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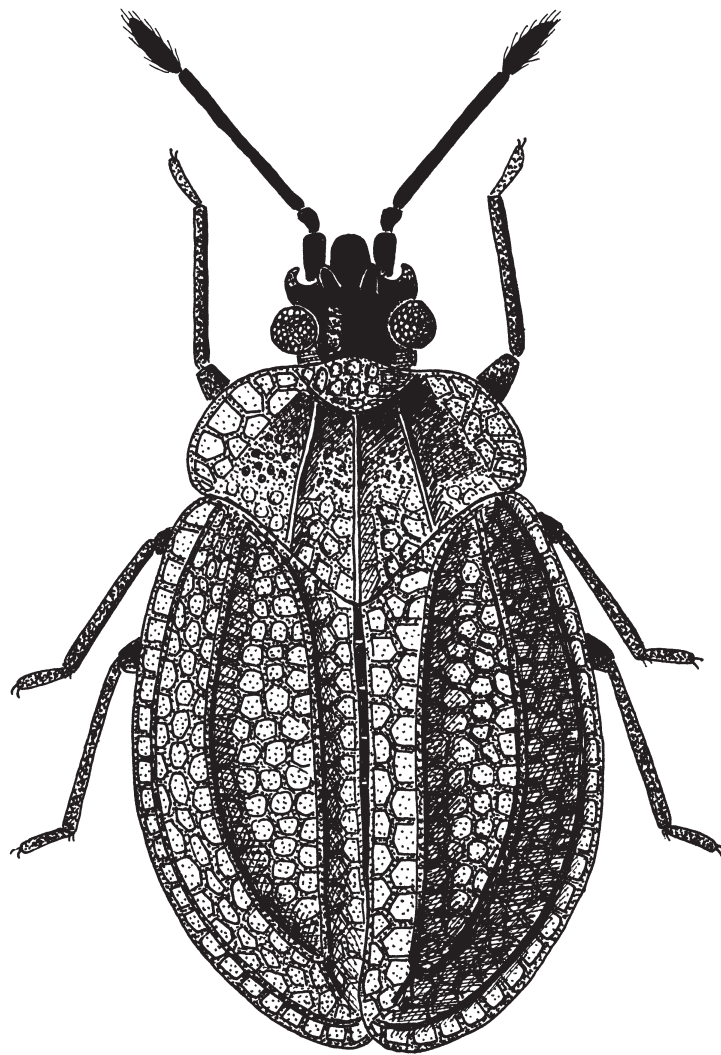
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ECOLOGICAL IMPLICATION OF PLANT TRAITS, STRATEGIES AND COMPETITIVE ABILITIES OF HERBS

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Abstract. Natural selection generally favors plants whose forms and physiology tends to maximize their net rate of growth, because plant uses resource to reproduce and compete for additional space. Above ground characteristics of a plant often greatly affect its competitive abilities and population dynamics rather than below ground because drymass of a plant depends on leaf strategy, where as root in the top soil is responsible for exploitation, competition through depletion of a common pool of resources so fine roots are also important. The use of root/shoot ratios to describe allocation of dry weight to structures for capturing soil resources and light is limited due to other functions of the root and shoot such as storage and support. Dry matter allocation, specific leaf area, root length and different ratios were determined for 17 herb species by extraction of soil cores from an intact forest. Interspecific values of RLA were considered. It has been hypothesized that the co-variation among species in leaf trait and other morphological adaptations particularly leaf and growth rate reflects a set of mutually supporting traits that interact to determine plant identity in similar climatic conditions.

Keywords: *Herbs, above ground characteristics, dry matter allocation, SLA, RLA, root length, root: shoot ratio*

Introduction

The recognitions in ecology of the significance of individual species level of reduction or what species do in ecosystem is important [21]. Although, It is well recognized that plant species differ in taxonomic status and ecological strategy [7, 17]. The presence of a plant species in a forest community is determined by many ecological traits of the target species, as well as those of surrounding species as its competitors [19] because of species interacts with both its biotic and abiotic environments [35, 36]. Both biotic and physical factors determine the growth of plant species in any habitat and the composition of plant communities. The relative importance of these two categories of factors may vary with the successional position of the community and the time scale in which it is considered. Perhaps so the fundamental niche of a species in the contribution and resources allow the species to maintain viable population [4]. Parrish and Bazzaz (1982) [26] also have observed that physical factors such as disturbance, creates, have shaped the niches of early successional species, and biotic factors, including competition have relatively more impact on the evolution of niches of successional species.

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Earlier, Hall (1974) [11] stated that the growth of plants in a multi-specific community in typically influenced in some or all stages of development by biological and physical processes which are frequently due to competition, which results in physical effects. Variation in relative competitive abilities among environments has been proposed to control the species composition of plant communities. Since the relative performance of different species in any given interaction in a given environment is governed to a large degree by plant traits [6, 16], a logical extension of the comparative approach is the utilization of plant traits across several species for predicting species performance [34]. A major spectrum of variation runs between species with traits that favor nutrient conservation and those with traits that allow rapid short term growth [39]. Wilson and Tilman, (1995) [38] stated that within any given habitat, species with a range of leaf traits may coexist.

Few experimental studies have succeeded in partitioning the effects of roots and shoot competition in an intact sward [8]. However, Tilman (1988) [32] assumed that the competition intensity is unaffected by plant productivity though the ratio of above ground to belowground varies and further he stated that root/ shoot ratio may be used to quality allocation to structure for capturing light and soil resources, but this does not focus the other functions of both the root and shoot such as storage and support system. Much attention has been directed towards the edaphical nature of energy allocation pattern in plant under various environmental studies. The distribution of biomass among the vegetative parts is also subject to variation [1] suggested that the pattern of allocation will depend on the nature of the living factor.

The purpose of the present study was to investigate through using a range of 17 herbaceous plant species of several ecophysiological traits measured for each of the species, the degree to which the various types of interactions outlined above were related to particular traits, and which combinations of traits may have potential for preceding outcomes of interactions involving plant species and the ultimate goal was to investigate the range values of root length/ leaf area ratios for number of representative species growing at study site.

Materials and methods

Study site

The samples were taken from the evergreen oak (Quercus leucotrichophora) forest at 2200m altitude with 290 23' N latitude and 79026'-79027'E longitude with south facing slope.

Oak forest soil is residual brown earth with acidic pH-6, 4.5% carbon, 0.5% nitrogen content and 56-79% water holding capacity The annual rainfall is 248 cm of which 75% occur in rainy season, Mean daily temperature ranges from 7.8 to 21.20°C (Poonam Mehrotra, 1998).

Herb species were selected for the detailed study on the basis of their high density (> 4.0 plants/ m²) at the site (Poonam Mehrotra 1988), following Misra (1968). Plant species characteristics were sampled during the first week of October (peak of growing season) and ten individuals were randomly sampled for each species. The plants were carefully excavated from the surroundings and separated into different components viz., belowground, main root, fine roots, stem, leaves, and fruit / flower. Each component was dried at 800°C until constant weight and weighed.

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The categorization of forms in different growth forms, except fern and sedge, species were categorized into different growth forms following Givinsh (1987); Poonam Mehrotra (1998) [13, 28]. The categorization was based on the appearance of aerial stem, presence of branching, lateral branching and foliar arrangement on supporting systems—1.- Erect form: Attains peak growth after summer. Here the plant has scattered leaves along a vertical, indeterminate axis and finally have a multilayered crown. 2.- Basal form: These plants have very short aerial shoot; leaves emerge near the ground level with well developed petioles. 3.- Arching form: This growth form differs from erect ones in having a long arching stem and is present generally at hill slopes with acute slope angle. 4.- Sprawling mat: these are characterized by small leaves placed in close proximity to the ground surface. 5.- Umbrella forms: Species arrange thin foliage in a variety of umbrella like structures including branching and lateral branching. 6.- Special umbrella form; this is slighty different to that of umbrella growth form. In them, an umbrella is formed by compound leaves which are directly attached to with the stem through minute petioles.

Results

Seventeen herb species at the site were studied. Artemisia nilagirica var. septentrionalis (Clarke) (Pamp.), Arisaema concinnum (Schott), Gerbera gossypina (Royle) (G. Beauv.), Onychium cryptogrammoides (Wall. ex Hope), Anemone vitifolia (Buch.-Ham) (DC.), Calamintha umbrosa (Linn.), Carex cruciata (Wahlenb), Thalictrum foliolosum (DC.), Cnicus argyranthus (Clarke), Geranium wallichianum (D.Don), Strobilanthus alatus (Muell.-Arg.), Sonchus asper (Garsault), Valeriana wallichii. (DC.), Ainsliaea aptera (D.C.), Roscoea procera (Wall.), Hedychium spicatum (Smith) and Aralia cachemrica (Decne).

These herb species showed a wide range of taxomonic placements as well (Table 1) i.e. from primary family Ranunculaceae to Asteraceae an advanced family in dicots and Aracaeace and Zingiberaceae in monocots. These species varied in leaf longevity i.e., Arisaema, Roscoea were among the deciduous habit and others like Aralia, Artimisia, Cnicus Strobilanthus, Gerbera, Sonchus, Valeriana approaching towards the evergreen habit (leaf life span <310 days) [28]. These seventeen herbs belonged to different growth forms. (Table1). Four herbs belonged to erect form, seven to basal, and two to umbrella one each to arching, special umbrella, sedge and fern growth forms respectively.

Percent allocation of drymass in different components

Givnish, (1987) stated that the allocation of dry mass of a plant depends on leaves strategy because leaf provide outstanding opportunities for competitive studies and their extraordinary variation in form, physiology and phenology have implications for not only carbon exchange but also water loss, allocation to above vs. below ground tissues, interactions with competitors and whole plant growth.

It had been observed that herbs wich like shady places differed from the others in having a greater proportion of their dry weight (above 60%) in above ground parts. i.e., Arisaema, Calamintha Strobilanthus, Roscoea, Geranium with the exception of Hedychium and Onychium (Fig.1). These two were rhizomatous plants and differed

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Table 1. *Characteristics of the seventeen herb species.*

S.N.	Species	Family	Growth form
1	Artemisia vulgaris	Compositeae	Erect
2	Anemone vitifolia	Ranunculaceae	Erect
3	Calamintha umbrosa	Labiateae	Small erect
4	Roscoea procera	Zingiberaceae	Erect
5	Geranium wallichianum	Geraniaceace	Basal
6	Ainslaea aptera	Compositaea	Basal
7	Gerbera gossypina	Compositaea	Basal
8	Sonchus asper	Compositaea	Basal
9	Valeriana wallichianum	Valaerianaceae	Basal
10	Cnicus argyranthus	Compositaea	Basal erect
11	Thalictrum foliolosum	Ranunculaceae	Umbrella
12	Strobilanthus alatus	Acanthaceae	Umbrella
13	Arisaema cancinnum	Araceae	Special umbrella
14	Aralia cachemerica	Araliaceae	Sprawling-mat
15	Hedychium spicatum	Zingiberaceae	Arching
16	Carex cruciata	Cyperaceae	Grass
17	Onychium contiguum	Polypodiaceae	Fern

Table 2. *Values of fine root length, BGR (Belowground ratio), RLA (ratio of fine root length to leaf area) and Fine root weight. Values in parentheses indicate standard error (±).*

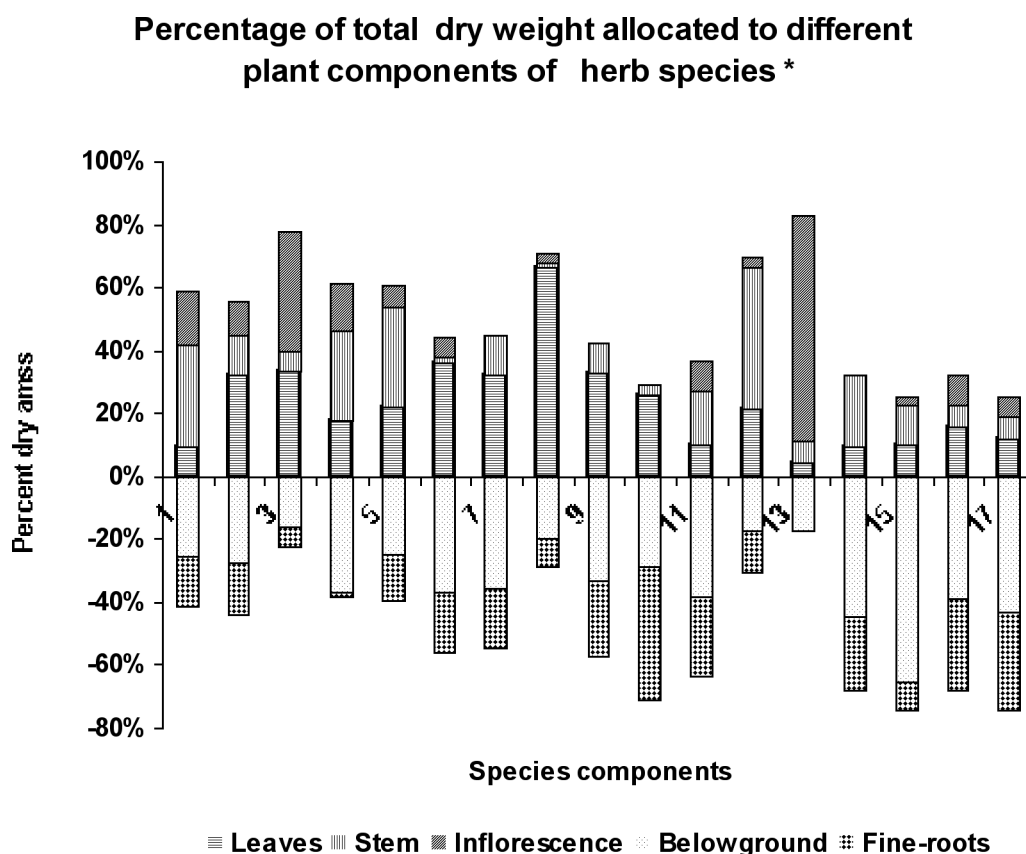| S.N.	Species	Fine root length (cm)	BGR (g/g)	RLA (cm/m²)
1	Artemisia	7.14±0.315	0.38	0.030
2	Anemone	22.44±1.7	0.39	0.404
3	Calamintha	19.16±1.324	0.265	2.514
4	Roscoea	15.06±0.735	0.379	14.678
5	Geranium	9.532±0.542	0.281	0.549
6	Ainslaea	11.9±0.314	0.462	1.226
7	Gerbera	11.42±0.99	0.438	0.293
8	Sonchus	8.94±0.635	0.211	0.262
9	Valeriana	17.1±2.531	0.48	0.289
10	Cnicus	24±1.619	0.498	0.216
11	Thalictrum	4.13±10.334	0.51	3.473
12	Strobilanthus	22.32±1.262	0.203	0.416
13	Arisaema	5.84±0.816	0.17	0.260
14	Aralia	6.88±0.428	0.57	0.356
15	Hedychium	18.7±1.333	0.73	0.0486
16	Carex	12.61±0.76	0.552	0.101
17	Onychium	7.21±0.475	0.628	0.097

Percentage of total dry weight allocated to different plant components of herb species *

≡ **Leaves** ⫼ **Stem** ▨ **Inflorescence** ⋯ **Belowground** ⊞ **Fine-roots**

Figure 1*: *Species:1 Artemisia, 2 Anemone, 3 Calamintha, 4 Roscoea, 5 Geranium, 6 Ainslaea, 7 Gerbera, 8 Sonchus, 9 Valeriana, 10 Cnicus, 11 Thalictrum,12 Strobilanthus, 13 Arisaema, 14 Aralia, 15 Hedychium, 16 Carex, 17 Onychium.*

from the others allocating greater proportion of their dry weight to below ground part (more than 55% of dry mass). Thalictrum, Carex and Onychium had well-developed absorptive system and allocated a significant dry mass to fine roots. The allocation of drymass to different above ground parts are shown in Fig. 1. The percent of drymass allocation to above–ground vegetative components ranged from 11 (Hedychium) to 73% (Sonchus) for leaves and from 1(Sonchus) to 52% (Strobilanthus) for stem.

Among the growth forms erect, basal, umbrella forms had greater investment to the above parts significantly to the photosynthetic component (leaf) except special umbrella (Arisaema) which allocated its >70% dry matter to its reproductive efforts. The reproductive efforts of species ranged from 3 (Hedychium) to 72% (Arisaema).

The maximum drymass allocation for leaves of Sonchus might be due to its basal habit having very low plant heights, to harvest more filter light for its survival through spreading its leaves. Whereas Hedychium, having well developed long massive stem by which it kept high leaf height, did not face any competition for light. So it allocated minimum drymass to its photosynthetic component as compared to supporting system.

Dry matter partitioning within root system

In general, the main root systems were thick with few, sparsely branched thin lateral/vertical secondary roots. Tables 2 and 3 show characteristic of the root system of dif-

ferent herb species. All 17 herbs differed in below ground system having tap root /adventitious /tuber / rhizome. Table 2 and 3 shows that, the main root in some species had greater mass accumulation for storage function than in the fine roots (absorption). The percent of allocation of dry mass in secondary roots ranged from 0.07 (Arisaema) to 74% (Cnicus).

Fine root length ranged from 4.13 (Thalictrum) to 22.44 cm (Anemone) (Table 2). The growth of secondary roots totally depends on habitat conditions. Generally the harsh or low water potential promotes a minimum growth of secondary roots. This trend is just opposite to present study. It might be due to that the roots of herbaceous plants generally go 25-30 cm deep expect xerophytes and they want to utilize every drop of natural water. So they tend to increase the surface area of below ground system for the absorption of more water and due to this, plants growing at low water potential site show a significant growth of secondary roots.

Arisaema likes mesic as well as filter light area. So in this area soil moisture content in microclimate always remains more than enough. Hence plants never feel scarcity of water.

Exchange surface: mass

This gives thee explanation for the ratio of investment in structure for light capture for photosynthesis and the absorption of soil resources by a plant. This ratio can be measured by specific leaf area and specific root length. These express the size of exchange surface (leaf surface) attained by investment of unit dry weight [24]. Values of SLA are shown in Table 4 and specific root length of fine root length for all plants is given in Table 3. Among the species SRL (fine roots) ranged from 0.243 cm/mg (Cnicus) to 0.255 cm/mg (Roscoea). The low value of SRL for fine roots suggests low surface area and limited investment of dry matter. Cnicus showed the lowest SRL, which is a primary succession-al species and representative of harsh degraded site, their root length became more than their accumulation of mass. To tolerate the adverse conditions, their storage system becomes more massive than the absorptive system. Like, in case of Roscoea, which prefers mesic conditions, where soil moisture content is not a limiting factor. So in these species belowground absorptive component was well developed and the number of fine roots as well as their length became reduced. Similar observation has been seen int he exemple of other plants which like mesic conditions, such as Calamintha, Geranium and Valeriana.

The specific leaf area denotes the dry mass cm^2 in photosynthetic tissue. It is recip-rocal to species leaf mass, which is directly correlated with leaf longevity of herb plants [27]. In the study, this trend was reverse to earlier reports, in which the plants growing at harsh site had more SLM than SLA.

In general, mesic conditions favor the low investment to leaf thickness of a plant because they devoid of external growth, hair, spines, a layer of fibers etc. These mor-phological adaptations may promote large SLA but low SLM in a plant. The plant with long leaf longevity, growing at harsh soil conditions (low water potential) allocate dry-mass to their photosynthetic system as well as supporting organs. Across species max-imum SLA were represented for Artemisia (697.369 cm^2/g) and minimum for Ainslaea (8.09 cm^2/g) (Table 4).

The maximum SLA of Artemisia showed also its low investment of dry mass to photo-synthetic components and tended to increase number and area. Whereas Ainslaea had small surface area, which might be due to that this species has two times flowerings, which are in

Table 3. *Characteristics of the root system for the herb species. Main root dry wt., Fine root weight and SRL (specific root length) of herbs and values in parentheses indicate standard error (±).*

S.N.	Species	Type of root system	Main root dry wt (gm/plant)	Fine root weight (gm/plant)	SRL (cm/mg)
1	Artemisia	Tap root	10.82±0.227	17.323±0.154	0.007
2	Anemone	Rhizome	8.526±1.34	17.519±0.926	0.019
3	Calamintha	Rhizome	5.272±0.042	3.088±0.471	0.126
4	Roscoea	Rhizome	13.85±1.196	0.046±0.005	0.255
5	Geranium	Tap root	1.885±1.34	2.72±0.356	0.177
6	Ainslaea	Tuber	22.02±1.596	11.6±0.635	0.029
7	Gerbera	Rhizome	9.982±0.593	11.612±0.29	0.010
8	Sonchus	Tuber	5.437±0.572	4.886±0.735	0.016
9	Valeriana	Rhizome	5.3882±0.766	9.12±1.38	0.074
10	Cnicus	Taproot	28.008±6.18	228.17±3.076	0.243
11	Thalictrum	Taproot	7.514±.234	15.43±1.085	0.003
12	Strobilanthus	Taproot	6.151±2.10	39.511±3.647	0.005
13	Arisaema	Tuber	31.324±0.89	0.129±0.005	0.146
14	Aralia	Tap root	12.58±1.019	14.746±1.595	0.013
15	Hedychium	Rhizome	223.82±6.108	34.841±2.035	0.004
16	Carex	Taproot	8.882±0.351	24.768±0.377	0.055
17	Onychium	Rhizome	12.229±0.74	29.728±0.943	2.04

winter and second during cold early spring [28]. So perhaps it required a significant amount of organic mass in photosynthetic component for its extra reproductive efforts.

The low values of SRL for coarse root of a plant may reflect the role of these roots as storage organs. The ratio of fine root length to leaf area of plant will be influenced by the characteristics of the plant and its environment.

Root length: leaf area

The leaf area together with pigment concentration plays a key role in ecosystem functioning. Gholze et al., (1982) [11] suggested that leaf area is of great importance to model ecophysiological process and growth efficiency, and leaf enlargement is highly sensitive to water stress. It is one of the first growth processes to be affected by a decrease in leaf water potential [18].

RLA is influenced by the changes of condition. In winter the ratio becomes infinite because due to the above ground shoots die back completely. The ratio of fine root length to leaf area of plant will be influenced by the characteristics of the plant, its environment and the characteristics of site i.e., fertility and moisture content and longevity of the plant both significantly effect the RLA of plants.

Across, species RLA ranged 0.03 (Artimisia) to 2.514 cm/cm^2 (Calamintha)(Table 2). Calamintha is a short herb which generally prefers shady places, whereas Artemisia is a primary successional species growing at open eroded-xeric sites but the leaf area (per leaf) was higher in Artemisia than Calamintha. This result did not follows the thumb rule of Cowling and Cambell (1980), Anderson (1961) and Givnish (1984) [2, 9, 12], where they stated that the effective leaf size increased with shade as well as along

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Table 4. *Characteristics of total dry weight, TLA (total leaf area/plant), LAR (leaf area Ratio), SLA (specific leaf area), and LWR (leaf weight ratio) of seventeen herb species. Values in parentheses indicate standard error (±).*

S.N.	Species	Total dry weight (gm/plant)	TLA (cm²)	LAR (cm²/gm)	SLA (cm²/gm)	LWR (gm/gm)
1	Artemisia	74.49±2.4	7506.4±1755.0	100.700	697.360	0.144
2	Anemone	69.123±5.8	817.0±71.8	11.819	44.138	0.267
3	Calamintha	31.895±2.8	147.84±14.4	4.635	8.297	0.558
4	Roscoea	38.06±4.5	554.4±45.6	14.560	14.560	0.187
5	Geranium	6.554±1.3	1294.0±66.3	78.969	216.270	0.365
6	Ainslaea	93.282±7.5	347.68±333.8	3.727	8.090	0.458
7	Gerbera	48.524±1.7	1377.0±148.6	28.377	72.360	0.392
8	Sonchus	49.854±4.7	638±36.8	12.797	17.620	0.726
9	Valeriana	30.515±3.8	918.86±61.7	30.110	74.720	0.402
10	Cnicus	633.415±12.6	7649.3±663.6	12.066	265.160	0.045
11	Thalictrum	45.433±4.6	439.54±335.8	9.674	76.040	0.127
12	Strobilanthus	190.20±12.2	5030±283.7	26.430	108.200	0.245
13	Arisaema	179.29±5.6	382.06±23.9	2.130	50.934	0.040
14	Hedera	46.711±4.1	516.26±51.7	10.990	90.930	0.122
15	Hedychium	354.17±14.7	2744.1±156.1	7.747	70.590	0.109
16	Carex	60.881±3.7	4105±365.9	67.426	310.900	0.216
17	Onychium	65.26±2.7	1578.26±149.3	24.190	140.530	0.172

increasing soil fertility, and decreased with irradiance and disturbance.

The RLA differences are expected to be particularly higher for with low height [22]. The lowest values of RLA were found in species found in disturbed fertile soils whilst the highest values were at infertile soils [23]. Grazing is another factor, which greatly influences leaf area. In general, low altitude species had RLA values in the range of 240-500m/m², whilst high altitude species had values in the range of 1000-2000m/m².

