ГОДИШНИК НА МИННО-ГЕОЛОЖКИЯ УНИВЕРСИТЕТ "СВ. ИВАН РИЛСКИ", Том 55, Св. II, Добив и преработка на минерални суровини, 2012
ANNUAL OF THE UNIVERSITY OF MINING AND GEOLOGY "ST. IVAN RILSKI", Vol. 55, Part II, Mining and Mineral processing, 2012

POTENTIAL FOR BIOREMEDIATION OF CALCAREOUS SOILS BY RHIZOSPHERIC BACTERIA AND HUMIC ACIDS

Svetlana Bratkova[1], Katerina Nikolova[1], Konstantin Chakalov[2]

[1] University of mining and geology "St. Ivan Rilski", 1700 Sofia
[2] Romb ltd.

ABSTRACT. Calcareous soils, which are typical for warm and dry areas contain minerals such as calcite and dolomite, which increase pH of the soil and worsen the conditions of plants nutrition with microelements and partly with phosphorus and nitrogen. The potential for bioremediation of such soil by rhizospheric bacteria and humic acids were studied in pot vegetation test. Test plant was Medicago sativa (Alfalfa). The strains of species B. subtilis and B. amiloliquefaciens were isolated from rhizospheres of Cichorium intybus (Common chicory), inhabiting the area with carbonate soil. The used humic acids were extracted from leonardite. There isn't significant effect of microbial cultures on physical-chemical properties of the carbonate soil. The test shows that microorganisms alone or in combination with humic acid decrease the active calcium with 5 to 20%. The composition of the soil organic carbon, after treatment with humic acid, was changed to humate type. The biomass of alfalfa, after treatment of soils with microbial culture alone or in combination with humic acid, was increased in the range of 5 to 10 times.

ВЪЗМОЖНОСТИ ЗА БИОРЕМЕДИАЦИЯ НА КАЛКРЕТНИ ПОЧВИ ЧРЕЗ РИЗОСФЕРНИ МИКРООРГАНИЗМИ И ХУМИНОВИ КИСЕЛИНИ

Светлана Браткова[1], Катерина Николова[1], Константин Чакалов[2]
[1] Минно-геоложки университет "Св. Иван Рилски", 1700 София
[2] Ромб ООД

РЕЗЮМЕ. Калкретните почви, характерни за райони с топли аридни условия съдържат калциеви и магнезиеви карбонатни минерали, които повишават pH на почвите и влошават условията за хранене на растенията с микроелементи и частично с фосфор и азот. В публикацията се описва изследване на възможността за биоремедиация на такива почви чрез третирането им с ризосферна микрофлора и хуминови киселини. Проведен е съдов опит с почва от с. Скалица, Югоизточна България. Тест културата е люцерна *(Medicago sativa)*. Щамове на видовете *B. subtilis* и *B. amiloliquefaciens* са изолирани от ризосфери на диворастяща цикория (*Cichorium intybis*) обитаваща района. Хуминовите киселини са изолирани от леонардит. Опитът показва, че третирането на калкретната почва с микробната култура практически не променя физикохимичните свойства на средата. Прилагането на микробния инокулат самостоятелно или с хуминови киселини води до намаляване на активния калций от 5 до 20%. Съставът на почвения органичен въглерод при третиране с хуминови киселини се измества към хуматен тип. Биомасата на люцерната при третиране на растенията с изолираните ризосферни бактерии и с хуминови киселини поотделно или в комбинация нараства от 5 до 10 пъти.

INTRODUCTION

Carbonate soils of different texture and composition are widely exposed in the Thracian plane (Dimitrov et al. 2009). The carbonate soils are product of a specific depositional facies, which is common for warm and dry areas, usually with rainfall under 1000 mm. The main constituents of the calcrete are the minerals calcite and dolomite, although a variety of other minerals such as gypsum or chalcedony may occur in minor amounts. Critical factors in the formation of calcrete are climate; topography; vegetation; presence of carbonate and oxalic phases; carbonate content, texture, porosity and permeability of the hosting substrate; action of microorganisms as well as exposure time (which may range between a few tens of thousands and millions of years) (Ehrlich, 2001; Reith et al., 2009).

