CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences

CHEMISTRY OF LICHENS OF SOUTHWEST MOJAVE DESERT

DIPLOMA THESIS

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I hereby declare that this diploma thesis is completely my own work and that I used only the cited sources.

Prague, 30. 4. 2012 ……

ABSTRACT

The aim of the thesis was to analyze secondary metabolites of 139 lichen specimens that were collected from the investigated area, the southwestern part of the Mojave Desert in California. Samples were processed by TLC (Thin Layer Chromatography) analysis that is one of the most commonly used micro-chemical methods for identification of lichen compounds. More than 50 types of secondary metabolites were detected in analyzed material, 40 substances were determined concretely. Based on the results of TLC analysis it will be possible to confirm the taxonomic classification of individual samples. Another goal of the thesis was designing a database for gathering gained results and information about specimens. An annotated checklist of lichen substances was created as output of the database. Within the thesis new methods for collecting sufficient data for ecological statistical analysis was drawn up. A new dimension of utilization environmental information about lichens was discovered. The methodology of collecting data and its application in statistical analysis offer possibilities for biomonitoring through lichens and their sensitivity to air pollution and climate changing.

Key words: California, checklist, database, lichens, secondary metabolites

ABSTRAKT

Cílem této práce bylo zanalyzovat sekundární metabolity celkem 139 vzorků lišejníků, které byly nasbírány v zájmové lokalitě, v jihozápadní části Mojave Desert v Kalifornii. Vzorky byly zpracovány metodou TLC (Thin-layer Chromatography) analýzy, jednou z nepoužívanějších mikrochemických metod, pomocí níž je možné identifikovat sekundární metabolity obsažené ve vzorcích. Při analýze bylo zjištěno více než 50 typů sloučenin, z toho bylo konkrétně identifikováno 40 látek. Na základě této analýzy bude možné potvrdit taxonomické zařazení jednotlivých vzorků. Jako jeden z hlavních cílů byla navržena internetová aplikace pro shromažďování získaných dat a informací o jednotlivých vzorcích. Z databáze byl vygenerován komentovaný seznam sekundárních metabolitů. V rámci práce byl navržen postup sběru dat vhodných pro ekologicky statistickou analýzu. Tímto byl navržen nový směr využití environmentálních dat o lišejnících. Předpokládá se, že metodika sběru dat a jejich následné využití ve statistické analýze, bude vodítkem pro biomonitoring skrze lišejníky a jejich citlivost na znečištění ovzduší a klimatické změny.

Klíčová slova: Kalifornie, komentovaný seznam, databáze, lišejníky, sekundární metabolity

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INTRODUCTION

Lichens are fascinating organisms with a marked sensitivity to any changes in the ecosystem of which it is component. Although lichens seem to be a single organism, they are actually a symbiosis formed between a fungus and one or several algal or cyanobacterial units.

The high valuation of lichens consists in their relatively steady reactions on environmental stressors like air quality and climate change (Jovan 2008). Many research findings, that are checking species diversity, continue with examination of air quality and climate. Mapping and defining lichen indicator species is suitable for estimating the environmental conditions (Jovan 2008).

Lichens play one of the key roles in weathering and soil formation. The weathering of lichen rock substrates can be accelerated by presence of some lichen species (Chen et al. 2000). On the other hand lichens are inseparable part of biological soil crusts which are key features in reducing erosion, increasing water retention, and increasing soil fertility (Belnap et al. 2001).

The originality of lichens is unassailable. Many question marks hang over them. Many questions have been already answered, some incorrectly, and many other questions are still awaiting their answers. Lichen compounds have been investigated from the early beginnings of modern chemistry, because lichens with their secondary metabolites seemed to be quiet unique, mysterious, and scientifically attractive organisms. This fact connects the object of interest with this diploma thesis.

Secondary metabolites are one of the main objects of the research. Lichen compounds were investigated by one of the most frequently used method for determining secondary metabolites - Thin Layer Chromatography (TLC). Results in the thesis show the spectrum of secondary metabolites presented in lichen species from southwestern part of the Mojave Desert - Joshua Tree National Park in the south of California. Afterwards TLC results were used as chemo-taxonomic clue for species determination of selected specimens. The TLC analysis was a part of inventory research in Joshua Tree National Park organized by a lichen specialist from California, Kerry Knudsen, and a Czech lichenologist doc. RNDr. Jana Kocourková, CSc.

The crucial practical part of the thesis research was performed at Gdansk University in collaboration with a lichen-chemistry specialist, Dr. Martin Kukwa. During one month in fall of 2011 all specimens, chosen by Kerry Knudsen, were analyzed, reported in protocols, and documented by camera. All steps were

remarked; all detected substances were marked and noted. This thesis includes the whole step by step methodology of gathering TLC results.

Important part of the thesis was fieldwork in California during December 2011. During this phase of the research I explored the locality and habitats of analyzed specimens. This phase of the project was beneficial in terms of gaining knowledge of the living conditions of studied species in the desert. This knowledge is naturally included in the text.

Methodology of TLC analysis used for the project and for designing new ways for following researches is described in following sphere - lichen chemistry, collecting specimens in chosen localities, biomonitoring, and database for gathered results. The superior result above all results and goals of the thesis is described in the discussion. It shows the methodology and several procedures for managing new data for significant ecological analysis.

The structure of the text was organized to understand issues of lichen chemistry, TLC, and studied area. The methodology is described in such close details as possible. The results are arranged in a manner providing maximal lucidity and illustrative style through the whole text. Discussion part summarizes all knowledge gained and brings up new instructions for consequent researches. Altogether 3 appendixes include annotated checklist, sheets with individual samples and their determined substances, and manual for using the database designed specially for this thesis.

1 THE MAIN OBJECTIVES OF THE THESIS

The master thesis is part of the project working on lichen inventory of localities in the southwestern part of the Mojave Desert, in Joshua Tree National Park. The intention of the project is to make an inventory of lichens, lichenicolous fungi, and saxicolous microscopic fungi. The master thesis should contribute to elucidation of some samples which are problematic to determine.

The main goal of the thesis was to analyze and identify secondary metabolites in selected Mojave Desert lichen species. Specimens were collected from experimental localities in Joshua Tree National Park, in southwestern part of Mojave Desert. 139 specimens were chosen to be analyzed by Thin Layer Chromatography (TLC), the chemical method for identifying lichen species and their secondary metabolites. Some of chosen specimens were previously sent to the laboratory in New York Botanical Garden. Afterwards results of TLC analysis running in the laboratory in Gdansk (University of Gdansk, Department of Plant Taxonomy and Nature Conservation) were compared with results from New York laboratory. If there were found differences in results between both TLC analyses it was the stimulus for discussion about the results and the subsequent determination. The comparison of the results from both laboratories confirms their accuracy.

Selected specimens for TLC analyses were chosen by Kerry Knudsen. They were the specimens for which it was necessary to confirm their classification by TLC. In this case, TLC was applied as a chemical method for identifying lichen species.

On the base of TLC results from the thesis the incompletely defined specimens could be determined exactly. Finally the checklist from the inventory localities will be complete and it will be the first official basic overview material of lichen flora for Joshua Tree National Park. The checklist will be published as the first official checklist for the park.

The second goal of the thesis is to prepare an interactive database for analyzed lichen species and their secondary metabolites. Database is designed as an internet application to access information for other projects or research teams. The internet application could be primarily used to add or check new data and results online.

The annotated checklist generated from the database is the last of three main goals of the thesis. The checklist includes all substances which were found in

analyzed specimens. It shows all identified and unidentified substances which were contained in collected specimens from Joshua Tree National Park.

2 OVERVIEW OF CURRENT KNOWLEDGE OF LICHEN CHEMISTRY

2.1 HISTORY OF STUDIES OF LICHEN CHEMISTRY

Secondary lichen compounds have been studied since 18th century. A scientist Hoffman was one of the first who applied chemical analysis on several lichen species in 1787. In 90's of 18th century Westring specialized in utilization of lichens as dyes. In all his publications he aimed at the methodology of obtaining the various types of colors (Thomson 1817). First researches regarded colorful substances which make lichens characteristically colored. Swedish chemist Berzelius was the first chemist who begun to study colorless but no less important lichen substances. A chemist Gmelin continued after him and he gave an overview about known lichen compounds in that time (Jarkovský 1978). A botanist Zopf and a chemist Hesse are other great German scientists who published their own work at the turn of the 19th century. In the 20th century Japan chemists Asahina and Shibata described structures of many unknown lichen substances and they also described their synthesis (Asahina et Shibata 1954). They identified these compounds using microcrystallization and they introduced the new method into lichenology. Chiquita Culberson also contributed significantly to lichenology in 1976 with her summarized data containing about 430 lichen substances (Huneck et Yoshimura 1996). In 1989 Ch. Culberson together with Elix published work on recognizing the key role of *para*-depsides as potential precursors (biosynthetic intermediaries), metadepsides, depsons, biphenyl ethers, dibenzofurans and depsidons (Elix et Stocker-Wörgötter 2008 in Nash 2008).

2.2 LICHEN CHEMISTRY

Chemistry of lichens plays the very important role in the life strategy of lichens. With its specific secondary compounds lichens are able to withstand such extreme conditions. Within testing, most tested species survived without damage during an immersion into liquid nitrogen at the temperature of -196°C. This high resistance at very low temperatures is based on similar structural and biochemical traits such as resistance to desiccation (Glosser 2008).

2.2.1 Primary metabolites

In connection with lichen chemistry secondary metabolites are the main feature. Primary metabolites are standard products of metabolism. These includes substances stored in individual cells of lichen symbionts such as proteins, amino acids, polyols, polysaccharides, carotenoids and vitamins bound in cell walls. All of these substances are water soluble and they can be extracted with boiling water (Elix et Stocker-Wörgötter 2008 in Nash 2008). Most isolated lichen compounds belonging to the primary metabolites occur also in non-lichenized fungi, algae, and higher plants. They are synthesized by the fungus and algae as well so it is not always clear where the product was biosynthesized (Elix et Stocker-Wörgötter 2008 in Nash 2008).

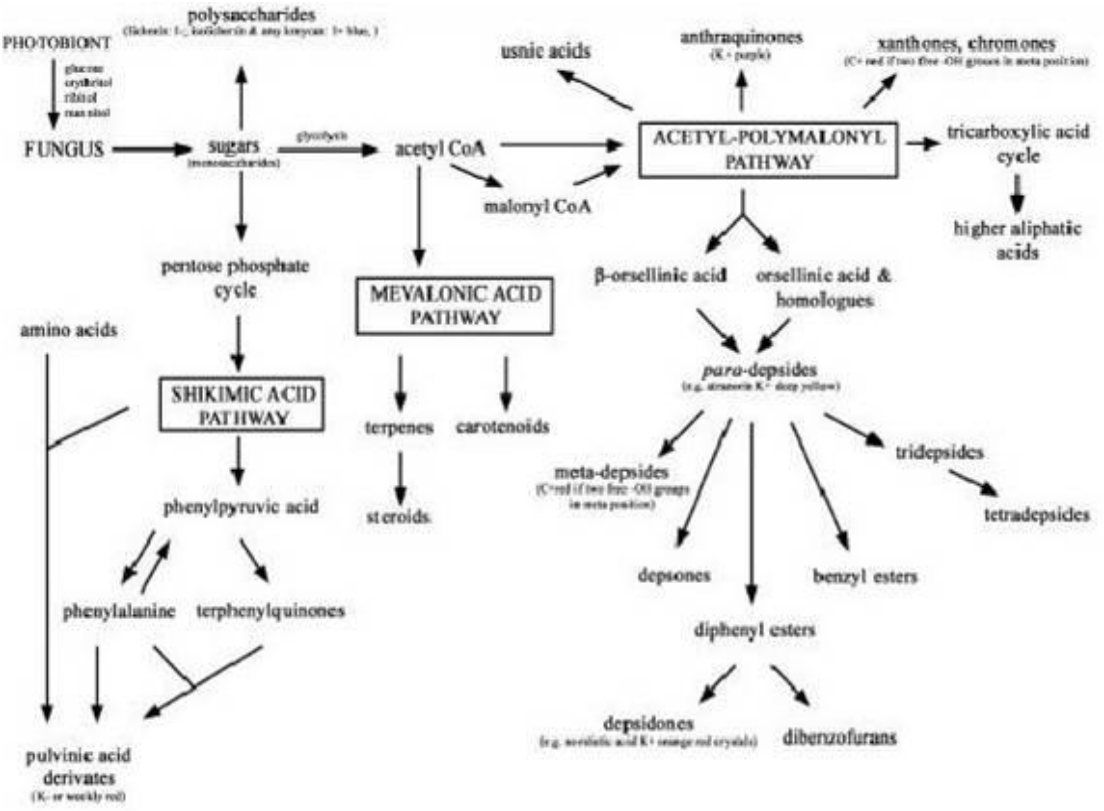
Primary products of metabolism have importance for studies of lichens. For example today carotenoids are the subject of studies of evolutionary lichens relationships. Glucans, lichenans, and isolichenans occurring in the cell walls have taxonomic significance (Egan 2010). There is evidence that lichen thallus can fulfill its physiological function only if it is sufficiently saturated with water (Glosser 2008). Therefore in mycobiont's cells and also in photobiont's cells must be consistently maintained a high concentration of osmotic active compounds, especially sugars (sucrose, trehalose), polyhydric alcohols (sorbitol, mannitol), and amino acids (proline) (Glosser 2008). Dehydrins are protective proteins which are ever-present in primary lichen metabolites. These substances help lichens to maintain their vital function without fatal consequences when water significantly decreases (Glosser 2008).

2.2.2 Secondary metabolites

Lichens produce a wide range of secondary metabolites enabling them to make life easier on habitats. Many of lichen compounds serve to deter herbivores or decomposers (Nash 2008). No wonder that secondary products of lichen metabolism have been under study for several centuries. So it is possible to say that interest in investigation of secondary metabolites of lichens exceeds the interest in research of secondary metabolites of higher plants (Jarkovský 1978).

Generally there are three biosynthetic pathways by which secondary metabolites are sectioned: acetic-polymalonyl pathway, mevalonic acid pathway, and shikimic acid pathway (see the Table 1) (Karunaratne et al. 2005). The scheme is illustrated on the Figure 1.

Figure 1: Biosynthetic pathways of lichen compounds (Nash 1996, 2008)



2.3 IDENTIFICATION OF LICHEN COMPOUNDS

2.3.1 THE ROLE OF LICHEN COMPOUNDS

Lichen compounds play important role in lichen chemotaxonomy. Lichenologists introduced nomenclature of "chemical species" - "Chemovars". Afterward it was generally used the name „Chemical Strains"or simply „Strains", „les lignées chimique" in French literature (Jarkovský 1978 Egan 2010). New chemical methods introduced into the taxonomy of lichens gradually found that chemicals can change the classification of the species. On the other hand squamatic acid, which is abundantly contained in lichens, can be derived by simply oxidation or by reduction the thamnolic acid, baemycetic acid, or barbatic acid. As a result of this fact these lichen acids occur together in some species, so their presence does not prove anything taxonomically significant (Jarkovský 1978).

Lichen compounds content is related to dry mass of thallus. Compared to higher plants, the concentration of secondary metabolites in lichens is much higher (Jarkovský 1978). Colored substances are stored in the crust and colorless substances in the marrow (Elix et Stocker-Wörgötter 2008 in Nash 2008).

Table 1: The main classes of secondary metabolites (numbers of compounds with known structure in brackets) (Nash 1996, 2008)

1. Acetyl-polymalonyl pathway
   - 1.1 Secondary aliphatic acids, esters and related derivatives (45)
   - 1.2 Polyketide derived aromatic compounds
     - 1.2.1 Mononuclear phenolic compounds (19)
     - 1.2.2 Di- and tri-aryl derivatives of simple phenolic units
       - 1.2.2a Depsides, tridepsides and benzyl esters (185)
       - 1.2.2b Depsidones and diphenyl ethers (112)
       - 1.2.2c Depsones (6)
       - 1.2.2d Dibenzofurans, usnic acids and derivatives (23)
     - 1.2.3 Anthraquinones and biogenetically related xanthones (56)
     - 1.2.4 Chromones (13)
     - 1.2.5 Naphthaquinones (4)
     - 1.2.6 Xanthones (44)
2. Mevalonic acid pathway
   - 2.1 Di-, sester- and triterpenes (70)
   - 2.2 Steroids (41)
3. Shikimic acid pathway
   - 3.1 Terphenylquinones (2)
   - 3.2 Pulvinic acid derivatives (12)

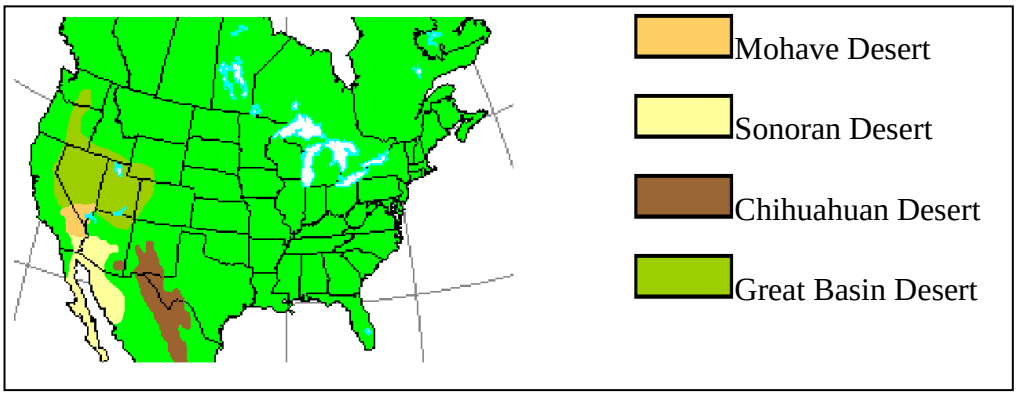
2.3.2 ANALYSIS BY TLC (THIN LAYER CHROMATOGRAPHY)

The analysis made by Thin Layer Chromatography (TLC) is a type of chromatography where finely pulverized particles of the stationary stage are applied onto inert carrier (aluminous plate or glass plate). The mobile stage rises due to capillary suction forces. Silicagel is often used as stationary stage (Kodíček 2007).

Standard validated method was introduced by Chiquita Culberson in 1970's (Culberson 1972, Culberson et Amman 1979). This method is fast and relatively cheap and it has been successfully applied till today (Elix et Stocker-Wörgötter 2008 in Nash 2008). Its standards have not undergone any significant change since its presentation except the partial change introduced by Ch. Culberson herself. She started using the MTBE instead of the diethylether (Huneck 2001) in the solvent systems which are used during TLC analysis to produce the standardized R_f values of lichen substances. Generally TLC is one of the most useful methods in chemotaxonomy allowing determination of taxons.

3 DESCRIPTION OF THE MOJAVE DESERT

Figure 2: North American Deserts (Woodward 1996)



North America has four different desert ecosystems distinguished by their different plant associations and climatic conditions. The Mojave Desert, the Chihuahuan Desert, and the Sonoran Desert are hot deserts. The Great Basin Desert is only one which is considered a cold desert (Woodward 1996).

3.1 WHAT & WHERE IS THE MOJAVE DESERT

The Mojave Deserts, also informally called as "a high desert" (Michaelsen 2009), is the smallest of the North America's deserts and lies between the Great Basin Desert to the north and the Sonoran to the south (NPS 2012b). The desert covers a huge part of southeastern California, and portions of central California, southwestern Utah, southern Nevada, and northwestern Arizona. The Mojave Desert landscape encompasses over 125,000 square kilometers (USGS 2003) and is composed of four national park units and six major military training bases (USGS 2003). The Mojave Desert region is characterized by diverse topography, complex geology, and indicator plant communities; it is a land with striking contrasts (Mac et al. 1998).

Figure 3: Mojave Desert boundary (yellow), Southern California (USGS 2003)

The Mojave Desert region has a distinctive beautiful landscape. Next to the major national parks there are other protected areas in the desert such as the Big Morongo Canyon Preserve or Red Rock Canyon National Conservation Area.

List 1: Protected areas located in the Mojave Desert region

Antelope Valley California Poppy Reserve
Arthur B. Ripley Desert Woodland State Park
Death Valley National Park
Desert National Wildlife Refuge (Nevada)
Joshua Tree National Park
Mojave National Preserve
Providence Mountains State Recreation Area
Red Rock Canyon State Park
Red Rock Canyon National Conservation Area (Nevada)
Saddleback Butte State Park

The Mojave Desert can be divided into three subregions that have indistinct boundaries but are quite different (Michaelsen 2009). The boundaries of the western Mojave, which comprises the triangular Antelope Valley, are defined by the two largest faults in California (the San Andreas and the Garlock) (see the Figure 4) (Mazzucchelli et al. 1967). The two fault zones join in the western end of the triangle and form the boundary of the Mojave Desert Region. The central Mojave boundaries are consistent with the Mojave River Valley (Michaelsen 2009). It includes the region between Victorville and Barstow and it extends to east to Soda dry lake and Baker and south to Joshua Tree National Park. The east Mojave is situated in the centre of the area and it makes up the East Mojave National Preserve. Its edges blend indistinctly into the Basin and Range region to the north and the Colorado Desert region to the south (Michaelsen 2009).

Figure 4: San Andreas and Garlock Faults (USGS 2000)

3.1.1 HISTORY

In history, the territory of the Mojave Desert was occupied by the Mojave tribe of Native Americans, which gave its name to the Mojave Desert (Healy et Orenski 2003). The Mojave Desert was colonized for the first time approximately 10,000 years ago, perhaps earlier and it has been used by many groups through the time (Webb et al. 2009). The climate was completely different than it is today. It was colder and wetter in early Holocene (Webb et al. 2009). There were lakes, streams and marshes, and plentiful vegetation and animal life. From the early to middle Holocene human population densities were low concentrated near dependable water sources (Webb et al. 2009). Late Holocene archeological records show increasing the number of human sites. Estimates of human population in the time period before 1492 are controversial. After Europeans arrival the human population density started to increase dramatically. In few decades of the mid-nineteen century, human occupation in the region changed rapidly from small scattered groups of hunter-gathers and primitive farmers to mining settlements joined by railroads and ranches (Webb et al. 2009). Mojave Desert region is the home to over one million people today, including the nation's fastest growing city, Las Vegas (USGS 2003).

3.1.2 ELEVATIONS

The main factor for all extreme environmental effects is the elevation. While the north Mojave Desert is mountainous, the southern part of the Mojave is the transitional zone merging into the lowland Sonoran Desert (Mazzucchelli et al. 1967). Elevations are generally between three and six thousand feet, although Death Valley National Park includes both the highest of 11,049 feet at Telescope Peak and the lowest point in the United States at Badwater (NPS 2012b). The dispersion of elevations, from 282 feet below sea level to over 11,000 feet, determines the range of habitats.

3.1.3 CLIMATE

The climate regime in the Mojave Desert falls under the Mediterranean regime of winter precipitation (Woodward 1996). Deserts are caused by patterns of hot and cold fronts which affect rainfall. The Mojave Desert region is typical of a rain shadow desert (NPS 2012b).

Annual precipitation is not regular but annual precipitation typically falls during the winter season from November through March. Occasionally additional precipitation comes in October or April in some localities (Hereford et al. 2004). The cool season precipitation period is the most important and appreciable source of rain in the Mojave Desert region. It arises from extratropical cyclones of the North

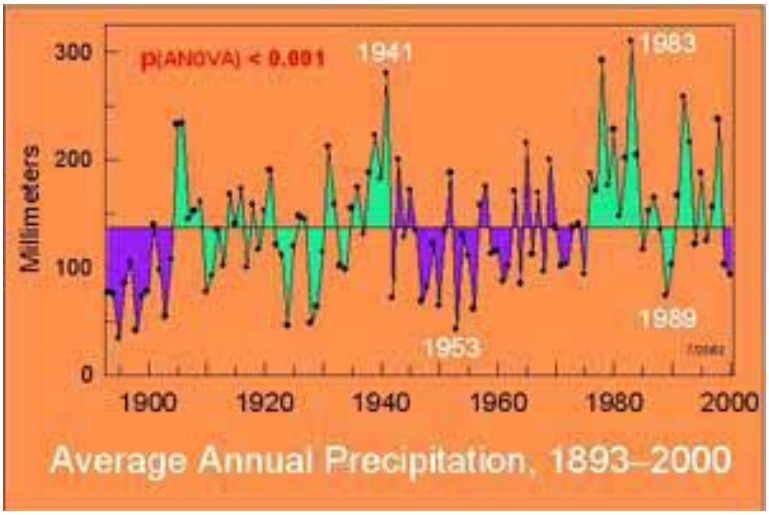
Pacific Ocean that come together with large synoptic and planetary scale tropospheric depressions and with the polar and subtropical jet streams (Hereford et al. 2004). In the west part of the Mojave Desert the precipitation mostly comes from large winter storms which move from west to east of the Pacific. By contrast in the eastern Mojave Desert a chief fraction of the annual total arrives with summer thunderstorms generated by the Arizona monsoon, a flow of warm, humid sub-tropical air that moves north out of Mexico into the Southwestern U.S. during mid and late summer (Michaelsen 2009).

Distribution of winter precipitation is different in different portions of the Mojave Desert. The annual average precipitation is approximately 200 mm and less throughout of the Mojave Desert (Michaelsen 2009). Generally eastern portions of the Mojave have less rainfall than in western portions. In the central part of the Mojave Desert summer rainfall contributes more towards the annual total, 25% coming in summer July-October period and 55% coming during the winter period (December-March). In the east part the two seasons are almost equal in consequence, with around 40% coming in each season (Michaelsen 2009). In mountain ranges more rain falls than in low lands because their presence can initiate convection and cloudiness. Annually rain falls in mountain ranges is 250 to as much as 750 mm (Webb et. al. 2009).