Poorfer and Remkes 1990 [29] investigated the early growth of seedlings of 24 species grown on solution-culture of optimal nutrient supply. Negative relation between growth rates vs. RLA can be observed, since grasses had values of RLA in the range of 3000-10,000m/m², forbs had values in the range of 1000-4000m/m².

Korner and Renhardal (1987) [20] used the ratio of root length to leaf area (RLA) to express the relative sizes of structures. Interspecific plasticity in RLA has been shown for a number of grass species grown under high nutrient concentrations having a lower value of RLA by Boot and Mensink (1991) [3] and concluded that species characteristic on infertile soils exhibit greater plasticity in RLA than on fertile soil. They reported a negative correlation between RLA and nutrient availability.

Discussion

Natural selection would generally favor plants whose form and physiology tend to maximize their net rate of growth because such plants use resources with which to reproduce and compete for additional space [25].

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The forbs, which all have rosette form, showed little investment in stems [24] because species have a rosette of more or less horizontal wide leaves and are effective in casting shade. They also stated that graminoides show low percent allocation to belowground structure and no obvious differentiation of tissues for storage and differ from the forbs in having a greater proportion of their dry weight in above ground parts. Species with massive belowground parts – nutrients stored in the root system – may provide gerater part of energy for aboveground productivity [23].

Among the growth forms, the observation for different parameters showed that at erect forms, photosynthetic as well as absorptive systems were well developed i.e. they showed maximum value for leaf area ratio, specific leaf area, ratio of fine root length to leaf area and specific root length. They had relatively large sizes of above and below ground exchange surface, and this feature made them well adapted for any situation.

The short plants like basal forms as rosette, faced extreme hot and cold condition as compared to other. During hot weather, the tolerate high temperatures and evaporation by keeping leaves overlapped, and in winter – with leaves just above soil surface – they can maintain optimum temperature for their physiological activities.

The maximum drymass allocation for leaves of Sonchus might be due to its basal habit having a very low plant height, so, by spreading its leaves, it tres to harvest more filter light for survival. In addition, their absorptive system was also well developed, compared to others to tolerate the extreme situations of environment.

In arching form, which prefers well developed soil profile with mesic conditions – but they may occur between boulders under filter light conditions – their below ground rhizomatous system was well developed, they accumulated more than 70% of their energy in it. However, they also can be found in dim/ filter light. So light factor can be a competitive factor for them, but their arching, long, supporting system helps during adverse light /conditions. So these plants do not face any danger for their existence and used only 5-10% of their energy for reproduction. They occur generally under canopy between boulders but on mesic soils. So they do not face any harsh condition during their life cycle, so they invest much of their energy to storage system rather than other systems. Thus, they allocated only 9-10% of drymass to their reproductive systems.

The well branched plants of umbrella forms inhabited shady places mostly under canopy area where they have almost optimal water potential conditions of soil. They spend much of their energy to their supporting system for intercepting every ray of light. These preferred dim light, well developed soil profile with mesic conditions. So they develop well-branched supporting system to use every ray of light in deep forests. In special umbrella form, the pattern of the allocation of dry mass to reproductive components showed the highest reproductive efforts (Arisaema), living on mesic soil and under dense canopy. This helps the plant to avoid harsh, unfavorably extreme conditions, since it is very short aboveground life span ends before extreme cold could weather commences. So both biotic and physical factors determine the growth of plant species in any habitat and composition of plant communities. The relative importance of these two categories of factors may vary with the successional position of the community and the time scale in which it is considered. It is assumed that physical factors, such as disturbance, have shaped the niches of early successional species, while biotic factors, including competition have had relatively more impact on the evolution of niches of successional species. Tilman and

Downing, (1994) [33] state that annuals should have lower competitive effect than perennial

The above ground characteristics of species often greatly affect their habitat tolerance, competitive abilities, and population dynamics. The morphology of underground structures also has important ecological consequences [3] because species with massive belowground parts – nutrients stored in the root system – may provide greater part of energy needed for aboveground productivity but Reynolds and Dantonio (1996) [30] disagree with this, because they indicate that most of the above ground and below ground plant traits are not closely related to competitive ability. Wilson and Tilman (1995) [93] stated that root/shoot ratios do not vary significantly with habitat but do vary among species. Givnish (1987) [13] already declared that the dry allocation mass of a plant depends on leaf strategy because leaf is a central array of the plant, which outstanding opportunities for competitive studies and implications for competition.

Tilman (1987) [31] stated that water is essential for plant growth and higher level of soil water generally supports higher plant biomass and leaf area. The effect of water on competition for light differs from the effect of soil nutrients such as Nitrogen, because increased water availability, increases shade tolerance, thus allows the survival of plants in the under storey. In addition to the above-mentioned, he also stated that because of inherent physiological differences between species, the ranking of growth rates among a group of species depends on the relative abundance of different resources. Such a growth rate advantage may or may not result in long-term dominance by a particular species, depending on its other life history characteristics and the frequency and type of disturbances in that environment. So it is clear that shoot length may not be so important in situations where the interaction of nutrient availability and soil moisture content have great importance in determining species abundance. This study is also consistent with Grime and Curtis (1976) [15].

Wardle et al., (1998) suggested that individual plant species effects are important determinants of ecosystem properties since these effects may override the importance of abiotic factors. He also provided evidence for the existence of strong linkages between plant ecophysiological traits, biotic interactions involving plants and ecosystem-level properties and processes. The ability to predict the outcome of competition by investigating the relationship between traits and competition ability has long been an objective for ecologists (Grace, 1990) [14]. So while considering plant traits, the different competitive mechanisms of leaves and fine roots must be considered. Leaves act not just as photosynthesis organs, but also as a part of the plant weaponry against their neighbors, denying them access to light. Roots in the topsoil, on the other hand, engage in exploitation competition through depletion of a common pool of resources.

We hypothesize that co-variation among species in leaf area, SLM, and other morphological adaptations particularly in leaf and growth rate reflects a set of mutually supporting traits that interact to determine plant behaviour and production in similar atmospheric conditions.

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COMPARATIVE STUDY OF HERB LAYER DIVERSITY IN PINE FOREST STANDS AT DIFFERENT ALTITUDES OF CENTRAL HIMALAYA

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Abstract. Species richness of pine forest along with elevation varying from 1800 m (site A) to 1500 m (site B) at central Himalaya was compared to judge the ecological significance. A total of 56 species comprising 51 genera and 28 families were recorded. Alpha-diversity and equitability or evenness was significantly higher at site A as compared to the site B. The number of families, genera and species observed in site A were also higher than site B. Very few herb species were found common in both the forest sites and indicated lower values of similarity index. Asteraceae and Lamiaceae were the most dominant family at both the sites. The percentage contribution of annuals and perennials were 48.1% and 51.9%, respectively. Species diversity at site A was more than at site B. This may be due to more gentle slope at site A than at site B. The ability of retention of more water in the soil provides favourable condition for plant growth. The study revealed that the forest site A is more diverse, old and stable in comparison to site B.
Key words: *herb species, family, pine forest, species diversity, species-area curve*

Introduction

The species presently inhabitating Earth are the result of over 3 billion years of natural selection likely favoured efficiency, productivity and specialization (Tilman, 2000) and natural communities differ greatly in the proportion of species performing different ecological functions (Haola and R. Dhand, 2000). Mohamed et al. (2000) also stated that the variety and variability of plant and animal species are the most distinguished feature of life, which reflects the complexity, uniqueness and intactness of natural ecosystems.

Most theoretical and empirical work on functional consequences of changing biodiversity has focused on the relationship between species richness and ecosystem functioning [2]. Several studies using experimental species assemblage have shown that annual rate of primary productivity and nutrient retention increases with increasing plant species richness. Changes in species evenness deserve increased attention, because they usually respond more rapidly to human activities than do changes in species richness and because they have important consequences to ecosystem long before a species threatened by extinction [2]. The different bio-geographic zones with the diverse climate and edaphic factors not only have been a storehouse of a variety of plant species, but also a centre of diversification of taxa. The diversification of taxa has a multitude of facets. The most commonly considered facet of biodiversity is species richness. Even these two attributes of biodiversity, species richness and endemism have attracted particular attention from the international conservation community: [1], because these two attributes reflect the complexity, uniqueness and intactness of natural ecosystems. It is well known that the abundance of a particular species changes over time in progress, which may be due to dispersal but others will be the product of initial condition [4]. Every factors and mech-

anisms, which stimulate and accelerate speciation and maintenance of high species diversity such as habitat heterogeneity, favourableness, and local medium disturbances, are active.

The unique pattern of spatial and temporal species distribution has attracted the attention of several workers [9, 10, 11, 13, 14]. Diversity indices have been developed to convey the extent to which individuals are distributed evenly among species. Most but not all combine evenness with species, losing information by reducing two dimensions to one. Therefore, evenness is another important factor of biodiversity. Present study deals with two levels of diversity namely alpha and beta and its three concepts i.e. species richness (S), equatibility (Ec) i.e. species per log index and synthetic diversity of Shannon-Wiener (H), which incorporates the measure of species richness and equitability and, species composition between two pine forests in different altitudinal range.

Study-sites: The study-sites are located between 1500-1800 m altitudes in central Himalaya (29°22' N latitude and 79°26' E longitude). Both the sites are dominated by chir-pine (Pinus roxburghii) forest. Site A is located between 1700-1800 m altitudes and site B at 1580-1700 m altitudes. The climatic condition is monsoon temperate, and annual rainfall of the area is 2668 mm/year. The mean monthly temperature ranges from 11.5 °C in winter to 18.5 °C during summer. Limestone and quartzite are the principle rock types. Soil texture is sandy loam and it was acidic in nature (5.6-5.9). The physio-chemical properties of soil are given in Table 1.

Methods

A general reconnaissance was made from June -September 2000. Based on frequent surveys of the area, sites were selected for sampling, which was also carried out in the following year.

The phytosociological analysis of herbaceous flora at two sites was carried out by using random sampling quadrat method. The species-area curves were developed for both the sites by using nested plot technique [7]. It was found that gradual increase in the number of species occurring up to 64 m^2 (8mx8m) size (plot) for site A, while for site B the curve showed gradual increase in the number of species up to 32 m^2 (4mx8m) size (plot) (Figure 1). At each site, the quadrats were placed randomly. The herbaceous vegetation was analysed by 30, 1 mx1 m randomly placed quadrats at the time (during rainy season because maximum species were present in that season) of peak herbaceous cover in the first week of September 2000. Each shoot of herb was considered as an individual plant [13].

The index of diversity was calculated by using Shannon-Wiener information index.

$$H' = \sum_{n=1}^{i} (Ni/N) \log2 (Ni/N)$$

where, Ni is the total number of species i and N is the number of individuals of all species in that site. Concentration of dominance was measured by Simpson's index [12] where Ni and N were the same as for the Shannon-Weiner information function.

$$E = S / (\log Ni - \log Ns)$$

Table 1. Certain important soil characteristics of the study sites

Characters	Site A	Site B
Elevation	1700-1800	1580-1700
Forest site	Chir-pine	Chir-pine
Slope (0)	50	42
Aspect	East	South-west
Dominant tree species	P. roxburghii	P. roxburghii
Sand (%)	61.67	62.37
Silt (%)	21	20.3
Clay (%)	15.3	17.8
Moisture (%)	32.8	32.3
pH	5.6	5.9
C (%)	1.4	1.9
N (%)	0.22	0.19
Organic matter (%)	4.67	4.43

where, S is the total number of species, Ni is the number of individuals of most important species, Ns is the number of individuals of least important species and E is the evenness index.

Beta diversity was calculated by following Whittaker (1975) [18] as:

$$\beta = Sc / s$$

where, Sc is the total number of species encounter in all quadrats and s is the average number of species per quadrat.

Results and Discussion

The sites were dominated by chir-pine forests with varying different altitudes. Slight differences in physio-chemical properties of soil has been obtained in site B than site A. Percentage of nitrogen content and organic matter were observed maximum at site A than site B.

A total of 56 species comprising 51 genera and 28 families were recorded from the two study sites (Table 2). Across the sites, 42 species were encountered belonging to 22 families and 37 genera (Tables 2 and 3). Asteraceae and Lamiaceae were the most dominant family (with eight species), followed by Rubiaceae (with three species), Apiaceae, Cyperaceae, Poaceae and Fabaceae (with two species). The remaining 15 families were represented by one species each. Taxonomically, Lamiaceae (with eight genera), were the most dominant family followed by Asteraceae (with seven genera) was, Apiaceae, Poaceae, and Fabaceae (with two genera each) and remaining 16 families were represented by a single genus only at site A.

At site B, a total of 29 species belonging to 17 families and 29 genera (Tables 2 and 3) were observed. Taxonomically, well-represented families were Asteraceae (eight species), followed by Lamiaceae (three species), Apiaceae, Cyperaceae and

Table 2. Herb species composition of the study-sites

Species	Family	Site-A	Site-B
Agrimonia pilosa	Rosaceae	+	-
Arthraxon prionodes	Poaceae	+	-
Anaphalis contorta	Asteraceae	+	+
Ageratum houstonianum	Asteraceae	-	+
Ajuga parviflora	Lamiaceae	+	+
Bupleurum tenue	Apiaceae	+	-
Bidens biternata	Asteraceae	+	+
B. pilosa	Asteraceae	+	-
Begonia picta	Begoniaceae	-	+
Conyza japonica	Asteraceae	+	+
Carex nubigena	Cyperaceae	+	+
C. condensata	Cyperaceae	+	-
Carpesium cernuum	Asteraceae	+	-
Commelina benghalensis	Commelinaceae	+	+
Carum anathifolium	Apiaceae	+	+
Cassia mimosoides	Caesalpiniaceae	-	+
Centella asiatica	Apiaceae	-	+
Crotalaria sessiliflora	Fabaceae	+	-
Campanula colorata	Campanulaceae	+	-
Erigeron karvinskianus	Asteraceae	+	+
E. bonariensis	Asteraceae	+	-
Flemingia bracteata	Fabaceae	+	-
Galium rotundifolium	Rubiaceae	+	-
G. aparina	Rubiaceae	+	+
Gerbera gossypina	Asteraceae	+	+
Sedum sinuatum	Crassulaceae	+	+
Justicia simplex	Acanthaceae	-	+
Leucas lanata	Lamiaceae	+	-
Lindenbergia indica	Scrophulariaceae	-	+
Lepidium virginianum	Brassicaceae	+	+
Micromeria biflora	Lamiaceae	+	+
Neanotis calycina	Rubiaceae	+	+
Origanum vulgare	Lamiaceae	+	-
Oxalis corniculata	Oxalidaceae	-	+
Plectranthus japonicus	Lamiaceae	+	-
Pouzolzia hirta	Utricaceae	+	+
Polygonum hygropiper	Polygonaceae	+	+
Setaria homonyma	Poaceae	-	+
Swertia tetragona	Gentianaceae	+	-
Stachys sericea	Lamiaceae	+	+
Cynoglossum lanceolatum	Borginaceae	+	-
Scutellaria angulosa	Lamiaceae	+	-
Setaria glauca	Poaceae	+	-

Seigesbeckia orientalis	Asteraceae	+	-
Thalictrium foliolosum	Ranunculaceae	+	-
Torenia cordiflora	Scrophulariaceae	-	+
Teucrium royleanum	Lamiaceae	+	-
Roscecea procera	Zingeberaceae	+	-
Polycarpa corymba	Caryophyllaceae	+	-
Urena lobata	Malvaceae	-	+
Nervillea crispata	Orchidaceae	-	+
Platystemma violoides	Gosneriaceae	-	+
Artimesia nilagarica	Asteraceae	+	-
Calamintha umbrosum	Lamiaceae	+	-
Satyrium nepalensis	Orchidaceae	+	-
Viola canascens	Violaceae	-	+
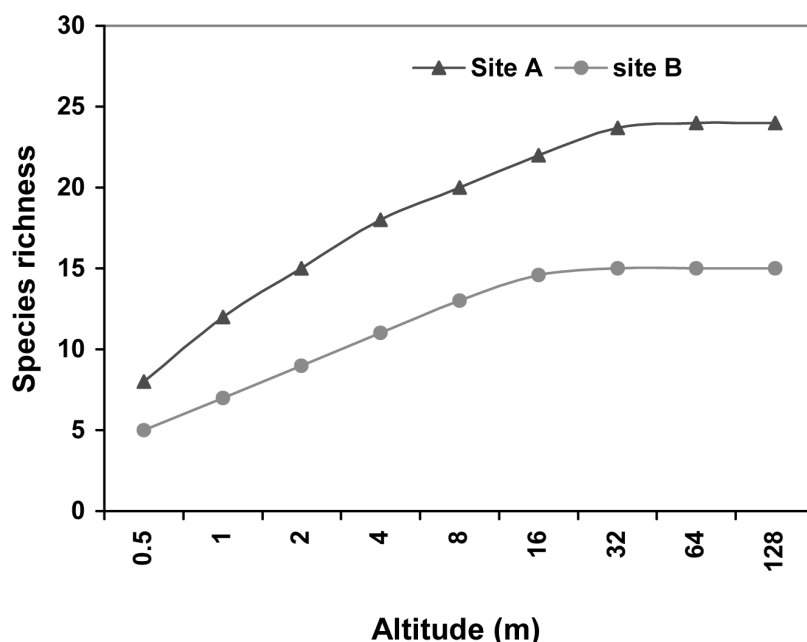
Figure 1. Species-area curve for site A and site B

Scrophulariaceae (two species each), whereas twelve families were represented by a single species. Taxonomically Asteraceae was the most dominant family (with eight genera), followed by Lamiaceae (with three genera), Apiaceae and Scrophulariaceae (with two genera each), and the remaining eight families were represented by single genus only.

The proportions of families to genera, families to species and genera to species were observed higher in case of site A than the site B (Table 4). The percentage of growth form and life forms of herbaceous flora are given in Figures 3. and 4.

Between the sites, site A showed higher maximum species richness than site B. The number of families was more in site A than site B. Between the sites, Asteraceae and

Table 3. *Family-wise distribution of species in the study-sites*

Species	Site A		Site B	
	Genus	Species	Genus	Species
Asteraceae	7	8	8	8
Acanthaceae	0	0	1	1
Apiaceae	2	2	2	2
Borginaceae	1	1	0	0
Begoniaceae	0	0	1	1
Brassicaceae	1	1	1	1
Commelinaceae	1	1	1	1
Crassulaceae	1	1	0	0
Caryophyllaceae	1	1	0	0
Companulaceae	1	1	0	0
Cyperaceae	1	2	1	2
Fabaceae	2	2	0	0
Gentianaceae	1	1	0	0
Gosneriaceae	0	0	1	1
Lamiaceae	8	8	3	3
Malvaceae	0	0	1	1
Orchidaceae	1	1	1	1
Oxalidaceae	0	0	1	1
Poaceae	2	2	1	1
Polygonaceae	1	1	1	1
Rosaceae	1	1	0	0
Rubiaceae	3	3	1	1
Ranunculaceae	1	1	0	0
Scrophulariaceae	0	0	2	2
Utricaceae	1	1	0	0
Violaceae	1	1	1	1
Caesalpiniaceae	1	1	0	0
Zingiberaceae	1	1	0	0

Lamiaceae were the most dominant families, and comprising maximum species richness in both sites.

The number of family, genera and species present at site B were less than that of site A (Tables 2 and 3). This was also reflected by the species-area curve (Figure 1). Though Asteraceae and Lamiaceae showed maximum number of genera and species at both the sites, it was also observed that a few species were dominant in both the sites.

Species richness (per m^2) was higher (7.23) at site A as compared to site B (5.7) (Table 4). However, β-diversity and Shannon-Wiener index values were more or less similar for both sites. Simpson's index value was higher (0.62) for site B than site A (0.11). (Table 5). Evenness or Equitability ratio of species diversity was higher for site A (28.77) than site B (13.80) (Table 4), which indicates maximum diversity at that site.

Percent contribution of annuals at site B was greater than at site A, while contribution of perennials was comparatively higher at site A than at site B (Fig 2). Annuals are able

Table 4. Ratio of species and family in the study-sites

Forest site	Family: genera	Family: species	Genera: species
Site A	1.86	1.95	1.05
Site B	1.57	1.00	1.00

Table 5. Comparison in diversity parameters in the study-sites

Parameters	Site A	Site B
Total number of species present in the site	42	29
Species richness (per m^2)	7.23	5.7
Beta-diversity	2.82	2.60
Shannon-Wiener index	3.92	3.37
Concentration of dominance	0.11	0.62
Evenness / Equitability	28.77	13.80
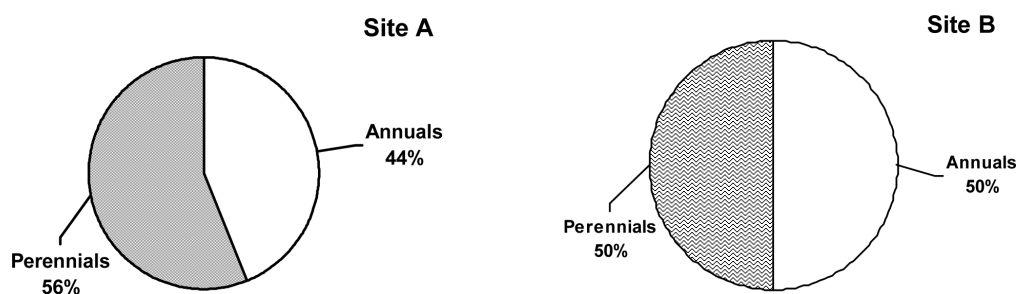
Figure 2. Growth form composition of the study-sites
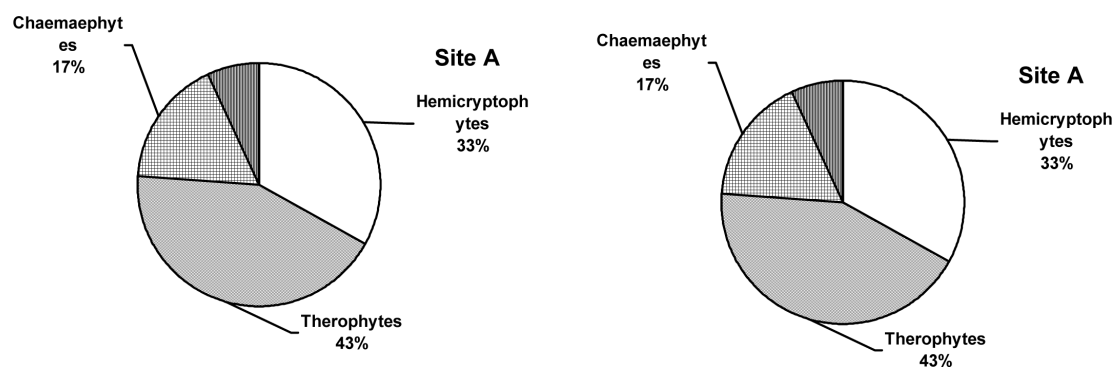
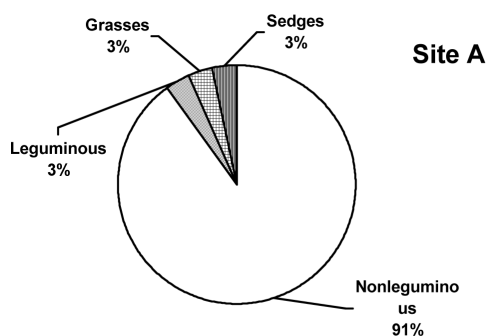*Figure 3.* Growth form composition of the study-sites

Grasses
3%

Sedges
3%

Site A

Leguminous
3%

Nonlegumino
us
91%

Figure 4. (a)

Grasses
3%

Sedges
3%

Site A

Leguminous
3%

Nonlegumino
us
91%

Figure 4. (b)

Sedges
3%

Site A

Grasses
8%

Other forbs
89%

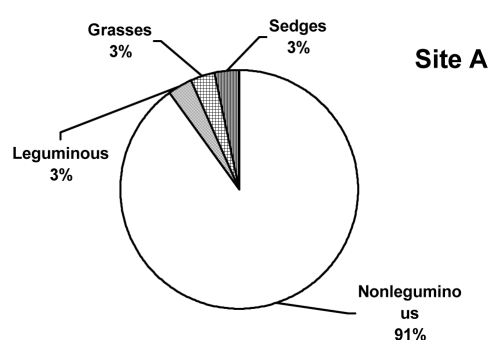*Figure 4. (c)*

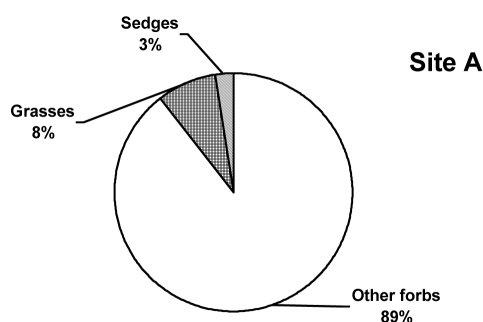Grasses
5%

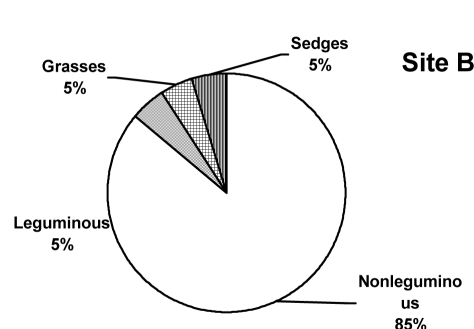Sedges
5%

Site B

Leguminous
5%

Nonlegumino
us
85%

Figure 4. (d)

Figures: (Fig. 4 (a-d): Life form composition of the study-sites

to produce large number of seeds to disperse them efficiently. They contribute their life span in short duration due to escaping nature from harsh conditions. The perennials occupied the study sites and shared dominance with the annuals. Perennial grasses have ability to conserve soil. The extensive root systems of perennial grasses also add more organic matter to the soil than annuals can do and this may due to more favourable conditions for plant growth for site A. This reason may be the main cause for the higher diversity at site A than at site B.

