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Proceedings of the International Conference

AGRI-FOOD SCIENCES, PROCESSES AND TECHNOLOGIES

AGRI-FOOD XXV

Celebrating the
XXV th Anniversary of the beginning of Food Industry and Agronomy Higher Education in Sibiu

SIBIU,
May 24-25, 2015

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Scientific Committee

Organizing Committee

Distingueshed guests, dear colleagues and students,

I have the great pleasure to welcome you on the Transylvanian realms, at the Faculty of Agricultural Sciences, Food Industry and Environmental Protection of Sibiu. Every time we see each other in these situations, there is a feeling of emotion and pleasure, generated by the need of knowing and of establishing of camaraderie relationships, beneficial to human development and professional achievements. Today we celebrate 25 years of continuity for the higher education in food industry at Sibiu, continuing a more than 70 years one in Romania. It is a beautiful evolution and a confirmation of the teaching and research quality within our Faculty, which may be seen and taken for granted, but in fact encompasses a lot of work, dedication and even sacrifice.

It has been, during human evolution, a permanent quest for improving the standard of living, mainly by food quality and diversification, and this will hold as long life will exist on Earth. Soil and plant agronomy will enable the obtaining of raw materials in accordance with the actual quality requirements and their industrial processing will define and ensure the right nutrition, also respecting their traceability. Romania has always had exquisite conditions for obtaining raw materials with the highest quality which, carefully processed, have provided an excedent of the domestic, then being a "hard currency" for Romanian exports. Now there are good conditions for obtaining organic food in Romania, but these conditions should be more exploited in our own benefit, to contribute more and to enlarge the national GDP. These are facts that, corroborated with the scientific potential of our researchers and manufacturers' practical expertise can make the difference in competiveness within a society in continuous move of the areas of influence and food markets.

The Faculty of Agricultural Sciences, Food Industry and Environmental Protection intents to stay connected to the European requirements and standards, by educating specialists able to adjust themselves to the advanced system of technological and scientific values. Situated in a city with old cultural traditions and historical memories, it pursues the work of carving and shaping the human and spiritual values, giving a new dimension to scientific knowledge, in a climate of normality. By its team of academic staff, students and doctoral candidates we have managed to keep steady our Faculty among the first ones within the Lucian Blaga University of Sibiu in what concerns scientific research and obtaining European funding, of paramount importance for structuring a successful education activity, comparable with other European similar institutions.

Nowadays we have as priority providing a favourable climate for creativity and novelty of higher education in agronomy and food industry, by encouraging research and teaching, for the integration into the world scientific community. Within this context we have created and

developed study programs that are interested for the license, master and doctoral students, with the appreciation of industrial stakeholders.

The year 2015 has an exquisite importance for the academic community of Sibiu, being a celebration year for our University that has since 20 years as spiritual father a multivalent personality of Romanian culture, the poet and philosopher Lucian BLAGA. It is a happy destiny that our faculty shares with the university and local community we belong to.

I would like to thank in this way to all that during the past time has contributed to the creation of the present of us all – colleagues, students and partners – for the edification of the future. I wish you all success, health and happiness.

With esteem and friendship,

Dean of Faculty of Agricultural Sciences, Food Industry and Environmental Protection,

Prof.Eng. Ovidiu TIȚA, Ph.D

SUMMARY

BIOSAFETY FRAMEWORK – LOCAL CONSERVATION
MINIREVIEW

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Abstract
The scope of this article is to review biosafety measures for the marginal villages in the South East Transylvania where exists a myriad of landraces considering the need also to respond to the Plant Treaty requirements for the survey of each plant genetic resources for food and agriculture. The results of our study revealed that at the local level the major constraint is due to the lack of communication both for biosafety and landrace conservation to which a major contribution may have political regime change.

Keywords: Biosafety, landraces, South East Transylvania, marginal villages

Genetically modified plants (GMPs) entered the EU market place for cultivation with the acceptance of the maize MON810 in 1998 (Tinland et al., 2007). Still, the European regulation framework, which is considered as one of the most restrictive at the global level, constantly needs to adapt and develop accordingly based on new scientific evidences, environmental constraints and socio-economic issues (Gehring and Faude, 2014).

Romania developed its own biosafety regulatory framework in 2000 through the adoption of the Governmental Emergency Ordinance 49/2000, which was harmonized with the EU former regulatory framework (Badea and Pamfil, 2009). Before the EU accession the entire biosafety regulatory framework was changed accordingly (Antofie and Baz, 2006).