The climate of the Mojave Desert region had changed many times in history. The desert climate was significantly much cooler and less arid than it is today (Hereford et al. 2004). It was related to richer vegetation and animal diversity than recently. Human occupation persisting for thousands years illustrates this fact. Now, there are some endemic species which were able to adapt to new extreme climatic conditions (Mac et al. 1998).

The precipitation of the Mojave Desert region and its historical results indicate that precipitation has varied substantively during the 20th century (Hereford et al. 2004). Dry periods were changed by relatively wet epochs (Figure 5). Moreover, long-term changes were in conjunction with global-scale climate fluctuations which have a moderate degree of predictability (Hereford et al. 2004). Recent models of global climate say that the heavily dry seasons since the end of the nineties imply the time of drought conditions similar to the mid-century drought (ca. 1942-77) in the Mojave Desert (Hereford et al. 2004).

Figure 5: One century of Mojave Desert precipitation data, horizontal line summarized the long-term average, below the line are noted dry years, and wet years represented above the line. Drier years indicated by purple and wetter years indicated by green. Long periods correspond to the Pacific Decadal Oscillations which affected weather throughout the western U.S. (Hereford et al. 2004)**.**



Temperatures in the Mojave Desert are determined by elevation. Wide day-night temperature fluctuations and seasonal strong winds are characteristic for the Mojave region (Michaelsen 2009; Webb et al. 2009). Temperature fluctuations are much larger near the surface. This event could be explained by considering the flows of energy to and from surfaces (Webb et al. 2009).

Daily maximum temperatures during summer seasons get around from 44°C at 290m in Baker to about 30^{o}C above 1,500m in the eastern Mojave. Cool season nighttime minimum temperatures are less variable, from 4^{o}C at the low elevations to -5^{o}C at the higher elevations (Michaelsen 2009). Although snow is uncommon phenomenon, during cool winter season it falls in some parts of the Mojave Desert.

Along the western end of the Mojave (of the Antelope Valley), there are high winds, often above 50 miles per hour (NPS 2012b). They are also a weather factor because in addition to elevation, these strong westerly winds coming in the summer afternoon have slightly moderating effect on the maximum temperatures. They bring a bit marine influence to the western end of the Mojave Desert because these winds are not common toward the east (Michaelsen 2009; NPS 2012b).

3.1.4 BIODIVERSITY

The Mojave Desert is sandwiched between the Great Basin shrub steppe to the north and the Sonoran Desert (the Low Desert) which is warmer to the south. So the Mojave Desert is the physical transition region that hosts a various biodiversity and diversity of landscape. The Mojave Desert presents critical habitat for many

specific animal and plant species (approximately between one and two thousand) (Bowers 1998; Mazzucchelli et al. 1967).

The Mojave Desert is distinguished by the presence and abundance of its specific plant species. Most of plants are annuals or have special adaptations to survive in desert (dual root system, for example). An annual life cycle helps them to avoid the problem of water defection by remaining in the form of seeds until rains come to vitalize them (BLM 1999). The main characterizing feature of the floristic regions is the presence of probably 10,000 – 11,000 years old creosote bush in the Mojave Desert which survives by cloning (Webb et al. 2009). Various vegetation zones in the Mojave Deserts are determined by elevation and annual precipitation. The cycle of winter rains influences desert wildflowers and their blooming (Bowers 1998). In wet years the deserts are carpeted with wildflowers.

Joshua tree (*Yucca brevifolia*) is the woody species growing exclusively in the Mojave Desert and at higher elevations, from 700 m to 2100 m (Gilliland et al. 2006). Joshua trees require 200 - 250 mm of rainfall per year. Climate changes in the Mojave Desert region have an effect on the distribution of vegetation (BLM 1999). For example pinyon-juniper woodlands need the precipitation between 250 450 mm a year. In the past these woodlands covered vegetation zones in localities, about 1000 m lower than today (Webb et al. 2009).

The growing human population in the Mojave Desert has caused degradation of air quality, and depletion of water resources in this habitat (BLM 1999). The Mojave Desert is studied to understand how the population and land use changes (USGS 2003). Results should answer the questions about physical and biological processes that influence vulnerability of the ecosystem to disturbance and its ability to recover (USGS 2003).

Endemic plant species, the federally listed desert tortoise, and several other prominent species for the Mojave Desert are affected by the impact of human activities in the region (Mac et al. 1998). So it is necessary to apply successful land management which results from the availability and application of scientific information regarding biological and physical resources (Webb et al. 2009).

Soil moisture is the most important limitation in the Mojave Desert ecosystem. Texture and structure of surficial deposits regulate the moisturizing (USGS 2010). Covering of plant communities and their composition depend on water residing (USGS 2010). Another desert aspect are biological soil crusts populations - “desert skin” - which are important soil stabilizers and have considerable influence on soil in the Mojave Desert (Belnap et al. 2001; Williams et

al. 2010). Biological soil crusts prevent desertification and are sensitive to disturbance effects (Belnap et al. 2001; Williams et al. 2010)

3.1.5 GEOLOGY

The Mojave Desert is called the Basin and Range Province, a landscape with mountain ranges and their adjacent basins (NPS 2004). It lies near the Pacific and North American plate boundaries (USGS 2010). The intersection represents two different tectonic forces and the results of their activities are visible as jamming together of different rock strata to the south in the San Bernardino Mountains and other east-west trending Transverse Ranges north of Los Angeles (USGS 2000). Two bounding fault zones form many long linear features, such as valleys, sag ponds, and slope breaks (Michaelsen 2009).

All three types of rock are presented in the Mojave Desert: igneous, sedimentary, and metamorphic. The oldest rock substrate is metamorphic Precambrian gneisses which composes mostly desert slopes (NPS 2004). Granite is another rock type which is represented abundantly in the Mojave rock structure (NPS 2004).

Geologists are working with hydrologists, biologists and geographers in several locations in the west part of the Mojave region. These studies could bring new information about the processes governing desert ecosystems (USGS 2010).

4 DESCRIPTION OF JOSHUA TREE NATIONAL PARK

In 1936 President Franklin D. Roosevelt proclaimed the Joshua Tree National Monument to protect its extraordinary natural values. It became one of the units of the national park system. Eventually the monument boundaries were modified and in 1994 the Joshua Tree National Monument was redesigned and extended as Joshua Tree National Park in 1994 (NPS 2011). The change from a monument to a national park affords a higher level of protection of natural features and wilderness.

Today the park contains nearly 800,000 acres; nearly 600,000 acres are legislated as wilderness or potential wilderness (NPS 1995; 2011; 2012a). This fact makes Joshua Tree one of the largest wilderness areas in southern California (NPS 2011). It includes sand dunes, dry lakes, flat valleys, rugged mountains, granitic monoliths, and oases located together in one park (NPS 2011; 2012a). The park is located along the east-west trending range of the Little San Bernardino Mountains in southern California (NPS 2011). The area represents the unique confluence of natural resources formed by the conjunction of two Californian ecosystems, The Sonoran (Colorado Desert) and the Mojave. The first desert ecosystem is represented by the Colorado Desert. It occurs in the southern and eastern parts of the park and is determined by the western extension of vast Sonoran Desert (NPS 2012a). One of the main factors which play the role in forming biotopes is the elevation. In contrast with the Sonoran Desert the Mojave Desert is higher, more moist, and slightly cooler, while the Sonoran Desert is generally lower and dryer (NPS 2011). The elevations range from 900 feet to over 5,000 feet above sea level (NPS 2000; 2012a). The biodiversity of local communities found in the park is unique.

4.1 ENVIRONMENTAL FACTORS

Joshua Tree National Park has a fascinating and apparently unchanging landscape. However, the land is constantly being transformed by wind, rainfall and erosion (NPS 2012a). Human activities (especially nitrate and ammonia pollution from urban areas) and wildfire have radically changed some parts of the landscape. Throughout the park dynamic it is possible to observe environmental factors and their influence on the desert ecosystems. The visible contrast between a less-disturbed ecosystem in the park and in the urban areas like big cities nearby is striking (NPS 2012a). Changes in air quality, effects of nitrogen deposition, wildfires, and invasions of nonnative species, all these factors are monitored to maximize the park protection (NPS 2011; 2012a).

Ozone's levels are currently relatively stable. However the recent trend shows that they can be expected to rise from development around the park borders (NPS 2011). Also the air quality is threatened especially by contributions from Los Angeles basin, where live more than 12 million people (NPS 2012a). Another risk factor could be dust (both natural and resulting from land use change) in the future due to Salton Sea water loss (NPS 2011). The national park in cooperation with the UCR (University of California, Riverside) are studying how the air pollution affects soil nutrients, carbon cycling, and the nitrogen supply in the park. Effects of different types of pollutants are controlled by observation of species sensitive to high levels of pollutant concentrations (NPS 2012a). Generally good indicators of environment affection are lichens. The study of indicators, sensitive organisms to environmental changes, is necessary for evaluation the environmental impact of environmental changes.

Polluted air and its particulate matter draw down nitrates on to the soil. Native desert plants which are not adapted to grow in nitrogen-rich soils cannot compete with non-native species which prosper under nitrogen deposition (NPS 2012a). Natural quiet in the park is another threatened factor which is altered under current conditions. Military activities, surrounding land use, aircraft, and other similar activities in adjacent regions could have negative effect on the soundscape in the park (NPS 2011).

Today fire has become a regular feature in all North American deserts (Emming 2005). Lighting-caused fires in Joshua Tree National Park have occurred for centuries. And the fire regime has devastating impact for the desert ecosystem and especially for long-growing Joshua Tree population (NPS 2012a). Fire is not as common in deserts as in forests. It is the aridity of desert ecosystems, which protect them against experiencing devastating fires (Emming 2005). Trees and shrubs in deserts are normally widely spaced in deserts and grasses are not as near as in wetter localities (NPS 2012a). Flames normally cannot spread from one plant to another in cases of sparse vegetation. But non-native species, especially grasses, colonizing desert ecosystems often fill in the empty space between typically spread native plants and supply fuel for wide-spread devastating fires (Emming 2005).

Non-native invasive species affect the function of a healthy natural ecosystem (NPS 2011; 2012a). They change conditions for natural competition between native species. Very often these species compete for nutrients and water, which are extremely scarce in desert conditions (NPS 2012a). The most dangerous invasive species are Eurasian salt cedar (*Tamarisk*), perennial fountaingrass, and cheatgrass and red brome (NPS 2012a).

4.2 HISTORY

Near the end of the Ice Age, all California's deserts were green environments with a lot of water. There were rivers and lakes. So Joshua Tree area was a very different place than it is today (Kaiser 2008). The cultural history of Joshua Tree National Park reaches back to early Holocene period (NPS 2011; 2012a). The first inhabitants, the Pinto Culture, lived here between 7,000 - 10,000 years ago. Several thousand years later new groups of inhabitants occupied the Joshua Tree area. New tribes are known today as the Serrano and Mojave, the Chemehuevi, and the Cahuilla (NPS 2011; 2012a). Desert Indians were generally hunters. They hunted large animals (bobcats and bighorn sheep) as same as smaller animals such as jackrabbits and rodents (Kaiser 2008).

After the European colonization by Spanish soldiers, Europeans decided to explore the coast of California. After their expansions, European people occupied southern California including the Mojave Desert including the Joshua Tree area (Kaiser 2008). During the 18^{th} century till the last quarter of the 19^{th} century, the area was rich of grass. Euroamerican surveyors, cattlemen, miners and homesteaders came to these lands to live alongside native people. They created a new social and cultural heritage. They had extensive herds of cattle and they built water impoundments for them. Miners looking for gold dug tunnels and pits. Marks of their activities across the desert are still visible (NPS 2012a).

Joshua Tree National Park protects over 700 archeological sites (NPS 2012a). Numerous of them are associated with one of the earliest prehistoric culture of California Desert, the Pinto culture (NPS 2011). Other historic sites are associated with other ethnographic Native cultures. Sites of recent European-American history (cattle ranching, homesteading, mining camps) are also preserved (NPS 2011; 2012a).

4.3 ECOLOGY

The convergence of the two desert ecosystems (Mojave and Sonoran deserts) forms unique and biologically rich ecosystem in Joshua Tree National Park. The park's iconic communities are Joshua tree woodlands, native palm oases, and vast expanses of creosote scrub (NPS 2011). The plant diversity corresponds with the diversity of animals. Joshua Tree National Park is the stand for the unusual combination of species of two deserts (NPS 2012a).

Elevations in the park range from a low of 536 feet to a high of 5,814 feet at Quail Mountain (NPS 2011; 2012a). The eastern Colorado part includes no more than 2,000 feet above the sea level while the western portion average is mostly above

4,000 feet (NPS 2011). Extreme elevation differences range from the lowest position of 536 feet above sea level to the highest peak of 5,814 feet at Quail Mountain (NPS 2012a)

The park seems to be a barren and lifeless landscape. But it counts hundreds of vascular plants (NPS 2011) and many reptile species, mammal species, and more than two hundreds of bird species (NPS 2011; 2012a). Fall and winter precipitations together with spring temperatures and elevations allow the spring wildflowers blooming period. In lower elevations and along the south boundary first flowers are blooming during late winter (January). At higher elevations, up to in localities above 5,000 feet, wildflowers are blooming in March and April (NPS 2012a).

Over 250 birds occurring in the park have been recorded especially in winter time (NPS 2012a). There are 40 cold-blooded reptile species, 41 species of mammals in very low density, as well as spiders, insects, scorpions and other invertebrates (Kaiser 2008).

4.3.1 PLANTS

Plants in the park have many ways of adaptation for living in desert conditions. Some of them hoard sufficient amount of water for long periods of time by keeping it in their bodies. They use available water quickly anytime it rains. Typical water hoarders are cacti (Kaiser 2008). A second group of plants in the Joshua Tree National Park are drought tolerant species. Their strategy is maintaining a slow metabolic idle for most time of the year but they are never in true dormant stadium or hibernation (Pavlik 2008). When it rains they immediately suck up enormous amounts of water. Shrubby plants, creosotes and brittlebush are typical representatives of drought tolerators (Kaiser 2008). The third type of adaptation to drought is drought avoiding. Drought-avoiding plants wait in one part of their life cycle for optimum conditions before they appear. Their seeds can stay in dormant stadium for months or even years until it starts to rain (Kaiser 2008, Pavlik 2008)

Joshua Tree National Park affords a habitat for more than 800 species of higher plants (NPS 2011, 2012a). A characteristic component of the Mojave Desert ecosystem is represented by a world-renowned, undisturbed population of Joshua trees (NPS 2011). The Joshua tree is a Mojave Desert endemic (Turner 1982 in Brown) which is in the public eye the iconic species for the park. The namesake's habitat lies across the western part of the park including the southern boundary of the Mojave Desert. Stands of Joshua tree are located in the elevation above 4,000 feet (NPS 2012a). Joshua tree woodland represents a symbolic feature of a healthy desert ecosystem (NPS 2011). Today Joshua trees confront climate change, fires and

limited seed distribution. They are slow-growing species and their population trends are unknown today (NPS 2011).

The park also preserves native palm oases and vast expanses of creosote scrub that are perfectly adapted to extreme conditions. No other unit in the national park system has as much palm oases as in the Joshua Tree National Park (NPS 2011). Palm oases are threatened by invasive plants, unknown hydrogeology limits, and urban growth (NPS 2011).

Spike-like ocotillo plants and "jumping" cholla cactus occupy the southern and eastern parts of the park where the western Sonoran Desert reaches, below 3,000 feet (NPS 2011; 2012a).

One species of plants, the triple-ribbed milk vetch (*Astragalus tricarinatus*), has status as endangered species and another species, Parish′s daisy (*Erigeron parishii*), is registered as threatened (Sanders 1998; NPS 2011). Some wildflowers from Joshua Tree National Park are listed bellow Table 2 .

Table 2: Wildflowers in Joshua Tree National Park (examples) (Kaiser 2008)

English Name	Latin Name
Barrel Cactus	*Ferocactus cylindraceus*
Beavertail Cactus	*Opuntia basilaris*
Bladderpod	*Isomeris arborea*
Brittlebush	*Encelia farinosa*
Calico cactus	*Enchinocereus angelmanii*
California juniper	*Juniperus californica*
Canterbury bellis	*Phacelia campanularia*
Claret cup cactus	*Enchinocereus triglochidiatus*
Creosote Bush	*Larrea tridentata*
Desert dandelion	*Malocothrix glabrata*
Desert lavender	*Hyptis emoryi*
Desert mallow	*Sphaerlcea ambigua*
Sacred datura	*Datura wrightii*

4.3.2 ANIMALS

Animal diversity in the park is very high despite the impression that the park is without any life, especially in daytime. During the day usually birds, lizards, and ground squirrels are active. The birds concentrate commonly near oases, by reason of their daytime activity (Miller et Stebbins 1964). But the main animal activity is visible at night. Early morning and late evening give the best opportunities to see nocturnal animals like snakes, bighorn sheep, kangaroo rats, coyotes, and black-tailed jack rabbits (NPS 2012a).

Overall the park includes 41 mammal species, 40 reptile species, more than 250 species of birds, and other amphibians, and invertebrates. Some examples of unique species include the threatened desert tortoise, the Californian tree frog, and the desert bighorn sheep (NPS 2012a).

The General Management Plan includes extra priorities how to protect the desert tortoise (NPS 1995; 2011). Their populations in recent years are in very low numbers. The main current and potential threats consist in growing of urbanization, invasive species, and climate changing (NPS 2011).

Animals living in desert environment need to be specially adapted to live in extreme conditions with limited water and food resources and high summer temperatures. Ectothermic (cool-blooded) reptiles are perfectly adapted to desert conditions and they need very little water (NPS 2012a). Mammals need energy to regulate their body temperature. So for the sake of food and water resources, their population densities are very low (Kaiser 2005).

4.3.3 CLIMATE

Strong winds, unpredictable torrents of rain, and climatic extremes are typical aspects for the area of Joshua Tree National Park (NPS 2012a). Joshua Tree National Park lies in southern California. Most mountain ranges in North America run in north-south direction. But Transverse Ranges, on the border of the park, runs east-west way (NPS 2012a). High mountains cause the rain shadow effect because they block the moving wet winter storms. The east side of the land is drier and it brings consequences in a desert environment (NPS 2012a).

Annual precipitation in Joshua Tree is largely represented by tropical storms coming from the south of California. They come occasionally at the end of August or the beginning of September. These storms could bring a considerable amount of rainfall in a very short time (NPS 2012a). The rain shadow effect is conclusively the main aspect which keeps the climate.

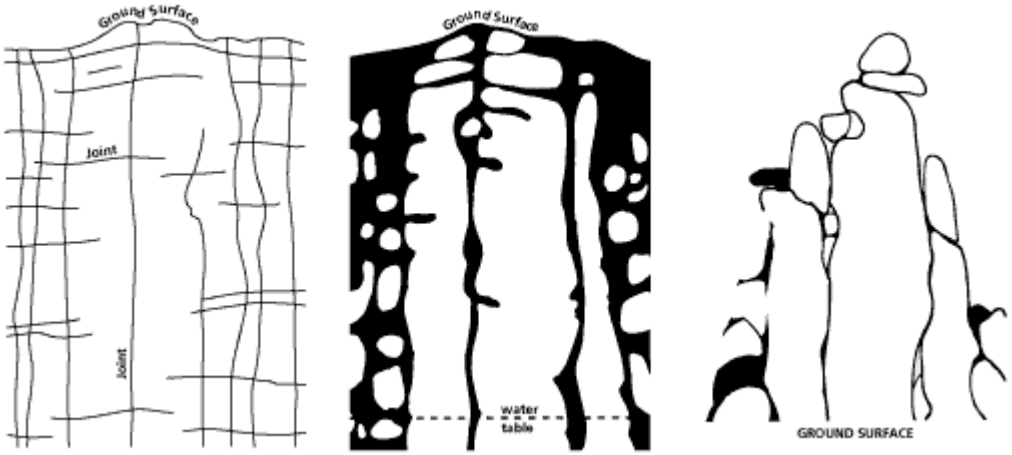
4.3.4 GEOLOGY

The landscape formation begins 100 million year ago (Kaiser 2005; NPS 2012a). Joshua Tree National Park is geological wonderland. Geologic processes continually works and their presence is noticeable in the occasional earthquake. The park lies on geologically active zone along one of the most active earthquake faults of the world, the San Andreas Fault. The fault is nearly 28 million years old (Lynch 2010). Hundred of faults and raw rocks are physical evidences of tectonic activity in Joshua Tree National Park (NPS 2012a).

Tectonic activity has played a major role in shaping characteristic topography, the mountains, valleys, and basins of the park (NPS 2011). It shows the most interesting effect of plate tectonics, volcanism, mountain-building, and stark erosion in California's desert areas. Exposed granite monoliths, eroded monzogranite boulder formations are world-renowned natural features that represent unique natural values of Joshua Tree National Park (Kaiser 2005, NPS 2011, 2012a).

North America is moving towards the west at one or two inches per year over the Pacific Plate (NPS 2012a). Fault zones create natural springs. Four fault-caused oases represent typical habitats for the native palm tree California Fan Palm, *Washingtonia filifera*. These oases bring important food and water resources to many species of wildlife and act as a bridge between the park's geology history and its wildlife habitat (NPS 2012a).

Figure 6: The rock formation (NPS 2012a).



Granitic rock, monzogranite, was created by plutonic intrusions when magma oozed up toward and cooled while it still was below the surface (NPS 2012a). These rock formations (called tors), which are so characteristic for Joshua Tree National Park, are illustrated in the Figure 6.

5 METHODS & ANALYSIS

139 specimens were processed in the project for the analysis of secondary metabolites. The specimens came from the different localities and altitudes in southwestern part of Mojave Desert in Joshua Tree National Park. TLC was used as method for the reliable analysis of lichen secondary metabolites.

5.1 METHODS OF COLLECTING THE SPECIMENS

Specimens were collected between 2005 and 2011. The main collector was Kerry Knudsen, a curator of lichens at UCR (University of California Riverside) Herbarium. One specimen of the collection was found by Nicole Pietrasiak (UCR, Ph.D. program - Soil and Water Sciences). All of the studied specimens are a property of UCR Herbarium. All material was collected, preserved, and stored by standard and considerate techniques.

The collecting equipment consisted of a notebook, a pencil, paper bags, a map, GPS, a stone chisel and hammer, a jackknife. Lichen specimens found together on one substrate were separated and put into bags individually. Every specimen got the number which is used as its ID in the UCR database. Every time a sample was collected, the information about the substrate, elevation and GPS coordinates was logged on the sample envelope (paper bag). Specimens were collected subjectively and qualitatively according to the field observations to include all habitat types found in the area of interest. *The Lichen flora of the Greater Sonoran Region* [Nash et al. 2002, 2004, and 2007 (2008)] was the main source of literature used as the taxonomy key.

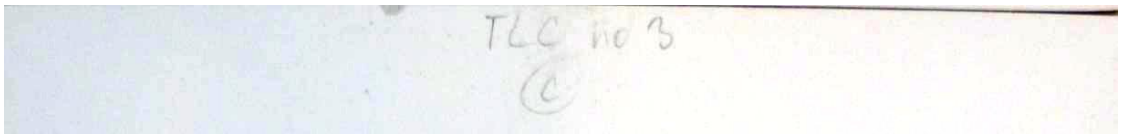
5.2 METHODS OF GAINING TLC RESULTS

TLC analyses were made in the laboratory at University of Gdansk, Faculty of Biology, Department of Plant Taxonomy and Nature Conservation, during the fall of 2011 under supervision of Dr. Martin Kukwa, who supervised the rigorousness and accuracy of the procedure. Special care was taken during the analysis and during manipulation with the samples to protect them from any damage, so they could be properly returned to the UCR Herbarium.

Each TLC set was marked by number and type of solvent. For example the first TLC proceeded on a plate prepared for the solvent system A was marked as “TLC no 3 C”. “3” stands for analysis number and “C” stands for solvent system type (see the Figure 7). The same way was applied for the rest of the sets. Altogether

32 analyzes were performed. For the last two runs only two plates were used, because there were only two samples for analyses. Plates were cut in three pieces, each for individual solvent system (A, B, and C).

Figure 7: Illustration of analysis number „3“ for solvent system „C“



5.2.1 ESSENTIAL EQUIPMENT AND COMPONENTS

TLC plates

TLC plates were 20 × 20 cm in size and aluminum coated with thin layer of silica gel on surface. This type of plates was the most convenient for the analysis. In total, 12 sets of analysis were performed.

Solvent systems

The provisional identification must be confirmed by running the unknown substance in three different solvent systems together with a sample known to contain the studied metabolite, or preferably with a pure sample of the substance if such is available.

Solvents A, B’ and C were selected as the most suitable solvent systems for the identification of lichen compounds in this type of analyses. These three solvents systems are used for routine analyses and provide requisite visualization for sufficient results. The very stable (several weeks) solvent C provides the best discrimination ability for many lichen substances. Solvent A and B are also generally used as routine solvent systems for the lichen analysis.

SOLVENT A (Orange et al. 2001)**:**
toluene / dioxane / acetic acid (180 : 45 : 5)

SOLVENT B (Orange et al. 2001)**:**
hexane/methyl *tert*-butyl ether (MTBE)/formic acid 140:72:18

SOLVENT C (Orange et al. 2010):
toluene/acetic acid 170:30

Fume cupboard

Some parts of the procedure were performed in the fume cupboard to prevent against dangerous vaporization. A fume cupboard is required for the preparation in order to meet the applicable safety rules. The fume cupboard was absolutely essential for example when using 10 % sulphuric acid.

Developing tanks

All the time during the procedure developing tanks must be placed in fume cup board. Then it is necessary to have the tanks signed with a name of solvent system to provide against any confusion.