High pH and $CaCO_3$ levels in calcareous soils are predominantly responsible for low availability of plant nutrients. The presence of $CaCO_3$ in soils directly or indirectly affects the availability of N, P, Mg, and K (Brady and Weil, 2002). The alkaline pH of calcareous soil also greatly reduced the solubility of microelements Fe, Zn, Mn and Cu, which are necessary for plant growth.

Soil microorganisms play an important role in biogeochemical cycles. The rhizosphere is the zone of soil surrounding the root which is affected by it. There are many reports on plant growth promotion and yield enhancement by plant growth-promoting rhizobacteria (PGPR) (Lugtenberg et al. 2009). PGPR show direct and indirect mechanisms of plant growth stimulation such as: increasing of the fixation and administration of the atmospheric nitrogen; transformation of hard to dissolve phosphoric compounds into easily assimilated ones (Vazquez et al., 2000); production of siderophores, chelation of iron into a biologically absorbable form; microbic synthesis of physiologically active substances (phytohormones - auxins, cytokinins, gibberellins, vitamins, aminoacids, and others); synthesis of exopolysaccharides; biocontrol of infected patogenic plants, through synthesis of substances with

antibiotic and fungi-toxic action; change of membrane permeability of root cells, and an increase in the absorption capabilities of the plant roots.

The other major components of soil are humic substances (humic and fulvic acids). The humic substances in the soil might have both direct and indirect effects on plant growth (Chen and Aviad, 1990). Indirect effects involve improvement of soil properties such as aggregation, aeration, permeability, water holding capacity, ions transport and availability through pH buffering. Humic materials have an abundance of carboxyl groups and weakly acidic phenolic groups, which contribute to their complexation and ion-exchange properties. They exhibit both hydrophobic and hydrophilic characteristics and can bind to soil mineral surfaces. Humic mateials are able to complex various cations and serve as a sink for polyvalent cations in the soil.

The objectives of this study were to investigate some soil parameters and the structure of microbial communities in the depth of agricultural soil, affected by formation of calcrete; to determine the effect of plant growth-promoting bacteria and humic acids (separately and mixed) on some agrochemical parameters and the growth of *Medicago sativa* (Alfalfa) in calcareous soil.

MATERIALS AND METHODS

Sampling

The sampling site for this study is located about 1 km northwest of the village Scalitsa (WGS84, Lat/Lon: 42.275224° N, 26.242694° E). The sampling point is located at about 170 m height above sea level. The topography is dominated by gentle hills. The petrocalcic horizon occurs 25 - 35 cm under the surface and its thickness is between 1.0 and 3.0 m. This layer contains various amounts of calcite and dolomite. Soil samples were collected with a bore-hole equipment at depths 0-20, 20-40 and 40-60 cm.

Application of rhizospheric bacteria and humic acid

The soil used in this study was collected from 0-40 cm depth of the field in summer. Air-dried soil samples were passed through 4 mm-sieve. The soil was homogenized and put into plastic pots with volume 10 dm^3. Basal fertilizer was applied to the pots before planting. Each pot was treated with 100 ml solution, consisting macroelements nitrogen, phosphorus and potassium in concentration KH_2PO_4 – 1 g/l and NH_4NO_3 – 2 g/l.

The rhizospheric bacteria and humic acids are applied according the scheme, represented in Table 1. Each application consists of three replications.

Table 1.
The scheme of applications

Variant		Quantity per pot
1	control	-
2	CI R1 - CI R4	100 ml (10 ml/l)
3	Humic acids	100 ml (50 g HA/l)
4	CI R1 - CI R4 + Humic acids	100 ml (10 ml/l + 50g HA/l)

The strains CI R1, CI R2, CI R3 and CI R4 were isolated from rhizospheres of two plants *Cichorium intybus* (Common chicory). 16S rRNA gene nucleotide sequences were used for identification of four strains (Bratkova et al. 2012). The strains CI R1 and CI R3 were found to belong to the species *Bacillus subtilis* and the strains CI R2 and CI R4 to the species *Bacillus amyloliquefaciens.* For the purpose of the study the strains CI R1, CI R2, CI R3 and CI R4 were growth on microbial medium consisting nutrient broth, glucose and yeast extract. They were incubated for 24 h on a rotary shaker at 200 rpm at 30°C. The obtained microbial cultures were mixed and diluted a hundredfold. The variant 2 was treated once with 100 ml diluted microbial solution.