The percentage of growth forms was shown maximum by forbs which was followed by sedges and grasses at site A, and, forbs showed also maximum contribution but followed by grasses and sedges at site B (Fig. 4: a-d).

The contribution of therophytes was maximum at both of the study sites followed by hemicryptophytes, chaemaephytes and cryptophytes (Fig. 3). The higher therophytic character of both the sites is undoubtedly due to strong periodic climate and grazing which keeps the communities open for further invasion by therophytes. The nonleguminous forms were followed by leguminous, grasses, sedges and ferns at site A whereas, at site B, only nonleguminous forms were present (Fig. 4).

The species diversity of the forest ecosystem of this region is influenced by; topography, soil characteristics, climate and geographical location of the area. Results show that

species had dissimilarity in both the sites. It can be stated that species composition changed with respect to altitude and varied in similar forest types.

The species diversity at site A was higher than site B. This may be due to more gentle slope at site A than at site B. The ability of retention of more water in the soil provides favourable condition for plant growth. The Western part of the Himalayan region is characterised by maximum diversity of plant species in comparison to other parts. This region also represents maximum diversity of leguminous flora, which in general diminishes as the altitude increases. As a pioneer species on many disturbed areas, various species in addition to their fast growth and multiple uses play role on providing   fertility to land on which other species will colonize later. Therefore, any change in their community structure and function in changing climate at different elevations will influence other vegetation profoundly.

These observations also indicate that at site A forest trees were older and more stable than at site B. Variation in herb species composition in both sites may be due to change in local abiotic factors such as soil, temperature, moisture and altitude. The above study shows that the herb species diversity was higher at site A was more than at the site B, which may be due to the effects of many physical factors. The herb diversity data analysis of pine forest will serve as base-line information for the researchers.

Conclusion

Distribution is one of the most significant objectives. Spatial heterogeneity and species richness in particular, is an obvious feature of the natural world. Understanding of its determinants will impinge on applied issue for the role of biodiversity in ecosystem process (Gaston, 2000).

Earlier Gaston et al., (1996) stated that a substantial proportion of regional variation in species richness can be explained in terms of a few experimental variables. The numbers of species were also influenced by birth, death, immigration and emigration rates in an area (Gaston, 2000). Species composition has strong effects on ecosystem processes by directly mediating energy and material fluxes or by altering abiotic conditions that regulate the rates of these process (Hooper et al., 1997; Tilman, et al., 1997). This is because species alteration can change the availability of limiting resources, by which disturbance regime and the climate can have particularly strong effects on ecosystem processes. Species composition of an area is important because it seems that the multiple factors doubtlessly contribute to, or influence the biodiversity of an area. So all concerned will need to remember that no single process can adequately explain a given pattern.

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ELECTROCHEMICAL DETECTION AND CATALYTIC OXIDATION OF PHENOLIC COMPOUNDS OVER NICKEL COMPLEX MODIFIED GRAPHITE ELECTRODE

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Abstract. A Novel Ni(II) mixed ligand complex modified graphite electrode is developed for detection and oxidation of the phenolic compounds based on simple cyclic voltammetric technique. The Ni(II) mixed ligand complex with thiocyanate and macro cyclic ligand 1, 4, 8, 11 tetraaza cyclotetradecane (cyclam) was synthesised and used for catalytic oxidation of phenolic compounds viz. phenol, nitro-phenol and chloro-phenol etc. The Ni(II)-cyclam-thiocyanate mixed ligand complex was synthesised in typical molar ratio of 1:2:5:: Ni(II):cyclam:thiocyanate and crystallized followed by characterization and catalytic applications. The electrochemical study of the complex mediated graphite electrode revealed its fast electron-transfer property, as formation of higher oxidation state of central Ni(II) metal ion as Ni(III) on applying a positive potential, which catalyses the oxidation of the target species. The Ni(II) complex modified graphite paste electrode enhanced the oxidation current of phenolic compounds many times relatively at lower oxidation potential in comparison to simple graphite paste electrode. It is also found to be sensitive for the detection of various phenolic compounds in the range of 20 mg/L to 0.5mg/L in aqueous solution. The Ni(II)-complex stability at higher potential and ambient condition proved its potential for sensing and detoxification of phenolic compounds released in the ecosystem from polluting industries.

Keywords. *Cyclic voltammetry, chemically modified electrode, mixed ligand complex, phenol oxidation, detoxification.*

Introduction

The determination of phenolic compounds is of great importance due to their toxicity and persistency in the environment [1–2]. Natural waters are ultimately contaminated by inputs from all compartments of the environment. Effluents are discharged into rivers, lakes and seas, while superficial and ground waters receive leachates and run off from the land, which is contaminated by industrial waste compounds [3]. Phenolic compounds are the most common organic pollutants, which are highly soluble in water. Phenols and substituted phenols are important contaminants in medical, food and environmental matrices [4]. Phenolic compounds are a class of polluting chemicals, easily absorbed by animals and humans through the skin and mucous membranes. Their toxicity affects directly a great variety of organs and tissues, primarily lungs, liver, kidneys and genito-urinary system [5].

Phenol appears in the effluents of many industries such as explosives, coke ovens, phenolic resins, plastic, rubber reclamation, various chemical and pharmaceutical manufacturing processes and oil refineries [6]. Particularly in the case of coal conver-

sion, phenolic residues are considered an acute environmental problem [7]. Soil and surface water from sites around coal production and processing plants were frequently contaminated by phenols, which may cause considerable pollution of groundwater resources [8]. Many industrial processes generate wastewater flows with a high concentration of phenols and other related compounds. About 26.3% of phenol eventually ends up in air, approximately 73.3% in water and about 0.2% in terrestrial soil and aquatic sediments [1]. Many phenols, especially chlorophenols, are known for their persistency in the environment and propensity for bioconcentration and biomagnification. The estimation and detoxification of phenols from wastewaters is, therefore of great importance. Many methods and techniques are available for the determination of phenolic compounds, including gas chromatography, spectrophotometric analyses [9,10] and sensors/bio-sensors [11,12]. However, these methods suffer from complicated process, sample pre-treatment, unsuitable on-site monitoring and do not having the potential for detoxification.

Conventional processes have proven to be efficient in the detoxification of phenolic compounds. However, these processes have certain disadvantages and limitations. The high cost and disposal of contaminated media are the disadvantages of solvent extraction [13] and activated carbon adsorption while biodegradation has been connected with operational problems and bacterial inhibitions [14]. Chemical and wet air oxidation processes have the limitations such as treament effectiveness, cost (main reason), ease of handling, compatibility with precedent or subsequent treatment steps, oxidising agents and nature of the oxidation operation [15,16].

In order to solve this problem, electrochemical techniques have been used [17-19], however, there is still a need to improve the electrode system in order to enhance the sensitivity, simplicity and energy efficiency [20]. Significant attention has been paid for these problems hin the last few years using chemically modified electrodes [21,22]. Although the idea of chemically modified electrodes was first demonstrated systematically in the early 1970's, this approach has assumed a dominant position in modern electrochemistry [21]. There are different directions by which chemically modified electrodes can benefit analytical applications. These include acceleration of electron transfer reactions (catalytic process), preferential accumulation, or selectivity and stability. Such steps can impart higher selectivity, sensitivity and stability to electrochemical devices [21-24]. Moreover, chemically modified electrodes also offer the possibility of adjustable physical and chemical properties (i.e., charge, polarity, surface area, permeability etc.).

Coordination compounds containing macrocyclic ligands especially "synthetic macrocycles", viz. phthalocyanines, metalloporphyrins and crown ethers etc., are well known for chemically modified electrodes and development of electrochemical sensors [18,20-24]. However, the stability and electroactivity of the complexes are the major problem in their technological applications. Recently, modified cyclam complexes viz. polymerization or substitution of the cyclam ring or mixed ligand complex have been reported for the construction of chemically modified electrodes in several applications [25-28]. We have recently developed Ni(II)-cyclam-thiocyanate mixed ligand complex and investigated its structure, stability, spectral and electrochemical properties [20].

In the present paper, we report the optimized ratio, construction and application of Ni-cyclam-thiocyanate in the chemically modified graphite paste electrode for catalytic oxi-

dation and low concentration estimation of phenolic compounds using simple cyclic voltammetric technique.

Materials

Materials and Instruments: 1,4,8,11 tetraaza cyclotetradecane (cyclam), graphite powder (2-4 mm) and nujol were obtained from Aldrich Chemicals Company Inc., U.S.A. Potassium thiocyanate and $NiCl_2$ (AR) were obtained from Glaxo Chemicals, Mumbai, India. Tris (hydroxymethyl) aminomethane was obtained from Sisco Research Laboratories, Mumbai, India. All the other chemicals used in this investigation were of analytical extra pure grade.

Electrochemical studies of mixed ligand complex of nickel in solution were performed in a single compartment cell using three-electrode system viz. Glassy carbon (as working electrode), platinum (as auxiliary electrode) and Ag/AgCl (3 M KCl) (as reference electrodes), using Potentiostat/Galvanostat, PG 30, interfaced with a PC, obtained from Autolab, The Netherlands. The Teflon body of graphite electrode or modified graphite electrode was constructed in the laboratory with the help of Electronics Company, ELICO Pvt. Ltd. Hyderabad, India and used for the phenol oxidation and estimation studies.

Methods:

1. Complex Formation: Ni(II)-cyclam complex was prepared in the molar ratios of 1:2 (keeping the concentration of Ni(II) as 20 mM) in 0.2 M tris-buffer, pH 7.2. The
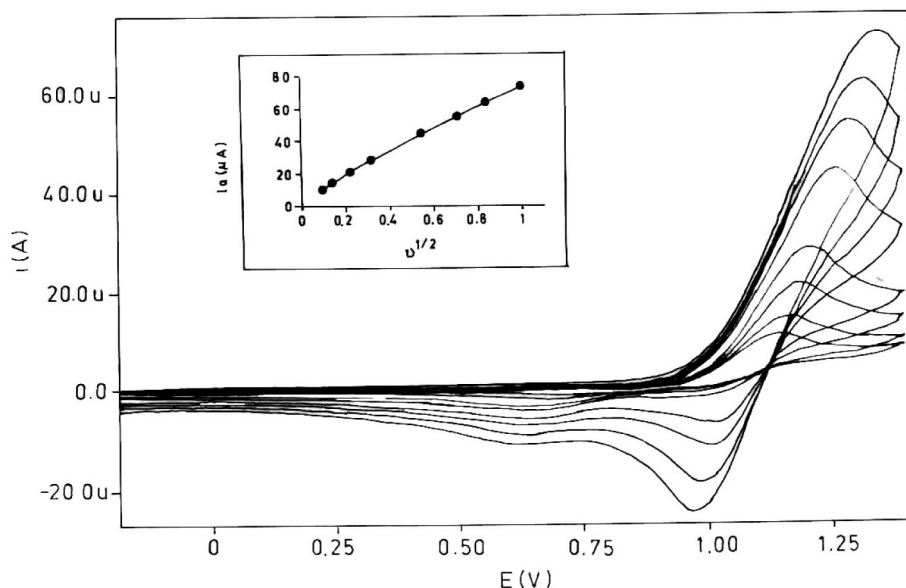

Figure 1. Cyclic voltammetry of Ni(II)-cyclam-thiocyanate complex in dichloromethane (2 mg/ml) using 0.1 M tetrabutyle ammonium perchlorate as supporting electrolyte at various scan rates from 10 mV/s to 1000 mV/s. Inset: A linear plot for current vs. square root of scan rates.
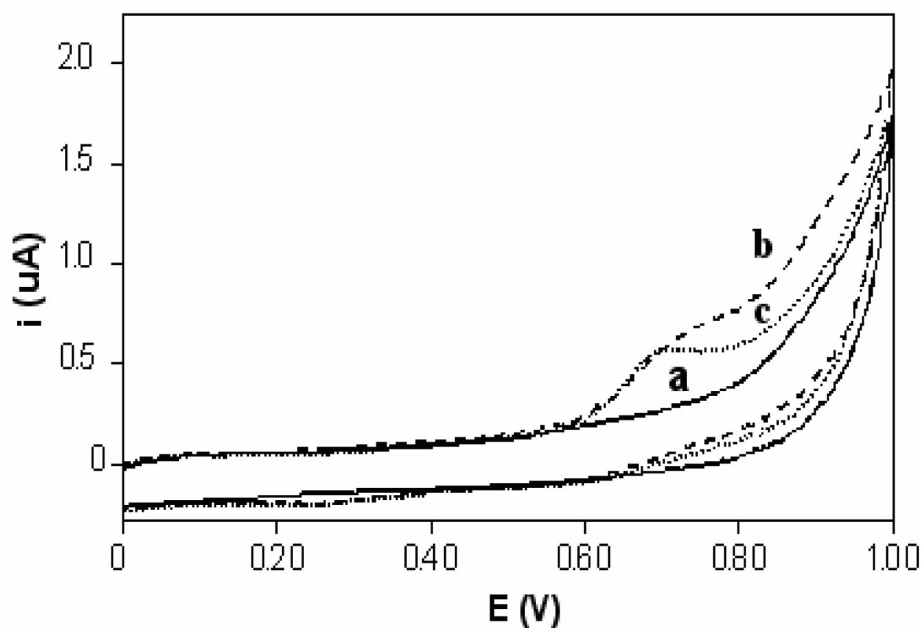
Figure 2. *Cyclic voltammogram in 100 mg/l phenol solution (in 0.2 M Tris-HCl buffer at pH 7.2) for potential range 0.0 V to 1.0 V over (a) 0.0 % (b) 0.5 % and (c) 0.25% Ni(II)-cyclam-thiocyanate complex modified graphite paste electrode.*
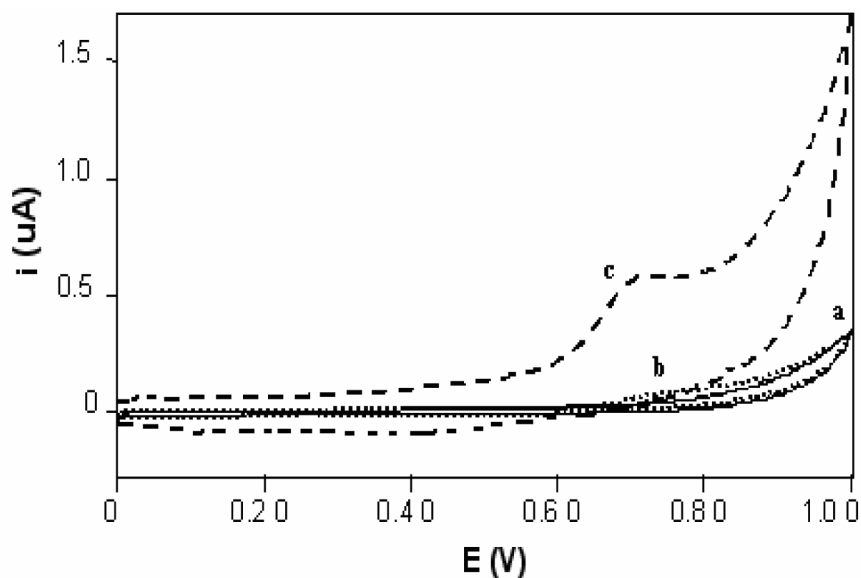

Figure 3. *Cyclic voltammogram in (a) 0.2 M Tris-HCl buffer at pH 7.2 in the potential range 0.0 V to 1.0 V, (b) 100 mg/l phenol solution over graphite paste electrode and (c) 100 mg/l phenol solution over 0.25% Ni(II)-cyclam-thiocyanate complex modified graphite paste electrode.*
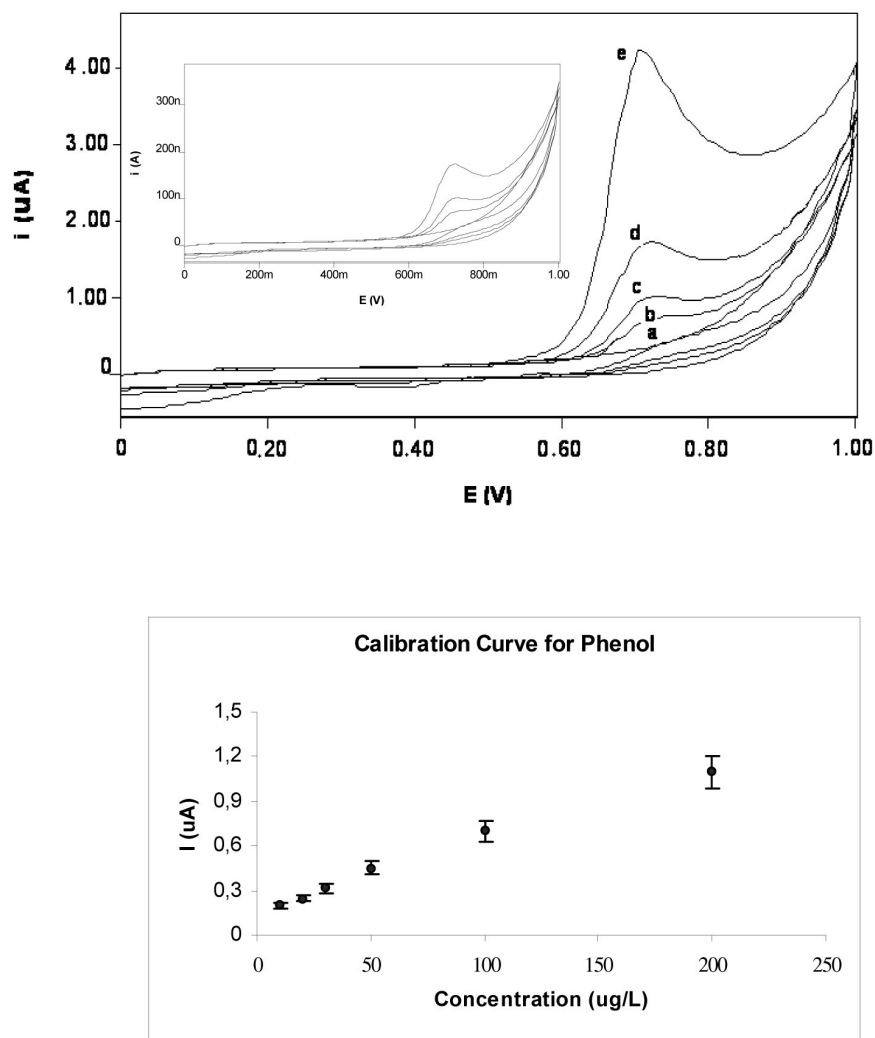
Figure 4. (a) Cyclic voltammogram (100 mV/s scan rate) over 0.25% Ni(II)-cyclam-thiocyanate complex modified graphite electrode for 0, 50, 100, 200, 500 mg/l and in Inset for 0, 10, 20 and 30 mg/l phenol concentration of phenol in 0.2 M Tris-HCl buffer at pH 7.2 (b) calibration curve for phenol from 10 mg/l to 200 mg/l.

complex formation was monitored by change in the absorption spectra (in the range of 300-800 nm) and electrochemical behaviour. Mixed ligand complex formation of nickel was carried out by adding thiocyanate solution in 0.2M tris buffer (pH 7.2) to the Ni(II)-cyclam complex in the molar ratios of 1:2:5 and crystalline product was characterized by spectral and electrochemical behaviour as described earlier [20].

2. Preparation of Graphite Paste Electrode and Modified Graphite Paste Electrode: Graphite paste electrode (GPE) was prepared by filling the paste of graphite powder (80 mg) mixed thoroughly with mineral oil (20 ml) into the bottom hole of Teflon body (hole diameter 2 mm and depth 2.5 mm) electrode. This electrode was used for the analysis
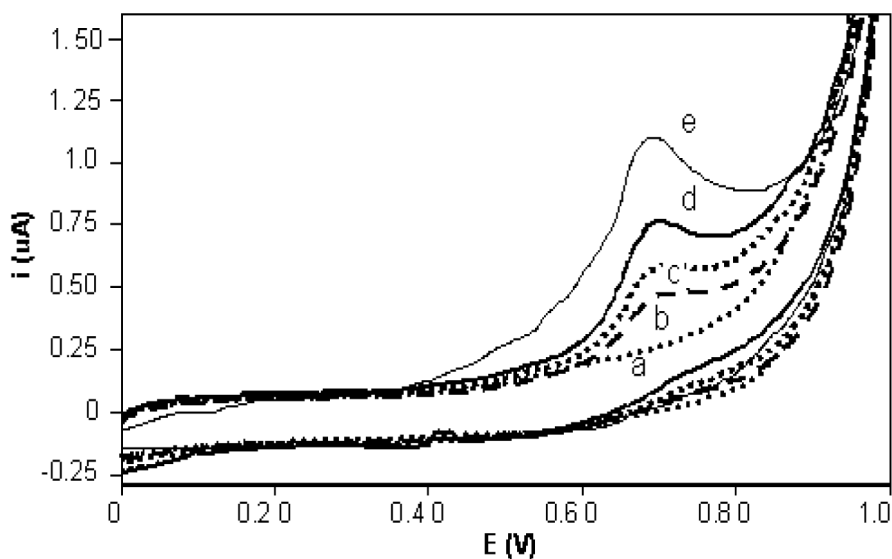
Figure 5. *Cyclic voltammogram (100 mV/s scan rate) over 0.25% Ni(II)-cyclam-thio-cyanate complex modified graphite electrode for 0, 50, 100, 200 and 500 mg/l concentration of 2,4-dichloro phenol in 0.2 M Tris-HCl buffer at pH 7.2.*

and oxidation of the phenolic compounds. The catalytic effect of the Ni(II)-cyclam-thio-cyanate complex on the electrochemical oxidation of phenol was studied by incorporating the complex in the graphite paste electrode. This is now termed as Modified Graphite Paste Electrode (MGPE). MGPE was prepared by taking various amounts of complex mixed thoroughly with graphite paste and an optimum amount of 0.25% of the complex in graphite paste (by weight) was found to be optimum for the study.

3. Electrochemical Studies: Cyclic voltammetry of GPE and MGPE was performed in the wide range of potential –0.2 V to 2 V vs. Ag/AgCl (3 M KCl) in 0.1M tris buffer, pH 7.6 at room temperature (25°C ± 1°C) and also in the chloroform solution (2 mg/ml) using tetrabutyl ammonium perchlorate (TBAP) as supporting electrolyte at various scan rates.

The cyclic voltammetric estimation of phenolic compounds was also done along with the platinum counter electrode and Ag/AgCl (3 M KCl) reference electrode in 0.1 M tris HCl buffer in the range of 0 V to 1 V. The data was recorded on the computer in the software provided with the Potentiostat.

Results and Discussion:

Cyclic Voltammetry (CV) of Ni(II):Cyclam complex (in solution phase) did not reveal any defined redox peak in –0.2 to 2.0 V vs Ag/AgCl range in 0.1 M tris-buffer, pH 7.6, however, the Ni(II)-cyclam-thiocyanate crystalline complex dissolved in chloroform using 0.1 M TBAP as supporting electrolyte exhibited two well defined oxidation peak at 1.15 V along with reduction peak at 1.1 V vs. Ag/AgCl and an irreversible oxidation peak at 1.65 V vs. Ag/AgCl. The CV of Ni(II)-cyclam-thiocyanate complex in chloroform recorded at various scan rates further reveals a pseudo reversible redox

Ni(II) → Ni(III) system with high stability of the complex as shown in Figure 1. A linear plot was observed for the current vs. square root of scan rates (Fig. 1-inset). Indeed, the formation of Ni(II) → Ni(III) are reported in several systems viz. Ni(II)-peptide [28] and Ni(II)-porphyrin [29]. The CV studies for several cycles and at higher potential provided the information regarding reversible redox system and stability of the complex.

The effect of the Ni(II)-cyclam-thiocyanate complex on electrochemical oxidation of the phenol was studied over carbon paste electrode by incorporating with various amounts of complex. The variation of the complex percentage in the carbon paste was studied with the change in the peak height and peak sharpness for 100 mg/l phenol oxidation in 0.1 M Tris-HCl buffer at pH 7.6 in the potential range 0.0 V to 1.0 V vs. Ag/AgCl. A comparison of the CV of MGPE with 0.5% and 0.25% (by weight) Ni(II)-cyclam-thiocyanate complex is shown in the Figure 2. A relatively sharp and at lower oxidation potential (0.7 V vs. Ag/AgCl) peak was observed with 0.25% complex, which was found to be optimum and used for the phenol analysis and oxidation studies.

