, Asian and European taxa.

Keywords: Students' Palace Park, Constanta city, woody taxa, trees, shrubs, lianas.

1. Introduction

The Students' Palace Park, one of the largest green area of Constanta city is located near Tăbăcăriei Lake, in the northern part of Constanta city between Soveja Blvd. and Primăverii Street. The total surface of the Park is approximately 10 ha.

The great variety of trees and shrubs delight the city inhabitants over entire seasons of the year. Unfortunately, many interesting woody species of the park are less known or unknown by the citizens of the city.

The paper includes both an inventory of trees, shrubs and lianas of Student Park and taxonomic and phytogeographic considerations about the identified taxa.

2. Material and methods

The woody species of the Students' Palace Park were identified during the spring and summer of the years 2008 and 2009. The trees and shrubs have been assigned at three sub-categories of phanerophytes depending on their height: megaphanerophytes (MPh) - trees over 15 m height, mesophanerophytes (mPh) - trees or shrubs with a height between 2 and 15 m, nanophanerophytes (nPh) - shrubs up to 2 m height). The trees and shrubs taxa belong to the category of phanerophytes (Ph) and the lianas to epiphytes (Ep). For each taxa of floristic inventory has been mentioned the biological forms and phytogeographic origin.

The nomenclature of taxa is according to Ciocârlan, 2009 [1], Săvulescu et al., 1952-1976 [2], Tutin et al., 1964-1980 [3] and Zanoschi et al., 1996-2004 [4-7]. The botanical families, life forms and phytogeographic origin of woody taxa are generally given according to Ciocârlan, 2009 [1].

3. Results and Discussions

The field researches carried out in the area of the Students' Palace Park led to identification of 71 taxa among which 37 tree species, 30 shrubs and 4 lianas.

The inventoried taxa within area of the Student Park are the following (between brackets are given the biological forms and phytogeographical origin of taxa): *Abies concolor* (Gord. et Glend.) Lindley ex Hildebr (MPh, North America), *Acer campestre* L. (mPh, Europe), *Acer negundo* L. (MPh, North America), *Acer platanoides* L. (MPh, Europe), *Acer pseudoplatanus* L. (MPh, Central-Europe), *Aesculus hippocastanum* L. (Mph, Balkan), *Ailanthus altissima* (Miller) Swingle (MPh, China), *Albizia*

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julibrissin Durazz. (mPh, SV Asia), *Amorpha fruticosa* L. (mPh, North America), *Berberis thunbergii* DC. - dracila japoneza (nPh, Japan), *Betula pendula* Roth. (MPh, Eurasia), *Buxus sempervirens* L. (mPh, Mediteranean), *Campsis radicans* (L.) Seemann (syn. *Tecoma radicans*) (Ep, North America), *Catalpa bignonioides* Walter (MPh, North America), *Celtis australis* L. (MPh, Mediteranean), *Cerasus avium* (L.) Moench (MPh, Submediteranean), *Cerasus serrulata* (Lindley) G.Don cv. *kiku-zakura* (mPh, SE As.), *Chaenomeles japonica* (Thunb.) Spach. - gutui japonez (nPh, Japan), *Chamaecyparis lawsoniana* (A.Murray) Parl. (MPh, North America), *Cotoneaster horizontalis* Decne. (nPh, China), *Cotoneaster simonsii* Baker (nPh, E.India), *Cupressus sempervirens* L. (MPh, East Mediteranean), *Eleagnus angustifolia* L. (mPh, Asia), *Euonymus japonicus* Thunb. (nPh, East Asia), *Forsythia suspensa* (Thunb.) Vahl (nPh, China), *Fraxinus excelsior* L. (MPh, Europe), *Fraxinus ornus* L. (mPh, Submediteranean), *Hedera helix* L. (Ep, Atlantic-Mediteranean), *Hibiscus syriacus* L. (nPh, Asia), *Juniperus chinensis* L. var. *columnaris* (mPh, SE.Asia), *Juniperus horizontalis* Moench (nPh, V.Asia), *Juniperus sabina* L. (nPh, V.Asia), *Koelreuteria paniculata* Laxm. (mPh, China), *Laburnum anagyroides* Medik. (mPh, Central-Europe), *Ligustrum vulgare* L. (nPh, Eur.), *Liriodendron tulipifera* L. (MPh, N.Am.), *Lonicera japonica* Thunb. (Ep, East Asia), *Lonicera tatarica* L. (mPh, West Asia), *Mahonia aquifolium* (Pursh) Nutt. (nPh, N. America), *Morus alba* L. var. *pendula* (nPh, China), *Morus nigra* L. (mPh, Mediteranean), *Partenocissus quinquefolia* (L.) Planchon (Ph (liana), N.Am.), *Paulownia tomentosa* (Thunb.) Steud. (mPh, Japan), *Philadelphus coronarius* L. - iasomie (mPh, Caucaz), *Philadelphus inodorus* L. (mPh, N.Am.), *Picea pungens* Engelm. cv. *argentea* (MPh, North America), *Pinus nigra* Arnold (MPh, South-East Europe), *Platanus hispanica* Miller ex Muench (syn. *P. acerifolia*) (MPh, North America), *Populus alba* L. (MPh, Eurasia), *Populus x canadensis* Moench. s.l. (*P. deltoides* x *P. nigra*) (mPh, in cultivation), *Populus nigra* L. cv. *italica* (MPh, Eurasia), *Populus nigra* L. (MPh, Eurasia), *Populus simonii* Carriere (mPh, China), *Prunus cerasifera* Ehrh. var. *pissardi* (Carriere) C.K.Schneid. (mPh, Pontic-Balkan), *Quercus robur* L. (MPh, Eur), *Ribes aureum* Pursh – cuisor (nPh, N.Am.), *Robinia pseudacacia* L. (MPh, North America), *Rosa multiflora* Thunb. - trandafir urcător (nPh, Cult.), *Salix fragilis* L. (mPh, Eurasia), *Sambucus nigra* L. (mPh, Europe), *Sophora japonica* L. (MPh, East Asia), *Spiraea chamaedrifolia* L. (syn. *S. ulmifolia* Scop.) (nPh, Eua), *Spiraea x vanhouttei* (Briot) Zabel (nPh, China), *Symphoricarpos orbiculatus* Suksd. (nPh, North America), *Syringa vulgaris* L. (mPh, Carpathic-Balkan-Anatolic), *Tamarix tetrandra* Pallas ex Bieb. (mPh, South-West Asia), *Taxus baccata* L. (mPh, Atlantic-Mediteranean), *Thuja orientalis* L. (mPh, China), *Thuja occidentalis* L. (mPh, North America), *Tilia platyphyllos* Scop. (MPh, Central-Europe), *Tilia tomentosa* Moench (MPh, Balkan-Panonic), *Ulmus procera* Salisb. (MPh, Europe).

Among the inventoried taxa 36,61% are megaphanerophytes (MPh), 29,57% mesophanerophytes (mPh) and 28,16% nanophanerophytes (nPh). The percentage of lianas (Ep) is 5,63 % (Fig. 1).

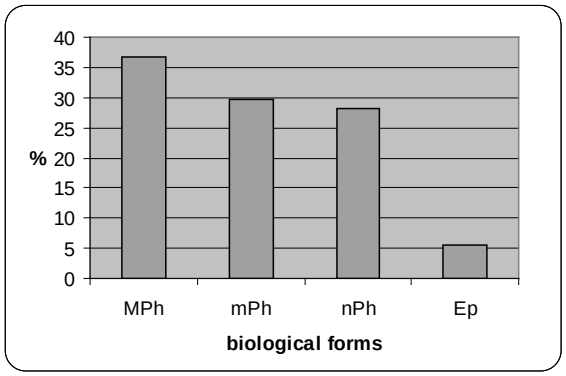

Fig. 1 – The biological forms spectrum of Students'Palace Park flora

Regarding phytogeographical origin of taxa, the majority of species have Asian origin (inclusive China and Japan) (33,08%), North American (19,71%), European (14.94%), Mediteranean and Sub-Mediteranean (8,45%), Eurasiatic origin (7,04%) or are obtained in cultivation (7,04%). Other phytogeographical categories (Atlantic-Mediteranean, Balkan, Pontic-Balkan, Caucasian, Carpathian-Balcan-Anatolic) have a lower rates and totalize 9,85% (Fig. 2).

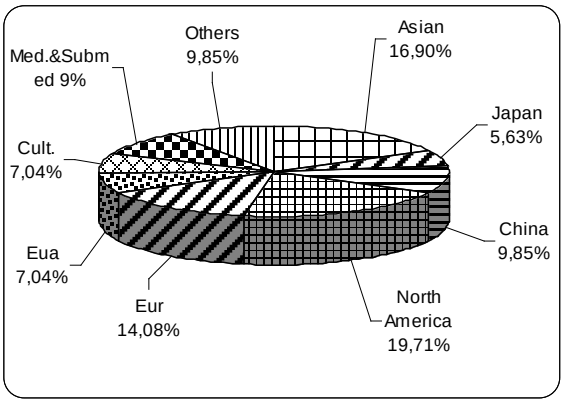

Fig. 2 – Spectrum of phytogeographical elements

The inventoried taxa belong to 30 families, 22 orders, 5 subclases and 1 class (*Magnoliopsida*).

The best represented botanical families as number of species are the following: *Rosaceae* (10 taxa), *Cupressaceae* (8 taxa), *Salicaceae* (5 taxa), *Oleaceae* (5 taxa), *Caprifoliaceae* (4 taxa), *Fabaceae* (4 taxa), *Aceraceae* (4 taxa), *Caprifoliaceae* (4 taxa), *Pinaceae* (3 taxa) (Tab. 1).

The following botanical orders have a high number of woody taxa: *Pinales* (12 taxa), *Rosales* (12 taxa), *Sapindales* (6 taxa), *Fabales* (5 taxa), *Oleales* (5 taxa), *Urticales* (4 taxa), *Dipsacales* (4 taxa), *Scrophulariales* (3 taxa) (Tab. 1).

Tab. 1 – Taxonomical analysis of identified taxa

Orders	**Families**	**Number of taxa**
Pinales	*Pinaceae*	3
	Cupressaceae	8
	Taxaceae	1
Magnoliales	*Magnoliaceae*	1
Saxifragales	*Hydrangeaceae*	2
Rosales	*Rosaceae*	10
	Hydrangeaceae	2
Salicales	*Salicaceae*	5
Fabales	*Fabaceae*	4
	Mimosaceae	1
Hammamelidales	*Platanaceae*	1
Urticales	*Moraceae*	2
	Ulmaceae	2
Sapindales	*Aceracea*	4
	Hippocastanaceae	1
	Sapindaceae	1
Dipsacales	*Caprifoliaceae*	4
Fagales	*Betulaceae*	1
	Fagaceae	1
Oleales	*Oleaceae*	5
Malvales	*Tiliaceae*	1
	Malvaceae	1
Rutales	*Simaroubaceae*	1
Rhamnales	*Vitaceae*	1
Berberidales	*Berberidaceae*	2
Eleagnales	*Eleagnaceae*	1
Scrophulariales	*Bignoniaceae*	2
	Scrophulariaceae	1
Tricoccales	*Buxaceae*	1
Celastrales	*Celastraceae*	1
Tamaricales	*Tamaricaceae*	1
Umbelliflorales	*Araliaceae*	1

Some trees and shrubs of the Park are remarkable as plants, very nice especially in the flowering period:

- *Liriodendron tulipifera* (tulip tree) – with yellow (rarely white) big flowers and an orange band on the tepals like a tulipe;
- *Cerasus serrulata* cv. *kiku-zakura* (Japanese flowering cherry) – the flowers are produced in pink racemose clusters at the same time with the new leaves;
- *Chaenomeles japonica* (Maule's Quince or Japanese Quince) – with the big red lowers; the species flowering in late winter or early spring;
- *Albizia julibrissin* (Persian silk tree) – with bipinnate leaves divided into 6–12 pairs of pinnae, each with 20–30 pairs of leaflets and flowers produced in the summer in dense pink or white inflorescences;
- *Paulownia tomentosa* (Empress Tree, Princess Tree or Foxglove Tree) – with violet flowers produced before the leaves in early spring, grouped in 10-30 cm long panicles;
- *Catalpa bignonioides* – with large cordiforme lives and big white flowers with yellow spots inside grouped in panicles of 20-40;
- *Berberis thunbergii* (Japanese barberry or Thunberg's Barberry) – dense and spiny shrub with pale yellow flowers; flowering is from mid spring to early summer; the fruits is a glossy bright red to orange-red;

- *Taxus baccata* (common yew, or European yew) – a conifer native in Europe, protected by law in Romania

4. Conclusion

As a result of our researches carried out in the Students' Palace Park of Constanta, 71 taxa were identified among which 37 trees, 30 shrubs and 4 lianas

The inventoried woody taxa belong to 30 botanical families, 22 orders, 5 subclases and 1 class.

The best represented botanical families are the following: *Rosaceae, Cupressaceae, Salicaceae, Oleaceae, Pinaceae, Caprifoliaceae, Fabaceae, Aceraceae, Caprifoliaceae, Pinaceae*.

Amog different sub-categories of phanerophytes, the megaphanerophytes (high trees) are most well represented biological forms.

The Asian, North American and European origin taxa are prevalent in the area of the Students' Palace Park.

Some trees and shrubs within the Park are remarkable as decorative species: *Liriodendron tulipifera, Cerasus serrulata* cv. *kiku-zakura, Chaenomeles japonica, Albizia julibrissin, Paulownia tomentosa, Catalpa bignonioides, Berberis thunbergii.*

5. References

[1] CIOCÂRLAN V., 2009 – Flora ilustrată a României (Pteridophyta et Spermatophyta), Edit. Ceres, Bucureşti, 1141 pp.

[2] SĂVULESCU, TR. (eds), 1952-1976, Flora R.P.R – R.S.R., I – XIII, Edit. Acad. Române, Bucureşti.

[3] TUTIN T.G. HEYWOOD V.H., BURGES N.A., MOORE D.M., VALENTINE D.H., WALTERS S.M. & WEBB D.A. (eds), 1964-1980. Flora Europaea, Vols. 1-5, Cambridge, Cambridge University Press.

[4] ZANOSCHI V., SÂRBU I., TONIUC ANGELA, 1996 – Flora lemnoasă spontană şi cultivată din România, Vol. I, Edit. "Glasul Bucovinei", Iaşi, 309 pp.

[5] ZANOSCHI V., SÂRBU I., TONIUC ANGELA, 2000 – Flora lemnoasă spontană şi cultivată din România, Vol.II, Edit. Univ. "Al. I. Cuza", Iaşi, 458 pp.

[6] ZANOSCHI V., SÂRBU I., TONIUC ANGELA, 2004 – Flora lemnoasă spontană şi cultivată din România, Vol. III, Edit. Univ. "Al. I. Cuza", Iaşi, 305 pp.

[7] Zanoschi V., Sârbu I., Toniuc Angela, 2004 – Flora lemnoasă spontană şi cultivată din România, Vol. IV, Edit. Univ. "Al. I. Cuza", Iaşi, 340 pp.

Fig. 2 – *Liriodendron tulipifera*

Fig. 3 – *Cerasus serrulata cv. kiku-zakur*

Fig. 4 – *Chaenomeles japonica*

MORPHOLOGICAL OBSERVATIONS ON *ACER CAMPESTRE* F. *LOBATUM* PAX LEAVES

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Abstract: The large polymorphism of the leaves, determined us to introduce the morphometrical method analyse them not only by morphological but also by the morphometrical criterions, a less tackled subject in the morphological studies. The morphometrical measurements were performed for *Acer campestre* L. f. *lobatum* (a spontaneous species) using the literature cited. Some morphometrical observations indicate that the linear measurements of *Acer platanoides* leaves possess medium values concerning the length leaf (L), the width leaf (l), the apex length (A), the petiole length (Lp) and the lobes incision deepness (Ls).
The percentage report indicates that the apex leaves (A/I-I') is acute (delicate). The angular measurements of this species blade, exhibit that the emergent angle of the primary lateral veins (ω) are large. No emergent angle of the tertiary veins (γ) is present. The blade of *Acer campestre* f. *lobatum* possesses a large number of secondary pairs of veins (Np) and has a large surface (S). Biometrical measurements may represent distinctive features for the morphological analyse of *Acer campestre* L. species but also for any plants genus and species as well.

***Keywords*:** actinodromous, angle, morphomety, blade, pinati-craspedodromous, petiole, sinus, venation, *Acer campestre* f. *lobatum*

1. Introduction

The leaf, as a vegetative organ of a plant, may be defined not only by morphological and anatomical features but also by morphometrical criterions, which is not an easy task allowing for their polymorphism, that is why we introduced this method also for the spontaneous plants leaves [1]. This methodology has been used on *Acer campestre* L. f. *lobatum* Pax. leaves (a spontaneous species). The hedge maple is a small deciduous tree (15 m height) or large shrub with a round, dense crown, often branched to the ground. It grows frequently in mixture woods (associated with ash, hazel and oak), boscage and forest margins hedge or street tree usage. The annual stems are light brown, with lenticels and fissures, early pubescent and soon become glabrous [2, 3].

The opposite simple, palmate leaves, usually, have 3 to 5 lobs with rounded edges. The hermaphrodite flowers appear, with leaves, late in April to Mid-May. They are small-green, widely spaced in erect corymbs. The fruits are double samaras

We consider that this methodology, used for the leaves characterization - the biometrical method – will completes the studies of this taxon.

2. Material and Methods

The morphological and morphometrical observations and measurements were performed on 40 mature leaves collected from Fantanita near Constantza City The calculation of the morphometrical measurements follow the calculations of Mouton (1966/76), Roth and Dilcher (1978), Givulescu (1999), Givulescu and Soltesz, (2000): L- the length leaf, l- the width leaf, h- the height of the maximum width; A- the apex length, I-I'- the apex width; La- the lobe length, Ls- the incision deepness; Lp- length of petiole; L/l- the length-width ratio; A/L- the acuminate ratio, h/L- the oval ratio; A/I-I'- the apex finesse, s- the relative

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sinus deepness, α- the apical angle, β- the emergent angle of the secondary veins with primaries, γ- emergent angle of the tertiary veins, ω- the emergent angles between the primary lateral veins and other measurements such as: S- surface (cm^2), Np- the semi sum of secondary pairs of veins and T- teeth/cm.

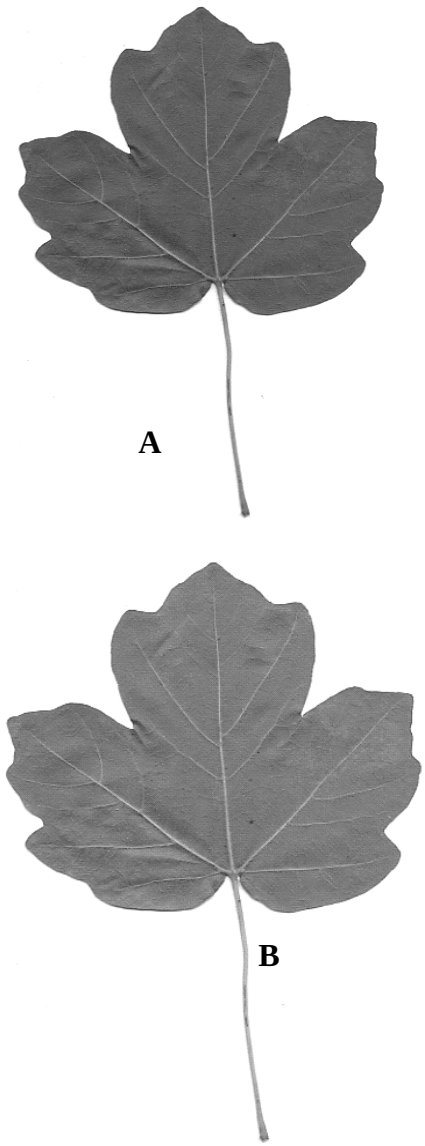

Fig. 1 – Fig. 1. *Acer campestre* L. f. *lobatum* Pax. leaf. The upper (A) and lower (B) surface of the blade.

