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Comparative analysis of the dynamics and functions of rhizosphere soil microbial community in two ecosystems of the Chatkal Biosphere Reserve

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Content

1. Introduction

1.1. Chatkal Biosphere Reserve

Uzbekistan is globally and regionally important due to its location between the European, Middle Eastern, and Asian biogeographical regions. The Republic is located in the centre of the Eurasian continent and its varying landscapes of high mountain ranges, wide steppes, deserts and riparian wetlands has resulted in a diversity of habitats. In the east and southeast, the extensive Tien-Shan and Gissar-Alai mountain systems, which occupy 15 percent of the territory, flank the deserts. The nature and diverse flora of Western Tien Shan is one of the most interesting places in Central Asia. The basic vegetation pattern of mountain forests consists of trees and shrubs alternating with steppe and meadow areas or bare rocks. Mountain deciduous forests occupy small areas (altogether about 218,200 km^2), ranging between 800 and 2,800 m above sea level. Soils are brown and climate is continental.

Western Tien-Shan is regarded as one of the key eco-regions in the world representing a natural zone distinguished by its high level of plant and animal species concentration. The flora of this region is very diverse and characterized by a high level of local endemism. Almost all types of vegetation of Central Asian Mountains grow here. Chatkal Biosphere Reserve is protected territory in Western Tien Shan within Uzbekistan that plays a significant part in the conservation of its unique biological diversity, because they provide important habitats for endemic and rare plant species. Major ecosystem type mixed mountain and highland systems. Major habitats and land cover types Juniper forest with *Prunus sogdiana, Acer turkestanicum,* and *Malus kirghisorum*; and highland areas with herb and grass meadows. Vegetation cover of this region is represented with unique plant communities, and invaluable genetic pool of indigenous flora. The flora of Western Tien Shan significantly surpasses the other areas by the absolute number of endemics and the percentage of endemism. Over 2500 species of vascular plants presumably grow here (Cherepanov 1981). Over 40 species of rare and endemic plant species included into the Red Data Book of Uzbekistan (1988) are protected in Chatkal Nature Reserve, which constitutes 47% of endangered plants of Western Tien Shan. Some of the species are *Salsola titovii* Botsch., *Thesium minkwitzianum* B. Fedtsch., *Adonis leiosepala* Butk., *Allium baschkyzylsaicum* Krassovskaja, *Atsragalus abolinii* M.Pop., *Crocus alatavicus* Regel et Semen., *Dracocephalum komarovii* Lipsky, *Eremurus lactiflorus* O. Fedtsch., *Ferula juniperina* Korov., *Geranium*

baschkysylsaicum Nabiev, *Oxytropis fedtschenkoana* Vass., *Paeonia hybrida* Pall., *Prangos tschimganica* B. Fedtsch., *Restella albertii* (Regel) Pobed, *Salvia tianschanica* Machmedov, *Trollius altaicus* C.A.Mey., *Tulipa butkovii Z*. Botsch and etc. (Red Data Book of Republic of Uzbekistan (1998).

1.2 Herbal plants

The herbal plants from Tien-Shan mountains (including Chatkal Biosphere Reserve) was introduced into the West in the early part of the twelfth century by Avicenna. About 70% herbal plant species of Uzbekistan are growth here in protected Biosphere reserve. Knowledge of these medicinal plants is very important because not only is there the potential to discover new alternatives for the treatments of illnesses, but also from a conservation point of view. The traditional use of medicinal plants in Uzbekistan is widespread with over 80% of Uzbek households using medicinal plants for centuries.

If certain plant species are found to be under threat due to a high demand for plant medicines then measures can be implemented to try and ensure sustainability of the plant species. Some of the things make up our modern culture were codified by the Greeks: information not only about the therapeutic use of medicinal plants, but about the plants themselves and their propagation (Furnell., 1985). Central Asian medicine is not as widely known or understood as medical systems developed in neighboring countries (Mamedov et al., 2004). Plant-derived medicines have been part of traditional healthcare in most parts of the world for thousands of years and there is increasing interest in plants as sources of agents to fight microbial diseases (Chariandy et al., 1999). Uzbekistan has an excellent historic research base of herbal medicine. "The Book of Healing" is a whole epoch in the history and classic consolidated work written by Avicenna (born near Bukhara, in Uzbekistan (980–1037) and that has created him a world reputation and he is considered by many to be "the father of modern medicine'. Few sources of information in the literature are available about the medicinal plants of Uzbekistan especially from nature reserve (Sezik et al., 2004; Mamedov et al., 2004). As traditional remedies in Uzbekistan have never been deeply examined scientifically, the compounds in these remedies are very likely may contain new medicinal compounds (Kogure et al., 2004). Shuikishima et al. (2001; 2002) reported that compounds isolated from various plants collected in Uzbekistan showed anti-HIV activity in vitro and preventive effects on the generation

and release of inflammation agents such as TNF-a and IL-2 in vitro. Kogure et al., (2004) found various new antioxidants, some of which had a unique mechanism of action, in Ferula, Inula, Prangos and Rheum plants collected in Uzbekistan as seeds used in medicine. The flora of Central Asia includes more than 6500 species of higher plants and over 600 of these plants are used in traditional or conventional medicine (Shreter 1986; Mamedov 2004). Most of them growth in Chatkal Biosphere Reserve and there is also a specific group of relic endemic plants, which are nearly to disappear from the face of the earth (*Ostrrowskkia magnifica, Callispepla aegacanthoides; Otostegia bucharica, Spirostegia bucharica* and etc.).

Those plants contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties. From ancestral traditions to the most advanced research, natural products have always fascinated scientists in microbiology. The research on natural products may offer substantial advantages, like discovery of new drugs with a new mode of action, high selectivity and activity. It is clear that from all microorganisms the procaryotes whose secondary metabolism shows the highest flexibility, are the most suitable.

1.3 Soil as Microhabitat

Soil contains more genera and species of micro organisms than other microbial habitats, as soil is exposed to and eventually receives essentially all microbes present on Earth. Microbes, perhaps more than any other organisms, are highly adaptable, both physiologically and genetically, to varying conditions (Stozky, 1997).

These are the conclusions from the Darwinian dictum that the 'fittest survive', which leads to organisms specifically adapted to their particular habitat, and from the Gaussian principle of competitive exclusion', which leads to the elimination of species, thus reducing the complexity of ecosystems. In general, each long term, continuous culture would have its resident organism well adapted to handling the particular problems of that environment. (Koch, 1985)

Soil is fundamental and irreplaceable; it governs plant productivity of terrestrial ecosystems and it maintains biogeochemical cycles because microorganisms in the soil degrade, sooner or later, virtually all organic compounds including persistent xenobiotics and naturally occurring polyphenolic compounds. The living population inhabiting soil includes macro fauna, mesofauna, micro fauna

and microflora (Nannipieri et al., 2003). Even if the available space is extensive in soil, the biological space, that is, the space occupied by living microorganisms, represents a small proportion, generally less than 5% of the overall available space (Ingham et al., 1985). Another peculiarity is the presence of 'hot spots', zones of increased biological activity, such as aggregates with different physicochemical properties from the bulk of the soil (Sexstone et al., 1985), zones with accumulated particulate organic matter (Parkin, 1987) or animal manures (Petersen et al., 1996), and the rhizosphere (Lynch, 1990; Pinton et al., 2001; Nannipieri et al., 2003) . Variation in space is particularly important in soil because soil is a heterogeneous matrix composed of many different microhabitats. Soil is usually aggregated, and the centre and exterior of the aggregates differ greatly in availability of oxygen, moisture, and other factors. A different microorganism is commonly found in the soil including bacteria, fungi, actinomycetes, protozoa, and algae. Of these bacteria is by far the most common type of soil bacteria, possibly because they can growth rapidly and have a great influence on many organic matter oxidation, hydrolysis and degradation, and these in turn reflected in the natural cycles of carbon, nitrogen, phosphorus and other elements (Glick, 1995).

1.3.2. Soil enzyme activities

Soil enzyme activities are involved in soil nutrient cycling dynamics and can catalyze the conversion of nutrient from unavailable to forms readily assimilable by plants and microorganisms. Soil enzyme activities are believed to be able to discriminate between soil management treatments (Dick, 1993) probably because they are related to microbial biomass, which is sensitive to such treatments. The enzyme activity was stimulated by increasing organic matter content (Pathak and Rao (1998).

Enzyme activity is generally higher in rhizosphere than in bulk soil, as a result of a greater microbial activity sustained by exudates, actively or passively released by roots, or due to the release of enzymes from plant roots (Pinton et al., 2001). Among soil enzyme activities, hydrolase activities of rhizosphere are supposed to play an important role in plant nutrition. For example, the hydrolysis of organic phosphate monoesters by phosphomonoesterases can account for 30-80% of P taken up by plants in agricultural soils (Tarafdar and Jungk, 1987; Gilbert at al., 1999).

Enzymes are potential indicators of the extent to which soil disturbance by a given activity may affect the immediate environment (Pascual et al., 2000).

Dehydrogenase activity has correlated well with other measures of microbial activity in some studies but has been poorly correlated in others. Enzymes are potential indicators of the extent to which soil disturbance by a given activity may affect the immediate environment (Pascual et al., 2000). Hydrolase activities in the soil can be quantitatively important for plant nutrition; for example the hydrolysis of organic phosphate monoesters by phosphomonoesterases can account for 30-80% of P taken up by plants in agricultural soils (Tarafdar and Jungk, 1987; Gilbert at al., 1999).

Measurement of soil hydrolases provides an early indication of changes in soil fertility, since they are related to the mineralization of such important nutrients elements as N, P, C (Ceccanti and Garcia, 1994). Protease activity is involved in the hydrolysis of N compounds to NH_4, using low-molecular-weight protein substrates and urease is responsible for breaking down urea into ammonium. Phosphotases catalyse the hydrolysis of organic phosphorus compounds to phosphates and b-glucosidase catalyses the hydrolysis of the ends unreduced chains of b-D-glucoside to form b-D-glucose, and indicates the potential for soil organic matter decomposition.

1.3.3. Soil microbial activities

Microbial activity in soil is controlled by several environmental factors, such as availability of C, mineral nutrients and growth factors, availability water, favourable temperature and pH, composition of soil microflora and ecological interactions between microorganisms (Nannipieri et al., 2003). Microorganisms play an important role in soil fertility because they oxidise organic matter and promote the biogeochemical cycles of C, N, P, and S (Balloni and Favilli, 1987). Soil enzyme activities are involved in soil nutrient cycling dynamics and can catalyze the conversion of nutrient from unavailable to forms readily assimilable by plants and microorganisms. Soil enzyme activities are believed to be able to discriminate between soil management treatments (Dick, 1993) probably because they are related to microbial biomass, which is sensitive to such treatments. Soil enzyme activities are involved in soil nutrient cycling dynamics and can catalyze the conversion of nutrient from unavailable to forms readily assimilable by plants and microorganisms. Enzyme activity is generally higher in rhizosphere than in bulk soil, as a result of a greater microbial activity sustained by exudates, actively or passively released by roots, or due to the release of enzymes from plant roots (Pinton et al., 2001). Among soil enzyme activities, hydrolase activities of rhizosphere are supposed to play an

important role in plant nutrition. For example, the hydrolysis of organic phosphate monoesters by phosphomonoesterases can account for 30-80% of P taken up by plants in agricultural soils (Gilbert at al., 1999).

Indeed, rhizosphere microorganisms can both mobilise and immobilise plant nutrients (C, N and S) and can produce growth promoting substances, such as phytohormones, as well as phytotoxins. Beneficial bacteria such *Pseudomonas, Bacillus, Arthrobacter* are major component of the microbial flora which live in close association with various types of plants. Their association with plant materials has been related both to their antagonistic activities towards pathogens and to their ability to colonise and produce plant growth promoting compounds within the rhizosphere (Cook et al., 1995). In order to understand microbes behaviour in a certain habitat, it is necessary to know their physiological characterisation. It is important to measure the soil biochemical and biological parameters related to microbial activity in order to evaluate soil quality and productivity (Garcia et al., 1998). Because microorganisms play a important role in establishing biogeochemical cycles and facilitate the development of plant cover and has been assessed frequently through biological and biochemical parameters such as biomass C and enzyme activities (Caravaca et al., 2005).

Soil microorganisms are important components in the natural soil sub-ecosystem because not only can they contribute to nutrient availability in the soil, but also bind soil particles into stable aggregates, which improve soil structure and reduce erosion potential (Shetty et al., 1994). Studies of soil microbiota enabled soil biologist to set up a new understanding of soil as a combination of various ecological microenvironments. This concept explains the unique character of soil as a specific biotope, possessing the highest life density and diversity of all environments of the earth. The free –living components of the soil biota are the bacteria, fungi, algae and the fauna. Within each of the free living components, there exist a broad range of morphological and physiological characteristics that has led tom the naming of a large number of taxa for each group (Paul and Clark, 1988).

In the great majority of natural communities, the primary producers depend heavily on microorganisms. These include the organisms that decompose and mineralize organic detritus, others that promote plant growth and suppress plant pathogens and some that build soil structure. They help the roots take up nutrients, brings nutrient elements into the ecosystems from atmosphere or mineral reserves,

break down detritus, release mineral elements in soluble forms and protect the roots from pathogens. They also hold soil aggregates together, creating channels through which roots grow, soil animals move and water percolates. Bacteria occupy a wide variety of habitats and perform many ecological functions. They produce a vast array of chemical metabolites, many of which affect other organisms (Lynch 1976). Some fix carbon dioxide into organic compounds and are part of the primary level of the food chain. Many are extremely important in cycling nutrients in the soil and in water, making the nutrients more or less available to plants and other organisms. Air, water, and soil quality can be altered by the activities of bacteria. Soil microorganisms play important roles in maintaining soil quality and plant production, and they have a great influence on soil hydrolysis reactions and these in turn reflected in the natural cycles of carbon, nitrogen, phosphorus and other elements. 80-90% of the processes in soil are reactions mediated by microbes (Coleman & Crossley, 19960). The study of diversity, distribution, and behaviour of microorganisms in soil habitats is essential for a broad understanding of soil health. The physiological response of bacterial populations following introduction into the natural soil environment is poorly understood. Yet information on the physiology of bacteria in soil in relation to their resistance to soil factors is required to obtain a better in their survival strategies so that effectiveness of bacterial releases can be predicted (Overbeek et al., 1995).

Bacteria exhibit many ways of exploiting their environments. Species differ greatly in their biochemical capabilities and utilize many different substrates. Some form mutualistic associations with other bacterial species or other organisms. Most bacteria are exposed to gradients either of substances such as nutrients or of physical factors such as temperature and radiation, and they often have adaptations which allow them to find or remain in the location in these gradients which best meets their needs (Schlegel and Jannasch 1981).

Bacterial cells introduced into soil should be able to rapidly adapt to soil conditions in order to persist and reproduce. Survival strategies depend on the physiological adaptation in the introduced cells, such as adaptation to nutrient-limited conditions and/or other physical chemical conditions, efficient utilization of root-released compounds or specific interactions with plants. Therefore bacteria which are well adapted to the soil environment, i.e. that survive and persist, probably have an efficient response to stressful soil conditions, activating molecular mechanisms necessary for their adaptation and survival (Overbeek and elsas, 1997). Soil contains a

high number and diversity of microorganisms withy a wide range of metabolic activities and physiological properties.

The distribution of bacteria is governed by limiting factors, and each species will exist where levels of factors such as temperature, moisture, nutrient availability, and pH fall within its specific range of tolerance (Dommergues et al. 1978). Because soil is a complex system, the determination of where a soil microorganism would be found based on limiting factors must take into account interactions between environmental factors, alterations of the organism's range of tolerance by other factors, and variations of environmental factors in both time and space. The range of environmental conditions over which micro organisms can maintain growth and activity increases their importance. Extreme conditions may reduce the activity of some micro organisms but will stimulate that of others, and in many extreme environments micro organisms are the only contributors to nutrient cycling. Two conditions stressful to bacteria in soil are nutrient (mainly carbon) starvation and low water activity. The availability of organic nutrients in soil largely depends on the soil sites occupied by the bacterial cells or microcolonies. Oligotrophy is probably locally dispersed through most soils. Therefore, soil in general can be regarded as a grossly oligotrophic environment ((Morita and Moyer 1989). Both starvation and low water activity represent typical stress conditions general in most soil. Other stress conditions like extreme temperatures and pH values or toxic compounds may be more specifically related to the climate geographic location or soil type and site (Overbeeek and Elsas, 1997). The majority of known bacterial species re chemoorganotrophic and are commponly referred to as heterotrophs. Both energy source and carbon source are useful for describing basic physiological differences among bacteria as well as among organisms generally. The some functional groups are (1) heterotrophs, (2) nitrogen fixers, (3) nitrifyers, (4) denitrifyers, (6) phosphorus, calcium, and potassium solubilizers.