A comparison of GPE alone and MGPE was done for the oxidation of 100 mg/l phenol in 0.1 M Tris-HCl buffer at pH 7.6 using cyclic voltammetry in the potential range 0.0 V to 1.0 V vs. Ag/AgCl and the data was recorded as shown in Figure 3. A very large difference of oxidation peak current (more than 10 times), relatively at lower potential (0.2 V) was observed for 100 mg/l phenol over MGPE in comparison with simple GPE. The large enhancement of the oxidation current revealed the catalytic effect of the complex, which is probably due to the reversible Ni(II) to Ni(III) conversion at low potential, which serves as a fast electron-transfer mediator for the oxidation process. It has been already reported that electrochemically formed nickel porphyrin coated electrodes exhibit good electrocatalytic activity for the oxidation of carbohydrates and benzyl alcohol [29].

The cyclic voltammogram were recorded over same MGPE for 0 mg/l to 1000 mg/l phenol solution in 0.1 M Tris-HCl buffer at pH 7.6 in the potential range 0.0 V to 1.0 V and a well defined oxidation peak at 0.7 V vs. Ag/AgCl (3M KCl) were observed as shown in Figure 4 (inset for lower concentration of phenol). The calibration curve was plotted with peak current vs. phenol concentration showed a linear response with detection limit of 20 mg/l phenol, which saturated over 200 mg/l. The detection of phenol was also done over simple graphite electrode or graphite paste electrode and no any defined relation were found below 500 ppb of phenol using cyclic voltammetry.

The other phenols viz. chloro or nitro phenols were also studied over MGPE and nearly the same results were observed with 50mg/l detection limit. A typical voltammogram of 2,4-dichloro phenol of 0, 50, 100, 200 and 500 mg/l concentration is shown in Figure 5. The complex modified graphite electrode exhibits the potentiality for the catalytic oxidation for organic materials especially for phenolic compounds.

Conclusion

The reversible Ni(II) to Ni(III) conversion at low potential and fast electron-transfer mediator property of the Ni(II)-cyclam-thiocyanate complex is demonstrated and the property was used for the catalytic application. The enhanced oxidation for phenolic compounds was observed over the complex modified graphite electrode. Thus, nickel complex based electrodes offer an indisputable advantage for developing new electrode materials in the field oxidation of phenolic compounds and also for other organic deriv-

atives. The use of simple cyclic voltammetry makes the technique simple and easy to be used for the application of sensing and detoxification of phenolic compounds and construct the portable devices. These descriptive results constitute the first step in our studies that are under investigation to evaluate this complex in the field of analysis and detoxification of organic pollutants in the industries.

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KINETICS STUDY OF METHYLENE BLUE DYE BIOADSORPTION ON BAGGASE

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Abstract. The adsorption potential of agricultural (sugarcane) by-product, the baggase was investigated in batch experiments with two different forms i.e., raw and chemically activated forms, for the removal of Methylene Blue dye, with different parameters like dye concentration, contact time, temperature and adsorbent dose. The removal is better and more effective with chemically activated baggase in comparison to the raw baggase. An average percent removal difference between the two adsorbents of around 18% was achieved under the different conditions in the experiment. The data fit well in the Freundlich isotherm.
Key words: Adsorbent, Adsorption, Methylene Blue dye, Raw Baggase, Chemically Activated Baggase.

Introduction

Of various pollutants contained in industrial wastewaters, colour is considered to be very important from the aesthetic point of view and is stated as 'visible pollutant'. Almost every industry uses colouring matter to colour their products. Unspent colouring materials are usually discharged, with/without treatment into the aquatic environment. Dyes are highly coloured polymers and low biodegradable. Color/dye being one of the important recalcitrant, persist for long distances in flowing water, retards photosynthetic activity, inhibit the growth of aquatic biota by blocking out sunlight and utilizing dissolved oxygen and also decrease the recreation value of stream.

India produces more than 400 million tonnes (MT) of agricultural waste annually which include a very large percentage of the total world production of rice husk, baggase, jute, stalk and coconut fiber. India became the largest producer of sugar cane/sugar in the world, producing 285.4 MT of cane representing about a little more than 20% of cane sugar production. Presently, there are over 450 mills with installed capacity of 20.2 MT with average size of 2150 TPD with some units of 10000 TPD and few of 5000 TPD. Sugarcane production has increased in last decade and has reached to a maximum production of 300.1 MT in 2001-02 [www.indiaonestep.com] [1]. It have been analysed that after sugar extraction, one third of the waste product generated is baggase. Thus baggase production in India has reached a little above 100MT in 2001-02. Per capita consumption of sweetner in India, (prepared form sugarcane) is 24.4 kg, which is higher compared to world average of 20.4 kg.

Burning of baggase also releases large quantities of smoke, soot, ash and carbon dioxide into the air with detrimental environmental effects. Most efficient as well as balanced mills should be able to save baggase to the extent of 10% of its production, which can be used as captive fuel in the mill as power. Total production of baggase if used for power generation can generate 4000 MW surplus eco-friendly energy. Power generation

from baggase comes under clean power and is very much eligible to get carbon emission benefits. But still in India, use of baggase for power generation is considered as the secondary option.

Literature Review

There is an ever-increasing demand of fabrics and food in the country for the rapid expanding population, which is growing at a rate of 11.04 %. Nearly 10-15% of the synthetic textile dyes, used yearly are lost to waste streams and about 20% of these losses enter the environment through effluent from wastewater treatment plant. The wastewaters discharged from dying processes exhibit high BOD, high COD are highly coloured, hot, alkaline and contain high amounts of dissolved solids [2]. The disposal of coloured wastes such as dyes and pigments into receiving waters damages the environment, as they are carcinogenic and toxic to humans and aquatic life [3, 4]. Besides the problem of colour, some dye impart non-visibility and can be modified biologically to toxic or carcinogenic compounds. Nowadays concern has increased about the long-term toxic effect of water containing these dissolved pollutants.

Basic dyes are the brightest class of dyes [5] and are applied widely in small scale industries like textile, carpets and wool industries whose discharge bearing dyes throught effluents are added into natural streams [6]. In dying industry above 30-60 litres of water is consumed per kg of cloth dyed and large quantities of effluents are released during process [7]. Its amount is to be about 16% of the total water consumed in the mill [8].

Various treatment methods for removal of dyes from industrial effluents like coagulation using alum, lime, ferric chloride, ferric sulphate, chemical oxidation methods using chlorine and ozone; and membrane separation methods are in vogue [8]. Many of them do not operate at low concentration of coloured compounds in the effluent. Special measures therefore are necessary to be taken to remove them from the effluents.

Adsorption has received considerable attention for colour removal from wastewaters as it offers the most economical and effective treatment method. Low cost adsorbents like flyash, coal, peat, sawdust [9], sawdust [10] lignite and wood have received considerable interest because of their local availability and their practically low cost [6]. Use of bio-adsorbent like rice husk [11], coconut coir, banana pith [12] wheat straw, baggase, saw dust [9,11,13], used tea leaves, cow dung [14] have been found to be highly effective, cheap and eco-friendly

Objective of present study was to explore equilibrium as well as the rate of adsorption of basic dye (Methylene Blue) in aqueous solution and efficiency of cellulose based adsorbents prepared from Baggase with its different states i.e., Raw and Chemically Activated form.

Materials and Methods

Preparation of Adsorbents

Baggase was obtained from a nearby sugarcane mill. Two parts, of the total cellulosic material by weight, were made and one part of the baggase was processed to obtain chemically treated baggase carbon. Four parts of raw baggase were treated with three parts by weight of concentrated Sulphuric acid and kept in air tight oven, maintained at

150°-160°C for a period of 36 hours. The carbonized material was washed well with distilled water to remove the free acid and dried at 110-115°C for 24 hours. This material was grounded and sieved to obtain carbon particle size £ 0.33 mm and was used as chemically activated baggase adsorbent.

Another part of cellulosic baggase was soaked in distilled water for forty-eight hours with repeated change of distilled water, every 12 hours. It was then treated with alkali for 12 hours to make it lignin free, thoroughly washed with distilled water and then was treated with formaldehyde to prevent any further colour interference during treatment (adsorption) process. The material was now dried in an oven at 50-60°C for 24 hours and this dried material was pulverized to convert it into fine powder and was used as raw baggase adsorbent. Table 1 gives the physical properties of prepared treated adsorbent.

Table 1. Physical properties of chemically Treated Sawdust Adsorbent

Parameters	Characteristic Value
pH 6.5	
Moisture (%)	12.1
Bulk density (g/ml)	0.33
Matter soluble in water (%)	Nil
Matter soluble in acid (%)	Nil
Surface area,m2/g	1.713
Pore volume (ml/g)	0.11
Ash content (%)	36.62
Iodine Number	75
Ion exchange capacity (meq/g)	1.06

Dye and Chemicals

Methylene Blue [3,9-bis dimethyl- aminophenazo thionium chloride], a cationic dye (figure 1), is a G.R. Product of Merck, Germany and was used as received. All chemicals used were of analytical reagent grade and supplied by Merck Germany.

Adsorption Studies

To evaluate the efficiency of adsorbents, laboratory batch mode studies were conducted. 0.1 to 1 gm of adsorbents, taken separately, were shaken in 100ml aqueous solution of dye of varying concentration for different time periods and temperatures at natural pH. At the end of pre-determined time intervals, adsorbent was removed by centrifu-

**Methylene Blue
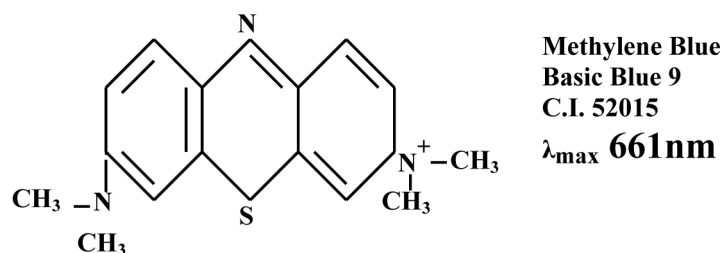Basic Blue 9
C.I. 52015**

λ_{max} **661nm**

Figure 1. Methylene Blue [3,9-bis dimethylaminophenazo thionium chloride]

gation at 2000 rpm for 10 minutes and supernatant was analyzed for the residual concentration of Methylene Blue, spectro-photometerically at 665 nm wavelength. All experiments were carried out in triplicate with respect to each condition and mean values are presented. The maximum deviation was 4%.

Results and discussion

Effect of Initial Dye Concentration and Time

Effect of initial dye concentration on the rate of adsorption by baggase (raw and chemically activated) was achieved as presented in Table 2. The data table of amount of dye adsorbed at various intervals of time indicates that the removal of dye (adsorbate) initially increases with time but attains an equilibrium within 45-60 minutes. The adsorption process was found to be very rapid initially, and a large fraction of the total concentration of dye was removed in the first 30 minutes. Though it was observed that adsorption of dye increased with an increase in dye concentration in the solution, which shows that removal of dye is dependent upon the concentration of the dye solution. But as a whole the percent removal decreases with the increase in dye concentration as observed in the plot.

Table 2. Effect of Initial dye concentration on MB removal by Raw and Chemically Activated Baggase

Conc. → Time ↓ (mins)	50ppm		100 ppm		150 ppm		200 ppm		250 ppm		300 ppm	
	RB	CAB	RB	CAB	RB	CAB	RB	CAB	RB	CAB	RB	CAB
15	61.3	71.54	56.4	66.32	44.48	61.64	38.72	55.49	28.51	42.96	20.58	31.85
30	79.8	80.26	69.81	77.91	58.84	67.52	48.03	60.32	35.48	48.93	33.71	36.55
45	83.96	89.54	77.52	82.95	66.22	72.58	54.67	64.91	41.24	54.79	37.98	39.23
60	86.28	92.46	83.90	85.67	70.28	78.92	56.62	67.95	47.36	57.29	39.93	42.67
90	88.72	94.84	86.28	87.03	74.29	82.99	61.26	69.67	49.25	59.28	42.39	44.59
120	90.11	96.12	88.90	90.11	78.26	84.91	66.19	72.03	51.76	61.54	44.35	46.32

For a particular experiment, the rate of adsorption decreased with time, it gradually approached a maximum adsorption, owing to continuous decrease in the concentration driving force and it also indicate that the adsorbent is saturated at this level. In addition it is observed that initial rate of adsorption was greater for higher initial dye concentration because as the resistance to the dye uptake decreased, the mass transfer driving force increased. The time variation adsorption increases continuously and seems to be smooth which, is indicative of the formation of monolayer coverage on the surface of adsorbent [15].

Raw (RB) and Chemically Activated baggase (CAB) could remove a maximum of 83.93% and 85.67% respectively, of Methylene Blue at initial dye concentration of 100 ppm, while for dye concentrations of 50 ppm, the adsorption of the dye was well above 90% and 96% in two hours. Both cases were studied at natural pH, temperature 250°C and adsorbent dose of 4 gm/l.

Effect of adsorbent dose and time

The effect of varying the RB and CAB mass on aqueous dye solution are presented in Table 3. Data show a decreasing trend in dye concentration at a faster rate as the adsorbent mass is increased. Of the two adsorbents, CAB gave the greater removal at all levels of the adsorbent dose. An equilibrium percentage removal rate of 77.91% and 69.81% was achieved with 4 gm/l in 30 minutes by CAB and RB respectively of adsorbate concentration of 100 ppm. Initially the rate of increase in the percent dye removal has been found to be rapid which slowed down as the dose increased. This phenomenon can be explained, based on the fact that at lower adsorbent dose the adsorbate (dye) is more easily accessible and because of this, removal per unit weight of adsorbent is higher.

Table 3. Effect of adsorbent dose on MB removal by RB and CAB

Dose → Time ↓ (Mins)	0.1 gm		0.2 gm		0.4 gm		0.6 gm		0.8 gm		1.0gm	
	RB	CAB	RB	CAB	RB	CAB	RB	CAB	RB	CAB	RB	CAB
15	20.56	38.98	31.56	54.28	56.4	66.32	70.64	78.62	84.98	87.28	96.01	95.09
30	25.77	45.78	41.98	62.82	69.81	77.91	82.52	84.28	92.44	92.91	98.68	97.52
45	27.24	50.75	48.35	69.59	77.52	82.95	88.85	88.35	94.70	94.79	99.23	98.73
60	29.36	54.17	51.49	75.48	83.93	85.67	90.58	91.47	96.46	93.71	99.47	98.61
90	31.17	57.67	53.61	79.55	86.28	87.03	92.08	93.25	97.16	97.68	99.52	99.54
120	33.11	60.45	54.72	80.62	88.90	89.46	93.98	95.34	97.80	98.06	96.56	99.63

The initial rise in adsorption with adsorbent dose is probably due to bigger driving force and lesser surface area. Larger surface area of the adsorbent and smaller size of adsorbate favour adsorption. The rate of adsorption is higher in the beginning as sites are available and unimolecular layer increases. Adsorption and desorption occur together and rate become equal at a stage called adsorption equilibrium, when isotherms are applied. The subsequent slow rise is observed in percent removal, which states that adsorption and intra-particle diffusion taking place simultaneously with dominance of adsorption. With rise in adsorbent dose, there is less commensurate increase in adsorption, resulting from lower adsorptive capacity utilization of adsorbent. [16].

Thus, the results obtained from this section of experiment indicate that chemically activated baggase has a large potential as an adsorbent for dye removal than raw baggase.

Effect of temperature

Temperature dependence of adsorption process is a complex phenomenon. Thermodynamic parameters, like heat of adsorption and energy of activation play an important role in predicting the adsorption behavior and both are strongly dependent on temperature. Temperature rise affects the solubility and chemical potential of the adsorbate, the latter being a controlling factor for adsorption. It have been reported that if solubility of the adsorbate increases with increase in temperature, then chemical potential decreases and both of these effects work in the same direction, causing a decrease in adsorption [17]. On the other hand if temperature has the reverse effects on the solubility than both the said effects will act in the opposite direction and adsorption may increase or decrease depending upon the predominant factor.

In the present experiments the adsorption rate at three different temperatures (30°, 40°, 50°C) have been analysed as presented in data Table 4. In case of raw baggase, the rate of dye uptake decreases with an increase in temperature from 74.26% to 55.92% at 30°C to 50°C with 4 gm/l dose in 30 minutes for 100 ppm dye solution, indicating that the process is exothermic in nature [18]. This may be due to a tendency of dye molecules to escape from the solid phase to bulk phase with an increase in temperature of the solution [19]. Whereas in the case of Chemically activated baggase rate of dye uptake increases rapidly from 78.09% to 86.35% with rising temperature at 30°C to 50°C with 4 gm/l dose in 30 minutes from 100 ppm dye solution, since the adsorption rate increase as the diffusion coefficient rises with temperature. [5].

Table 4. *Effect of Temperature on MB removal by Raw and Chemically Activated Baggase*

Temp.	30°C		40°C		50°C	
Time	RB	CAB	RB	CAB	RB	CAB
5 Minutes	64.38	61.04	57.39	65.25	46.48	71.22
10 Minutes	67.21	68.84	60.72	72.85	50.16	76.28
15 Minutes	70.56	74.54	64.09	78.06	53.63	81.5
30 Minutes	74.26	78.09	67.28	83.54	55.92	86.35
45 Minutes	76.59	80.21	69.52	86.19	56.85	90.16
60 Minutes	77.72	81.55	70.25	88.94	57.73	92.42

For adsorption of dye using baggase (raw and chemically activated) adsorbent there is the possibility of intra-particle diffusion. This was also investigated and is presented in Fig 2 and 3. The graph is plotted with log (% adsorption) vs. log (time), for adsorption at three (30°, 40°and 50°C) temperatures resulting in straight lines, which indicate the occurrence of intra-particle diffusion. The adsorption at higher temperature becomes more dependent on intra-particle diffusion, which would be the rate-determining step [20].

Adsorption isotherm

The analysis of equilibrium data for the adsorption of the Methylene blue on adsorbents baggase (RB and CAB) have been done in the light of the Freundlich isotherm model [21].
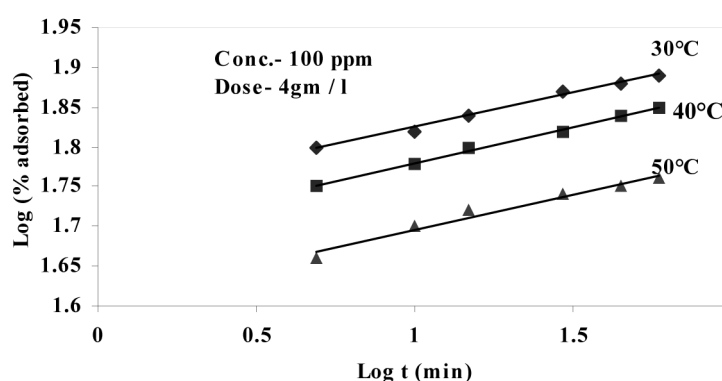

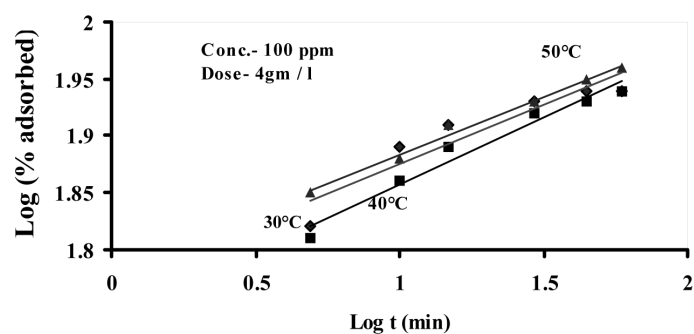*Figure 2.* *Kinetics of Adsorption of MB Dye on Raw Baggase at Different Temperatures*

Figure 3. *Kinetics of Adsorption of Dye by Chemically activited Baggase at Different Temperatures*
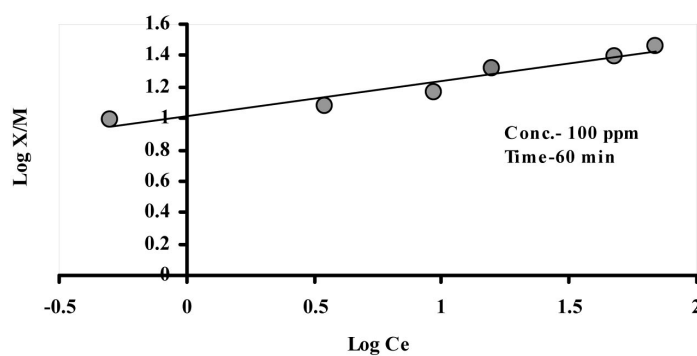

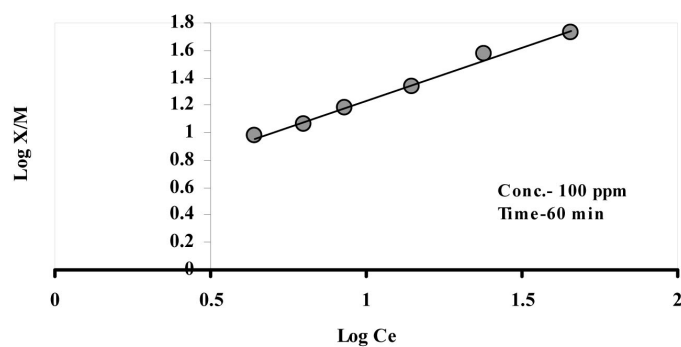*Figure 4.* *Freundlich Isotherm for Adsorption rate of MB Dye on Raw Baggase*

Figure 4. *Freundlich Isotherm for Adsorption rate of MB Dye on CA Baggase*

$$\log_{10} \frac{x}{m} = \log_{10} K_f + \frac{1}{n} \log_{10} C_e$$

(Eq. 1)

Where, Ce is the equilibrium concentration (mg/l), x is the amount of dye adsorbed (mg), m is the adsorbent dose used (g) and Kf and n are constants, incorporating all factors affecting the adsorption process such as adsorption capacity and intensity, respectively. Linear plots of log10 (x/m) vs. log Ce shows that adsorption follows Freundlich isotherm model. Fig. 4 and 5.

These preliminary studies state that adsorbents prepared from baggase can be used effectively for the adsorption of dyes. Cost analysis for the preparation of activated carbon of baggase has not been done since Baggase is available abundantly and can be obtained for nominal price as agricultural by-product in the country. The present method has been adopted for further analytical kinetics study of other agricultural by-products for the removal of dyes and heavy metals from aqueous solutions and industrial effluents.

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TAXONOMIC CHARACTERISATION OF ACTINOBACTERIA ISOLATED FROM THE ATMOSPHERE SURROUNDING CHAMOMILE PLANTS

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Abstract. Many different microbes, including actinobacteria thrive in the conditions offered by moisture nutrient materials, which are needed for microbial growth. The actinobacteria isolated and identified from the atmosphere surrounding chamomile plants cultivated at El-Fayoum Governorate, Egypt reached a maximum concentration during harvesting period of chamomile plants. The main components of the air-borne bacteria in the atmosphere of the studied area were Streptomyces, Micrococcus , Rhodococcus, Arthrobacter, Microbacterium, Cellulomonas and Nocardia. These bacteria have an important role in the spreading of allergic diseases among the population during the harvesting of the chamomile plants and indicate a potential risk of occupational respiratory disease.
Key words: characterisation, actinobacteria, atmosphere, chamomile plant.

Introduction

The long-term inhalation of organic dusts may cause inflammation of the respiratory tract in reposed agricultural workers as a result of specific reactions [38, 4 and 21]. Mostly, microorganisms associated with organic dusts induce these reactions. These microorganisms have been identified as common causative agents of respiratory disorders due to inhalation of organic dusts, such as allergic alveolitis, asthma and organic dust toxic syndrome [16,19,20,27 and 23]. The harmful effect of allergens and endotoxins may caused by Gram – negative bacteria [8, 19, 24, 30 and 32], actinomycetes [18 and 21] and filamentous fungi [21, 33 and 34].

Grain handling workers (harvesting, threshing, loading, unloading and shuffling) who are associated with heavy exposure to grain dust is regarded as emplyoees in hazard occupation [4, 22, 25, 35, 40, 3 and 5]. Work – related symptoms have been reported in 20-44% of farmers handling grain in England, Poland and Canada [4, 8 and 37] and in 44.8 –89.4% of grain soil workers in Poland, U.S.A., Canada and Hungary [15, 36, 8, 7 and 31].These effects of grain dust are largely due to the action of microorganisms associated with grain and their products which occur in great concentrations in air polluted with grain dust [6, 9 and 17]. Of particular importance are actinobacteria and fungi which develop in incorrectly preserved and overheated grain. It was reported as a cause of allergic alveolitis and other diseases in many countries [21, 19 and 20].

The aim of the present work was to characterise the actinobacteria communities isolated from the atmosphere surrounding the chamomile plant from the beginning of cultivation till harvesting, it to assess the effect of the exposure to grain dust in the popula-

tions of the Egyptian farmers engaged at the harvesting of the plant.