3. Results and Discussion

The results of the morphometrical measurements of *Acer campestre* L. f. *lobatum* leaves indicate that the leaves possess dark green color above, paler below, palmately 5-lobed and deeply cordate base. *A. platanoides* blades lobes are equal in shape and size, Membranous texture. The blade venation is actinodromous perfect basal with 5 evident primary veins. All leaves are included in mesophyll size class (Fig. 1).

The linear measurements indicate that *Acer platanoides* leaves possess medium values concerning the length leaf (L), the width leaf (l), the apex length (A), lobes incision deepness (Ls) and the petiole length (Lp).

The percentage report indicates that the apex leaves (A/I-I') is acute (delicate). Concerning the leaves angular measurements, the emergent angle in between the primary and secondary veins are acute. The emergent angle of the primary lateral veins (ω) are large such us the angle between the primary lateral veins. The emergent angles of the tertiary veins (γ) are absent. *A. campestre* L. f. *lobatum* possesses a great number of secondary pairs of veins (Np).

A. platanoides blades have a medium surface (S).

The morphological characterization of the leaves [9, 10], attended by morphometrical measurements (mathematical calculations) on spontaneous plants may be useful for taxonomical identification. Nevertheless, the plants species, varieties and forms may be distinguishable by morphometrical criteria.

The biometrical measurements performed on the 40 leaves represented the mathematical calculation base of *Acer campestris* f. *lobatum* average values.

Mathematical calculation base of *Acer campestris* f. *lobatum* average values measurements:
(*n = 40*)

$$\overline{L}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{L_{ACL}}{n} = \frac{L_1 + ... + L_n}{n} = \frac{70 + ... + 50}{40} = 69mm$$

$$\overline{l}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{l_{ACL}}{n} = \frac{l_1 + ... + l_n}{n} = \frac{83 + ... + 75}{40} = 86mm$$

$$\overline{h}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{h_{ACL}}{n} = \frac{h_1 + ... + h_n}{n} = \frac{27 + ... + 25}{40} = 26mm$$

$$\overline{A}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{A_{ACL}}{n} = \frac{A_1 + ... + A_n}{n} = \frac{6 + ... + 5}{40} = 4mm$$

$$\overline{I - I'}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{(I - I')_{ACL}}{n} = \frac{(I - I')_1 + ... + (I - I')_n}{n} = \frac{15 + ... + 8}{40} = 9mm$$

$$\overline{La}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{La_{ACL}}{n} = \frac{La_1 + ... + La_n}{n} = \frac{33 + ... + 30}{40} = 35mm$$

$$\overline{Ls}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{Ls_{ACL}}{n} = \frac{Ls_1 + ... + Ls_n}{n} = \frac{18 + ... + 18}{40} = 19mm$$

$$\overline{Lp}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{Ls_{ACL}}{n} = \frac{Lp_1 + ... + Lp_n}{n} = \frac{65 + ... + 50}{40} = 66mm$$

$$\overline{\frac{L}{l}}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{\left(\frac{L}{l}\right)_{ACL}}{n} = \frac{\left(\frac{L}{l}\right)_1 + ... + \left(\frac{L}{l}\right)_n}{n} = \frac{84,3 + ... + 78,6}{40} = 80\%$$

$$\overline{\frac{A}{L}}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{\left(\frac{A}{L}\right)_{ACL}}{n} = \frac{\left(\frac{A}{L}\right)_1 + ... + \left(\frac{A}{L}\right)_n}{n} = \frac{8,5 + ... + 8,4}{40} = 5,7\%$$

$$\overline{\frac{h}{L}}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{\left(\frac{h}{L}\right)_{ACL}}{n} = \frac{\left(\frac{h}{L}\right)_1 + \ldots\ldots\ldots + \left(\frac{h}{L}\right)_n}{n} = \frac{38{,}5 + \ldots\ldots\ldots + 42{,}3}{40} = 37{,}6\%$$

$$\overline{\frac{A}{I-I'}}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{\left(\frac{A}{I-I'}\right)_{ACL}}{n} = \frac{\left(\frac{A}{I-I'}\right)_1 + \ldots\ldots\ldots + \left(\frac{A}{I-I'}\right)_n}{n} = \frac{40 + \ldots\ldots\ldots + 62{,}5}{40} = 44{,}4\%$$

$$\overline{s}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{s_{ACL}}{n} = \frac{s_1 + \ldots\ldots\ldots + s_n}{n} = \frac{45 + \ldots\ldots\ldots + 40}{40} = 5{,}7\%$$

$$\overline{\alpha}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{\alpha_{ACL}}{n} = \frac{\alpha_1 + \ldots\ldots\ldots + \alpha_n}{n} = \frac{83 + \ldots\ldots\ldots + 82}{40} = 80^{\circ}$$

$$\overline{\beta}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{\beta_{ACL}}{n} = \frac{\beta_1 + \ldots\ldots\ldots + \beta_n}{n} = \frac{42 + \ldots\ldots\ldots + 39}{40} = 38^{\circ}$$

$$\overline{\omega}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{\omega_{ACL}}{n} = \frac{\omega_1 + \ldots\ldots\ldots + \omega_n}{n} = \frac{174 + \ldots\ldots\ldots + 180}{40} = 161^{\circ}$$

$$\overline{Np}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{Np_{ACL}}{n} = \frac{Np_1 + \ldots\ldots\ldots + Np_n}{n} = \frac{11 + \ldots\ldots\ldots + 29}{40} = 32\ p/2 \text{ of secondary veins}$$

$$\overline{S}_{\text{ACLi}} = \sum_{i=1}^{n} \frac{S_{ACL}}{n} = \frac{S_1 + \ldots\ldots\ldots + S_n}{n} = \frac{43{,}16 + \ldots\ldots\ldots + 30{,}71}{40} = 44{,}74 cm^2$$

Size class = MESOFIL

3. Conclusion

The results of the morphometrical measurements of *Acer campestre* L. f. *lobatum* leaves indicate that the leaves possess dark green color above, paler below, palmately 5-lobed and deeply cordate base. *A. platanoides* blades lobes are equal in shape and size, Membranous texture. The blade venation is actinodromous perfect basal with 5 evident primary veins. All leaves are included in mesophyll size class.

The linear measurements indicate that *Acer platanoides* leaves possess medium values concerning the length leaf (L), the width leaf (l), the apex length (A), lobes incision deepness (Ls) and the petiole length (Lp).

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are large such us the angle between the primary lateral veins.

The emergent angles of the tertiary veins (γ) are absent. *Acer campestre* L. f. *lobatum* possesses a great number of secondary pairs of veins (Np) and a medium surface (S).

The morphological characterization of the leaves, attended by morphometrical measurements (mathematical calculations) on spontaneous plants may be useful for taxonomical identification. Nevertheless, the plants species, varieties and forms may be distinguishable by morphometrical criteria.

4. References

[1] BERCU RODICA, 2005 – Morphometrical and anatomical observations of some *Acer* L. species leaves, Edit. Ex Ponto, Constanta, Belgrade, 275 pp.

[2] SAVULESCU, TR. (eds), 1958 – Flora R.P.R, vol. VII, Edit. Acad. R.S.R, Bucureşti.

[3] CIOCARLAN V., 2000 – Flora ilustrată a României (Pteridophyta et Spermatophyta), Edit. Ceres, Bucureşti, 1139 pp.

[4] MOUNTON J.A., 1966 – Les types biologiques foliaires de Raunkiaer. Etat actuel de la question, *Bull. Soc. Bot*. France, 125: 145-158.

[5] MOUNTON, J.A., 1976 – La biométrie du limbe mise au point de nos connaissances, *Bull. Soc. Bot.* France, 113: 28-36.

[6] ROTH L.L., DILCHER D.L. 1978 – Some considerations in leaf size and leaf margin analysis in fossil leaves, Frankfurt am Mein, *Cour. Forsh. Inst*. Senckenberg, 30: 265-171.

[7] GIVULESCU R., 1999 – Flora mică ilustartă a terţiarului din România, Edit. Casa Cărţii de Ştiinţă. Cluj-Napoca, 171 pp.

[8] GIVULESCU R., SOLTESZ A., 2000 – Observaţii de ordin biometric şi anatomic asupra frunzelor unor specii de Tilia, *Nymphaea*, *Folia nature Bihariae*, Oradea, XVIII: 83-89.

[9] ANDREI M., 1997 – Morfologia generală a plantelor, Edit Enciclopedică, Bucureşti, 400 pp.

[10] BUIA AL., PÉTERFI, ŞT., 1965 – Botanica agricolă. Morfologia, Vol I, Edit. Agro-Silvică, Bucureşti, 339 pp.

Table no. 1 **Biometrical observations on *Acer campestre* L. f *lobatum* Pax leaves**

number	L mm	l mm	h mm	A mm	I-I' mm	La mm	Ls mm	Lp mm	L/l %	A/L %	h/L %	A/I-I' %	s %	α^0	β^0	γ^0	ω^0	Vp	T/ cm	S cm^2	Size class
1	70	83	27	6	15	33	18	65	84,3	8,5	38,5	40	45	83	42	-	174	11,4	-	43,16	Mesofil
2	67	80	35	3	8	31	18	70	84	4	52	37,5	41,9	93	42	-	181	19		43,51	Mesofil
3	73	91	41	5	16	33	20	80	80	6,8	56	31	39	106	47	-	179	31	-	52,93	Mesofil
4	75	98	27	3	6	35	8	93	76,5	4	36	50	77	89	41	-	177	25	-	51,95	Mesofil
5	60	68	35	5	10	32	17	74	88	8,3	58	50	46	91	35	-	86	15	-	29,45	Notofil
6	80	97	35	3	5	45	20	90	82	375	43,7	60	55	65	37	-	160	39	-	63,37	Mesofil
7	68	92	30	3	12	37	22	55	73,9	4,4	44	25	40	109	35	-	205	31	-	43,42	Mesofil
8	81	104	35	3	5	45	20	97	77,8	3,7	43	60	55	60	30	-	145	42	-	66,83	Mesofil
9	80	97	31	6	11	40	25	76	82	7,5	38,7	54,5	37,5	93	35	-	145	39	-	53,37	Mesofil
10	82	110	30	3	5	50	25	70	75,4	3,6	36	60	50	78	30	-	153	40	-	65,92	Mesofil
11	53	63	18	5	8	30	15	35	84	9	33,9	62,5	50	88	36	-	180	34	-	28,58	Notofil
12	70	93	25	5	14	35	18	60	75,2	7	35	35,7	48,5	115	35	-	140	31	-	45,65	Mesofil
13	75	88	30	4	5	37	20	76	85	5	40	80	45,9	66	35	-	155	46	-	46,54	Mesofil
14	66	94	22	15	3	35	20	80	70	2	33	50	42,8	85	36	-	170	39	-	45,82	Mesofil
15	59	80	22	4	7	29	18	55	73	6	37	57	72	58	37	-	150	33	-	34,60	Mesofil
16	60	83	21	3	9	33	18	55	72	5	35	33	45	110	36	-	180	36	-	40,53	Mesofil
17	75	100	25	3	6	35	22	65	75	4	33	50	37	82	44	--	165	31	-	51,29	Mesofil
18	63	79	36	2	4	37	17	53	79	3	57	50	54	82	41	-	138	22	-	39,02	Notofil
19	75	92	25	3	5	33	20	72	81	4	33	60	39	70	35	-	160	33	-	50,17	Mesofil
20	90	115	25	3,5	7	57	35	105	78	3	27	50	38,5	80	41	-	150	38	-	82,17	Mesofil
21	80	105	30	5	10	38	26	106	76	6	37,5	50	31,5	70	40	-	154	30	-	55,56	Mesofil
22	61	66	17	4	6	30	9	40	90	6	27,8	66	70	75	36	-	130	28	-	28,93	Notofil
23	65	72	32	5	8	37	18	35	90	7	49	62,5	51,3	65	30	-	155	40	-	33,80	Notofil
24	52	68	11	2	6	25	15	53	76	3,8	21	33	40	87	39	-	180	21	-	31,40	Notofil
25	65	90	20	3	7	31	21	52	72	4,6	30	42	32	65	40	-	180	40	-	43,61	Mesofil
26	58	80	20	6	12	25	15	50	72,5	10	34	50	40	82	37	-	180	25	-	36,72	Notofil
27	68	91	25	3	6	35	20	92	74,7	4	36	50	42,8	78	37	-	180	40	-	47,46	Mesofil
28	67	83	22	5	10	34	20	46	80	7	32	50	41	75	35	-	180	40	-	28,19	Notofil
29	75	90	29	5	7	35	22	70	83	6	38	71	37	82	35	-	88	30	-	47,06	Mesofil
30	82	89	30	5	9	45	25	70	92	6	36	55	44	83	35	-	180	34	-	53,21	Mesofil
31	77	86	20	10	17	35	20	60	89	12	25,9	58,8	42	75	39	-	154	45	-	48,84	Mesofil
32	70	89	24	4	13	36	22	75	78	5,7	34	30	38,8	105	40	-	180	27	-	43,39	Mesofil
33	80	95	31	7	12	40	21	73	84	8,7	38,7	58	47,5	80	37	-	120	33	-	43,39	Mesofil
34	63	88	24	3	4	35	20	50	71	4,7	38	75	42,8	62	45	-	180	40	-	52,35	Mesofil
35	72	93	26	7	12	36	20	63	77	97	36	58	44	84	40	-	180	40	-	52,35	Mesofil
36	52	68	20	3	8	25	14	53	76	5,7	38	37,5	44	85	40	-	180	20	-	27,56	Notofil
37	58	71	28	5	9	30	18	53	81,6	8,6	48,2	55	40	75	40	-	180	19	-	28,69	Notofil
38	55	69	20	7	17	27	16	56	79,7	12,7	36,3	41	40	87	38	-	180	21	-	29,27	Notofil
39	67	77	22	5	9	36	21	53	87	7,4	32,8	55	41,6	80	35	-	123	36	-	40,29	Mesofil
40	59	75	25	5	8	30	18	50	78,6	8,4	42,3	62,5	40	82	39	-	180	29	-	30,71	Notofil

DATA REGARDING THE QUALITY STATE OF TASAUL LAKE UNDER THE IMPACT OF INCREASED ANTHROPOGENIC IMPACT

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Abstract: This paper presents the results of the study made on the Tasaul Lake, one of the most important ecosystem changed by a complex man-made canal system from saline into freshwater and threatened by various pollutants (eutrophication) and fishing over-exploitation. The aim of this study is to identify the main pollution sources and to show the evolution of the water quality during this period 2007-2009 and to find some measurements for the protection and rehabilitation of Tasaul Lake. During the perioad 2007-2008 were measured the main nutrients (phosphates, nitrates), and starting with february 2009 were measured all the physico-chemical parameters (turbidity, dissolved oxigen, p.H, chlorophyll, salinity) using YSI multiparamter sonde and also there were made Microbiotests for the determination of water toxicity using the *Vibio fischeri* bacteria. The study also presents the main results obtained in the IRES Project, between Wisconsin University Milwaukee and Ovidius University Constanta, regarding the influence of anthropogenic impacts from Casimcea watershed on Tasaul water quality. Because the main activities are represented by the use of land in agriculture, the study was concerned about the accumulation of some fertilizers in soil and in the surface and ground water.

Keywords: Tasaul Lake, anthropogenic impacts, nutrients, microbiotests;

1. Introduction

Tasaul Lake, formed on Casimcea Valley is a sea-river estuary that feeds from both surface water and underground water. It has an area of 2335 ha, a maximum depth of 3.5 m, an average depth of 2.2 m, length of 11.5 km, average width of 2.0 km, maximum 4.0 km, and a catchment of 872 km ^{2} formed mostly by Casimcea River (755 km ^{2}).

By a pipe receives water from Siutghiol and the excess it is release through a channel in Lake Corbu (Gagarlâc Lake). Lake Tasaul is related to the Black Sea only through Lake Corbu.

It has a fishery utility, the fish productivity being directly related to the freshwater coming from Siutghiol Lake.

The catchment of Tasaul Lake, represented mainly by Casimcea, it has a characteristic feature, rarely seen in nature: the overlapping of surface reception area with the ground one. In these circumstances, the reserves of groundwater will be drained all to Lake Tasaul. Water regime of Casimcea is typical continental with great variation between extreme flows (maximum flow near the river flow into the lake 222 m^3 / s and least flow 0380 m^3 / s in Cassian Point, located upstream.

Lake Tasaul is proposed for a Ramsar site. The lake is located in the path of migratory bird populations, being one of the largest lake in the South Cape Midia. In Constanta County there are 22 Bird Protection Areas (SPA), reported by GD 1284/2007 regarding the special birds protection areas, as a part of European ecological network Natura 2000 in Romania [1].

As main sources of pollution around the lake are household waste water coming from rural areas (Piatra, Luminita, Sibioara) and Navodari city. Domestic waters become loaded with pollutants of organic and biological origin (bacteria, viruses, eggs of parasites) or with some chemicals used increasingly in households [2]. Then the wastewaters from animal husbandry - pig farm from Sibioara and farm ducks.

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A classification of livestock waste can be done as: plant biomass, manure, slurry and pasty semi-liquid waste feed, dead bodies.

Important sources of contamination are big animal farms, especially those of large and diseased specimens, which can discharge into the water leptospire, brucella, and other organisms to which the animals serve as the host [3]. Waste water from animal farms and birds, generally have urban waste water features, primary pollutants of organic substances in large amount and materials in suspension.

PetroMidia Năvodari-refining and petrochemical pollute mainly through emissions in the atmosphere. So rain water become contaminated by the washing of air at the beginning of fall precipitation. The main sources of pollution from petrochemical and oil refining are SO_2, NO_x, CO, O, H_2S, dust sediments, phenols, cresols, mercaptans, acids, ketones, aldehydes, hydrocarbons.

Career limestone-quarries are a primary source of pollution, seasonal, inorganic, which may act on terrestrial biotic environment, resulting from the emission of particulate phases of the technological process (decopertation, processing, loading, transport) and by products of burning fuel. At high temperatures, in drought conditions, with winds predominantly from the east and northeast are created large quantities of dirt, dust and other nuisance which make unbreathable the air, affecting health of inhabitants, the state of adjacent land, gardens and trees of village. By precipitation air and soil are washed, all the water reaching Tasaul Lake.

Agriculture - administration of chemical fertilizers, nitrogenous and phosphate fertilizers enter the lake Tasaul basin, being washed by rainfall or irrigation and thus can reach also the groundwater.