Capillary tubes

Capillary tubes are equipment for transferring small amounts of extracted lichen compounds. Lichen extract is applied onto the TLC plate with these tubes.

Stereomicroscope

When one analyzed sample was together with another one species on the same substrate or when specimen was too small to catch it, the stereomicroscope was very useful. It is necessary equipment for precise preparation of pure piece of specimen.

5.2.2 PREPARATION

SPECIMENS

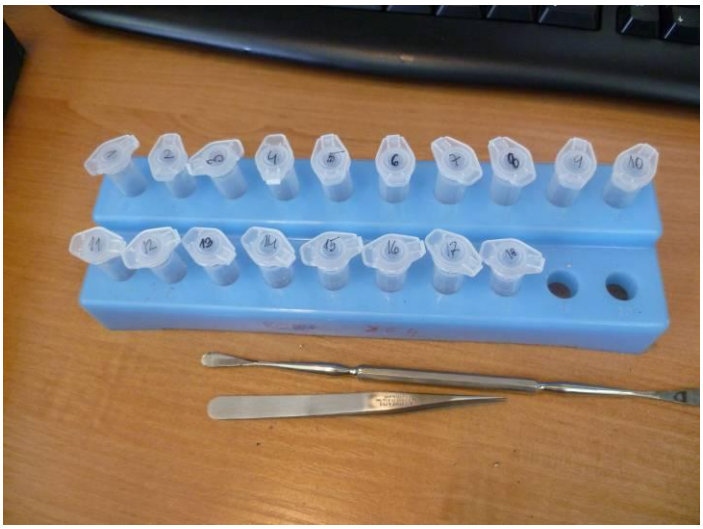
It was important to have samples carefully prepared before each analysis. A set of micro-tubes with a lid (1.5 ml) was placed into the rack. Each tube has the same number as a lichen sample analyzed in the same TLC set. Examined lichens were numbered and recorded into prepared Excel sheet. The same form of the data sheet was used for each TLC run. This sheet is maintained to ease the control of the analysis procedure and to allow reviewing of the analysis results. A table listed in the published work *Microchemical Methods for the Identification of Lichens* [Orange et al. 2001] was used as the protocol musters for results of analysis.

Control samples with their substances were selected for each TLC run depending on the analyzed specimens. For the laboratory are supplied appropriate mixtures of species with useful control substances. In this analysis, number and type of control samples dependent on the analyzed lichen species which were predefined, e.g., if there was a specimen probably containing the norstictic acid, a control species containing norstictic acid was chosen for the analysis. The same type of procedure was also applied in repeated analyses with ambiguous results. Most of controls were supplied from the Dr. Kukwa's collection of control samples. Some controls came from University of Gdansk herbarium. The checklist of controls used in analyses is in tables in chapter 8 "Results".

Small fragments of herbarium fragments in sufficient quantities were placed in micro-tubes for the testing (Figure 8). It was important to ensure that the taken pieces of samples were not a mixture of species. Sometimes it was necessary to use

stereomicroscope to prevent unintended mixing of species when working with samples of crustose lichens. Fragments of the samples were removed carefully. Crusts on rock were scraped away by scalpel on a clean slip of paper to collect necessary amount of fragments. Foliose, fruticose and easily separable species were removed with the tweezer. Samples like these should be pushed well down into the bottom of the micro-tube to make the maximal effect with the solvent for extraction of lichen compounds. It is possible to re-use those samples for the second time for another sampling as some sufficient amount of lichen compounds in samples is still presented in analyzed pieces of specimens. They are stored in signed bags with number of TLC run. Samples were completely dried before they were saved in bags.

Figure 8: Numberd micro-tubes with equipment for separation lichen samples.



SOLVENTS AND DEVELOPING TANKS

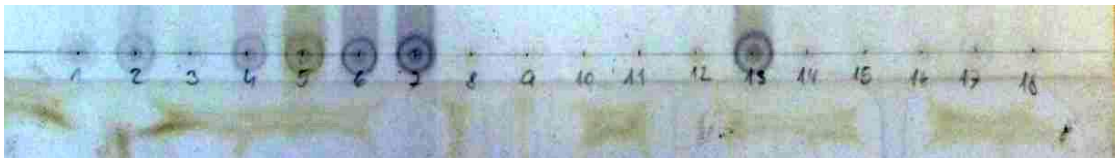
Tanks with solvent systems were placed into the fume cupboard during the whole procedure. The preparation of precise solvent systems was preceded in following steps:

- Glassware used during the procedure should be clean and absolutely dry.
- Solvents were prepared in small quantities, because they lose their reliability with time (especially solvent system B). Consequently they could give misleading results.
- Prepared solvents were filled into assigned tanks up to 15 mm.
- Lid should fit tightly to the tank to avoid disruption to the solvent. For this purpose a thin layer of silicone grease was used which was equally spread over the edges of tank.

PLATES

Firstly on a plate a starting line was drawn by soft pencil 20 mm from the base of the plate. The line was marked with 10 mm intervals. Intervals gave starting positions for mostly eighteen samples per plate (Figure 9). In TLCs number 8, 9 and 10 9 mm intervals were used to save space on plates. Twenty samples were analyzed on those TLC plates. According to the results of analyses of twenty samples it was possible to confirm that results were not affected. Last two analyses were done on plates cut by knife to three smaller equal sizes because there were used only four samples.

Figure 9: Illustration of marked line at 15 intervals 20 mm from the base of the plate.



5.2.3 PROCEDURE

Each procedure begins with extraction of secondary compounds by acetone at room temperature. Two or three drops of acetone were added to each lichen sample in microtube. When it was necessary (extra absorbent species), a few more drops were added.

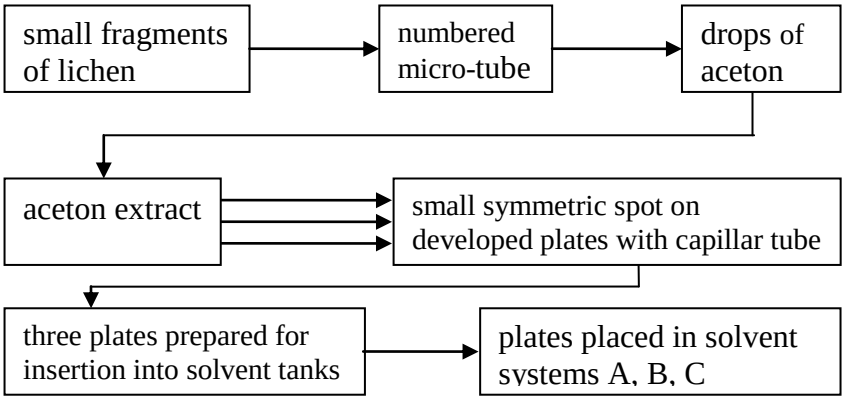
For each sample a clean capillary tube was used for transferring the acetone extract from a micro-tube with extracted samples to the numbered starting spots on prepared TLC plate. It was important to ensure that the numbered spot on the TLC plate corresponded with number of micro-tube with extracted samples.

The procedure was as follows:

- The tip of a capillary tube was submerged into the micro-tube with extracted samples to be analyzed.
- A small amount of extract was soaked up into about third of the tuber.
- Then the extract drop was placed on the starting point. The spotting extract evaporated in few seconds.
- When the acetone evaporated, another application of the same extract was applied on the same position. This step was repeated mostly three times per spot, but for better results more of the extract was applied. For the best results it was absolutely necessary to make small (about 5 mm) and maximally regular spots. It was important to avoid spreading and merging of spots on TLC plates.

- After acetone extract application the capillaries were not used again and were disposed.

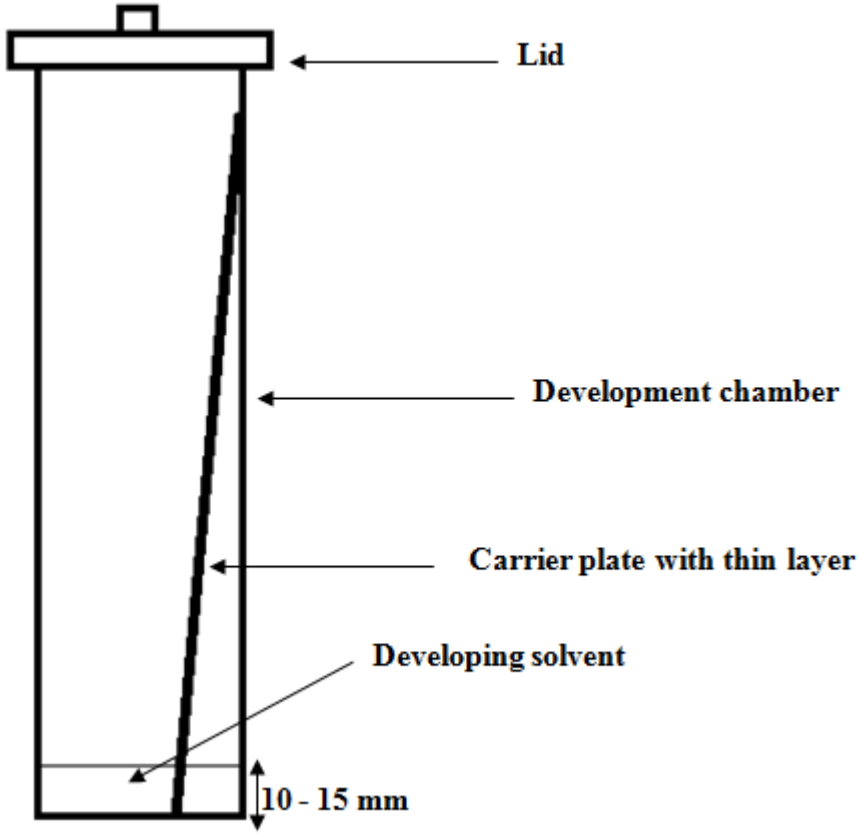
Figure 10: Procedure (scheme)



Prepared plates were carefully put into a developing tank with solvent system. Each plate was placed approximately 20 mm from the rear wall of the tank and the line with starting points was a few millimeters above the solvent surface. It was necessary to ensure that the whole edge of the plate was placed at the same distance from the rear wall of the tank to make the representative experiment. If it is placed unevenly (one side of plate is banked more than the second side) the solvent could be absorbed unevenly by the plate leading to distorted results. After that three developing tanks with solvent systems A, B and C in fume cupboard were left for the developing process. After 45 - 50 minutes plates were taken out.

After removal plates were dried in a fume cupboard. For this purpose a hair drier was available in the lab. Usually plates were dried for about 15 minutes. It was necessary to make plates completely dry as prevention against evaporation of acetic acid or formic acid. Traces of acid could create a mottled effect when sprayed with water; those could be mistaken for fatty acids.

Figure 11: Developing tank with a plate in developing solvent (illustration)



MARKING COMPOUNDS

pigments

Completely dry plates were examined for pigments in day light. Their positions were marked with a soft pencil by a convex line drown over the top of the colored spot and capital letter "P". Afterwards plates were inspected under short UV wave. All invisible spots, which were invisible by day light, were noted in the same way (convex line drawn over the top of the spot) at this stage.

In a next step plates were examined under long UV wave. All spots visible under the long wave UV were marked as pigments except the letter "P". Instead they were noted with the resulting fluorescence colors on the plate by hand in capital letters indicating the specific color (for example "OR" as orange).

After marking spots under UV lamp, a search for fatty acids was made. Dry plates were sprayed with water and then dried. Fatty acids appeared as brightly white dry spots due to their hydrophobic properties. They were marked with dotted line all around the spot. When it was necessary (lot of fatty acids were present per plate) the stage with watering plate was repeated.

After watering and when plates were dry again, 10% sulphuric acid was applied on plates by a paint brush. Plates were spread with sulphuric acid on the surface where reagents worked. If some fatty spots previously unnoted appeared they were also marked. After that, plates were thoroughly dried with hair dryer in fume cupboard and were placed in preheated oven at 110 °C for 3 minutes. After 3 minutes the spot colors were completely developed.

After removing plates from the oven, they were examined under long wave UV. Some lichen samples contained compounds which were not properly visible under long wave UV. By these samples the visibility under long wave UV was improved by application of sulphuric acid (confluentic acid). It was very important to take a picture of plate in UV by a camera to note colors of all spots as the colors change in time.

INTERPRETATION OF PLATES

Developed plates were compared with the published TLC data, primarily using the *Lichen Flora of the Greater Sonoran Region* [Nash et. al 2002, 2004, and 2007 (2008)], as a key for the identification of spots. The R_f, color, and fluorescence characteristics of the final spots were used as determining elements. Information got from visible colors and spots on developed plates were compared with published scientific resources. R_f values were controlled with freeware application called Wintab.

Unknown substances were confirmed in three different solvent systems together. For each run there were three plates to compare them together. Some pairs of substances had almost identical TLC characteristics. Chosen three solvent systems were used to separate them. Identification of lichen substances in chromatogram depends on individual experiences. In many cases, many lichen substances were easy to determine because they were contained in lichen specimens very often. However in some cases there was necessary to use help from Dr. Martin Kukwa as supervisor of this project.

R_f value

Figure 12: Calculation of R_f value

$$R_f = \frac{\text{distance of the spot on the TLC-plate}}{\text{distance of the solvent front}}$$

The R_f value expresses the distance between starting point on the plate and the solvent front. It is usually expressed in scale from 1 to 100 (or multiplied by

100). Each lichen substances have specific R_f values. R_f values are also differed according to the type of used solvent system. One substance has different R_f values in solvent systems A, B and C. In Table 3 there are provided typical control substances and their R_f values:

Table 3: Relative R_f values (Mietzsch et al. 1994).

	Relative Rf values (x100)		
solvent system:	A	B	C
ATRANORIN	75	73	79
NORSTICTIC ACID	40	32	30
USNIC ACID	70	65	71
STICTIC ACID	32	9	18
SALAZINIC ACID	10	7	4

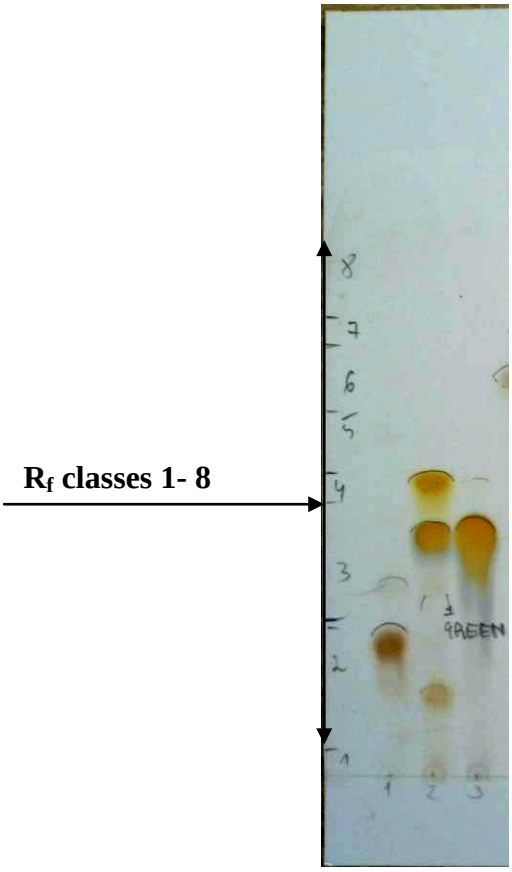
R_f values are only relative because the absolute R_f value could be influenced by some external conditions, for example atmospheric conditions, the age of the solvent, the tape of plate in use, and so on. That is the reason why standardized procedure to distinguish lichen compounds is using not only one but three different solvent systems as well as in this case. The differences between the R_f values of substances are in principal more or less constant. For a refinement of the standardized R_f values there was another reason to use more control substances with assigned standard R_f values. Therefore, a suitable selection of control substances depended on examined specimens and their predicted R_f values. Detected substances were expressed as a relative R_f values by comparison with the control substances.

R_f values are used as one of the important diagnostic matter. As good control R_f values is the reference to substances selected as standards (controls) and their R_f values. For determination of R_f values "Wintab" (Mietzsch et al. 1994) program was used as it was said above.

Rf classes

The R_f class is not the same character as R_f value. It is a method which standardizes R_f with reference to norstictic acid and atranorin controls. R_f Class 4 includes substances with an R_f the same as norstictic acid. Substances belonging to R_f Class 7 have a similar R_f to atranorin. An R_f of 0, these substances are Class 1. There are two classes between 1 and 4; the distance between them is divided into two equal parts and borders between these two parts are borders between classes 2 and 3 as well. The area between Classes 4 and 7 is divided into Classes 5 and 6 as well. Class 8 is above class 7 (Orange et al. 2001). The R_f classes are shown on Figure 13.

Figure 13: R_f class marked on TLC plate for illustration.



Color

The color of a spot on the chromatogram is also important diagnostic character for each lichen compound. The color is mostly influenced by several important factors. The main one is the concentration of the substance; another factor is the solvent system used, and the last important factor is the degree of heating of the plates after acid treatment. When there is a colored spot surrounded by a halo it is sign of a large quantity of a substance (Orange et al. 2001).

Structural information

TLC method generally does not inform well about chemical structure of lichen compounds. Position and appearance of the spots on the chromatogram could provide some information on the chemical structure of the identified compound. However, no significant details of the structure can be obtained using this method. Chemical structure is rather related to the color of the spot which could give some top level image of the chemical structure (Culberson 1972).

Identification of spots

For precise identification of unknown spots their characteristics were used (R_f, colour, and fluorescence) and compared with published TLC data. The special program was the very important tool for the most precise identification of spots.

Firstly, it was necessary to know predicted substances of the samples. The information (color and R_f value) of predicted substances were found in published materials. To ensure the identity of the substance, TLC was run again for that metabolite together with a control substance.

6 CURRENT STATE OF SOLVED PROBLEMS

6.1.1 LICHENS OF THE UNITED STATES OF AMERICA

Lichens and their studies are not unusual in United States. In 2005 the U. S. Department of Interior launched an online database that combines information about lichens and national park units (USGS 2005). The database contains over 26,100 records of lichens that were found in 144 concrete U. S. National Parks but this database is no longer funded (Bennett et Wetmore 2005; USGS 2005). Monitoring of lichen community is also a part of the Forest Inventory and Analysis National Program (USDA 2005). The program gathers data gained from the Forest Health Monitoring (FHM) program that run from 1990's: 1996 in Idaho; 1997 in Wyoming; 1998 in Washington, Oregon, and California; 1999 in Utah, and Nevada; and in 2003 in Idaho again (Neitlich et al. 1999a,b; Neitlich 2000; Neitlich et Rosentreter 2000**,** Neitlich et al. 2003). The studies monitored an estimate of air pollution level and other human impacts on environmental (USDA 2005).

The California Lichen Society (CALS) is another institution seeking to promote the study of lichens. It was established in 1994 as nonprofit corporation by originally nine founding members. Nowadays, the group of roughly 200 members of the Society does occasional small scale non-professional lichen surveys. The focus of the group includes the whole western part of the North America (CALS 2012).

The oldest nation's organization The American Bryological and Lichenological Society was established in 1898. The organization's goal primarily is the scientific study of all aspects of the biology of bryophytes and lichen-forming fungi (ABLS 2011).

Theoretically lichen biodiversity is well known today in the U.S. The assembled database of lichens of the U.S. National Parks comprises 63% of species and 73% of genera of the North American flora, respectively (Bennett et Wetmore 2005). Many studies of lichen diversity in forests of U.S. have been realized since the last century (Neitlich et al. 1999b). Currently, many websites are available providing information about lichens such as bioindicators of air pollution and climate, and

checklists of states (WSH 2005). But large parts of California and North America are unexplored for lichens or are only superficially surveyed.

6.1.2 PREVIOUS RESEARCHES OF LICHEN DIVERSITY IN THE MOJAVE REGION

Research on lichen diversity of two localities in Mojave Desert region was published in 2002 (Knight et al. 2002). Forty lichen species in 24 genera were described from two localities in the protected Mojave National Preserve in Southern California. The specimens were collected from north-facing basalt flows, rock, soil, and dead wood. The results of lichen diversity should show expected correspondence with environmental conditions in the Mojave National Preserve (Knight et al. 2002). The Mojave National Preserve has one reference within the Database of Lichens in the U. S. National Parks. 39 records of taxa have been verified in the park location. The Mojave is registered as the park researched from 26% to 50% (Bennett et Wetmore 2005). Number of records shows the low level of identified lichen species in 2005.

In the southwestern part of the Mojave Desert the situation is not much better. The inventory of lichens in Joshua Tree National Park was not carried out until a few years ago on two selected areas (Keys Ranch and Eureka Peak). The results (Knudsen et La Doux 2005, 2006). These studies showed that there are unique species of lichens in the park. Thus, it is also very likely that there are new species to the science. Due to the scientific interest in these initial projects, the current inventory was developed from the field observation made to characterize new Joshua Tree National Park’s localities and their lichen diversity.

7 RESULTS

7.1 TLC ANALYSIS

The practical part of the thesis, consisting of the TLC analysis at Gdansk laboratory, took one month in total. All obtained results are important completion of the checklist of lichens in Joshua Tree National Park. The results show substances contained in analyzed material.

Names of specimens were not significant for the analysis. Results are connected primarily with sample's ID. The determination was another part of the project not including goals of this thesis. Specimens were predetermined by Kerry Knudsen before the analysis in Gdansk; however, it provided just a basic guideline for identification secondary metabolites of samples. Thus, the final determination of samples was done by Kerry Knudsen after obtaining TLC results.

11 tables summarize general information about each run of TLC. It includes: number of analyzed specimen, TLC solvents used for the run, names of control substances, names of analyzed genera, names of determined substances, and additional notes (if they were some). 11 protocols summarize the whole TLC run and show the results of determined substances in conjunction with ID of specimens. In each protocol it is noted the date when the TLC was performed, which type of plates was used, which solvents were used and other procedural notes. Pictures taken for fixing developed plates are included in following documents. They are ordered in the same manner as the TLC's protocols. The size and ledges of the pictures were modified before they were added into this document. The nomenclature of specimens in the following tables and pictures has been taken over from Kerry Knudsen.

Table 4: TLC no 1, summarizing table.

TLC no 1	
Nuber of analyzed samples:	16
Solvents:	A, B, C
Control substances:	norstictic acid, atranorin, rhizocarpic a.
Analyzed genera:	*Acarospora* (*A. obpallens, A. rosulata, A. socialis*)
Determined substances:	gyrophoric acid, lecanoric a., rhizocarpic a.
Notes:	some unidentified substances and pigments

Table 5: TLC protocol no 1.