Material and Methods

Sampling Site

The samples were taken form El-Fayoum Governorate (~100 km south west of Cairo). It is a residential area cultivated with chamomile plant, temperature ranged from 15-30°C and relative humidity varied between 62-72%.

Sampling methods

Air samples (5 samples collected form the study area, from January to March, 2003) were collected from 11 a.m. to 2 p.m. Each set of samples consisted of 5 Petri dishes (10 cm diameter). The gravitational method [29 and 26] was employed for collection of actinomycetes. Petri plates contained 3% malt extract agar (Difco, Detroit, Mi) were exposed to air for 15 min. The samples were taken at 1.5 m above the ground level from vegetated area. Plates were incubated at 25°C for 4-7 days .The colonies obtained were counted and expressed as colony forming units for plate per hour (CFU/plate /h).

Identification of Actinobacteria

Actinobacteria were isolated from the atmosphere samples and subjected to purification. Adequate phenotypical test set and chemo-taxonomical investigations were used for the identification of the strains (colony and micro-morphological characteristics, pigment production tests, whole cell sugar pattern, cell–wall chemotype, oxidase, catalase, aminopeptidase, benzidine test, OF glucose, lecithinase, lipolysis, proteolysis, hydrolysis of pectin, chitin, hippurate, casein, esculin, gelatine, soluble starch, degradation of xanthine, elastin, arbutin, growth with 2,3,4,5,6,7 and 7.5 % NaCl utilisation of sucrose, m-inositol, D-melibiose, fructose, xylose, galactose, glycerol, ribose, salicin, glucose , arabinose, NO_3- reduction, H_2S production [39 and 12].

Results and Discussion

There is no doubt that the pollution caused by microbial aerosols presents specific hazards to the human occupants. Organic particulate components of the air include plant pollen grains, spores from 20.000 to 40.000 species of fungi, actinomycetes and bacteria [28]. A sedimentation method has used to gain insight into the quantity and quality of actinobacteria in the investigated area. The mean counts (CFU/plate/h) of actinobacteria at the atmosphere surrounding the chamomile plants at El-Fayoum Governorate were shown in Table 1. It increases slightly from the beginning of cultivation period (November) to the 1. of January. Remarkable increase is noticed during the end of January and the beginning of March (harvesting period) followed by a drastic decrease at the late harvesting of the chamomile plants (end of March). These results are in agreement with those of [8 and 11] who reported that crop harvests have been coincided with increased concentration of microorganisms.

A total of 311 actinobacteria colonies from the atmosphere under consideration were obtained and characterised. The distribution of the isolates into different actinobacteria morphotypes is shown in Table 2. The isolated strains fell into 7 genera and 13 species.

Table 1. Mean monthly count in CFU of actinobacteria from the atmosphere surrounding chamomile plants

Month	Mean actinobacteria counts CFU/Plate/h
November	93
1st January	103
30th January	213
5th March	314
25th March	129

The dominant genus was identified as Streptomyces (229 isolates and it represents 73.63%). The dominant group with 143 (45.98%) strains could be identified as Streptomyces anulatus. Members of this group have rectiflexibiles or occasionally spiral spore chains, the spore surface is smooth. Melanoid pigments are generally not produced, but a few strains are positive, particularly on tyrosine agar. The members of this group belong to the yellow and grey colour series. Based on a broad spectrum of phenotypical characters this species has been delineated at 81% SSM by Williams and Co-workers [39]. 52 strains (16.72%) are members of Streptomyces albus. This group have spiral spore chains, the spore is smooth and it belong to white series. Melanoid pigments are not produced. 23 strains (7.39%) identified as Streptomyces californicus and 11 strains (3.54%) were identified only at the genus level as Streptomyces sp. Streptomyces californicus have spore chain rectiflexibiles and the surface is smooth. The spore mass is red, yellow to grey. Melanin pigment is not produced. This variable species is widespread in nature.

Table 2. Total count of genera and species of actinobacteria isolated from the atmosphere surrounding chamomile plants and the percentage of its occurrance

Genera and species	Number of isolates	% of occurred species
Streptomyces anulatus	143	45.98
Streptomyces albus	52	16.72
Streptomyces californicus	23	7.39
Streptomyces sp.	11	3.54
Micrococcus roseus	27	8.68
Micrococcus luteus	17	5.47
Micrococcus sp.	6	1.93
Rhodococcus sp.	10	3.21
Arthrobacter globiformis	4	1.29
Arthrobacter sp.	4	1.29
Microbacterium sp.	5	1.61
Cellulomonas sp.	5	1.61
Nocardia sp.	4	1.29
Total isolates count	311	100.00

The specific components or metabolites in the spores of streptomycetes should be considered the most probable causative agents of allergic and/or immunostoxic respiratory disorders. For example, streptomycetes have complex lipid–sugar structures in their cell walls [2]. These bacteria also produce a vast variety of bioactive compounds as secondary metabolites [1]. Streptomyces anulatus and Streptomyces albus are actinomycetes common in soils and dust which may cause allergic alveolities [10]. In addition, the spores of Streptomyces californicus were also found cytotoxic to mouse macrophages [1].

The percentage of the total non streptomycetes isolates was 26.37%. 40 strains of non streptomycete actinomycetes were belonging to the genus Micrococcus which represents 16%. Micrococcus is Gram positive, non sporing, colonies usually pigmented in shades of yellow or red colours and catalase positive. They occur in soils, but commonly they are isolated from air. The first cluster comprises Micrococcus roseus. The second one identified as Micrococcus luteus and six strains could be identified as Micrococcus sp. Several species from Micrococcus are known to have allergic properties [13]. The growth conditions present in the study area regulate its secondary metabolism such as toxin production.

10 strains could be characterised as Rhodococcus (3.21%). Rhodococcus have rods to branched mycelium and Gram positive. They are widely distributed, but particularly abundant in soils.

8 strains are members of genus Arthrobacter (2.58%). Arthrobacter cells are irregular rods, often V-shaped but there are no filaments, Gram positive and catalase positive. It represents marked rod-coccus growth cycle. This genus is widely distributed in the environment, principally in soils. Four of them are Arthrobacter globiformis. Much less is known about the potentially pathogenic properties of corynebacteria associated with organic dust. Nevertheless, cases of allergic alveolitis caused by Arthrobacter globiformis [23] and the involvement of peptidoglycan produced by these bacteria in causing allergic disease can not be excluded.

With the help of our determinative key, Microbacterium sp. (1.61%), Cellulomones sp. (1.61%) and Nocardia sp. (1.29%) could be identified.

Microbacterium have irregular rods, no marked rod-coccus cycle and Gram positive. It is commonly found in soils.

Cellulomonas also have irregular rods, no mycelium is found, Gram positive, catalase positive and it showed cellulolytic activity. It seems to be common in sugar based biopolymer rich habitats, weather on surface of leaves, weather in soil and decaying vetable matter.

Nocardia have rod-shaped to coccoid elements and Gram positive. They are widely distributed and abundant in soils.

The bacteria triggered the production of pro-inflammatory mediators at lower concentration indicating that inflammation may be the primary response in lungs. These results imply that bacterial species need to be considered as causative agents for adverse inflammatory effects in this environment. We can conclude that during harvesting of chamomile plants, microorganisms are released onto the air and high concentration of airborne bacteria may occur inside the harvesting area.

In conclusion, the obtained results indicate that farmers exposed to large concentrations of grain dust and associated microorganisms during harvesting period are under increased risk of work – related pulmonary disorders, such as allergic alveolitis, asthmas, chronic bronchitis and orgasnic dust toxic syndrome.

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STRUCTURE OF DANUBE SHAD (Alosa pontica Eichwald, 1838) SPAWNER FLOCKS MIGRATING FOR REPRODUCTION IN DANUBE RIVER
(MIGRATION OF FISHES IN ROMANIAN DANUBE RIVER № 2)

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Abstract. The main purpose of this paper is to actualize last data on Danube shad (Alosa pontica Eichwald, 1838) migration process in Danube River. This species was widely studied last time in Danube River, but there are still some biological and ecological aspects to be revealed. Study of periodical changes in the total amount of capture and spawners structure could bring more and relevant information on presumed cyclic decrease and increase of stocks and also about the main causes of their interesting population dynamics. We try to examine some other aspects related to the spawning biology as well as to the biometry of different age-classes and also to link these issues to the last changes in environment conditions.
Keywords: *Danube shad, Migration, Captures, Stocks structure*

Introduction

Danube shad (*Alosa pontica* Eichwald, 1838) migration in Danube River is a yearly event quite impatiently expected by fishermen and biologists. First ones expect big captures of this species of fish which is highly appreciated by a certain number of customers regionally because it is a Christian custom of local people to eat Danube shad in the Lent. Second ones are trying to improve their database with new aspects regarding the interesting migration of this species.

The interest in this species is even more accentuated by its specific biology. Danube shad is a migratory species that is spread only in Black Sea and Low Danube River hydrologic basins. It could be considered as a species formed by only one population that use to live, eat and spend its winter season in the South-West part of Black Sea coming up into Danube just for spawning. This population is structured in one stock containing spawners of different ages and another one formed by the juveniles that migrate at a certain moment from Danube to feed and grow up in Black Sea.

From commercial aspects, Danube shad is a very important fish for fisheries in the Danube Delta, because in these places were registered some times 90% from the total amount of annual Danube shad capture on the whole Danube, and also for other upstream fisheries especially in the early spring season when other fish species are less available.

Materials and methods

We have a large amount of data for the study of Danube shad migration. We used samples collected from both experimental fishing campaign and commercial fishing on many different locations on Danube River.

Within experimental fishing period from 2000 to 2001 we used the same fishing technologies and similar fishing gears as commercial fishermen. We had been fishing on the same places during these years and we also collected samples from a few different locations known as preferred places for juveniles crowding. Fishing gears specialized for Danube shad were used: drift gillnets and appropriate boats operated by two fishermen.

We have collected biometric data from 1072 individuals randomly taken in different places on Danube starting from the Danube Delta to Bazias Town. The main biological parameters registered for each individual were: total length, standard length, individual mass, age and sex.

For age estimation 4 to 6 scales situated on the first half of the upper part of fish body have been collected and annual growing ring marks on the degreased scales were read in a laboratory. The sex of each individual has been established through dissection, where the gonads were sought.

We used also new and older statistic data regarding capture's level, data on water levels variability and water temperature dynamics in order to find any new relation between the intensity of migration and the fluctuation of main physical environment.

There have been considered also some information on ecological issues related to the local behavior of Danube shad such as best reproduction spots and preferred places of the offspring to crowd.

Results

Statistic data show that for a longer period of time the level of Danube shad captures could be very different in Romanian Danube River. If we look at the capture data taken in Danube Delta area (Sector No 1) and also captures upstream (Sector No 2) from 1991 to 2001, the total amount of yearly capture in Danube River had a very wide range. There
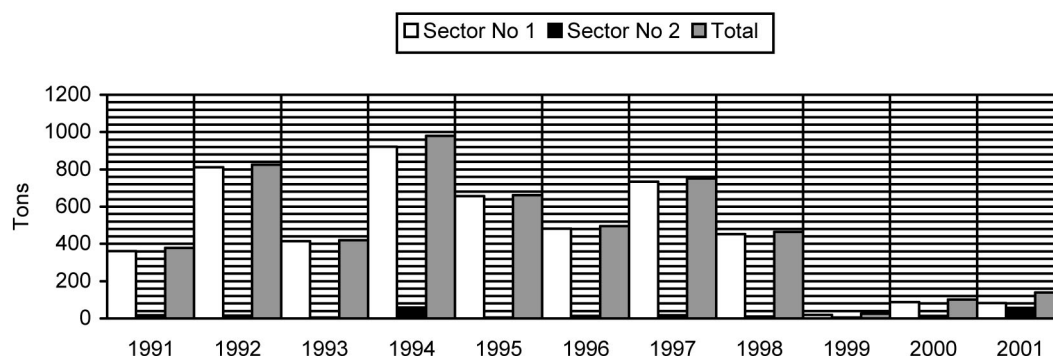

Figure 1. Danube shad capture dynamic in Romanian side on Danube River from 1999 to 2001.

is a maximum of 980 tons in 1994 and a minimum of only 24.6 tons in 1999 (Fig.1) We have to underline that even if there had been changes in fishing effort and even if some unfortunate errors happened or incomplete data registered, these factors could not bring a reasonable explanation of such a huge difference between the levels of annual amount of capture.

We do not consider the 1999 amount of capture in official statistics as real or credible because its unusual low value. It is easy to observe the big difference between annual data: from more than 980 tons in 1994 to less than a half of this amount (379 tons) in 1991. However in the next two years the capture dropped to about its third, if we compare the average yearly capture until 1999 with the values from 2000 and 2001.

In 2000 Danube shad started to migrate in March when water levels were increasing and water temperature stabilized at about 6°C, the maximum of migration intensity took place in April, and the migration ended in July.

The main capture of about 86% of total has been registered in Danube Delta area - especially on Saint George Branch.

Study of experimental fishing capture shows spawners belonging to 6 classes of age (Tab. 2), quantitatively dominated by those of 3 and 4 years old that represented together 83.5% of total annual amount of capture.

Table 1. *Danube shad percentage of sexes and classes of age estimated within 2000 and 2001 fishing campaigns (NA: not available).*

Year	Danube shad	Number of Fish	%	Class of age percentage (%)					
				2 years	3 years	4 years	5 years	6 years	7 years
2001	Males	402	53.5	87.8	4.2	34.0	29.8	18.8	14.5
	Females	350	46.5	12.2	45.8	66.0	70.2	81.2	85.5
	Total	752	100	5.2	50.2	33.3	5.0	3.2	1.2
2000	Males	148	46.25	72.2	51.2	18.4	11.2	5.3	NA
	Females	172	53.75	27.9	48.8	71.6	88.8	94.7	NA
	Total	320	100	21.5	41.2	30.3	4.1	2.9	NA

Males/females sex ratio (M/F) calculated on 752 individuals of about 1.15 is considered to be a normal one because the M/F of this species is usually around 1.0.

Average mass and length values (Tab. 3) show a normal condition of spawners in each class of age and a particularly good growing up and development of the younger, 2 years old individuals that explained a higher presence in the 2001capture than usual.

Because of the relative high water temperature of 5°C to 7°C in February 2001, Danube shad migration started earlier than 2000 and lasted until the end of July with a peak of migration intensity in the second decade of April when the daily average water temperature was between 10-12°C.

Table 3. Biometric data of Danube shad spawners (TL: total length, ST: standard length, W: weight).

Age (Years)	Number of Fish	Biometric Data	Minimum Value	Maximum Value	Average Value
2	39	TL (cm)	20,4	31,6	27,2
		SL (cm)	19,2	17,4	23,0
		W (g)	98	238	175
3	378	TL (cm)	20,5	36,5	28,2
		SL (cm)	19,5	31,1	24,0
		W (g)	101	381	201
4	264	TL (cm)	23,4	37,4	30,1
		SL (cm)	19,9	32,1	25,6
		W (g)	124	418	236
5	38	TL (cm)	25,9	36,6	32,8
		SL (cm)	22,1	31,1	28,2
		W (g)	184	389	288
6	24	TL (cm)	33,4	38,5	35,2
		SL (cm)	26,0	32,8	30,1
		W (g)	268	434	348
7	9	TL (cm)	37,8	38,0	37,9
		SL (cm)	32,4	32,9	32,6
		W (g)	438	475	456

Similar to 2000, individuals of 3 and 4 years old are the most frequent representatives having together a percentage of 71.4%. We have noticed a bigger number of 2 years old spawners (21.5%) also in 2001, dominated by younger males as much as 72.1%. This time we did not find any individual of 7 years old. The possible reason for that could be the smaller number of individuals investigated and the reduced percentage of this class of age in total amount of capture (about 1%).

In 2001, sex ratio decreased under the value measured in 2000, showing a relative dominance of females. Calculated M/F for a number of 320 individuals was 0.86.

Discussion

Even there is not any reasonable explanation on this topic, some biologists [5] believe there is cyclic dynamism of Danube shad migration that includes a maximum every 10-11 years. This idea is based on the statistic analysis of yearly capture starting in 1920 [1], data which could not be considered very accurate for a certain period of time. This supposition considers year 1994 when the total amount of capture was more than 980 tons as the peak of the last cycle as well as 1999 being the year with the minimum value of this supposed cycle.

Probably there is another cycle: the intensity of migration changes every 3 to 4 years

[4]. The explanation of this fact could be the age structure of Danube shad flocks in which spawners of 2 (not so often) 3 and 4 years old are predominating as of more than 85%. That means a successful migration and reproduction, which will result in a large amount of offspring [2] and will form the core of flocks coming up for reproduction next time. In the capture dynamism it is easy to see that there is a peak of migration intensity every 2-3 years.

However, the graph of yearly captures (Fig. 1) shows an evidently decreasing tendency meaning a dramatic drop of the number of spawners migrating for reproduction and we do not have any reasons to suppose in a couple of years this situation will become better.

Causes of decline of migration could be multiple [6], including changes in evolution of seasonal temperature, hydrologic regime of Danube River, habitat and water quality degradation, all of them potentially affecting spawners status and offspring survival. Yet, the most important is the increasing fishing effort on almost all species of fish in Danube River and particularly on Danube shad.

Biometric data bring up some interesting information related to the average sex ratio and its distribution in the classes of age. Estimated M/F ratio as of 1.15 in 2000 and of 0.86 in 2001 are considered normal, their average value of about 1.0 being very close to the M/F = 1.1 ratio calculated within a wider period of time [3].

It is also very interesting to notice the very different values of M/F ratio for younger and older spawners. In the class of 2 years old individuals this value has an average of almost 2.6, for the individuals of 6 and 7 years old to be clearly represented almost just by females (M/F=0.1-0.18).

Conclusions

The recent dramatic fall in the number of Danube shad migrating for reproduction in Danube River is a real issue that evidently should concern interested persons and involved institutions, companies and organizations from both commercial and ecological point of view.

Last data on the quite low annual amount of capture does not bring up any hope concerning eventually further positive changes on Danube shad migration intensity, until environmental conditions will be more favorable.

Even if we consider the unfortunate changes of some ecological aspects related to the environmental factors such as lower water level, water temperature and pollution that could actually affect the success of the Danube shad reproduction, the most important cause of the decreasing of the stocks is the overfishing mainly in Danube Delta area, which provides about 90% of total amount of capture in Danube River.

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SIMULATION EXPERIMENTS TO EVALUATE THE ROBUSTNESS OF THE OPTIMAL CONSTRUCTION OF MONITORING NETWORKS

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Abstract. OptiNet is a PC program for optimal network selection. The aim of this study is to test the program by simulation experiments and to investigate the robustness of the optimal selection. One of the most important results is that the area should not be represented by an equidistant grid to calculate the maximum Kriging prediction variance, it is sufficient to investigate the boundary points only. Effects of the parameters of the covariance function, the number of selected points and of a possible factor were also investigated. All simulations are based on the Gauss-Krüger coordinates with 1 m raster in the area of Brandenburg.
Keywords: *spatial statistics*, *optimal design, simulation, kriging, covariance function*

Introduction

In agriculture there is often need for using monitoring networks to detect changes within an agro-ecosystem. Monitoring is usually an expensive and time consuming work. Therefore monitoring networks should be planned and organized in an optimum way. To do this OptiNet, a program for optimal network selection was developed by BioMath GmbH in Rostock [6]. This is a PC program for:
- Constructing an optimal network in a given area.
- Extending an existing network in a given area.
- Reducing an existing network in a given area.
- Graphical representation of the candidate (starting) points and the selected points.

The selection is based on the optimality criterion of the maximal Kriging prediction variance in the area, which is based on an exponential covariance function. A network is optimal if it minimizes this criterion within a given area (Brandenburg in our case) over the set of all possible networks. To find the maximum prediction variance, the area should be represented by a regular (equidistant) grid with a given distance D between neighbouring points in a row and a column.

Several problems, which up to now have not been solved theoretically, were investigated by simulation experiments. During the simulations, components of OptiNet were used and tested.

Our aim was to answer the following questions:

- How large can D be chosen so that a wrong network would be selected with a small probability?
- How can we interpret the parameters of the covariance function for practical purposes?
- What is the influence of incorrectly selected parameters of the covariance function?
- Is there any optimal number of selected points for a network?

The area examined

The simulation was based on the Gauss-Krüger coordinates with 1 m grid in the area of Brandenburg. This means that locations can be identified as different only if at least one of their coordinates differs by more than 1 m.

The sites were selected from an existing monitoring network for ground water level in Brandenburg. Most of the simulations were done with two sets of starting points, one with 20 sites, the other one with 24 sites. *Figure 1.* shows the 20 selected sites with circles and the squares indicate the additional points. Later in some parts of the simulation further points were added up to 32 or 40.
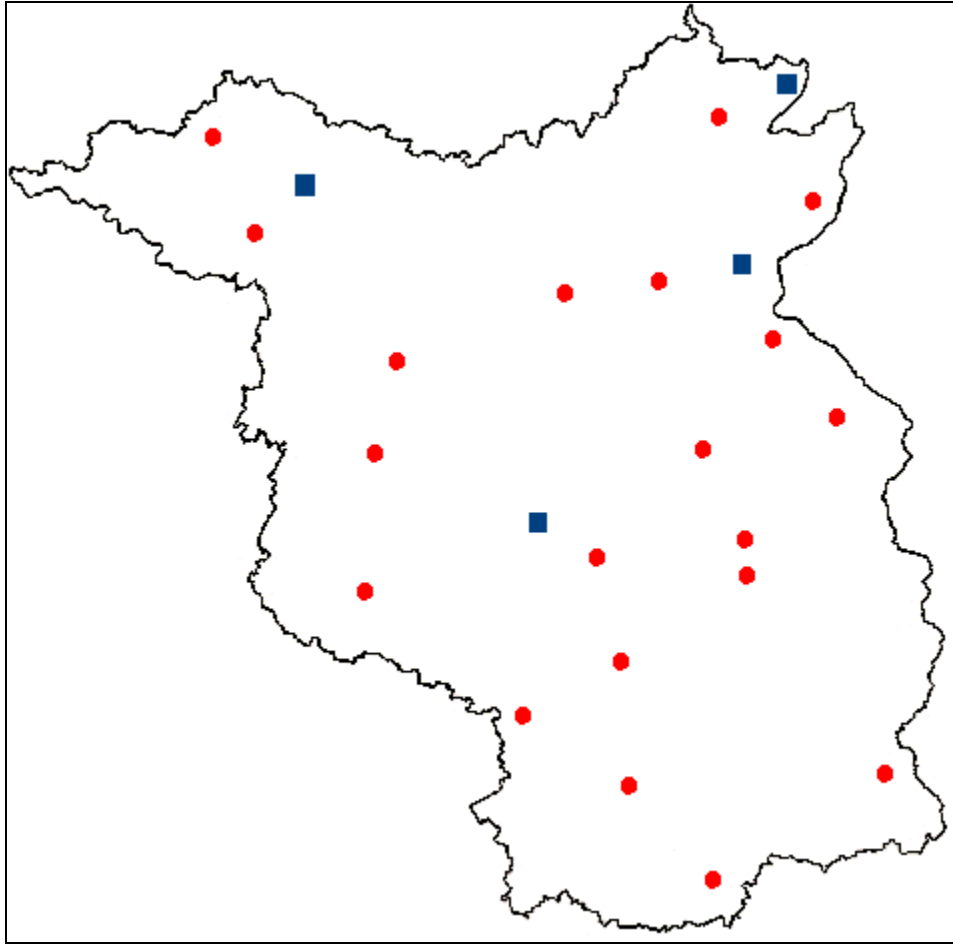
Figure 1. *The selected sites in the region Brandenburg*
(the circles indicate the 20 points, the squares the additional points).

The covariance function

To calculate the Kriging prediction variance, the exponential covariance function was chosen. It is given by:

$$\text{cov}(h_{ij}) = \alpha + \beta \cdot e^{\gamma h_{ij}}; \quad \alpha, \beta > 0, \quad \gamma < 0, \ i \neq j; i, j = 1,..., N \tag{Eq.1}$$

where h_{ij} represent the Euclidean distances between the pairs of candidate points P_i and P_j.

The exponential function in (Eq.1) is an intrinsically non-linear function [7] with linear parameters α and β and non-linear parameter γ.