Still, of major importance in the biosafety domain in Romania as well as in other countries it was considered during time the constantly need to harmonize the scientific achievements with the existing regulatory framework embedded in the current realities (McLean et al., 2002). Therefore after 2007, the year of Romania' accession to the European Union, from scientific point of view it was recognized the need for re-evaluating scientific environmental assessment (Ramessar et al., 2007), socio-economic criteria (Ohl et al., 2007) and conflicts with protected areas (Henle et al., 2008). All these topics, aside others (e.g. the thresholds for seed labeling Marceau et a. 2013) have been concluded based on a long experience in implementing the biosafety regulatory framework in different countries (De Bièvre et al., 2014).

As it was already mentioned above, in case of Romania, the regulatory framework developed in accordance with the EU regulation through the adoption also of the Governmental Decision (GD) 256/2006 for framing the implementation of the EU Regulation

1829/2003 and GD 173/2006 for framing the implementation of the EU Regulation 1830/2003. In case of the Directive 2001/18/EC it was adopted the Governmental Emergency Ordinance 43/2007 repealing the former GEO 49/2000. Thus, in Romania the scientific risk assessment is realized according to procedures lay down by the Directive 2001/18/EC and based on the EO 43/2007 established the principles for organizing and functioning of the Romanian Biosafety Commission too (Antofie et al., 2010).

After 2007 Romania complies with the EU regulatory framework regarding environmental risk assessment which is centralized in the European Food Safety Authority (EFSA) for genetically modified organisms based on the provisions of the Regulation No 1829/2003 and Directive 2001/18/EC. Still procedures for environmental risk assessment cannot fit perfectly for each of the member states due to peculiarities of the countries even at the general level it can be considered that they are harmonized. In this respect, specific general guidelines are provided by the EFSA authority that are supporting the member states in harmonizing the implementation of the regulatory framework giving clarifications for specific requirements (i.e. notification, dossier analysis, implementation and monitoring) (Devos et al., 2014). The authorization in case of plant genetic resources is granted for maximum 10 years, issued by the Member State where the notification is submitted and furthermore after all Member States expressed their support. At the general level this seems to be very clear when only political or regulatory framework is involved in the EU context (Poting et al 2014). The daily working experience with this regulatory framework however is becoming complicated by the peculiarities of agro-ecosystems for the local level (Jank and Gaugitsch, 2001). In other words, risk assessment procedures are to be implemented at the ecosystem level and other issues became more relevant at the local level. In case of Romania it became clear that difficulties in the countryside are more than a burden for authorities in charge with agriculture and environment. Subjects such as landowners and arable land atomization which are specific for Romania are becoming much important in case of also applying the regulatory framework for crops landraces conservation. In this case, the South East of Transylvania and not only, represents a crop genetic pool for landraces conservation in marginal rural area (Antofie, 2012). The arable land fragmentation became a major issue for authorities dealing with the implementation of decisions regarding the cultivation of genetically modified plants (GMPs). In this case considering as valid *the scientific evaluation* still risk assessment procedures have to comply with *socio-economic criteria* and the national regulatory framework for *protected areas*. For South Eastern Transylvania based on the

National Statistics, exists a high level of vulnerabilities due to increased migration of the people and population ageing. Moreover, during the last 25 years (i.e. after 1989), important land use changes has been produced and landowners' structure changed accordingly. Thus, if before 1990, all landowners belonged to a local community, during the last 25 years the situation changed through embedding foreign landowners among the local. It can be considered that the people owning small parcels in a specific agro-ecosystem encounter today a barrier regarding biosafety communication which is also recently recorded at the international level (Dickmann et al., 2015). Mixed agricultural practices and management measures as well as land abandonment are among the negative issues of the lack of communication encountered for the last 25 years in Romania. Under these circumstances in many rural areas it can be assisted to a hazardous infusion of modern crop varieties and mixture of practices without crop management communication among neighboring landowners. Most threatened are for example maize landraces, first described in 1928 by Andronescu (Antofie, 2011), which are still present in the rural areas in Transylvania, in small marginal villages (i.e. Holbav from Brasov county). The cultivation of maize hybrids may become among the major threats for eliminating these landraces only due to the implementation of inappropriate crop management at the rural landscape level. This is also the case of a the so called "Moldavian maize" as creole varieties cultivated in Codlea city starting with 1960 which is affected by the modern hybrids cultivation in the area. On one hand the hybrids cultivators are not aware about the existence of landraces and on the other hand the rural community is not aware on the hybrids cultivation in the next garden which may have a negative impact on their landraces conservation. The risk communication for biosafety issue at the local level may become a real barrier for the proper implementation in harmony of both biosafety and landraces conservation regulatory frameworks in line with the commitments taken under the European Union. Unfortunately these issues have not been addressed yet at the national level, being highly costly. Still, at the European Commission level the harmonization in between different regulatory frameworks acting in the same ecosystems poses problems at the local level which cannot be solved at the European Level but only at the local level and under the national context constraints (Poting et al., 2014).