Tasaul Lake is a lake with predominant supply of surface drainage. Given the dependence of precipitation fell, the intake varies depending on the rainy or dry periods. Bringing various soluble salts in the tributary, surface drainage brings large amounts of nutrients. Their presence gives the qualities of the lake food web and create optimal conditions for fish farming development in the allowable limits of mineral water [4].

Very significant amounts of nutrients enter the lake from the catchment of Casimcea River.

Casimcea River has a length of 69 km, with an area of 740km ².

2. Material and Methods

The study of Tasaul lake water quality was done in two periods and consisted in water sampling of 3 stations, namely Pescom, Dig Midia, Sibioara, the sampling frequency being seasonal, in July 2007, October 2007 and April 2008, including almost a full seasonal cycle.

Water sampling was done using a device comprising on a support on which is fixed by a clip a sterile plastic bag. On the bag was noted the sample collection date. At the sample point, we measured water temperature and dissolved oxygen using Consort Z82 Oxygenometer, making a previously device calibration.

We filtered a small amount of water, for which we determined the amount of nitrates and phosphates using Merck field kits strips RQ Flex.

Samples were brought to the laboratory where was made their analysis. All samples were filtered - a quantity of 150 ml for each sample. Analysis of samples was done with YSI 9100 Spectrophotometer, which provides a modern way for water analysis.

On February 2009, we conducted a second study to measure all physical-chemical parameters using YSI multiparameter sonde (dissolved oxygen, turbidity, chlorophyll, pH, conductivity), but also the analysis of key nutrients. There were taken in consideration 5 stations, respectively: Pescom,, Case, Cariera, Dig Midia, Sibioara (Fig. 1).

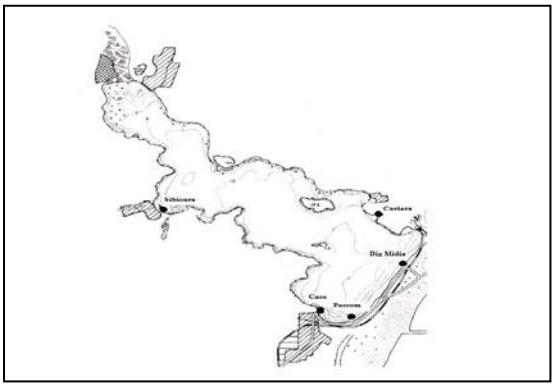

Fig.1 – Sampling stations (February 2009)

For all 5 stations were made also microbiotests for determining water toxicity using Toxkit Microbiotests. The microbiotest for toxicity measures the drop of luminiscence at some bacteria that are placed in a contaminated environment. The amount of light produced by the bacteria is directly proportional to the intensity of cell respiration and is measured by luminometer in "relative light units" (RLU).

Inhibition of bacterial respiration under a toxic stress automatically lead to a decrease of bioluminescence. Decreased bioluminescence in water is measured after a short exposure time (30min) and is compared with a decrease of luminescence in non-toxic environment (an uncontaminated water). The magnitude of decrease light energy in the analyzed water is an indicator for the degree of sample toxicity.

3. Results and Discussions

Within the IRES project was analyzed the influence of anthropogenic impacts of Casimcea catchment on Lake Tasaul water quality. Because the main activities are the use of land for agricultural purposes, the study followed the nutrient accumulation in soil and groundwater and surface waters. The amount of fertilizers used per hectare has increased dramatically in the communist era. On an area of 326 hectares of crop, in a period of approximately 40 years have been applied more than 1 million kg of fertilizer in Casimcea catchment basin (Fig.2).

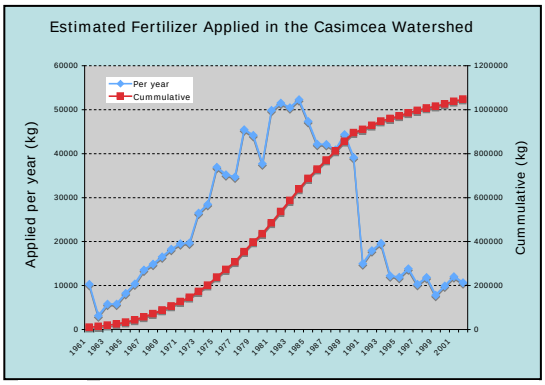

Fig. 2 – Assessing the degree of accumulation of fertilizers in the catchment of Casimcea River

Using an estimation method of how to reduce the amount of fertilizers in the soil, the study showed that there are needed 20 years to no longer apply any fertilizer so that their level in soil to reach the maximum limit allowed.

There were taken samples of water from private wells in Cheia and Gradina and also soil samples collected from 6 locations: Valley Dobrogea in an area of grassland, at Gradina (plantation of sunflower), 3 samples were collected from the dam area on Casimcea and a sample from Cheia village (private garden) (Fig. 3).

Their location was done with GPS equipment for accurate determination of geographical coordinates and then the results of the quality from these locations were introduced into a GIS database.

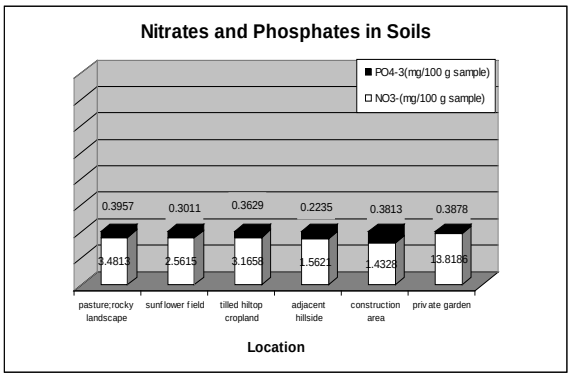

Fig. 3 – Areas of soil sampling (IRES Project, 2007)

Analysis of water samples collected from wells have revealed overruns of extremely high nitrites, so water consumption from these wells was prohibited.

The study realized under IRES project, based on documentation, the study of statistics on the quantities of fertilizers applied in agriculture along Casimcea valley, especially in Cheia village and Gradina, until 1989, showed a continuous accumulation of them in soil, within about 40 years so far. After sampling the soil in the catchment area of Casimcea and laboratory analysis of soil samples, resulted the fact that the largest amounts of phosphorus and nitrogen was applied in private gardens, followed by pasture. Extremely high concentrations of nitrites present in water sample from wells is then correlated with historical accumulation of soil fertilizers in groundwater of

localities in Casimcea Valley, creating a serious risk to public health but also for animals.

In the period July 2007-April 2008 the amount of dissolved oxygen was highest in April for all three sampling stations, putting the lake in I class of quality as Ord. 1146/2002, the minimum value being recorded in July 2007 (fig.4). The values of nitrates were high in April 2008 for all 3 stations, the minimum being recorded in October (fig.5), they putting the lake in class II of quality. Phosphate concentration was maintained throughout the study in I class of quality for all stations considered (fig.6). According to the Order 1146/2002 overall quality of lake water Tasaul is included, in terms of key indicators of eutrophication process, in hypertroph type.

For February 2009, the data obtained with the help of YSI sonde showed that at the station Sibioara is recorded the highest quantity of dissolved oxygen (fig.7), this also because of the lowest temperatures recorded in this station but also because of the mixing of waters at the mouth of Sibioara river. In which regards the turbidity and the chlorophyll concentration, these records the highest values at Case station (figs.8, 9).

The analysis of water samples showed a very high concentration of phosphates at Case station (fig.11), this indicating a pollution with fecal phosphorous or detergents, while the highest quantity of nitrates is recorded at Sibioara station, nitrates coming mainly form washing of agricol soils by precipitations but also form livestock waters (fig.10).

Table 1 – Degree of toxicity in 5 monitored stations

LOCATIE	Control Medie	Sample Medie	B/A	**Tox. %**
	(A) RLU t0- RLU t30	**(B) RLU t0- RLU t30**		
CASE	1444.5	1069.5	0.740	7.4
SIBIOARA	814.0	-442.0	-0.543	-5.43
DIG	642.0	351.0	0.546	5.46
PESCOM	2884.5	-959.0	-0.332	-3.32
CARIERA	-611.5	-520.0	0.850	8.50

LOCATIE	control		sample	
	RLU t0	RLU t30	RLU t0	RLU t30
CASE I	4664	3184	5231	4032
CASE II	4656	3247	5184	4244
Sibioara I	3225	2580	4024	4556
Sibioara II	3540	2557	3448	3800
Dig I	4126	3784	4348	4254
Dig II	4678	3736	4089	3481
PESCOM I	5399	5497	5296	6167
PESCOM II	5148	4778	6168	7215
Cariera I	3394	4018	3777	4302
Cariera II	3581	4180	5028	5543

Results of microbiotests applied showed that areas with toxicity are stations Case, Cariera and Dig, while the other 2 areas have toxicity close to 0, RLU values increasing from time t0 to t30 (Table1).

4. Conclusions

Hypertrophy and overcoming of maximum permitted values for key nutrients, that are involved in the development of water eutrophication in 2007-2008, is due to direct human impact of the many pollution sources around the lake, such as farming and livestock (pig farm Sibioara and duck farm) and the immediate vicinity of the lake with human settlements (Năvodari city, village Sibioara) etc.

Analysis of the results of the Project IRES and Project Estrom shows a correlation between the use of land in the catchment area of Casimcea and nutrients reached the lake.

Tests conducted with multiparameter YSI probe showed the strong impact of human settlements on Lake Tasaul, the station Case recording the highest values for parameters: chlorophyll, turbidity.

Analysis of water samples showed a very high concentration of phosphate in station Case, indicating a pollution with fecal phosphorus or detergents, while the largest amount of nitrates is recorded at station Sibioara, nitrates originating mainly from the washing of agricultural soils by rainfall but also from livestock (manure).

In which concerns the results of Toxkit mircobiotests, they showed values ranging between 7-9%, for three of the 5 stations.

Lake Tasaul so far as not been studied sufficiently to allow an accurate forecast of its response to ongoing contributions of nutrients and pollutants.

There are needed investments to protect Lake through research and environmental reconstruction.

5. References

[1]. BABOIANU, Gr., 2003 – Directory of Azov - Black Sea Coastal Wetlands, Ucraina

[2]. CHIRILA, E., 2003, Analiza poluanţilor – Controlul Calităţii Apelor, Ed. Universităţii Transilvania, Braşov

[3]. BARNEA, M., 1987 – Poluare şi protecţia mediului, Edit. Ştiinţifică şi Enciclopedică, Bucureşti

[4]. BREIER, A., 1976 – Lacurile de pe litoralul românesc al Mării Negre, Edit. Academiei Republicii Socialiste România, Bucureşti

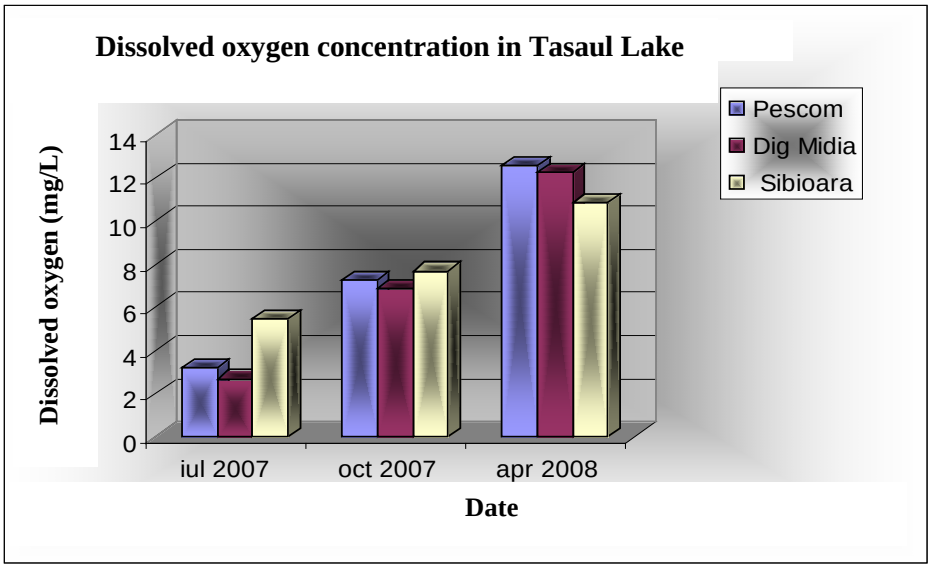

Fig.4 – Dissolved oxygen concentration in Tasaul Lake (2007-2008)

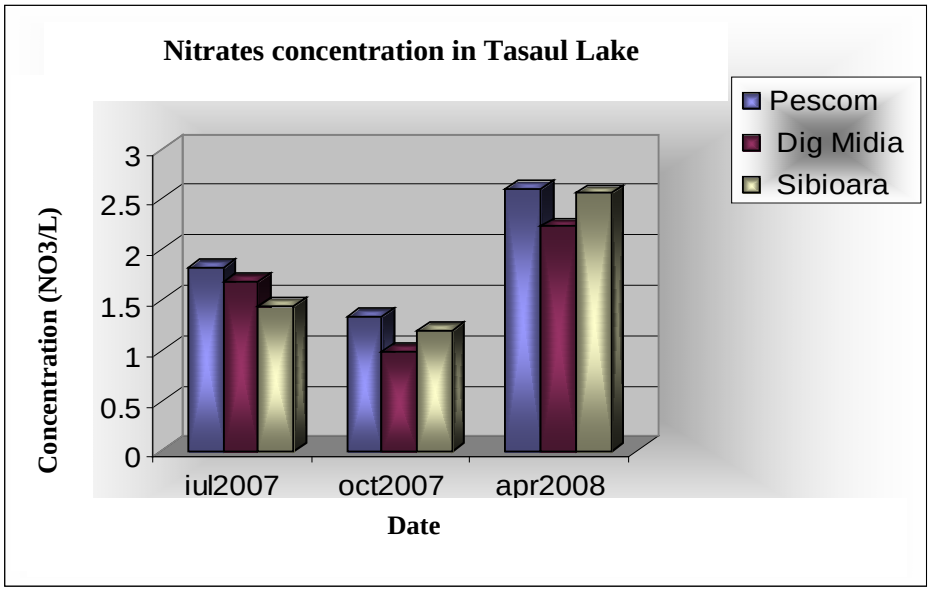

Fig. 5 – Nitrates concentration in Tasaul Lake (2007-2008)

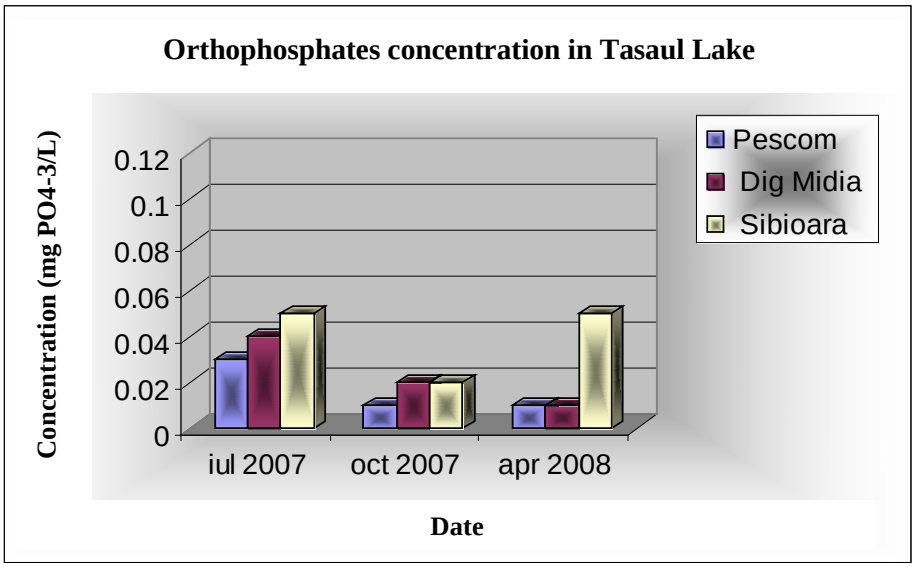

Fig.6 – Orthophosphates concentration in Tasaul Lake (2007-2008)

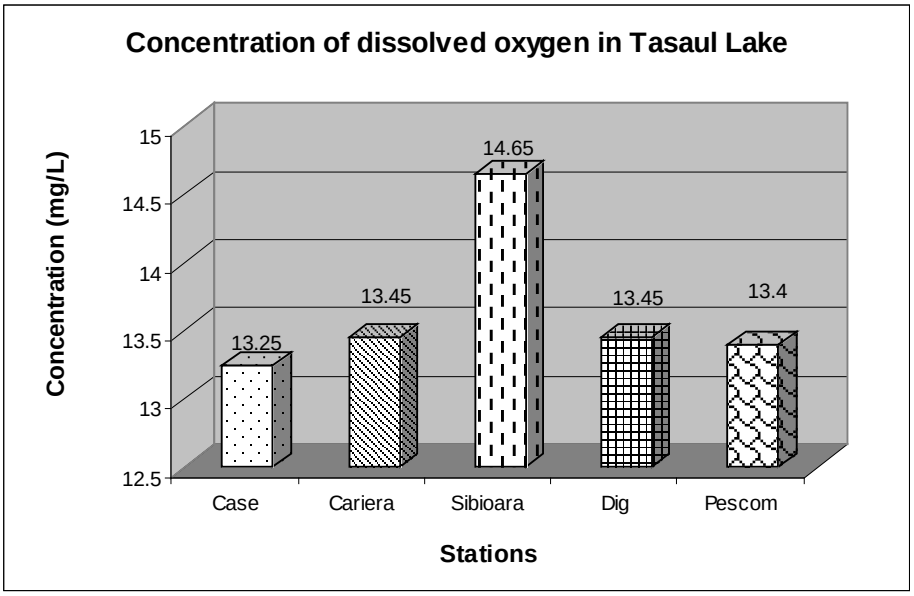

Fig. 7 – Concentration of dissolved oxygen in Tasaul Lake (February 2009)

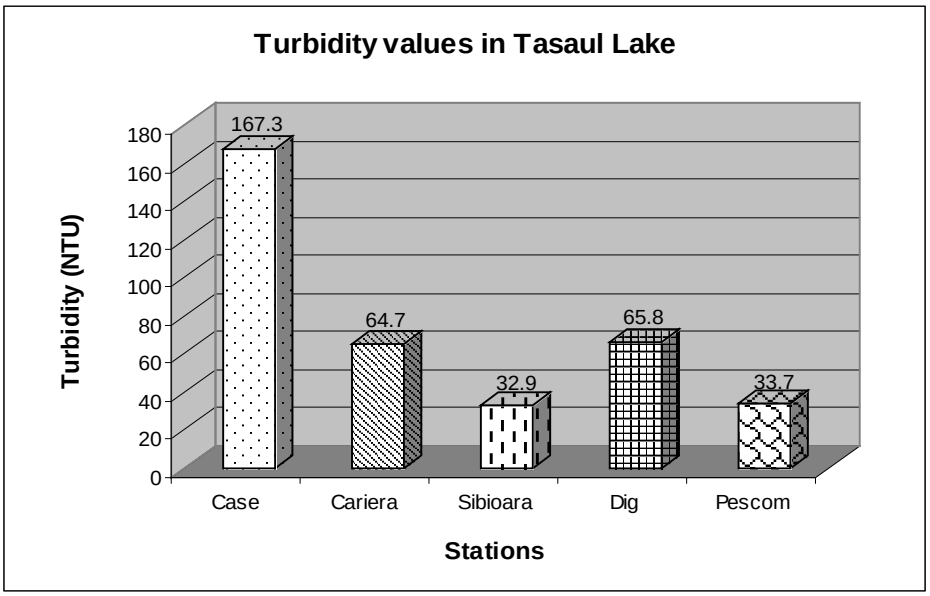

Fig. 8 – Turbidity values in Tasaul Lake (February 2009)