Figure 2. shows the graphs of three exponential covariance functions with different γ values. The value of γ depends on the dimension in which h is measured. As the scale of x-axis (representing the distances) is changed from kilometres to meters, the value of γ must be divided by 1000 as it can be seen from (1) because the product γh must be invariant due to rescaling. During the calculations the coordinates, and by this the distances were given in meters. But in this reports we use the km-scale.
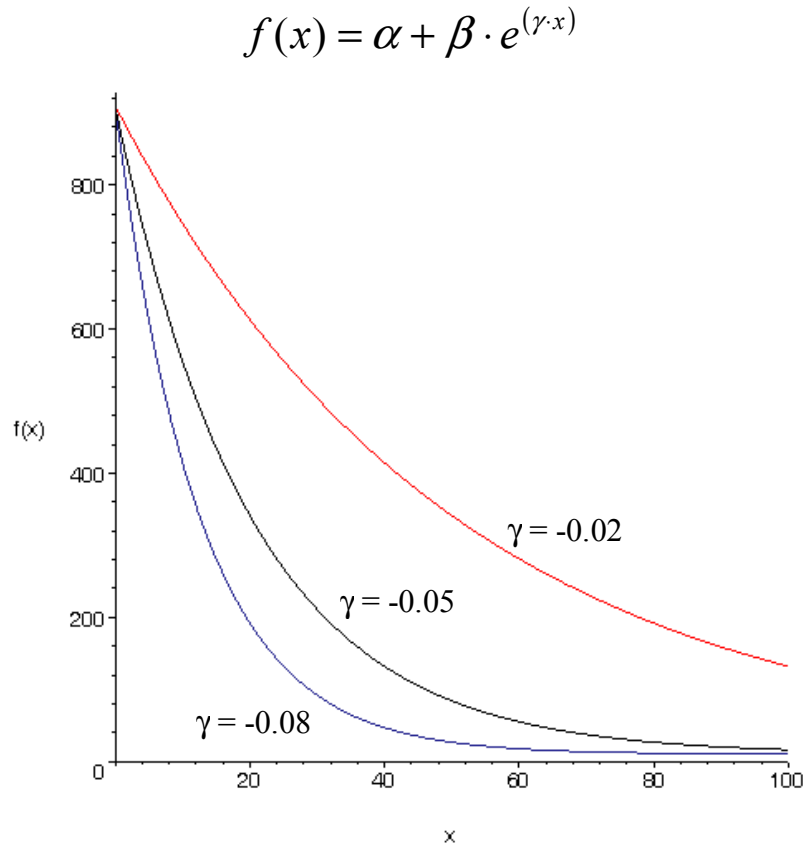
$$f(x) = \alpha + \beta \cdot e^{(\gamma \cdot x)}$$

Figure 2. The graph of the exponential covariance function
with $\alpha = 10$, $\beta = 900$ and different γ values.

The value of $\alpha + \beta$ represents the variance (covariance for the distance 0), α is the asymptote for x to infinity, and γ describes the spatial correlation. In the examples above, we can consider the middle one ($\gamma = -0.05$) as a general case, while the other two represent extreme situations. For practical purposes we can say that in case of $\gamma = -0.05$ the spatial correlation has effect within about 60 km distance, in case of $\gamma = -0.02$ also in more than 100 km, while in case of $\gamma = -0.08$ only within 30-40 km.

Simulations and results

Grid distances

Based on the former study [6], the area should be represented by an equidistant grid with a given distance D to find the maximum prediction variance. The grid-sizes were examined with D-values of 5, 10, 15, 20, 25 and 30 km. A special case, labelled with 00, with only four grid-points out of the region was applied additionally. The parameters of the covariance function were $\alpha = 10$, $\beta = 900$ and $\gamma_1 = -0.05$, $\gamma_2 = -0.02$ and $\gamma_3 = -0.08$. The number of the candidate points were $N = 20$ or 24, and in both cases we selected $n = 12$ points for the network. The main results were the followings:

- The time needed for the calculation depends on N and n (according to the $\binom{N}{n}$ possible selections) and on the grid-size, too. For example in case of $N = 24$, $n = 12$ the total number of possible selections is almost 3 million (2704156). In our computer the running times were for $D = 10$ km 4 days, in case of $D = 5$ km more than 17 days.
- The values of the prediction variance usually increase as the grid-sizes decrease, but it is not a monotonous change.
- If γ has lower (more negative) value, the standard deviation of the criterion values is much lower, however the criterion values are higher.
- Comparing the different grid-sizes, the results of 5, 10 and 25 km grids proved to be similar.
- The grid-points, where we can find the lower criterion values, are located in almost every case on the boundary of the region or near to it, if there is no point on the boundary (like in case of the grid with 30 km).

Calculations based on the boundary points

Since in former simulations the maximum criterion values were always found on the boundary of the region, in our calculations we were searching the maximum prediction variance for evaluating different subsets on the boundary only instead of the looking at the whole grid.

This in this time heuristical approach is in the meantime justified by a paper of Haberl [3]. There it is shown that even in the case of a non-convex region (like Brandenburg) the maximum of the prediction variance is always on the (outer) boundary of the region and a gap inside the region (Berlin in Brandenburg) plays no role.

The distances between the points on the boundary were 5, 10, 15, 20, 25 and 30 km. These calculations take significantly less time than in investigating the whole grid.
We can also observe that:

- Results obtained with 5 or 10 km distances can be considered as equal.
- As in case of the grids, if γ has lower value, the standard deviation of the criterion values is much lower and the criterion values are higher.
- We can say that γ has an effect on the selected sets.

Effects of α and β

As we know that γ has an effect on the selected sets, it is interesting whether the two other parameters, α and β have also some influence or not. The covariance function was used with $\gamma = -0.05$ and with the following parameters:

a) $\alpha = 10$, $\beta = 900$
b) $\alpha = 5$, $\beta = 450$
c) $\alpha = 5$, $\beta = 200$
d) $\alpha = 5$, $\beta = 900$
e) $\alpha = 20$, $\beta = 900$

Regarding these parameters we got the following results:

- The values of α and β do have no effect on the selected sets, we get always the same results. This was expected because these parameters are linear parameters of the intrinsically non-linear exponential function.
- The criterion values are determined only by β (in this case more or less equal to the value of β).
- As β is increasing, the difference between the minimum and maximum values of the criterion is also increasing.
- The results of $D = 5$ and $D = 10$ km can be considered as equal, also the minimum and maximum criterion values are the same, but in case of $D = 5$ the standard deviations are a little bit higher.
- With the same β, the criterion values were a little bit higher in case of $N = 20$ than in case of $N = 24$.
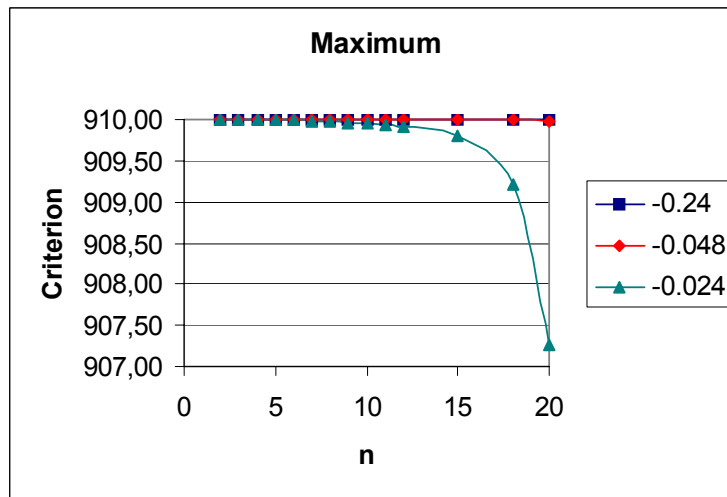
Effect of the selected sets

One of the most important questions is, whether there are an optimal number of selected points for a network. To answer this, a series of selections were made from $N = 24$ points, with the parameters $\alpha = 10$, $\beta = 900$, $\gamma_1 = -0.24$, $\gamma_2 = -0.048$ and $\gamma_3 = -0.024$ (the different γ values indicate that the spatial correlation may be significant within 10, 50 or 100 km). The distances between the points on the boundary were 10 km, as we could see now, that it gives the same results as $D = 5$ km, but the calculation is quicker. Simulations showed that:

- When the number of the selected points (n) increases, the criterion values decrease.
- The change is more rapid in case of the minimum of the criterion values, and not significant in case of the maximum criterion values until $n \leq N/2$. It means also that if the number of the selected points increases, the differences between the minimum and maximum values of the criterion (which shows the mistake in case of a completely wrong selection) are also increasing in the first part, but then they decrease a little bit, when $n \approx N$.
- When we suppose that the spatial correlation exists only within a very short distance (in this example γ_1), the criterion values are the same for all selections and we cannot find an optimal subset of points.
- In case of larger distances (the γ value is near to zero) the differences are larger, so the optimal selection is really important.
- When we select 12 or more points (from 24), the change in the minimum value of the criterion is not important (*Figure 3/a*). This could mean that there is no reason to select more points for an optimal network, or we can select them arbitrarily. However, in the *Figure 3/c* we can see, that the difference between the minimum and maximum values of the criterion is still high (especially in case of γ_3), does not decrease with n, and the selection of additional points is also important.
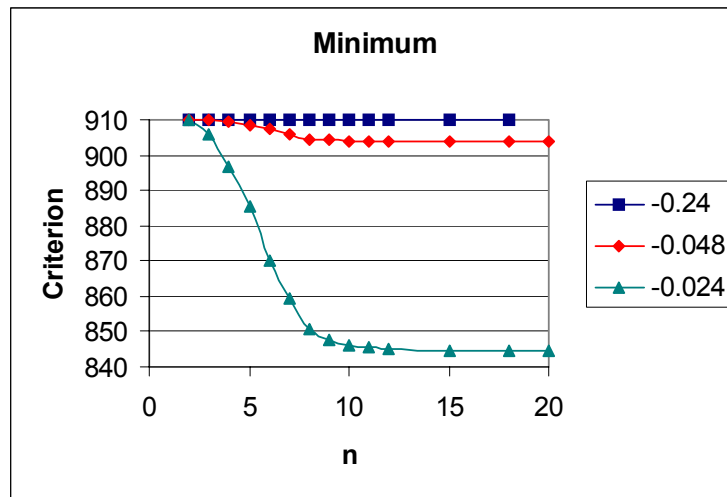
a)

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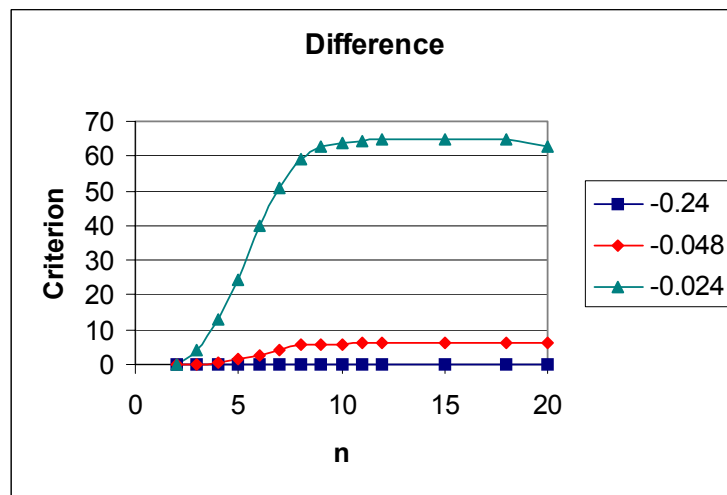
c)

Figure 3. *The change of the criterion values by the selected sets (n), in case of different* γ *values (N = 24, D = 10 km,* α *= 10,* β *= 900).*

Table 1. shows a series of selected sets. It can be seen that if we want to select a small set, the optimal selection is very important, as the selected points are changing. Than it seems that the formerly selected points remain, only we have additional selections too.

Table 1. *Selection series in the case N = 24 (D = 10 km, α = 10, β = 900, γ_3 = -0.024).*

N	Candidate points																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
5		X										X		X						X			X	
6	X			X								X			X					X			X	
7	X		X					X		X		X						X	X					
8	X					X					X					X		X	X				X	
9	X	X				X						X				X		X	X				X	
10	X	X				X						X				X		X	X	X			X	
11	X	X				X			X			X		X			X	X	X	X			X	
12	X	X		X		X			X			X		X			X	X	X	X			X	X
15	X	X	X	X		X			X			X		X		X	X	X	X	X			X	X
18	X	X	X	X		X	X	X	X	X		X		X		X	X	X	X	X			X	X
20	X	X	X	X	X	X	X	X	X	X		X	X	X		X	X	X	X	X			X	X

From this simulation we cannot conclude that the change of the criterion values is not significant selecting more than 12 points (half of the starting points). Therefore we repeated the simulation with more starting points, like N = 32 and N = 40, using only γ = -0.024. It seems that when we select from many points, the minimum of the criterion values changes more rapidly, but the maximum changes slower, so the differences are higher.

As the running time does not allow calculating all the possible selections (in case of N = 40 and n = 8 the running time is more than 300 hours, in case of N = 32 and n = 9 more than 8 days), we suggest fitting a non-linear curve to estimate the missing values. The tangent hyperbolicus function proved to be good for these fittings (*Figure 4.*).

Based on the fitted curves we can conclude that the change of the minimum values of the criterion is not important when $n \geq N/2$.

Effect of a possible factor

All the previous simulations supposed that the area examined is more or less homogeneous. Since in most cases we have at least one environmental factor (like climate, soil type) defining different conditions at the candidate points, we also carried out a simulation supposing that we have a factor with four levels. The candidate points were divided into four groups (the whole area was divided into four sub-regions with equal number of sites in each). The sites for the network must be selected so that we have points from all sub-regions. (When it is possible with the same number of points from each sub-region.)
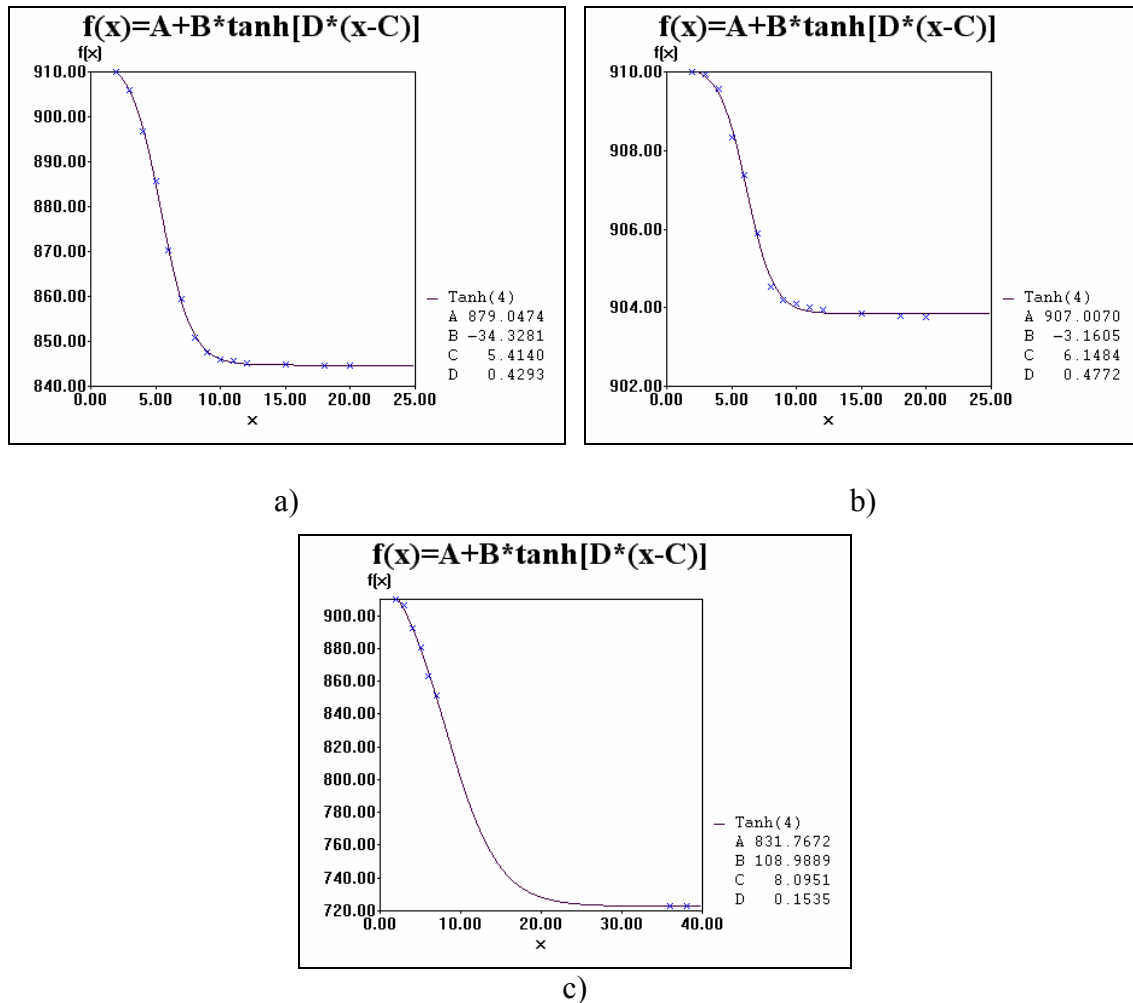
a)

b)

c)

Figure 4. *Change of the criterion values by the selected sets (n) after fitting.*
a) N = 24, γ = -0.024, b) N = 24, γ = -0.048
c) N = 40, γ = -0.024

As formerly, a series of selections were made from $N = 24$, $N = 32$ and $N = 40$ points, with the parameters $\alpha = 10$, $\beta = 900$ and $\gamma = -0.024$. We found that:

- The minimum values of the criterion are in almost every case the same as without sub-regions, but the maximum values and also the differences decrease more rapidly.
- Using sub-regions or not, the selected sites are in almost every case the same (therefore are the minimum values of the criterion the same).
- The calculation time is shorter, because we have a restricted number of possibilities due to blocking in sub-regions.

An example for utilising the results of simulation experiments

A monitoring character for Genetically Modified Plants

Genetically modified plants (GMP's) - once they have been placed on the market - have to be observed for suspected adverse environmental effects in accordance to

Annex VII of the EU Directive 2001/18/EC and the Monitoring Guidance Note 2002/811/EC.

The aims are to monitor dissemination tendencies of the GMP's and of outcrossing events concerning the transfer of recombinant DNA to wild relatives (formation of recombinant hybrids). An example for a crop species possibly tending to disseminate and, additionally, having a number of wild relatives living in natural habitats could be rape (*Brassica napus* L.) [1]. Since genetically modified crop species - as the non-modified lines - will be cultivated in an agricultural environment the supposed GM crop depending environmental effects have to be monitored in ecosystems typical for agricultural landscapes.

Dissemination tendencies of GM crops and of hybrids of GM crops and wild relatives could be expected if the recombinant traits offer some ecological advantages to the recombinant plants. The new traits may change the behaviour of the species in a way the recombinant descendants becoming feral. As a consequence, recombinant crop species may get the opportunity to emerge not only in agro-ecosystems but also in special natural habitats. Mainly, more or less frequently disturbed habitats (so-called "ruderal" sites) will be appropriate for the growth of feral GM crops and of GM hybrids out of cultivated fields [5].

Consequently, to observe the behaviour of recombinant feral crops and hybrids special target (cultivated fields) and non-target ecosystems (natural and semi-natural habitats) typical for agricultural landscapes have to be investigated in a GMO monitoring. Accordingly, a monitoring network has to consider the landscape structure of agricultural landscapes concerning cultivated fields and natural habitats.

Neemann et al. [4] found the proportion of annual weeds of the plant communities, especially of the non-agricultural ecosystems, to be a suitable indicator for the differentiation of habitats of greater or minor importance for the dissemination of GM crops. Higher proportions of annual weeds are typical for ecosystems being frequently disturbed. Additionally, the more frequent natural ecosystems in agricultural landscapes are disturbed, the more often feral crops or wild relatives are found, too. To differentiate disturbed habitats of higher or minor importance for the dissemination of feral GM crops and wild hybrids a ranking system was developed. However, some agro-ecosystems (e.g. fields cultivated with summer annual crops) may also be of importance for the reproduction of feral GM crops.

As a consequence, for the implementation of a GMP monitoring in agricultural landscapes a character is needed offering information about the quality of the agricultural and non-agricultural ecosystems for dissemination. Additionally, the character must give information about the quantitative importance of disturbed (ruderal) sites and of fields cultivated with summer annual crops within specific landscapes. Considering the ranking system the character summarizes the area-based proportion of disturbed habitats of great importance for dissemination and of the area-based proportion of fields cultivated with summer annual crops. Accordingly, the character may be described as "dissemination character".

An optimal monitoring network for Genetically Modified Plants

The construction of an optimal network for agricultural landscapes considering the dissemination character firstly means to analyse landscape sectors quantitatively being important for GM crops and GM hybrids dissemination. This work has to be done by environmental specialists. Secondly, to reduce the monitoring expenditure within a

landscape the optimal numbers of the selected sectors and the optimal places for them have to be found.

For the optimization example calculated below the composition of agro- and non-agro-ecosystems were investigated in six agricultural landscapes of the German regional state of Brandenburg. 22 landscape sectors representing the habitat composition of the six landscapes were analysed in detail concerning their habitat and field characteristics. Each of the sectors had an extension of 3 km x 3 km.

For the agricultural landscapes considered the optimal places of monitoring sectors have to be found by the optimization procedure. The distribution of the exponential covariance function was determined from measured values representing the "dissemination character" from the 22 habitat sectors. The model parameter ß was estimated from the range of the values to be 38.23 %. The parameter γ describing the magnitude of the spatial dependency was – due to the results of the simulation study – fixed to -0.05. With this model it is possible to construct an optimal network for monitoring the dissemination within habitats, i.e. to find the optimal number and location of habitat sectors of 3x3 km from possible candidate points. For the region Brandenburg we took the 22 candidate points from which 6, 12 or all points should be selected for a future monitoring network – considering the factor "landscape". In *Table 2*. the X characters represent the best selection for a given number of selected sets and the criterion values can be seen, too.

Table 2. *Results of the selection series.*

LOCATION				Selected points		
Name	Coord. x	Coord. y	Landscape	6	12	22
Dahnsdorf-Ost	5341244	5778382	Fläming			X
Dahnsdorf-West	5338225	5777500	Fläming		X	X
Illmersdorf	5381900	5752046	Fläming			X
Hohenseefeld	5385900	5752200	Fläming	X	X	X
Schönefeld	5387244	5773400	Nuthe-Urstromtal		X	X
Stülpe	5384300	5771300	Nuthe-Urstromtal	X	X	X
Bliesdorf	5443525	5842237	Oderbruch	X	X	X
Altlangsow-Nord	5458844	5828555	Oderbruch		X	X
Altlangsow-Süd	5461855	5826730	Oderbruch			X
Mallnow	5464644	5817600	Oderbruch			X
Rädikow	5432400	5842800	Barnim-Lebuser-Platte		X	X
Frankenfelde	5435600	5838400	Barnim-Lebuser-Platte			X
Seelow	5455844	5822900	Barnim-Lebuser-Platte			X
Schönfließ	5464244	5809400	Barnim-Lebuser-Platte	X	X	X
Postlin	5283231	5899400	Prignitz	X		X
Blüthen	5284131	5896000	Prignitz		X	X
Pirow	5293200	5904600	Prignitz			X
Burow	5296400	5903200	Prignitz		X	X
Falkenhagen	5416531	5916200	Uckermark		X	X
Ellingen	5421831	5914500	Uckermark			X
Göritz-Malchow	5427531	5922281	Uckermark	X	X	X
Dauer	5428417	5917900	Uckermark			X
criteria[*]				**2,716**	**3,285**	**3,335**

[*] criteria = (estimation of variance - optimality criterion) * 10^3

The optimality criterion is the maximal prediction error for the dissemination character in the Brandenburg region. With increasing the number of selection points the optimality criterion decreases, i.e. the prediction error for sites without measurement will become smaller. By selecting 12 points (two per landscape) the criteria enhances by 20.95 % compared to the selection of only 6 points. Selecting all (=22) points the criteria enhances only by 1.52 % (with reference to 12 points).

The example shows that with the knowledge about the features of the covariance function and the distribution of the characteristic under examination it is easy to select different numbers of optimal sites from networks. This is helpful for example in designing time and cost effective monitoring plans for example for GMO´s in balancing the number of sites to be maximised and the prediction error to be minimised.

Discussion

Construction of monitoring networks is often needed in agriculture and environmental sciences. The aim of this study was to test the OptiNet program developed by BioMath GmbH [6]. One of the most important results is that the area should not be represented by an equidistant grid to calculate the maximum Kriging prediction variance, it is sufficient to investigate the boundary points only, that makes the selection more rapid. In the meantime it was justified by a paper of Haberl [3], too.

The optimality criterion of the maximal Kriging prediction variance in the area, based on an exponential covariance function is effective especially in case we select less then half of the candidate points ($n \leq N/2$).

It is also very important that when we suppose that the spatial correlation exists only within a very short distance, the criterion values are the same for all selections and we cannot find an optimal subset of points.

A case study regarding the monitoring of GMP's was also demonstrated. It showed that the program is a helpful tool in designing time and cost effective monitoring plans.

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ECOLOGY OF THE ACALYPTA SPECIES OCCURRING IN HUNGARY (INSECTA: HETEROPTERA: TINGIDAE)
DATA TO THE KNOWLEDGE ON THE GROUND-LIVING HETEROPTERA OF HUNGARY, № 3.