If in case of the European Member states according to EFSA, the GMPs for cultivation have at least two major characteristics important for a detailed risk assessment such as resistant against a specific pest or specific herbicide –tolerant (Arpaia et al., 2014). Later, it is recognized that the production of pesticides may have harmful effects on the non-target fauna

and for Romania, in South Eastern Transylvania may have a negative impact on the fauna belonging to protected areas. In this case, over 50% of the area is covered with protected areas belonging to the current ecological network Natura 2000, which is different compared with other regions (Geacu et al., 2012). Thus, in case of approving the cultivation of GMPs in Romania it should be developed studies regarding the impact of such plants on the non-target fauna as well as on possible presence of landraces which are protected by the Plant Treaty in line with the provisions of art. 5 (Antofie, 2012). From scientific point of view it can be accepted the new monitoring methodologies for invertebrates published in peer review articles and peer reviewed journals either on the plant covering either in soil, even the current regulatory framework is not covering standards for soil analysis or for monitoring invertebrates (Todd et al., 2008). In case of herbicide tolerant GMPs the biosafety regulatory framework is not harmonized with the pesticide regulatory framework regarding the production of substances against the herbicide which according with some studies may result in the production of weed resistant to such pesticides (Kumar et al., 2008). Taken under consideration that it is possible to have crop landraces and modern varieties in the same place and belonging to the same species, it is more than obvious that it is possible that the existing landraces to become contaminated and to become genetically modified based on natural mechanisms this time. Thus, in South Eastern Transylvania, it is important to clearly address this issues in order to find solutions in the harmonization of four regulatory frameworks: [1] biosafety; [2] landrace conservation; [3] Natura 2000 and [4] pesticide use. The things are becoming more complicated if we are taking into consideration the current political commitments regarding the declaration of this region as a traditional region (Kamau and Winter, 2009).

Long term monitoring In case of Biosafety domain it is not a clear definition in relationship with *long term* effects of the GMPs on environment (Séralini et al., 2014). Still, from scientific point of view, a landrace should be a crop lasting for more than 100 years in the same ecosystem (Zeven, 1998). In case of more than 30 years such landraces are called creole varieties. If the regulatory framework for biosafety is recognizing the value of landraces than long term effect should go further 30 years at least for the value of creole varieties. In this case almost all risk assessment not going for more than 10 years are rare, excepting the case of maize MON810, which is monitored for more than 20 years. Thus, long term effect monitoring in biosafety continue to be an issue and references should be done

according to the regulatory frameworks the biosafety domain is harmonizing (i.e. landrace conservation, pesticides, environmental).

Socio-economic Even it is considered that genetically modified plants (GMPs) are the subject of public controversy because of their advantages and disadvantages (Habden et al., 2003)there is no recognition regarding the development of guidelines for risk communication to the local level. The major barrier is still the not recognized risk communication issue. As mentioned above this may become a barrier when the local community is confronted with high socio-economic and climate change vulnerabilities fueled by political regime change like it is the case for Romania or other former communist countries. In case of Romania the atomized arable land belonging to over 4 mil landowners became dramatic in applying co-existence measures according to the European Union guidelines. Thus, for Romania the "public controversy" as it is called as the major problem for the European Union according to EFSA, may not be valid due to other vulnerabilities not encountered by the majority of the European Member States (Jank and Gaugitsch, 2001). In this case risk communication at the local level between landowners owning very small parcels in agro-ecosystems positioned inside or in the buffering zones of protected areas may become problematic for the implementation of biosafety framework.

