Volume 22(2), 8- 13, 2018
JOURNAL of Horticulture, Forestry and Biotechnology
www.journal-hfb.usab-tm.ro

The activity of cellulosolithic microorganisms from soils covered by invasive plant species

Borozan Aurica Breica[1]*, Nistor Eleonora[1], Madoșă E.[1], Mișca Corina Dana[2*], Dogaru Diana[2], Popescu Sorina[1], Bordean Despina Maria[2], Dumbravă Delia[2]

[1]Banat's University of Agricultural Sciences and Veterinary Medicine, "King Michael of Romania" from Timisoara, Horticulture and Silviculture Faculty; [2]Banat's University of Agricultural Sciences and Veterinary Medicine, "King Michael of Romania" from Timisoara, Food Technology Faculty

*Corresponding authors. E-mails: borozan_a@yahoo.com; c9misca@yahoo.com

Abstract The interest in finding microorganisms capable of degrading plant residues, which is an interesting topic in agriculture and biotechnology, determined us to first evaluate the activity of cellulosic microorganisms in the soils occupied by invasive plants which fill larger areas in abandoned pastures. In addition, cellulose is considered a natural, renewable source of the biosphere.

For the evaluation of cellulosic activity, soil samples from the rhizosphere were taken from three invasive species, the nail of the bear (*Carduus acanthoides L),* thistle *(Carduus nutans L*) and wild mint (*Menta spicata var. viridis)*, developed on the degraded pastures of the Experimental Didactic Resort, within the University of Agricultural Sciences and Veterinary Medicine of Banat "King Michael the I[st] of Romania" from Timisoara. For the control and for each plant species 4 samples of soil were harvested. The standard method used has undergone some modifications by Stefanic and his cooperators (1994).

The results obtained revealed that the cellulosylium microflora in the plant rhizosphere is in most cases quantitatively reduced compared to the control variant, but it gives us the possibility to isolate and identify species that have a high degradation potential. The reduction of microorganisms can work together primarily with plants and humidity, but also with other abiotic and biotic factors.

Key words

cellulosolithic microorganisms, soil, *Carduus acanthoides L, Carduus nutans L, Menta spicata var. viridis*

In recent years, many abandoned pastures are covered by invasive plants, considered nutritionally non-nutritive and in competition with fodder plants. Of these plants, it is found very often, in degraded meadows, species like *Carduus nutans L.* (thistle), *Carduus acanthoides L.* (nail of the bear) and *Menta spicata var. viridis* (wild mint), either isolated or in the form of ankles (Radulescu et al. 2013; Daehler, 2003; Young et al. 2011; Blank and Morgan, 2012; Chengchou and Stephen, 2014).

Cellulose is the essential structural component of the cell wall of green plants, algae and oomycetes, and a renewable, energy-rich source of abundance in nature (Bikash, 2017/https://www.lap-publishing.com/.../cellulolytic-microbes-in-.), being considered the most important carbon source of the biosphere (Bakare et al., 2005; Feng et al., 2007).

In their studies, Post and collaborators (1982) report that the soil contains about twice as much carbon as it is found in the atmosphere, and research on the decomposition of organic matter in soils and sediments is essential for controlling climate change and atmospheric modeling (Jenkinsun et al., 1991).The cellulose content of dried plants is 45% and wood is 40-50%. (https://www.merriam-webster.com/dictionary/cellulolytic).

Degradation of cellulosic material is accomplished through the interaction of a microbial community (Vishnivetskaya et al., 2012). Cellulosic microorganisms are represented by bacteria (López-Mondéjar et al., 2016), along with actinomics and micromycetes (Acharya et al., 2012) aerobic and anaerobic (Gautam et al., 2012), mesophile and thermophile (Bagnara et al., 1985), involved in the biotransformation of organic matter, forestry and agricultural areas, ecological conservation (Avellaneda-Torres et al., 2014), the carbon circuit in nature (Sailendra et al., 2014), but also obtaining biofuels (Arifoglu and Ögel, 2000). Cellulosic microorganisms are also used for the production of enzymes (Mach and Zeilinger, 2003; Haki and Rakshit, 2003), which may be of interest to biotechnologies (Avellaneda-Torres et al., 2014). Among the cellulosic

fungi are mentioned in particular *Trichoderma sp., Penicillium sp* and *Aspergillus spp*.