Heterotrophs

Heterotrophic bacteria have been assumed to be the dominant types among soil bacteria, since the consumption and mineralization of organic materials represent most of the energy flux though the soil biota. For this reason, all attemptsto culture the majority of soil bacteria has concentrated on the heterotrophic bacteria (Bakken 1997). For heterotrophic bacteria, it is the distribution of their organic

substrates which primarily determines the occurrence of pockets of activity, sometimes referred to as microhabitats. The majority of the organic substrates entering soil are either insoluble or are soluble but packaged in cells. Nevertheless, some soluble materials will diffuse into the soil water and be leached out of the surface horizons (Nedwell and Grey, 1987).

The other components of the microbial carbon cycle are the conversion of organic compounds into other organic compounds, and the breakdown of organic compounds with the accompanying release of energy and CO2. Those bacteria that fix CO2 will also break down organic matter in respiration (Atlas and Bartha 1987). Many bacteria are able to grow heterotrophically, obtaining all their energy and cellular carbon from pre-existing organic material. They are extremely important in the formation of humus in soil, the cycling of other minerals tied up in organic matter, and the prevention of buildup of dead organic materials. Heterotrophic bacteria are found in many diverse groups, can be aerobic or anaerobic, and are capable of utilizing a broad array of organic compounds. Microorganisms are unique in their ability to carry out anaerobic or fermentative degradation of organic matter (Atlas and Bartha 1987), although C turnover is greater under aerobic conditions (Boyd 1984). They are also responsible for the digestion of polymers such as cellulose and lignin, which multicellular organisms are unable to utilize. Some are able to break down man-made synthetic materials (Atlas and Bartha 1987). Microbial decomposition is favored by an adequate supply of nitrogen and phosphorus, neutral to alkaline conditions, good aeration, and adequate moisture (Boyd 1984). Physical breakdown of materials by insects, earthworms, and other animals also favors decomposition by exposing more surface area to microbial attack. Good soil structure is extremely important for plant growth, water penetration, gas exchange, and resistance to erosion (Gray and Williams 1971). Organic matter is also produced within the soil by plant roots. In 1 ha of a 50 year old pine forest, the soil to a depth of 200 cm may contain about 4 tones of living roots less than 0.3 mm in diameter, about 50% of which are replaced each year.

Heterotrophic bacteria play an important role in maintaining soil structure by their involvement in the formation and stabilization of water-stable soil aggregates. Polysaccharides are one of the major agents of soil aggregation, and it is thought that the soil polysaccharides are likely to be of microbial origin and are more resistant to breakdown than animal and plant polysaccharides (Gray and Williams 1971; Lynch 1976; Tisdall and Oades 1982). Bacteria also release other organic compounds which

affect aggregate structure and other soil properties such as water holding capacity. Bacterial cells themselves can hold soil particles together by adhesion or mechanical binding, and even after the bacteria die, their remains continue to bind the particles (Lynch 1976, Tisdall and Oades 1982). Many heterotrophic bacteria are specialized to the environmental conditions under which they are found.

Plant species had strong influence on soil microbial organisms and their activity. According to Merckx et al., (1987), obviously the input of nutrient by the roots into surrounding soil as well as the mineral nutrients levels in the soil are of considerable importance. Rovira (1965) was convinced that root exudates play a key role in the selective stimulation of microorganisms and the view has shared by others (Atkinson et al., 1975). Plants have an important effect on soil microbiology, due releasing different nutrients and organic compounds into the soil (Grayston et al., 1998).

Nitrogen fixers

Nitrogen (N) is an extremely important element to all forms of life. It is found in amino acids and many other organic compounds. It exists in stable valence states from -3 to +5 (Atlas and Bartha 1987, Blackburn 1983, Sock, Koops and Harms 1989). Large amounts of N are in N2 gas in the atmosphere, but utilizable combined forms are limiting in many ecosystems. Ammonium (NH4+) and nitrate (NO3-), inorganic salts of nitrogen, are water-soluble, and are the main forms used by organisms. Nitrogen Fixation ($N_2 - NH_3$) is carried out only by prokaryotes which may be symbiotic or free living. Nitrogen fixation requires a considerable amount of energy and it has been calculated that in bulk soil there is not enough available carbon (Postgate, 1982). However round the roots there may be sufficient exudates to allow some fixation of nitrogen, the production of which could be available to the plant.
Most of oligonitrophilic bacteria have ability to fix N_2. In nitrogen poor soils they are widely distributed to compare to other soil.

Oligotrophic bacteria

By general definition, an oligotrophic bacterium predominates in a nutrient poor environment, is isolated using a low nutrient medium and shows relatively high

growth rates in culture at low concentrations of energy yielding substrates (Hirsh et al., 1979).

One of the earliest attempts to relate the growth and substrate utilization of soil microbes was made by Winogradskiy (1924). He distinguished between the "zymogenous" microbes, which existed mainly in a resting phase with brief periods of activity in the presence of available substrates, and the "autochtonous" microbes, which were more or less continually and slowly active.

Despite the current paucity for the occurrence of strictly oligotrophic bacteria in soil, it is often regarded as a nutrient poor environment. Oligotrophic bacteria (oligotrophs) are microorganisms that grow in extremely nutritionally deficient conditions in which the concentrations of organic substances are low.

Nitrifying bacteria

In nitrification process, the NH3 or NH4 + is oxidized to nitrite (NO2-) and then to nitrate (NO3-). NO3- is readily taken up by plants and because of its negative charge, moves freely through soil. Both NO3- and NO2-are also more susceptible to leaching than is NH4+. They can become a health hazard when they reach groundwater. Nitrification can also prevent nitrogen losses in soils where NH3 volatilization is a major factor (Bock, Koops and Harms 1989). Nitrification is limited to species of a few genera of aerobic autotrophic bacteria (Focht and Verstraete 1977). Nitrifies are found in most aerobic environments where organic matter is mineralized, and on rocks where NH4 + is liberated through weathering (Bock, Koops and Harms 1989). Many are restricted to specific environments. The oxidation of NH3 and NH4 +to NO2 - is carried out in most soils by species of *Nitrosomonas,* while the oxidation of NO2 - to NO3 - is carried out by *Nitrobacter* (Bock, Koops and Harms 1989). Species of *Nitrosospira, Nitrosococcus,* and *Nitrosolobus* can also be involved in the former oxidation, while species of *Nitrospira.* and *Nitrococcus* can be involved in the latter. The two processes are usually closely coupled. Both oxidations are energy yielding, and the bacteria involved are chemolithotrophic, utilizing the energy to fix CO2. Because the energy yields of the oxidations are small, large amounts of N are turned over for relatively small cell yields (Blackburn 1983, Focht and Verstraete 1977). Nitrification is inhibited by anaerobic conditions or high acidity.

1.4. Plant rhizosphere

Hiltner in 1904 recognised the potential importance of microbial activities associated with root systems in plant nutrition and coined the term "rhizosphere"to describe the zone of intense microbial activity around roots of the leguminousae. This term is now used in a more general sense to describe soil influenced physically and or chemically by any root system.(Chanway C.P. 2002). The rhizosphere is first of all a unique hot spot in the soil at the viewpoint of microbial ecology as soil micro organisms are considerably stimulated in the vicinity of the roots, as a consequence of the release by roots of a range of C-compounds (Jones et al., 2004; Hinsenger et al., 2006). Depletion occurring as a consequence of the sink-effect of the absorbing roots of higher plants has been observed for P (Hinsinger, 2001) and for other major nutrients such as K and nitrate-N, which are substantially more mobile than P in the soil (Jungk, 2002; Hinsinger et al., 2005). The region of soil surrounding and including the plant root (the rhizosphere) is of crucial importance for plant health and nutrition (Marschner, 1995). It has a high level of microbial activity, particularly because of nutrients secreted by plant roots in the form of soluble exudates as amino acids, organic acids and other photosynthates. The rhizosphere is relatively nutrient rich because 40 % of the photosynthates moving into roots are lost to the soil in the form of soluble exudates, mucilage, and shed. It is a habitat for a vast interactive community of rhizotrophic microorganisms whose activities largely determine the physico-chemical properties of the rhizosphere soil. Exudates from roots have long been recognised as a major potential source of energy for many saprophytic bacteria in soil, being the prime cause of the rhizosphere effect. There is considerable amount of evidence suggesting that loss of soluble organic substances from roots is significantly stimulated by the presence of microbes around them (Lynch, 1977).

Understanding the complexity of this environment and how the microbial community adapts and responds to alterations in the physical, chemical and biological properties of the rhizosphere remains a significant challenge for plant and microbial biologists (Handelsman and snabb, 1996; Rainey, 1999).

The interactions between microbes and roots are now considered to occur in the outer, invaded cortical layer, on the rhizoplane when this can be distinguished and also in the surrounding soil, the rhizosphere. Because all soil borne nutrients obtained by the plant rot must pass through the rhizosphere, the potential for microbes to alter these compounds in a way that will affect plant growth is great (Chanway 2002).

The term “endorhizosphere” may now appropriately be used to describe the multilayered microenvironment, which includes a mucoid layer of plant or microbe derived polysaccharide, the epidermal layer including the root hairs, and the cortical layer. The rhizoplane (root) surface should be defined as the epidermal layer, including its associated polysaccharide matrix. By comparison ectorhizosphere comprises the rhizosphere soil, which usually extends a few millimetres from the root surface (Sorensen, 1997). The important role of the root cap is its production of mucilage (polysaccharide), which covers epidermal cells and acts as a lubricant while the root advances through soil. It is interesting that the mucilage layer on the young root may contain the chemical components of importance in host-pathogen or host symbiont recognition system. In the young plant root, the uptake of nutrients and water occurs through an intact epidermal layer including the root hairs. Normally, this layer is quite short-lived as a result of the mechanical tension or desiccation of the rhizosphere (Christensen 1995). Living epidermal cells and root hairs become densely colonised by micro organisms, notably bacteria, which depend completely on simple organic molecules exuded from the plant cells (Sorensen, 1997). The exudates are typically carbohydrate monomers (sugars), amino acids, and organic acids, which are suitable substrates for a wide range of rhizobacteria.

Roots form an unstable habitat for micro-organisms for the interfaces between the root, soil and microbes are continually changing. Healthy, vigorous root tip elongate so rapidly that bacteria and fungi cannot grow fast enough to colonize them from pre-existing root parts and they are colonized from the soil. The rhizosphere soil contains more soluble sugars but less insoluble material than the surrounding soil and there is less nitrogen but more polyphenols (Campbell 1985).

1.5. Rhizosphere micro organisms

In early work on the rhizosphere research the ratio of organism count in rhizosphere soil to count in root-free soil were determined for different plant species and for single species in different soils, under differing climatic regimes and at different stages of phenology Total microbial counts were commonly found to be increased 10 to 50 fold in the rhizosphere. The rhizosphere harbours a large and diverse community of prokaryotic and eukaryotic microbes that interact and compete with each other and with the plant root. The concentration of bacteria that is found

around the roots of plants (in the rhizosphere) is generally much greater than the bacterial density, or concentration, that is found in the rest of the soil (Lynch 1990).

Many bacteria are intimately associated with plants roots. In a zone surrounding the root known as the rhizosphere, the populations of microorganisms differ from those in the surrounding soil both in total number and in species distribution (Atlas and Bartha 1987). The plant alters rhizosphere populations through root exudation and the sloughing of root cells. Most plants also interact with specific fungi to form associations known as mycorrhizae, and these also have considerable effects on populations of rhizosphere bacteria (Meyer and Linderman 1984).

The rhizosphere is known to harbour proportionately more G- bacteria *Pseudomonas*, *Achromobacter* and denitrifiers and fewer G+ and Gram variable forms (*Bacillus*, *Arthrobacter*). Increases in the rhizosphere micro flora are accompanied by heightened faunal activity, especially in those groups that are grazers on the micro flora or on roots (Paul and Clark, 1988). The rhizosphere harbours a large and diverse community of prokaryotic and eukaryotic microbes that interact and compete with each other and with the plant root. Activity of any one member of this community affects the growth and the physiology of the others, and also affects the physical and chemical properties of the soil. A continuous interaction exists between the plant roots and the rhizotrophic microorganisms and within the different groups of these microorganisms which has an important influence on plant growth (Saxena and Tilak, 1994). The microbial composition in the rhizosphere often differs greatly from that of the surrounding soil and from one plant species to another, as a result of diverse plant microbe interactions. There are different groups of micro organisms playing in the rhizosphere. Some of them may be deleterious and others may be supporting the plant growth and some others may not affect the plant developments at all. They are mainly bacteria, fungi, and actinomycetes.

The microbial composition in the rhizosphere often differs greatly from that of the surrounding soil and from one plant species to another, as a result of diverse plant microbe interactions. Some bacteria, such as *Pseudomonas* and *Flavobacterium* spp. tend to be more predominant in the rhizosphere than others, such as *Arthrobacter* and *Bacillus* spp. (Alexander, 1977).

Because of the wide range of microbes that is stimulated in the rhizosphere, it is difficult to assess the influence of rhizosphere microbial activity on plant growth. The

interaction between bacteria and the roots of plants may be beneficial, harmful, or neutral for the plant. Rhizosphere microbes can both mobilise and immobilise plant nutrients, and can produce growth promoting substances, such as gibberellins, as well as phytotoxins. Rhizosphere microbes, such as species of the fungi *Fusarium, Gaeumannomyces* and *Rhizoctonia* can also be plant pathogens, as well as antagonist against those pathogens.

The challenges for the next decades include understanding of the behaviour of microbes in their natural and often complex habitats, such as the rhizosphere (Lugtenberg et al., 2002). The overall in the rhizosphere, that is on the plant root or its close vicinity, bacteria are abundantly present, most often organized in microcolonies. Some of these rhizobacteria not only benefit from the nutrients secreted by the plant root but also beneficially influence the plant in a direct or indirect way, resulting in a stimulation of its growth (Bloemberg and Lugtenberg 2001). Rhizosphere bacteria depend on plant root exudates- which are low molecular weight, easily oxidizable compounds – for much of their nutrition (Rovira, 1963). Microbial processes in the rhizosphere of various crop plants are crucial to agriculture. Many rhizosphere colonizing bacteria, including *Bacillus, Azospirillum, Pseudomonas*, typically produce substances that stimulate plant growth or inhibit root pathogens (Glick, 1995). Previous studies of bacteria in salt-affected soils lies behind those on halophiles in extremely saline soils (Quesada, *et al*. 1982) and also some bacterial related to the nitrogen cycle. Culturability of micro organisms on laboratory media has been the basis for the majority of studies on microbial populations in the rhizosphere. Even cell viability has often determined from estimates of colony forming units (CFU) as percentage of total counts. This figure is variable, but estimate of 10% viability of bacterial population in rhizosphere soil are common (Rovira et al., 1974).

1.5.1 Diversity of rhizobacteria

Microbial diversity studies are important in order to understand the microbial ecology in soil and other ecosystems (Wilcinson et al., 1994; Atlas et al. 1991; Garland, and Mills, 1994). Diversity can be regarded as the amount and distribution of genetic information in a natural community. A representative estimate of microbial diversity is a prerequisite for understanding the functional activity of microorganisms in such ecosystems (Simon, et al., 1991). Studies in microbial

diversity are important in order to understand the role of microorganisms in the ecology of soil and also in other ecosystems (Atlas, 1991). Analysis of the genotypic and phenotypic characteristics of indigenous rhizobacteria can help to clarify the mechanism of interactions between them and plant roots (Tripathi, et al. 2002).

Alexandre (1977) has made a brave attempt to summarise a great number of classic taxonomical studies of bacteria prevalent in soil and presents the following ranges (%): *Arthrobacter* 5 – 60%, *Bacillus* 7-67%, *Pseudomonas* 3-15%, *Agrobacterium* up to 20%, *Alcaligenes* 2-12%. *Flavobacterium* 2-10%. The arid and semi arid ecosystems may provide an excellent natural research field and information on microbial physiology, diversity and their role in the ecology of soil as affected by gradients of environmental conditions combined with anthropogenic activities (Atlas et al., 1984).