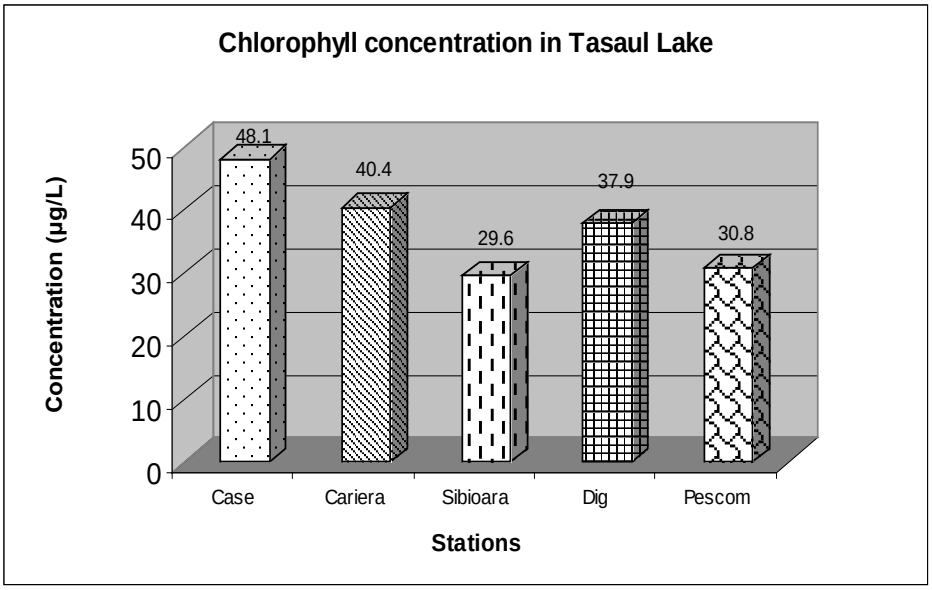

Fig. 9 – Chlorophyll concentration in Tasaul Lake (February 2009)

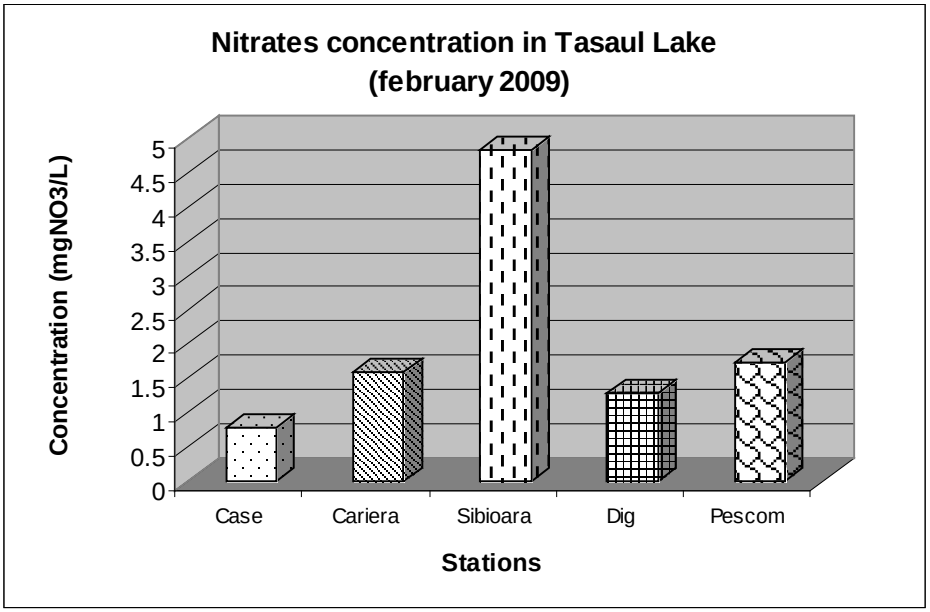

Fig.10 – Nitrates concentration in Tasaul Lake (February 2009)

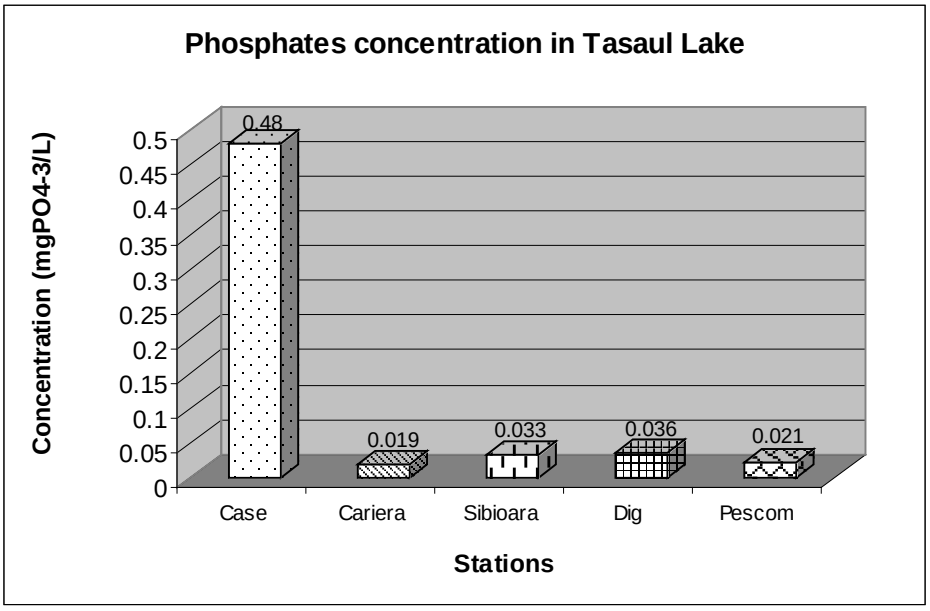

Fig. 11 – Phosphates concentration in Tasaul Lake (February 2009)

THE INTEGRATE ECOLOGICAL MONITORING ASPECTS PRESENTATION FOR SIUTGHIOL (MAMAIA) LAKE USING GIS TECHNOLOGY

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Abstract: The coastal lakes (Tăbăcărie, Siutghiol, Taşaul and Corbu), situated between the Chituc sandbank and Constanţa municipality contain complex interacting ecosystems with diverse ecological communities. These lakes are also of economic significance because of exploitable natural resources and specific human activities. Siutghiol Lake is situated in the northern part of Constanţa municipality and it represents an important attraction for tourism, its eastern shore being parallel to the important summer resort of Mamaia. The lake provides socio-economic value to the surrounding localities in addition to its importance for agriculture and industrial activities. Other ecosystem services of the lake include it being a significant area for aquatic migratory birds and its fisheries resources. Our research program focused on the aspects of biological/ecological monitoring, which could be incorporated into and analyzed using a GIS database to obtain a general view of the current status of Siutghiol Lake. The methodology of work combines the creation of a geospatial database of various direct field observations of environmental parameters (e.g. phosphates, nitrates, dissolved oxygen) and collection of information from different institutions involved in the management of this lake.

Keywords: Siutghiol Lake, ecological monitoring, GIS technology

1. Introduction

Coastal lakes from the area between Chituc sandbank and Constanta municipality (i.e. lakes Tăbăcărie, Ovidiu, Taşaul, Corbu) represent a complex of interacting ecosystems that are significant economically, for natural exploitable resources, ecological communities and for the specific human activities.

Coastal lake ecosystems are areas most affected by stress, through the complexity of interrelations between humans and the natural environment, and the consequences of the demographic increase (particularly during summer tourist season, but also of the population growth in general), in the background of climate change, will cause the amplification of human impact on aquatic ecosystems [2]. Siutghiol Lake is located to the north of Constanta and it together with Tăcărie Lake an aquatic complex because of the close relationship between them [1]. The lake is a major area of attraction for tourism, its socio-economic value is also given by the localities around it and by the importance of lake water for specific agricultural and industrial activities. The lake is a significant area for migratory waterfowl and for exploitable fish resources. Given the importance of this lake, research activity was focused on issues of environmental monitoring, which were built and analyzed using a GIS database in order to obtain an overview of the current situation of Siutghiol Lake. Work methodology combines the creation of a geospatial database with field observations, laboratory tests (e.g. phosphates, nitrates) and data collection from institutions involved in the management of the lake.

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2. Material and Methods

Water samples have been collected from five collection points. (Table no.1) during three seasons: spring (May), summer (August) and autumn (November). Samples have been collected using a water sampler with plastic bags then transported in a frozen chamber, processed and analyzed immediately in the laboratory. We have determined the concentrations of orthophosphates and nitrates using YSI 9100 Photometer, closely following the working methods described in its manual [4].

For the determination of dissolved oxygen concentration we have used Z621Consort oxygen-meter, offering the possibility to achieve field determinations. We determined the degree of bacterial contamination of water sample collected in the summer season at station no. 3 using the Microbiotests Inc. company product (toxicity screening kit), from Belgium [3].

3. Results and discussions

3.1. Presentation of data obtained from the institutions responsible for monitoring coastal lakes

According to reports on environmental conditions in Constanta County, conducted by the Environmental Protection Agency of Constanta, for Siutghiol Lake were established four monitoring sections: Neptune wharf, N-E Ovidiu, C.E.T. Ovidiu and the lake center, with a sampling frequency of four times per year. Parameters monitored and recorded values in the years 2003-2007 are presented in Table 2. Quality conditions as set in STAS 4706/88 led to the assessment of Siutghiol Lake as an eutrophic one during 2003.

In 2004, based on values for monitoring indicators, Siutghiol Lake was classified as a hypertrophic lake.

For 2005, values for the entire lake system for oxygen regime indicators and nutrients meet class II, the indicators for the mineralization degree correspond to IIIrd quality class, and metals to Ist quality class. The overall water quality for the lake corresponds chemically to the IIIrd quality class. Total mineral nitrogen indicators values of 3405 mg/L, total phosphorus of 0.218 mg/L and phytoplankton biomass of 7.745 mg/L put the lake in the eutrophic type.

According to the Order 161/2006, global quality of lake water in the year 2006, is framed in from the chemical point of view in IIIrd quality class, corresponding to a moderate ecological status. Phytoplankton biomass, total mineral nitrogen and total phosphorus values frame Siutghiol Lake in the category of hypertrophic lakes.

Table 1 – Geographical coordinates of the sampling points

Location	Latitude	Longitude
Station 1	44º15'32.41''	28º37'02.39''
Station 2	44º14'05.47''	28º37'17.51''
Station 3	44º13'10.31''	28º37'38.65''
Station 4	44º14'56.62''	28º34'26.93''
Station 5	44º14'06.86''	28º35'34.97''

Table 2 – Evolution of indicators monitored by Constanţa EPA for Siutghiol lake between 2003-2007

Indicator	Average value (mg/l) **2003**	Average value (mg/l) **2004**	Average value (mg/l) **2005**	Average value (mg/l) **2006**	Average value (mg/l) **2007**
Chloride	356,03	-	-	-	-
Fixed rezidium	1528,4	-	-	-	-
Dissolved O_2	10,8	-	-	8,35	-
CBO5	6,97		-	2,5	-

CCO-Mn	14,79	21,2	-	-	-
Total N	3,64	3,27	3,405	1,724	2,19
Total P	0,14	0,361	0,218	0,1620	0,11
Phytoplankton biomass	16,43	15,49	7,745	11,251	9,88

Interpreting the values of indicators for eutrophication process, we fit Siutghiol Lake in 2007 in the hypertrophic type, and by physicochemical determinations, according to Order 161/2006, in the fourth grade of quality, corresponding to a weak ecological status.

3.2. Presentation of own results

Comparing the data obtained (Table 3) for the month of May with existing standards for surface waters, we can say that Siutghiol Lake falls within the Ist quality class in terms of the amount of dissolved oxygen in water and nitrate (Fig. 1). Regarding the amount of phosphates, the quality category is II.

In the summer season the values obtained frames Lake Siutghiol within class I, based on the concentration of dissolved oxygen in water and nitrates. Regarding the orthophosphate indicator, the values obtained indicate the IInd quality category.

Compared with results obtained in spring and autumn seasons, the ones for the summer season have shown a significant increase in the amount of orthophosphates. The highest growth was recorded at station no. 3 five times higher than in May.

Table 3 – Tests results

Location name	**Sampling period (2008)**	**Phosphates (mg/l)**	**Nitrates (mg/l)**	**Dissolved oxygen (mg/l)**
Station no.1 Butoaie	Mai	0,05	2,40	12,60
	August	0,12	2,50	8,10
	Noiembrie	0,02	1,2	10,00
Station no.2 Cazino	Mai	0,05	2,21	12,43
	August	0,10	2,80	7,20
	Noiembrie	0,02	1,25	10,20
Station no.3 Debarcader	Mai	0,04	2,32	12,30
	August	0,20	3,40	8,70
	Noiembrie	0,047	1,47	10,70
Station no.5 Ovidiu	Mai	0,06	2,60	12,50
	August	0,08	2,40	8,40
	Noiembrie	0,04	1,3	9,90
Station no.4 Palazu	Mai	0,06	2,40	11,90
	August	0,06	2,60	8,20
	Noiembrie	0,04	1,35	10,20

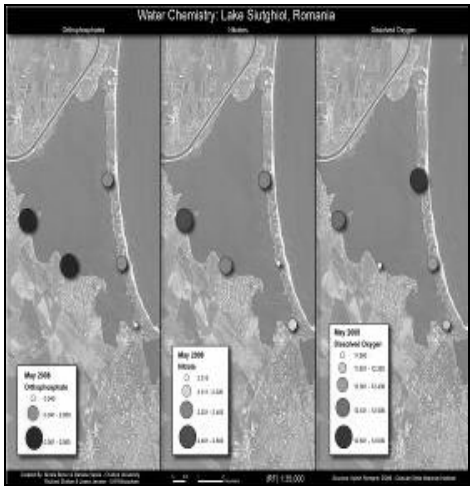

Fig. 1 – GIS representation of parameters monitored in spring (May 2008)

Also values for nitrate indicator increased, but not as significant as those for orthophosphate.

For dissolved oxygen indicator it was observed a decrease compared with the values of the spring season.

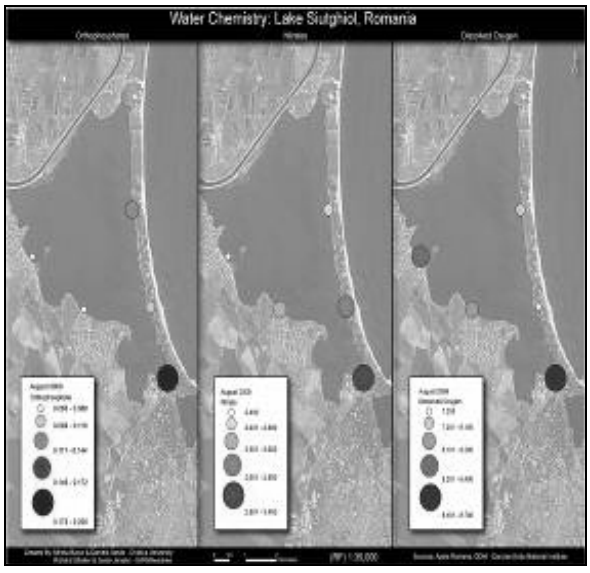

Fig. 2 – GIS representation of parameters monitored during summer season

During summer season the test to determine the degree of bacterial contamination was conducted for water samples taken from station no. 3, where there were higher values for the parameters of orthophosphates and nitrates. It was obtained a value of 6187 RLU, indicating the presence of relatively few bacteria in water (<10,000 RLU).

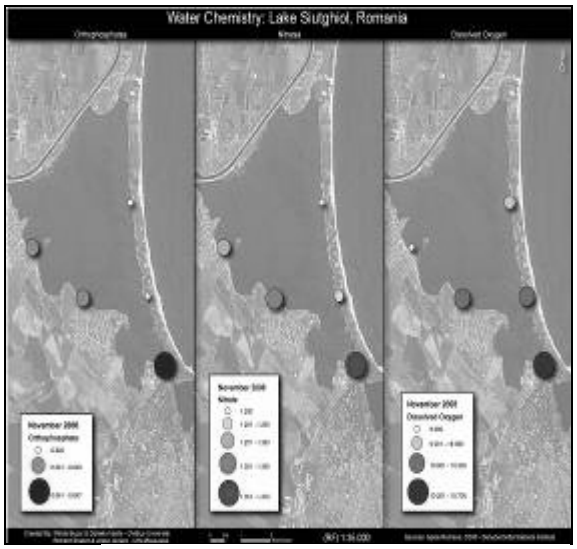

Fig. 3 – GIS representation of parameters monitored during autumn

By comparison with the limits imposed by water quality standards, we can say that in autumn Siutghiol Lake falls into the category I in terms of concentration of oxygen dissolved in water (> 7 mg/L), nitrates (<3 mg/L) and orthophosphates (<0.1 mg/L).

4. Conclusions

One may say that the highest values for othophosphates parameter were recorded in summer and autumn seasons at station no. 3 (Figs. 2, 3), yet fits in the IInd quality class. In this area is felt the influence of wastewater discharged by various operators in the Holiday Village in the channel connecting the Siutghiol Lake to Tăbăcărie Lake. The lowest values were recorded in the spring season.

For nitrate parameter, the highest values were recorded in summer, according to which, the quality class was II. In this season the highest value was recorded at station no. 3 (Fig. 2). The lowest values were recorded in autumn. It is necessary to establish more stations, with a higher frequency of monitoring.

Given the importance of the lake ecosystem and taking into account that in recent years there were carried out activities (high-speed racing boats) and launched proposals for new projects (navigation channel in the north, the creation of artificial islands), which can affect the future health of Siutghiol Lake, the adoption of management strategies that aim at reducing pressure exerted by human socioeconomic systems is required.

5. References

[1] NIEMI G., WARDROP D., BROOKS R., ANDERSON S., BRADY V., PAERL H., RAKOCINSKI C., BROUWER M., LEVINSON B., McDONALD M., 2004 – Rationale for a new generation of indicators for coastal waters, Environmental Health Perspectives.
[2] GÂŞTESCU P., 1971 – Lacurile din România. Limnologie regională, Edit. Academiei Române, Bucureşti, 1971.
[3] *** Manual Fotometru YSI 9100.
[4] *** Manual Bacterial Contamination Screening Kit.

CHARACTERISTICS OF WORKFORCE IN SOUTH DOBRUDJA PLATEAU

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Abstract: In South Dobrudja Plateau, after 1990, old branches of the economy developed, the primary and secondary sectors, lost employees because they were subject to restructuring. Throughout entire South Dobrudja Plateau, the employed population has low levels: 27% in rural areas, 36% in urban areas, given that, in 2002, the rural population represented only 23% of the total population of the plateau. Urban Employment in South Dobrudja Plateau is small. In 2007 the employment rate of labor (ratio of employed population and total population) was 58.8%, falling well below the 70% target set by the Lisbon Strategy for 2010 across the EU.

Keywords: South Dobrudja Plateau, workforce, sustainable development, unemployment, employees.