According to the estimates made by Bakare and collaborators (2005) annual pulp production reaches 4.0 x 107 tons. According to some authors, cellulose wastes from industry and agriculture are not exploited enough (Kim et al., 2003; Lee et al., 2008).

Cellulosic activity is influenced by two chemical elements, nitrogen and phosphorus, but also abiotic and biotic factors.

The enzyme complex that mediates this process is at a high cost (Olson et al. 2011; Klein et al. 2012), which is why a number of researches in the field aim to find more efficient cellulosic microorganisms and capable of synthesizing a large amount of enzymes Johnvesly et al., 2002; Pattana et al., 2000; Subramaniyan and Prema, 2000), which adds the desire to find alternative energy variants, which makes the study of cellulosic microorganisms have a significant global position.

Starting from the importance of cellulolytic activity and interest in finding microorganisms of interest for agriculture and biotechnology, this paper proposes as a first step the evaluation of the activity of cellulosic microorganisms in quantitative terms from the soils covered with invasive plants.

Materials and Methods

Soil samples were taken from the rhizosphere of the bear's nail species (*Carduus acanthoides),* thistle*(Carduus nutans*) and wild mint (*Menta spicata var. viridis)*, developed on the pastures of the Experimental Didactic Resort, within the University of Agricultural Sciences and Veterinary Medicine of Banat "King Michael the Ist of Romania" from Timisoara. For the control variant and for each plant species 4 samples of soil were harvested. The harvest depth was between 0-20 cm.

After soil processing in the Microbiology Laboratory, the humidity of each sample was determined and the experiments for the cellulosic capacity determination were determined, according to the method of Vostrov and Petrova (1961), with the modifications introduced by Ştefanic et al. (1994), (Borozan, 2001; Borozan, 2003). The incubation period was 3 weeks, during which periodic humidity checks were performed. The optimum incubation temperature was 28 °C. For the calculation of this biotic activity the humidity coefficient was taken into account (Ştefanic, 2006).

Results

The results on cellulosynthetic activity are intense in the control samples. In contrast, in soil samples from parcels covered with the three invasive crops, cellulosic activity is lower.

From the studies conducted by Cojocariu et al. (2017), it was observed that microorganisms in the rhizosphere from species *Carduus nutans L* dominated the control version.

Cellulosic microorganisms with intense degradative capacity are aerobic, and only a small percentage is representated by anaerobic microorganisms (5-10%). (Ehhalt, 1976; Vogels 1979).