Microbial diversity is a general term used to include genetic diversity, that is, the amount and distribution of genetic information, within microbial species; diversity of bacterial and fungal species in microbial communities; and ecological diversity, that is, variation in community structure, complexity of interactions, number of trophic levels, and number of guilds (Nannipieri et al., 2003).

The microbial population in soil is very diverse. Torsvik et al., (1996) calculated the presence of about 6000 different bacterial genomes per gram of soil by taking the genome size of *Esherichia coli* as a unit. Microbial diversity is a general term used to inclue genetic diversity, that is, the amount and distribution of genetic information, within microbial species, diversity of bacterial and fungal species in microbial communities; and ecological diversity, that is, variation in community structure, complexity of interactions (Nannipieri, 2003). Determining the presence and abundance of specific groups of bacteria can provide useful insight into ecosystem functions. Identification of which species representing a functional group is/are present could enable the choice of indicator species to be monitored during ecosystem disturbances. Species diversity can also be an indicator of soil quality because of its obvious relationship to functional diversity (Visser and Parkinson 1992).

1.5.2. Secondary metabolites

The research on natural products may offer substantial advantages, like discovery of new drugs with a new mode of action, high selectivity and activity. It is clear that from all microorganisms the procaryotes whose secondary metabolism shows the highest flexibility, are the most suitable. Many bacteria are synthesis secondary metabolites such as certain extracellular enzymes and other bioactive compounds.

A diverse group of soil microorganisms are capable of producing physiologically active compunds that may have pronounced effects on plant growth and development (Frankenberger and Arshad, 1995). According scientific reports 86% of the bacterial isolates from the rhizosphere of various plants produced phytohormones, and also different vitamins. Rhizosphera bacteria produce growth promoting substances in culture media, in the rhizosphere and in the rhizoplane of forage grasses and many economically important cereals like wheat, barley and vegetables, tomato and bean plants under cultural conditions (Frankenberger and Arshad, 1990).

No scientific information is available about microbial diversity and functions in these ecosystems, especially little is known about the bacterial ecology associated with plants grown in Chatkal Biosphere Reserve which protected territory in Western Tien Shan. The study of microbial populations and their biochemical activities associated with herbal plants growth in such unique environment may thus provide valuable information on microbial distribution, their biochemical activities and their role in plant establish and development.

In this report we describe about plants growth in Chatkal Biosphere Reserve and their microbial association within those ecosystems. We also investigated soil biological properties and microbial activity using sites with different elevation (at the 2000 m) in various ecosystems located in Chatkal Biosphere Reserve of Uzbekistan. The biochemical and physiological properties of bacteria associated with some herbal plants such *Ziziphora capitata, Salvia sclarea* and *Calamagrostis epigeios L*. will be reported.

2. Materials and Methods

2.1. Climate

Chatkal Biosphere Reserve, established in 1947, is situated in Ugam-Chatkal National park, to the East from Tashkent within the Chatkal mountain range of the West Tien-Shan Mountain. The high point of Chatkal range on territory of Uzbekistan - Large Chimgan peak (3309 m). Bordering Kazakhstan and Kyrgyzstan, the park has an approximate area of 5,746 km2 and includes Chatkal Biosphere Reserve (452 km^2) within its borders. The reserve is situated at the territory of Tashkent oblast, Bostanlik, Parkent and Akhangaran districts.

Photo 1a

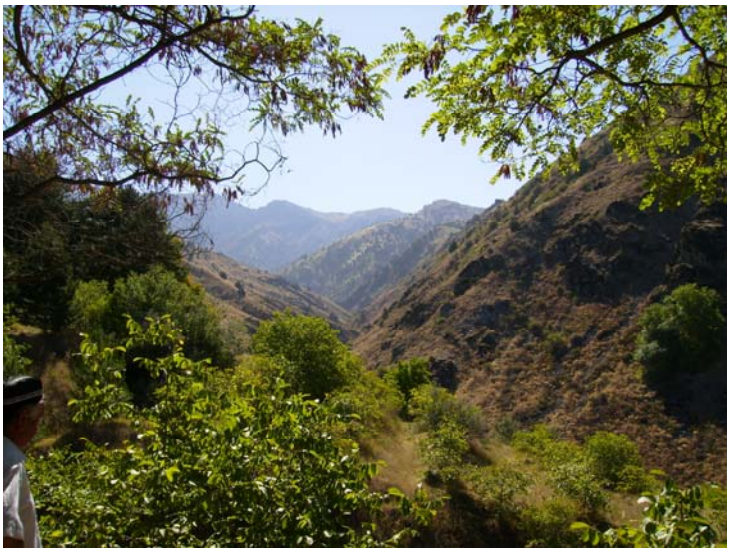


Photo 1b.

The geographical location effects on the climate of the region, intensity of the solar radiation, originality of atmospheric circulation and relief of place. Here is many sun days, considerable daily and annual variation of temperature, not much a precipitation. In summer the sun in Uzbekistan very high stayed over horizon. In warm half-year entering the solar radiation in republic so large, that other powerful climate forming factor (atmospheric circulation) played subordination role. The average temperature of air in January can is lowered to -3^{o}C and below. In summer the air becomes to dry and hot, is saturated by small-sized dust. The average temperature of air in July is 31oC (Table 1).

Photo 1c Chatkal Biosphere reserve site

Table 1. Climatic characterisation of Chatkal Biosphere Reserve

Season	Precipitation , mm	Humidity, %	Temperature, ^{o}C
January	127.3	51	-3.6
February	135.6	59	-2.3
March	152.6	66.3	2.3
April	123.7	62	19.3
May	176.4	60.3	19
June	38.6	49	26.6
July	40.3	44	31.3
August	4.06	37	29.6
September	12.4	42.3	22.6
October	24.4	48	14.6
November	120.3	58.3	3.6
December	77.0	54	-1

The spring is moist, short with non-sustainable weather. For the fall characterized the clear weather and slow low of the temperature of air at the crossing to winter. In mountain the air is fresh cool, more the cloudy days. The loss of main masses of atmospheric precipitation is in the fall-winter-spring period and sharp decreasing in summer season. In summer the precipitation drops out only 4 mm. In high mountains the snow stayed annual. In the mountain the annual quantity of precipitation ranges about 1000 mm. The main part of precipitation in Chatkal is on winter-grade and spring months (30 % drops out in winter-grade months and 40 % - in spring), that connect with activation of cyclone actions in cold season of year. In mountains the snow cover is powerful and can to last a 2-6 months. The thickness of snow covered in mountains more, then 1 m.

The summer usually cold, sometimes drop out the rains. The height and location of the mountains ranges on attitude to sun and direction of the air flows influence on distribute of temperature in mountain regions. On measure of rise up the temperature of the air lowered. The amplitude of the average annual temperatures is about 14oC. The relative humidity is around 40% summer and in winter about 66%. In mountain regions the direction of wind depends from relief, which is around 6 m/c. However, the strengthening of wind to 6 m/c, against a background of the quickly drying soil, promotes to develop the dust storms.

2.2. Vegetation

In formation of natural vegetation communities of these zone participate representatives of different vital forms of plants: plantation of trees, shrubs, semi-shrubs, grassy with different vegetation cycle. More than 70% three species of Uzbekistan are growth in Chatkal Biosphere Reserve area. They are located at altitudes from 800 to 2,000 m and contain relict forests of walnut (*Juglans regia*) mixed with wild apple, apricot, plum, and other fruit tree species. Subalpine and alpine meadows are located at altitudes between 2,800 m and 3,700 m.

The basic vegetation pattern consists of trees and shrubs alternating with steppe and meadow areas or bare rocks. The largest areas of deciduous species are concentrated in the Western Tien- Shan Mountains. The significant part of shrubs kind form of growth is widespread in foothills and mountain region. Semi-shrubs make the most the numerous group of plant diversity in mountain. Arboreal vegetation is submitted as archa, broad-leaved forests and shrubs vegetation is presented with thin out

plantation of shrubs from different systematic groups such St.-John's worth, many flower cornel-tree, kinds of sweetbrier etc. The development and distribution of many-grassy meadow, in top zone accepts here a creep and short shrubs, for example, turkestan archa has a creep form, founded a honey suckle, cornel-tree, sweetbrier etc., as well as are advanced a pillow type mountainous xerophytes.

The plant cover includes: *Hypericum perforatum*, *Salvia sclarea, Geranium tuberosum, Ziziphora capitata, Ephedra eqisetina Achillea millefalium, Juglans regia, Persica vulgaris, Amygdalus spinosissima, Prunus sogdiana Vess, Astragalus sieversianus, Verbascum songaricum, Tussilago farfara, Juniperus turkestanica, Ulmus densa, Hardeum leporinum, Hordeum bulbosum, Cynodon dastrylon, Jagea stipitata. Juglaus regia, Polygonum coriarum, Cerasus erythrocarpa, Convolvulus arvensis, Malus sieversii, Prunus sogdiana, Elaeagnus angustifolia.*

Meadow vegetation is dominated by *Polygonum*, *Prangos*, and *Ferula*. The zone of high mountains starts from 3,500 m. Vegetation is represented by "carpet" meadows of dwarf grasses that are characteristic of the alpine belt of glaciated high mountains. The rich flora of Uzbekistan is represented by at least 4,500 species of vascular plants belonging to 115 families and 650 genera.

A top zone takes mountainous slopes higher 3000-3200 m, a top border beyond to eternal snow band. Here dominate a dry stone slopes with thin out steppe or meadow-steppe cover, where dominate a oatmeal, or mountainous xerophytes. Short-grass cover meadows are taking the small areas and cereal meadow, geranium, fox tail and other cover take large cover.

One of characteristic types for high-hills is mountainous-xerophytes, non-uniform on structure ecological types and biological forms, that is prickly-grassy and prickly-pillow forms, a significant part of cover make a steppe plants, in some cases with it associated the elements of meadow vegetation. The modern condition of vegetation cover is characterized with rarefaction and distribution of derivative secondary communities, formed under influence of anthropogenic factors.

2.3. Plant collection

Plants were collected in May 2005 from Chatkal Biosphere Reserve from different ecosystem, which is single protected area in Western Tien Shan within Uzbekistan. Over 40 species of rare and endemic plant species included into the Red Data Book of

Uzbekistan (1988) are protected in Chatkal Nature Reserve, which constitutes 47% of endangered plants of Western Tien Shan. Sub alpine and alpine meadows are located at altitudes between 2,800 m and 3,700 m. Meadow vegetation is dominated by *Polygonum*, *Prangos*, and *Ferula*. The information on name, part used, purpose, mode of administration, etc. was recorded in the field notebooks. The nomenclature of the plants listed follow with identification in Institute of Botany.

Photo 2. Plant collection from study site.

2.4. Soil

In mountain the generic diversity of soil pursuant with planetary laws depends from their high-altitude location. With increase of absolute height of district the increase of quantity of precipitation, increase the water-supply of mountainous slopes, strengthening of dismembered of relief, decrease of average temperatures of air, increase its humidity and consecutive change of vegetation from ephemeral-semi-desert to different-grass-steppe, juniper-sparse growth of trees, woody, meadow-steppe, steppe and meadow is usual observed.

All diversities of soil of foothill-mountainous areas of republic with vertical zone is contained in three main soil-climatic zones, each of which is characterized of unique peculiarity of soil structure and structure of the soil covered. It, in accordance with

increase of height of district, the zone of greyish, zone of mountainous brown soils and zone of light-brown meadow-steppe hightail soils.

Photo 3. Serozem soil of Chatkal Biosphere Reserve

The greyish zone is located between absolute heights from 250-400 to 900-1500 m. Its lower border divides the foothill plains, relating to area with vertical zone from more low and remote mountainous massifs of plains. In accordance with increase of absolute marks in foothills and low hills the greyish and connected with them poly-hydromorphic and hydromorphic soils are distributed on foothill and mountains

On heights from 900-1500 to 1300-2900 m, in average hills zone prevail the mountainous brown soils. Main their areas are widespread under covered of dense different grass, leaf-bearing woods trees. Soils are more humidified so differ with high humus content. The most the humidified sites in this high-altitude zone take brown and mountainous-woody soils. In high mountain high-altitude zone, on heights more than 2300-2900 m are advanced the light-brown meadow-steppe soils. Soils of

high hills frequently change with large massifs of exits on surfaces radical rocks and covers.

2.4.1.Soil sampling

Soil samples will be taken from different ecosystems and sites; forest area and highland ecosystem area (grasses, herb plants) grown in Chatkal Biosphere Reserve at different altitudes (500 – 2000 m) (Photo 1,2,3,and 4). The site characterisation is described in Table 2.

Photo 4. Soil sampling for chemical and physical analysis.

Soils were sampled in spring and autumn from 5 sites with various altitudes ranges from 500 – 2000 m, where soils are typical serozem, dark serozem, brown carbonate soil, typical brown, leached brown soil. A plot 1 m^2 was pegged out at each area and triplicate samples (1 kg each) were collected from each plot to a depth of 0-30 cm using a soil corer (3,5-cm diameter).

Soils were sampled in spring and autumn from 5 sites with various altitudes ranges from 500 – 2000 m, where soils are typical serozem dark serozem, brown carbonate soil, typical brown, leached brown soil. A plot 1 m^2 was pegged out at each area and

triplicate samples (1 kg each) were collected from each plot to a depth of 0-30 cm using a soil corer (3,5-cm diameter).

Table 2. Ecological characterization of studied soil.

Site	Typical serozem	Dark serozem	Brown carbonate soil	Typical brown	Leached brown
Altitude from sea level, m	500-700	600-1100	900-1300	1300-1600	1600 -2000
Climate	Dry, hot and warm winter	Dry, hot and warm winter	Moist, humid, warm	Humid and warm	Humid and cold winter
Average of annual precipitation, mm	78-422	100-600	400-700	1040-700	1060 and high
Average of annual temperature, C^0	13-16	12-14	11-13	9-11	9-10
Snow cover, см	10-20	30-80	80-100	100-110	110-120
Vegetation	Grassland, wormwood, fowl grass, viviparous barley,	Ephemeral vegetation	Forest and shrubs ephemeral, gramineae	Forest threes, bushes, gramineae	Meadow steppe motley grass
Relief	Plain, foothills, river valley	Foothills terrace	Mountain bed	Foothills, mountain range	Mountainous rocky range
Soil formation	Loess, loess like loam				
CO_2 carbonate, см	27	120	48	35	20
Upper limit of gypsum, см	140	86	60	75	80

These consisted of 15 soil samples taken randomly from the marked plots using a tube sampler and transported in sealed plastic bags separately to the laboratory in unsieved and field moist condition to limit the disturbance to the microbial community. In the laboratory they were bulked, sieved (2 mm) and stored in polyethylene bags at 4^oC for soil biochemical analyses afterward. Microbial and biochemical analyses were carried out on field-moist samples while soil chemical analyses were performed on air-dried subsamples.

2.4.2. Soil physical and chemical analysis

Air-dried samples were analysed for the total C, N, P, K and Mg content. Soil texture was determined according to DIN 19683. Contents of soil carbon (C_t), and total nitrogen (N_t) contents were determined after dry combustion using a CNS elemental analyzer (LECO Corporation, St. Joseph, MI) according to DIN ISO 10694 (1996), DIN ISO 13878 (1998), and DIN ISO 15178 (2001), respectively. Soil water contents were determined gravimetrically (105°C, 24 h). The potential and the effective cation exchange capacity (CEC) was analyzed according to DIN ISO 13536 (1995) and 11260 (1994) protocols, respectively. Soil pH were measured potentiometrically in a 1:5 (w/v) aqueous solution.

The molybdenum blue method were used to determine the total phosphorus content in soil. Potassium content where determined using the flame photometric method (Riehm, 1985).

2.5. Soil enzyme activity

Phosphotase activities were assayed according to Tabatabai and Bremner (1969) and catalase activity as reported by Galstyan (1956), dehydrogenase activity by Lenard (1962). Urease activity was measured using 0.1 phosphate buffer at pH 7, as reported by Nannipieri et al. (1974). All enzyme activities were assayed 37°C for 1 h, after centrifugation of soil mixtures at 6000*g* at 4°C. Concentration of p-nitrophenol (*p*-NP) produced in the assays of phosphotase activities was calculated from a *p*-NP calibration curve after subtracting the absorbance of the control at 400nm wavelength using a UV-VIS spectrophotometer Lambda 2 (Perkin Elmer). The NH_4^+ produced by urease and protease *N*-BAA-hydrolysing activities was extracted using 2M KCl and quantified by a flow injection analyzer (FIAStar, Tecator, Sweden). To account for the NH_4^+ fixation by soils, NH_4^+ solutions with concentrations in the range of those released by urease and protease activities were incubated with these soils. The recovery of NH_4^+ ranged between 93 and 96% (data not shown). Invertase activity were measured by methods of Kuprewich (1951).