1. Introduction

Initially, sustainable development was originally meant to be a solution to the environmental crisis caused by intense industrial exploitation of resources and environmental degradation to primarily seek preservation of environmental quality, in recent years the concept has expanded upon quality of life in its complexity, nurturing in time with an economic and a social dimension.

The concept of sustainable development covers all forms and methods of socio-economic development, not only in the short or medium term, but also in long term, whose background is mainly represented by assuring a balance between these socio-economic and natural capital items.

The objective of this paper is to present employment situation, but also employment policies in South Dobrudja Plateau, in terms of sustainable development, using objective data - statistical indicators provided by the Statistics Directorate Constanta County.

2. Material and Methods

Data on unemployment and employees of South Dobrudja Plateau are provided by the Statistics Directorate Constanta County, incorporated in the tables on which were carried out various graphics. We'll analyze evolutionary trends during the 17 years of study of each wage group, the average and total plateau, to analyze their variations. The received data had as the first reference year 1991, the last being in 2007.

3. Results and Discussions

Throughout the history of the region, environmental conditions, with social and economic, demographic movements and the natural and migration (Figure 1), enabled the emergence in South Dobrudja Plateau, a total of 11 cities and 37 communes, consisting of a mosaic of demographic, ethnic, cultural, so specific and so famous Dobrudja.

Unemployment

Correlations to be made within a region are huge: the supply of labor versus labor demand, labor demand relative to demand for goods and services and their technological level of production, scientific and technical progress in relation to the quality and quantity of labor work required.

These correlations are difficult to existing data currently in the Departments of Statistics county or even national level, we tried an important detail, full correlation between unemployment, total population, employment, or the active and unoccupied Plateau

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Offline South Dobrudja, trying to outline one of the suitability of the currently available to this region, namely the huge labor force.

Analyzing the unemployment rate (Fig. 1) by age and sex (2002) notes:

• very large group 15-19 years (most components below 18 years going to different forms of education) - 50%;

• 20-24 years group is high - 27-28%;

• decreases the group 55-59 years - 10%;

• group 60-64 years - 3-4%.

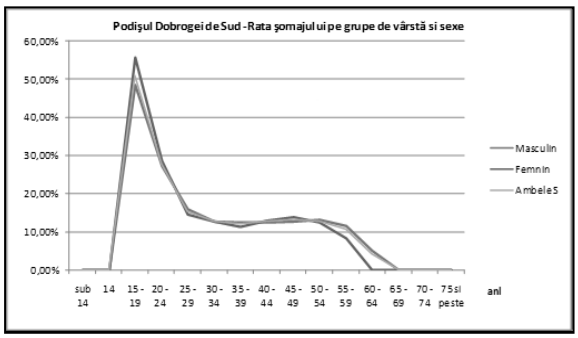

Fig. 1 – Values of unemployment in South Dobrudja Plateau, by age and sex (2002)

It is amazing the similarity values for the two areas - urban and rural areas so that we can say that in South Dobrudja Plateau, the proportion of unemployment urban / rural, is balanced (Table 1).

Table 1 – Values of unemployment, the average South Dobrudja Plateau (2002)

Environ-ment	Total active	Unem-ployed	Unemployment rate
Urban	207.082	30.809	14,88%
Rural	118.472	17.390	14.68%

Studying the unemployment rate by age and sex see higher net values in all age groups for males, the maximum being 55-59 years and 78% 25-29 years 64%.

If the female population, the highest values belongs to age group 40-49 years, maybe due to the new demands of the private sector, where young people prefer, with or without experience (Fig. 2).

Youth unemployment, when the low values is the greatest achievement of demographic and labor market policies of any region, regardless of their economic development.

Other indicators for sustainable employment analysis of a region are given by the active population, employment, working age population and a variety of indicators drawn by combining them.

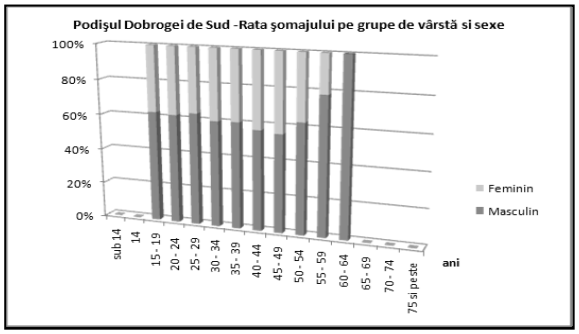

Fig. 2 – Unemployment in South Dobrudja Plateau, by age and sex (2002)

On the whole plateau, in both sexes, the maximum activity rate is 77% - 78% in groups 30-39 years, the lowest weight being 60-64 years, only 4.57%, resulting an average activity rate of 42.09% on the plateau.

Looking at the graph in Fig. 3, we see that in urban areas, population inactive retirees prevail, the proportion of 56% followed by those households 16%, 15% students and dependents of state or private organizations 11%.

Maintained by another person or persons assigned to the cases not quantify than 1% each of the two categories.

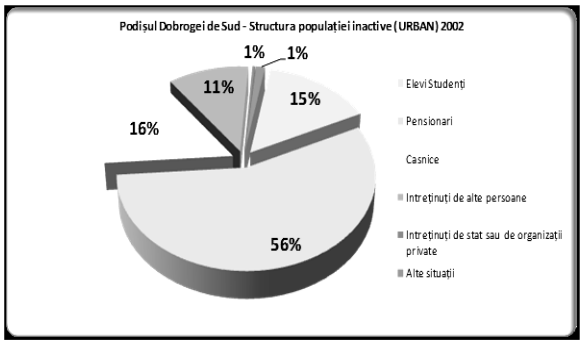

Fig. 3 – Structure of inactive population in urban areas

Structure of inactive population in rural areas is quite different from that of the urban environment, with approximately equal sectors retired: 29%, housekeepers: 26%, students: 24%, maintained by others: 18% (Fig. 4.).

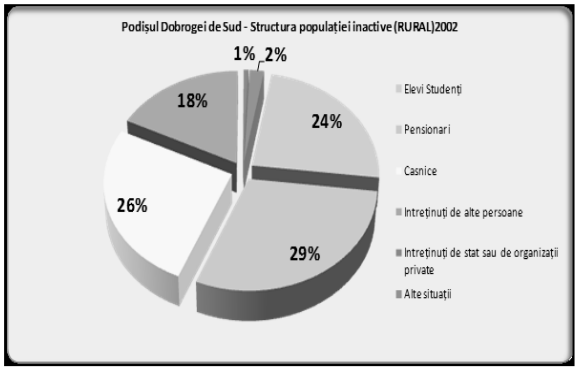

Fig. 4 – Structure of inactive population in rural areas

Employees

In comparison, the branches of the tertiary sector (trade, financial, banking and insurance activities, public administration) became major recipients of labor in all three components, the number of employees doubled.

In trade, financial, banking, insurance and public administration was constantly rising trend. In education, health and social care course has been stagnant, with only small ups and downs.

Sectors that have had dramatic developments, although the number of students varied that the patients for 17 years, are education and health, they can be classified as balance of society in this regard.

Looking at the average situation, comparisons can not do much, because the difference in the number of employees between the two areas is very high. The only activity in that compromising situation is agriculture.

In its case, developments in the average number of employees and total Plateau had the same trajectory, and the numerical differences were not so great. On the other hand, however, agriculture has registered the largest drop in Chapter employees, values of 20,000, 30,000 employees, amounting to fewer than 5,000 today.

4. Conclusions

All youth unemployment indicators show large scale loss of young human resources and labor power in total, representing a significant proportion of the total number held by the South Dobrudja Plateau.

On all indicators of unemployment to the highest values recorded male population, perhaps because there are still black market labor and industries where illegal employment practices are applied mainly by men: construction, plumbing, agriculture, etc.. Another reason could be the growth of the services segment, which does not require physical work which gave rise to a competition between the two sexes.

The increasing of women's employment rate but not men offset lower employment. Such a warning is made necessary by local authorities, because, if not used, this part of the population will migrate to work in other countries or regions with much higher potential for employment, leaving the plateau, and so many impoverished and harsh failures and not a valuable treasure: a young workforce.

Judging the future possibilities of economic recovery in a sustainable way, a plateau exists, and hence the possibility of increasing the percentage of active population in total population.

5. References

[1] RĂBOACĂ GH, 2003 – Labour market and sustainable development, Tribune Publishing House, Bucharest, p. 17.

[2] PREDA D, 2002 – Employment and Sustainable Development, Economics Publishing Bucharest, pp. 165.

[3] ERDELI G, Dumitrache L, 2001 – Population Geography, Corint Publishing, Bucharest, pp. 173.

THE USE OF METHODS TO OBTAIN AND INVESTIGATE BACTERIAL BIOFILMS IN THE STUDY OF SURFACE COLONIZATION

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Abstract: Bacterial biofilms are communities of microorganisms surrounded by a layer of exopolysaccharides secreted by themselves in which bacterial cells forms (bacilli, cocci, spirillum and filamentous) attach to surfaces and form microcolonies that attracts other organisms (eukaryotes) and determine micro and macrofouling fomation in harbor areas. The accurate investigation of bacterial biofilms was accomplished after the appearance of new microscopy techniques, such as electronic and confocal microscopy due to these techniques advantages. The methods for the cultivation and harvesting of biofilms have known a considerable development over la last decades, but no standard protocol has been yet created or generalized method that could be used in any laboratory protocols. The aim of this study was to observe some of the main methods for bacterial biofilm fomation in laboratory conditions static or continuous and the new microscopy techniques that are more reliable on biofilm formation and temporal dynamics.

Key words: bioreactor, exopolysaccharides EPS, chemotactism, biofilm, confocal microscopy,

1. Introduction

Biofilms are formed on different types of surfaces which allow the growth of microorganisms in all aquatic ecosystems [1, 2, 3]. These communities of microorganisms and exopolysaccharides have a structural and functional architecture which is influenced by the internal metabolic processes of microorganisms, the formation of biofilms themselves occurs due to the existence of organic nutrients at the level of interfaces in the liquid medium[4, 5] These multicellular structures display resistance to antimicrobial agents and other toxic factors, being a defense mechanism of the microorganisms against their environment [5, 6]. Bacterial growth on various surfaces is due to the adhesion phenomenon and currently requires an interdisciplinary study which interests microbiologists, engineers, ecologists and chemists. In order to understand how biofilms form and how they can be controlled, techniques and methods were developed for this purpose [1, 5]

Taking into account the importance of biofilms in aquatic ecosystems and in technology, it is necessary to realize a study which presents the main methods used currently, with advantages and disadvantages, as well as the possibility to use and adapt them for the study of biofilms and in microbiology laboratories at national level.

The perfecting of such methods was accomplished after a long process of interdisciplinary research at national and international level, which has known a real development especially over the past twenty years.

2. Material and Methods

2. 1. Obtaining biofilms

The study of biofilms enjoyed particular attention and obtained significant popularity, especially in the last decade. However, there are problems: anybody who wishes to approach the research field of biofilms or to compare their results to those obtained by other

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laboratories encounters problems of the limited number of standard methods [2, 7]

There are numerous strategies for the study of biofilm formation, although they generally involve cells in stationary stage. These models use static systems which are recommended for the study of biofilms in initial phases and chemo static systems with continuous circuit for the examination of biofilms in the mature stage [7, 8]

Over time, numerous in vitro models were realized for the study of bacteria which can be classified as follows: bacteria can adhere to plane surfaces such as plastic tubes, Petri dishes, plates for tissues or rooms for chemotaxy)[7, 9].

A popular static method for obtaining biofilms is represented by the culture on plates of micro titration, by which the cells are cultivated on a minimal medium. Serial dilutions are realized and the samples are incubated. The samples are colored with violet crystal 0.1% which will be turned soluble with dimethyl sulfoxide and the optical density of the coloring matter will be analyzed [7] subsequently, an analysis of the percentage of bacteria attached to the substrate and the quantity of Crystal Violet absorbed can be made [9].

The testing in conditions of continuous circuit of the biofilm formation processes by the use of special models such as: the circuit with perfusion cells, the device with rotating disc, the room with radial circuit or the Robbins device (which can modify by the use of catheters as surfaces for the growth of biofilms) allowed researchers to obtain new data about the formation of biofilms. The changing of the material used as substrate helps to use this device in laboratory conditions, but also to determine the effect of antibiotics on the biofilms [7] The same kind of device can be realized in a simpler manner, from cheap materials such as: a modified Erlenmeyer glass with two orifices (entry and exit), a syringe, and a Pasteur dropper introduced in a rubber cork and capillary tubes connected to a peristaltic pump which permit the existence of permanent laboratory cultures in the chemostat conditions [10].

Pedersen [11] studied biofilms in conditions of continuous circuit with the direct use of water from a natural environment (taken from a gulf from a distance of 40 m from the shore). This was used in a special installation in which the biofilms were cultivated in rooms with continuous flux under the direct action of the seawater on the glass microscope slides used as substrate.

When such a model is realized in vitro for the study of bacterial adherence, the circulatory factor should be taken into account. Thus, the speed of the water flux, including the selection of bacterium type and the preparation of the testing surfaces is extremely important in laboratory conditions [12].

Also the researchers used “in vitro” conditions for most frequent microorganisms which belong to the classic strains of the type *Staphilococcus aureus, Staphilococcus epidermis, Esherichea coli* or *Proteus* and a few other common anaerobe bacteria. The bacteria can be obtained either from pure cultures resulted from laboratory collections or by isolating them from the natural environment.

In 1997, Yuehuei et al. [12] recommends that the purpose of the research should justify the careful choice of bacteria in order to obtain optimal and interpretable results. Thus, they must take into account certain essential aspects:

1. Significant modifications of the number of bacteria adherent to surfaces that can be isolated from the natural environment compared to the possibility to cultivate them in laboratory conditions;
2. The considerable influence of the growing conditions on the production of extra cellular polymers;
3. The centrifuging and washing of the samples can detach the bacterial capsule of the biofilm bacteria;
4. The adhesion of one bacterial species in the natural environment could be influenced by the presence of other pioneer species which could not adhere in laboratory conditions.

2. 2. Test surfaces

The solid surfaces can have a number of features that are important in the adhesion process. Thus, it was observed that the percentage of adherent bacteria increases with the level of roughness of the surfaces. This phenomenon determines a reduction of the

friction forces and an increase in the surface colonization [5, 13].

The preparation of testing surfaces for the analysis of biofilms is based on the existence of the two types of surfaces with different properties:

1. Surfaces with high energy (these are charged negatively and are hydrophile: glass, metal or minerals); Surfaces with low energy (these are charged either poorly positive or poorly negative and are hydrophobe: plastic made of organic polymers) [8, 14]. Most studies proved the existence of a large number of bacteria on this type of surfaces compared to those in the first category [5]

Because of these physic-chemical characteristics of the materials used as substrate, but also because of the bacterial cultures used, the microorganisms will attach differently to the substrate. Thus the choice of material should depend on the following:

1. The purpose of the chosen study;
2. Knowledge of certain materials used previously as adhesion surface by other researchers;
3. The possibility of bacterial adhesion to the respective surfaces [12, 15]

The materials used in the experiments can be represented either by simple plane surfaces such as glass slide, the interior of a plastic culture tube, catheters and Petri dishes etc. The use of such surfaces for the tissue culture has certain advantages:

1. Most of these surfaces are clean from the optical point of view, straight and uniform;
2. The attached bacterial layers are easy to obtain and analyze under an optic microscope;
3. Due to the high familiarity of these surfaces, they are often used in the laboratory, being standardized;
4. They are more accessible and economic. [12, 16]

The surfaces used as substrate can be modified according to the study. Thus, a classical method is to cut the tested material. The parts obtained can be in turn modified by the use of surface treatments such as grinding by means of sand paper, wrapping in macromolecules or surfactants. [17]

The sterilization of surfaces is essential and the materials must be thus cleaned or treated chemically before they are used. Plastic and the polymers are usually cleaned by means of detergents or distilled water in order to eliminate fat and waste. For metals, more complex sterilization methods are used such as passivisation by phosphation. [16]

Many studies ignore the need to characterize the surfaces, which does not allow the comparison of results with those of other researchers. Thus, it is important to characterize the surface in terms of roughness, physical configuration, hydrophobicity and even chemical composition. All these should be analyzed prior to the experiments. [18]

The position of the test surfaces is essential and they should be in permanent contact with the bacterial suspension, especially in circuit condition. Also, the physical forces must be strong to permit the attachment of bacteria to the substrate [19]. The vertical or horizontal positioning is also very important because the sedimentation process intervenes in the case of horizontal surfaces. In the case of cells cultivated in systems with continuous flux, these are pushed by the flux in the contact area with the test surface. Thus, the main advantages of a circuit system are:

1. A controlled transport of the micro-organisms and nutrients;
2. A high density of the adherent bacteria during the experiments;
3. The lack of air-liquid interference which should be crossed by the bacteria [12]

In conditions of bioreactor, the bacterial growth was accomplished as microcolonies separated by canals, these structures being maintained over time. It was observed that a number of internal canals of considerable proportions start from the external parts of the biofilm and move towards the inferior ones forming a network-like structure. Depending on the type of substrate in the bioreactor, the shape of bacterial colonies differs. Those that form on active granular carbon are shaped like a ball if treated with toluidine or can look like a coral reef if treated with BTEX (a mixture of petroleum derivates) [20].

The studies in natural environment and in laboratory-made bioreactors do not show how the microorganisms respond to the signals from their environment and how the complex structures of the bacterial communities are formed [21]. Such signals can be observed on laboratory cultures (in the case of rooms with continuous circuit and of plates of

microtitration). The great advantages of these systems consist in:

1. The control of available nutrients accessible to the bacterial cells;
2. The possibility to determine the composition of microorganisms (including the genetic variety of the strains in biofilms);
3. The ability to watch the phases of biofilm formation from colonization to the detachment from a substrate.[12]

2.3. Washing, drying and staining

Once obtained, the samples need to be washed and stain. The washing is an essential part of the bacterial adhesion studies. Its purpose is to eliminate the bacteria that are not attached to the substrate. There is no standard protocol, so each laboratory makes one for itself [20]

Among the liquids normally used in the washing process the following can be counted: sterile water, saline solution and buffer saline solution. The washing process is followed by drying at room temperature or by means of a light flux of air and nitrogen.[22]

There are methods by which biofilms can be detached from the substrate, among which homogenization, sonication, the use of surfactants. After drying, the coloring process can take place with various coloring matters depending on the desired results and microscopic investigation method. [12]

2.4. Methods for the investigation of biofilms

In 2009, Hirshfield [7], with a humoristic tone states: as it always occurs in science, the development of technology determines the appearance of new ideas. However, once accepted in the world of science, these ideas bloom and such a history is at the basis of the existence of biofilms.

An essential aspect of the study of biofilms is represented by the fact that at the moment there are many methods for the analysis of biofilms at international level, and each has advantages and disadvantages. Among the main advantages of these methods (culture in Petri dishes, on glass slides, in plates of microtitration or metallic plates etc), one is that a various number of biofilms can be obtained on different surfaces, while the disadvantages are represented by the environmental conditions which are different from the natural ones [17]

In the case of cultivation methods in bioreactors or in installations with continuous flux, the biofilms are formed in conditions similar to those in the natural environment (dynamic conditions), but the disadvantage is the expensive materials used for the experiment 12].