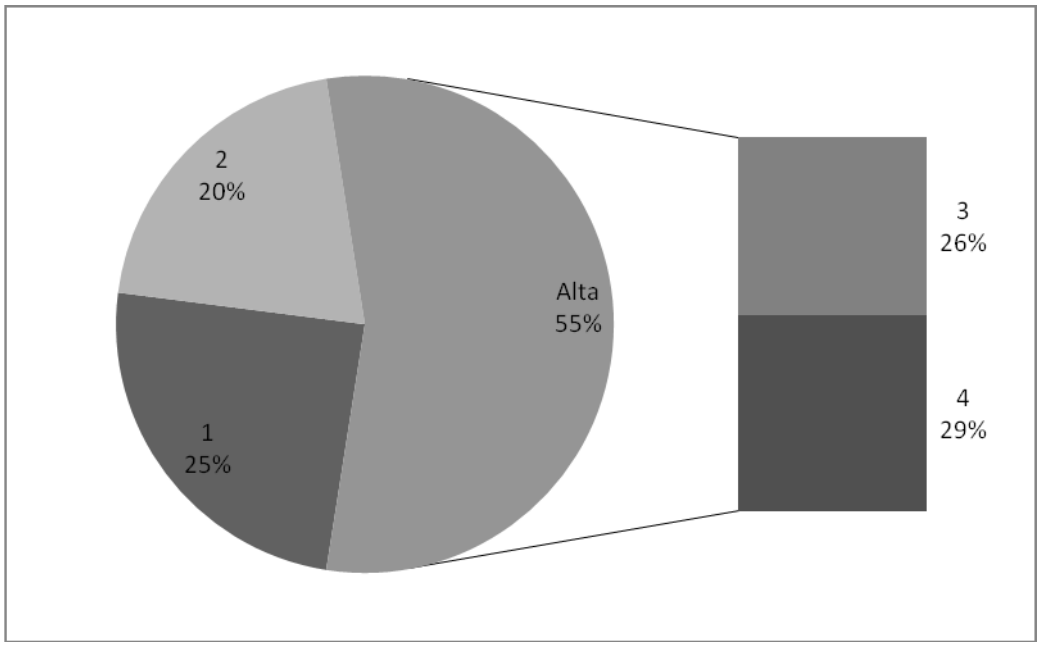


Fig. 1. Cellulosic activity in rhizosphere of spiked mint plants (1,2,3,4) and the witness samples (other)

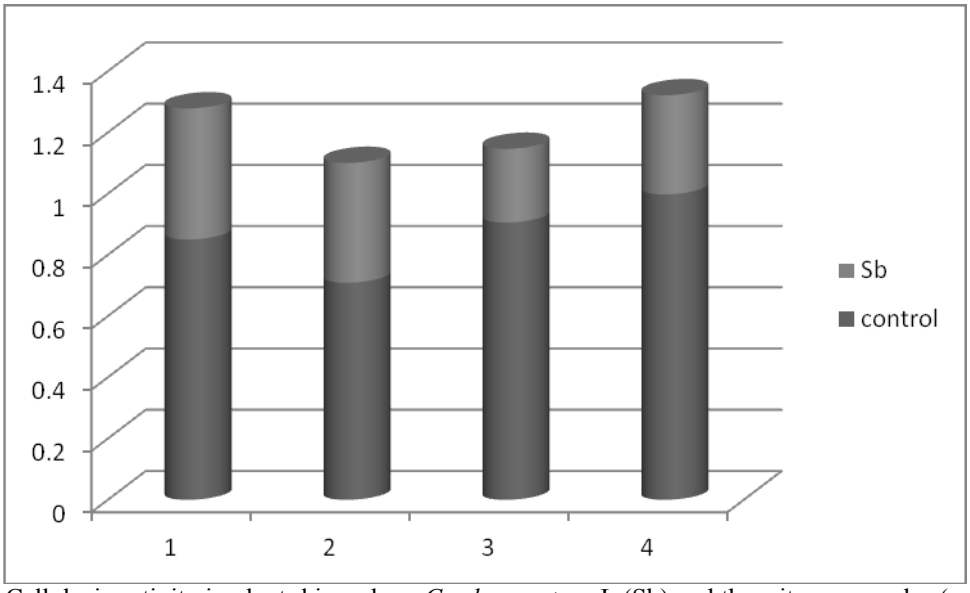
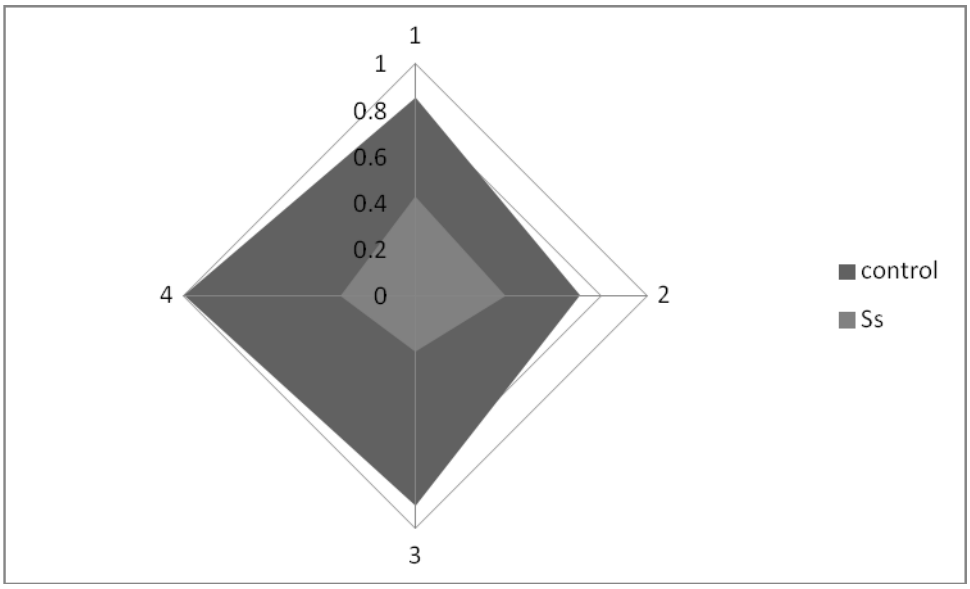