The associated costs for GMPs as products or in products thereof, regarding their labeling and monitoring from the field to the forks, in a society facing at the national level political regime change and at the local level high vulnerabilities, may become rather a barrier to the society development (Jank and Gaugitsch, 2001). The rapid acceptance of the GMPs may result in the complete removing of valuable landraces which are not recognized today, but that may became valuable for developing adaptation measures to the climate change according to the Bali Decision under the Plant Treaty (Esquinas-Alcázar et al., 2013). Based on the historical existence in an ecosystem, landraces should be first accepted and officially recognized for their value (Mercer et al., 2012). After that, the risk assessment for GMPs should be applied taking into consideration both socio-economic vulnerabilities and nature protection. In other words the biosafety domain is new and may fit or not based on the agricultural management in place which may be scientifically proved (Jacobsen et al., 2013). Also, the local tradition knowledge has to be recognized in accordance to the provisions of art. 8j of the Convention on biological diversity and become part of the existing local agricultural practices (Rahman and Mamun, 2015). Traditional knowledge in Romania exists mainly in relationship with the conventional agricultural practices. Sometimes they are acting

as a perfect mixture in between. An additional element – such as biosafety agricultural practices should be carefully integrated and evaluated in close connection with the conservation of traditional knowledge related to existing landraces, conventional knowledge related to conventional agricultural practices and mainly to the need for on farm preservation of landraces for ensuring food security for the future (Nijar, 2013).

In conclusion biosafety framework continues to be an important debating issue for Romania. If before the 2007, Romania was one of the most important countries for GM soybean cultivation today it changed the soybean with maize cultivation (Lombardi et al 2014). At the local level the major issues not taken into consideration by the current regulatory framework is the protection of landraces. This issue may become important for marginal villages, where local people still cultivate old crop varieties. In such cases the lack of biosafety communication together with the lack of communication for new agricultural practices may negatively influence the preservation of genuine landraces (Dickmann et al., 2015). Under these circumstances, it is a great need for revising the harmonizing between the biosafety and landrace conservation regulatory frameworks as they are provided by the European Union. Moreover, the presence of protected areas, may limit the cultivation of genetically modified plants in the buffering areas or inside protected areas. Considering the political commitments Romania took under the Plant Treaty, all plant genetic resources for food and agriculture should be conserved either in situ or ex situ (Antofie, 2011).

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RESEARCHES CONCERNING THE PRESENCE OF SOME BACTERIAL STRAINS OF BIOTECHNOLOGICAL INTEREST IN COPŞA MICĂ CONTAMINATED PROXIMITY

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Abstract
The coterminous area of Copsa Mica town, occupying an inhabited, agricultural and forest area of about 20,000 ha, presents a historical contamination with Pb, Cd, Zn and Cu ions, which exceeds the maximum permitted levels. Despite the closure of non-ferrous metal processing plants (Sometra SA) and Carbosem Company, producing carbon black, organic acids, plastics, phosphoric and oxalic acids, Copşa Mică town is considered among the most affected settlements in Europe, which continues to face severe pollution problems. Other still unsolved issues are the industrial ash and slag dumps and the areas exposed to wind and rain corrosion, chronically contaminated.
Soil samples were collected from four urban and suburban areas: from outside the polluted area, considered as control (M), from grass areas (CM1), from the rhizosphere of cultivated areas (CM2), and from poorly fertile areas (CM3), with the highest representation for the groups of aerobic facultative anaerobic heterotrophic bacteria and for the strictly anaerobic heterotrophic ones.
The presence of 8 physiological groups of bacteria was identified by cultivation on selective media. The high density, even in the samples from heavily contaminated areas, reveals the adaptation level of the microbiota. From the analyzed samples, several strains were isolated and identified down to the genus and species level, by conventional, biochemical and molecular methods; their ability to synthesize products of biotechnological interest was tested as well.
The present work is a signal as to the presence of a specific microbiota in chronic polluted areas, this formed microbiota being a result of the selection achieved under contamination stress conditions.

Key words: contamination/pollution, bacterial strains, biotechnology

Introduction

Copsa Mica town and its surrounding areas are a case study in terms of historical pollution, in particular with Zn, Pb, Cd, Cu metal ions, as well as with SO_2 and carbon black. Until a few years ago, in the center of the town, two industrial plants functioned: Sometra, founded in 1936-1939 (1), which provided 7% of Romania's ferrous metal production, supplying in 200,1 50000 tons of metallurgical Zn, 18,000 tons of refined Pb and 40,000 tons of refined Zn, and Carbochim plant, which manufactured carbon black, organic acids, plastics, phosphoric and oxalic acids, formaldehyde, etc.

As a result of the processing of metal ores, representative deposits of residual material accumulated in the industrial area in the form of slag heaps and industrial ash, which occupy an area of over 15 ha, located in the valley between two watercourses: Valea Visei and Tarnava Mare, the accumulation of heavy metals recorded at ground level down to depths

below the rhizosphere layer, exceeding the maximum permissible values from a few dozen times to more than 130 times.