THIN-LAYER CHROMATOGRAPHY			**PLATE NO 1**	
Plate:	**aluminium**	**Spray:**	**water, sulphuric acid**	
Solvent:	**A B C**	**Operator(s): M. Kukwa, M. Michalová**	**Date:** 10. 10. 2011	
Subject:	**Chemistry of Lichens in South-Western Mojave Desert**			
specimen	**substances detected**			
1 13280	gyrophoric acid (with lecanoric acid)	unidentified substances in traces		
2 13480	gyrophoric acid (with lecanoric acid)	unidentified substances in traces	unidentified pigments	
3 13056	gyrophoric acid (with lecanoric acid)			
4 13238.2	gyrophoric acid (with lecanoric acid)	unidentified substances in traces	unidentified pigments	
TEST	**NORSTICTIC ACID (Rf cl. 4)**	**ATRANORIN (Rf cl. 7)**		
6 12920.2	gyrophoric acid (with lecanoric acid)	unidentified substances in traces	unidentified pigments	
7 12918	gyrophoric acid (with lecanoric acid)	unidentified substances in traces	unidentified pigments	
8 13135	rhizocarpic acid			
9 12706	rhizocarpic acid			
10 13428	rhizocarpic acid			
11 12888	rhizocarpic acid			
12 13001	rhizocarpic acid			
TEST	**RHIZOCARPIC ACID**			
14 13553	rhizocarpic acid			
15 12854	rhizocarpic acid			
16 12907.1	rhizocarpic acid			
17 12758	rhizocarpic acid			
18 13537	rhizocarpic acid			

Figure 14: TLC no 1 in solvent A

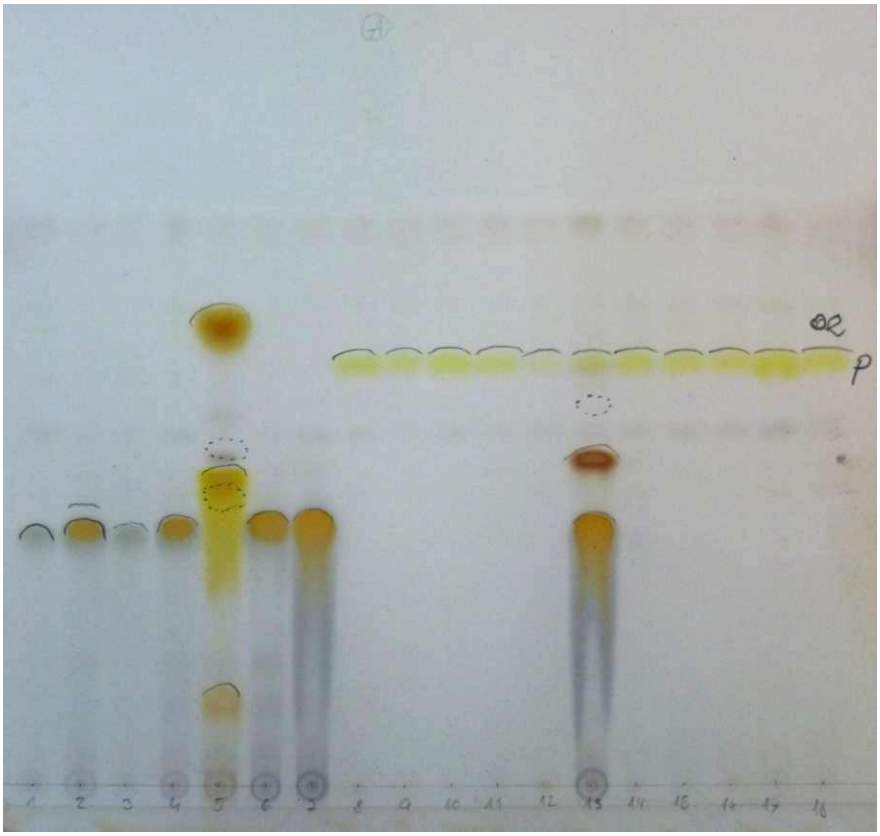


Figure 15: TLC no 1 in solvent B

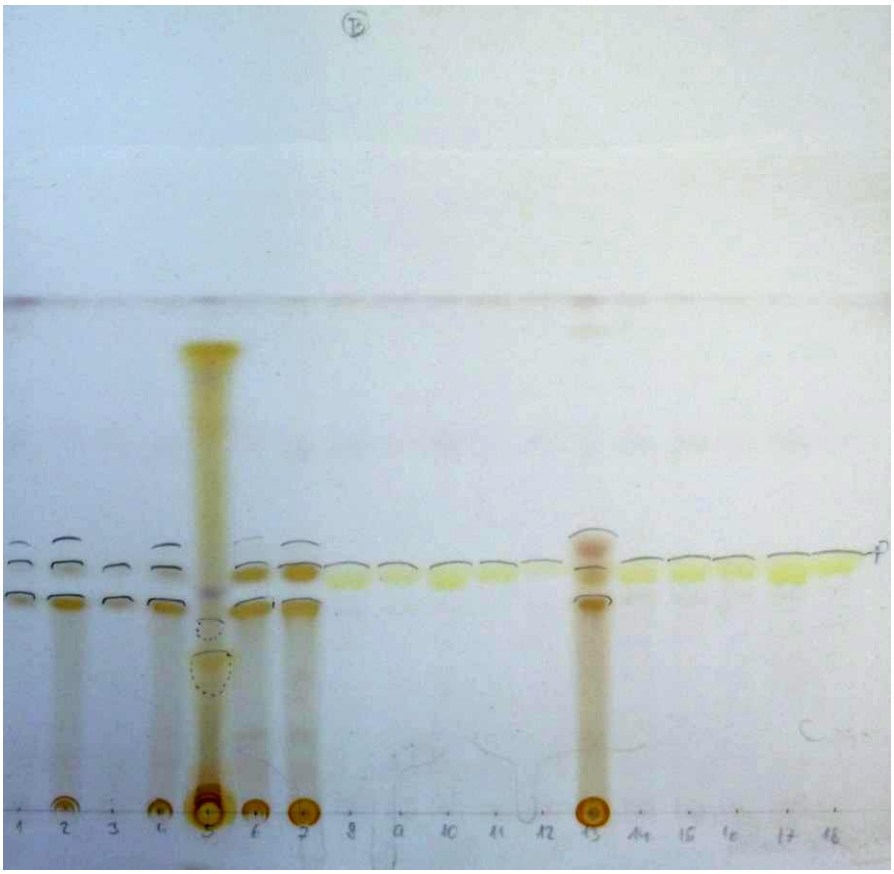


Figure 16: TLC no 1 in solvent C

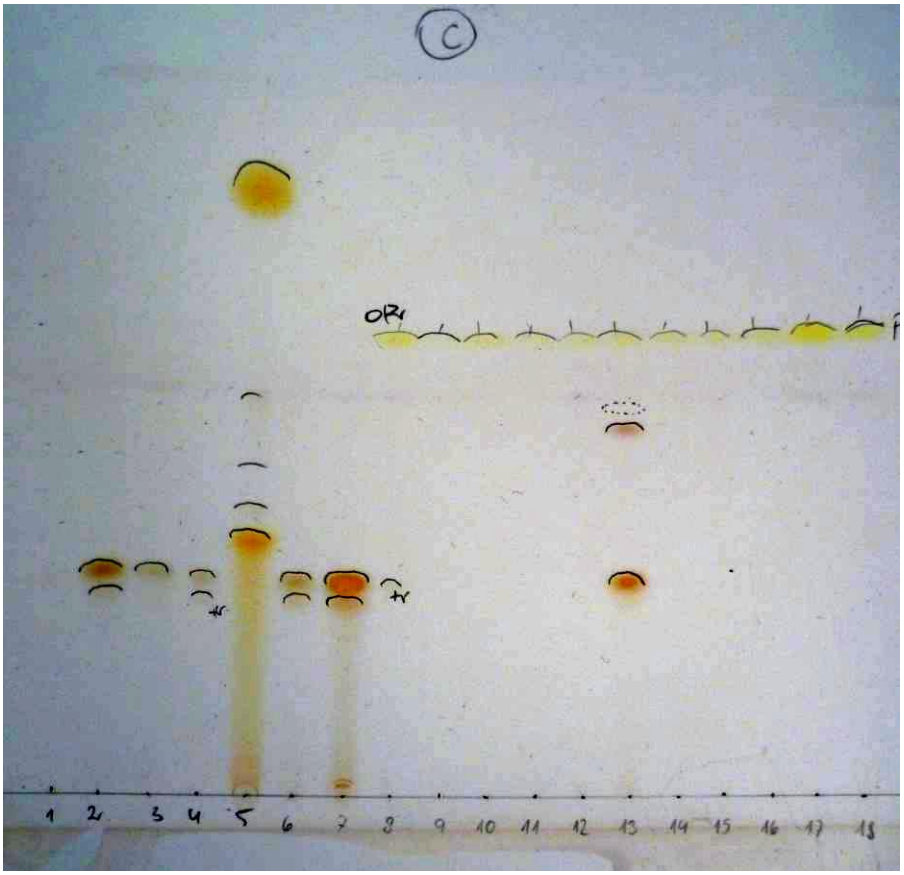


Table 6: TLC no 2

TLC no 2	
Nuber of analyzed samples:	16
Solvents:	A, B, C
Control substances:	norstictic acid, atranorin
Analyzed genera:	*Aspicilia*, *Melanelia*, *Rhizoplaca* (*R. chrysoleuca, R. subdiscrepans*), *Lepraria*, *Physconia* (*P. fallax*), *Physcia* (*P. dimidiata, P. biziana*)
Determined substances:	4-oxypannaric acid 6-methyl ester, atranorin, conorstictic acid, conpsoromic a., gyrophoric a., lecanoric a., mixture of lecanoric and gyrophoric a., norstictic acid norstictic acid, annaric a. 6-methyl ester, placodiolic a., pseudoplacodiolic a., psoromic a., substictic a., usnic a.
Notes:	unknown substance, Rf classes: A4, B5, C4; some unidentified substances; unknow substance with ice blue colour in before 366nm UV before sulphuric acid. Rf classes: A2-3, B2, C2.

Table 7: the TLC protocol no 2

THIN-LAYER CHROMATOGRAPHY			**PLATE NO 2**	
Plate: glas/ **aluminium**		**Spray:** sulphuric water		
Solvent: **A B C**		**Operator(s): M. Kukwa, M. Michalová**		**Date:7.,10.10.2011**
Subject:	**Chemistry of Lichens in South-Western Mojave Desert**			
speciment	substances detected			
1 13476	substictic acid (no control available)			
2 12969	norstictic, conorstictic acids	mixture of lecanoric (trace) and gyrophoric acids		
3 13493	gyrophoric a	lecanoric a.	trace of unknown substance, Rf classes: A4, B5, C4	
4 12845	usnic a.			
5 12787	usnic a.	aliphatic a. (fatty acid)		
6 TEST				
7 13137	pseudoplacodiolic acid			
8 12910	pseudoplacodiolic acid	usnic acid (in low concentration)		
9 Nicole Pietrasiak	usnic acid	psoromic acid with conpsoromic acid (trace)	unidentified substances and fatty acid	
10 3577	placodiolic acid			
11 13477	pannaric acid 6-methyl ester	4-oxypannaric acid 6-methyl ester		
12 13362.1	atranorin	unknow substance with ice blue colour in before 366nm UV before sulphuric		
13 12968	atranorin			
14 TEST				
15 13220	atranorin			
16 13219	atranorin			
17 12846	atranorin	unknow substance with ice blue colour in before 366nm UV before sulphuric		
18 13051	atranorin			

Figure 17: TLC no 2 in solvent A

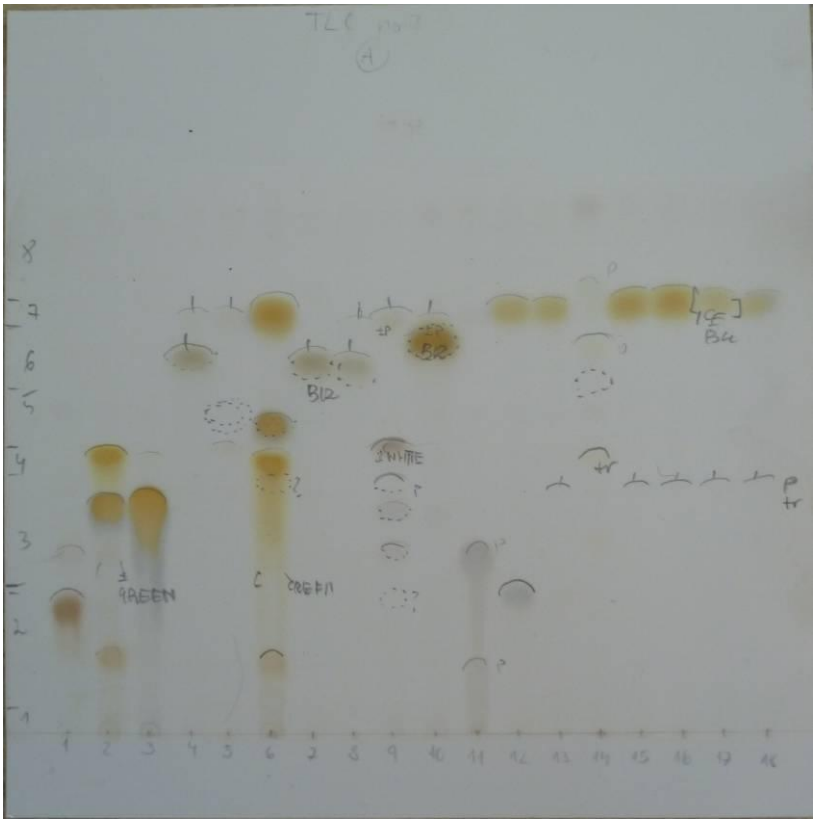


Figure 18: TLC no 2 in solvent B

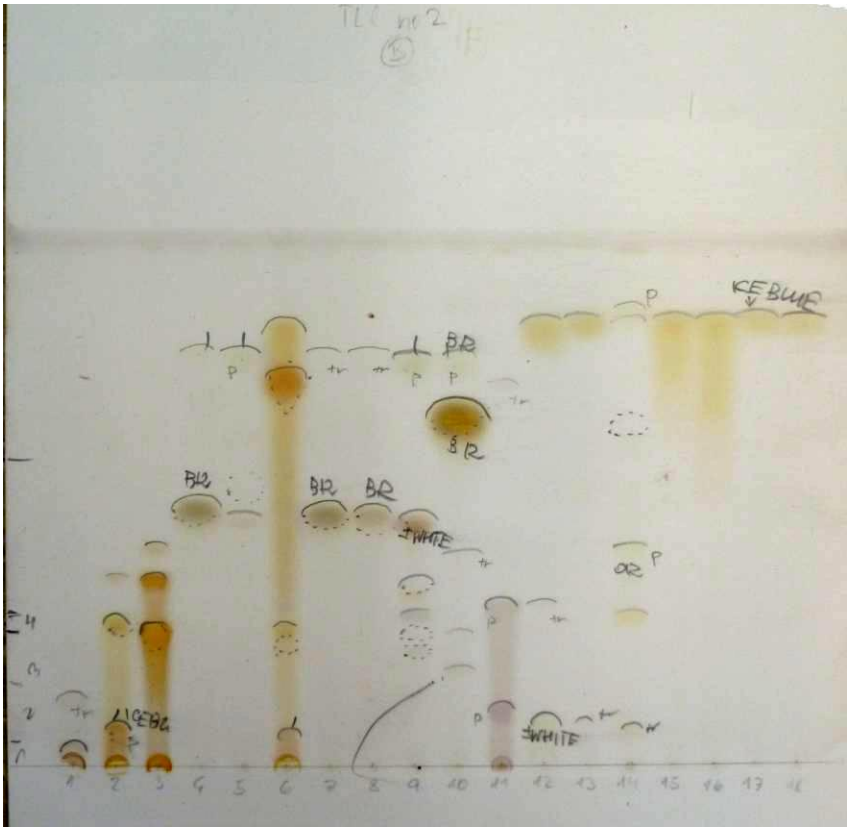


Figure 19: TLC no 2 in solvent C

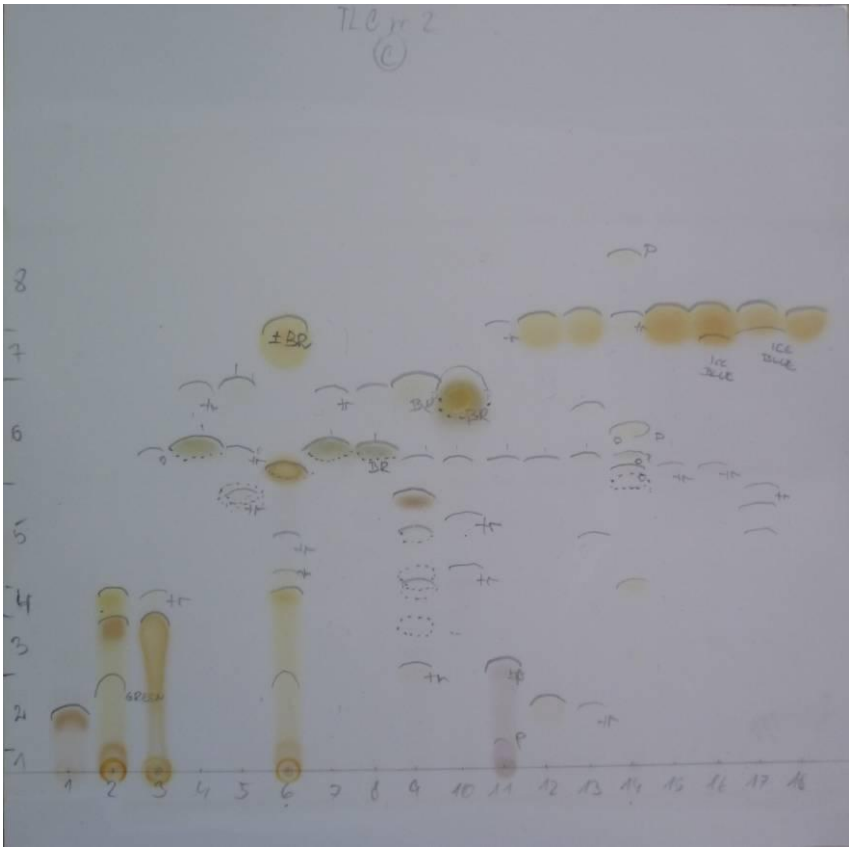


Table 8: TLC no 3

TLC no 3	
Nuber of analyzed samples:	15
Solvents:	A, B, C
Control substances:	norstictic acid, atranorin, psoromic a., pseudoplacodiolic a.
Analyzed genera:	*Lecidella* (*L. patavina*), *Miriquidica* (*M. scotopholis*), *Rhizoplaca* (*R. subdiscrepans*, *R. chrysoleuca*), *Lecanora* (*L. varia*), *Lecidea* (*L. leprarioides*), *Circinaria* (*C. arida*), *Lobothallia* (*L. praeradiosa*)
Determined substances:	connorstictic acid, fatty acid (prob. aspicilin), miriquidic a., norstictic a., unknown pigment, up to 5 unidentified substances, zeorin
Notes:	in some specimens were no substances

Table 9: the TLC protocol no 3

THIN LAYER CHROMATOGRAPHY			PLATE NO 3	
Plate:	**aluminium**	**Spray:**	**water, sulphuric acid**	
Solvent:	**A B C**	**Operator(s): M. Kukwa, M. Michalová**	**Date: 11.10.2011**	
Subject:	**Chemistry of Lichens in South-Western Mojave Desert**			
specimen	**substances detected**			
1 12625	trace of zeorin			
2 13035	trace of zeorin			
3 13319	trace of zeorin			
TEST	NORSTICTIC ACID	ATRANORIN	Rf cl. 4	Rf cl. 7
5 13355	miriquidic acid	up to 5 unidentified substances		
6 13392	miriquidic acid	up to 5 unidentified substances		
7 13062	miriquidic acid	up to 5 unidentified substances		
8 3577 Nicole Pietrasiak s.	usnic acid	psoromic acid with conpsoromic acid (trace)	unidentified substances and fatty acid	
TEST	PSOROMIC ACID			
10 12910	usnic acid (low concentration)	pseudoplacodiolic acid		
TEST	PSEUDOPLACODIOLIC ACID			
12 3577	placodiolic acid			
13 13163	fatty acid (probably aspicilin)			
14 13011.1	no substances			
15 12898.1	fatty acid (probably aspicilin)			
16 12989	no substances			
17 3607	fatty acid (probably aspicilin)			
18 12962	norstictic and connosrtictic acids	trace of pigment		

Figure 20: TLC no 3 in solvent A

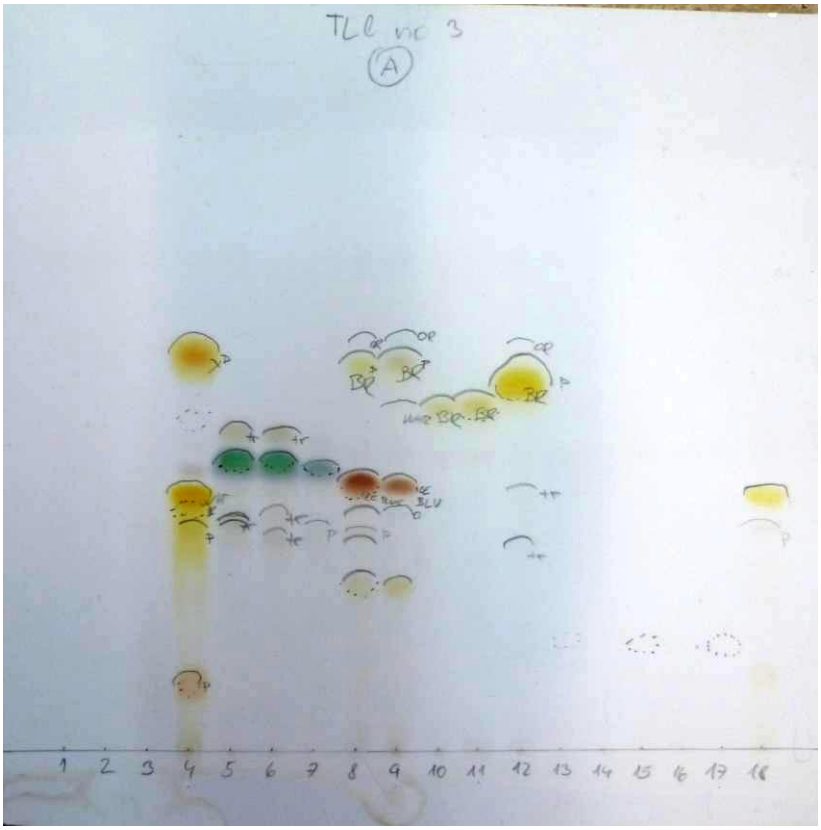


Figure 21: TL TLC no 3 in solvent B

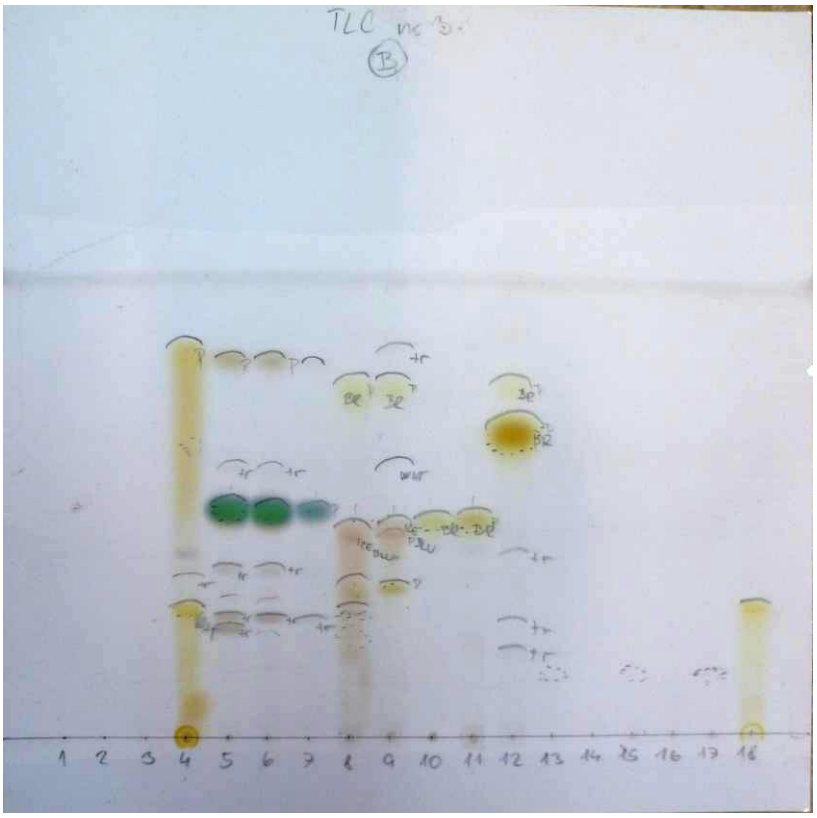


Figure 22: TLC no 3 in solvent C

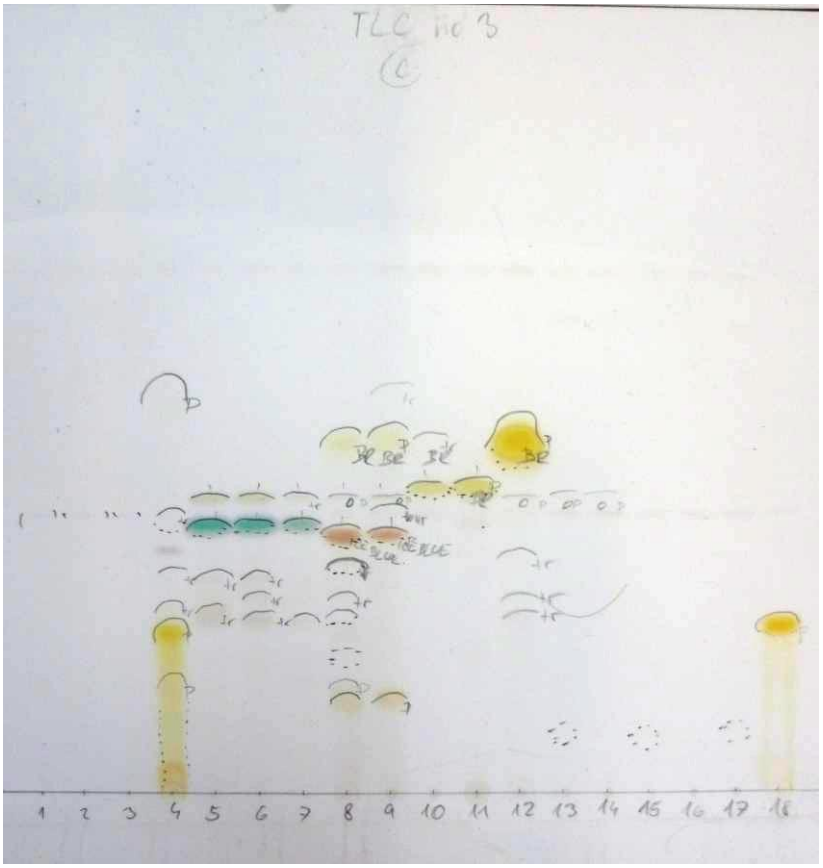


Table 10: TLC no 4

TLC no 4	
Nuber of analyzed samples:	17
Solvents:	A, B, C
Control substances:	norstictic acid, atranorin
Analyzed genera:	*Lecanora* (*L. muralis*, *L. saligna*, *L. sierrae*, *L. laxa*), *Acarospora* (*A. succendens*), *Rhizocarpon* (*R. geminatum*, *R. disporum*)
Determined substances:	consalazinic acid, gyrophoric a., isousnic a., lecanoric a., norstictic a., placodiolic a., salazinic a., stictic a., substictic a., usnic a., zeorin, variolaric a., stictic a. complex (stictic a. is a major substance)
Notes:	in some specimens wer unknown substances, fatty acid(s), pigment(s), terpenoid(s)