The humic acids were derived from leonardite. The obtained potassium humate were applied to variant 3 as 100 ml solution at the dose 50 g/l humic acids. The last variant of the experiment was treated with 100 ml solution containing both microbial species and humic acids at the same concentrations.

0.5 g alfalfa (*Medicago sativa)* were sown per pot at a depth of 5 mm into the soil. After a two month vegetation period, the shoot biomass was carefully cut off.

Study methods

Chemical analysis. The pH determination was performed according to the International Standart БДС ISO 10390 - an instrumental method for the routine determination of pH using a glass electrode in a 1:5 (V/V) suspension of soil in water (pH-H_2O).

Total carbon concentrations were determined by dry combustion via an elemental analyzer (The International Standart ISO10694). The carbon presented in the soil was oxidized to carbon dioxide (CO_2) by heating the soil to at least 900°C in a flow of oxygen-containing gas that was free from carbon dioxide. The amount of released carbon dioxide was then measured by infrared detection method.

The organic carbon content in soil was determined due to the International Standart БДС ISO 14235 by oxidation in a mixture of dichromate solution and sulfuric acid at a temperature of 135°C.

Total nitrogen (ammonium-N, nitrate-N, nitrite-N and organic N), content in soil was determined by Kjeldahl digestion, according the International Standart БДС ISO 11261 l.

Total phosphorus was extracted using БДС EN 13346 and determinated by spectrophotometrical analysis.

Water-soluble fraction of calcium and magnesium was established with БДС ISO 11048, using a titrimetric method of a 1:5 (m/V) suspension of soil in water.

Available phosphorous and potassium were determinate by the Egner-Riehm method, where calcium lactate was used as the extracting agent.

Exchangeable cations sodium, potassium, calcium and magnesium (Na, K, Ca and Mg) were extracted with ammonium acetate at pH 7.0 (Scholenberger and Simon 1945). Na and K were determined by Flame photometer and Ca and Mg by by edta complexometric titration.

Active calcium carbonate was determined with 0.01 M NM4-oxalate using a 1:25 soil:solution ratio and shaking 2 h at 250 opm on a reciprocating shaker (Drouineau 1942).

Microbiological analysis. The soil sample (5g) was suspended in 50 ml of a 0,9% NaCl solution. The suspension was incubated for 2 h on a rotary shaker at 200 rpm to detach cells from substratum. The turbid suspension was diluted in 10-folds steps to 10^{-8}. Count of viable microbial cells was determined by the plate- or liquid media count methods. Aerobic heterotrophic bacteria, fungi, actinomycetes, amylolytic microorganisms and nitrogenfixing bacteria were counted by plating on agar. For estimating the number of anaerobic heterotrophic bacteria, bacteria fermenting sugars with gas production, cellulose-degrading microorganisms, amonifying bacteria, denitrifying bacteria, Fe^{3+}-reducing bacteria, sulphate-reducing bacteria and nitrifying bacteria, a three-tube most-probable number technique was applied.

RESULTS AND DISCUSSION

General geochemical properties of the soil substrate

The pH of the soil (Table 2) in the Scalitsa site is in the range 8.41 to 8.85, as the higher pH values were found in depth, that is closer to the calcrete hardpan. High values of total carbon (72.6 – 86.2 g kg^{-1}) were found at a depth of 0 to 60 cm. Significant part of it is inorganic and are concentrated in calcrete nodules, which are common in the soils developed on top of calcrete. The amount of organic carbon is higher - 20 g kg^{-1} at the top layer, and it is reduced as soil depth increases to 3.3 g kg-1. Total nitrogen in the surface layer (0 to 20 cm) is about 1.8 g kg^{-1}. In depth, the nitrogen content decreases as in the 40 to 60 cm layer from the surface it is barely 0.19 g kg^{-1}. The phosphorus is similarly distributed in the soil profile. Highest phosphorus content – 1.9 g kg^{-1} was found in the top surface layer and the lowest (0.19 g kg^{-1}) - in the 40 to 60 cm layer.