2.6. Microbiological analysis

Plate dilution methods on different agar media where used for determining culturable microbial populations from the soil samples taken under various plants. Soil (10 g) where shaken with 90 ml of ster.-distilled water. From this suspension the serial dilution (1:10) will be prepared; plate counts will be replicated six times and incubated until the occurrence of growth (usually 3-7 days). CFU of important physiological croup of microorganisms where enumerated on agar plated mediums. CFU of bacteria that growth in nutrient rich medium was enumerated on meat extract agar. Fungal populations and actinomycetes were determined on Chapek agar. Microbial density was expressed as colony forming units (CFU). CFU of ammonifying bacteria were enumerated on glycerin peptone agar. Oligotrophic bacteria on soil agar containing 900 ml water, 100g soil, 18g agar L^{-1}, oligonitrophilic bacteria was determined on Eshbi agar containing 0,2 g K_2HPO_4, 0,2 g $MgSO_4$, 0,2 g of NaCl, 0,1 g K_2SO_4, 5 g $CaCl_2$, 20 g sacharosa, agar 15 gl^{-1}. Microbial density was expressed as colony forming units (CFU). The data were analyzed using the statistical analysis of variance by (ANOVA).

Photo 5. Soil sampling for microbiological analysis

The rhizosphere bacterial strains were isolated from the rhizosphere of *Ziziphora capitata, Salvia sclarea* and *Calamagrostis epigeios L*. grown in Chatkal Biosphere Reserve Mountains at the altitudes 1500 m. The plants were taken together with the root systems. Plant roots were chopped into 10-mm lengths and three replicates of the same crop were used for microbiological analysis. Root samples (1 g) were shaken well with 9 ml. of sterilised distilled water and 10^{-1} to 10^{-7} serial dilutions were made in 0.2% saline. Before isolating bacterial strains we have determined the natural colonization of bacteria in the rhizosphere of plants. From each dilution 0.1 ml of suspension was spread in triplicate onto LC medium, KB medium and 20 times diluted TSA medium agar plates and the number of colony forming units (cfu) was determined. The plates were incubated at 28°C for 2-3 days. After incubation, the isolated strains were purified, by twice streaking on LC agar medium. The pure cultures were grown on LC medium at 28°C for 24 h and the colonies were collected for further examination.

2.7. Physiological and biochemical analysis

Antagonistic activity of the bacterial isolates was tested in vitro to select those with inhibitory effects against *Fusarium oxysporum, Botrius cinera, Phytium ultimum, Fusarium culmorum, Fusarium solani, Alternaria alternate and Gaeumannomyces graminis* f. sp. *tritici*

using a plate bioassay with Chapek agar. Fungal strains grown in Chapek agar plate at 28°C for 5 days Disks of fresh culture of the fungus (5 mm diameter) were cut out and placed in the centre of a 9 cm Petri plate with Chapeck agar. Bacteria (grown in peptone agar plates), were streaked on the test plates perpendicular to the fungi. Plates were incubated at 30°C for 7 days, until the fungi had grown over control plates without bacteria. Antifungal activity was recorded as the width of the zone of growth inhibition between the fungus and the test bacterium. The antibiotic resistance abilities of strains were determined by plating of bacterial strains on KB agar supplemented with Km (kanamycin).

The production of IAA (indole acetic acid) was determined according to the method of Bano and Musarrat, 2003). Tested bacterial strains were grown in LC medium without and with tryptophan (500 µl/ml) and incubated at 28°C. A 2 ml

culture was removed from each tube and centrifuged at 10,000 rpm for 15 min. One ml of supernatant fluid was transferred to a fresh tube to which 100 µl of 10 mM orthophosphoric acid and 2 ml of reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) were added. After 25 min, the absorbance of the developed pink color was read at 530 nm. The IAA concentration in culture was determined by using a calibration curve of pure IAA as a standard (Bano and Musarrat, 2003). The IAA production were measured after 2, 4, 6, 8 and 10 days.

For testing HCN production by bacterial strains, the isolates were grown in Kings'B medium. A sterilized filter paper saturated with a 1 % solution of picric acid and 2 % sodium carbonate was placed in the upper lid of a Petri dish. The Petri dish was sealed with parafilm and incubated at 28 ^{o}C for 3 days. A change the color of the filter paper from yellow to dark blue was recorded as an index of HCN production.

For determination of protease activity, the bacteria were grown in KB medium containing 5 % of milk. After 5 days, the degradation of milk respectively, can be seen as a clear halo around the bacterial inoculum. ß glucanase activity were detested using the glucan substrate lichenan in top agar plates. Degradation of the substrate can be seen as clear zone (Walsh et al., 1995). Cellulase activity was detested using the substrate carboxymethylcellulose in top-agar plates.

3. **Results**

3.1. Plants growth in Chatkal Biosphere Reserve

Chatkal Biosphere Reserve is plays a significant part in the conservation of its unique biological diversity, because they provide important habitats for endemic and rare plant species. Major habitats and land cover types Juniper forest with *Prunus sogdiana, Acer turkestanicum,* and *Malus kirghisorum*; and highland areas with herb and grass meadows. Vegetation cover of this region is represented with unique plant communities, and invaluable genetic pool of indigenous flora. (Photo1,2,3,4,5).

The list of the medicinal plant species growth in Chatkal Biosphere Reserve is presented in Table 3. For each species, the following ethnobotanical and pharmacognostic elements are provided: botanical name; voucher specimen; local names; ailments treated.

Photo 6a

Photo 6b

Photo 6c

Photo 6d

Photo 6e. Vegetation of Chatkal Biosphere Reserve.

Table 3. Medicinal plants growth in Chatkal Biosphere Reserve

Plant species	Local name	Medicinal use
APOSYNACEAE *Vinca erecta*	Burigul [a] Periwinkles [b]	Headache, blood pressure, hypertonic disease, diarrhoea, astringent, diabetes
BERBERIDACEAE *Berberis oblonga* *Barberis vulgaris*	Kora Zirk [a] Oddiy zirk [a] Barberries [b]	Lowering blood pressure, heart disease, stomach disease, chronic hepatitis [4]
BETULACEAE *Betula pendula* Roth	Okkayin [a] Silver birch [b]	kidney stone, skin eruption, eczema, internal maladies, diuretic, wound [4]
BETULACEAE *Betula verrucosa Ehrh.*	Kayin [a] Birch [b]	diuretic drug, urinary infections kidney stones, skin eruption, dropsy eczema, wounds

BORAGENACEAE *Lithospermum officinale l.*	Ilonchup [a] Alkanets [b]	headache, pain killer, ulcer, inflammations, burnings [4,31]
CAPRIFOLIACEAE *Loniceria korolkovil* *Loniceria tatarica*	Korolkov uchkati [a] Honeysuckles [b]	diuretic, digestive, inflammation, influenza, fever, headache, coughing spasm
CARYOPHLLACEAE *Herniaria glabra L.*	Saminchup [a] Rupturewort [b]	lung disease, stomach disease, urinary infection, hepatitis, kidney inflammation, dropsy, cardiac [4]
CHENOPODACEAE *Chenopodium album*	Lebeda [a] Goosefoot [b]	wounds, allergic rashes, skin infections [31]
CHENOPODIACEAE *Spinacia tuukestanica*	Ismalok [a] Spinat [b]	improving blood formation, hypertonic [31]
CONVOLVULACIAE Convolvulus purpureus	Pechak [a] Bindweeds [b]	Cathartic, purgative action, pain, colic, scan disease [31]
COMPOSITAE *Achillea millefolium*	Buymaradon [a] Yarrow [b]	diaphoretic, tonic stimulant, eruptive disease, kidney disorders, bleeding piles, inflammation, rheumatism, anaemia, haemorrhoid [4]
COMPOSITAE *Calendula officinalis*	Kalendula [a] Marigold [b]	cuts and abrasions, wounds, diaphoretic, chronic ulcer, varicose, pain swellings, inflamed eyes
COMPOSITAE *Matricaria chamomilla*	Romashka [a] Chamomille [b]	Inflammation, tonic, sedative, neuralgic pain, stomach disorders [4]
COMPOSITAE *Helichrysum arenarium*	buznoch [a] life everlasting [b]	liver disease, skin burn, eye disease, chronic gastritis [4]
COMOSITAE *Bidens tripartita*	Ittikanak [a] Agrimony [b]	diuretic, astringent, fevers, stone and bladder in kidney, blood vessels, bleeding, liver disease [31]
COMPOSITAE *Tanacetum vulgare*	Pijma [a] Tansy [b]	Hepatitis, tonic, stimulant, worms, hysteria, kidney disorders, diaphoretic [4]
COMPOSITAE *Artemisia absinthium*	polin gorkaya [a] polin osennaya [a]	increasing food digestion, blood circulation, tonic stomachic,

Artemisia setorina –	wormwood [b]	dysentery, pain, anti parasitic, heart disease [4,5]
COMPOSITAE *Tussilago farfara*	Mat i macheha [a] coltsfoot [b]	cold and inflammation, heart ailments, cardiac, cough,, chest infection, tonic, bronchitis [4,5]
COMPOSITAE *Artemisia taurica Willd.*	Polin [a] Mugwort [b]	Tonic, stimulant, diuretic, diaphoretic, epilepsy, digestion [4,5]
COMPOSITAE *Tanacetum vulgare*	Dastrabosh [a] Tansy [b]	liver disease, stomach disease tonic stimulant, worms, fever [4,5]
COMPOSITAE *Cichorium intybus* L.	Sachratki [a] Chicory [b]	wounds, allergic rashes, skin infections, laxative diuretic [4,31]
CONIFERAE *Juniperus turkestanica* *Juniperus zerawschanica*	Archa [a] Juniper [b]	allergic rashes, skin infections, renal colic, dyspepsia, disorders of the prostate gland, diuretic, stomachic, kidney and bladder disease, cardiac and hepatic dropsy [4,31,5]
COVOLVULACIAE *Convolvulus subhirsutus*	mingbosh [a] bindweed [b]	astma, against nose and ear pains, poisoning [4]
CRUCIFERAE *Capsella bursapastoris*	Gag'gag [a] Shepherds purse [b]	liver disease, bleeding, urinary infections, uterus, stomach haemorrhage, wounds, chronic diarrhoea, dysenteric, dropsy [31]
ELAEAGNACEAE *Elaeagnus angustifolia*	Jiyda [a] Loh [b]	gastrointestinal disease diarrhoea [4]
EQUISETACEAE *Equisetum arvense L.*	Kirkbugim [a] horsetail [b]	Blood circulation, bleeding, liver disease, skin disease, cough, gastric ulcer, gallstones, kidney bladder
GRAMINACEAE *Hordeum bulbosum*	Piezli arpa [a] Barley bulbous [b]	tonic, skin disease [4]
GRAMINACEAE *Agropyrum ancheri*	Tukli bugdoyoek [a] Twitch grass [b]	diuretic, catarrhal disease, rheumatism [4]
GERANIACEAE *Geranium tuberosum*	Yarongul [a] Wild cranesbills [b]	tonic, internal bleeding, leucorrhoea diarrhoea, cholera [4,5]

GERANIACEAE *Geranium collinum*	Erongul [a] Cranesbills [b]	diarrhoea, bleeding,. tonic, styptic, leucorrhoea [4,3]
GENTIANACEAE *Gentiana gris*	Gazakut [a] Gentian [b]	stimulant, tonic, antiseptic [4]
HUPERICACEAE *Hypericum perforatum L.*.	Dalachoy [a] St. Jones Worth [b]	wounds, gastric, pain, cuts and scalds, skin infections, expectorant, pulmonary, dysentery, diarrhoea, nervous depression, lung disease [4,31]
JUGLANDACEAE *Juglans regia* L.	Oreshnik engok [a] Walnut [b]	Inflammation, diarrhoea, skin disease, mouth wash, dysenteric, congestions, syphilis, old ulcers [4,5]
LABIATAE *Salvia sclarea L.*	Shalfey [a] Clary [b]	wounds, allergic rashes, skin infections, antispasmodic, tonic, stomachic digestion, eye inflammation, headache [4,3]
LABIATAE *Origanum vulgare L.* *Origanum tyttanthum*	Tog rayhoni [a] Marjoram [b]	whooping cough, lunge disease, skin infections, antiseptic, carminative, diaphoretic, tonic, dyspeptic colic complains [5]
LABIATAE *Melissa officinalis*	Lemon balm [b]	epilepsy, mental illness [5]
LABITAE *Thymus vulgaris*	Thyme [a] Chabrez [b]	allergic rashes, skin infections, antiseptic, tonic, gastric fermentation, spasm, colic [31]
LABIATAE *Scutellaria galericulata L.*	Shlemnik [a] Skullcap [b]	against nervous disorders, vascular disease, tonic, antispasmodic, astringent, headache, neuralgia [31,4]
LABIATAE *Leonurus turkestanicus*	Pustirnik [a] Motherwort [b]	diaphoretic, antispasmodic, tonic, strengthening heart, nervous disorders [4]
LEGUMINOSAE *Galega officinalis*	Goats rue [b]	wounds, allergic rashes, skin infections, diaphoretic [31]

LEGUMINOSAE *Sophora pachycarp*	Indigo [b]	wounds, allergic rashes, skin infections, typhus, purgative, emetic stimulant, astringent, antiseptic [31,4]
LEGUMNIOSAE *Melilotus officinalis L.*	Kashkarbeda [a] melilot [b]	skin disease, after burn, lung disease, antiseptic, antibacterial activity, digestive, rheumatic pains [4]
LEGUMINOSAE *Medicago sativa*	Beda [a] Lucerne [b]	Stomach ulcers [5]
LEGUMINOSAE *Glycurrhiza glabra*	Lakriza [a] Liquorices [b]	wounds, allergic rashes, skin infections, bronchitis, cough, sore throat laryngitis, bronchitis, chest infections, stomach ulcers [2,3,4]
LEGUMINOSAE *Sophora pachicarpa*	Achchik miya [a] Indigo [b]	typhus, stimulant, antiseptic [4]
LEGUMINOSAEA *Psoralea drupaceaBge.*	Danakli okkuray [a]	Eczema, pigment disorders, skin disease, infections, antibacterial against skin disease [3,4]
LILIACEAE *Allium pskemense* *Allium aflatunense* B. Fedtsch.	Onion pskemskyi [a]	diaphoretic, diuretic, expectorant, stimulant, antiseptic, wounds, burn, skin, cough, antiseptic, diarrhoea.
LILIACEAE *Asparagus officinalis*	Sarsabil [a] Asparagus [b]	kidney, urinary tract, gall bladder infections, diuretic [5]
LINACEAE *Linum humile Mill*	Zigir [a] Flax [b]	lung inflammation, stomach disorders, gastritis, diuretic, tonic, diabetes, dropsy, kidney disease [4,5]
OLEACEAE *Frxinus excelsior*	shumtol [a] ash [b]	dropsy, malaria fever, diuretic [5]
PAEONIACEAE *Paeonia hybrida* Pall.,	peony [b]	dissolve stones from bladder and kidney
PAPAVERACEAE *Papaver rhoeas*	kizgaldok [a] Poppy [b]	angina, asthma, sleeping drug
PLANTAGINACEAE *Plantago ovata*	Plantain [b]	wounds, allergic rashes, skin infections, diarrhoea, dysenteric,

		inflammation, urinary tract [1,4]
PLANTAGINACEAE *Plantago major*	Zubturum [a] Plantain [b]	stomach disease, against blood solidity, headache, pains, skin infections [3,4]
PLANTAGINACEAE *Plantago psyllium*	Podorojnik [a] Plantain [b]	cough, bleeding, inflammation, wounds, headache, rheumatism skin infections, digestive ailments, wound bleeding. [4,5]
RANUNCULACEAE *Adonis tianschanica L.*	Tyanshan adonisi [a] adonis [b]	heart disease, kidney disease
RHAMNACEAE *Rhamnus cathartica L.*	Itjumrut [a] Buckthorn [b]	against various cancer disease , tonic, cathartic
ROSACEAE Potentilla	Gozpanja [a] Five fingers [b]	diarrhoea, leucorrhoea, antiseptic activity, diarrhoea gastrointestinal disease [4]
ROSACEAE *Sorbus tianschanica*	Chetan [a] Ash tianshan [b]	diarrhoea, leucorrhoea [4]
ROSACEAE *Prunus sogdiana* *Prunus divaricata L.*	Dikayaolcha [a] Cherry wild [b]	tonic, pectoral, bronchitis, nervous cough, dyspepsia [4]
ROSACEAE *Rosa canina*	Namatak [a] Dog rose [b]	digestion, against hypertonic, kidney cleaning purpose
ULMACEAE *Ulmus densa*	Karagach [a] Elm [b]	digestive ailments, intestinal infectious, diarrhoea, skin infections, stomach ailments, gastric ulcers [4,31]
URTICACEAE *Urtica dioica l.*	Gazanda [a] Nettle [b]	liver disease, stopping blood flow, skin disease. gastrointestinal disease, diuretic [3,4]
UMBELLIFERAE *Ferula moschata* *Ferula assa-foetida* *Ferula juniperina*	Kowrak [a] Asafetida [b]	tuberculosis, diabetes, stomach disorders, gastrointestinal disease, dyspepsia, allergic rashes, skin infections, stimulant, asthma [4,31]

ZYGOPHYLLACEAE *Peganum harmala* L.	isirik [a]	nervous disease, nervous inflammation, arthritis, cold

[a] Uzbek, b English

1.Khodjimatov K. KH. Aprasidi G.S., A.K Khodjimatov. Wilde type medicinal plants of Central Asia. Tashkent, 1995, 2. Newton SM, Lau C, Wright CW. A review of antimycobacterial natural products. Phytother Res 2000;14:303–22, 3. Dennis Furnell, 1985.Health from the Hedgerow, Batsford Ltd., 4. A Modern herbal By M. Grieve 1998 tiger books International, London, Reid and Betts (1979)

In table 3 presented some of medicinal plant description, however there are a much more medicinal plants growth in Chatkal Biosphere Reserve which identified and keep in Institute of Botany in Uzbekistan. But not all plants are studied deeply from that Biosphere Reserve and also their microbial association. We have studied the microbial population of different soil type from different ecosystems, and microbial physiology also investigated.