Air pollution, diminished after the closure and decommissioning of plants, could not be completely removed due to wind dispersion, which brings in dust from the areas with no vegetation, even desertified. It was found that the process of humification was disrupted in an area of over 18,000 ha of agricultural land and 3,000 ha of forests.

Thus, the entire urban community as well as the crops and the livestock had suffered several generations and continue to be affected, due to the fact that the contamination still remains above the permissible limits.

All these aspects, briefly mentioned in the introduction to this paper, were developed in detail in reports, essays and synthesis papers.

Starting from the chronic conditions offered by the Copsa Mica area, we collected soil samples in order to assess the microbial diversity and to isolate bacterial strains tolerant to high concentrations of contaminants.

At the same time, out of the strains isolated and identified down to genus and species level, several strains were selected capable of synthesizing biologically active products, with potential biotechnological applications.

Our investigations are still going on, the following steps aiming at the characterization of the synthesized bacterial metabolites by gas chromatographic and Fourier transform infrared spectroscopy techniques. Simultaneously, we intend to expand our researches by both electron microscopic observations (visualization and comparison of bacterial cells and their intracytoplasmic inclusions, under contamination stress) and molecular biology experiments for an as rigorous identification of bacterial strains under study, on one hand, and on the other hand, for establishing the intimate mechanisms for the synthesis of bio-products.

The partial results presented in this paper supplement the picture of Copsa Mica case study with microbiological information, the indigenous microbiota thus characterized may represent a pattern for areas contaminated with heavy metals. By in-depth studies of these microorganisms, different bacterial species can be selected that can be used as indicators for metal ions pollution.

Material and methods

Our researches started with the collection of soil samples from the Copsa Mica urban and suburban area, considering as control the samples taken from an area somewhat outside

the polluted zone (CM) and other three soil samples: CM1, from a grassy plot, CM2, from a crop field whose soil showed metal ions values above the allowed limit, and CM3, from an poorly fertile and heavily eroded area.

Soil pH measurements were performed with portable pH-meter Digi-Sense.

Samples were processed by serial dilutions and cultured on selective media specific for the different physiological groups. The selective media used were: S Postgate, Vinogradsky, LB, VL, Postgate, 9K, medium 4 recommended by Manning, and nutrient agar (2). Isolated bacteria werw characterized and identified by classical techniques of bacterial taxonomy and by the BIOLOG method.

Experiments were also conducted for assessing the ability of isolated bacteria to synthesize products with biotechnological potential such as surfactants and polyhydroxyalkanoates, respectively. The assessment consisted in "colorimetric" reactions: the strains which have the ability to secrete polyhydroxyalkanoates appear fluorescent under UV light if, after they were grown on Blue Nile containing medium (3), and strains that secrete surfactants produce lysis of red blood cells (4).

Results and discussions

The pH of the sampled soils ranged between 5.25 and 5.7 for the contaminated area and 4.8 for the area outside the perimeter of pollution. Values that fall within the land values in Romania (5). The difference between the pH of contaminated sites and the area outside the perimeter of pollution may be due to presence of various pollutants, but this is only an assumption because the differences are not significant.

A first analysis of the samples was performed by culturing in selective media; the results being presented in Table. 1 which shows the presence of the main physiological groups of bacteria in the soil samples collected from the Copsa Mica urban and suburban area.

Table 1. Cultivation on selective media samples

Selective cultivation media	From outside the contaminated area - CM	From the contaminated area		
		CM1 Grassy plot	CM2 metal ions above the permissible limit	CM3 poorly fertile and heavily eroded area
pH	4.80	5.25	5.20	5.7
Vinogradski	$1.4x10^4$	$1.1x10^6$	$1.4x10^4$	$2.2x10^3$
LB	$3.5x10^8$	$1.1x10^6$	$1.6x10^4$	$3.5x10^8$
VL	$4.5x10^8$	$4.5x10^4$	$3.5x10^8$	$4.5x10^4$
Postgate	$1.1x10^6$	$1.1x10^6$	$1.5x10^5$	$3.0x10^4$

Lethan	$0.9x10^2$	$2.5x10^2$	$0.4x10^2$	$2.5x10^2$
9K	$1.1x10^6$	$4.5x10^2$	$2.5x10^2$	-
Medium 4	$1.4x10^4$	$4.0x10^4$	$1.4x10^4$	$1.1x10^5$

High concentrations were noticed of up to $4.5x10^8$ bacterial cells/ml of analyzed sample of aerobic heterotrophic bacteria for the area surrounding the contaminated habitat, and of $3.5x10^8$ aerobic facultative anaerobic heterotrophic bacteria, a value that is maintained for the same physiological group of bacteria isolated from the heavily contaminated area with low fertility CM3.