Fig. 2 Cellulosis activity in plant rhizosphere *Carduus nutans* L (Sb) and the witness samples (control)

By evaluating cellulosic activity in control samples and those influenced by wild mint roots, there was a similar evolution of cellulose in a control sample as determined in one of the rhizospheric samples. Otherwise, the degrading activity was more intense in the control samples against the soil under the influence of wild mint plants (figure 1).

One of the things that should be mentioned here is that the plant influences the micromedium in the root area, and thus the plant reserves the selection of a specific microbiota (Dennis et al, 2010; Marschner et al., 2011; Pasca et al., 2012; Pasca et al., 2012; Pasca et al., 2013; Pasca et al., 2012; Pasca et al., 2012, Borozan et al., 2014), which may have happened in the case of these invasive plants.

The cellulosic activity in the control samples is close to that determined in the soil influenced by the roots of the thistle plants (figure 2).



Fig, 3 Cellulosis activity in plant rhizosphere *Carduus acanthoides* (Ss) and the witness samples (control)

According to figure 3, the cellulosylitic activity is reduced in the plant rhizosphere *Carduus acanthoides L.*, compared to the blank. Values from the 4 soil samples taken from the rhizosphere area of the species *Carduus acanthoides L.* are almost similar.

However, studies on the cellulosylitic activity in the soils covered with the three invasive plant species have not been carried out so far, which is why it is not possible to report data obtained by other authors, it can still be taken considering the fact that

the differences in the growth of microorganisms, the ascending or descending evolution of this biotic activity in the plant rhizosphere depends on a multitude of factors among which are mentioned: temperature, humidity, pH, carbon sources, macronutrients and micronutrients, incubation time (Ahamed and Vermette, 2008; Arifoglu and Ögel, 2000; Grigorevski de Lima et al., 2005; Hanif et al., 2004; Ögel et al., 2001).

Conclusions

Degrading processes in the soil can be a way of recycling organic matter and a nutrient base for soil and plant organisms, and studies can help find species with a high degradation capacity, which can serve humans in their activities agricultural and biotechnology.

In our research it was noticed that in general the cellulosylitic activity in the rhizosphere of the three invasive plants is reduced in value compared to the control variant, being mentioned in many bibliographic references that the microorganisms in the rhizosphere are influenced by the root exudates, to which are added other factors abiotic and biotic, which is certainly true in this case.