3.2. Soil chemical and physical analysis

The physical and chemical analysis of soil samples taken from different altitude of Chatkal Biospere Reserve showed hat they differ from each other with their mineral content also with texture. Soil physical content demonstrate that sand content of typical serozem soil higher compare other soil types. Leached brown soil has more clay than sand content (Table 4).

Table 4. Soil physical characterization (%) of Chatkal Biosphere Reserve

Soil	Soil depth, cm	1-0,25	0,25-0,1	0,1-0,05	0,05-0,01	0,01-0,005	0,005-0,001	<0,001
Typical serozem	0-20	6,6	6,7	16,0	31,5	13,7	13,3	12,2
	20-30	6,7	6,2	15,7	28,5	14,2	13,7	15,0
	30-45	5,9	5,7	10,2	33,4	15,5	13,9	15,4
	45-70	4,3	3,7	9,5	28,6	15,9	14,0	24,0
	70-120	2,4	2,1	7,4	32,4	15,7	13,6	26,4
Dark serozem	0-20	0,2	0,1	8,8	50,4	15,1	18,0	7,4
	20-30	0,2	0,1	6,5	43,6	17,7	19,1	12,8
	30-45	0,2	0,2	2,1	51,2	10,1	22,7	13,5
	45-70	0,1	0,3	0,9	47,9	15,3	22,8	12,7
	70-120	0,3	0,2	2,1	47,0	13,4	23,1	13,9
Brown carbonate	0-20	0,3	0,6	4,4	40,1	21,6	21,6	11,7
	20-30	0,6	1,7	1,7	39,4	23,5	21,0	12,1
	30-45	0,4	1,6	9,0	42,6	20,4	18,4	7,6
	45-70	0,8	2,0	10,7	40,1	19,7	17,2	9,5
	70-120	0,6	7,9	11,8	34,2	16,9	16,8	11,8
Typical brown	0-20	1	1,3	7,0	44,0	18,1	20,1	8,5
	20-30	0,9	1,0	6,8	40,0	15,3	22,8	14,0
	30-45	0,8	0,8	6,3	39,7	16,8	21,2	14,4
	45-70	1,6	0,6	6,5	38,6	15,9	21,3	16,1
	70-120	1,5	0,5	6,7	37,0	15,2	21,1	19,0
Leached brown	0-20	1,4	2,0	10	40,1	20,4	17,8	8,3
	20-30	0,2	2,0	4,1	38,8	22,3	26,1	8,3
	30-45	0,3	0,4	3,9	31,8	22,3	24,8	16,5
	45-70	0,4	0,7	5,6	30,5	17,7	23,5	21,6
	70-120	0,9	1,8	7,7	25,4	16,5	24,2	23,5

The chemical characterisation of soil samples taken from different sites and altitudes of Cahtakl Biosphere Reserve showed that typical brown soil has more C and N content to compare typical, dark serozem, brown carbonate soil and leached brown soil. C/N rate ranges between 10 and 12, which shows that those soils are more fertile

and productive (table 5a,b; 6a,b; 7a,b; 8a,b; 9a,b). This type of soil has typical humus horizon. Distribution of humus in soil profile has definite appropriateness. Upper horizon of soil has more root residues and it includes more humus content. In typical brown soil the humus content riches 3.47%, where typical other soils has 0.5 to 1 % of humus content.

Nitrogen (N) content of soil changes in accordance with humus content. The typical brown soil has much more nitrogen than other types, it ranges between 0.162 to 0.201 %, where typical serozem soil has 0.057-0.090%. N content decreases with deepening soil layer (from 0-30 to 70-120 cm soil depth). C/N ration most wide in typical brown soil (10.01-12.27), lower in brown carbonate soil (3.59). Most significant ratio C/N riches on upper turf horizon, and with deeper magnitude its decreasing. In spite of poor organic matters and nitrogen of soil, the phosphorus content is high in studied soils. Higher phosphorus content was observed in typical brown soil which was taken from 1300 – 1600 m height. The potassium content also differ regarding soil types. The high content of potassium observed in typical brown soil (2.400%), where serozem soils have 1,30-1,40 %, and leached brown soil has 2,00% potassium content. Distribution of carbonate in soil profile perform important index. Carbonate profile of soil serve as diagnostic sign for eroded soils (Горбунов и др., 1972). Our investigations demonstrated that in brown carbonate soil the carbonate content is high and ranges between 20-25% where typical serozems has low carbonate content (10-14%).

Table 5a. Soil characterization of typical serozem soil (500-700m).

Soil depth, cm	Humus content %	%			mg/kg		C:N	SO_4 gypsum %	CO_2 carbonate %
		N	P	K	P_2O_5	K_2O			
0-20	1,009	0,090	0,150	1,90	50,0	240,0	6,50	0,143	6,211
20-30	0,887	0,080	0,132	1,80	42,0	220,0	6,43	0,150	6,230
30-45	0,712	0,075	0,120	1,80	26,0	180,0	5,50	0,149	6,270
45-70	0,465	0,050	0,080	1,60	10,0	140,0	5,39	0,153	6,340
70-120	0,335	0,040	0,072	1,40	8,0	120,0	4,85	0,162	6,500

Table 5b. Soil chemical analysis of typical serozem (500-700 m).

Soil depth, cm	HCO_3 %	Cl %	SO_4%	Anions %	Ca %	Mg %	Na,%	K, %	Kations %
0-20	0,048	0,007	0,037	0,092	0,020	0,009	0,001	0,003	0,033
20-30	0,036	0,007	0,017	0,060	0,010	0,006	0,0007	0,002	0,0187
30-45	0,032	0,007	0,017	0,059	0,010	0,06	0,0007	0,002	0,0187
45-70	0,034	0,007	0,018	0,069	0,010	0,006	0,0007	0,001	0,0186
70-120	0,042	0,007	0,020	0,069	0,010	0,009	0,0007	0,001	0,0207

Table 6a. Soil chemical analysis of dark serozem (600-1100 m)

Soil depth, cm	Humus content %	%			mg/kg		C:N	SO_4 gypsum, %	CO_2 carbonate %
		N	P	K	P_2O_5	K_2O			
0-20	1.254	0.057	0.084	1.30	16.1	180.0	7.49	0.01	13.10
20-30	0,543	0,035	0,065	1,35	14,1	160,0	8,99	0,01	14,20
30-45	0,481	0,026	0,050	1,30	10,0	120,0	9,32	0,01	13,98
45-70	0,124	0,012	0,030	1,10	7,0	90,0	7,19	0,01	14,86
70-120	0,080	0,010	0,012	1,00	5,0	60,0	4,64	0,01	15,08

Table 6b. Soil chemical analysis of dark serozem (600-1100 m)

Soil depth, cm	HCO_3 %	Cl %	SO_4%	Anions %	Ca, %	Mg %	Na,%	K, %	Kations %
0-15	0,132	0,037	0,010	0,028	0,076	0,015	0,009	0,0007	0,002
15-40	0,090	0,025	0,007	0,018	0,050	0,010	0,006	0,0007	0,002
40-75	0,087	0,020	0,007	0,018	0,045	0,010	0,006	0,0007	0,002
75-107	0,085	0,022	0,007	0,020	0,049	0,010	0,006	0,0007	0,001
107-135	0,085	0,022	0,007	0,018	0,047	0,010	0,006	0,0007	0,001
135-175	0,083	0,018	0,007	0,018	0,043	0,010	0,006	0,0007	0,001

Table 7a. Soil chemical analysis of brown carbonate soil (900-1300m)

Soil depth, cm	Humus %	%			mg/kg		C:N	SO_4 gypsum, %	CO_2 , carbonate%
		N	P	K	P_2O_5	K_2O			
0-20	1,024	0,165	0,080	1,40	18,2	310,0	3,59	0,01	15,45
20-30	0,899	0,070	0,075	1,15	8,0	280,0	7,44	0,01	12,32
30-45	0,564	0,038	0,070	0,95	7,2	110,0	8,60	0,01	16,17
45-70	0,502	0,035	0,065	1,00	5,7	100,0	7,66	0,01	25,08
70-120	0,181	0,031	0,060	1,00	4,7	90,9	3,38	0,01	25,52

Table 7b. Soil chemical analysis of brown carbonate soil (900-1300m)

Soil depth Cm	HCO_3, %	Cl, %	SO_4 %	Anions %	Ca, %	Mg %	Na, %	K, %	Kations, %
0-20	0,060	0,007	0,040	0,107	0,020	0,009	0,001	0,006	0,036
20-30	0,045	0,007	0,021	0,073	0,015	0,006	0,0007	0,003	0,0247
30-45	0,040	0,007	0,021	0,068	0,015	0,006	0,0007	0,003	0,0247
45-70	0,036	0,007	0,020	0,063	0,015	0,006	0,0007	0,002	0,0237
70-120	0,036	0,007	0,020	0,063	0015	0,006	0,0007	0,002	0,0237

Table 8a. soil chemical analysis of typical brown soil (1300-1600 m)

Soil depth, cm	Humus %	%			mg/kg		C:N	SO_4 gypsum %	CO_2 carbonate %
		N	P	K	P_2O_5	K_2O			
0-20	3,47	0,201	0,198	2,400	21,3	490,0	10,01	0.02	6,00
20-30	3,10	0,162	0,183	2,312	12,1	460,0	11,09	0.01	6,05
30-45	2,18	0,103	0,154	2,100	9,0	380,0	12,27	0.03	6,21
45-70	1,74	0,090	0,131	1,913	7,0	210,0	11,21	0,04	6,25
70-120	0,30	0,031	0,090	1,412	4,0	80,0	5,61	0,05	6,55

Table 9b. Soil chemical analysis of typical brown soil (1300-1600 m)

Soil depth cm	HCO_3, %	Cl, %	SO_4 %	Anions %	Ca, %	Mg %	Na, %	K, %	Kations, %
0-20	0,100	0,045	0,003	0,017	0,065	0,015	0,006	0,003	0,0030
20-30	0,122	0,045	0,003	0,022	0,070	0,015	0,006	0,003	0,0027
30-45	0,107	0,045	0,003	0,017	0,065	0,010	0,006	0,002	0,0027
45-70	0,075	0,036	0003	0,010	0,049	0,010	0,003	0,002	0,0023
70-120	0,027	0,036	0003	0,019	0,058	0,010	0,006	0,002	0,0022

Table 9a. Soil analysis of leached brown soil (1600-2000 m)

Soil depth, cm	Humus %	%			mg/kg		C:N	SO_4 gypsum %	CO_2 carbonate %
		N	P	K	P_2O_5	K_2O			
0-20	0,700	0,076	0,080	2,00	20,0	200	5,34	-	8,10
20-30	0,473	0,048	0,055	2,20	16,8	140	5,71	-	10,08
30-45	0,400	0,028	0,035	1,95	15,1	130	4,55	0,080	12,88
45-70	0,360	0,020	0,030	1,70	13,2	120	4,06	0,150	13,20
70-120	0,345	0,015	0,024	1,40	12,8	115	2,70	0,152	14,10

Table 9b. Soil analysis of leached brown soil (1600-2000 m)

Soil depth cm	HCO_3, %	Cl, %	SO_4 %	Anions %	Ca, %	Mg %	Na, %	K, %	Kations, %
0-20	0,051	0,010	0,030	0,091	0,015	0,009	0,0007	0,004	0,0287
20-30	0,030	0,007	0,018	0,061	0,010	0,006	0,0007	0,003	0,0197
30-45	0,029	0,007	0,018	0,053	0,010	0,006	0,0007	0,003	0,0197
45-70	0,027	0,007	0,020	0,054	0,010	0,006	0,001	0,002	0,019
70-120	0,025	0,007	0,020	0,052	0,010	0,006	0,001	0,002	0,019

Our results showed that pH of typical serozem soils ranges between 7,17 to 7,30, dark serozem soil, and brown carbonate soils 7.00-7,30, leached brown soils 6,6 -7,20. The pH index rises in deeper soil layer, which has relations with effect soil formation processes. In anion content predominate SO_4^{--} and Cl^- , typical serozem soil has 0.150 % $SO_4^{--}$and Cl content was higher in typical brown soil. Increase of dry residue in deeper horizon in soils occurs due to sulphates and also minor chlorides.

In kation content predominate Ca^{++} and Mg^{++} where in typical brown soil has Ca^{++} 0,060 to 0,075 %, and Mg^{++} from 0.010 to 0.015%. Thus in mountain soils it is typical the lack of salinisation especially in upper part profile where solid residue not exceed from 0,125 %. Our investigations showed that absorbent complex of mountain soils such typical serozem, dark serozem, brown carbonate soil, typical brown, leached brown) saturated with Ca^{++} and Mg^{++} with suppression prevalence in upper horizons ions Ca^{++}. Calcium content is decreasing in deeper soil profile, where Mg increases. In typical serozem soil absorbed Ca and Mg constitute from 87.95 to 88.81 %.

3.3. Soil enzyme activities

The catalase, urease phosphotase, protease, dehydrogenase, invertase activities was generally higher in typical serozem soil taken from 500-700 m height in mountain and typical brown soil taken from 1300-1700 m elevation (Table 10). The catalase activities in typical serozem soil ranges between 3.14 to 4.30 cm^3 O_2 1 g soil /1 min, where urease activities ranges between 3.05-6.90 (100 g soil / 3h). The dehydrogenase activity was very high to compare other soil samples which range 7.26 to 8.86 (10g soil for 24

h.). The brown carbonate soil and dark serozem soil has lower enzyme activities. The catalase, urease, phosphotase and invertase avtivities were very low in such type of soil. Low soil enzyme activities might be due to the increase of clay dispersion (Lax and Garcia Orenes, 1993) which may leave the extracellular enzymes unprotected and therefore more susceptible to degradation. Kandeler et al. (1999) indicated a close relationship between enzyme activities and particle-size fractions.