Table 11 the TLC protocol no 4

THIN-LAYER CHROMATOGRAPHY			PLATE NO 4	
Plate:	aluminium	Spray:	water, sulphuric acid	
Solvent:	A B C	Operator(s): M. Kukwa, M. Michalová	Date: 13.10. 2011	
Subject:	Chemistry of Lichens in South-Western Mojave Desert			
specimen	substances detected			
1 12685	usnic acid	placodiolic acid	fatty acid	terpenoids (low concentration), zeorin
2 13340	isousnic acid	traces of two unknown substances		
3 12790.1	usnic acid	placodiolic acid	fatty acid, terpenoids (low concentration), zeorin	unidentified pigments in traces, salazinic acid and consalasinic acid
TEST	NORSTICTIC ACID	ATRANORIN		
5 12851	usnic acid	placodilic a.	fatty acid (same as in n. 1)	terpenoids (low concentration), zeorin
6 12951	usnic acid	unidentified terpenoids, zeorin	fatty acid	unknown substance (variolanic acid?)
7 12974	isousnic acid			
8 13073	isousnic acid	some unknown subsances in traces		
9 13236.1	usnic acid	placodiolic acid	fatty acid	terpenoid, trace of pigment
10 5246	usnic acid			
11 12876	gyrophoric, lecanoric acid			
12 5232	unknown substances			
13 2615	stictic acid			
14 13489	norstictic acid	stictic acid complex (stictic acid is a major substance)		
15 13401.2	contamination of unknown lichen			
16 12963	stictic acid	substictic acid	trace of pigment	
17 13040.1	trace of pigment			
18 13083	trace of stictic acid	trace of salazinic acid		

Figure 23: TLC no 4 in solvent A

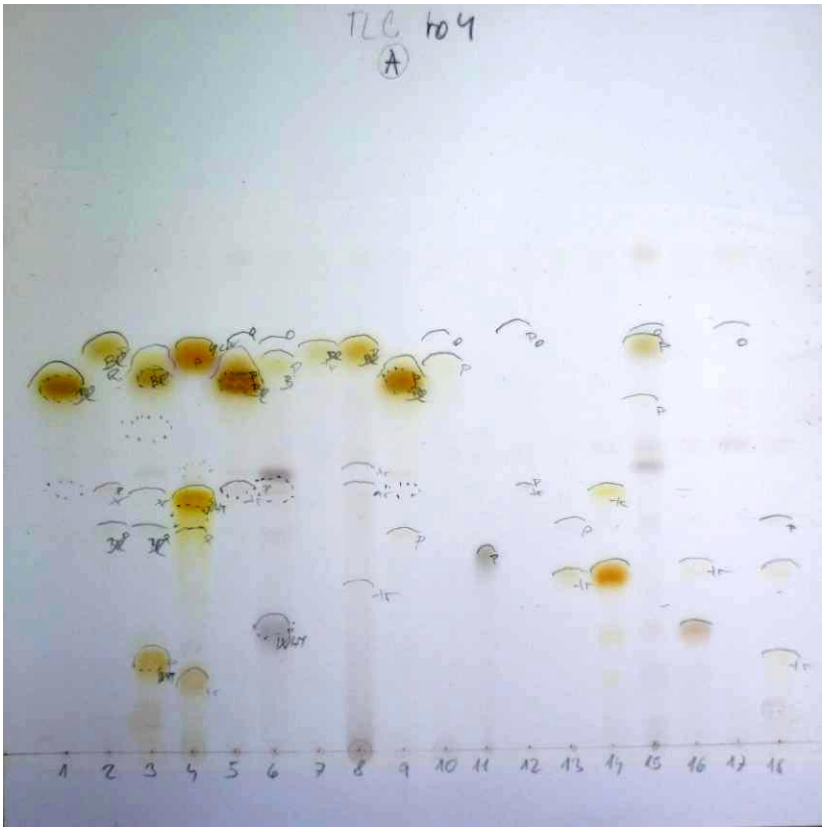


Figure 24: TLC no 4 in solvent B

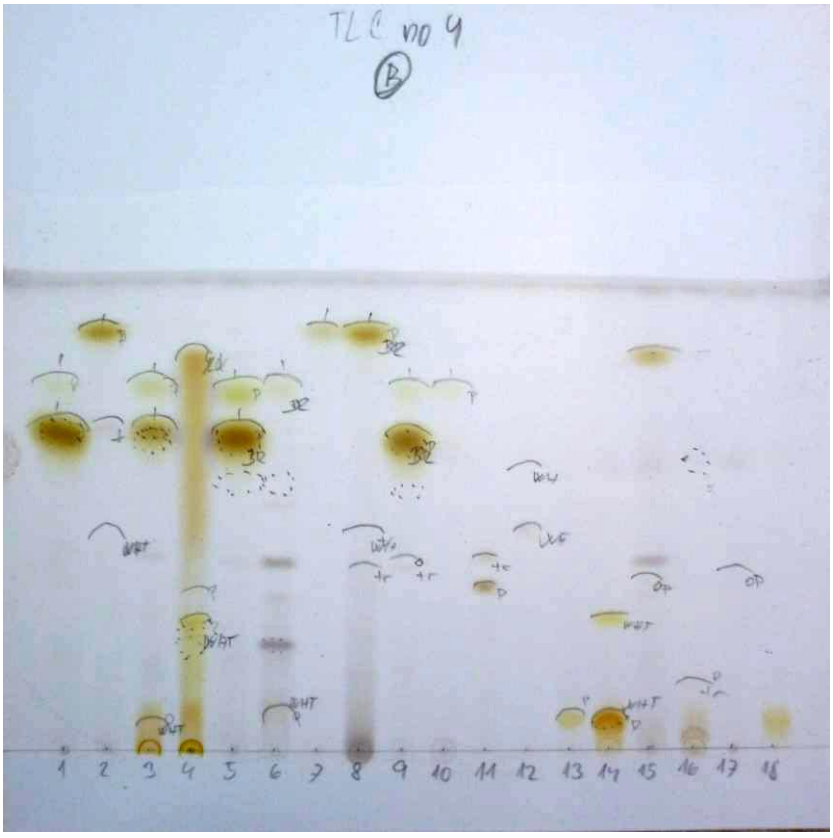


Figure 25: TLC no 4 in solvent C

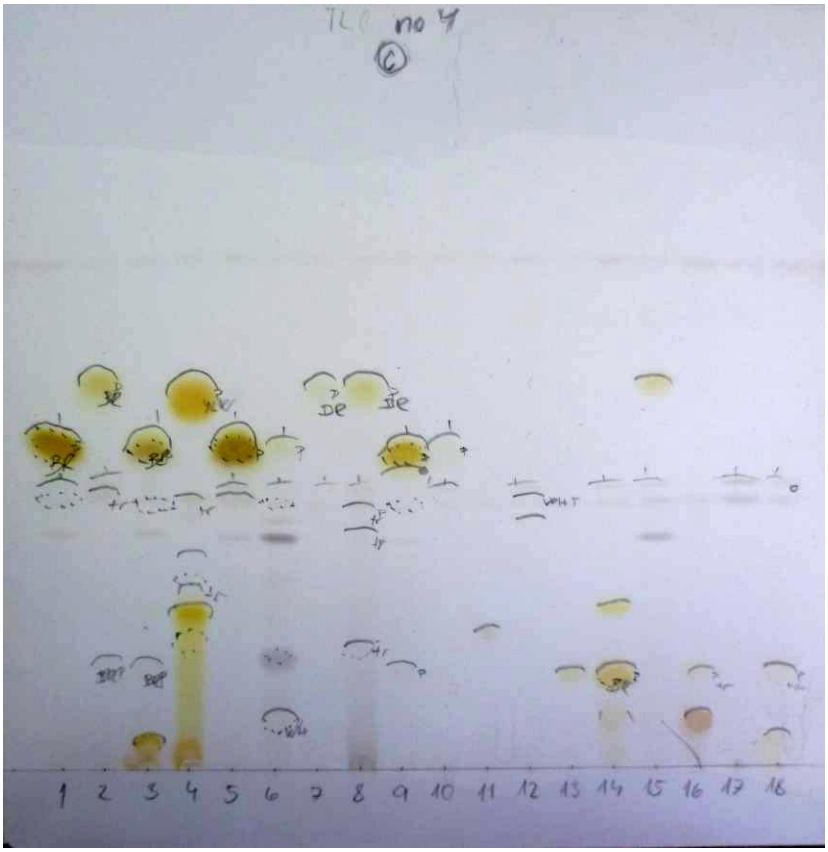


Table 12: TLC no 5

TLC no 5	
Nuber of analyzed samples:	14
Solvents:	A, B, C
Control substances:	norstictic acid, atranorin, zeorin, variolic a.
Analyzed genera:	*Lecidea* (*L. confluens*, *L. hassei*, *L. laboriosa*), *Lecanora* (*L. muralis*), *Rhizocarpon* (*R. disporum*)
Determined substances:	2'-*O*-methylperlatolic acid, schizopeltic a., 4-*O*-demethylplanaic acid
Notes:	in some specimens were no substances, unknown pigment(s), unknown substance above 2'-*O*-methylperlatolic acid

Table 13: the the TLC protocol no 5

THIN-LAYER CHROMATOGRAPHY			**PLATE NO 5**	
Plate:	**aluminium**	**Spray:**	**water, sulphuric acid**	
Solvent:	**A B C**	**Operator(s): M. Kukwa, M. Michalová**	**Date: 14. 10. 2011**	
Subject:	**Chemistry of Lichens in South-Western Mojave Desert**			
specimen	**substances detected**			
1 13187	2'-O-methylperlatolic acid	unknown substance above		
2 13480.2	2'-O-methylperlatolic acid	unknown substance above (trace)		
3 13482.1	2'-O-methylperlatolic acid	unknown substance above (trace)		
TEST	NORSTICTIC ACID	ATRANORIN		
5 12709	schizopeltic acid			
6 12926	schizopeltic acid			
7 12760	schizopeltic acid			
TEST	ZEORIN			
9 12951	usnic acid	fatty acid	variolaric acid (compared with *Ochrolechia microstictoides*)	unidentified terpenoids with zeorin
TEST				
TEST	VARIOLIC ACID			
12 12665	schizopeltic acid			
13 13241	4-O-demethylplanaic acid (no control available)			
14 13401.2	no substances	trace of pigments		
15 13174	no substances			
16 13564	no substances			
17 13529	schizopeltic acid			
18 13123	trace of pigment			

Figure 26: TLC no 5 in solvent A

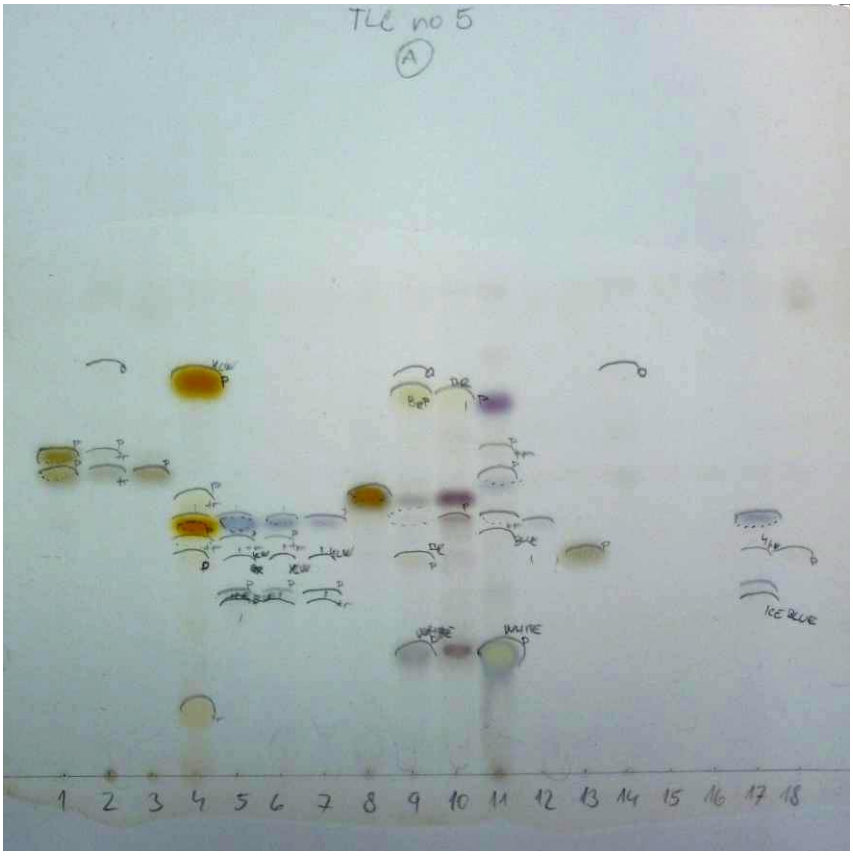


Figure 27: TLC no 5 in solvent B

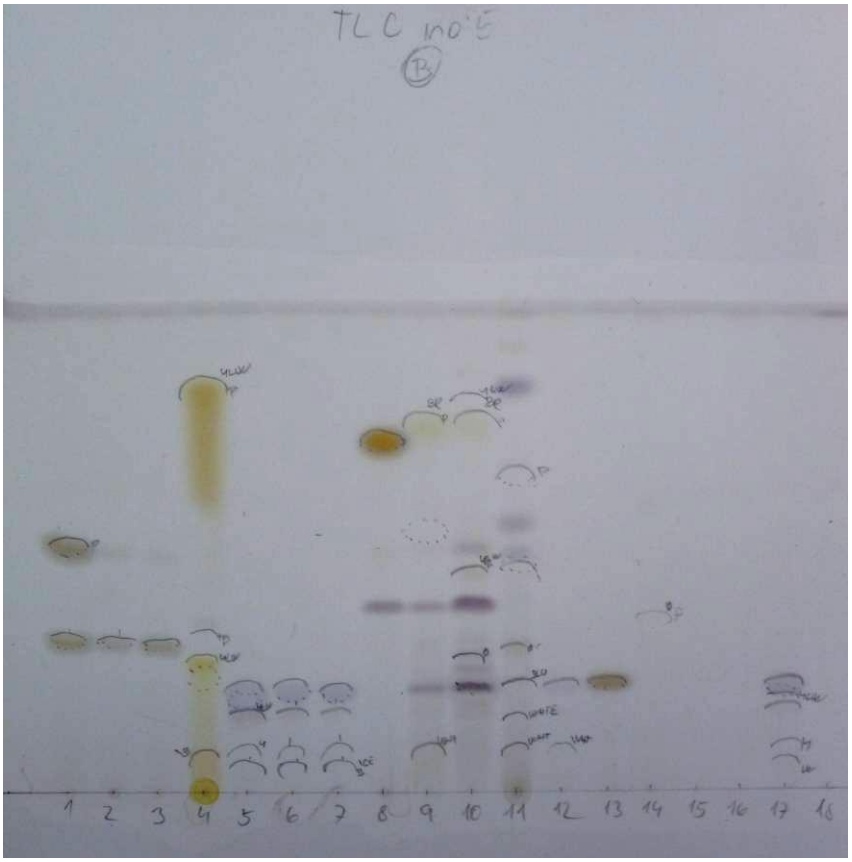


Figure 28: TLC no 5 in solvent C

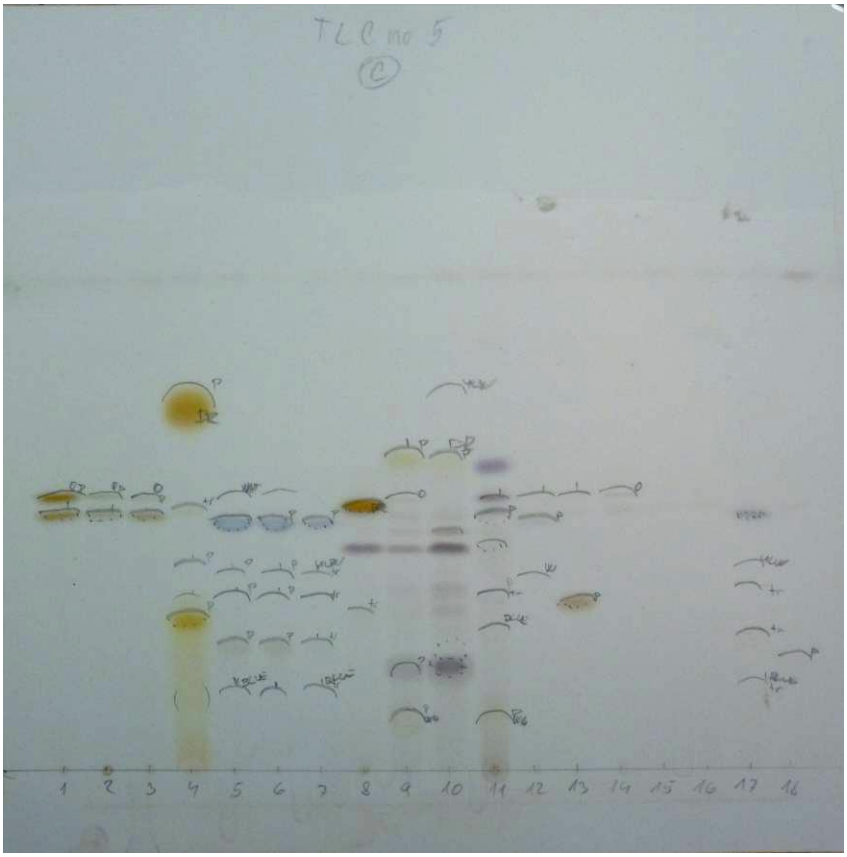


Table 14: TLC no 6

TLC no 6	
Nuber of analyzed samples:	17
Solvents:	A, B, C
Control substances:	norstictic acid, atranorin
Analyzed genera:	*Xanthoparmelia* (*X. plitti, X. mexicana, X. verruculifera*)
Determined substances:	5-*O*-methylhiascic acid, bourgeanic a., connorstictic a., fumarprotocetraric a., gyrophoric a., lecanoric a., menegazziaic a., menegazziaic a., protocetraric a., salazinic a., stictic acid complex (menegazziaic acid), usnic a.
Notes:	in some species found some unknown fatty acids, pigments and other unknown or unidentified substances, unknown sunstance related to norstictic acid Rf cl. A2-3, B3, C2

Table 15: the TLC protocol no 6

THIN-LAYER CHROMATOGRAPHY				PLATE NO 6	
Plate:	**aluminium**	**Spray:**	**water, sulphuric acid**		
Solvent:	**A B C**	**Operator(s): M. Kukwa, M. Michalová**		**Date: 17. 10. 2011**	
Subject:	**Chemistry of Lichens in South-Western Mojave Desert**				
specimen		**substances detected**			
1	**12881**	salazinic acid	fatty acid	usnic acid	trace of norstictic acid
2	**13172**	norstictic acid, unknown sunstance related to norstictic acid Rf cl. A2-3, B3, C2	conorstictic acid	unidentified substance	
3	**12848**	norstictic acid (minor)	stictic acid complex (menegazziaic acid)	usnic acid	fatty acid
TEST		NORSTICTIC ACID (Rf cl. 4)	ATRNORIN (Rf cl. 7)		
5	**13095**	salazinic acid	norsticic acid	unidentified substances	trace of protocetraric acid (most probably)
6	**12829**	salazinic acid, norstictic acid	unidentified substances	fatty acid in solvent B	trace of protocetraric acid (most probably)
7	**13360**	norstictic acid (minor)	stictic acid complex (menegazziaic acid)		
8	**13402**	usnic acid	bourgeanic acid	salazinic acid	
9	**13494.3**	usnic acid	fatty acid	salazinic acid	
10	**12632**	usnic acid	norstictic acid, unknown sunstance related to norstictic acid Rf cl. A2-3, B3, C2	conorstictic acid	unidentified substance
11	**12871**	fumarprotocetraric acid	protocetraric acid (precursor for fumarprotocetraric acid)	usnic acid	
12	**13080**	salazinic acid	norstictic acid (minor)	fatty acid	
13	**12770**	norstictic acid, unknown sunstance related to norstictic acid Rf cl. A2-3, B3, C2	usnic acid	conorstictic acid	unknown substance
14	**12733**	usnic acid	fatty acid	salazinic acid	
15	**13491**	usnic acid	fatty acid	salazinic acid	traces of two pigments
16	**13084**	lecanoric acid	gyrophoric acid	traces of pigments	cf. 5-O-methylhiascic acid
17	**13400**	lecanoric acid	gyrophoric acid	traces of pigments	cf. 5-O-methylhiascic acid
18	**13081**	lecanoric acid	gyrophoric acid	traces of pigments	cf. 5-O-methylhiascic acid

Figure 29: TLC no 6 in solvent A

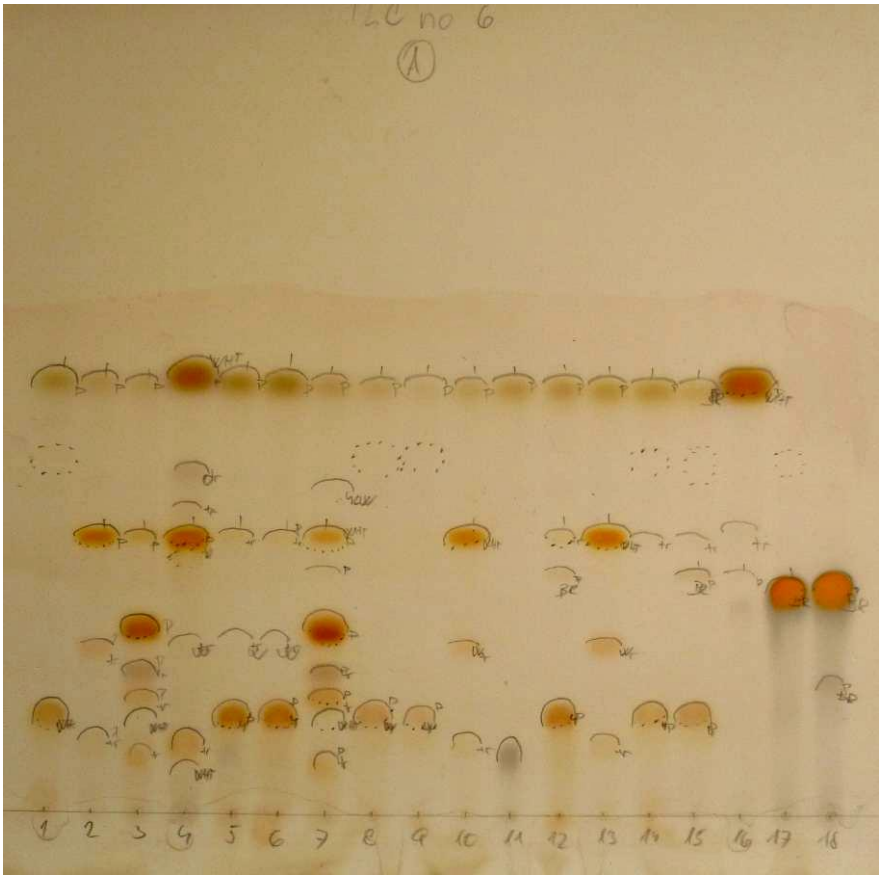


Figure 30: TLC no 6 in solvent B

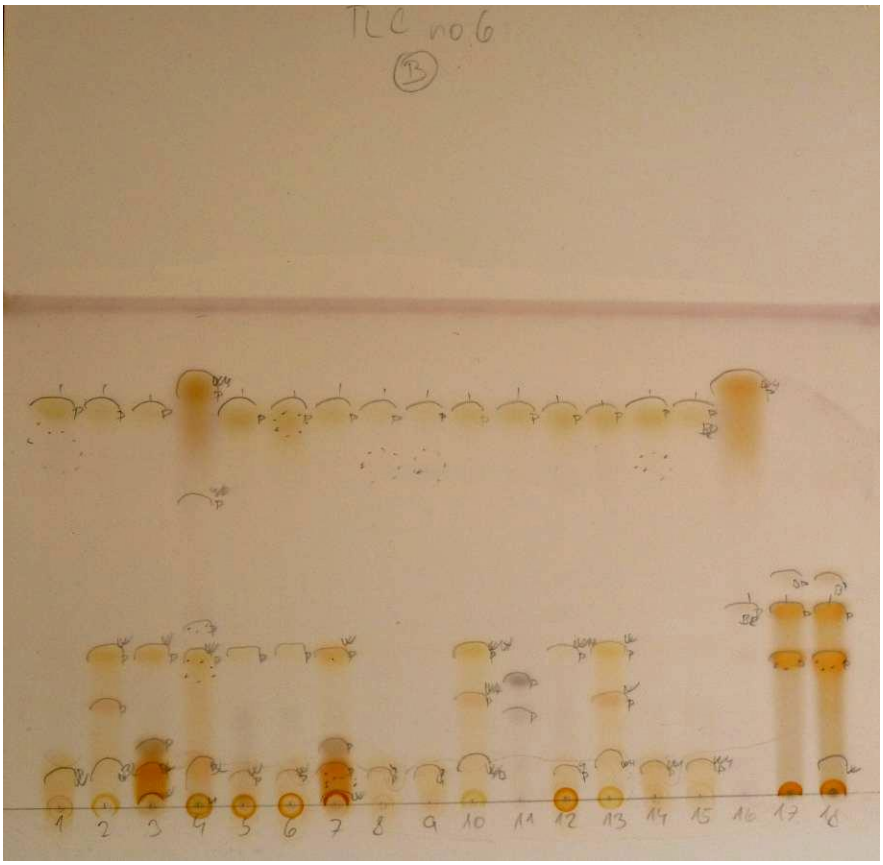


Figure 31: TLC no 6 in solvent C

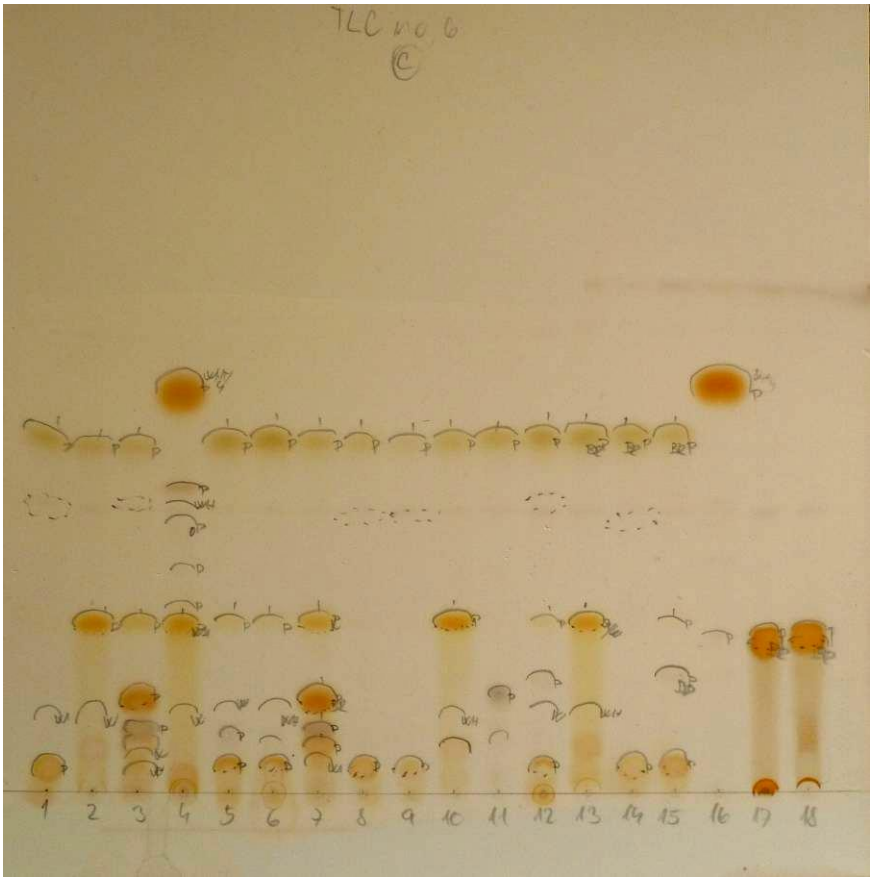


Table 16: TLC no 7

TLC no 7	
Nuber of analyzed samples:	13
Solvents:	A, B, C
Control substances:	schizopeltic acid, gyrophoric acid
Analyzed genera:	*Umbilicaria* (*U. phaea*), *Lecidea* (*L. hassei, L. laboriosa, L. manii*), *Xanthoparmelia* (*X. verruculifera, X. subramigera*), *Dimelaena* (*D. oreina*)
Determined substances:	atranorin, gyrophoric acid, lecanoric a., menegazziaic a., norstictic a., protocetraric a., salazinic a., chizopeltic a., tictic acid complex, umbilicaric a., usnic a.
Notes:	All detected substances were determined.