References

1.Acharya A., Joshi D.R., Shrestha K., Bhatta D.R. (2012) *Isolation and screening of thermophilic cellulolytic bacteria from compost piles.* Scientific World, Vol. 10, No. 10, p43-46

2.Ahamed A, Vermette P. (2008) *Enhanced enzyme production from mixed cultures of Trichoderma reesei RUT-C30 and Aspergillus niger LMA grown as fed batch in a stirred tank bioreactor.* Biochem Eng J 42:41-46

3.Arifoglu N., Ögel Z.B. (2000) *Avicel-adsorbable endoglucanase production by the thermophilic fungus Scytalidium thermophilum type culture Torula thermophila*. Enzyme Microb Technol 27:560-569

4.Avellaneda-Torres Lizeth Manuela, Guevara PulidoClaudia Patricia, Torres Rojas (Esperanza 2014) *Assessment of cellulolytic microorganisms in soils of Nevados Park, Colombia*, Brazilian Journal of Microbiology 45, 4, 1211-1220

5.Bagnara C., Toci R., Gaudin C., Belaich J.P. (1985) *Isolation and Characterization of a Cellulolytic Microorganism, Cellulomonas fermentans sp. nov. c.* International Journal Of Systematic Bacteriology, Vol. 35, No. 4, p. 502-507

6.Bakare M., Adewale I., Ajayi A., Shonukan O. (2005) *Purification and characterization of cellulase from the wild-type and two improved mutants of Pseudomonas fluorescens*. Afr. J. Biotechnol. 4:898-904

7.Bikash Chandra Behera (2017) *Cellulolytic Microbes in Mangrove & Their Biotechnological Application*. LAP Lambert Academic Publishing/ https://www.lap-publishing.com/.../cellulolytic-microbes-in-...

8.Blank R.R., Morgan T. (2012) *Suppression of Bromus tectorum L. by established perennial grasses: potential mechanisms—part one*. Appl Environ Soil Sci 2012:1–9

9.Borozan Aurica (2003) *Influenţa erbicidelor sulfonilureice asupra activităţii microorganismelor celulozolitice pe modele în laborator,* Biotehnologie şi Biodiversitate, USAMVB Timişoara, 159

10.Borozan Aurica (2001) *L'effet des sulfonylureides sur l'activite cellulosolitique du sol,* Biotehnologie şi Biodiversitate, USAMVB Timişoara, 103

11.Borozan Aurica Breica, Luminiţa Cojocariu, Horablaga M., Mişcă Corina Dana, Moldovan Camelia, Raba Diana, Dogaru Diana (2014*) Bacterial variation response depending on cultivated plant,* Journal of Horticulture, Forestry and Biotechnology, ISSN-2066-1797, vol 18(4), 24-26

12.Chengchou Han, Stephen L. Young (2014) *Root Growth of Two Perennial Grass Types and Musk Thistle (Carduus nutans) in Temperate Grasslands of North America.* Invasive Plant Science and Management 7:387–397

13.Cojocariu Luminita, Bordean Despina Maria, Borozan Aurica Breica, Nita Simona, Horablaga Adina, (2017) *Carduus nutans L and the Effect on the Heavy Metals and Microenvironment Biota.* Rev.Chim.(Bucharest), 68, No. 7

14.Daehler C.C. (2003) *Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoratio*n. Annu Rev Ecol Evol Syst 34:183–211

15.Dennis P.G., Miller A.J., Hirsch P.R. (2010) *Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities?,* FEMS Microbiol. Ecol., 72 (3):313-27

16.Ehhalt D.H.. (1976) *The atmospheric cycle of methane* In *Microbial Produclion and Utilization of Gases,* ed. HG Schlegel, G Gottschalk, N Pfennig, pp. 13-22. Grttingen: Goltze