Cultural community structure analyses in soil

Highest number of aerobic and anaerobic heterotrophs (more than 10^6 cells/g) was found at depth of 0-40 cm, an area with higher humidity and highest rates of organic carbon presence (Figure 1). In the rest of the soil profile the number of heterotrophic bacteria drop to $2{,}6.10^2$ cells/g. The highest number of all organic polymers-degrading bacteria had the amylolytic bacteria ($5{,}8.10^6$ - $2{,}5.10^7$ cells/g), with a maximum in the layer 0-40 cm. The number of cellulose-degrading bacteria from the surface to 20 cm depth was about 10^3 cells/g. The number of actinomycetes was in the range $4{,}8.10^4$ - $9{,}6.10^4$ cells/g in organic layer of soil profile. About the bacteria involved in the transformation of nitrogen in soil samples from 0 – 40 cm was counted a large amount of nitrogen-fixing bacteria (more than 10^6 cells/g). The number of amonifying bacteria was in the range 10^2-10^4 cells/g. The most part of denitrifying bacteria ($7{,}2.10^4$ cells/g) was found in the area 0-20 cm, as in the rest of the soil profile, their number decrease. The nitrifying bacteria were characterized with a low number (10^2 cells/g) and they were found only at the depth 0 – 20 cm. The number of sulfate-reducing bacteria was higher also from the surface to 40 cm depth. Their number in barren massive calcrete was fall to 10^1 cells/g.

According to data shown in Figure 1, it is obvious that the numbers of all investigated microbial groups in organic soil layer is 2 to 4 orders higher than those in barren massive calcrete.

Effects of application of rhizospheric bacteria and humic acids on some soil parameters and plant growth.

The data about the effects of application of microorganisms and humic acids on cation exchange capacity and exchangeable cations are given in Table 3.

Table 2.

Basic soil parameters from the Scalitsa site found during the spring sampling

Parameter	Sampling depth, cm		
	0-20	20-40	40-60
pH(H_2O)	8.41	8.69	8.85
Specific conductivity, mS/cm	0.137	0.113	0.102
Total carbon content, g/kg	72.6	71.3	86.2
Organic carbon content, g/kg	20.0	13.0	3.3
Total nitrogen content, g/kg	1.8	1.5	<0.2
Total phosphorus content, g/kg	1.9	1.3	0.19
Water-soluble hydrogen-carbonates, meq /100g	0.70	0.56	0.42
Water-soluble calcium, meq/ 100g	0.84	0.55	0.58

Table 3.

Cation exchange capacity and exchangeable cations in calcretious soil, meq/100g

	Treatment	CEC	K^+	Na^+	Ca^{2+}	Mg^{2+}	Σ cations
	Control before vegetation	10,00	0,36	0,22	34,70	2,10	37,38
1	control	9,80	0,32	0,27	34,70	2,10	37,39
2	CI R1 - CI R4	9,93	0,29	0,27	34,50	2,30	37,36
3	Humic acids	10,08	0,99	0,27	33,90	2,40	37,56
4	CI R1 - CI R4 + Humic acids	9,95	1,34	0,27	34,10	1,80	37,51