Table 17: the TLC protocol no 7

THIN-LAYER CHROMATOGRAPHY			PLATE NO 7	
Plate:	**aluminium**	**Spray:**	**water, sulphuric acid**	
Solvent:	**A B C**	**Operator(s): M. Kukwa, M. Michalová**	**Date: 18. 10. 2011**	
Subject:	**Chemistry of Lichens in South-Western Mojave Desert**			
specimen	**substances detected**			
1 12966	lecanoric acid	gyrophoric acid	trace of umbilicaric acid	
2 13530	lecanoric acid	gyrophoric acid	trace of umbilicaric acid	
3 13389	lecanoric acid	gyrophoric acid	trace of umbilicaric acid	
TEST	SCHIZOPELTIC ACID			
5 12709	schizopeltic acid			
6 13241	4-*O*-demethylplanaic acid (no control available)			
TEST	SCHIZOPELTIC ACID			
8 13080	usnic acid	norstictic acid	salazinic acid	consalazinic acid
TEST				
10 13402	bourgeanic acid	usnic acid	salazinic acid	
TEST				
12 13400	lecanoric acid	gyrophoric acid	traces of pigments	cf. 5-*O*-methylhiascic acid
13 13084	lecanoric acid	gyrophoric acid	traces of pigments	cf. 5-*O*-methylhiascic acid
TEST	GYROPHORIC ACID			
15 12783	stictic acid complex	menegazziaic acid	trace of norstictic and usnic acid	
16 12913	stictic acid complex	menegazziaic acid	trace of norstictic and usnic acid	
17 12871	usnic acid	trace of norstictic acid	salazinic acid	trace of protocetraric acid, trace of atranorin
18 13398.1	schizopeltic acid	lecanoric acid	gyrophoric acid	

Figure 32: TLC no 7 in solvent A

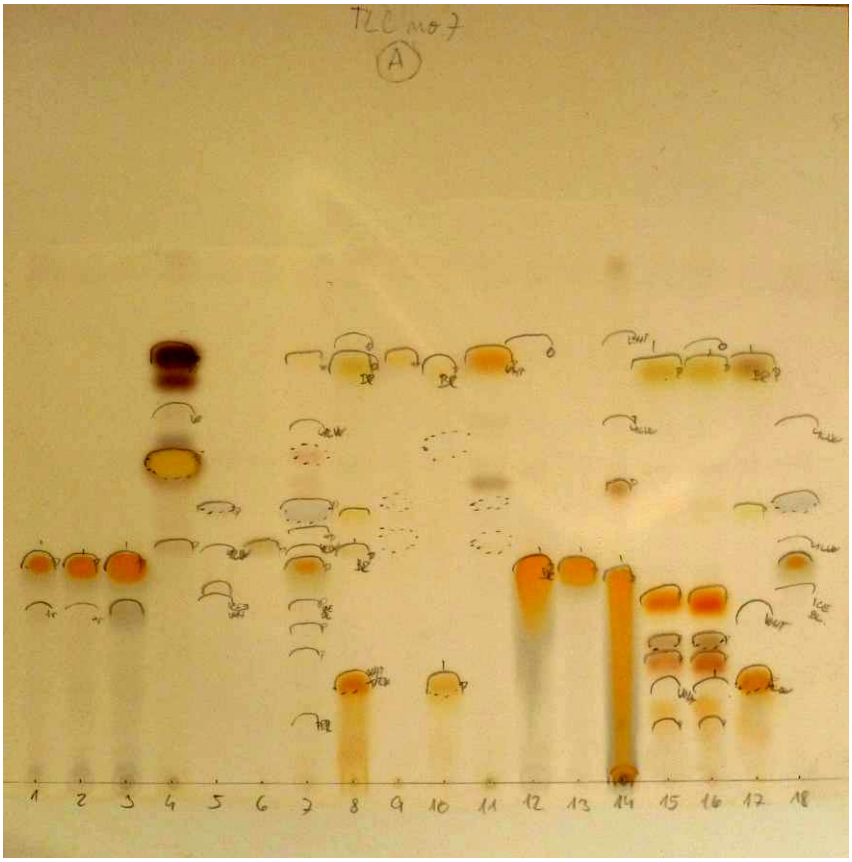


Figure 33: TLC no 7 in solvent B

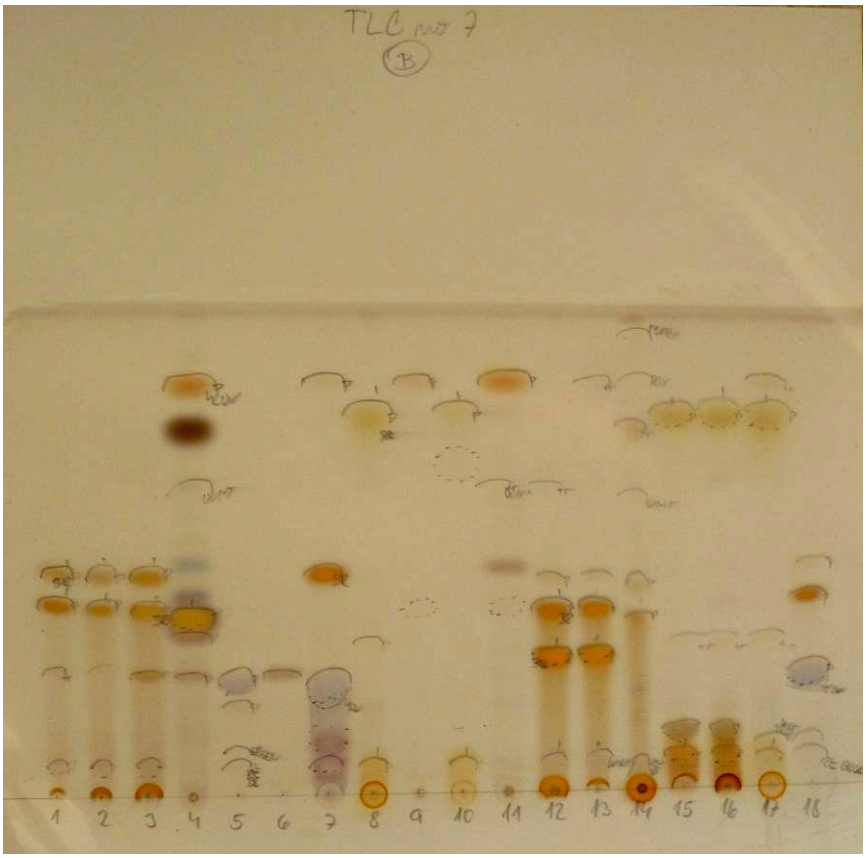


Figure 34: TLC no 7 in solvent C

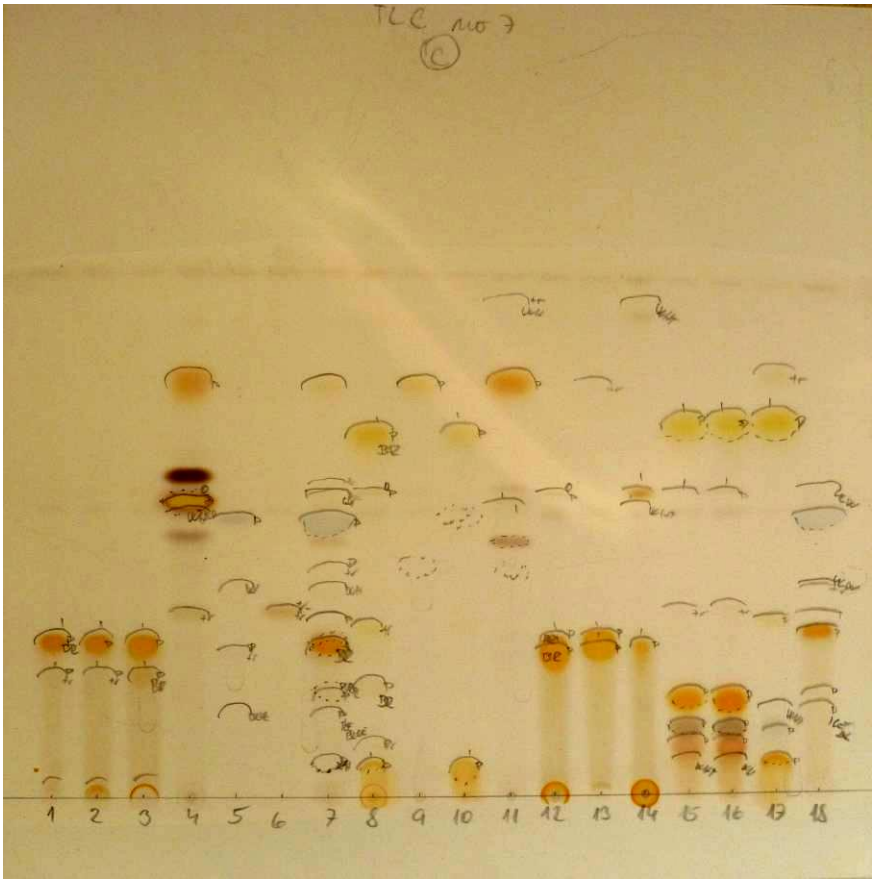


Table 18: TLC no 8

TLC no 8	
Nuber of analyzed samples:	16
Solvents:	A, B, C
Control substances:	gyrophoric acid, bourgeanic acid, norstictic acid
Analyzed genera:	*Dimelaena* (*D. thysanonta*, *D. oreina*), *Buelia* (*B. abstracta, B. nashii, B. spuria*), *Xanthoparmelia* (*X. mexicana*), *Lecanora* (*L. argopholis, L. garovaglii*), *Lichenothelia*, *Lepraria*, *Lobothalia*(*L. praeradiosa*)
Determined substances:	atranorin, bourgenic acid., connorstictic a., epanorin, hypostictic a., menegazziaic a., norstictic a., pannarin, pseudoplacodiloic a., sphaerophorin, stictic acid complex, zeorin, usnic a.
Notes:	in some specimens were unknown substances, fatty acids, terpenoid(s)

Table 19: the TLC protocol no 8

THIN-LAYER CHROMATOGRAPHY			PLATE NO 8	
Plate: aluminium	Spray: water, sulphuric acid			
Solvent: A B C	Operator(s): M. Kukwa, M. Michalová		Date: 19. 10. 2011	
Subject: Chemistry of Lichens in South-Western Mojave Desert				
specimen	**substances detected**			
1 13049	sphaerophorin	traces of additional substances		
2 13354	sphaerophorin	traces of additional substances		
TEST	GYROPHORIC ACID			
4 13406	norstictic acid	conorstictic acid		
5 13167	norstictic acid	conorstictic acid		
6 13402	bourgeanic acid	usnic acid	salazinic acid	
TEST	BOURGEANIC ACID			
8 13732	bourgeanic acid	zeorin with additional terpenoids	epanorin	atranorin
9 13742	trace of terpenoid			
10 13719	pseudoplacodiolic acid	traces of unknown substances		
11 13709	pannarin	zeorin with additional substances		
12 13713	norstictic acid	connorstictic acid		
13 12993.1	stictic acid comlex	hypostictic acid		
14 12943	atranorin	norstictic acid (minor)	stictic acid complex (major)	hypostictic and conorstictic acids traces
15 12943	atranorin	norstictic acid (minor)	stictic acid complex (major)	hypostictic and conorstictic acids traces
TEST	NORSTICTIC ACID			
17 2596.2	atranorin	norstictic acid (minor)	stictic acid complex (major)	hypostictic and conorstictic acids traces
18 13346	atranorin	norstictic acid (minor)	stictic acid complex (major)	hypostictic and conorstictic acids traces
19 13717	usnic acid	sticti acid complex	menegazziaic acid	trace of norstictic acid

Figure 35: TLC no 8 in solvent A

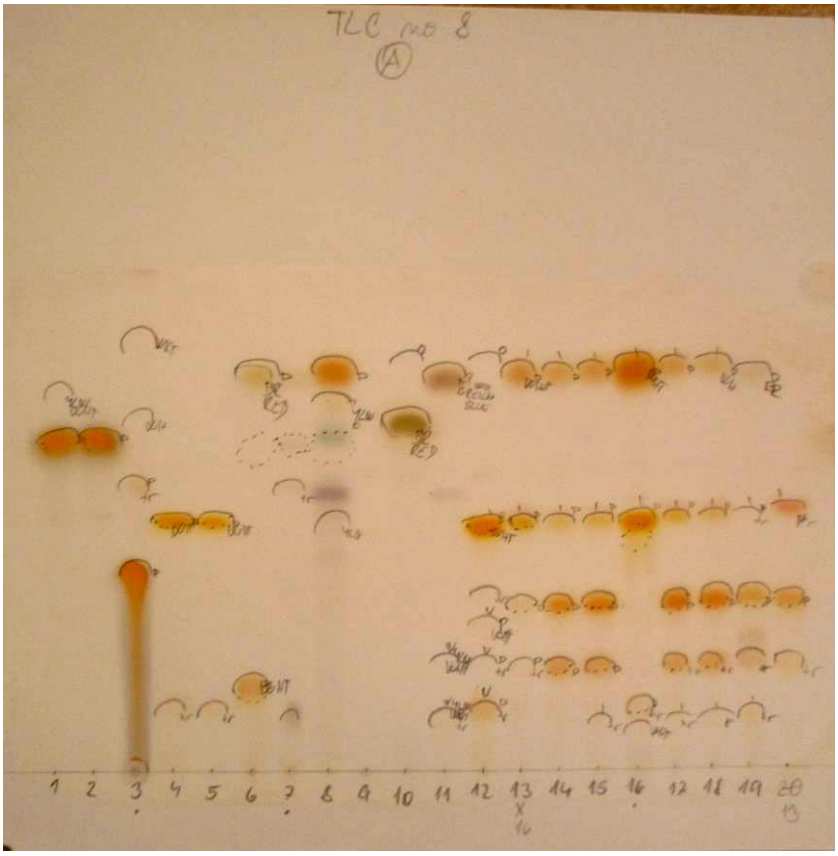


Figure 36: TLC no 8 in solvent B

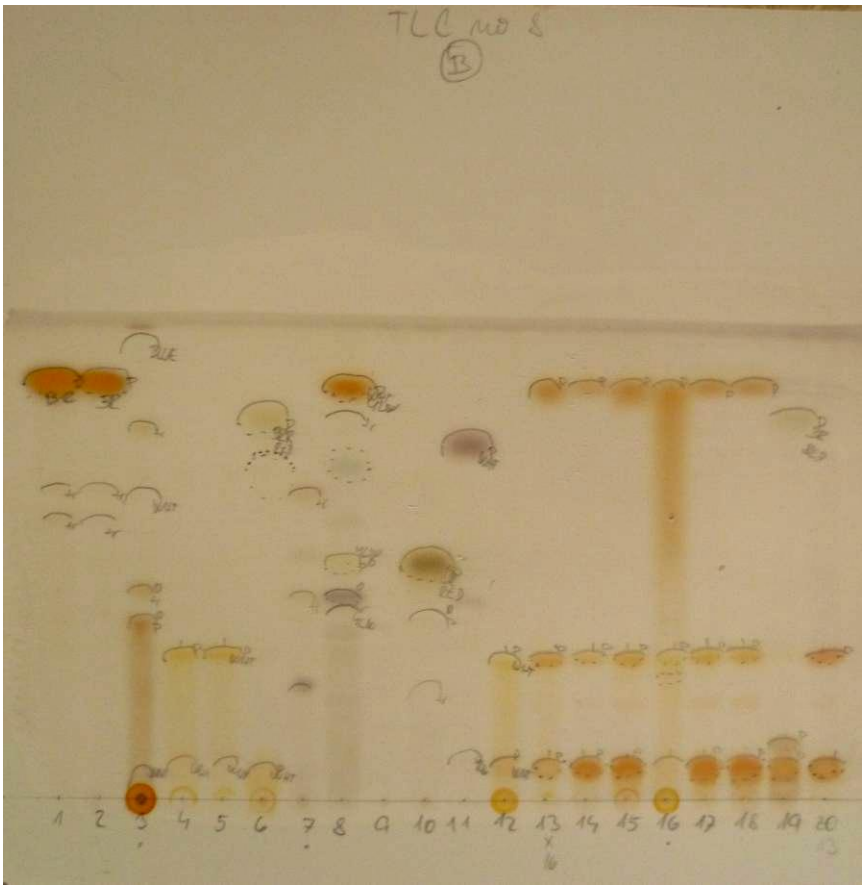


Figure 37: TLC no 8 in solvent C

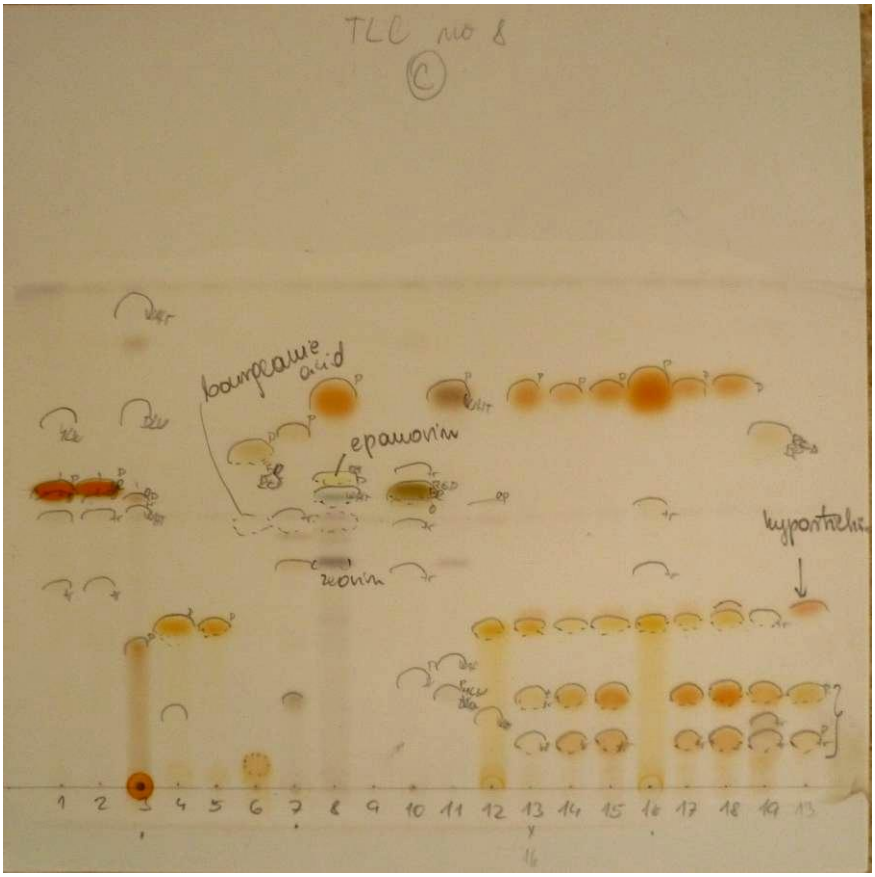


Table 20: TLC no 9

TLC no 9	
Nuber of analyzed samples:	17
Solvents:	A, B, C
Control substances:	atranorin, 2'-*O*-methylperlatolic acid, pannarin, norstictic a.
Analyzed genera:	*Buellia* (*B. dispersa*), *Lepraria*, *Xanthoparmelia* (*X. mexicana*)
Determined substances:	2'-O-methylperlatolic acid, atranorin, bourgeanic a., connorstictic a., confluentic a., consalazinic a., cryptostictic a., cf. hyposalazinic a., hypostictic a., norstictic a., pannarin, salazinic a., stictic a., variolaric a., usnic a., zeorin
Notes:	additional substances, two unidentified substances related with pannarin

Table 21 the TLC protocol no 9

THIN-LAYER CHROMATOGRAPHY			PLATE NO 9		
Plate:	**aluminium**	**Spray:**	**water, sulphuric acid**		
Solvent:	**A B C**	**Operator(s): M. Kukwa, M. Michalová**		**Date: 19. 10. 2011**	
Subject:	**Chemistry of Lichens in South-Western Mojave Desert**				
specimen		**substances detected**			
1	**3628**	norstictic acid			
2	**5193**	norstictic acid			
3	**5756**	variolaric acid	some additional substances in trace		
TEST		ATRANORIN (minor)	2'-*O*-METHYLPERLATOLIC ACID		
5	**13111.1**	pannarin	two unidentified substances related with pannarin		
6	**13474**	confluentic acid	2'-O-methylperlatolic acid		
7	**13097.1**	hypostictic acid	stictic acid	cryptostictic acid	trace of atranorin
8	**13472.2**	confluentic acid (minor)	2'-O-methylperlatolic acid (major)		
9	**12804.1**	confluentic acid (minor)	2'-O-methylperlatolic acid (major)		
10	**2620**	confluentic acid (minor)	2'-O-methylperlatolic acid (major)		
11	**13709**	pannarin	zeorin	two unidentified substances	
TEST		PANNARIN			
13	**12791**	confluentic acid (minor)	2'-O-methylperlatolic acid (major)		
14	**13063**	confluentic acid (minor)	2'-O-methylperlatolic acid (major)		
15	**13492**	confluentic acid (minor)	2'-O-methylperlatolic acid (major)		
16	**5259**	norstictic acid	conorstictic acid		
17	**12797**	confluentic acid (minor)	2'-O-methylperlatolic acid (major)		
TEST		NORSTICTIC ACID Rf. Cl. 4	ATRANORIN (Rf cl. 7)		
19	**13762**	usnic acid	norstictic acid	conorsticic acd	cf. hyposalazinic acid
20	**13748**	usnic acid	bourgeanic acid	trace of norstictic	salazinic and consalazinic acid