17.Feng Y., Duan C.J., Pang H., Mo X.C., Wu C.F., Yu Y., Hu Y.L., Wei J., Tang J.L., Feng J.X. (2007) *Cloning and identification of novel cellulase genes from uncultured microorganisms in rabbit cecum and characterization of the expressed cellulases.* Appl Microbiol Biotechnol 75:319-328

18.Gautam S.P., ,Bundela P.S., Pandey A.K., Jamaluddin, Awasthi M. K., Sarsaiya S (2012), *Diversity of Cellulolytic Microbes and the Biodegradation of Municipal Solid Waste by a Potential Strain*, International Journal of Microbiology, Volume 2012, Article ID 325907, 12 pages

19.Grigorevski de Lima A.L., Pires D.O., Nascimento R., Da Silva Bon E.P., Coelho R.R.R (2005) *Streptomyces drozdowiczii cellulase production using agro-industrial by-products and its potential use in the detergent and textile industries*. Enzyme Microb Technol 37:272-277

20.Haki G.D., Rakshit S.K. (2003) *Developments in industrially important thermostable enzymes: a review.* Biores. Technol. 89, 17-34.
21.Hanif A., Yasmeen A., Rajoka M.I. (2004) *Induction, production, repression, and de-repression of exoglucanase synthesis in Aspergillus niger,* Bioresour Technol 94:311-319.
22.Jenkinsun D.S., Adams D.E., Wild A. (1991) *Model estimates of CO~ emissions from soil in response to global warming .Nature* 3:51:304—6
23.Johnvesly B., Virupakshi S., Patil G.N., Naik G.R. (2002) *Cellulase-free thermostable alkaline xylanase from thermophillic and alkalophillic Bacillus sp. JB-99.* J Microbiol Biotechnol 12:153–156
24.Kintl A., Nawrath A., Elbl J., Tůma I., Muchová M., Brtnický M., Kynický J. (2016) *Nitrogen and phosphorus availability effect on activity of cellulolytic microorganisms in meadows,* Volume 64, Number 4
25.Klein M.D., Oleskowicz P.P., Simmons B.A., Blanch H.W. (2012) *The challenge of enzyme cost in the production of lignocellulosic biofuels.* Biotechnol Bioeng 109:1083–1087
26.López-Mondéjar R., Zühlke Daniela, Becher Dörte, Riedel Katharina, Baldrian P. (2016), *Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems* , Scientific Reports | 6:25279 | DOI: 10.1038/srep2527
27.Ögel Z.B., Yarangümeli K., Dündar H., Ifrij I. (2001) *Submerged cultivation of scytalidium thermophilum on complex lignocellulosic biomass for endoglucanase production*. Enzyme Microb Technol 28:689-695.
28.Olson D.G., McBride J.E., Shaw J.A., Lynd L.R. (2011) *Recent progress in consolidated bioprocessing*, Curr Opin Biotechnol 23:1–10
29.Mach R.L., Zeilinger S. (2003) *Regulation ofgene expression in industrial fungi: 103 References Trichoderma*, Appl. Microbial. Biotechnol. 60, 515-522
30.Marschner P., Crowley D., Rengel Z., (2011) *Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis-model and research methods*, Soil Biology and Biochemistry, vol. 43, Issue 5, 883-894
31.Pattana P., Khanok R., Khin L.K (2000) *Isolation and properties of a cellulosometype multienzyme complex of the thermophilic Bacteroidessp. strain P-1*, Enzyme Microb Technol 26:459–465
32.Pasca Marinel, Luminita Cojocariu, Despina Bordean, Roxana Popescu, Marioara Nicoleta Filimon, Vasile Gherman, Marinel Horablaga, Aurica Breica Borozan,(2013) *Rhizodeposid a disturbing factor of soil microbial balance,* Water Resources, Forest, Marine and Ocean Ecosystems, Conference Proceedings, 13th International Multidisciplinary Scientific Geoconference SGEM 2013, 16-22 June, 2013, Albena Co., Bulgaria, ISSN 1314-2704, ISBN 978-954-91818-7-6, DOI:10.5593/SGEM2013/BC3/S13.023, p:649-654