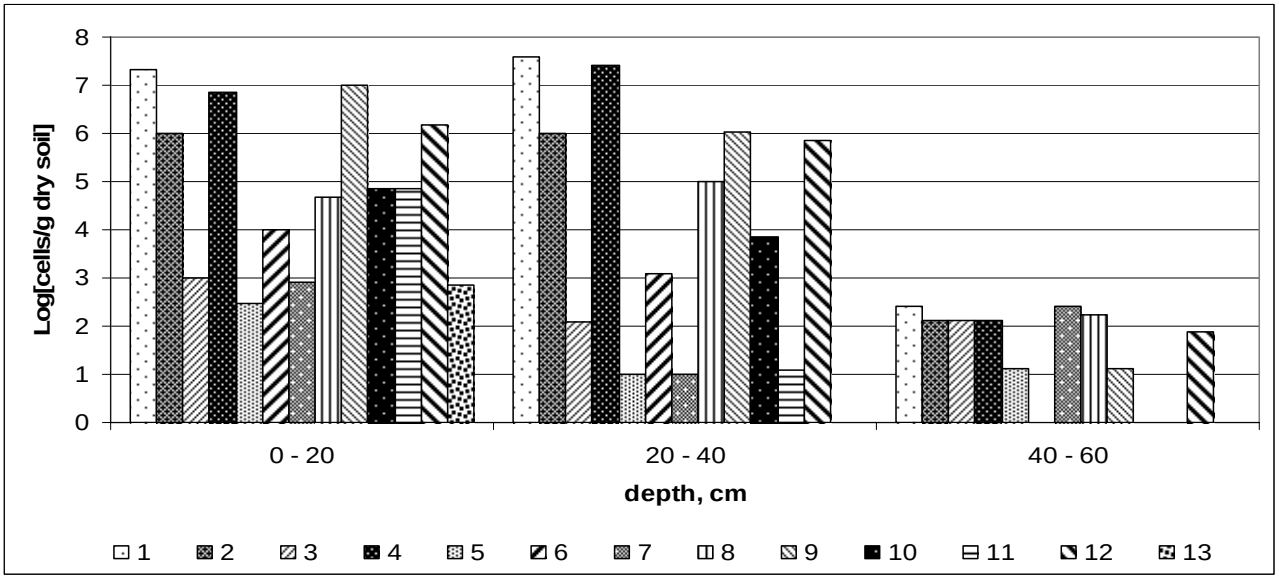

Fig. 1. Different groups of microorganisms in the soil profile of the study sites, counted during spring sampling. 1. Aerobic heterotrophic bacteria; 2. **A**naerobic heterotrophic bacteria; 3. **C**ellulose-degrading microorganisms; 4. **A**mylolytic microorganisms; 5. **B**acteria fermenting sugars with gas production; 6. **A**monifying bacteria; 7. Fungi; 8. Actinomycetes; 9. Nitrogenfixing bacteria; 10. Denitrifying bacteria; 11. Fe^{3+}-reducing bacteria; 12. Sulphate-reducing bacteria; 13. Nitrifying bacteria

According to the analysis results, the treatment with potassium humate increase exchangeable K^+ in the range of 3 to 4 times (figure 2), but this effect doesn't change cation exchange capacity of the soil. It was found, that the increase of exchangeable K^+ is related with decrease of exchangeable Ca^{2+}(figure 3).

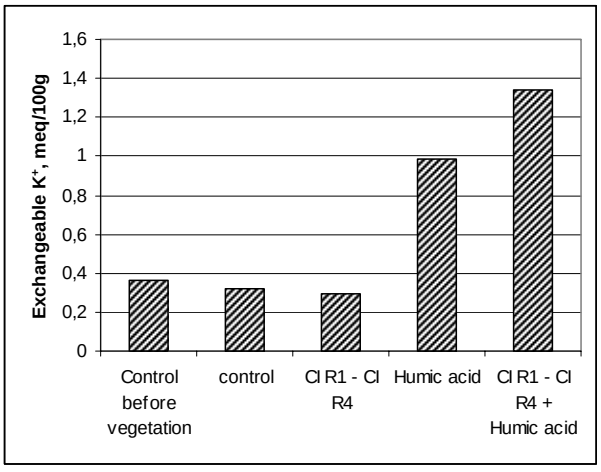

Fig. 2. The effects of application of microorganisms and humic acids on exchangeable K^+

The specific electroconductivity of calcareous soils saturation extract characterizes this substrate as poor in salts. The insignificant increase of EC was found in all treated soils (Figure 4.).

The soils affected by formation of calcrete have low content of available P_2O_5 and available K_2O. The treatment with potassium humate raises the level of available K to value beneficial for plants growth.