Figure 38: TLC no 9 in solvent A

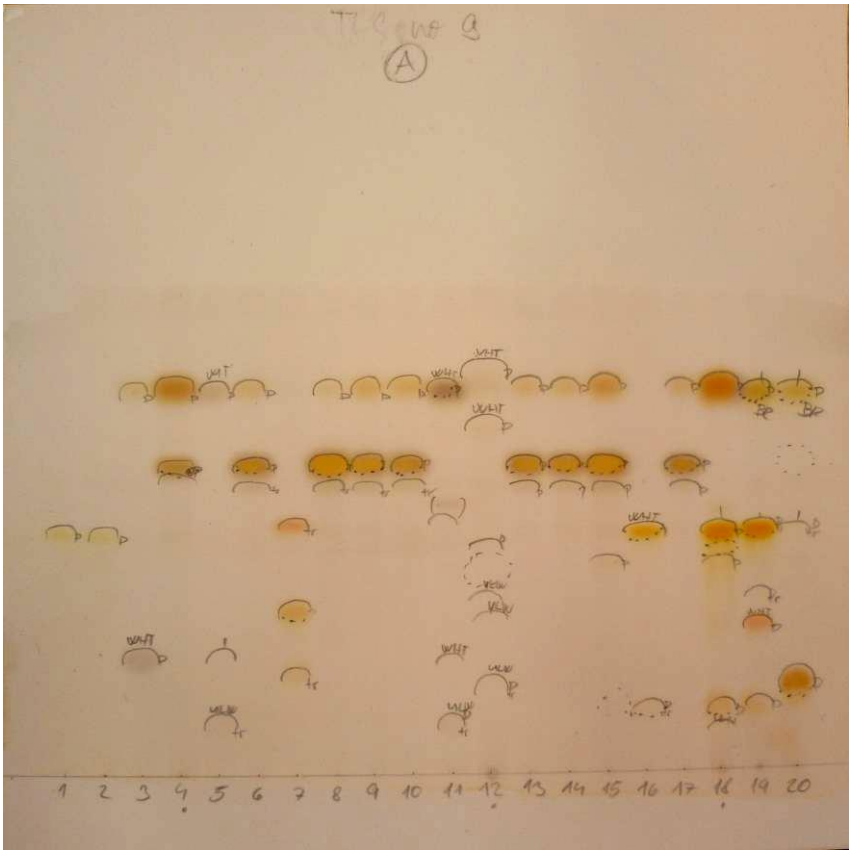


Figure 39: TLC no 9 in solvent B

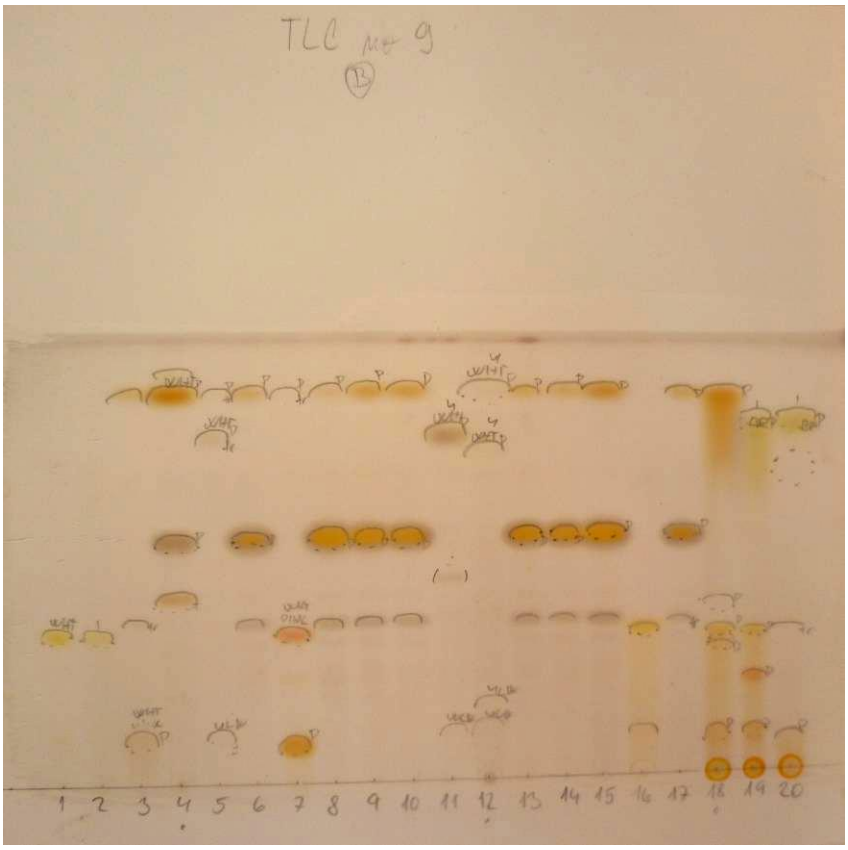


Figure 40: TLC no 9 in solvent C

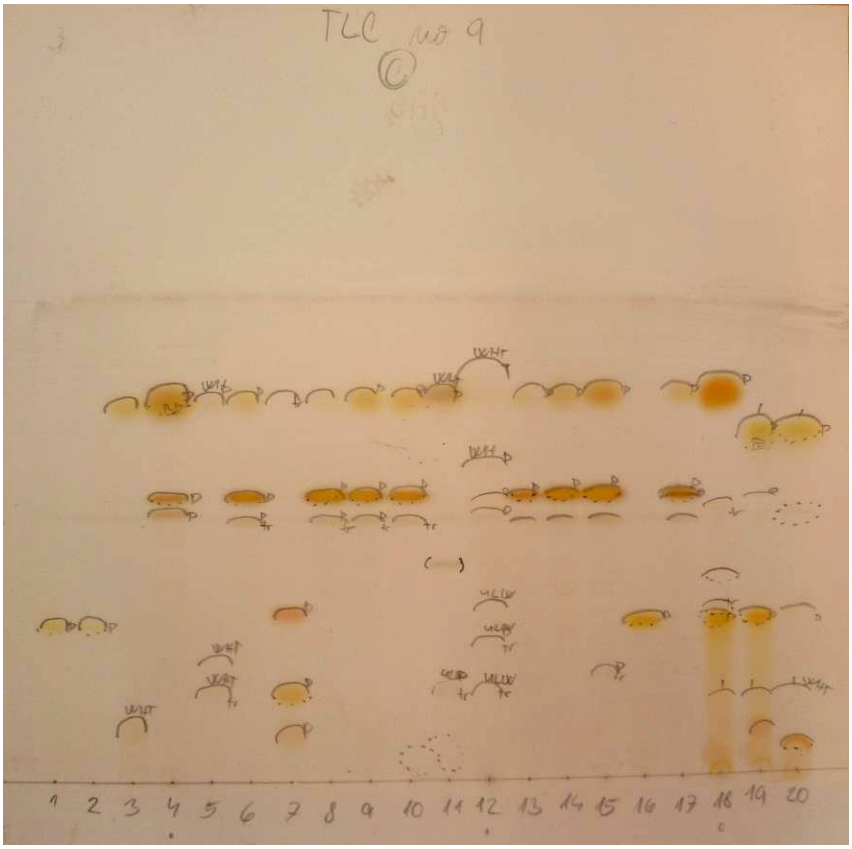


Table 22 TLC no 10

TLC no 10	
Nuber of analyzed samples:	16
Solvents:	A, B, C
Control substances:	rhizocarpic acid, confluentic a., norstictic a., variolaric a.
Analyzed genera:	*Pleopsidium* (*P. flavum*), *Lecidea* (*L. confluens*), *Buellia* (*B. dispersa*), *Xanthoparmelia* (*X. subplitti*), *Aspicilia*
Determined substances:	2'-O-methylperlatolic acid, 4-O-demethylplanaic a., acaranoic a., acarenoic a., fumarprotocetraric a., protocetraric a., protocetraric a., rhizocarpic a., schizopeltic a., substictic a., usnic a.,
Notes:	unidentified substances, unknown substance above 2'-O-methylperlatolic acid

Table 23 the TLC protocol no 10

THIN-LAYER CHROMATOGRAPHY			PLATE NO 10	
Plate: aluminium		Spray: water, sulphuric acid		
Solvent: A B C		Operator(s): M. Kukwa, M. Michalová		Date: 19. 10. 2011
Subject: Chemistry of Lichens in South-Western Mojave Desert				
specimen	substances detected			
1 13373	rhizocapric acid	acaranoic acid	acarenoic acid	
2 12921	rhizocapric acid	acaranoic acid	acarenoic acid	
TEST	RHIZOCARPIC ACID			
4 368	rhizocarpic acid	acaranoic acid		
5 2320	rhizocarpic acid	acaranoic acid	acarenoic acid	
6 13187	2'-O-methylperlatolic acid	unknown substance above		
TEST	CONFLUENTIC ACID			
8 13691	no substances			
9 13691	no substances			
10 13689	4-O-demethylplanaic acid (no control available)			
11 13683	schizopeltic acid	unidentified substances in traces		
TEST	NORSTICTIC ACID			
13 13685	schizopeltic acid	unidentified substances in traces		
14 13692.1	2'-O-methylperlatolic acid	unknown substance above (trace)		
15 13759	schizopeltic acid	unidentified substances in traces		
16 5756	variolaric acid	some additional substances in traces		
TEST	VARIOLARIC ACID			
18 13703	usnic acid	fumarprotocetraric acid	protocetraric acid	
19 13684	no substances			
20 13476	substictic acid (no control available)			

Figure 41 TLC no 10 in solvent A

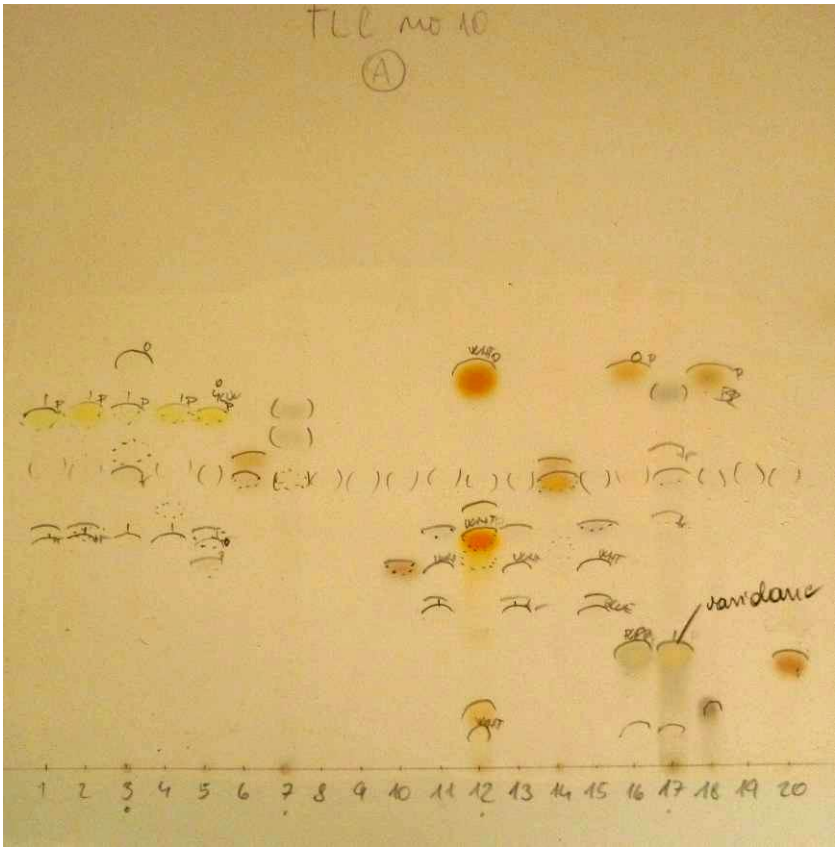


Figure 42 TLC no 10 in solvent B

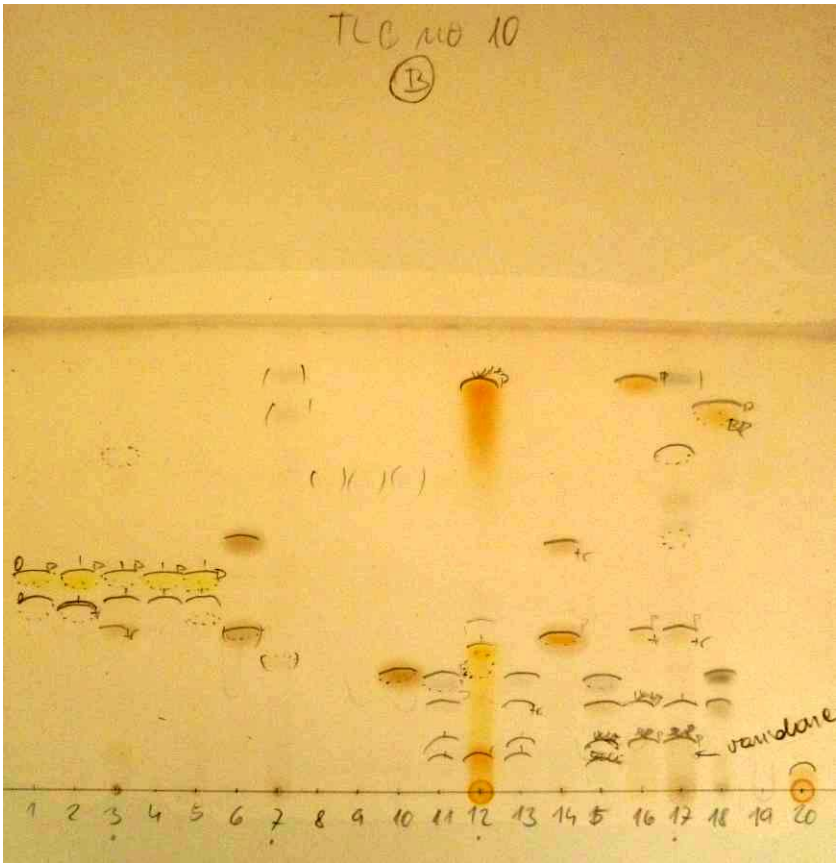


Figure 43 TLC no 10 in solvent C

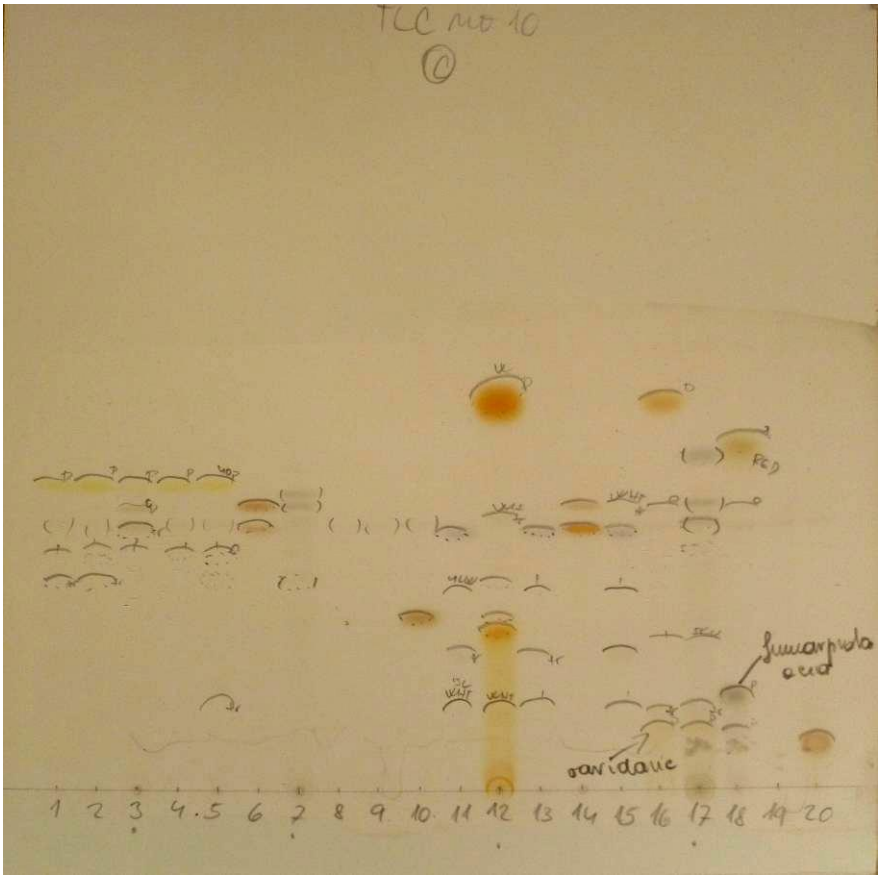


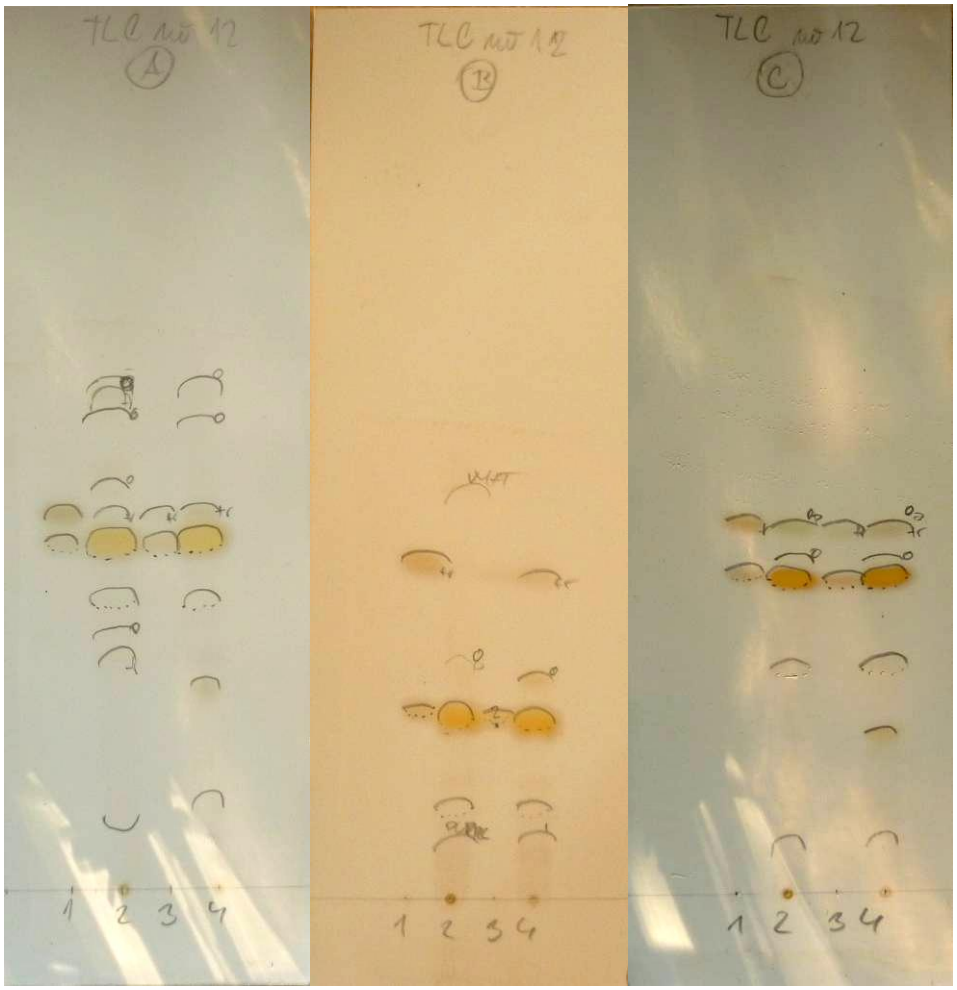
Table 24 TLC no 12

TLC no 12	
Nuber of analyzed samples:	2
Solvents:	A, B, C
Control substances:	confluentic acid, 2'-O-methylmicrophyllinic a.
Analyzed genera:	*Lecidea* (*L. confluens*)
Determined substances:	2'-O-methylperlatolic acid
Notes:	unknown substance above

Table 25 the TLC protocol no 12

THIN-LAYER CHROMATOGRAPHY			PLATE NO 12		
Plate:	aluminium	Spray:	water, sulphuric acid		
Solvent:	A B C	Operator(s): M. Kukwa, M. Michalová		Date: 25. 10. 2011	
Subject:	Chemistry of Lichens in South-Western Mojave Desert				
specimen	substances detected				
1 13187	2'-O-methylperlatolic acid	unknown substance above			
TEST	CONFLUENTIC ACID	2'-O-METHYLMICROPHYLLINIC ACID			
3 13692.1	2'-O-methylperlatolic acid	unknown substance above (trace)			
TEST	CONFLUENTIC ACID	2'-O-METHYLMICROPHYLLINIC ACID			

Figure 44 TLC no 12 in solvent A, B, C



The TLC number 11 is absent; because it was not applied in proper way and therefore results were not clear. In that case the analysis was repeated in the same form by the TLC number 12, which run successfully and results are visible and significant.

7.1.1 THE CHECKLIST OF DETERMINED SUBSTANCES

The checklist summarizes all 40 concretely identified substances found in analyzed specimens from Joshua Tree National Park.

2'-O-methylperlatolic acid
4-O-demethylplanaic acid
4-O-pannaric acid 6-methyl ester
5-O-methylhiascic acid
acaranoic acid
acarenoic acid
atranorin
bourgeanic acid
connorstictic acid
conpsoromic acid
consalazinic acid
cryptostictic acid
epanorin
fumarprotocetraric acid
gyrophoric acid
hyposalazinic acid
hypostictic acid
isousnic acid
lecanoric acid
menegaziaic acid
miriquidic acid
mixture of lecanoric and gyrophoric acids
norstictic acid
pannaric acid 6-methyl ester
pannarin
placodiolic acid
protocetraric acid
pseudoplacodiolic acid
psoromic acid
rhizocarpic acid
salazinic acid
schizopeltic acid
sphaerophorin
stictic acid
stictic acid complex
stictic acid complex (stictic acid as major substance)
substictic acid
umbilicaric acid
usnic acid
variolaric acid
zeorin

7.1.2 THE CHECKLIST OF UNDETERMINED SUBSTANCES

Following substances couldn't be determined because their chemical structure has not been described yet. TLC analysis showed these unknown types of substances in following categories:

additional substance(s)
fatty acid (probably aspicilin)
fatty acid(s)
pigment(s)
terpenoid(s)
two unidentified substances related with pannarin

uknown substance; **Rf cl.: A4,B5,C4**

unidentified substance(s)

unknown substance above 2'-*O*-methylperlatolic acid

unknown substance related to norstictic acid; Rf cl.: A2-3, B3, C2

unknown substance with blue colour in before 366nm UV before sulphuric acid

7.2 LICHEN DATABASE APPLICATION

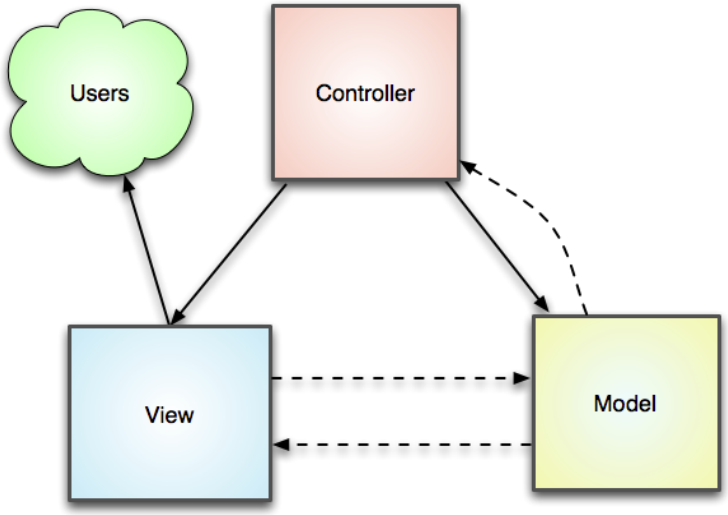
A database application was developed as a part of the thesis project. The aim of the database is to provide a platform to store the information of the analyzed samples which can be accessed online and shared among other people interested in lichen analysis.

The application was developed in PHP language and is based on the Zend Framework. This framework provides an advanced Model-View-Controller (MVC) implementation which makes the development more efficient. As a result of using the Zend Framework the application can be enriched of new functions such as user accounts etc. with only minor changes to the currently implemented code.

7.2.1 MODEL-VIEW-CONTROLLER STRUCTURE

This concept splits the application code into three parts: presentation, business logic and data access. Each part covers different functionality with different concerns and keeps this functionality consolidated and separated from the other part of the application code. This concept allows developers to keep the code structured and well organized. Moreover this separation allows developers to make significant changes in one part of the application without making any changes in the rest of the application.

Figure 45: MVC concept structure (ZF 2012)



Model - This part of the application defines the basic functionality using a set of abstractions. Data access routines and parts of business logic are usually defined in the model (ZF 2012).

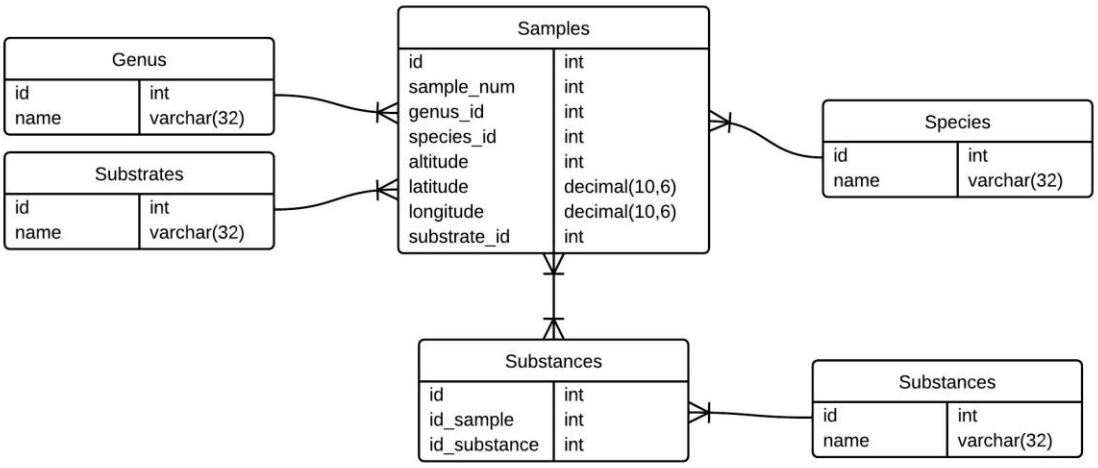
View - Views define exactly what is presented to the user. Usually controllers pass data to each view to render in some format. Views often collect data from the user and pass them to controller (ZF 2012).

Controller - Controllers bind the whole pattern together. They manipulate models and decide which view to display based on the user's request. Controllers pass along the data that each view will need or hand off control to another controller entirely (ZF 2012).

7.2.2 APPLICATION DATABASE SCHEME

The database scheme for this application is simple, consisting of six tables. The scheme is shown in the figure bellow. The database is easily expandable, if more information is required to be stored in the database.

Figure 46: Scheme of the application‘s database



8 DISCUSSION

8.1 TLC ANALYSIS AND SECONDARY METABOLITES

The results of the TLC analysis show explicit variability of lichen secondary metabolites presented in samples found in Joshua Tree National Park. It is possible to say that most of lichen substances were identified concretely. In case of substances with unknown structure, they were described as concretely as possible (e.g.:"unknown substance with blue color in before 366nm UV before sulphuric acid"). The description of unknown or unidentified substances was used for later discussions or consultations with other specialist on lichen chemistry.