Calculation of cell density was based on serial dilutions according to McCready table (6).

The presence of iron-oxidizing and sulfate-reducing bacteria as well as acidophilic chemolithotrophic and acidophilic heterotrophic bacteria was also recorded. Of these bacterial cultures grown on the mentioned selective media, three strains were selected, CMM1, CMM3-1 and CMM2, strains that may be of interest to biotechnology.

The strains, morphologically characterized (Table 2), were subjected to classical bacterial taxonomy techniques and to the BIOLOG biochemical technique; thus, a presumptive belonging to the *Pseudomonas* genus was established.

Table 2. Morphological characterization bacterial strains

Strain	**CMM1**	**CMM3-1**	**CMM2**
Characterization of colonies	time of occurrence: 24 h at 37 ºC; appearance: rough; size: 0.4 mm color: yellowish-cream, opaque shape: circular, round margins: irregular profile: convex surface: smooth.	time of occurrence: 24 h at 37°C; size: >0.4 mm color: yellowish-cream; slightly transparent shape: round margins: straight profile: slightly convex surface: smooth	time of occurrence: weak growth after 24 h and abundant after 48 h at 37 °C; size: 0.4 color: white-creamy; shape: round; margins: smooth; profile: convex; surface: smooth

Out of the three strains of interest isolated from the samples collected from the Copsa Mica area, CMM3-1 and CMM2 strains showed the ability to synthesize polyhydroxyalkanoates. Fig. 1a shows a bacterial culture in Petri dish.

The CMM2 strain possesses the ability to synthesize metabolites of surfactant type, the test for assessing the presence of these biologically active substances being the lysis of erythrocytes (Fig. 1b) (7)

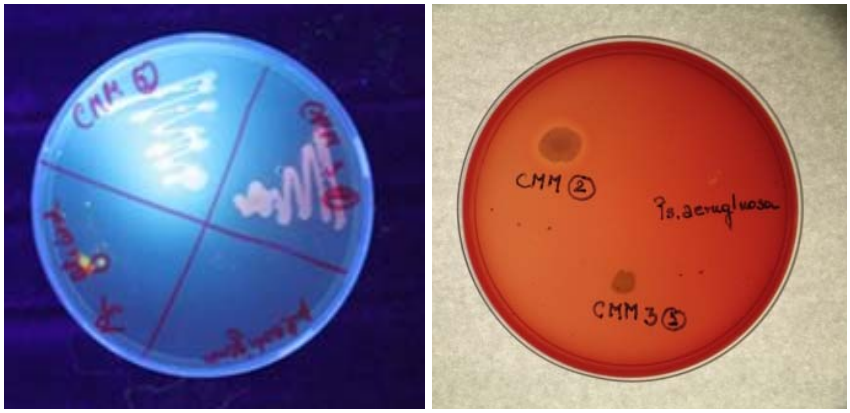

Fig. 1: Demonstration of the synthesis of polyhydroxyalkanoates (a) and surfactants (b)

The results of the undertaken researches confirm the fact that in strongly affected areas, the characteristic microbiota adapts to the contamination stress conditions, certain bacterial strains with potential for biotechnological applications showing a high versatility in terms of development under favorable and / or extreme conditions.

Conclusions

In the areas heavily polluted with metal ions and industrial waste there is a characteristic microbiota resistant to contamination stress conditions.

The indigenous microbiota, by the presence of certain bacterial species, may represent a pattern for areas contaminated with heavy metals.

Three bacterial strains of interest, belonging to *Pseudomonas* genus, were isolated, selected and characterized.

The ability of CMM2 bacterial strain to synthesize biologically active substances of surfactant type was demonstrated.

Corroborating the results obtained in these case studies can guide the development of effective strategies to reduce industrial pollution in areas with historical pollution.