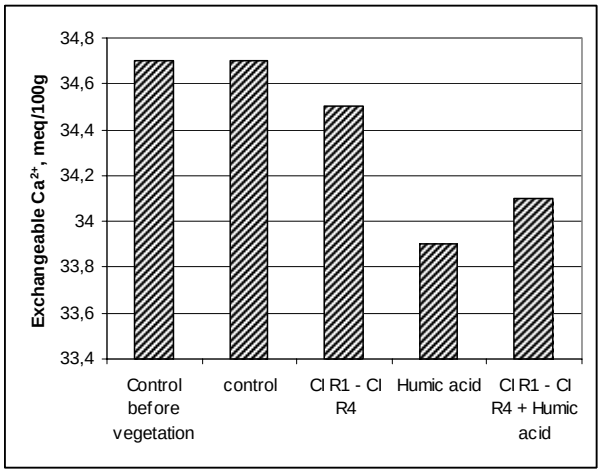

Fig. 3. The effects of application of microorganisms and humic acids on exchangeable Ca^+

The important effect of applied microorganisms and humic acids is decrease of concentration of active $CaCO_3$. The measurement of calcium carbonate equivalent (CCE) in calcareous soils is common and is useful for the evaluation of soil processes. Active calcium carbonate reactivity is related more highly at times than CCE to soil processes or properties. In alkaline soils phosphorus is fixed by Ca, which causes its low efficiency (Goldstein, 1986). This effect of reduced P availability in alkaline soil is driven by the reaction of P with calcium, with the lowest solubility of these calcium phosphate minerals at about pH 8. The data in table 4 shows that rhizospheric bacteria alone or in combination with humic acid decrease the active calcium with 5 to 20 %.

The biometric data (Table 5) demonstrates that the species Bacillus subtilis, Bacillus amyloliquefaciens, and humic acids have strong positive effect on plants growth.

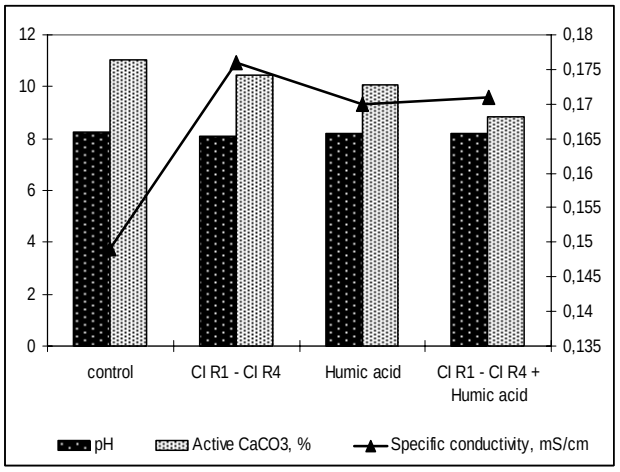

Fig. 4. Application of microorganisms and humic acids on pH, active $CaCO_3$ and specific conductivity

The formed shoot biomass of alfalfa in control was little because of low solubility of nutrients and the deficiencies of macro- and microelements. The results from this study show that the strains B. subtilis CIR1, B. subtilis CIR3, B. amyloliquefaciens CIR2 and B. amyloliquefaciens CIR4, isolated from phizosphere of chicorium belong to plant growth promoting rhizobacteria. Oliveira et al. (2009) reported that among the bacterial isolates, *Bacillus sp.* and *Burkholderia sp.* were the most efficient P-solubilizing strains from P-Ca source culture solution. Most of the soils contain the substantial reserves of total P; large part of it relatively remains inert and only less than 10% of soil P enters the plant-animal cycle. Phosphate solubilising bacteria (PSB) play an important role in enhancing phosphorous availability to plants by reducing soil pH and by microbial production of organic acids and mineralization of organic P by acid phosphatases (Awasthi et al. 2011). The isolated strains B. subtilis Cl R1 and Cl R3 also produce the enzymes alkaline and acid phosphatases. The strains B. amyloliquefaciens CIR2 and B. amyloliquefaciens CIR4 produce only alkaline phosphatase (data are not published).