Hirshfield (2009) [7] mentions the importance of a direction in the study and interpretation of their results by setting up a method for the quantification of the microscopic populations adherent to the surfaces, which will allow the existence of new data on the bacterial density after the analysis of surfaces by techniques of optic, electronic, confocal microscopy etc.

3. Results and Discussions

3. 1. Microscopy (Observation)

Light field microscopy is an essential method for the direct observation of biofilms or in histological section, as long as biofilms can be embedded in paraffin oil and sectioned by regular histological techniques. Scanning and transmission electronic microscopy cannot be used for the examination of biofilm structure [23].

Light field microscopy, transmission and confocal microscopy played an essential role in the concept of biofilm [7]

The methods for bacterium quantification are multiple and numerous synthesis papers were written about these methods, among which those of direct quantification such as epifluorescence microscopy, light field microscopy and electronic microscopy, but also of indirect quantification such as CFU counting, spectrophotometry for colored species, ATP and ribonucleic acids marking. Each method has advantages and disadvantages [12].

Optic microscopy is a fundamental method for the enumeration and observation of bacteria. The analyzed substrate must be transparent to be used in light field. Normally, the coloring is accomplished with Crystal Violet, Basic Fuchsine or Methylene

Blue. These techniques were also used for bacteria in the conditions of continuous flux in order to see the bacteria attached to the substrate in real time. The combination of the two techniques permits the determination of the number of attached cells, the covered area, the biovolume, the evaluation of bacterial growth in real time [23].

Over the past few decades, fluorescent coloring matters we used to realize systems for the image analysis which became very popular in the quantification of bacteria in natural samples, especially planktonic bacteria for opaque surfaces such as metal, plastic and ceramics. The method could be used due to epifluorescence microscopy [24].

The use of acridine orange permitted the emphasis of metabolically active cells colored in green (with DNA) and of cells with RNA (colored in orange-red). The advantages of this technique are given by the reduction by 85% of the quantification time compared to light field microscopy [23].

The analysis of images by epifluorescence microscopy is done by the CTC coloration method. The CTC coloring matter was used as an indicator for the transport of electrons in the observation of tumor cells and in order to visualize the respiration process in natural samples. After oxidation, CTC is not fluorescent, but when it is reduced by the electron activity, the coloring matter forms CTC- formasan. Using the image analysis by epifluorescence microscopy, the CTC coloration helped to visualize and quantify bacterial respiration on the surface of titanium plates [12].

To analyses the role of epifluorescence in the study of biofilm "in vitro" experiments regarding the temporal dynamics of biofilms in seawater was investigated in static conditions (in containers). All the experiments were accomplished at room temperature (22º C) in the Microbiology Laboratory within "Ovidius" University of Constanta.

The hydrophilic surfaces used for the analysis of the biofilms were represented by glass microscope slides, previously degreased with 70% ethanol and sterilized by heating at 180º C in the drying oven for one hour in order to avoid contamination with microorganisms and organic matter prior to the experiment [7]. To obtain bacterial microfilms in static conditions "in vitro" two sets of sterile plastic containers (100 ml) with 50g of marine sediment and 50 ml of sea water (culture medium) were used.

The slides were introduced according to the adapted Henrici method, where the slides were positioned in an oblique position, compared to the classical method with horizontal slides, in order to avoid the sedimentation phenomenon and the buried slide technique [13, 25]. The slides were positioned with one half in the sea water area of the containers and the other half in the sediment area [26].

To obtain data about the temporal dynamics of the biofilms, these were investigated for 18 days, with the harvesting of the slides every three days. For investigation under the bright field microscopy the slides were immersed for one minute in 1.0 % Crystal Violet solution in 100 ml distilled water, and washed twice with osmosis water, and left to dry at room temperature [27].

The slides were investigated under epifluorescence microscopy using the florochrome acridine orange 0,01% and washed twice with osmosis water, and left to dry at room temperature [27].

After harvesting, the slides were subjected to a thermal fixing in the flame for two minutes a process necessary to fix the biofilms on the hydrophilic surfaces of the microscopes slides

The slides were analyzed under Hund Wetzlar microscope in bright field and epifluorescence with 50X objective and 10X ocular. The number of bacteria was determined by means of the 10mm x 10mm micro-ocular grid (macroscopically) [28], investigating 10 microscopic fields per harvested slide. The calibration of the micro-ocular grid was realized for the objective and ocular mentioned previously and it was determined that the grid image on the microscopic field is $0.04mm^2$, a surface which represented the area for counting the cells attached to the glass slide for each microscopic field analyzed [29].

The analysis in epifluorescence of the biofilms formed on the hydrophilic surface of the glass slides collected from the containers with littoral seawater emphasized the existence of an important increase of bacterial cells density after a period of only three days from the immersion of the substrate into seawater.

Acridine orange is a nucleic acid selective fluorescent cationic dye useful for cell cycle determination. It is cell-permeable, and interacts with DNA and RNA by intercalation or electrostatic attractions respectively. When bound to DNA, it is very similar spectrally to fluorescein, with an excitation maximum at 502 nm and an emission maximum at 525 nm (green). When it associates with RNA, the excitation is maximum to 650 nm (red-orange) [12] (Fig. 1).

In figure one are the images of a biofilm formed in three different slide areas with adhered bacteria, microcolonies with exopolisaharides (EPS) the cells are dye in orange or green by the use florochrome and organic and inorganic is not sow individualized. In the sea water area there was a high percentage of green cell due to the nutrient concentration on the solid surfaces and the high number on bacteria in suspension (fig.1A, B), at the interface between seawater and sediment the orange cell form a thick layer (fig. C), visible macroscopically like line and on the sediment area a thin layer was formed and the number of adhered bacteria is much lower than in the other slide areas (fig.1 C, D).

The data about bacterial biofilm attachment and growth in static conditions was confirmed by Morató et al. after epifluorescence analyses of bacterial biofilms [30]. The rapid rhythm of biofilm formation in static conditions may be observed through the rapid formation of microcolonies in the first hour from the immersion of the slides into liquid medium.

The microcolonies are made up especially of cocci and bacilli, results confirmed by Meritt et.al. mentions that the formation of microcolonies is more favored by the static condition [22].

SEM (Scanning Electron Microscopy) is a classical observation method for the morphology of bacterial cells adherent to the surface of materials, as well as the relations between bacteria and surfaces. The technique is used especially for bacteria adherent to the surface of textile fabrics. Being a complex technique, the disadvantage is that the quantification duration is prolonged compared to other methods [23]. ESEM (Electro Scan E-3) is a modified SEM microscope where the sample room is different, as it allows the use of water vapors (10 torrs) and the hydration of the samples. ESEM permits the examination of intact biofilms through detailed analysis [24] (Fig.2).

The excess water from the immersed and washed samples permits the observation of the biofilm surface. The electron detector is based on the principle of gas ionization, thus the successive collisions among the gas molecules release more free electrons resulting a cascade of current in gas phase where the positive electron will serve to neutralize the excess electrons from the sample [23].

The microorganisms and their internal details, as well as the relations among them within the biofilm can be observed with the help of transmission microscopy (TEM) [12].The filters for biolayers were fixed in glutaraldehyde (2%), ruthenium red (2%), and cacodylate buffer solution (pH 7.2) for four hours 20 minutes. Subsequently, the samples were introduced 1/100, which is essential for optical sectioning using lens with aplano oil. In order to visualize the surface of the tube, a fluorescent aqueous solution 0.1% was used as negative coloring matter.. The bacteria were marked either with fluorescent isothiocyanate (FITC) or rhodamine isothiocyanate (RITC) aqueous solution 0.1%. For the images with fluorescence, optical filters with 488nm and 510nm were used, while for the images were transmitted by means of a processor to be analyzed [23].

A confocal microscope with argon laser, with a maximum emission of 488 nm and 514 nm wavelength, was placed in a superior position above an optical microscope. The images obtained by means of two different fluorescent coloring matters were overlapped using an excision filter and a mirror to direct the image in the photography multiplication 12].These filters can be changed without disturbing the optic arrangement of the microscope so that they permit visualization in one field in different spectral conditions. The advantages of this method include an increased resolution (Fig.3)

The ultrafine sections were then prepared for analysis at TEM microscope (Philips 300) and the samples were colored in LKB ultra coloring matter in order to observe the biofilm in detail [33] in polypropylene oxide for 20 minutes and in a mixture of polypropylene oxide/resin for one hour. The samples were placed in resin again for one hour and

in fresh resin over night at 70°C. [30].

The confocal microscopy represented a major advantage for the study of microfilms because they can be kept in a viable stage, but also hydrated intensely. Thus, it was discovered that complex structures of the biofilms exist as mushrooms or towers and are caught in the matrix crossed by canals [12] before being washed in cacodylate buffer solution (0.2 M) to pH 7.2. The samples were then postfixed in osmium tetraoxid (0.5%), and ruthenium red (0.5%) (pH 7.2) for one-two hours and then immersed gradually in 35%, 70% and 95% absolute alcohol.

Microscopy with atomic forces (AFM) is a form of sweep microscopy which uses a sample to make a continuous mapping of the samples. It is a type of field microscopy and has no resolution limits because of the diffraction effects (Fig.4). A normal AFM has a micro manufactured plate made up of silicone nitrite on a flexible console. During the normal operation mode, the sample plate is scanned so that the rejection forces should be minimal. The vibrations during scanning lead to a deviation of the console.

This is detected using a laser which reflects behind the console on a photo detector. A feed-back signal is then applied to a piezo-scanner to continuously adjust the height of the sample. Thus, the console remains fixed [33]. The voltage applied to the piezo-scanner during scanning is converted to an image with false color which shows the topography of the biofilm moisturized in excess can be deformed during the procedures for light field or electronic microscopy [12]. The careful planning of the samples before investigation is necessary because much time. The most frequent method is the indirect quantification one the images with rhodamine filters with 514nm and 580 nm were used [12] (Fig. 3).

The contrast microscopy is also an adaptation of the light field microscope. This adaptation allows the non-invasive visualization of the image without previous coloring or biofilm preparation. Images with biofilms can be obtained with high resolution leading to a 3D image as a result of the use of adequate techniques. The 3D image was obtained by the conversion of phase gradients, different from the opposed intensities of the image. A part of the object appears more luminous, while the other part appears dark on a grey background. The image must have a good contrast without forming artifacts such as halos which are often seen in phase microscopy [33].

This microscope can be modified by adding an objective with a special (modulating) filter. An opening is located in the focal plane of the condenser, which is partially covered with a polarized contrast control. The 40x contrast objectives with 0.5 opening and 100X with immersion oil were used in visualization [23]. The previously described microscope consists of the usual UV fluorescence microscope and the phase contrast microscope (DIC) with mercury lamp.

Some adaptations included the placing of the polarizing device on the opaque region of the sample in order to be better observed, the placing of a bigger support for the adjusting of the filters and the presence of mercury mirrors on the lamp rack to increase light intensity. DIC can be used to examine dense biofilms without their previous preparation.

The study objects were not covered so no slide was necessary. Thus, this technique offers essential topographic information because there is no compression of the biofilm [23]. Thus, the following coloration methods were used: acridine orange (AO), the test tubes were washed in 0.02 AO aqueous solution (BDH, UK) for five minutes before being rinsed twice in sterile distilled water. The test tubes were washed in 5mM of CTC aqueous solution for one hour before they were rinsed twice in sterile distilled water [23].

Confocal microscopie SCLM, nuclear magnetic resonance NRM, spectrophotometry with infrared ART – FTIR are a few of the most recent methods for the investigation of biofilm morphology. But access to such a technology is more difficult to obtain (Fig. 5).

3. 2. Spectrophotometry

Spectrophotometry represents a method based on the relation between the optical density obtained by different coloration techniques, generally by violet crystal or Congo red, and the counting of the colonies observed on the standard curve prepared for each bacterial species. There are two different measuring techniques:

1. The layer of attached bacteria is examined directly after coloration;

2. The bacteria are washed from the substrate and the coloration solution is examined afterwards at spectrophotometer. [12]

3. 3. The Coulter Counter Coulter

A new method to count and determine the size of bacteria is with the help of the Coulter counter/device, used initially in the counting of blood cells. This method has a few advantages compared to the optical methods:

1. the volume of each sample can be accurately counted;
2. due to small particles, the radio signals are stronger;
3. the signal is improved by a large filter of particles;
4. the counter is easy to use [12]

The Coulter device permits the determination of the total number of bacteria in a colony, while the direct counting method permits the determination of viable bacteria in samples, which is why the two methods are very often combined.

The number of adherent bacteria was estimated by the DNA measurement through a fluorimetric technique. The use of cell ureasis can be considered a marking method to observe the phenomenon of cellular adherence on various polymeric surfaces.

Confocal microscopy is more spread because of its use in the research of biofilms formed on biomaterial surfaces. Because of the wider use in the biomedical field, it was more and more used in research. Through this technique, biofilms can be investigated in situ in hydration conditions. This allows optical sectioning in order to observe the tridimentional structure of the biofilm [24]

The bacteria attached to surfaces or filtered on special paper can be colored by the method of immunofluorescence and analyzed under epifluorescence microscope. The specific antibodies are produced through the immunization of animals and are combined with a number of fluorescent coloring matters such as the content of a biofilm can be measured by a micro-test which permits the study of biofilm production by means of different types of coloring matters. The quantity of coloring matter associated to the attached bacterial cells was measured through spectrophotometry followed by the solubilization of toluidine blue with 0.2 NaOH at 85 ºC for one hour. This method is considered direct, simple and efficient for the quantification of glycocalyx [24].

An immunochemical method was developed to analyze the extra cellular film. The target antigens resulted from antibodies were located in the extra cellular matrix of bacterial cells. Thus, the tested bacterial cells were easily detached but also soluble in water. This can also be a diagnosis of bacteria that cause certain diseases [12]

3. 4. Identification

The specialized literature shows that the recognition of a biofilm was initially accomplished through the coloration of the biofilms formed on the inside part of plastic tubes with seraphim or trypan blue. This is a widely accepted and used method in the observation of biofilms. [12]

The determination of biofilm thickness and density are essential for the study of biofilms. The measuring of thickness by means of light field microscopy is widely used and it was accomplished through the focalization of the biofilm in different zones with the help of a system of lens and thickness estimation based on a curve of calibration. The use of a sample with the help of the SEM technique permits the observation of the biofilm thickness, but also of the layers of cells existing in the biofilm [36].

The samples can also be dried at 60º C, 103º C and 105º C for a few hours and weighed. The surfaces were also cleaned, dried and reweighed. The difference between the two measurements was the dry weight of the biofilm. The measurements of the thickness, surfaces and biofilm dry weight were useful in order to calculate the samples density [37].

3. 5. Quantification/Counting in Petri dishes

The method of quantification in dishes is the most used one and consists of the introduction of agar in Petri dishes and incubation at 37ºC. The number of final colonies should be around 50-300 [37, 38].

A version of this method is the method of surface spreading. This consists of 10 serial dilutions

of the initial sample. 100 µl of each dilution are sprinkled on three agar dishes. Inoculation occurs at 37ºC and then quantification. Many researchers use the method but it consumes isothiocyanate. This method can identify specific bacteria attached to the surface even in the presence of other colored species [37, 39].

4. Conclusions

The growth and harvesting methods for biofilms has known considerable development over time, but no standard models exist yet to be used in any laboratory;

The accurate investigation of bacterial biofilms could be accomplished with the development of technology and the appearance of new microscopy techniques such as electronic and confocalã.

The quantification of biofilms holds particular importance in the determination of the number of bacteria that form bacterial communities, as well as in the succession of microorganisms that form them and also in their temporal dynamics;

The light field microscopy can help when there is no confocal one, to determine the biofilm thickness. This can be accomplished by focusing on the biofilm in different areas which results in a calibration curve;

Mass and density determination can be accomplished by weighing the surfaces before and after harvesting. This method can be employed in Romania in case confocal microscopy is not available.

The analyzed methods are basic in the study of biofilms and remain a starting point for future research in the study of surface colonization

5. Refrences

[1] LAPPIN-SCOTT H.M., JASS J., COSTERTON J.W., 1993 – Microbial Biofilms: Formation and Control, Blackwell scientific publication, Oxford, pp. 336.

[2] PARSEK M.R., FUQUA C., 2004 – Biofilms 2003: Emerging Themes and Challenges Surface Associated Microbial Life, *J. Bacteriology*, 86, 4427–4440.

[3] COSTERTON J.W., LEWANDROWSKI, Z., DEBBEER, D., CALDWELL D., KORBER D., JAMES G., 1994 – Minireview: Biofilms, the customized microniche, *J. of bacteriology*, 176(8), pp. 2137-2142.

[4] NIELSEN T.T., MOLIN S., 2000 – Spatial Organization of Microbial Biofilm Communities, *Microb. Ecol.*, 40, pp. 75–84.

[5] DONLAN R.M., 2002 – Biofilms 2002: Microbial Life on Surfaces, 8 (9), pp. 881-891.

[6] LAWRENCE J.R., KOTBER D.R., HOYLE B.D., COSTERTON W., CALDWELL D.E., 1991 - Optical Sectioning of Microbial Biofilms, *Journal of Bacteriology*, 173 (20), pp. 6558-6567.

[7] CASSE, F., SWAIN, G.W., 2006 – The development of microfouling on four commercial anti-fouling coatings under static and dynamic immersion, *International Biodeterioration & Biodegradation*, 57, pp. 179-185.

[8] KUMAN A., PRASAD R., 2006 – Biofilms *Jk. Science*, 8 (1), pp. 14-17.

[9] SONAK S., BHOSLE B.N., 1995 – A simple method to assess bacterial attachment to surfaces, *Biofouling*, 9, pp. 31-38

[10] WHITELEY M., BROWN E., MCLEAN R. J.C., 1997 – An inexpensive chemostat apparatus for the study of microbial biofilms, *J. of Micro. Methods*, 30, pp. 125-132.

[11] PEDERSEN K.V., 1982 – Method for Studying Microbial Biofilms in Flowing - Water Systems, *Appl. Enviroment. Microbiol.*, 43, pp. 6-13.

[12] YUEHUEI H.A., FRIEDMAN R.J., 1997 – Laboratory methods for studies of bacterial adhesion, *J. of Microbiol. Meth*., 30, pp. 141-152.

[13] COSTERTON J.W., GEESEY G.G., CHENG K.J., 1978 – How Bacteria Stick, *Scientific American*, 238 (1), pp. 86-95.

[14] PRINGLE JH, FLETCHER M., 1983 – Influence of substratum wet ability on attachment of freshwater bacteria to solid surfaces, *Appl. Environ. Microbiol*., 45(8), pp. 11–17.

[15] MITTELMAN M.W., 1996 – Adhesion to biomaterials. In: Fletcher M, editor. Bacterial adhesion: molecular and ecological diversity. New York, Wiley-Liss, Inc., pp. 89–127.