Acknowledgement

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RESEARCHES CONCERNING THE EFFICIENCY OF MICROORGANISMS INVOLVED IN THE DEVELOPMENT OF BIOTECHNOLOGIES TO REDUCE THE CONCENTRATIONS OF HEAVY METALLIC IONS

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Abstract
Industrial wastes include a wide range of materials depending on the nature of the basic industry (rubber, salts, insoluble minerals, organic materials, metalliferous sludge, tannins), many with toxic properties. The infiltration of the washing/dissolution products out of waste into the soil and the surface water leads to diffuse pollution, difficult to control. Among the most common contaminants we find the heavy metals. The existence of different forms of impact upon the ecosystems has led to the reduction of the biodiversity. There is a direct conditioning between the pollution effects and soil microbiota. In this context, it became necessary studying the adaptation of the microorganisms, isolated from representative mining sites in Romania, at extreme conditions characterized by acidic pH and higher concentrations of heavy metallic ions and selecting culture with high resistance to the presence of these ions in the environment.

Keywords: clean technology, soil microbiota, metabolites, heavy metals

Introduction

Romania, like many other Eastern European countries, has to deal with serious problems of environmental pollution due to the intensive industrialization practiced in the past 25 years. Permanently, the environmental factors (water, air, soil) have been subjected to the human aggression and the progressive worsening in their quality has led to different social implications with repercussions on life quality.

Romania industrialization and agricultural development has brought negative effects on the possibilities of natural ecosystems restoration. Lead has a special place among the toxic metals, as form the atmosphere; it reaches into the ground through both irrigation water and fertilizers [1, 8, 13].

The presence of representative areas in Romania which were held for decades intense industrial activity that generated the chronic and historical pollution entails, give rise to liability for playing other destinations these sites strongly affected by the ion of Cr^{6+}, Cd^{6+}, Pb^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+} and Cu^{2+}, a program adequate management as regards to reducing the level of contamination, but equally to the soil protection and the study of its specific biodiversity. The investigations must focused, in specially, on the rhizosphere microbiota and

the external surface layer in studied area are aimed at the identification, characterization, observation of the action of the cellular versatility under the action of contamination stress and eventually exploitation of intra and/or extracellular metal ions accumulation [3, 10, 12].

Certain groups of microorganisms are considered bioindicators of soil pollution and knowledge specific metabolic mechanisms may constitute premises for possible biotechnological applications of biosolubilization, bioaccumulation and even attenuation harmful effects due to the presence of metallic ions excess [4, 14].

The degradation activity of microorganisms is a potential alternative solution to traditional environmental clean technology. Globally, they have major contributions to the transfer of biological elements in nature and locally prevent the accumulation of some waste materials by different environmental contaminants [2, 11].

The acid mine water is a major problem in the mining industry as the most valuable economic minerals are associated with deposits of iron and aluminum sulphate. The resistance of the microorganisms to heavy metals is due to several resistances mechanisms namely: (a) removing metals; (b) permeability barriers; (c) metallic ions active transport; (d) extracellular accumulation; (e) enzyme detoxification [5, 7, 13].

The present study shows how bacteria adapt themselves to extreme conditions of acidity in the context of the environment increasingly polluting with metallic ions due to the industrial activities of processing ore with high economic value. So over time, in our laboratory we managed to isolate microorganisms that have the ability to tolerate the presence of high concentrations of pollutants the type metal ions.

Materials and methods

1. Criteria for a successful system

The industrial development, especially in the perimeters with intense mining activity and also the agribusiness bring impressive volumes of wastewater contaminated with metal ions, sulphates, azotates/nitrates and other pollutants that seriously affect the environment. Legislations stipulate the permissible limits of such contaminants, but these levels are often exceeded, resulting, in case of accidents, in disasters, which are common in Romania and its neighbor countries [1, 7, 20].

On the whole, the accumulation of products with inhibitory activity and the antagonism phenomena contribute, together with the quality and quantity changes of the nutrients, to the

appearance of changes in the structure of the microorganism's community, underlying the succession of the populations in an ecosystem [3, 5, 13].

2. Development of the microbial system

Accidents occurred in Romania in 2000 and 2001, when waters contaminated with metallic ions and cyanide affected important rivers such as the Tisa and Danube in 2000 and the Aries River in 2001. These were generated by the waters of the decantation ponds in the Baia Mare area and Rosia Poieni copper exploitation, Alba County (the Valea Sesei decantation pond). Also were collected soil samples contaminated with Cd^{6+}, Pb^{2+} and Cu^{2+} from Copsa Mica area (Sibiu country) [1, 10, 11, 21].