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Abstract. As a third part of a series of papers on the ground-living true bugs of Hungary, the species belonging to the lace bug genus *Acalypta* Westwood, 1840 (Insecta: Heteroptera: Tingidae) were studied. Extensive materials collected with Berlese funnels during about 20 years all over Hungary were identified. Based on these sporadic data of many years, faunistic notes are given on some Hungarian species. The seasonal occurrence of the species are discussed. The numbers of specimens of different *Acalypta* species collected in diverse plant communities are compared with multivariate methods. Materials collected with pitfall traps between 1979–1982 at Bugac, Kiskunság National Park were also processed. In this area, only *A. marginata* and *A. gracilis* occurred, both in great number. The temporal changes of the populations are discussed. Significant differences could be observed between the microhabitat distribution of the two species: both species occurred in very low number in traps placed out in patches colonized by dune-slack purple moorgrass meadow; *Acalypta gracilis* preferred distinctly the Pannonic dune open grassland patches; *A. marginata* occurred in almost equal number in Pannonic dune open grassland and in Pannonic sand puszta patches.
Keywords. *Heteroptera, Tingidae, Acalypta, phenology, habitat preference, microhabitats*

Introduction

The lace bug genus *Acalypta* Westwood, 1840 is widely distributed in the temperate areas of the Northern Hemisphere and contains approximately 30 described species. The species belonging to the genus are primarily muscicolous, however some of them can also be observed on the stems of diverse plants. Much information are available on the ecological preferences of the common European species, based mostly on many years of field experience of heteropterists. However, quantitative data on the dynamics of the population and the preferences of the species are very rare.

As a third part of this series of publication on the Heteroptera occurring at the ground level in Hungary, in order to compare the habitat preferences of the Hungarian *Acalypta* species, the authors examined extensive materials of *Acalypta* collected with Berlese funnels during about 20 years all over Hungary. Because of the identical way of collecting, the samples can be well used for the characterization of the species and also

the habitats. The substrate preferences of the species as well as the seasonal changes of occurrence of the species were examined. Furthermore, the data of the numerous *Acalypta* specimens collected with pitfall traps between 1979–1982 at Bugac, Kiskunság National Park were also processed. The temporal changes of the populations and the microhabitat preference of the species were compared.

Review of literature

Out of the 18 species of *Acalypta* occurring in Europe [26, 27], the occurrence of 8 species were reported from Hungary [21].

After Butler's classic work [7], it was V.G. Putshkov [30] who summarized our knowledge on the biology of a part of the European species of Tingidae and he also presented numerous original data and observations. Later, Péricart [26] published a monograph on the Euro-Mediterranean lace bugs. In this work, he also synthetized the data available on the ecology of each species discussed. The Hungarian species were keyed and their biology and distribution briefly discussed by Vásárhelyi [49]. The latest Hungarian check-list was compiled by Kondorosy [21].

According to the literature, the species of the genus live primarily in mosses or Sphagnums and probably they also feed on these. Many authors observed them on the stems of short plants (see below); however, their trophic relations to these plants are very improbable [26, 30]. Most species occurring in Hungary most probably overwinter as adults or elder larvae [26, 30].

Acalypta carinata (Panzer, 1806), a Eurosiberian species, was reported from Sphagnums or other mosses – e.g., *Abietinella abietina* (Hedw.) Fleisch. [36] – growing on the ground, tree trunks etc. Typically, it prefers humid, shady, mostly woodland habitats [4, 26, 30, 37]; it was found in deciduous as well as in coniferous forests [37]. It can be collected throughout the year, oviposition in September, adults or elder larvae overwinter [30, 35]. In Hungary, for a long time the species was recorded only from a single locality [16]. Later it was found in the Bátorliget Nature Reserves [48], where several specimens were captured with pitfall trap and by sifting [50]. Recently, numerous new localities were reported [32]. The species must be relatively frequent in suitable places.

Acalypta platycheila (Fieber, 1844) is a closely related and very similar species to *A. carinata*, therefore, probably it also has similar life habits. However, the comparison of the ecological preferences of the two species based on literature data is impossible because of the numerous misidentifications [26]. In the Ukraine, it was collected among *Pleurozium schreberi* (Brid.) Mitt. growing on meadows and clearings [36]. Only a few specimens have hitherto been reported from Hungary [2, 32, 48, 49, 50]. Probably it occurs sporadically all over the country but it seems to be rare everywhere.

Acalypta nigrina (Fallén, 1807), a Eurosiberian species with boreo-alpine distribution, was collected among mosses – *Hylocomium splendens* (Hedw.) B.S.G., *Polytrichum* sp. [36] –, on the stems of short plants (*Calluna*, *Hieracium*, *Thymus* etc.) and also under stones [26]. It is known to be far less hygrophilous than the previous two species (also reported from dry sandy grasslands [13]), preferring also grassland and woodland habitats [4, 7, 12, 26, 30, 38]. It was recorded occurring together with *A. marginata* [30], *A. parvula* [24] or both [14]. It is univoltine in Northwestern Germany, copulation and oviposition take place in June–July and July–August, respectively [24]; adults and elder larvae

overwinter [24, 26, 30]. Only three places of collection in Hungary have hitherto been published [3, 13, 32, 48, 49]; it is one of the rarest *Acalypta* species in Hungary.

Acalypta marginata (Wolff, 1804), a Eurosiberian species, was observed to occur on the stems of diverse plants (*Artemisia*, *Calluna*, *Hieracium*, *Thymus*) by many authors; however, its trophic relations to these plants need verification [26, 30, 39]. It was found also in mosses (e.g. *Rhytidiadelphus* sp. [36]). It was captured in diverse biotopes, primarily in relatively dry ones [11, 37, 19] (in large numbers in dry sandy grasslands [13]), but also in humid places, even in swamps [4, 18, 19]. It can be collected throughout the year, oviposite in moss and also in detritus, adults or elder larvae overwinter [30]. In Hungary it is not frequent but occurs sporadically all over the country [49].

Acalypta parvula (Fallén, 1807) is a West-Palaearctic species also occurring in the Nearctic Region. It occurs in mosses and also on the stems of different plants (*Calluna*, *Genista*, *Thymus*, *Ulex*) and even on fungi. It can be found in diverse biotopes [37]; it seems to prefer relatively dry ones, but was also recorded from humid places [18, 26]. It is univoltine in Northwestern Germany, copulation and oviposition take place in August–September and September–October, respectively [24]; adults and eggs [24] or adults and elder larvae [26] overwinter. Jordan [19] mentioned it as the most common species in the former DDR. According to Vásárhelyi [48, 49], it is rare in Hungary, known only from a few places; however, a great number of localities were published recently [32].

Acalypta gracilis (Fieber, 1844), a Eurosiberian species, was reported from different mosses, e.g. *Pleurozium schreberi* (Brid.) Mitt., *Ptilium crista-castrensis* (Hedw.) De Not., *Tortula ruralis* (Hedw.) Gaertn. et al. [26, 30, 36]. It was been observed occurring on the stems of diverse plants (*Ajuga*, *Alkanna*, *Artemisia*, *Dianthus*, *Erodium*, *Hieracium*, *Plantago*, *Sedum*, *Thymus*) [3, 26]. It seems to occur primarily in relatively dry, sunny biotopes (e.g. dry sandy grasslands [13]), but it tolerates diverse habitats (forests, steep banks of rivers etc. [30]). Adults or elder larvae overwinter [26, 30]. Several localities are known from Hungary, mainly from the Great Hungarian Plain (Alföld) [2, 3, 13, 32, 49]; however, it seems to be not common, but locally frequent.

Acalypta musci (Schrank, 1781), a European species, occurs primarily in different mosses – *Abietinella abietina* (Hedw.) Fleisch., *Plagiomnium cuspidatum* (Hedw.) Kop., *P. undulatum* (Hedw.) Kop. [30, 36] –, but was also found on fungi growing on tree trunks (*Coriolus*, *Polyporus*, *Trametes*) [39, 44]. It seems to prefer highlands and was recorded to occur primarily in woodland habitats [26, 41]. It is mentioned in the literature as a species occurring in relatively humid places [4], frequently in mosses growing at the foot of trees [19, 37, 52]. Adults and nymphs can be found together throughout the year [26, 30, 35]. The species is frequent in Hungary [32, 49].

Acalypta pulchra Štusák, 1961 was reported to occur in Austria, Bulgaria and the former Yugoslavia. In the literature the species is also mentioned from Hungary [26, 27]. These records are based on Puton's historical collection – deposited in the Muséum National d'Histoire Naturelle, Paris –, in which there are four specimens labelled „Hongrie" [26]. However, these specimens were collected very probably on locality or localities today not belonging to Hungary. No other found of this species has hitherto been reported. In the latest check-list of the Heteroptera of Hungary, *A. pulchra* is also included [21], but the author only cited Péricart's work mentioned above, without seeing voucher specimens (Kondorosy, *pers. comm.*). Therefore, the occurrence of the species in Hungary needs verification. Practically nothing is known on its life habits.

Quantitative data on the dynamics of population and the preferences of *Acalypta* species were not published except Melber's paper [24] in which the author gave very

detailed data on the seasonal and microhabitat distribution of two syntopic species, *A. nigrina* and *A. parvula,* based on examinations in Northwestern Germany. In this study, *A. nigrina* was observed to prefer grounds densely covered by *Calluna vulgaris* (L.) Hull., while *A. parvula* occurred mostly in open and half-open places, most probably because of their different preference of humidity.

Materials and methods

The investigations were carried out on two materials:

1. Materials extracted with Berlese-funnels throughout Hungary

Material collected by the staff of the Department of Systematic Zoology and Ecology of Eötvös Loránd University, under the guidance of the late Dr. I. Loksa, between 1953–1974. The material was extracted with Berlese funnels from different substrata collected in all regions of Hungary (altogether 3657 samples). The materials were identified by D. Rédei (see also [31, 32]).

2. Materials taken by pitfall traps in the Kiskunság National Park

Material collected by the staff of the Department of Ecology of the University of Szeged under the leading of L. Gallé, Gy. Györffy, E. Hornung and L. Móczár between 1979–1982 with pitfall traps at Bugac, in the Kiskunság National Park. The research was carried out within the frame of the international program „Man And Biosphere" (MAB) [25].

The investigated area, mapped in *Figs. 1–2,* was not grazed at all after 1976, therefore, its vegetation is nearly natural, composed of a mosaic combination of the following plant communities developed on the topographically and microclimatically different patches of the moving sand [5, 9, 23]:

1. *Festucetum vaginatae* Rapaics ex Soó 1929 em. Borhidi 1996 (= *festucetosum vaginatae* seu *typicum*) — Pannonic dune open grassland. One of the most widely distributed endemic plant communities in Hungary. It can be found in many places in the sandy areas of the Great Hungarian Plain, most typical representatives in the area between the rivers Danube and Tisza [6]. In the areas at Bócsa and Bugac, in the Kiskunság National Park, almost native stands occur. It colonizes the thermic slopes and tops of dunes and constitutes the most widely occurring community in the investigated area. Its soil is characterized by very low water (2–7%) and humus (<1.5%) content [22].

2. *Potentillo arenariae-Festucetum pseudovinae* Soó (1938) 1940 — Pannonic sand puszta. Short, closed fescue pastures of sandy alluvial soils of the Pannonic basin, formerly widely distributed in the Hungarian plains, nowadays only local stands remained [6]. In the investigated area it occupies an intermediate position between Pannonic dune open grassland and dune-slack purple moorgrass meadow patches; as regards the water and humus content of soil, it is similar to the latter one [22].

3. *Molinio-Salicetum rosmarinifoliae* Magyar ex Soó 1933 — dune-slack purple moorgrass meadow. Pioneer grassland of Pannonic sands characterized by the juxtaposition of hygrophilous and xerophilous plant species. Occurs sporadically in the Great Hungarian Plain [6]. It colonizes the somewhat water-retentive depressions between dunes in the investigated area. Compared to Pannonic dune open grassland, its soil is characterized by relatively high water (3–15%) and humus (about 3.5%) content [22].

In the investigated area, the fauna was examined by diverse methods. The most extensively used method was pitfall trapping; the data of these were processed in course of the present study. The traps were placed out in 14–18 groups, each containing five of them. Traps were emptied and reset usually every month in 1979, every two weeks from 1980, between early spring and late autumn.

In order to establish the period of oviposition, the degree of maturity of the ovaria of females was investigated by dissection.

The materials were identified and preliminary faunistical data of the Heteroptera captured during the investigations were published by B. Harmat [13].
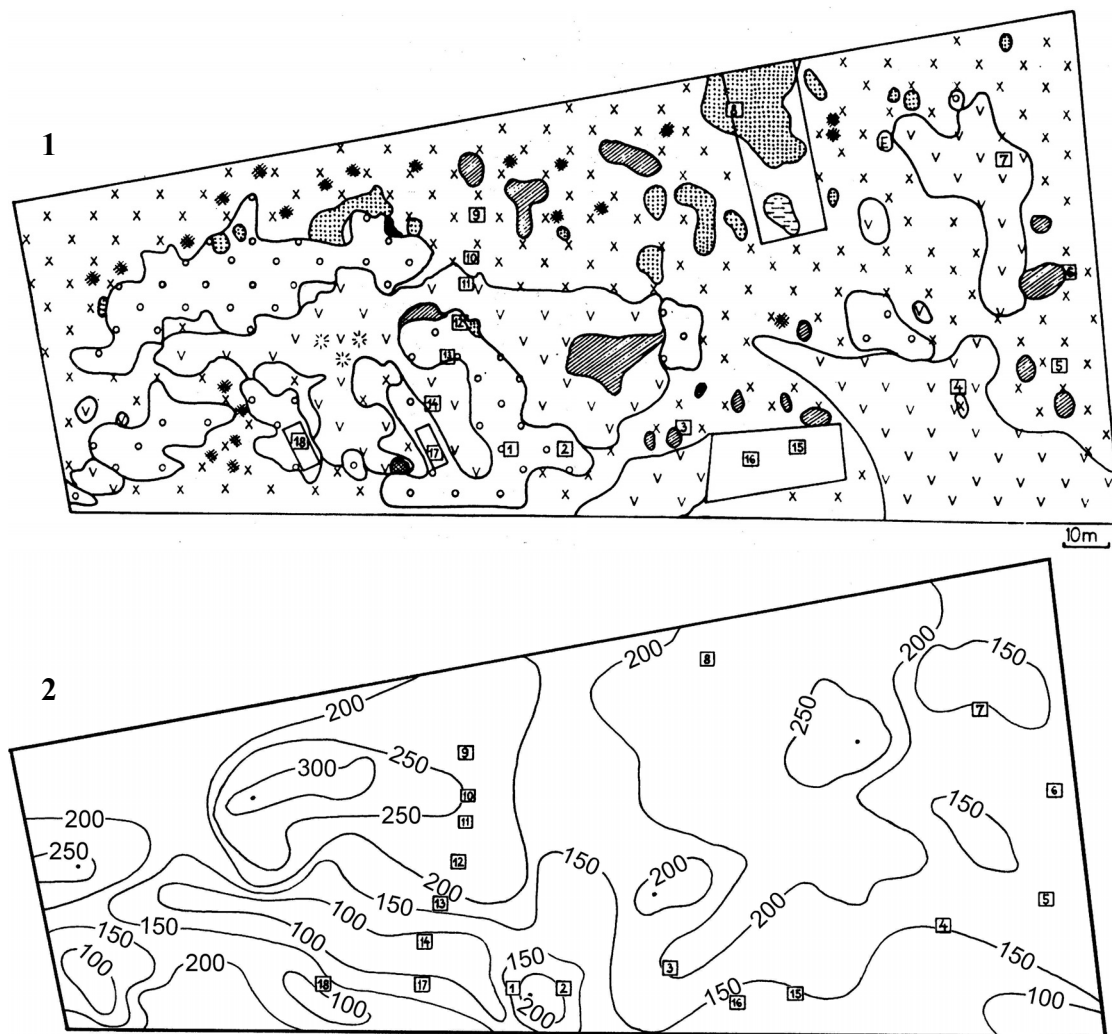
Figures 1–2. The vegetation (Fig. 1) and relief (Fig. 2) of the examined area (Bugac). ⬡ = Festucetum vaginatae; Ⓥ = Molinio-Salicetum rosmarinifoliae; ⊠ = Potentillo-Festucetum pseudovinae; ⊞ = Carex; ▨ = Stipetosum capillatae; ✳ = Holoschoenus romanus; ■ = Sedum; ⊠ = Brometum Secale facies; Ɛ = Euphorbia segueriana; ✳ = Salix; ① ... = No. of sampling site.

For the identification of adults, Vásárhelyi's [49], Wagner's [52] and Péricart's [26] works, of larvae Péricart's [26] monograph and Štusák's papers [40, 42, 43] were used. For distinguishing *A. carinata* and *A. platycheila*, the paper of Golub & Péricart [10] was also consulted. The nomenclature follows the *Catalogue of the Heteroptera of the Palaearctic Region* [27].

For the multivariate data analysis the program package SYN-TAX 5.1 [28, 29] was used. The diversity ordering was carried out with the program package NuCoSA 1.05 [45, 46, 47].

Results and discussion

1. Materials extracted with Berlese-funnels throughout Hungary

Species composition

A total number of 2542 specimens belonging to the genus *Acalypta* was found in the materials extracted with Berlese funnels. Each species recorded from Hungary before have been found except of *A. pulchra*, which occurrence is quite doubtful. The majority (74.4%) of the extracted specimens are larvae (*Table 1*).

Table 1. The number of Acalypta specimens extracted with Berlese funnels. L1…L5 = 1st…5th instar larvae.

Species	adult	L5	L4	L3	L2	L1	altogether
Acalypta carinata (Panzer, 1806)	66	390	141	47	19	—	663
Acalypta platycheila (Fieber, 1844)	11	—	—	—	—	—	11
Acalypta nigrina (Fallén, 1807)	5	—	—	—	—	—	5
Acalypta marginata (Wolff, 1804)	54	193	48	21	17	14	347
Acalypta parvula (Fallén 1807)	145	20	2	2	—	—	169
Acalypta gracilis (Fieber, 1844)	13	32	8	—	—	—	53
Acalypta musci (Schrank, 1781)	358	154	341	314	126	1	1294

Faunistical notes

A considerable part of the faunistical data of Loksa's pitfall trap material was published in different papers [1, 33, 34].

A list of the localities where samples were collected for extracting was published in the first part of this series of publications [32]. However, that paper contained only the data of the adult *Acalypta* specimens. In addition, a list of localities where larvae were found is given below. The names of species are followed by the recorded localities and their UTM codes in square brackets.

Acalypta carinata (Panzer, 1806): Alsópetény [CU60], Alsószuha [DU65], Ásotthalom [DS01], Bak [XM47], Balástya [DS24], Böhönye [XM84], Csaroda [FU03], Csongrád [DS37], Csöde [XM18], Fülöpháza [CS89], Gulács [FU02], Jánd [FU03], Kelebia [CS91], Kékkút [XM99], Marcali [XM86], Mátraszőlős [CU91], Mesztegnyő [XM85], Nagybajom [XM94], Nemesvid [XM75], Somogyaszaló [YM14], Somogytúr [YM17], Szendehely [CU50], Szenyér [XM84], Tiszaalpár [DS28], Vámosatya [FU03], Vindornyaszőlős [XM69]. — The species was rarely collected in Hungary before. However, a great number of it were collected by Loksa. The localities of the species in Hungary are depicted in *Fig. 3*.

Acalypta marginata (Wolff, 1804): Alsószuha [DU65], Ásotthalom [DS01], Balatonalmádi [BT71], Bélapátfalva [DU52], Bódvarákó [DU87], Csákvár [CT05], Csöde [XM18], Csővár [CT79], Esztergom [CT39], Fenyőfő [YN04], Harka [XN27], Jánd [FU03], Jósvafő [DU67], Kelebia [CS91], Kesztölc [CT38], Kosd [CT69], Kunbaracs [CT70], Mátraszőlős [CU91], Mesztegnyő [XM85], Nagykovácsi [CT47], Nagykőrös [DT00], Nemesvid [XM75], Piliscsaba [CT37], Piliscsév [CT38], Pilisszentkereszt [CT48], Pilisszentkereszt (Dobogókő) [CT48], Pilisszentlászló [CT48], Pusztavacs [CT82], Répáshuta [DU62], Sajókaza [DU64], Somló-hegy (hill) [–], Sövényháza (recent name =

Ópusztaszer) [DS25], Szabadszállás [CS69], Szentgotthárd [WN90], Szögliget [DU77], Tihany [YM19], Vajta [CS27].

Acalypta parvula (Fallén, 1807): Eger [DU50], Esztergom [CT39], Répáshuta [DU62], Szendehely [CU50].

Acalypta gracilis (Fieber, 1844): Bükkszenterzsébet [DU32], Cece [CS18], Pócsmegyer [CT58], Salgótarján (Somoskőújfalu) [DU13].

Acalypta musci (Schrank, 1781): Alsószuha [DU65], Bakony (mountains) [–], Bakonybél [YN03], Bélapátfalva [DU52], Bódvarákó [DU87], Dömös [CT39], Eger [DU50], Esztergom [CT39], Jósvafő [DU67], Kelebia [CS91], Kelemér [DU55], Kosd [CT69], Mónosbél [DU52], Nagykovácsi [CT47], Magymaros [CT49], Nemesvita [XM88], Oroszlány [BT96], Pásztó [DU00], Piliscsév [CT38], Pilisszentkereszt [CT48], Pilisszentkereszt (Dobogókő) [CT48], Pilisszentlászló [CT48], Putnok [DU54], Répáshuta [DU62], Sirok [DU30], Sopron [XN18], Szendehely [CU50], Szendrőlád [DU85], Szentendre [CT58], Szögliget [DU77], Tornakápolna [DU76], Vámosatya [FU03].
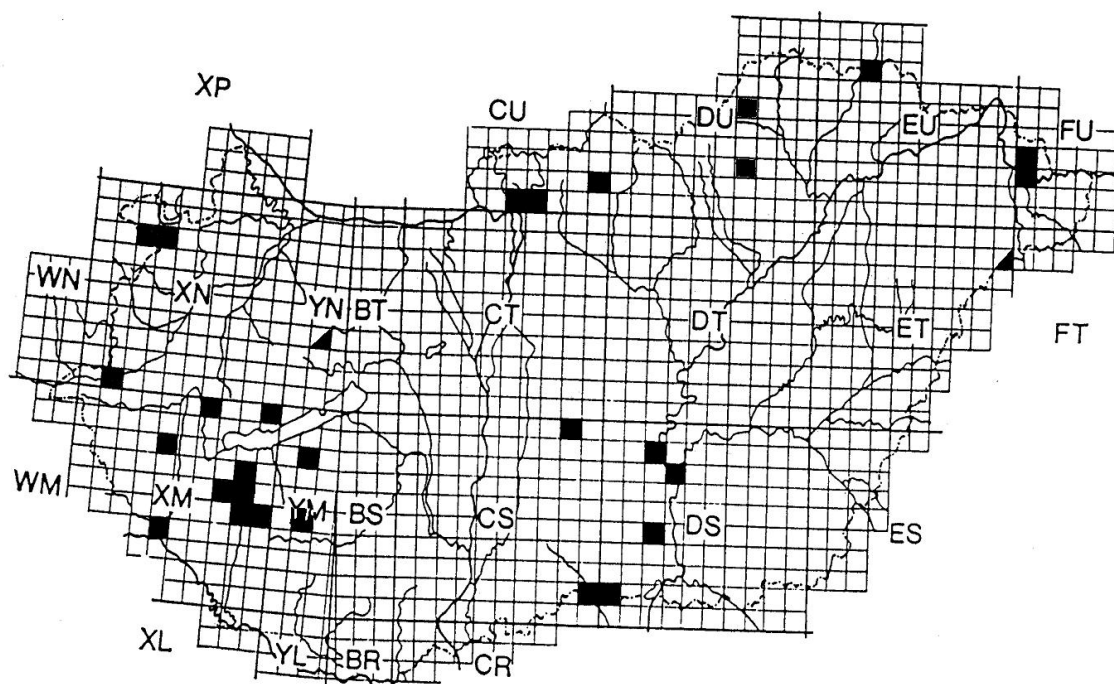

Figure 3. *Localities of Acalypta carinata in Hungary.* ▲ *= literature data,* ■ *= new data (partly published in [1] and [32]).*

Phenology

Because of the sporadic and non-quantitative collectings and the aggregative occurrence of the animals, the numbers of species collected in different dates cannot be compared. However, some qualitative establishments can be made based on the presence/absence of the species (*Fig. 4*).

Very few data are available both on larvae and adults of *A. platycheila*, *A. nigrina* and *A. gracilis*, therefore, these species are not discussed.