[16] FLETCHER M., LOEB G.I., 1979 – Influence of substratum characteristics on the attachment of a marine pseudomonad to solid surfaces. Appl Environ Microbiol, 37, pp. 67–72.
[17] RIJNAARTS H.H, NORDE W., BOUWER E.J., LYKLEMA J., ZEHNDE R., 1993 – Bacterial adhesion under static and dynamic conditions, *Appl. Environ. Microbiol*., 59(32), pp. 55–65.
[18] ZOBELL C.E., 1943 – The effect of solid surfaces on bacterial activity, *J. Bacteriol*., 46, pp. 39–56.
[19] HENRICI A.T., 1933 – Studies of Freshwater Bacteria: I. A Direct Microscopic Technique, *J. Bacteriol*., 25(3), pp. 277–287.
[20] NIELSEN T.T, MOLIN S. 2000 – Spatial organization of microbial biofilm communities. *Microb. Ecol*.: 40, pp. 75–84.
[21] KINNER N.E., BALKWILL D.L., BISHOP P.L., 1983 – Light and Electron Microscopic Studies of Microorganisms Growing in Rotating Biological Contactor Biofilms, *Appl. Environ. Microbiology*, 45 (5), pp. 1659-1669.
[22] MERRITT J.H., KADOURI D.E., O'TOOLE G.A., 2005 – Growing and Analyzing Static Biofilms, Current Protocols in Microbiology, 1B.1.1-1B.1.17.
[23] SURMAN S.B., WALKERB J.T., GODDARD D.T., MORTOND L.H.G., 1996 – Comparison of microscope techniques for the examination of biofilms, *Journal of Micro. Methods*, 2(5), pp. 57-70.
[24] SUCI P.A., SIEDLEKI K.J., PALMER R.J., WHITE D.C., AND GEESEY G.G., 1997 – Combined Light Microscopy and Attenuated Total Reflection Fourier Transform Infrared Spectroscopy for Integration of Biofilm Structure, Distribution, and Chemistry at Solid-Liquid Interfaces, *Appl. Environ. Microbiol.*: 63(11), pp. 4600–4603.
[25] GEESEY G. G. AND WHITE *D. C.* 1990 – Determination of bacterial growth and activity at solid liquid interfaces, *Annu. Rev. Microbiol.*, 44, pp. 579-602.
[26] LANGMARK J., ASHBOLT N. J., SZEWZYK U., AND STENSTRÖM T. A. 2001 – Adequacy of in situ glass slides and direct sand extractions to assess the microbiota within sand columns used for drinking water treatment, *Can. J. Microbiol*., 47, pp. 601–607.
[27] RUBIO C., 2002 – Comprehension des mecanismes d'adhesion des biofilms en milieu marin en vue de la conception de nouveaux motens de prevention, These de doctorat, Paris, pp. 216
[28] HULEA A., 1969 – Ghid pentru labortoarele de micologie şi bacteriologie. Edit. Agrosilvică, Bucureşti.
[29] FRY, J.C., 1990 – Direct methods and biomass estimation. *Meth. Microbiol.* 22, pp. 41-85.
[30] MORATÓ J., CODONY F., JORDI M., 2004 – Microscopy techniques applied for monitoring the development of aquatic biofilms, Current Issues on Multidisciplinary Microscopy Research and Education, pp. 93-100
[31] http://www.cdc.go
[32] http://www.nbtc.cornell.edu
[33] Lazar V., 2003 – Microbial Adhesion, Romanian Academy publishing house, Bucharest, pp. 216
[34] http://www.nanoset.net
[35] http:// www. bionewsonline.com
[36] ZARNEA GH., 1994 – General microbiology, Microbial ecology, Romanian Academy Publishing House, 5, Bucharest
[37] LAZAR V., HERLEA V., CERNAT R., BALOTESCU M., BULAI D., MORARU A., 2004 – General Microbiology, Laboratory Protocols, Bucharest University, pp. 320.
[38] CHARACKLIS W.G., 1973 – Attached microbial growths- II. Frictional resistance due to microbial slimes, *Water Res*., 7, pp. 1249–58.
[39] ZHENG D, TAYLOR G.A, GYANANATH G., 1994 – Influence of laminar flow velocity and nutrient concentration on attachment of marine bacterioplankton, *Biofouling*, 8, pp. 107– 120.

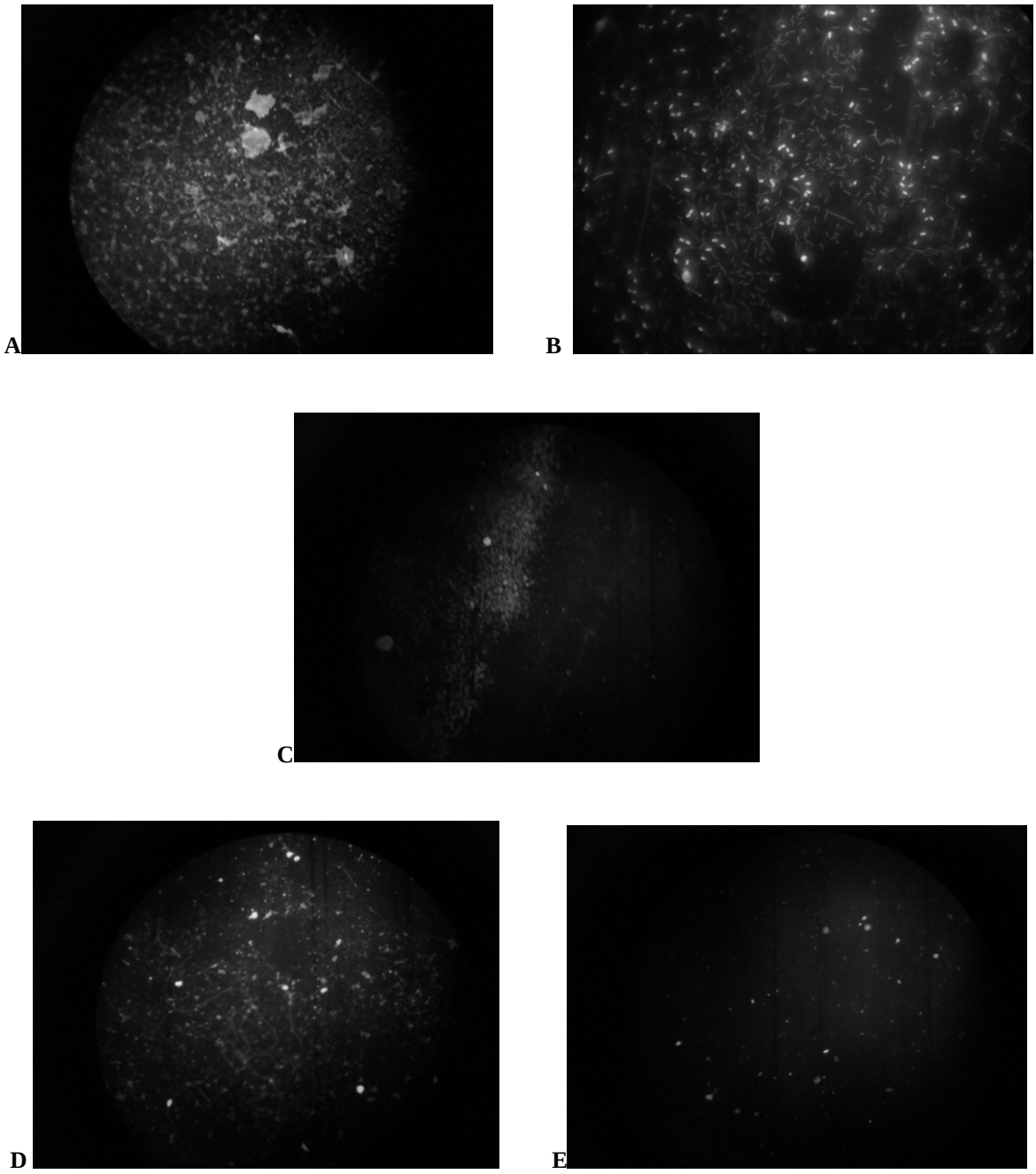

Fig. 1 – Biofilm formed after 18 days of immersion at in sea water area (A,B), interface (C) and sediment area (D,E) (500µm- Epifluorescence microscopy). Original photos

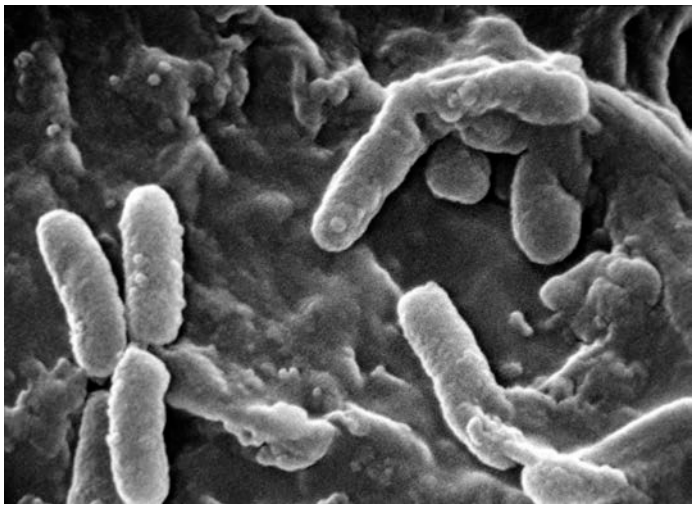

Fig. 2 – Image of a biofilm by means of electronic microscopy SEM (according to http:// www. cdc. go) [31]

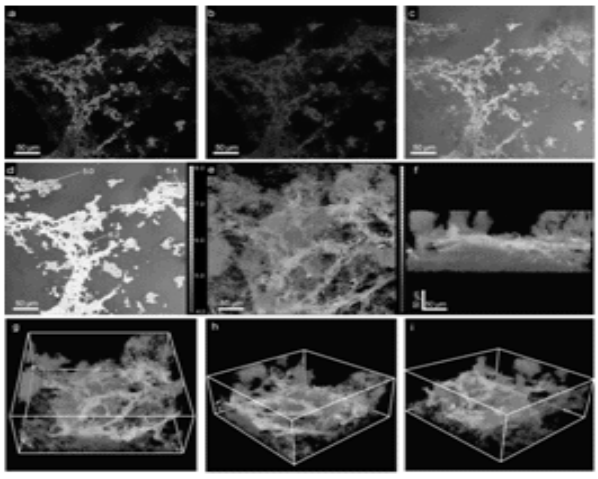

Fig. 3 – Tridimensional images of a biofilm obtained by means of confocal microscopy (according to http:// www.nbtc.cornell.edu) [32]

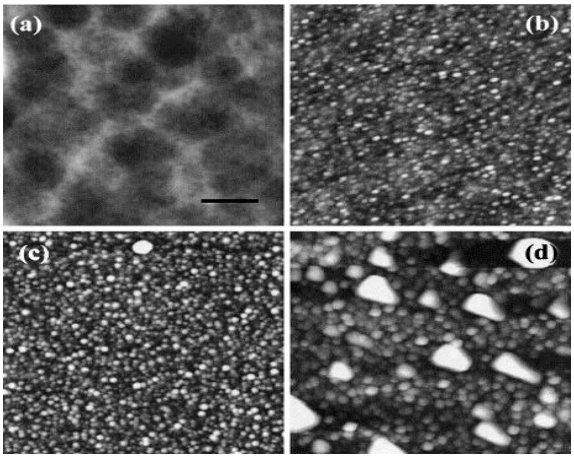

Fig. 4 – The image of a biofilm through atomic forces microscopy AFM (according to http://www.nanoset.net) [34]

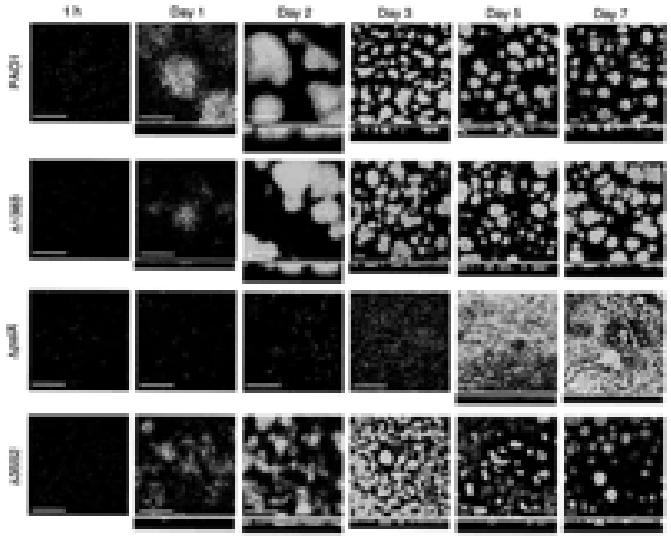

Fig. 5 – Image of a biofilm thorough confocal microscopy SCLM (according to http://www. bionewsonline.com) [35]

THE RESEARCH ACTIVITY OF THE SCIENTIST MARIA CELAN

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Abstract This paper presents the contributions to algology in particular and to the development of botany in our country in general, of scientist Maria Celan. We consider that by these contributions, Maria Celan enriched the patrimony of national knowledge and represented with integrity the Romanian science at international level.

Keywords: Maria Celan, research activity, algology

Maria Celan was born on November 20, 1898 in Salcea, the county of Soroca. She attends the Primary and Secondary School in Chişinău. Then she studies at the Faculty of Sciences – The Section of Natural Sciences within the University of Iaşi. She begins a didactic career in 1927 when she becomes an assistant professor at the Faculty of Sciences, following then the steps of university career, first at the Department of Botany within the Faculty of Agronomy and then at the Faculty of Natural Sciences in Iaşi. Her didactic activity is permanently paired by the scientific activity.

Maria Celan began her scientific activity in 1932-1933, when professor Ion Borcea, remarking her attraction for the research work, includes her in the first group of professors from Iasi invited at the Agigea Station in order to begin and organize the Romanian research activity at the Black Sea. The young assistant is entrusted the study of macrophytic algae, a field of science not yet researched in our country. She will dedicate her entire life to this study.

The results of Maria Celan's first observations in the field of macrophytic algae at our littoral materialize in articles published under the title "Notes sur la flore algologique du littoral rumain de la Mer Noire," between 1935 and 1938 in several volumes of the Bulletin and Memoirs of the Scientific Sections of the Romanian Academy [1], [2], [3] [4], [5].

In these first publications, Maria Celan presents a number of algae considered new for the Romanian littoral. These algae were harvested during field trips in different points along our littoral. However, the paper "Notes sur la flore algologique du littoral rumain de la Mer Noire IV. Deux Rhodophycees nouvelles pour la flore de la mer Noire: *Gelidiella antipae* et *Phyllophora brodiaei* (Turn) J. Ag.," published in 1937, brings more than the mere signaling of a species in our littoral waters. It brings the novelty of describing a species of red alga, new for science. The careful analysis of the macro- and microscopic characters of the thalli harvested from Cape Kaliakra, as well as the confirmation received from specialists in the field (the algologist M. J. Feldman), led to the conclusion that the alga is a new species of the genus *Gelidiella*, which M. Celan named *Gelidiella antipae*, as a tribute to the great scientist Grigore Antipa.

This is followed by the signaling of the presence of green algae (*Pseudopringsheimia confluens*, *Cladophora hutschinsiae*, *Cladophora dalmatica*), brown algae (*Ectocarpus caliacre*) and red algae (*Erytrotrichia bertholdii, E. subintegra, Acrochaetium virgatulum, A. hallandicum, A. mahumetanum*) collected from Cape Kaliakra, Agigea and Cape Midia and determined in the laboratory of the Museum of Natural History in Paris [5]. Otherwise, the lack of scientific papers in the field of algology in Romania, as well as her desire to know more in this domain, make Maria Celan take a trip for several months (between February and August 1943) to the Botanical Institute and Natural History Museum in Vienna. Then she works on her research for five years in France, at the Sorbonne University in Paris, where she approaches aspects related to the cytology and cytophysiology of

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red algae. The results of her work are materialized in 1940 by the defense of her doctoral thesis entitled: "Recherches citologiques sur les algues rouges" [6].

In the chapter "Les cellules axialles de quleques Floridees" of her thesis, she analyzes the axial cells of important genera of red algae such as *Asparagopsis, Furcelllaria, Ceramium, Delesseria, Polysiphonia, Halopitys, Laurencia*. They are described in detail and are accompanied by plates and explicative drawings with the disposition and composition of these cells: cytoplasm, vacuoles, plastids, nucleus, chondriosomes and plasmodesma.

Preoccupied by some characteristics connected to the reproduction of red algae, an important chapter of the thesis is dedicated to the study of cystocarps in certain Floridee: *Laurencia, Delesseria, Ceramium, Lomentaria, Gracillaria, Plocamium, Epilithon* (crusty alga) and *Halopitys pinastroides*. In the last one, the evolution and development of the cystocarp are described for the first time at international level. In the genera mentioned above, both the development and anatomy of the cystocarp are analyzed. The cytoplasmic content and all the cell organelles are described comparatively: plastids, chondriosomes, vacuoles, nucleus and plasmodesma. In the chapter entitled "Considerations generals," the scientist presents the results of her research on plasmodesma, nucleus and synapses in Rhodophyta in general, compared to the researches accomplished up to that date. An element of absolute novelty brought by Maria Celan through her doctoral thesis is the use of Feulgen coloration in the nucleus of red algae. The successful application of this technique contributed to the understanding of the structures of different types of cells in these algae, as well as their development and evolution. The value of the thesis was recognized by remarkable personalities of the time in the field of French botany and algology, namely the president of the doctoral commission, A. Guillermond, as well as the members of this commission. Moreover, even 25 years after the elaboration of the thesis, its value and novelty character were still acknowledged because, though her topic, Maria Celan set the bases for the cytological study of red algae.

As she returns to Romania with the doctoral title in sciences, with the qualifier "exceptionally endowed for research" obtained for the duration of her training stage in France, Maria Celan publishes a number of articles regarding the economical importance of algae in general and of the Black Sea algae in particular. These articles are meant to attract attention to these special riches of the seas and oceans which must be known and exploited [7], [8].

The continuation of the studies on the macrophytic algal flora means new contributions to the knowledge of this flora at our littoral. Marian Celan is the first algologist that signals the presence of *Rhizoclonium kochianum* in the Black Sea [9], as well as of a species of red alga, *Asterocystis ornata*, this one being noticed in natural culture where mussel valves were placed with the purpose of following the appearance and development of endolithic algal flora. The polymorphism of the species is signaled, as well as the fact that it is a rare alga, which explains, in the author's opinion, why it had not been signaled in the Black Sea before that date [10].

The more and more detailed studies on the biology and ecology of the Black Sea in general, accomplished at the Marine Zoology Station in Agigea, allow Maria Celan's further studies of the macrophytic algal flora. Thus, in 1962, the scientist publishes in the paper "New marine algae for the Romanian littoral of the Black Sea" a number of 18 new species from groups of blue algae (Cyanophyta) and green algae (Chlorophyta), [11]. In 1964, in "Note sur les algues brunes (Phaeophyta) du littoral roumain de la mer Noire," Celan describes brown algae mentioning the species *Ectocarpus caliacre* and *E. lebedii* f. *agigensis* as new for science and *Cystoseira bosphorica* and *Streblonema stilophorae* as new for the Black Sea [12], [13].

With the passing of time, the research methods in algology evolve and this requires a review of the species cited in the previous years. Thus, in 1967 Maria Celan publishes a paper in collaboration with A. Bavaru in which the authors bring new data on the morphology, anatomy, reproduction and distribution of the species of red algae *Dermatolithon cystoseire* and *Laurencia coronopus* [14]. The following year, in the festive volume of the "I. Borcea" Marine Research Station, new details are offered on the embryology of *Cystoseira* species at the Black Sea, a study that is absolutely necessary for the identification of species [15].