33.Paşca M., Cojocariu Luminiţa, Horablaga N.M., Bordean Despina-Maria, Filimon Mărioara-Nicoleta, Borozan Aurica- Breica, Marian F., (2012) *The Evolution Of Cellulolytic Potential Of The Far Rhizosphere Zone And Of The Near-Root,* Research Journal of Agricultural Science, 44 (1). 203-208
34.Paşca M., Cojocariu Luminiţa, Bordean Despina-Maria, Horablaga N.M., Popescu Roxana, Borozan Aurica Breica, Pălcuţ Manuela, Marian F. (2012) *The Study Of The Bacterial Community In The Root And Near-Root Zone Of Species Vicia Sativa*, Research Journal of Agricultural Science, ISSN: 2066-1843, 44 (1), 209 -215
35.Paşca M., Cojocariu Luminiţa, Horablaga M., Bordean Despina Maria, Filimon Mărioara Nicoleta, Popescu Roxana, Darlea Auruţa, Borozan Aurica Breica, (2013), *Plant-Soil Interrelationship, Factor Of Evolution For Bacterial Populations,* Review On Agriculture And Rural Development, Scientific Journal of University Of Szeged, Faculty of Agriculture, Volume 1. (1) Hódmezővásárhely, 346-351
36.Paşca M., Cojocariu Luminiţa, Horablaga M., Bordean Despina Maria, Dragos N., Filimon Marioara Nicoleta, Gergen Iosif, Borozan Aurica Breica (2012), *Changes In The Structure Of Actinomycete Populations In The Rhizosphere Of Vicia Sativa Species*, Review On Agriculture And Rural Development, Scientific Journal of University Of Szeged, Faculty of Agriculture, Volume 1. (1) Hódmezővásárhely, 352-357
37.Post W.M., Emanuei W.R., Zinke P.J., Stangenberger A.G. (1982), *Soil carbon pools and world life zones*, *Nature* 298: 156-59
38.Radulescu Cristiana, Stihi Claudia, Barbes Lucica, Chilian A., Chelarescu Daniela Elena (2013) *Studies Concerning Heavy Metals Accumulation of Carduus nutans L. and Taraxacum officinale as Potential Soil Bioindicator Species*, Rev. Chim. (Bucharest) 64 No.7, 2013, 754-760
39.Subramaniyan S, Prema P (2000) *Cellulase-free xylanases from Bacillus and other microorganisms*. FEMS Microbiol Lett 183:1–7
40.Sailendra Goyari, Shantibala S Devi, Mohan C Kalita, Narayan C Talukdar (2014), *Population, diversity and characteristics of cellulolytic microorganisms from the Indo-Burma Biodiversity hotspot*. SpringerPlus 2014, 3:700
41.Ştefanic G. (2006), *Metode de analiza a solului (biologica, enzimatica si chimica)*, Probleme de agrofitotehnie teoretica si aplicata. Institutul de cercetare-dezvoltare agricola Fundulea, XXVIII, 5-25.
42.Ştefanic G. (1994). *Biological definition, quantifying method and agricultural interpretation of soil fertility,* Romanian Agricultural Research, 2: 107-116
43.Vishnivetskaya T.A., Raman B., Phelps T.J., Podar M., Elkins J.G. (2012). *Cellulolytic Microorganisms*

from Thermal Environments/ Extremophiles: Microbiology and Biotechnology (Edited by: Roberto Paul Anitori). Caister Academic Press, U.K.
44.Vogels GD. (1979). *The global cycle of methane*. *Anionic van Leeuwenhoek J. Microbiol. Serol.* 45:347-52
45.****PartII:Degradation During Growth of Cellulolytic Microorganisms, (1960) journals.sagepub.com/doi/abs/10.../00405175600300 0403)