Larvae of *A. carinata* and *A. marginata* were collected in most months between February and December; however, adults were captured only from May to September or April to July, respectively. The overwintering of these species as larvae (also young and elder ones) is almost certain, however, adult specimens were collected only in summer and early autumn.
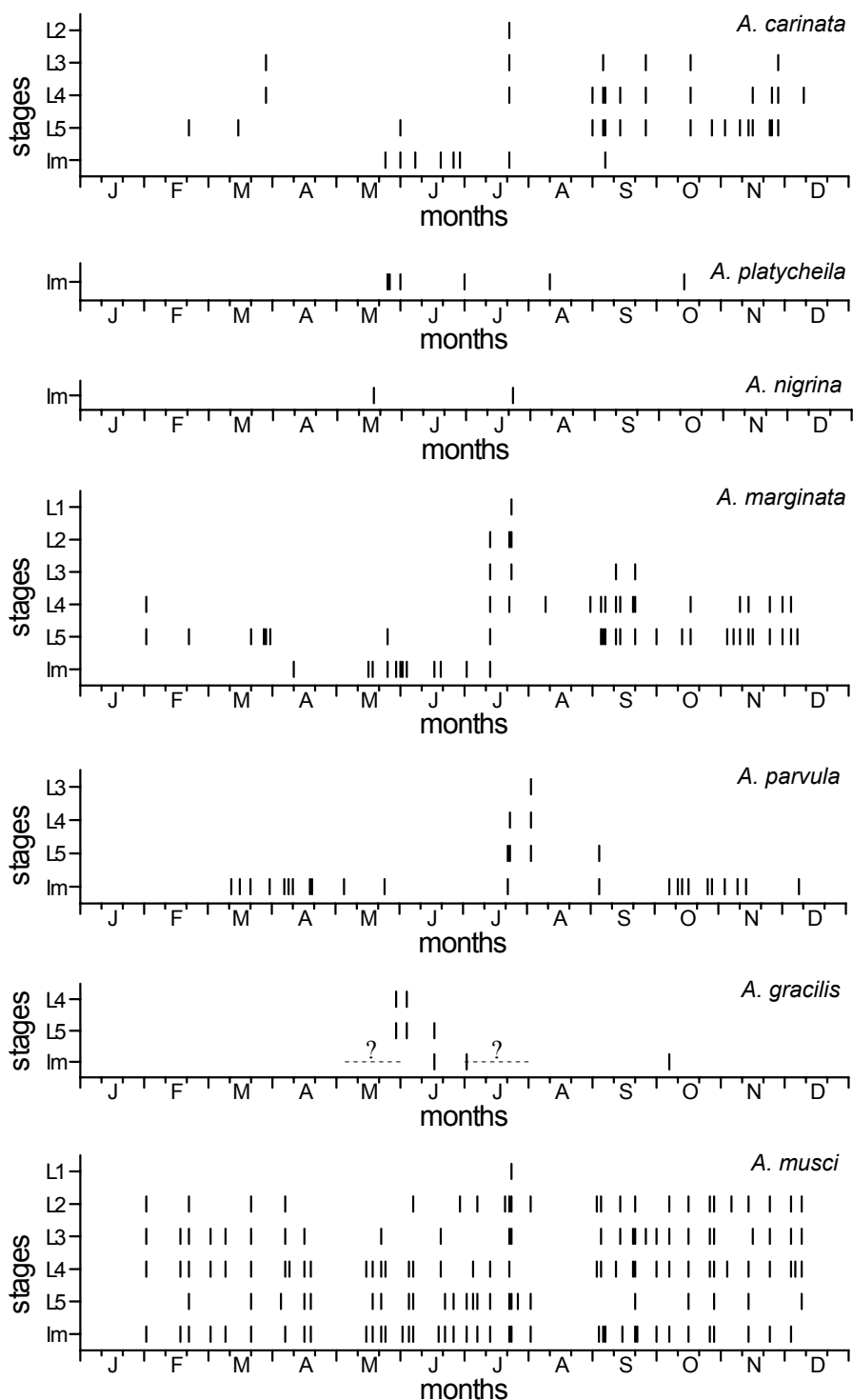
Figure 4. Dates of collection of different species of Acalypta based on many years' data from diverse localities in Hungary. In A. gracilis, '?' means month record only. Im = adults, L1...L5 = 1st...5th instar larvae.

Adults of *A. parvula* were collected from March to December, therefore, the species must overwinter as adults. Larvae were collected between July and September; however, because of the few data available, the overwintering in larval stage cannot be excluded.

Adults and larvae of *A. musci* were collected in great number throughout the year, between February and December. The species must overwinter as larvae (young and elder ones also) as well as adults, usually together.

Habitat preference

The number of *Acalypta* individuals collected in different plant community groups were examined (*Table 2*). The examined communities belonging to these groups were published in the previous part of this series of publications [31].

Table 2. *The community types examined.*

Community type		Community group
A humid treeless communities	1	reed beds and large sedge communities
	2	bogs and acidic fens
	3	humid grassland communities
B dry grassland communities	4	steppe and dry calcareous grasslands
C humid and riverine woodland communities	5	alder swamp woods
	6	riparian willow formations, stream ash-alder woods and other riverine forests
D mesophilous and relatively dry deciduous and coniferous forests	7	Medio-European beech forests and oak-hornbeam forests
	8	dry and mesophilous oak woods, mixed forests and deciduous thickets
	9	coniferous forests

The species occurring in greatest number in humid treeless communities (cenosis groups 1–3) was *A. carinata*; this species gave 90.0% of the total *Acalypta* specimens collected in such habitats. The species was extracted mostly from materials collected in *Caricetum elatae*, often in great number. In this type of communities, also *A. platycheila*, *A. marginata* and *A. musci* occurred but in far lower number.

In steppe and dry grassland communities (cenosis group 4), no species occurred in high number. *Acalypta marginata* and *A. parvula* were the species collected in greatest number of individuals; *A. carinata*, *A. gracilis* and *A. musci* occurred only in low number.

In humid and riverine woodland communities (cenosis groups 5–6), the species captured in greatest number was *A. carinata*. This species occurred in especially high number in *Querco-Ulmetum*. Also many specimens of *A. musci* were found in this community type, most of them in stream ash-alder woods. *Acalypta platycheila* and *A. marginata* occurred in very low number of individuals.

Each species except the extremely rare *A. nigrina* were collected in the group of mesophilous and relatively dry deciduous and coniferous forests (cenosis groups 7–9). (The five specimens of *A. nigrina*, collected in unrecorded community, are originated most probably also from deciduous forest.) The great majority (72.8%) of the individuals collected in such woodland communities belongs to *A. musci*; the species *A. carinata*, *A. marginata*, *A. parvula* and *A. gracilis* occurred in far less number, and only a single specimen of *A. platycheila* was collected.

Regarding the individual species, *A. carinata* was found in all groups of cenoses investigated, except in humid grasslands. However, latter absence may be due to the low number of samples collected in such communities. The species was found to be the dominant *Acalypta* species in large sedge communities (particularly in *Caricetum*) and

riverine woodland communities (particularly in *Querco-Ulmetum*). The species seems to tolerate a wide range of habitats, but shows definite preference to hygrophilous ones. The wooded or treeless character of the plant community seems to be a secondary condition since it was collected in great number in both types of habitats, but its real importance is difficult to establish because of the relatively low number of samples taken.

Virtually nothing can be established on the habitat preference of *A. platycheila* and *A. nigrina* because of the very few specimens available. However, it should be mentioned, that *A. platycheila* was collected mostly in the community types in which *A. carinata* was found to occur in relatively great number. In the southeastern part of Hungary, at Csaroda, this species was extracted at the same locality in two different occasions accompanied by *A. carinata*. In these occasions, the extracted substrata were mosses collected in *Dryopteridi-Alnetum*.

Acalypta marginata was found in relatively low number but in diverse plant communities. It was collected also in humid and more or less dry, woodless or wooded places; therefore, the species must tolerate a wide range of community types. However, it seems to prefer relatively dry habitats. *Acalypta parvula* and *A. gracilis* were found mostly in similar communities as *A. marginata* but in lower number; *A. gracilis* was also collected accompanied with *A. marginata* in some occasions in dry grassland communities, dry sand steppe oak woods (*Festuco-Quercetum*) and even pine woods planted on sandy soil.

The species collected in greatest number of individuals, *A. musci*, was also found to occur in most of the examined community types. It was captured in great number in Medio-European beech and oak-hornbeam forests (particularly in *Querco-Carpinetum*) as well as in dry and mesophilous oak woods, mixed forests and deciduous thickets (particularly in *Tilio-Fraxinetum*). Also a considerable amount of it was collected in riverine forests, but only a few specimens were found in woodless habitats.

The similarities of the species collected in different plant communities were studied with principal coordinate analysis (PCoA) and hierarchical clustering (both with Horn index). Because of the low number of data on some communities, for this analysis, the variables used were major coenosis types summarized from coenosis groups 1–9 (*Table 2*). The number of specimens collected in each major coenosis types was standardized with the total number of samples taken in that type.

In the pattern obtained (*Figs. 5–6*), *A. marginata*, *A. parvula* and *A. gracilis* show close resemblance and segregate significantly from the other species. This is undoubtedly due to the fact that each of them was collected in greatest relative number in dry grasslands, while the number of other species was the lowest in this type of communities. The segregation of *A. musci* from *A. carinata* and *A. platycheila* can be explained by the fact that *A. musci* had a high relative number in the group of mesophilous and relatively dry deciduous and coniferous forests besides low relative number in humid treeless communities, while this relation was inverse in case of the other two species. The PCoA and hierarchical clustering gave highly similar results.

The diversity conditions of the summarized data of the different plant community types were examined by Rényi's method (*Fig. 7*). It can be established that – except at the very low values of scale parameter – the assemblage of the humid treeless communities is the least diverse, with a very strong dominance of *A. carinata*; the
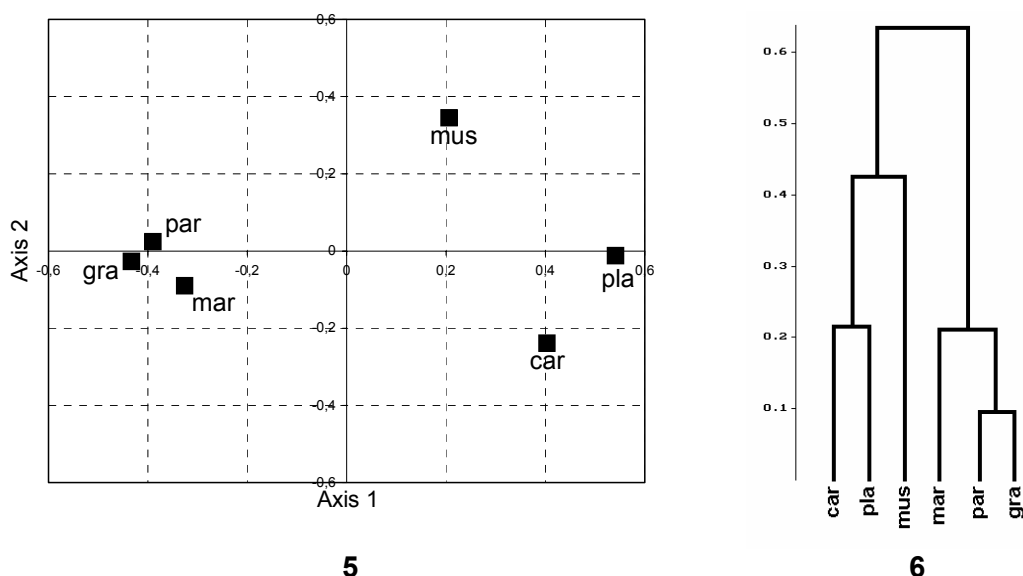
Figures 5–6. 5: *Similarity pattern (PCoA, Horn index) and* 6: *similarities (hierarchical clustering, single ling, Horn index as distance) of the species collected in different plant communities*
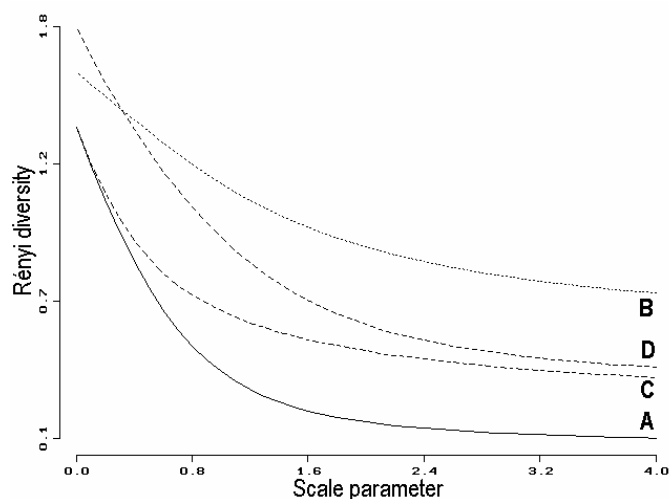

Figure 7. *Diversity profiles of the summarized Acalypta samples taken in different plant community types. A = humid treeless communities; B = dry grassland communities; C = humid and riverine woodland communities; D = mesophilous and relatively dry deciduous and coniferous forests.*

assemblage of the humid and riverine woodlands is more diverse, with a dominance of *A. carinata* and a relatively great number of *A. musci*; the assemblage of the mesophilous and relatively dry deciduous and coniferous forests is more diverse than the previousones with a dominance of *A. musci* and the presence of *A. carinata, A. platycheila, A. marginata, A. parvula, A. gracilis*; finally, the assemblage of the dry grassland communities is the most diverse, with greatest number of *A. marginata* and *A. parvula* and a smaller number of *A. carinata, A. gracilis* and *A. musci*.

Substrate preference

All species were extracted in greatest number of specimens from different mosses. Relatively great amount of *A. marginata* and *A. parvula* were taken from tussocks; the former species was collected in the greatest relative number in this type of substrata. The two most abundant species, *A. carinata* and *A. musci* were also collected in considerable amount from leaf litter. Surprisingly, a few specimens of *A. carinata*, *A. platycheila*, *A. marginata*, *A. parvula* and *A. musci* were taken also from soil.

Number of individuals pro sample

Regarding the adult specimens, most species were found only in very low number pro extracted sample. Even *A. marginata*, from which relatively great series were collected, was extracted always in low number – maximally four – pro sample. On the other hand, the relatively seldom collected *A. parvula* was found to constitute groups of more than five animals in 10.3% of the cases (17 on one occasion). The frequently captured *A. musci* was also often found in relative large groups, on one occasion 20 adults were extracted from a sample.

Regarding the larvae, *A. marginata* was found to constitute groups of more than five specimens in 19.5% of the cases (35 on one occasion). *Acalypta carinata* and *A. musci* was found in the greatest number pro sample; more than five specimens of larvae, usually together with adults, were collected in 40.3% and 30.3% of the cases, respectively. The greatest number of larvae extracted from the same sample was 37 in the case of *A. musci* (together with one adult), 101 in *A. carinata*.

2. Materials taken by pitfall traps in the Kiskunság National Park

Species composition

In the territory examined, only *A. marginata* and *A. gracilis* were found, both in great number of individuals.

Seasonal patterns of the activity

The number of adult individuals of *A. marginata* and *A. gracilis* collected with pitfall traps at Bugac during four different years are figured in *Fig. 8*. In each year, specimens were captured in greatest number during May and June; unfortunately, there are no pitfall trap data available before May. Ovipositing females were captured in great number with pitfall traps placed out between 3rd June and 1st July 1982. In the years 1980 and 1982, a second peak of activity of *A. gracilis*, with far less number of individuals, could also be observed around August. A few specimens of this species were collected even in October and November; however, no specimens of *A. marginata* were captured after middle of August. The number of captured individuals differed highly in the different years; the total number was smaller in the years 1979 and 1980 than in the years 1981 and 1982. Regarding the two latter years, *A. gracilis* was captured in far greater number than *A. marginata* in 1981; this ratio was inverse in the year 1982.

In the year 1982, *A. marginata* larvae were collected in great number. The number of larvae and adults of *A. gracilis* are figured in *Fig. 9*. The 2nd–5th instar larvae appeared before the adults, they were collected in May and June. The increase of the activity from younger to elder instars was observed. During the same year, the activity of *A. gracilis* larvae was very low.
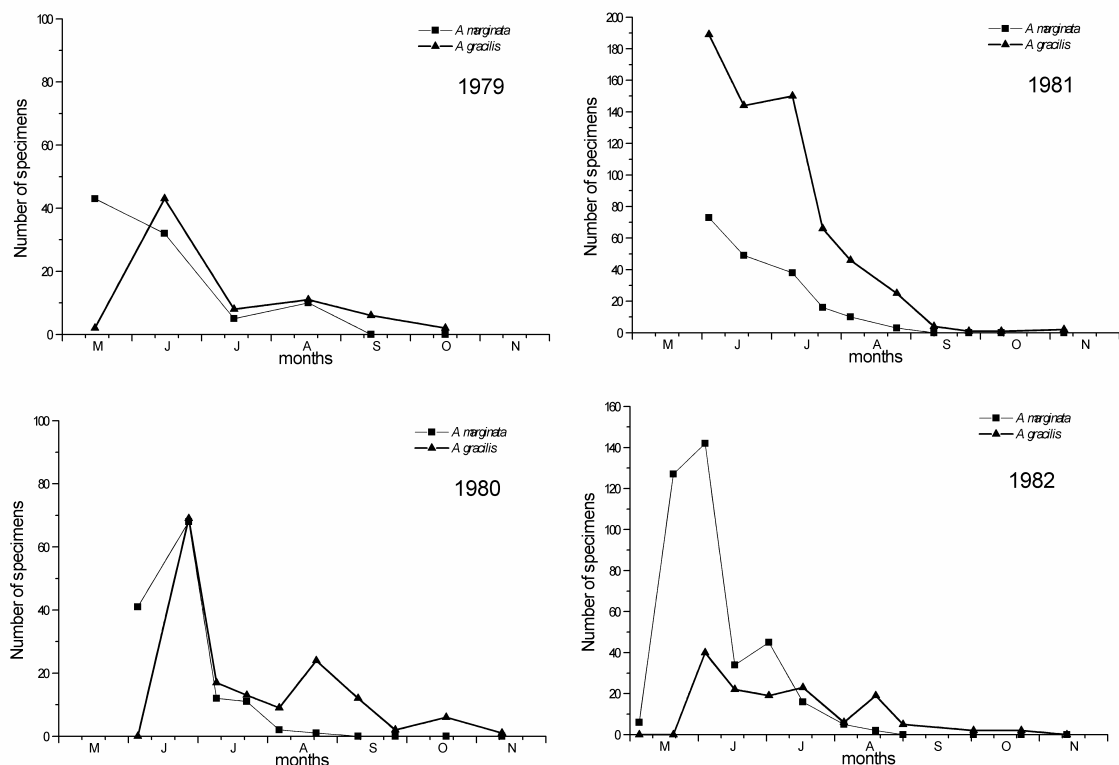
Figure 8. *The number of adult individuals of Acalypta marginata and A. gracilis collected with pitfall traps at Bugac during four different years.*

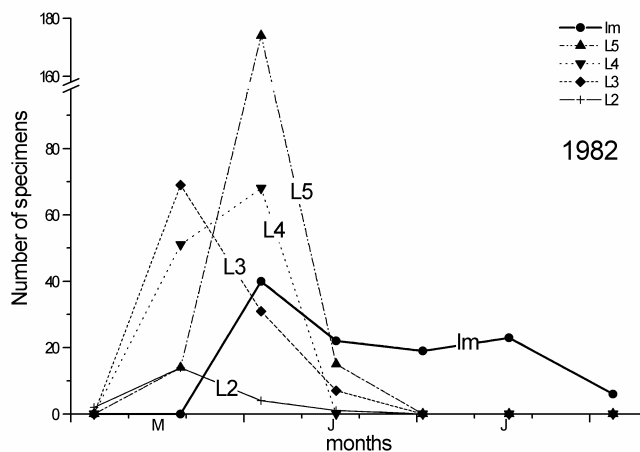**Figure 9.** *The number of larvae and adult individuals of Acalypta gracilis collected with pitfall traps at Bugac between May and early August 1982.*

Microhabitat preference

Considering the relative number of the adult individuals of *A. gracilis* and *A. marginata* collected in patches colonized by different vegetation (*Fig. 10*), the following observations can be made. In each year, much less specimens of both species occurred in the dune-slack purple moorgrass meadow patches than in the other two

xerophilous grassland ones. In case of *A. gracilis*, the greatest number was captured in the traps placed out in the Pannonic dune open grassland patches. In case of *A. marginata*, the difference between the number of individuals captured in the Pannonic dune open grassland and Pannonic sand puszta patches was small; in the years 1979 and 1982, the total number of individuals was somewhat higher in the former one, while in 1980 and 1981, it was higher in the latter one.
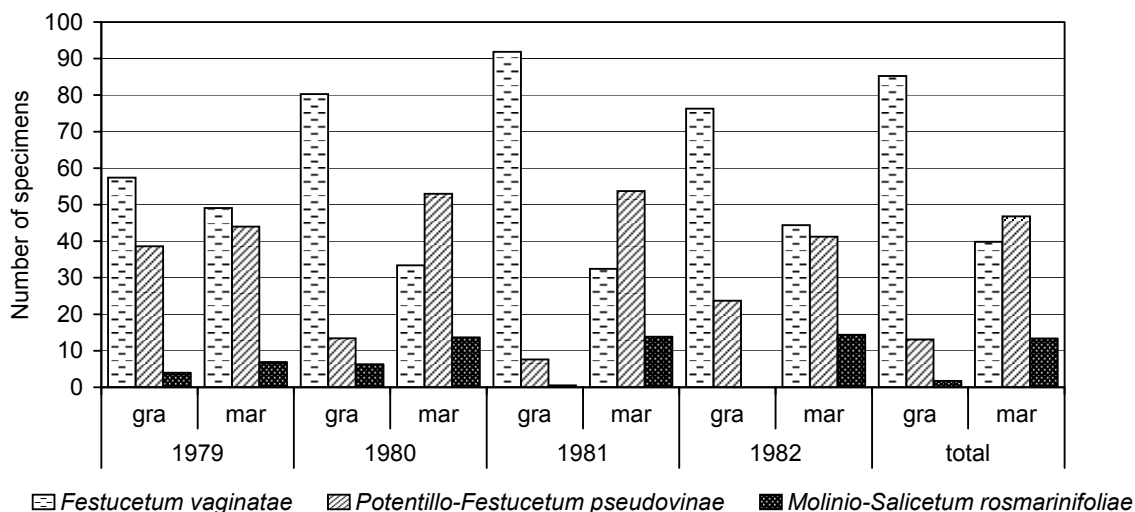

Figure 10. Percental ratio of the total number of adult individuals of Acalypta gracilis and A. marginata collected with pitfall traps in different plant communities at Bugac in the years 1979–1982 and the summarized data of the years (total of the specimens taken in all the three communities in one year altogether = 100%). The numbers of individuals were standardized with the number of traps placed out in each community.

Comparing pitfall trap samples with χ^2 test for independence, it can be proved that the number of the samples containing both species is significantly higher than the expected random distribution ($P = 0.0036$). Considering only the samples which contain any of the two species, sharp negative association can be observed: the number of samples containing only one species is significantly higher, that of samples containing both species is significantly lower than the expected random distribution ($P = 2 \cdot 10^{-77}$). Consequently, the differences between the microhabitat distribution of the two species as described above are significant.

Summary

Based on many years' sporadic collection data, the numbers of specimens of *Acalypta* species collected in diverse plant communities in Hungary were compared. *Acalypta marginata*, *A. parvula* and *A. gracilis* occurred in greatest relative number in dry grassland communities, while the number of *A. musci*, *A. carinata* and *A. platycheila* was the lowest in this type of communities. *Acalypta musci* had a high relative number in mesophilous and relatively dry deciduous and coniferous forests besides low relative number in humid treeless communities, while this relation was inverse in case of *A. carinata* and *A. platycheila*. In broad outline, these results agree well with the results of collecting experience of previous authors [26, 30].

All species were extracted in greatest number of specimens from different mosses. *Acalypta marginata* and *A. parvula* were taken relatively frequently from tussocks, *A. carinata* and *A. musci* from leaf litter.

The phenology of *A. marginata* and *A. gracilis* differs highly from that of *A. carinata* and *A. musci* in Austria, discussed by Ressl & Wagner [35]. According to these authors, *A. carinata* and *A. musci* shows two peaks of abundance, the first one in April–May, the second one around August (*A. carinata*) or October (*A. musci*). This pattern is similar to that of *A. parvula* in the same country [24]. These species must be univoltine, with copulation in late summer and autumn, oviposition in autumn. The phenology of *A. nigrina* differs from the species above; in Germany, it was observed to be also univoltine, with copulation in June–July, oviposition in July–August [24]. According to our observations, the seasonal pattern of the activity of *A. marginata* is similar to that of the taxonomically very closely related species, *A. nigrina*, because this species also has a peak of activity in late May and June, but oviposition most probably takes place somewhat earlier, in June and July. It is quite interesting, that – contrasted with the species *A. marginata* and *A. nigrina* – the phenologies of the taxonomically also closely related *A. gracilis* and *A. parvula* differ sharply: in the area examined, the activity of *A. gracilis* showed a temporal pattern similar to *A. marginata*. The numbers of *A. marginata* and *A. gracilis* showed great differences in the different years; in some years, one species occurred in greater number, in other years the other one.

Considering the differences of the relative number of *A. gracilis* and *A. marginata* captured in patches with different vegetation, the two species show significant differences. Both species occurred in very low number in the dune-slack purple moorgrass meadow, the most humid community of the three. *Acalypta gracilis* preferred distinctly the Pannonic dune open grassland, the most xerophilous, most exposed community of the three, while *A. marginata* occurred in almost equal number in this patches as well as in the Pannonic sand puszta. These observations suggest the xerophily of both species; the preference for xerophilous conditions of *A. gracilis* seems to be higher than that of *A. marginata*. This result agrees well with the results of the examination based on many years' collection data.

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