Table 5.

Effect of rhizospheric bacteria and humic acids on growth of alfalfa

	control	Cl R1-Cl R4	Humic acids	Cl R1 - Cl R4 + Humic acids
Fresh sooth biomass, g	0,39	2,19	2,97	3,39
% to control	100	563	762	1000
Dry sooth biomass, g	0,09	0,42	0,49	0,65
% to control	100	465	548	730

Bacillus subtilis is also used as a powerful biocontrol agent. It has the ability to produce endospores and also biologically active compounds (Nagoraska et al., 2007). Joseph et al. 2007 reported that strains related to genera Bacillus, isolated from rhizosphere of chickpea (Cicer arietinum L.) produce indoleacetic acid.

The applied potassium humate also enhance the growth of alfalfa. Humic acids influence plant growth both in direct and indirect ways. Indirectly, they improve physical, chemical and biological conditions of soil (Katkat A. et al. 2009). Humic acids sequester (chelate) soluble calcium and protect the phosphates. The amine functional groups on humic acids can adsorb the phosphate anions also.

Humic acid efficiently improves soil fertility and crop productivity on calcareous soils. (Rajpar et al. 2011).

The biomass of alfalfa, after treatment of soils with microbial culture in combination with humic acids, was increased 10 times. This effect is combination of soil chemistry changes, as a result of applied humic acids, and different microbial metabolites, produced from isolated rhizospheric bacteria.

Table 4.

The effects of application of microorganisms and humic acids on some agrochemical parameters

Variant	pH	EC mS/cm	Active $CaCO_3$, %	Total N, g/kg	Total P, g/kg	Organic carbon, g/kg	Available P_2O_5, %	Available K_2O, %
Control before vegetation	8,48	0,129	9,14	0,69	0,616	9,2	5,38	12,59
control	8,26	0,149	11,01	0,79	0,672	9,6	7,35	17,23
Cl R1 - Cl R4	8,11	0,176	10,46	0,84	0,821	9,6	6,60	14,58
Humic acid	8,19	0,170	10,09	0,78	0,769	9,6	4,60	46,38
Cl R1 - Cl R4 + Humic acid	8,22	0,171	8,84	0,82	0,789	10,3	5,95	54,33

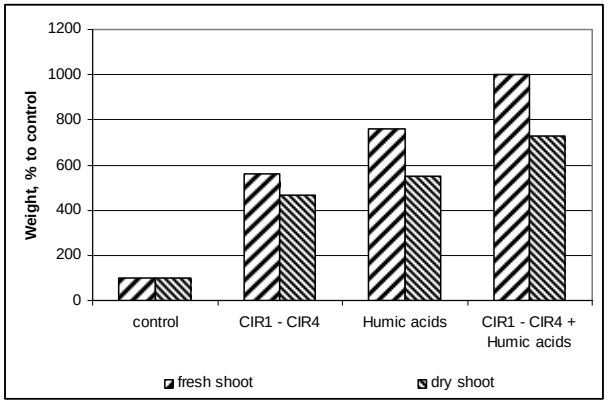

Fig. 5. Effect of rhizospheric bacteria and humic acids on shoots weight of alfalfa

CONCLUSIONS

The presence of calcrete is basic reason for alkalization of the soil, structure changes and low concentrations of available P, Mg, and K. This negative effect along with lower content of organic carbon and nitrogen fractions have unfavourable impact on microbial soil community, which reflects to the decrease of soil fertility. Phosphate solubilising bacteria are very effective for increasing plant available P in soil as well as the growth and yield of various crop plants. Humic acid efficiently improves soil fertility and crop productivity, especially on poorly fertile and alkaline-calcareous soils. Also the rhizospheric bacteria alone or in combination with humic acid decrease the active calcium with 5 to 20 %.

The fresh shoot biomass of alfalfa, after treatment of calcareous soils with plant growth-promoting rhizobacteria in combination with humic acids was increased 10 times.

REFERENCES

Awasthi R., Tewari R. and Nayyar H., Synergy between Plants and P-Solubilizing Microbes in soils: Effects on Growth and Physiology of Crops, International Research Journal of Microbiology, Vol. 2(12) pp. 484-503, 2011.