Table 10. Soil enzyme activities of various soil samples taken from Chatkal Biosphere Reserve

Soil	Soil depth, cm	Catalase, cm^3 O_2 1 g soil /1 min	Urease, 100 g soil / 3h	Phosphatase PO_4^{2-} 2 g soil for 18 h.	Protease mg tyrosine 1 g soil for 24 h	Dehydrogenase mg phormasan 10g soil for 24 h.	Invertasa, mg saharasa 1 g soil for 1 h
Typical serozem	0-10	4,30	6,90	2,60	0,73	8,84	0,191
	10-30	4,11	5,95	2,00	0,52	7,26	0,170
	30-50	3,14	3,05	1,80	0,51	5,12	0,140
	50-70	2,0	1,00	1,70	0,44	3,00	0,124
Dark serozem	0-10	1,40	4,10	1,50	0,31	5,98	0,122
	10-30	0,95	1,20	0,90	0,25	3,00	0,070
	30-50	0,60	0,50	0,43	0,10	1,70	0,040
	50-70	0,32	0,18	0,10	0,04	1,00	0,005
Brown carbonate	0-10	3,10	4,90	1,61	0,31	5,80	0,140
	10-30	1,85	3,00	1,70	0,18	3,00	0,110
	30-50	1,00	1,20	1,09	0,10	2,00	0,048
	50-70	0,50	0,20	0,80	0,05	1,02	0,020
Typical brown	0-10	4,50	6,70	3,10	0,52	8,95	0,183
	10-30	3,40	5,68	2,00	0,38	4,80	0,176
	30-50	1,30	3,45	1,70	0,23	2,10	0,150
	50-70	0,70	2,60	1,00	0,15	1,30	0,050
Leached brown	0-10	2,98	6,50	2,65	0,50	6,50	0,160
	10-30	2,50	5,35	2,40	0,45	4,00	0,140
	30-50	2,14	4,10	1,85	0,25	2,10	0,110
	50-70	1,10	1,30	1,10	0,16	1,70	0,080

Moreover, the reduction of organic matter content observed in the soils could also be a cause of reduced soil enzyme activity as the soil organic matter plays an important role in protecting enzymes through immobilization in organo-mineral complexes (Tabatabai, 1994).

3.4 Soil microbial population

Marked effects were found to have taken place on the bacterial populations under different ecosystems. This is clearly demonstrated by the total number of bacteria colony-forming units (cfu) recorded from the plates. Our results showed that microbial population was different in soils under different plant covers (Fig.1).

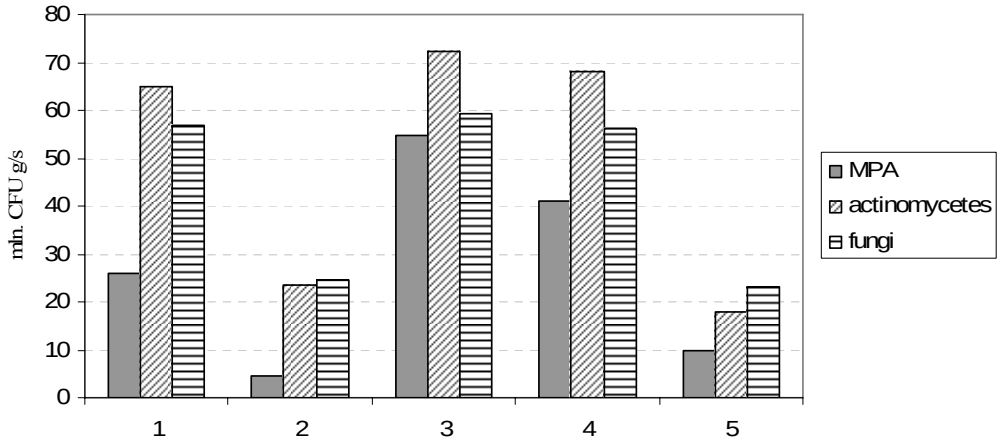


Fig. 1. The population of soil microorganisms in different soils (1- typical serozem soil, 2- dark serozem soil, 3-brown carbonate soil, 4- typical brown soil, 5- leached brown soil) in Chatkal Biosphere Reserve.

Soil microbial population analysis was studied in October (autumn), January (winter), April (spring), and July (summer). In this study, distinct differences in soil microbial populations in various soil depth, seasonal changes were analysed.

The total number of isolated bacteria varied in different samples of studied soils, and the range of variation comprised 10^5-10^8 CFU/g. Soil types had influence on soil microbial organisms, activity. The highest density of nitrifying bacteria was observed during spring, summer and the lowest in winter (Fig 2).

Among the studied soils brown carbonate and typical brown soils have the highest number of nitrifying bacteria, while leached brown soil appeared to have the lowest nitrifying bacteria (Fig.2).

In leached brown soil were found lower number of ammonifying, oligotrophic and oligonitrophilic bacteria. Bacterial density was the lowest in winter and increased gradually through spring and summer. The activity of microorganisms is known to depend on the presence of nutritive substrate, which is the source of energy and nutrients. For soil microorganisms, this substrate is presented by three major soil components: plant residues, soil solids, and soil solution. According to Entry et al., (1996) soil microbial biomass N was lower in leached brown soil.

Seasonal changes in the number of all group bacterial population showed that with increase in the atmospheric temperature, their numbers increasing (Fig.2,3,4,5). The highest number was found in spring and summer, and lowest in winter. Also Zou Li et al., (2000) found similar changes in microbial population during different seasonal time that in winter the total number of microorganisms decreased. The increased moisture content in soil in spring period and optimal temperature related to the higher atmospheric precipitation, and it enhances the development of microflora.

The number of nitrifying, oligotrophic, ammonifying, oligonitrophilic bacteria was higher at the 0-10, 10-20 cm than 20-30cm. Some authors suggest that the number of ammonifying bacteria, oligonitrophilic, oligotrophic bacteria and enzymatic activity decreased in soil sampled from deeper layers (Govedarica et al., 1995; Zou Li, et al., 2000).

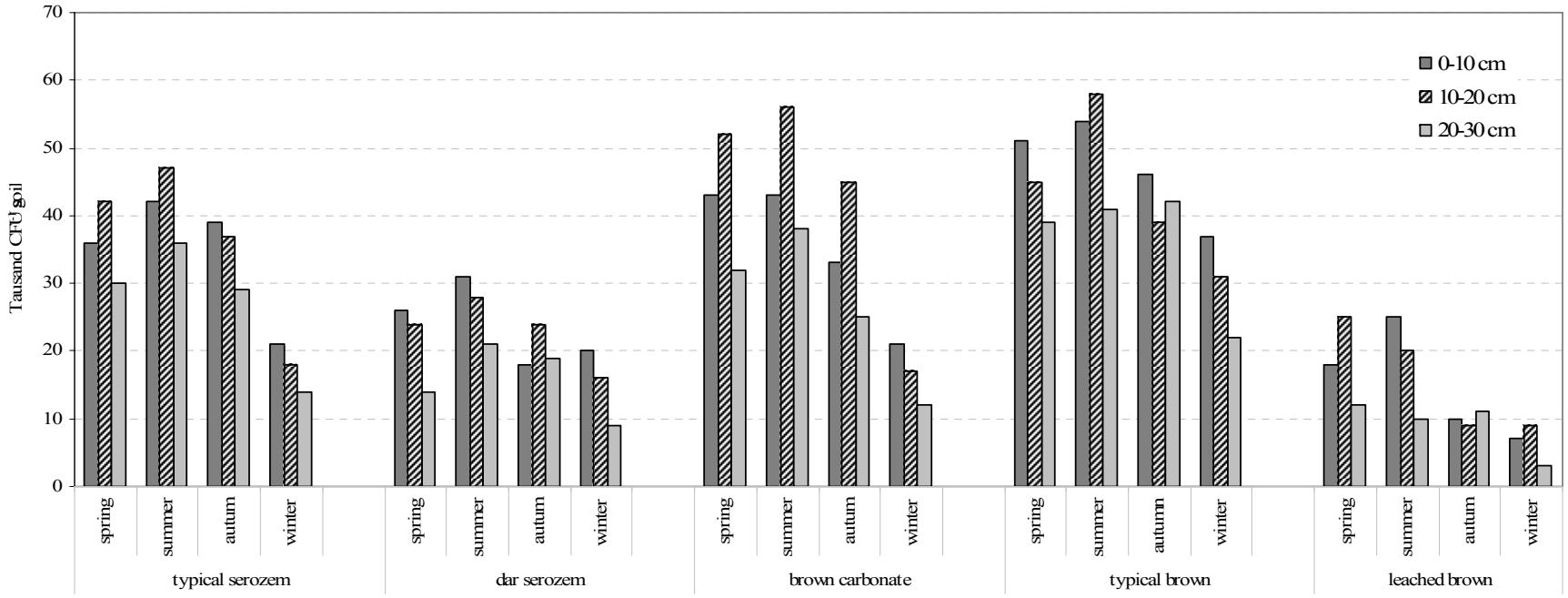


Fig.2. The numbers of nitrifying bacteria from various soils of Chatkal Biosphere Reserve.

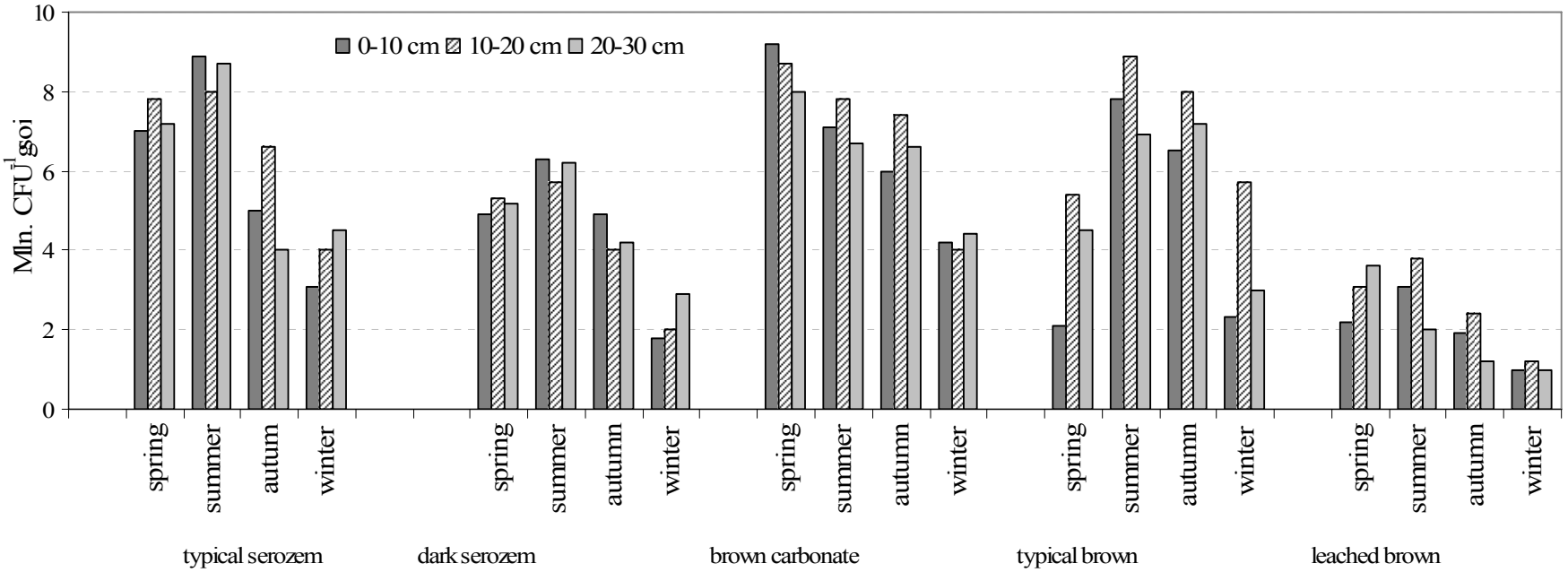


Fig. 3. The numbers of ammonifying bacteria from various soils of Chatkal Biosphere Reserve.

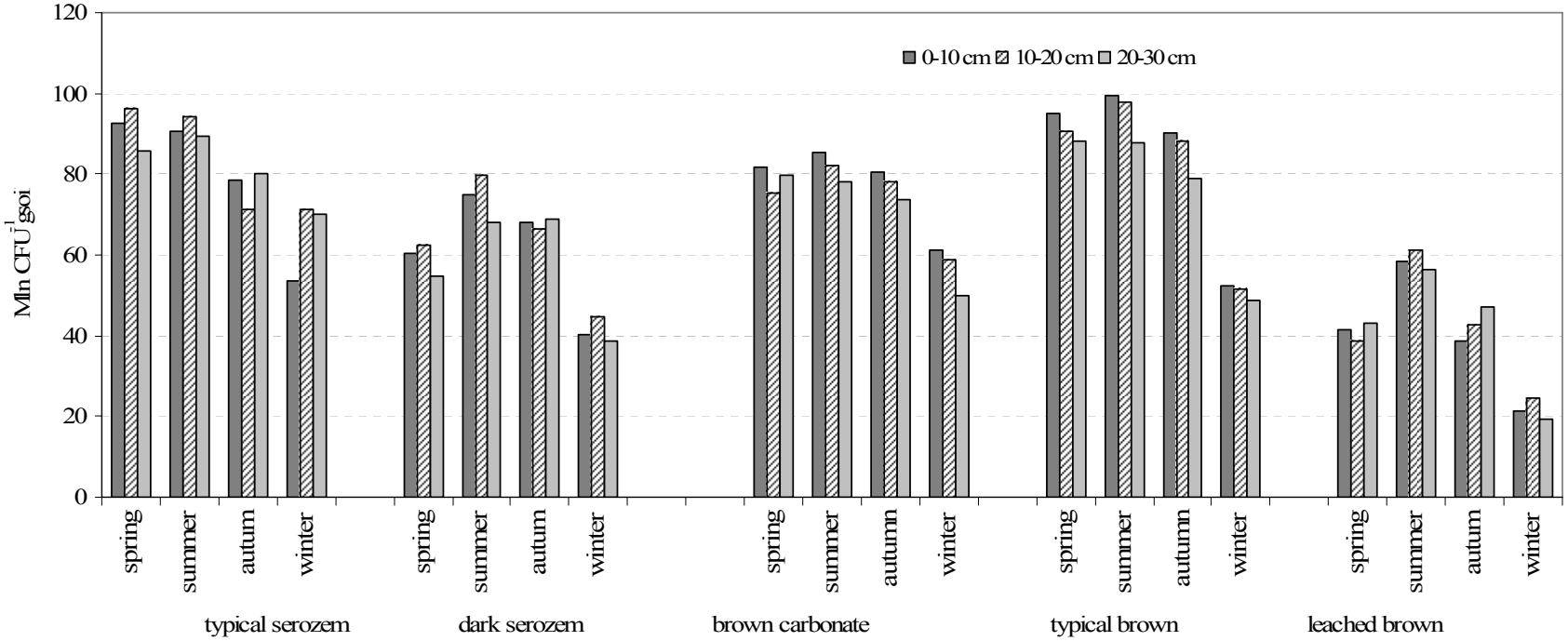


Fig.4. The numbers of oligotrophic bacteria from various soils of Chatkal Biosphere Reserve.

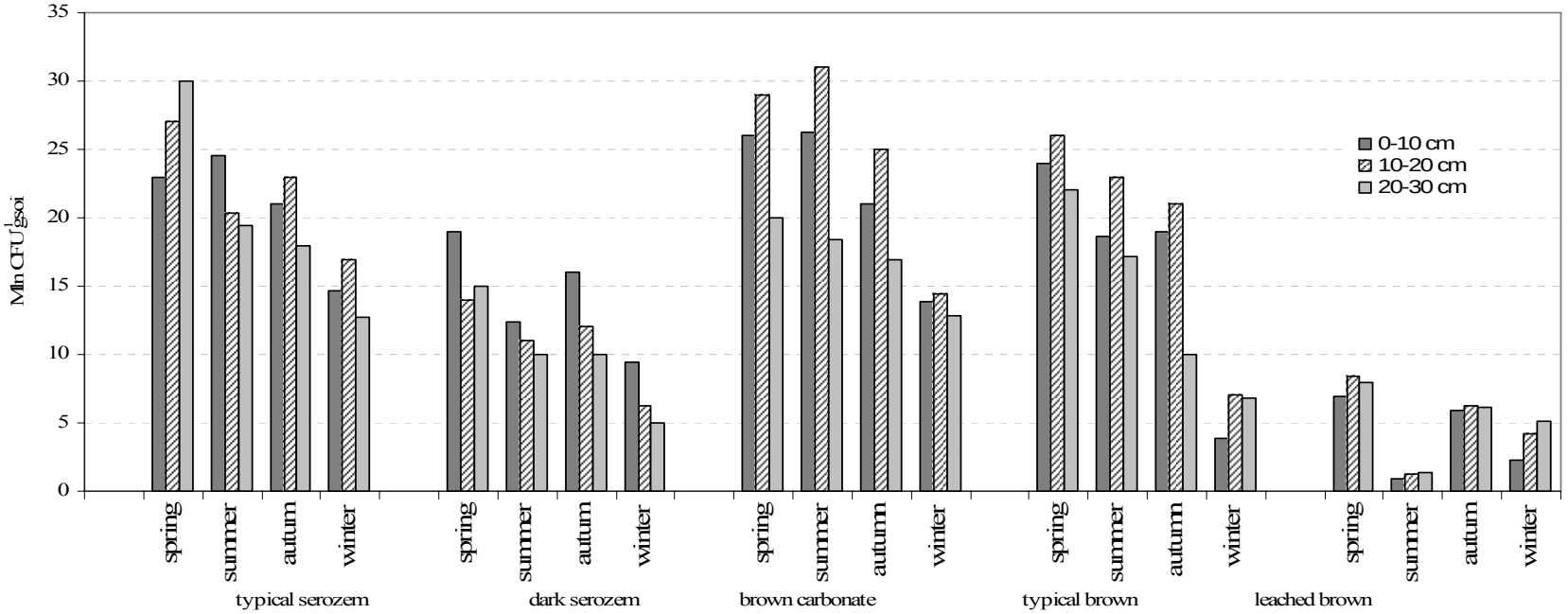


Fig.5. The numbers of oligonitrophilic bacteria from various soils of Chatkal Biosphere Reserve.