Among the algal phyla present at our littoral, the green algae represent a group of great interest due to the

presence of genera difficult from the systematic point of view and also due to the appreciable weights they can reach in certain environmental conditions. This is why this group of algae was intensely studied by Maria Celan who was particularly interested in the algae of the *Enteromorpha* genus.

The article "Observations sur les *Enteromorphes* du littoral roumain de la Mer Noire," published in 1975, brings important explanations regarding certain morphological characters of the stipes which facilitate the distinction between *Enteromorpha linza* and *E. intestinalis*. The article entitled "Nouvelles contributions a la connaisance des *Enteromorphes* du littoral roumain de la mer Noire," published the same year in collaboration with F. Vasiliu, describes in detail the macro- and microscopic features of the specimens collected from our littoral. The conclusion is that the *Enteromorpha* group at the Romanian sea shore displays many particular characters and the presence of numerous varieties and shapes is connected to the variability of ecological factors specific to the area [16], [17].

The morphological and anatomical characteristics, with their structural particularities and the presentation of the special ecological preferences of *E. linza*, are the object of a number of observations of Maria Celan's on this alga. Even though it is one of the species of algae identified a long time ago in the Black Sea, the author indicates that both the morphological and anatomical features are unstable as they are greatly influenced by the physical and chemical conditions of the environment [18]. The subsequent research confirms these statements. After a study on an algal population belonging to the *Enteromorpha* genus which developed abundantly in the summer of 1971 in Tomis marina, the results are that, from the point of view of the anatomical structure, namely the cell disposition, the specimens analyzed have intermediate characters between *E. compressa* (the cells are disposed regularly, in longitudinal rows) and *E. intestinalis* (in which there are rosettes made up of cells disposed in concentric circles [19].

Maria Celan was also interested in *Enteromorpha flexuosa*, with its variations present at the Romanian littoral (*E. flexuosa* f. *flexuosa*, E. *flexuosa* f. *paradoxa* [20]. Among the species of this genus, *E. flexuosa* subsp. *pilifera*, alongside another species of green alga, *Percursaria percursa*, are cited at the Romanian littoral for the first time [21].

The red algae are another group studied by Maria Celan. In the algal vegetation at our littoral, red algae of the genus *Ceramium* represent a particularly important component. Knowing how difficult it is to determine the species of this genus, Maria Celan studies in detail the algal material harvested from our littoral waters and divides them into two groups or natural complexes: "*elegans-diaphanum*" and "*rubro-circinatum*." Further on, in the delimitation of species, varieties and forms, a particular attention was paid to the ecological criteria, as well as to those of external morphology and anatomy, considering that there is a strong connection between the ecological factors and the morpho-anatomical particularities [22].

The subsequent studies on the species of the genus *Ceramium* at our littoral took into account the comparison between the "*Ceramium diaphnum – Ceramium strictum*" complex from the Mediterranean Sea, established by Feldman and the "*Ceramium elegans - Ceramium diaphanum*" complex cited for the Black Sea. The author remarks both the similitude and the differences between the two complexes, the differences being explained by the direct influence of the ecological factors characteristic to each geographical area [23]..

The specific features of the Black Sea, its particularities, so well known and so intensely studied, influence without a doubt the sea creatures and consequently, the macrophytic algae.

Thus, Maria Celan signals interesting and unusual aspects such as the mass development of certain species when others disappeared, like the case of *Desmotrichum undulatum*, an alga cited by E. Teodorescu in 1907, but which was subsequently considered disappeared at our littoral since it was no longer found here. In 1973, this alga was sighted again along the entire littoral and the observations accomplished over the next few years showed a luxuriant development in the cold periods of the year, the species being native to northern seas. The phenomenon could not be explained satisfactorily and it was supposed to be due to a number of favorable local factors. The author mentions that only further studies regarding the ecological preference of the species, as well as those connected to its reproduction type can offer explanations in this case, as well as in the case of

other algae in which similar "eclipse" phenomena were noticed [24].

Another species that aroused Maria Celan's interest was *Polysiphonia denudata*, particularly because of the increased variability of the species, depending on the ecological factors but also because of the seasonal dimorphism and the aspect of the thallus which differs in the cold season compared to the warm season [25]. A red alga belonging to the genus *Polysiphonia*, namely *Polysiphonia variegata*, attracts the scientist's attention through certain characters that were not signaled in the classical descriptions, namely the small size of the specimens developed in the pseudolittoral area and the unusual coloring (bright yellow), not signaled in this species before that date [26].

The great plasticity of certain algal species at our littoral was also observed in the brown algae. Thus, in *Ectcarpus siliculosus* modifications were noticed in relation to the aspect and number of sporangia and to the appearance of a large number of rhizoids, given that the thalli were subjected to very low temperatures [27].

Maria Celan was also interested in the origin of certain macrophytic algae in our littoral waters, therefore she begins the study of red algae of the genus *Laurencia*. In her paper, "*Laurencia caspia* A. Zin et. Zaberzh in our littoral lakes," the author considers that *Laurencia lacustris*, signaled as endemic in the Caspian Sea and present in the littoral lakes at the Romanian sea shore, can be considered a Ponto-Caspian relict, being the first among macrophytic algae to which this "status" is given [28].

Maria Celan was also interested in the study of algal associations, which she began in 1946, when she publishes the first paper in which she presents an overview of the autumn marine vegetation in Agigea [29].

More extended researches are accomplished between 1954 and 1958 in the Agigea-Eforie area. The results of these researches are published in 1958. The cited paper brings important contributions related to the characterization of the algal associations, whose configuration depends very much on the season, substrate and the hydro-meteorological conditions. Thus, the author mentions the presence of mixed associations in the cold season (spring and autumn), which are replaced by the unispecific ones, pure during the summer, as well as the qualitative scarcity of algal associations which, depending on the complex of ecological factors can be compensated by the quantitative richness. Maria Celan shares Feldman's opinion, according to which there is a major difference between the marine and terrestrial plants in terms of the interaction between the species of the same association and between them and the substrate. She also shares Borgesen's opinion which shows that different groups of algae can reunite in vast "communities" and live in similar ecological conditions, for which the term "formations" is proposed. Also, Maria Celan considers, as Morozova-Vodianitkaia, that a characterization of the algal associations cannot be accomplished without applying the methods for the quantitative evaluation of the components of the associations [30].

It is important to mention that Maria Celan signaled even at that time (1958) the qualitative impoverishment of the algal flora at our littoral, noticing the disappearance of certain species present abundantly between 1932 and 1933.

The aspect, evolution and modifications of the algal vegetation in the Agigea area represent a permanent preoccupation for the scientist and her collaborators. The paper published in 1969 signals again the reduced number of species compared to the situation existing 20 years before. However, she observes a tendency towards the restoration of the algal vegetation due to a favorable occurrence of hydrometeorological factors, to which man must contribute with a number of measures in order to weaken or eliminate the negative anthropogenic influences [31].

The study on algal associations is continued in the following period, as Maria Celan publishes alone [32] or together with her collaborators [33], [34], [35] a number of papers in which, apart from presenting the situation of the algal flora at that moment, she also signals the continuous impoverishment of the macrophytic algal flora, compensated in certain species by great biomasses and high productivity.

The more and more profound modifications of the ecological conditions in the Black Sea, especially in its north-western side, were signaled after 1970 and are due to hydrotechnical works, harbor constructions, and freshwater contributions with high amounts of

nutrients. These modifications influence considerably the algal flora. The comparative observations between the situation of 1960 and that of 1980 are the object of the following papers: “Sur l’etat actuel de la vegetation algale sur la cote nord- ouest de la mer Noire et les perspectives der recherches futures” and “Notice sur la flore marine du secteur sud du littoral roumain de la mer Noire,” published in 1981 and 1983, respectively. The latter, written in collaboration with A. Bologa, includes a list of species which comprises only 24 taxa, compared to 50 taxa cited between 1936 and 1938 [36], [37].

The description and knowledge of the algal vegetation are achieved as a result of a thorough and lengthy study. Often, problems are encountered and they are connected to the research type in marine aquatic environment, as well as the nature of the substrate, the hydro-meteorological conditions and the type of development accomplished by this vegetation: non-uniformity, seasonal occurrence, etc. Through her articles on the associations of macrophytic algae, the first of this type in the Romanian algological literature, Maria Celan opened the road to the difficult study of this important component of the littoral ecosystems. This road was then continued by her collaborators.

We cannot conclude this presentation of Maria Celan’s algological works without mentioning the paper published posthumously in 1989 and entitled “La flore algologique de la Mer Noire,” a true synthesis paper which concludes brilliantly an entire series of studies and researches dedicated with passion to the macrophytic algae of the Black Sea [38].

By her contributions to algology in particular and to the development of botany in our country in general, Maria Celan enriched the patrimony of national knowledge and represented with integrity the Romanian science at international level. Her scientific accomplishments were unanimously appreciated by Romanian and foreign researchers, and her papers represent even today landmarks and reference points for all those who study the vast, beautiful but difficult domain of the macrophytic algal vegetation

References

[1] CELAN M., 1935-1936a – Notes sur la flore algologique du littoral roumain de la Mer Noire I. Sur les *Cystoseira*, *Bull. Sect. Sci. Acad. Rom.*, Bucureşti, 17:81-96 +3 planşe (7 fig)

[2] CELAN M., 1935-1936b – Notes sur la flore algologique du littoral roumain de la Mer Noire II. Quelques mots sur le *Polysiphonia variegata* (Ag.) Zanard. de la Mer Noire, *Bull. Sect. Sci. Acad. Rom.*, Bucureşti, 17:176-183 (6 fig),

[3] CELAN M., 1937a – Notes sur la flore algologique du littoral roumain de la Mer Noire III. Quelques algues recoltees sur la portion du littoral Institut Biooceanographique du Constanţa-Cap Midia, *Mem. sect. Sci. Acad. Rom.*, ser 3:39-69, t-12

[4] CELAN M., 1937b – Notes sur la flore algologique du littoral roumain de la Mer Noire IV. Deux Rhodophycees nouvelles pour la flore de la mer Noire: *Gelidiella antipae* et *Phyllophora brodiaei* (Turn) J. Ag., *Bull. Sect. Sci. Acad. Rom.*, 19, 4-5:76-79

[5] CELAN M., 1938 - Notes sur le flore algologique du littoral roumain de la Mer Noire V. Quelques algues nouvelles pour la flore de la Mer Noire, Ag., *Bull. Sect. Sci Acad. Rom.*, 20, 4-5:84-89

[6] CELAN M., 1940 – Recherches citologiques sur les algues rouges. Thèse pour obtenir le grade de docteur es sciences naturelles, Univ. Paris, 168 pp

[7] CELAN M., 1943a – Importanţa economică a algelor. *Rev. St. ”V.Adamachi”*, 29,1:84- 86

[8] CELAN M., 1943b – Importanţa economică a algelor din Marea Neagră, *Rev. St.”V.Adamachi”*, 29, 2:204-205

[9] CELAN M., 1958– *Rhizoclonium kochianum* Kutz., algă nouă pentru Marea Neagră, *Contribuţii Botanice Univ „Babes-Bolyai”*, Cluj:55-58

[10] CELAN M., 1960- Însemnări cu privire la *Asterocystis ornata* (Ag.) Hamel, algă nouă pentru Marea Neagră , *Contribuţii Botanice Univ. ”Babeş - Bolyai”*, Cluj: 61-66

[11] CELAN M., 1962 - Alge marine noi pentru litoralul românesc al Mării Negre, *Analele Ştiinţifice ale Univ. ”Al. I Cuza”* Iaşi, sect II, Ştiinţe Naturale - Biologie tom 8, 1: 129-141

[12] CELAN M., 1964a - Note sur les algues brunes (Phaeophyta) du littoral roumain de la mer Noire, *Rev. roum. Biol. Seria Botanică*., 9, 1: 35-49

[13] CELAN M., 1964b- Notă asupra algelor brune (Phaeophyceae) de pe litoralul românesc al Mării Negre, *St. cercet. Biol. Seria Botanică* tom 16,1: 31-45

[14] CELAN M., BAVARU A., 1967 – Contribution à la connaisance des algues rouges (*Rhodophycees)* de la Mer Noire, *Rev. Roum. Biol.-Botanique* tom 2, 5: 345-36, Bucureşti

[15] CELAN M., BAVARU A., 1968 - Quelques observations sur l'embriologie des especes de *Cystoseira* de la mer Noire, *Lucr. Ses. Ştiinţifice a Staţiunii de Cercerări Marine "Prof. I. Borcea*" Agigea, vol. festiv al Univ "Al. I. Cuza Iaşi: 95-100

[16] CELAN M., 1975 – Observations sur les *Enteromorphes* du littoral roumain de la Mer Noire, *Rapp. Comm.int. Mer Medit.* 23, 2: 67-68

[17] CELAN M., VASILIU F., 1975- Nouvelles contributions a la connaisance des *Enteromorphes* du littoral roumain de la mer Noire, *Cercetări marine*, IRCM, 8: 83-89, Constanţa

[18] CELAN M., BAVARU A., 1967- L'*Enteromorpha linza* (Linne) J.Agardh. du littoral roumain de la Mer Noire, *Rev. Roum. Biol. Ser. Botanique*, tom.12,1: 23-26, Bucureşti

[19]. CELAN M., BAVARU A., 1977- Sur une population de l'*Enteromorpha linza* (Linne) J.Agardh du Port de Tomis, Constanţa - *Rev. Roumaine Biol., Biol. veg*. tome 22,1:33

[20] CELAN M., 1979a- Les *Enteromorphes* du groupe *flexuosa* Bliding sur le littoral roumain de la Mer Noire – *Cercetări marine* IRCM, 12:115-119, Constanţa

[21] CELAN M., 1979b - Deux algues vertes (*Chlorophyceae*) nouvelles pour le littoral roumain de la Mer Noire, *Cercetări marine* IRCM, 12: 109-113 Constanţa

[22] CELAN M., ŞERBĂNESCU G., 1959 - Sur les *Ceramium* de la Mer Noire- *Lucrările Sesiunii de Comunicări a Staţiunii Zool. Marit*. „Prof I.Borcea” Agigea, 1: 531-562, Constanţa

[23] CELAN M., 1981a – Sur le complexe *Ceramium elegans* Ducl.- *Ceramium diaphanum* (Lightf.) Roth. dans la mer Noire, *Rapp. Comm. int. Mer Medit*, 27, 2:231-232

[24] CELAN M., BAVARU A., 1976 – Sur le développment en masse sur le littoral roumain de l'algue brune *Desmotrichum undulatum* (J. AG.) Reinke, *Cercetări marine*, IRCM, 9: 95-99, Constanţa

[25] CELAN M., 1981b - Notice complementaire sur le *Polysiphonia denudata* (Dillw.) Kutz (Rhodophyceae, Rhodomelaceae), *Rapp. Comm .int. Mer Medit*, 27, 3:227-229

[26] CELAN M., BAVARU A., 1966 - O formă de pseudolitoral a speciei *Polysiphonia variegata* (Ag.) Zanardini, *St. şi cercet. Biol. Seria Botanică*, Bucureşti, tom.18, 4: 325-330

[27] CELAN M., BAVARU A., 1967- O formă curioasă de *Ectocarpus siliculosus* (Dillwyn) Lyngb., *St.şi cercet. biol. Seria Botanică* tom 19, 3: 215-219, Bucureşti

[28] CELAN M., BAVARU A., 1972 - *Laurencia caspia* A. Zin et. Zaberzh în lacurile noastre litorale *-Lucrările Ştiinţifice - Ştiinţe naturale Inst. Ped. de 3 ani*.: 95-100, Constanţa

[29] CELAN M., 1948 – Despre vegetaţia algală de la Agigea (Marea Neagră) în cursul lunilor Septembrie- Noiembrie 1946, *Bull. Inst. Polytech*. Iassy, 4: 340-351

[30] CELAN M., 1958-Noi contribuţii la cunoaşterea florei şi vegetaţiei Mării Negre *Analele Univ "C.I.Parhon"* Bucureşti,Seria St. Naturii,17:77-93

[31] CELAN M., BAVARU A., ELEFTERIU R., 1969 – Sur la vegetation algale à Agigea pendant le mois d'octobre 1968, *Lucrările Staţiunii de Cercetări Marine* "Prof. I. Borcea" Agigea 3: 59-64

[32] CELAN M., 1977 – Sur l'appauvrissement de la flore algale des côtes roumaines de la Mer Noire, *Hidrobiologia*, tom 15: 61-64, Bucureşti

[33] CELAN M., BAVARU A., 1973 - Aperçu general sur les groupements algaux des cotes roumaines

de la Mer Noire, *Rapp. Comm.int. Mer Medit*. 21, 9:655-656

[34] CELAN M., BAVARU A., 1978- Sur l'état actuel de la vegetation algale du littoral roumain de la Mer Noire, *Cercetări marine*, IRCM, 11: 85-90, Constanţa

[35] CELAN M., BAVARU A., BOLOGA A.S., 1979 - Sur l'état de la vegetation algale macrophyte du littoral roumain de la Mer Noire, pendant la periode estivale de l'annee 1977, *Cercetări marine*, IRCM, 12: 121- 128, Constanţa

[36] CELAN M., 1981c- Sur l'etat actuel de la vegetation algale sur la cote nord- ouest de la mer Noire et les perspectives des recherches futures, *Rapp. Comm. int. Mer Medit*, 27, 2:155-156

[37] CELAN M., BOLOGA A.S., 1983 – Notice sur la flore marine du secteur sud du littoral roumain de la mer Noire, *Rapp. Comm.int. Mer Medit*, 28, 3:215-217

[38] CELAN M., 1989 – La flore algologique de la Mer Noire, *Cercetări marine*, IRCM, 22: 5-106, Constanţa

INSTRUCTIONS TO AUTHORS

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Table 1 – Taxonomic analysis of identified species

Orders	**Families**	**Species number**
Pinales	*Pinaceae*	6
	Cupressaceae	6
	Taxaceae	1
	Taxodiaceae	1

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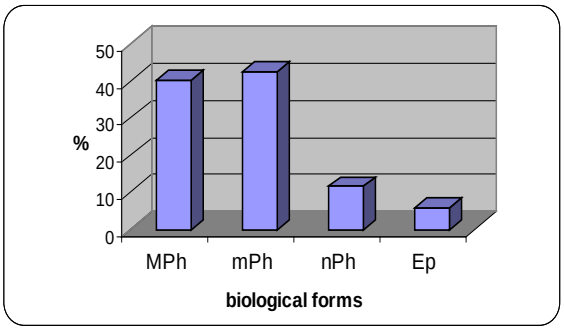

Fig. 1 – The life forms spectrum

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[1] BAKER C.D., REEVE M.R., 1974 – Laboratory culture of the Lobate Ctenophore *Mnemiopsis maccradyi* with notes on feeding and fecundity. *Marine Biology*, 96: 57-62.

[2] VALIELA I., 1995 – Marine Ecological Processes, 2nd edition, Springer-Verlag, New York, 686 pp.

[3] LUND J.W.C., 1966 – Summation., in C.H.Oppenheimer (ed.). Marine Biology, Vol. 2: 227-249, New York Academy of Science.

[4] PETROVA A.., VLADIMIROV V. (eds.), 2009 – Red List of Bulgarian vascular plants, *Phytologia Balcanica*, 15 (1): 63–94.

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