Brady NC, Weil RR (2002). The Nature and Properties of Soils. 14th ed. Prentice Hall, Upper Saddle River, New Jersey.

Banik S., Dey B.K., (1981) Phosphate-Solubilizing Microorganisms of a Lateritic Soil: I. Solubilization of Inorganic Phosphates and Production of Organic Acids by Microorganisms, Isolated in Sucrose Calcium Phosphate Agar Plates, Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Zweite Naturwissenschaftliche Abteilung: Mikrobiologie der Landwirtschaft, der Technologie und des Umweltschutzes 136:487-492.

Bratkova S., Nikolova D., Evstatieva Y., Dimitrov I., Nikolova K., Analysis of rhizospheric bacterial community in soils affected by the formation of calcrete, Journal of Geochemical Exploration 119–120 (2012) 44–50.

Chen, Y. and T. Aviad. 1990. Effects of humic substances on plant growth, in Humic Substances in Soil and Crop Sciences: Selected Readings. American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin.

Dimitrov, I., Panaiotova, M., Koleva-Recalova, E., Atanasova, E., 2009. Initial geochemical and physicochemical observations in areas affected by calcretization in the East Thracian plane. *Ann. UMG,* 52, Part I – Geol. and Geophys., pp. 55-60.

Ehrlich H. L., 2001. Geomicrobiology, Marcel Dekker, Inc., New York.

Goldstein AH (1986). Bacterial solubilization of mineral phosphates: historical perspective and future prospects. Am. J. Altern. Agric. 1:51–57.

Hayat R., Ali S., Amara U., Khalid R., Ahmed I., July 2010, Soil beneficial bacteria and their role in plant growthpromotion: a review, Ann Microbiol, Springer-Verlag and the University of Milan 2010.

Joseph B., Ranjan Patra R., Lawrence R., Characterization of plant growth promoting rhizobacteria associated with chickpea (Cicer arietinum L.), International Journal of Plant Production 1(2), September 2007.

Katkat A., Çelik H., Turan M. and Aýk B., Effects of Soil and Foliar Applications of Humic Substances on Dry Weight and Mineral Nutrients Uptake of Wheat under Calcareous Soil Conditions, Australian Journal of Basic and Applied Sciences, 3(2): 1266-1273, 2009.

Lugtenberg B.J., Kamilova F. (2009): Plant-growth-promoting rhizobacteria. Annual Review of Microbiology, 63: 541–556.

Nagorska K, Bikowski M, Obuchowskji M (2007). Multicellular behaviour and production of a wide variety of toxic substances support usage of Bacillus subtilis as a powerful biocontrol agent. Acta Biochim. Pol. 54:495–508.

Oliveira C.A., Alves V.M.C., Marriel I.E., Gomes E.A., Scotti M.R., Carneiro N.P., Guimara˜es C.T., Schaffert R.E., Sa´ N.M.H., 2009. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome, Soil Biology and Biochemistry. 41, 1782–1787.

Rahman M., Soaud A., F. Darwish, Golam F. and Sofian-Azirun M. Growth and nutrient uptake of maize plants as affected by elemental sulfur and nitrogen fertilizer in sandy calcareous soil, African Journal of Biotechnology Vol. 10(60), pp. 12882-12889, 5 October, 2011.

Rajpar I., Bhatti M., Zia-ul-hassan, Shah A. and Tunio S. HUMIC ACID IMPROVES GROWTH, YIELD AND OIL CONTENT OF BRASSICA COMPESTRIS L. , Pak. J. Agri., Agril. Engg., Vet. Sci., 2011, 27 (2): 125-133.

Reith, F., Wakelin, S. A., Gregg, A.I., Mumm, A. S., 2009. A microbial pathway for the formation of gold-anomalous calcrete, Chemical Geology, Vol. 258, Issues 3-4, 315-326.

Vazquez P., Holguin G., Puente M.E., Lopez-Cortes A., Bashan Y., 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon, Biol Fertil Soils 30:460–468.