The correct identification of secondary metabolites on TLC plates depends on experience of a chemist who analyzes samples. There is always a probability that some of the substances, which were not determined concretely, were not identified, or were described as unknown, could be determined more clearly by another chemist with different experience. For any cases in the TLC schedules following rule is applied: if there is a substance described as for example "terpenoid", it is necessary to check its position and other substances presented in the analyzed specimen.

Some of analyzed specimens chosen for TLC in Gdansk laboratory were analyzed for the second time. The first TLC run was performed in New York Botanical Garden (NYBG). The reason for comparison of these two sets of results was the necessity of verification and confirmation of gathered data. Most of the results were identical. Only the different result was noted in TLC no 12. Data from NYBG showed confluentic acid as one of determined substances in specimens numbers 13187 and 13692.1 predetermined by Kerry Knudsen as *Lecidea*. However, plates developed in Gdansk did not show the substances with same characteristics as the confluentic acid usually has. The spots of substances on the developed plates were not visible under UV after sulphuric acid application. And this is one of the most significant characteristics for confluentic acid. The found substances were determined as "unknown substance above 2'-*O*-methylperlatolic acid".

Pictures of developed plates were taken for subsequent verification of gained results. They were useful for verification and confirmation purposes and as a material for further discussions. Any differences within unknown or unidentified spots were noted. In this analysis they were split into following main categories: the unknown substance, unidentified, or related with some substances.

8.2 INTERACTIVE DATABASE

The internet application should help to organize results from TLC and information about lichen specimens. It was designed to make an interactive online platform, which helps to search through the records by given criteria, and will be useful for further research including lichen chemistry and consequential environmental analyses.

The stored data could be useful for the future utilization for ecological statistical analyses and GIS, giving the possibilities to making different lists of species or lists of lichen compounds. Some databases with information about lichens already exist, but these databases are understood as general biological databases with general information. This application can gather data usable for other analyses.

Big advantage of the application in connection with ecological statistical analysis is compatibility with a table prepared for program CANOCO used for multivariate analysis of ecological data. Although a table prepared for CANOCO in Excel application needs to undergo some minor conversions, it is possible to use the table as a data source for the internet application designed within the thesis. Installation guidelines are as a part of the APPENDIX 2.

8.3 ANNOTATED CHECKLIST

The checklist summarizing the results of TLC was generated from the application. The checklist represents one of goals of the thesis. It shows number of samples obtaining specific lichen compounds. The checklist is represented in the APPENDIX 1.

8.4 STATISTICAL ANALYSIS

The statistical analysis should answer the question about response of the lichen compounds to the environmental factors. These questions can be answered by proper explanation of the presence of lichen compounds in individual lichen species in specific parts of the park. Within gathered information about specimens it would be possible to do a multivariate analysis of ecological data by the application CANOCO (version 4.5 for Windows) and make resulting visualization using the application CanoDraw.

Multivariate analysis is perfect method how to see the relationship between lichens and their type of habitats, environmental conditions, and secondary metabolites. The main goal of statistical data processing like this is to investigate variation of lichen communities across a range of variable environmental conditions.

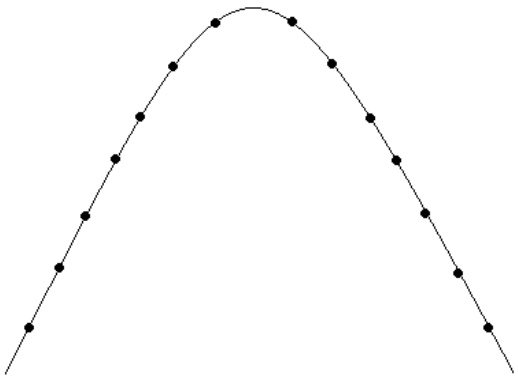
Large differences in compositions of species of studied communities could be predicted by variations of environmental factors (Lepš et Šmilauer 2003). Variables are correlated and the structure is the object of investigation. It makes the different view than the only one-dimensional case. Changes in the studied community compositions can be often related to differing, but partially overlapping, demands of the individual lichen species for environmental factors such as the altitude, the exposure, average value of air moisture or temperature, precipitation, or the nitrate depositions and other factors (Lepš et Šmilauer 2003). All these factors can affect the chemical substances contained in studied lichen species.

There are many reasons to consider hypotheses mentioned above. However during the work on the thesis many requirements were put on input data. One of them was a sufficient number of tested samples of one species. According to received data for TLC analysis it was impossible to apply the multivariate methods as statistical analysis within the actual thesis. Mostly there are represented as species with only few exponents. Only two species, *Acarospora socialis* and *Buellia dispersa* have been presented in relatively representative amount - ten or more samples. For the predicative statistic analysis is necessary to have at least 10 species with representative amount of samples. However, it is not the reason to exclude the statistical analysis definitively. Hypotheses and following responses have been still posed. The second problem with data was that many samples have only the genera name so they were not that suitable for application the multivariate statistical analysis missing one attribute. These two facts do not allow creating the analysis based on the relationship between samples (species) and their environment and chemistry. So results of statistical analysis would not be significant.

If there is a project to make an ecological analysis through enumeration lichens as type of biological indicators it is necessary to optimize the method of collecting data. It means to collect suitable information which will play the responsible role for the required results. Such information should consist of data regarding the site where the sample was picked up. It should be primarily coordinates (useful for GIS analyses), altitude/longitude, and type of substrate, and also exposure.

For the hypothesis resulting from this thesis the most important feature is the altitude. The correlation between environmental factors and lichen compounds could be in the first step expressed by the relationship of known altitude and known compounds connected with the concrete altitude. Collected species should be picked up equally. It means equally with the hill side. See Figure 47. This way of collecting data with regards to the altitude should provide reliable results on distribution of lichen compounds in the altitude context.

Figure 47: Illustration - distribution of collected data on a model hill (points represent collected samples)



TLC results are important feature for multivariate analysis too. However, it is necessary to make strict and unified records about identified substances. Special attention should be paid to amount of substances. For working with the CANOCO acquired data must be adjusted to a suitable form. TLC analysis cannot determine the exact amount of substances, but there is possibility to determine the range of amount of substances. The range is from nil (the quantity is null) through the level number 1 (trace amount) to the level 2 (standard amount). Such a scale is applied for input data table for the CANOCO.

Input data like these are should be appropriate for following statistic analysis. Respectively, results made of dataset collected by the way described above should show some significant trends within tested samples, environmental variables, and covariates. It is very likely that chemistry of lichens is not more or less random, but it is supposed to be influenced by some unidentified factors. The aim of this analysis would be to detect which factors play the main role in composition of lichen communities and their lichen compounds.

CONCLUSION

This work has been a part of the project running since 2005. The inventory of lichens in Joshua Tree National Park is the historically first one which was made in such extension for this locality. The estimate of the percentage of the known area by lichen diversity is the proof. Nowadays, the collection for preparing the new checklist for Joshua Tree National Park consists of about 2000 samples. 139 samples, which were analyzed by TLC, represent relatively high number of chemo-taxonomically analyzed specimens for the inventory. This was definitely very valuable contribution to the project.

It clearly indicates the purpose of TLC analysis. The TLC analysis for the project was used as a guideline for determination of selected specimens. Selected specimens were chosen according to the proposal of Mr. Kerry Knudsen. Material for TLC analysis in Gdansk had not been known till the real start of the analyzing process. In many cases the analyzed specimens were not determined completely, because there were some ambiguities for clear determination. Thus, the determination of lichen compounds was the way, how to bring explicit data for serious species determination. In some cases the specimens were chosen for confirmation of the species determination. And the third group of chosen specimens was analyzed to compare specimens analyzed in New York Botanical Garden with results from Gdansk. The results were in 99% identical.

55 types of substances were detected during the TLC analyses in Gdansk. 40 substances from this set are lichen compounds with known structure. Some substances could not been identified for various reasons mentioned in this paper. For example, the structure for some of these unidentified compounds is currently unknown, or the suitable literature sources were not available to use for the determination. Determination of lichen compounds depends on chemo-lichenologist's experience and knowledge. Therefore, all results were consulted with a specialist from University of Gdansk. Dr. Martin Kukwa. He verified the results and confirmed all of the developed plates and proved correctness of the results.

On the basis of gathered results and information about individual specimens (secondary metabolites, coordinates, elevation, coordinates, type of substrate) following proposal was made for similar research in the future. Statistical analysis based on gained results is a part of the future plans for the similar research. General information about specimens and their chemistry are relevant information for ecological statistic analysis. One of the main objectives of the research could be an attempt to answer the question about lichen chemistry. Available characteristics would

help to find the key for the relationship between lichen compounds, their presence or absence, and environmental conditions.

Basically, the idea of applying multivariate analysis on data gained by TLC analysis of collected lichens corresponds with the fact that lichens are indicators of environmental conditions. Lichens and their species composition could answer the questions about air pollution. Another question is: “Can the lichen chemistry affect the species composition? Is the chemistry influenced by environmental factors?” Among these speculations the spatial analysis performed by GIS are not omitted. The database was designed with presumption to be used as a basic data source for GIS spatial analysis.

Finally the goal of the thesis was not only to determine secondary metabolites and to design a database for gathering gained data. The goal of the thesis should show the future direction for lichen diversity and secondary metabolites research. The species determination and inventory represent only one piece of the real potential of these unique organisms.

Lichens are great bioindicators, they are sensitive organisms to air pollution and climate changes. But they will say more about the environmental situation and changes in ecosystems of which they are part.

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LIST OF FIGURES

APPENDIX 1

ANNOTED CHECKLIST OF IDENTIFIED SUBSTANCES

The list includes identified substances or categories of unidentified, unknown, or undetermined substances, and assigned specimens that obtain these substances. Substances are divided by types of compounds structure referred in *Identification of Lichen Substances* [Huneck et Yoshimura 1996]. The checklist corresponds with data from the interactive database. Species and genus names were determined by Kerry Knudsen. Used determination was used for TLC analysis in fall of 2011.

IDENTIFIED SUBSTANCES

ALIPHATIC AND CYCLOALIPHATIC COMPOUNDS

acaranoic acid

13373 *Pleopsidium flavum*

12921 *Pleopsidium flavum*

368 SPECIMEN FROM NY

232 SPECIMEN FROM NY

acarenoic acid

13373 *Pleopsidium flavum*

12921 *Pleopsidium flavum*

232 SPECIMEN FROM NY

bourgeanic acid

13402 *Xanthoparmelia*

13732 *Lecanora argopholis*

13748 *Xanthoparmelia mexicana*

DEPSIDES

2'-*O*-methylperlatolic acid

13187 *Lecidea confluens*

13480.2 *Lecidea confluens*

13482.1 *Lecidea confluens*

13692.1 *Lecidea*

4-*O*-demethylplanaic acid (10)

13241 *Lecidea laboriosa*

13474 *Buellia dispersa*

13472.2 *Buellia dispersa*

12804 *Buellia dispersa*

2620 *Buellia dispersa*

12791 *Buellia dispersa*

13063 *Buellia dispersa*

13492 *Buellia dispersa*

12797 *Buellia dispersa*

13689 *Lecidea*

5-*O*-methylhiascic acid (3)

13084 *Xanthoparmelia verruculifera*

13400 *Xanthoparmelia verruculifera*

13081 *Xanthoparmelia verruculifera*

atranorin (13)

13362.1 *Physconia phallax*

12968 *Physconia phallax*

13220 *Physcia dimidiata*

13219 *Physcia dimidiata*

12846 *Physcia biziana*

13051 *Physcia biziana*

12871 *Xanthoparmelia subramigera*

13732 *Lecanora argopholis*

12943 *Buellia*

2596.2 *Buellia spuria*

13346 *Buellia spuria*

13097.1 *Buellia dispersa*

confluentic acid (8)

13474 *Buellia dispersa*

13472.2 *Buellia dispersa*

12804 *Buellia dispersa*

2620 *Buellia dispersa*

12791 *Buellia dispersa*

13492 *Buellia dispersa*

12797 *Buellia dispersa*

13063 *Buellia Dispersa*

gyrophoric acid (15)

13280 *Acarospora obpallens*

13480 *Acarospora obpallens*

13056 *Acarospora rosulata*

13238.2 *Acarospora rosulata*

12920.2 *Acarospora rosulata*

12918 *Acarospora rosulata*

13493 NO DATA

12876 *Acarospora succendens*

13084 *Xanthoparmelia verruculifera*

13400 *Xanthoparmelia verruculifera*

13081 *Xanthoparmelia verruculifera*

12966 *Umbilicaria phaea*

13530 *Umbilicaria phaea*

13389 *Umbilicaria phaea*

13398.1 *Lecidea manii*

lecanoric acid (15)

13280 *Acarospora obpallens*

13480 *Acarospora obpallens*

13056 *Acarospora rosulata*

13238.2 *Acarospora rosulata*

12920.2 *Acarospora rosulata*

12918 *Acarospora rosulata*

13493 *NO DATA*

12876 *Acarospora succendens*

13084 *Xanthoparmelia verruculifera*

13400 *Xanthoparmelia verruculifera*

13081 *Xanthoparmelia verruculifera*

12966 *Umbilicaria phaea*

13530 *Umbilicaria phaea*

13389 *Umbilicaria phaea*

13398.1 *Lecidea manii*

miriquidic acid (3)

13355 *Miriquidica scotopholis*

13392 *Miriquidica scotopholis*

13062 *Miriquidica scotopholis*

mixture of lecanoric and gyrophoric acids (3)

12969 *Aspicilia*

sphaerophorin (2)

13049 *Dimelaena thysanota*

13354 *Dimelaena thysanota*

umbilicaric acid (3)

12966 *Umbilicaria phaea*

13530 *Umbilicaria phaea*

13389 *Umbilicaria phaea*

DEPSIDONES

connorstictic acid (14)

12969 *Aspicilia*

12962.2 *Lobothalia praeradiosa*

13172 *Xanthoparmelia*

12632 *Xanthoparmelia*

12770 *Xanthoparmelia*

13406 *Buellia abstracta*

13167 *Buellia abstracta*

13713 *Lobothalia praeradiosa*

12943 *Buellia*

12943 *Buellia*

2596.2 *Buellia spuria*

13346 *Buellia spuria*

5259 *Buellia dispersa*

13762 *Xanthoparmelia mexicana*

conpsoromic acid (1)

3577 Nicole Pietrasiak s. n. *Rhizoplaca subdiscrepans*

consalazinic acid (2)

12790.1 *Lecanora*

13748 *Xanthoparmelia mexicana*

cryptostictic acid (1)

13097.1 *Buellia dispersa*

fumarprotocetraric acid (3)

12871 *Xanthoparmelia*

13080 *Xanthoparmelia*

13703 *Xanthoparmelia subplitti*

hyposalazinic acid (1)

13762 *Xanthoparmelia mexicana*

hypostictic acid (5)

12993.1 *Buellia nashii*

12943 *Buellia*

2596.2 *Buellia spuria*

13346 *Buellia spuria*

13097.1 *Buellia dispersa*

menegaziaic acid (3)

12783 *Dimelaena oreina*

12913 *Dimelaena oreina*

13717 *Dimelaena oreina*

norstictic acid (26)

12969 *Aspicilia*

12962.2 *Lobothalia praeradiosa*

13489 *Rhizocarpon geminatum*

12881 *Xanthoparmelia*

12848 *Xanthoparmelia*

13095 *Xanthoparmelia*

12829 *Xanthoparmelia*

13360 *Xanthoparmelia*

12632 *Xanthoparmelia*

12871 *Xanthoparmelia*

12871 *Xanthoparmelia subramigera*

12770 *Xanthoparmelia*

12783 *Dimelaena oreina*

12913 *Dimelaena oreina*

13406 *Buellia abstracta*

13167 *Buellia abstracta*

13713 *Lobothalia praeradiosa*

12943 *Buellia*

2596.2 *Buellia spuria*

13346 *Buellia spuria*

13717 *Dimelaena oreina*

3628 *Buellia dispersa*

5193 *Buellia dispersa*

5259 *Buellia dispersa*

13762 *Xanthoparmelia mexicana*

13748 *Xanthoparmelia mexicana*

pannarin (2)

13709 *Lepraria*

13111.1 *Buellia dispersa*

protocetraric acid (6)

13095 *Xanthoparmelia*

12829 *Xanthoparmelia*

12871 *Xanthoparmelia*

12871 *Xanthoparmelia subramigera*

13080 *Xanthoparmelia*

13703 *Xanthoparmelia subplitti*

psoromic acid (1)

3577 Nicole Pietrasiak s. n. *Rhizoplaca subdiscrepans*

salazinic acid (12)

12790.1 *Lecanora*

13083 *Rhizocarpon disporum*

12881 *Xanthoparmelia*

13095 *Xanthoparmelia*

12829 *Xanthoparmelia*

13402 *Xanthoparmelia*

13709 *Xanthoparmelia*

12770 *Xanthoparmelia*

12733 *Xanthoparmelia*

13491 *Xanthoparmelia*

12871 *Xanthoparmelia subramigera*

13748 *Xanthoparmelia mexicana*

stictic acid (6)

2615 *Rhizocarpon geminatum*

12963 *Rhizocarpon disporum*

13083 *Rhizocarpon disporum*

12848 *Xanthoparmelia*

13360 *Xanthoparmelia*

13097.1 *Buellia dispersa*

stictic acid komplex (6)

12993.1 *Buellia nashii*

12943 *Buellia*

12943 *Buellia*

2596.2 *Buellia spuria*

13346 *Buellia spuria*

13717 *Dimelaena oreina*

stictic acid complex (stictic acid as major substance) (3)

13489 *Rhizocarpon geminatum*

12783 *Dimelaena oreina*

12913 *Dimelaena oreina*

substictic acid (2)

13476 *Aspicilia*

12963 *Rhizocarpon disporum*

variolaric acid (2)

12951 *Lecanora muralis*

5756 *Buellia dispersa*

DIBENZOFURANCES

4-oxypannaric acid 6-methyl ester (1)

13477 *Lepraria*

isousnic acid (3)

13340 *Lecanora*

12974 *Lecanora saligna*

13073 *Lecanora saligna*

pannaric acid 6-methyl ester (1)

13477 *Lepraria*

placodiolic acid (5)

3577 *Rhizoplaca subdiscrepans*

12685 *Lecanora*

12790.1 *Lecanora*

12851 *Lecanora muralis*

13236.1 *Lecanora sierrae*

pseudoplacodiolic acid (3)

13137 *Rhizocarpon chrysoleuca*

12910 *Rhizocarpon chrysoleuca*

13719 *Lecanora garovaglii*

schizopeltic acid (8)

12709 *Lecidea hassei*

12926 *Lecidea hassei*

12760 *Lecidea hassei*

12665 *Lecidea laboriosa*

13398.1 *Lecidea manii*

13683 *Lecidea*

13685 *Lecidea*

13759 *Lecidea*

usnic acid (27)

12845 *Rhizocarpon chrysoleuca*

12787 *Rhizocarpon chrysoleuca*

12910 *Rhizocarpon chrysoleuca*

3577 Nicole Pietrasiak s. n. *Rhizoplaca subdiscrepans*

12685 *Lecanora*

12790.1 *Lecanora*

12851 Lecanora muralis

12951 *Lecanora muralis*

13236.1 *Lecanora sierrae*

5246 *Lecanora laxa*

12881 *Xanthoparmelia*

12848 *Xanthoparmelia*

13402 *Xanthoparmelia*

13709 *Xanthoparmelia*

12632 *Xanthoparmelia*

12871 *Xanthoparmelia*

12770 *Xanthoparmelia*

12733 *Xanthoparmelia*

13491 *Xanthoparmelia*

12783 *Dimelaena oreina*

12913 *Dimelaena oreina*

12871 *Xanthoparmelia subramigera*

13717 *Dimelaena oreina*

13762 *Xanthoparmelia mexicana*

13748 *Xanthoparmelia mexicana*

13759 *Lecidea*

13703 *Xanthoparmelia subplitti*

PULVINIC ACIDS DERIVATIVES

epanorin (1)

13732 *Lecanora argopholis*

rhizocarpic acid (14)

13135 *Acarospora socialis*

12706 *Acarospora socialis*

13428 *Acarospora socialis*

12888 *Acarospora socialis*

13001 *Acarospora socialis*

13553 *Acarospora socialis*

12854 *Acarospora socialis*

12907.1 *Acarospora socialis*

12758 *Acarospora socialis*

13537 *Acarospora socialis*

13373 *Pleopsidium flavum*

12921 *Pleopsidium flavum*

368 *SPECIMEN FROM NY*

232 *SPECIMEN FROM NY*

TERPENOIDS

zeorin (9)

12625 *Lecidella patavina*

13035 *Lecidella stigmatea*

13319 *Lecidella stigmatea*

12685 *Lecanora*

12790.1 *Lecanora*

12851 *Lecanora muralis*

12951 *Lecanora muralis*

13732 *Lecanora argopholis*

13709 *Lepraria*

UNDETERMINED SUBSTANCE

fatty acid (prob. Aspicilin) (3)

13163 *Circinaria arida*

12898.1 *Circinaria arida*

3607 *Circinaria arida*

pigment (5)

12962.2 *Lobothalia praeradiosa*

13236.1 *Lecanora sierrae*

12963 *Rhizocarpon disporum*

13040.1 *Rhizocarpon disporum*

13123 *Lecidea*

pigments (4)

13491 *Xanthoparmelia*

13084 *Xanthoparmelia verruculifera*

13400 *Xanthoparmelia verruculifera*

13081 *Xanthoparmelia verruculifera*

fatty acid (10)

12685 *Lecanora*

12790.1 *Lecanora*

12851 *Lecanora muralis*

12951 *Lecanora muralis*

13236.1 *Lecanora sierrae*

12881 *Xanthoparmelia*

12848 *Xanthoparmelia*

13709 *Xanthoparmelia*

12733 *Xanthoparmelia*

13491 *Xanthoparmelia*

fatty acid in solvent B (1)

12829 *Xanthoparmelia*

terpenoid (1)

13742 *Lichenothelia*

additional terpenoids (1)

13732 *Lecanora argopholis*

terpenoid(s) (5)

12685 *Lecanora*

12790.1 *Lecanora*

12851 *Lecanora muralis*

12951 *Lecanora muralis*

13236.1 *Lecanora sierrae*

unidentified fatty acid (4)

12787 *Rhizocarpon chrysoleuca*

3577 Nicole Pietrasiak s. n. *Rhizoplaca subdiscrepans*

12790.1 *Lecanora*

13401.2 *Rhizocarpon disporum*

unidentified pigments (4)

13480 *Acarospora obpallens*

13238.2 *Acarospora rosulata*

12920.2 *Acarospora rosulata*

12918 *Acarospora rosulata*

two unidentified substances related with pannarin (1)

13111.1 *Buellia dispersa*

unidentified substance (2)

13172 *Xanthoparmelia*

12632 *Xanthoparmelia*

unidentified substances (11)

13280 *Acarospora obpallens*

13480 *Acarospora obpallens*

13238.2 *Acarospora rosulata*

12920.2 *Acarospora rosulata*

12918 *Acarospora rosulata*

3577 Nicole Pietrasiak s. n. *Rhizoplaca subdiscrepans*

13095 *Xanthoparmelia*

12829 *Xanthoparmelia*

13683 *Lecidea*

13685 *Lecidea*

13759 *Lecidea*

unknown substance (1)

12770 *Xanthoparmelia*

unknown substances (4)

13340 *Lecanora*

13073 *Lecanora saligna*

5232 *Rhizocarpon geminatum*

13719 *Lecanora garovaglii*

additional substances (4)

13049 *Dimelaena thysanota*

13354 *Dimelaena thysanota*

13709 *Lepraria*

5756 *Buellia dispersa*

unknown substance above 2'-O-methylperlatolic acid (4)

13187 *Lecidea confluens*

13480.2 *Lecidea confluens*

13482.1 *Lecidea confluens*

13692.1 *Lecidea*

unknown sunstance related to norstictic acid Rf cl. A2-3, B3, C2 (3)

13172 *Xanthoparmelia*

12632 *Xanthoparmelia*

12770 *Xanthoparmelia*

unknown substance, Rf classes: A4, B5, C4 (1)

13493 *NO DATA*

unknow substance with ice blue colour in before 366nm UV before sulphuric acid. Rf classes: A2-3, B2, C2 (2)

13362.1 *Physconia phallax*

12846 *Physcia biziana*

up to 5 unidentified substances (3)

13355 *Miriquidica scotopholis*

13392 *Miriquidica scotopholis*

13062 *Miriquidica scotopholis*

APPENDIX 2

INSTALLATION GUIDELINES

To publish the application online following steps are required. First of all you need to find an appropriate webhosting which runs MySQL, Apache server and ideally which supports Zend Framework. The Zend Framework support is not necessary, however if it is available it is not necessary to upload Zend libraries. If you have chosen the hosting server following issues have to be done:

Database creation

Create database on the hosting server using for example phpMyAdmin application. This application is free and is provided on the accompanied CD and allows you to manage the database and running the SQL scripts. Then you need to create the database tables. This can be done by running the script *createTables.sql* which can be found under *scripts* folder in the application main directory. Now the empty database is ready to use.

Set up the application

Open *application.ini* file under *application/configs* folder. Now you need to set the appropriate values to the following lines of code under `[production]` section:

`resources.db.params.host=`	Here specify database host address.
`resources.db.params.username=`	Username for the root access to the database.
`resources.db.params.password=`	Password for the root access to the database.
`resources.db.params.dbname=`	Database name.

Upload source files

Upload the whole application directory to the location you have designated on the hosting server. If the hosting server provides Zend Framework support you should not need to upload Zend libraries under *library* folder in the application main directory.

Now, everything should be done and you should be able to reach the application using the web browser on the appropriate URL.