The highest density of bacterial population which growth in nutrient rich medium was observed in typical serozem soi, brown carbonate and typical brown soils, where growth plants such *Hypericum perforatum*, *Salvia sclarea*, *Ziziphora capitata Achillea millefalium Calamagrostis epigeios L.*, *Juglans regia, Persica vulgaris, Juniperus turkestanica, Ulmus densa, Hardeum leporinum, Cynodon dastrylon, Jagea stipitata. Hypericum perforatum.* The lowest bacterial populations found in dark serozem and leached brown soil soil under *Ephedra eqisetina, Malus sieversii, Juniperus turkestanica*, *Juglaus regia, Polygonum coriarum, Cerasus erythrocarpa.* The fungal and actinomyces population also were very low in those samples to compare other soils.

3.5. Rhizosphere microbial population

We also studied the rhizosphere bacterial population of *Ziziphora capitata, Salvia sclarea* and *Calamagrostis epigeios L*. The soil microbial activity under those plants showed highest (CFU) number. The analysis of rhizosphere bacterial population in those plants showed that *Calamagrostis epigeios* has highest bacterial population in the rhizosphere to compare *Salvia sclarea* and *Ziziphora capitata.* We measured bacterial cfu in KB medium (for pseudomonades), LC medium and TSA medium diluted 20 times and consider nutrient poor medium.

TSA medium KB medium LC medium

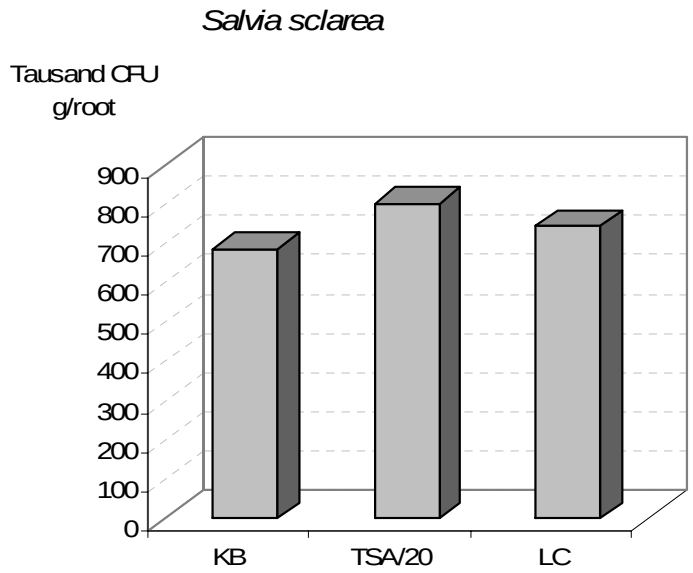


Fig.2. The rhizosphere bacterial population of *Salvia sclarea* grown in Chatkal Biosphere Reserve.

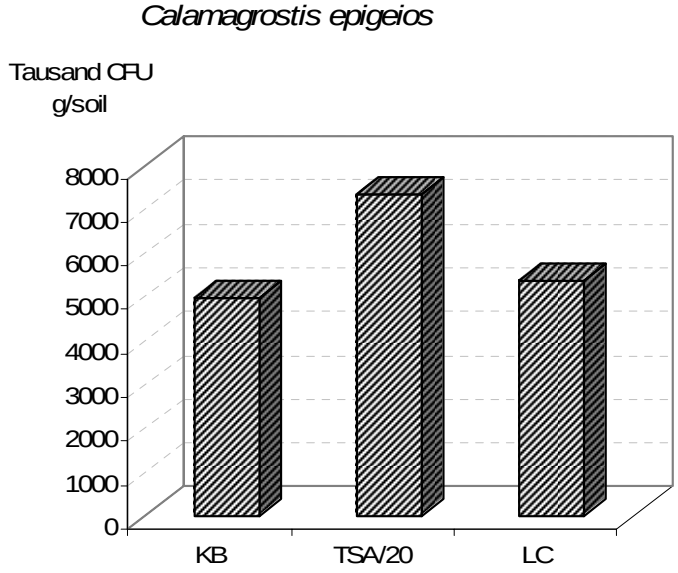


Fig.3. The rhizosphere bacterial population of *Calamagrostis epigeios* grown in Chatkal Biosphere Reserve

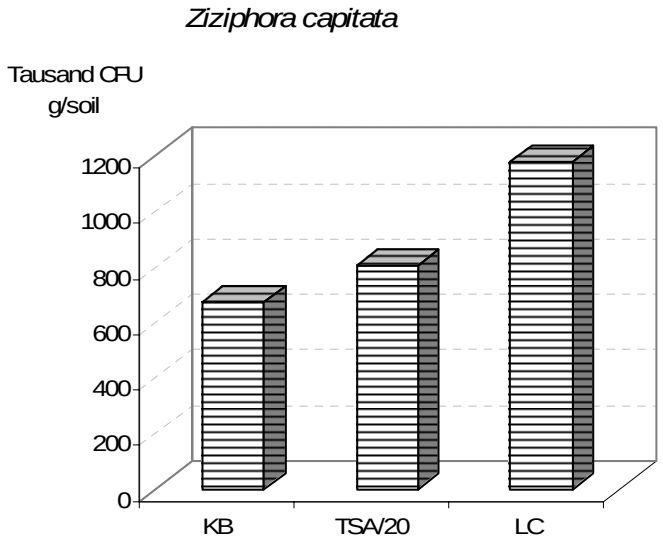
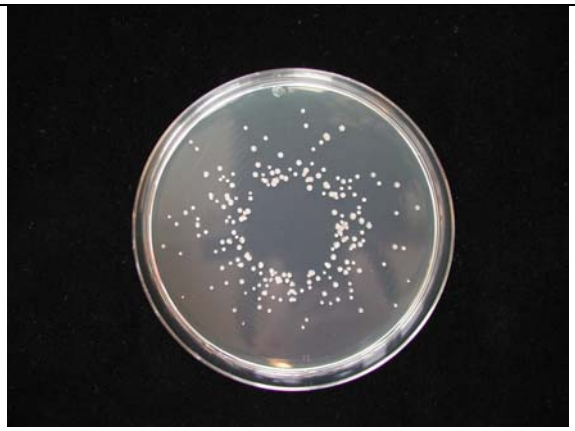


Fig. 4. The rhizosphere bacterial population of *Zizifora capitata* grown in Chatkal Biosphere Reserve.

Among the studied plants, *Ziziphora capitata* and *Salvia sclarea* tended to have similar number of culturable bacteria (Fig.2 and Fig.4). The bacterial strains isolated from the plates considering their morphological properties and purified for analysing their biochemical properties. Colony forming units of bacterial strains were shown in Picture 7.

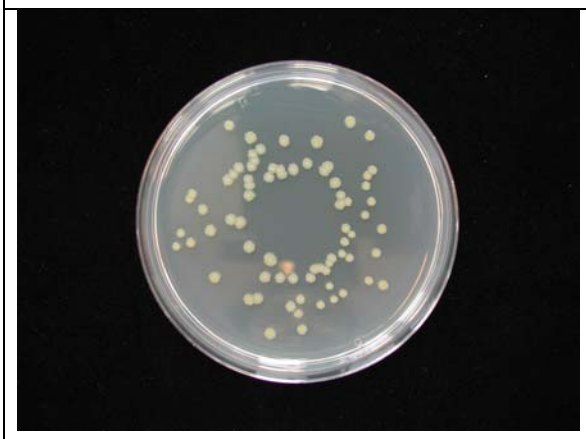
P. fluoro violaceus



Pseudomonas stutzeri

Acinetobacter spp.

Bacillus cereus



Pseudomonas denitrificans

P.`chlororaphis

Picture 7. The colonies of bacterial strains isolated from the rhizosphere of *Ziziphora capitata, Salvia sclarea* and *Calamagrostis epigeios L*

3.5.1. Bacterial diversity

From the rhizosphere of *Salvia sclarea* isolated 16 strains, from the rhizosphere of *Ziziphora capitata 15 bacterial strains,* and from the rhizosphere of *Calamagrostis epigeios L* 14 bacterial strains (table 11, 12, and 13)

Table 11. Bacterial diversity of *Salvia sclarea* grown in typical serozem soil in Chatkal Biosphere Reserve

Bacterial strains	Number	Origin
Arthrobacter simplex	S8	*Salvia sclarea*
Clavibacterium sp.	S15	*Salvia sclarea*
Bacillus licheniformis	S19	*Salvia sclarea*
Bacillus subtilis	S24	*Salvia sclarea*
Bacillus pumilus	S3	*Salvia sclarea*
Pseudomonas putida	S7	*Salvia sclarea*
Pseudomonas sp.	S2	*Salvia sclarea*
Pseudomonas `chlororaphis	S1	*Salvia sclarea*
Pseudomonas aurentiaca	S22	*Salvia sclarea*
Pseudomonas putida	S5	*Salvia sclarea*
Pseudomonas fluorescens	S18	*Salvia sclarea*
Pseudomonas corrugate	S4	*Salvia sclarea*
Pseudomonas extremophiles	S23	*Salvia sclarea*
Pseudomonas stutzeri	S14	*Salvia sclarea*
Microbacterium sp.	S25	*Salvia sclarea*
Microbacterium sp.	S13	*Salvia sclarea*

Salvia sclarea grown in typical serozem soil associated with bacterial species such *Bacillus, Pseudomonas, Microbacterium.* However *Pseudomonas* species are dominating in this species. The bacterial strains *Pseudomonas, Bacillus* common soil bacteria and have often been observed in rhizosphere studies (Kloepper et al., 1992a,).

The genus *Pseudomonas* encompasses arguably the most diverse and ecologically significant group of bacteria on the planet and is found in large numbers in all of the major natural environments and also in associations with plants. This universal distribution suggests a remarkable degree of physiological and genetic

adaptability (Spiers et al., 2000). Their association with plant materials has been related both to their antagonistic activities towards pathogens and to their ability to colonise and produce plant growth promoting compounds within the rhizosphere (Cook et al., 1995; De Weger et al., 1995). Pseudomonads, well suited to the rhizosphere because they are able to use a wide variety of carbon sources as nutrients. Like many rhizosphere bacteria, they play a role in nutrient cycling, can be plant growth-promoting rhizobacteria (PGPR), and may act as biological control agents (BCA). They exhibit a wide range of metabolic activities and are able to utilize a wide range of low molecular mass organic compounds, and some more complex compounds, as carbon and energy sources (Misco and Germida 2002). In early studies Stainer et al. (Steiner et al. 1966) described the remarkable capacity of *Pseudomonas* strains to degrade a wide range of substrates including aromatic compounds, halogenated derivatives and growth characteristics of 267 strains on 146 different organic compounds, plus a wide range of associated tests

Ziziphora capitata grown in typical brown soil associated with more bacterial diversity, which includes *Bacillus Pseudomonas*, *Arthrobacter, Microbacterium*, *Stenotrophomonas,* and *Clavibacterium.* This soil has more organic content and their enzyme activities higher to compare other soils (Table 12). Arthrobacters were considered to be prominent members of the autochthonous microflora of soil, maintaining population levels over long periods of nutrient shortage (Conn, 1948). The ability of arthrobacters to survive long periods of starvation or other adverse environmental pressures is well known (Gray, 1976). Most of *Arthrobacter* species refers as oligotrophs. Oligotrophs have been described as bacteria which are able to multiply in habitats of low nutrient flux (Poindexter, 1981).

Arthrobacter species are well distributed among the soil, rhizosphere, and rhizoplane fractions of various plants including sugar beet, canola and wheat (Germida et al., (1998). Similarly, Sato and Jiung (1996) found that one genus *Arthrobacter* comprised 50% of the rhizosphere population of wheat. In earlier studies Rouatt and Katznelson, (1961) suggested that *Arthrobacter* species do not appear to be stimulated in the rhizosphere and their numbers in samples of root zone soil adjusted to high moisture contents decreased, while those of pseudomonads increased (Peterson et al., 1965).

Table 12. Bacterial diversity of *Ziziphora capitata* grown in typical brown soil of Chatkal Biosphere Reserve

Bacterial strains	Number	Origin
Arthrobacter sp.	Z8	*Ziziphora capitata*
Arthrobacter globiformis	Z3	*Ziziphora capitata*
Bacillus pumilus	Z11	*Ziziphora capitata*
Bacillus amyloliquefaciens	Z13	*Ziziphora capitata*
Clavibacterium sp.	Z20a	*Ziziphora capitata*
Clavibacterium sp.	Z19	*Ziziphora capitata*
Bacillus subtilis	Z9	*Ziziphora capitata*
Bacillus pumilus	Z2	*Ziziphora capitata*
Pseudomonas aureginosa	Z12	*Ziziphora capitata*
Pseudomonas stutzeri	Z6	*Ziziphora capitata*
Pseudomonas aurentiaca	Z6a	*Ziziphora capitata*
Pseudomonas putida	Z14	*Ziziphora capitata*
Pseudomonas fluorescens	Z18	*Ziziphora capitata*
Microbacterium sp.	Z13a	*Ziziphora capitata*
Stenotrophomonas rhizophila	Z17	*Ziziphora capitata*

Calamagrostis epigeios plants associated with *Enterobacter* and *Acinetobacter* which there was not found in two other plant species (Table 13). Many of these bacteria are most often isolated from clinical and other sources (Krieg and Holt, 1984). Among of our identified strains *Pseudomonas aureginosa* reported humane pathogen bacteria, and are opportunistic pathogens causing urinary tract infections, septicaemia, and pneumonia.

Wilkinson et al., (1994) in their works isolated and identified *Acinetobacter* and *Staphylococcus saprofyticus* in low number from the rhizosphere of western australian orchids. A similar range of bacterial species such *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Staphylococcus* identified by Kloepper et al., (1992a,c) in the soil and rhizosphere of different plants.

However they are consider as humane pathogenic bacteria. The rhizosphere, rich with organic substrates stimulates microbial growth, can contain up to 10" cells gram of root in plants and the growth of human pathogens as part of this large microbial population is a major concern due to the potential effects on human health. Members of potentially pathogenic species survive and become enriched in the rhizosphere where they rapidly utilizing simple carbon sources (Gilbert , 1993)

Table 13 Bacterial diversity of *Calamagrostis epigeios L* grown in brown carbonate soil of Chatkal Biosphere Reserve

Bacterial strains	Number	Origin
Acinetobacter sp.	C15	*Calamagrostis epigeios L*
Bacillus licheniformis	C3	*Calamagrostis epigeios L*
Bacillus megaterium	C1	*Calamagrostis epigeios L*
Bacillus pumilus	C22	*Calamagrostis epigeios L*
Bacillus sp.	C7	*Calamagrostis epigeios L*
Enterobacter sp.	C40	*Calamagrostis epigeios L*
Microbacterium sp.	C14	*Calamagrostis epigeios L*
Micrococcus sp.	C9	*Calamagrostis epigeios L*
Pseudomonas sp.	C16	*Calamagrostis epigeios L*
Pseudomonas extromophilis	C42	*Calamagrostis epigeios L*
Pseudomonas stutzeri	C4	*Calamagrostis epigeios L*
Pseudomonas fluorescens	C15	*Calamagrostis epigeios L*
Pseudomonas fluorescens	C38	*Calamagrostis epigeios L*
Pseudomonas aureginosa	C6	*Calamagrostis epigeios L*

The genus *Bacillus* encompasses arguably the most diverse and ecologically significant group of bacteria on the planet and is found in large numbers in all of the major natural environments (in soils of all kinds, ranging from acid to alkaline, hot to cold, and fertile to desert). Their persistent spores readily survive distribution from natural environments to a wide variety of other habitats (Logan, 2002). This universal distribution suggests a remarkable degree of physiological and genetic adaptability. The majority of the termophilic bacteria investigated belong to the genus *Bacillus*, with strains that have been isolated from mesophilic and termophilic environments

(Sunna et al., 1997). Bacterial cells introduced into soil should be able to rapidly adapt to soil conditions in order to persist and reproduce. Survival strategies depend on the physiological adaptation in the introduced cells, such as adaptation to nutrient-limited conditions an/or other physical chemical conditions, efficient utilization of root-released compounds or specific interactions with plants.(Overbeek and Elsas, 1997). *Bacillus* species are also a major component of the microbial flora, which live in close association with various types of agricultural crops.

3.5.2. Antagonistic activity

All those bacterial isolates also were examined in the agar plate bioassay for their antagonistic activity against *Fusarium oxysporum, Botrytis cinerea, Phytium ultimum, Fusarium culmorum, Fusarium solani, Alternaria alternate and Gaeumannomyces graminis* f. sp. t*ritici* (Ggt) Table 14, 15, 16.

Table 14. Antagonistic activity of bacterial strains isolated from the rhizosphere of *Salvia sclarea*

Strain	*Fusarium oxysporum*	*Botrytis cinerea*	*Phytium ultimum*	*Fusarium culmorum*	*Fusarium solani*	*Gaeumanno-myces graminis*	*Alternaria alternata*
S8	+	-	-	+	+	+	+
S15	+	-	-	-	+	+	+
S19	+	-	+	+	-	+	+
S24	+	+	+	+	+	+	+
S3	-	-	-	+	-	-	+
S7	+	+	+	+	+	+	+
S2	+	-	-	+	-	-	-
S13	+	-	-	+	-	-	+
S22	+	-	-	+	-	+	-
S5	+	-	+	+	-	+	+
S18	+	-	-	+	-	-	+
S4	+	+	-	+	+	+	+
S23	+	-	-	+	+	+	+
S14	-	-	-	+	-	+	+
S25	+	-	-	+	-	+	+
S1	+	+	+	+	+	+	+

Sixteen bacterial strains isolated from the rhizosphere of *Salvia sclarea* tested for their antagonistic activities against pathogenic fungi and almost all bacterial strains showed antagonism against pathogenic fungi. *Bacillus subtilis* S24 and *Pseudomonas clororaphis* S1 showed antagonistic activity towards all plant fungal pathogens.

Table 15. Antagonistic activity of bacterial strains isolated from the rhizosphere of *Ziziphora capitata*

Strain	*Fusarium oxysporum*	*Botrytis cinerea*	*Phytium ultimum*	*Fusarium culmorum*	*Fusarium solani*	*Gaeumann omyces graminis*	*Alternaria alternata*
Z8	-	-	+	+	+	+	+
Z3	-	-	-	-	-	-	-
Z11	-	-	-	-	-	-	+
Z13	+	-	-	+	+	+	+
Z20a	-	-	-	-	-	-	-
Z19	-	-	-	-	+	-	-
Z9	-	-	-	-	-	-	-
Z2	-	-	-	-	-	-	-
Z12	+	-	+	+	-	+	+
Z6	-	-	-	-	+	-	-
Z6a	+	-	-	-	-	-	-
Z14	-	-	-	-	-	-	-
Z18	-	-	-	-	-	-	-
Z13a	+	-	+	+	-	+	+
Z17	+	-	-	+	+	-	-

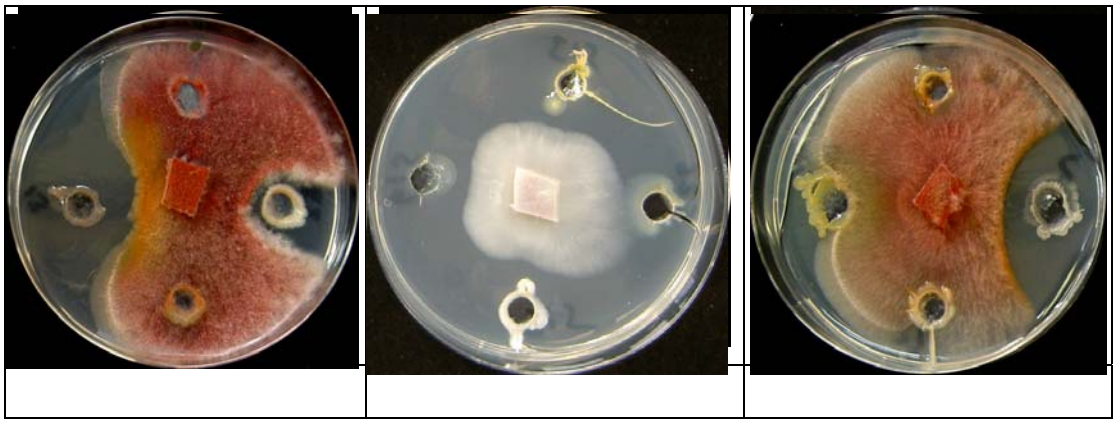
The bacterial strains isolated from Ziziphora capitata showed lower antagonistic activity . Arthrobacter sp. Z8, bacillus amyloliquefaciens Z13, Microbacterium Z13a, and Pseudomonas Z12 inhibited 5 fungal pathogens in plate assay.

Among strains isolated from *Calamagrostis epigeios L* only one strain were antagonist against six pathogenic fungi (Table 16).

Table 16. Antagonistic activity of bacterial strains isolated from the rhizosphere of *Calamagrostis epigeios L*

Strain	*Fusarium oxysporum*	*Botrytis cinerea*	*Phytium ultimum*	*Fusarium culmorum*	*Fusarium solani*	*Gaeumannomyces graminis*	*Alternaria alternata*
C15	-	-	-	-	-	-	+
C3	-	+	-	-	-	-	-
C1	-	-	-	-	-	-	-
C22	+	-	-	+	+	-	-
C7	+	-	-	+	+	+	+
C40	+	-	+	+	+	-	+
C14	-	-	-	-	-	-	-
C9	-	-	-	-	-	-	+
C16	-	-	-	-	-	-	-
C42	+	-	-	+	-	+	+
C4	+	-	-	+	+	+	+
C10	+	-	-	-	-	-	-
C38	+	-	-	-	+	-	-
C6	-	-	-	-	-	-	-
C25	+	-	+	+	+	+	+

However bacterial strains isolated from *Salvia sclarea* showed more antagonistic activity, they inhibit more pathogens with producing actively antifungal compounds. Antagonistic activity of some strains is shown in Picture 6. Most of bacterial strains showed wide zone of inhibition of pathogenic fungi in plate experiments. For example Strain *Pseudomonas putida* S7 and *Pseudomonas clororaphis* S1 produced large amount of antifungal compounds and inhibited the growth of *Fusarium oxysporum, Botrytis cinerea, Phytium ultimum, Fusarium culmorum, Fusarium solani, Alternaria alternate and Gaeumannomyces graminis* f. sp. t*ritici* (Ggt)



Picture 8. Antagonistic activity of bacterial strains isolated from the rhizosphere of *Ziziphora capitata, Salvia sclarea* and *Calamagrostis epigeios L* against pathogenic fungi.

3.5.3. Auxin production

Auxin production was tested in the absence and presence of the auxin precursor tryptophan. Results showed that bacterial strains isolated from 3 tested plants are able to produce phytohormone IAA in the absence and presence of the tryptophan in different amount. (Table 17). The presence of tryptophan in the medium substantially enhanced the IAA production. Low amount auxin production was detected in cells grown in the absence of tryptophan.

Table 17. Auxin production of bacterial strains isolated from S*alvia sclarea*

Bacterial strains	No Tryptophan.	Tryptophan.
Arthrobacter simplex S8	0.355	0.572
Clavibacterium sp. S15	0.336	0.371
Bacillus licheniformis S19	0.6	2.153
Bacillus subtilis S24	0.129	0.18
Bacillus pumilus S3	0.118	0.169
Pseudomonas putida S7	0.728	0.714
Pseudomonas sp. S2	0.158	0.581
Pseudomonas chlororaphis S13	0.142	0.427
Pseudomonas aurentiaca S22	0.514	2.062
Pseudomonas putida S5	0.262	1.048
Pseudomonas fluorescens S18	0.238	0.706
Pseudomonas corrugateS4	0.222	0.226
Pseudomonas extremophiles S23	0.116	0.175
Pseudomonas stutzeri S14	0.633	0.817
Microbacterium sp. S25	0.824	0.921
Microbacterium sp S13	0.666	0.929

Results obtained from 3 days old cultures isolated from *Ziziphora capitata* shown in Table 18. The strains *Bacillus amyloliquefaciens* Z13, *Microbacterium sp.* Z13a and pseudomonas sp. Z12, produced IAA wich ranges from 0.326 to 0.603 μg/ml cultural medium without tryptophan added. With tryptophan addition in cultural medium the amount of produced IAA is increase. *Arthrobacter globiformis* Z3 and *Bacillus pumilis* Z2 produced low amount of IAA, also with tryptophan.

Table 18. IAA production of bacterial strains isolated from *Ziziphora capitata*

Bacterial strains	No Tryptophan.	Tryptophan
Arthrobacter sp. Z8	0.14	0.127
Arthrobacter globiformis Z3	0.075	0.08
Bacillus pumilus Z11	0.085	0.089
Bacillus amyloliquefaciens Z13	0.603	1.969
Clavibacterium sp.Z20a	0.104	0.112
Clavibacterium sp. Z19	0.173	0.165
Bacillus subtilis Z9	0.108	0.113
Bacillus pumilus Z2	0.101	0.107
Pseudomonas sp Z12	0.428	0.871
Pseudomonas stutzeri Z6	0.129	0.13
Pseudomonas aurentiaca Z6a	0.182	0.273
Pseudomonas putida Z14	0.123	0.132
Pseudomonas fluorescens Z18	0.125	0.13
Microbacterium sp. Z13a	0.326	0.825
Stenotrophomonas rhizophila Z17	0.21	0.137

Table 19. IAA production of bacterial strains isolated from *Calamagrostis epigeios*

Bacterial strains	No Tryptophan	Tryptophan
Acinetobacter sp. C15	0.613	1.027
Bacillus licheniformis C3	0.174	0.408
Bacillus megaterium C1	0.113	0.099
Bacillus pumilus C22	0.343	0.33
Bacillus sp. C7	0.273	0.183
Enterobacter sp. C40	0.24	0.301
Microbacterium sp. C14	0.458	0.555
Micrococcus sp. C9	0.707	0.591
Pseudomonas sp.C16	0.099	0.097
Pseudomonas extromophilis C42	0.115	0.097
Pseudomonas stutzeri C4	0.263	0.309
Pseudomonas fluorescens C25	0.339	0.289
Pseudomonas fluorescens C38	0.401	0.287
Pseudomonas aureginosa C6	0.823	1.086

The bacterial strains isolated from *Calamagrostis epigeios* produced more auxin production (Table 19). A time course showed that the presence of tryptophan strongly stimulated auxin production in the bacterial strains and that *P. aeruginosa C6* and *Micrococcus sp. C9* produced auxin independent of exogenously added tryptophan (Table 19).

3.5.4. Phenotypic properties

The bacterial strains were tested for production of the volatile HCN and for their exo-enzymatic activities (Table 20, 21, 22). *Arthrobacter simplex* S3 and *Pseudomonas stutzeri* S14 produces hydrogen cyanide. Tree strains secrete the exoenzymes glucanase and protease (Table 20).

Table 20. Biochemical characterisation of bacterial strains isolated from the rhizosphere os *Salvia sclarea.*

Strains	cellulase	protease	glucanase	Km resistance	HCN
Arthrobacter simplex S8	-	+	-	-	+
Clavibacterium sp. S15	-	+	+	-	-
Bacillus licheniformis S19	-	-	-	-	-
Bacillus subtilis S24	-	+	-	+	-
Bacillus pumilus S3	-	+	-	-	-
Pseudomonas putida S7	-	+	-	+	-
Pseudomonas sp. S2	-	-	-	-	-
Pseudomonas chlororaphis S13	-	-	-	-	-
Pseudomonas aurentiaca S22	-	+	-	-	-
Pseudomonas putida S5	-	+	-	-	-
Pseudomonas fluorescens S18	-	-	-	-	-
Pseudomonas corrugateS4	+	+	+	-	-
Pseudomonas extremophiles S23	-	-	-	-	-
Pseudomonas stutzeri S14	+	-	-	-	+
Microbacterium sp. S25	-	+	-	-	-
Microbacterium sp S13	+	+	+	-	-

Some bacterial strains produce protease and glucanase enzymes and also hydrogen cyanide (HCN).

Table 21 Biochemical characterisation of bacterial strains isolated from the rhizosphere os *Ziziphora capitata.*

Bacterial strains	cellulase	protease	glucanase	Km resistance	HCN
Arthrobacter sp. Z8	-	+	-	+	+
Arthrobacter globiformis Z3	-	+	-	+	-
Bacillus pumilus Z11	-	-	-	+	-
Bacillus amyloliquefaciens Z13	-	-	-	-	-
Clavibacterium sp.Z20a	-	+	-	+	-
Clavibacterium sp. Z19	+	-	-	+	-
Bacillus subtilis Z9	+	-	-	+	-
Bacillus pumilus Z2	-	+	-	+	-
Pseudomonas sp Z12	-	-	+	-	+
Pseudomonas stutzeri Z6	-	-	+	+	-
Pseudomonas aurentiaca Z6a	-	-	-	-	-
Pseudomonas putida Z14	-	-	-	+	-
Pseudomonas fluorescens Z18	+	-	+	-	-
Microbacterium sp. Z13a	+	-	-	+	-
Stenotrophomonas rhizophila Z17	-	-	+	-	-

The bacterial strains *Pseudomonas extromophilis C42, Pseudomonas stutzeri C4, Pseudomonas fluorescens C38* isolated from *Calamagrostis epigeios and strains Microbacterium sp S13and Pseudomonas corrugate S4* produce tree cell wall enzymes such cellulase, protease and glucanase

Table 22. Biochemical characterisation of bacterial strains isolated from the rhizosphere of *Calamagrostis epigeios*

Bacterial	cellulase	protease	glucanase	Km resistance	HCN
Acinetobacter sp. C15	+	-	+	+	-
Bacillus licheniformis C3	-	+	-	+	+
Bacillus megaterium C1	-	-	-	-	-
Bacillus pumilus C22	-	-	-	+	-
Bacillus sp. C7	+	+	+	-	-
Enterobacter sp. C40	-	+	-	-	-
Microbacterium sp. C14	-	+	-	-	+
Micrococcus sp. C9	-	-	-	-	-
Pseudomonas sp.C16	-	-	-	-	+
Pseudomonas extromophilis C42	+	+	+	-	+
Pseudomonas stutzeri C4	+	+	+	+	-
Pseudomonas fluorescens C25	-	-	-	-	-
Pseudomonas fluorescens C38	+	+	+	-	+
Pseudomonas aureginosa C6	-	-	-	-	+

3. Conclusion

Chatkal Biosphere Reserve contains rich diversity of plants, most of them used for traditional medicine. Typical brown soil is rich with organic and nitrogen content where microbial communities higher in such soil. Soil enzyme activities are also high in typical brown soil to compare typical serozem, dark serozem, brown carbonate soil and leached brown soils. The brown carbonate and typical brown soils have the highest number of nitrifying , ammonifying, oligotrophic and oligonitrophilic bacteria, while leached brown soil appeared to have the lowest microbial population. Bacterial density was the lowest in winter and increased gradually through spring and summer. Microbial population was higher at the 0-10, 10-20 cm than 20-30cm soil depth. The rhizosphere bacterial population in plants taken from different ecosystems and altitude showed that *Calamagrostis epigeios* has highest bacterial population in

the rhizosphere to compare *Salvia sclarea* and *Ziziphora capitata.* They characterised with their high antagonistic activity, and production of phytohormone (IAA) auxin, cell wall enzyme which can protect plant from fungal pathogens and also stimulate plant growth of plants.

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