The experiments of the microbiological analysis revealing and quantitative estimating of the following physiological groups of microorganisms: macroscopic fungi (fungi and yeasts), heterotrophic aerobe and facultative anaerobe bacteria, sulphur- and iron-oxidizing bacteria. In the experiments of degradation, biosorption and bioaccumulation these cultures has been incubated, for 21 days, in the various experimental conditions: (1) temperature: 28^0C and 37^0C, acidity: pH – 2.0, 3.0, 4.0, 5.0, (3) static and stirring (150rpm) conditions. The microorganisms cultures have been grown in 100ml Erlenmeyer glasses containing 50ml growth medium and 5ml inoculums (isolates of 7 days old) [6, 10, 21].

Results and discussion

1. Selection of the field target

Sedimentary materials alter the basic vegetation and the bentall biomass, causing the modification of the microbial communities. If the effluent treatment is inadequate and its dilution in the emissary is too low, the spill area is ecologically compromised by the disappearance of most species from flora and fauna, except bacteria, protozoa, fungi and algae that form white and dark brown colonies on solid objects. The algae populations are reduced or disappear. Fish migrate or die and can be found only in distant downstream sections where natural conditions begin to recover. The extinct species are replaced by other species that form the non-specific pollution of microbiota in natural conditions [4, 6, 9].

2. Field tests

Compounds of heavy metals such as Cr^{6+}, Cr^{3+}, Cu^{2+}, $Zn^{2+}$and Ni^{2+} presents in industrial residual waters and mining tailings/coals have an obvious influence on plants, especially in

the neighborhoods of the metallurgical enterprises such as Rosia Poieni (Alba county), Ilba (Maramures County), Copsa Mica (Sibiu County), Baia (Tulcea County). In this case, our studies revealed that the heavy metal toxicity limits are usually set according to their influence on plants. Also, the toxicity of heavy metals often depends on the granulometric composition of the soil [4, 17, 19].

3. Current work

The transformation of heavy metallic ions is part of the "remediation by reduction" process, which can be applied to contaminated soils, resulting in environmental deterioration risk reduction. The reduction of metal ions in environments polluted with organic substances is a ubiquitous process, aerobic and anaerobic, involving different microorganisms that detoxify the environment [4, 12, 20].

One advantage in the reduction process is that its optimal temperature and acidity value coincides with the optimal development and enzymatic activity of the microorganisms involved in the remediation of areas polluted with heavy metals. In this process, an important role belongs to the microbiological analysis of waste waters loaded with metallic ions aiming to the isolation of the microorganisms with potential of reducing/sorption of metal ions existing in those sites.

Emphasis of thermo stable enzymes in relation to the specific physiological particularities of acidophilic microorganisms is important on the biotechnological potential of the bacteria present in the mining sites. In this case, the enzymatic activity of the acidophilic bacteria at ecosystem level is very important in limiting the development of some microorganisms populations and forming the soil structure, essential for water, air and nutrients circulation

The results obtained regarding the possibility reduction in the level contamination by organic and inorganic compounds through the accumulation and exploitation of metallic ions by using bionanotehnologic approaches to microorganisms are opportunities to play for contaminated habitats in their natural cycles and reducing their anthropization.

The prey-predator relationship between the mesophilic bacteria, heterotrophic and chemolithotrophic, and the forcedly acidophilic protozoa was observed and measured under laboratory conditions. It was found that Eutrepia-like flagella preferentially hunts the *Acidithiobacillus ferrooxidans* species as against the *Leptospirillum ferroxidans* in cultures containing both chemolithotrophic species [3, 5, 10].

Conclusions

The understanding of the influence of physico-chemical conditions on the enzymatic activity of acidophilic heterotrophic bacteria is very useful in establishing their action on the degradation of organic compounds in the polluted environments with inorganic substances. The starch-containing liquid media have several advantages over solidified media, including versatility and stability. The physic-chemical stability of the enzymatic structure allows the development of organic medium that can support the growth profile of acidophilic heterotrophic bacteria and the extraction of extracellular metabolites produced by these bacteria.

Our studies with pure and mixed cultures of microorganisms showed their ability to synthesize extracellular enzymes, in certain conditions, in significant amounts lead to the reduction of environmental pollution by organic substances in extremes conditions of acidity. On the other hand, it is important to increase the efficiency of biosorption, bioaccumulation and biosolubilization processes using microorganism's cultures in industrial laboratory conditions. In this context, the study mentioned above reveals that decreasing heavy metals pollution using the acidophilic microorganisms, heterotrophic and chemolithotrophic, isolated from industrial waste water collected from Baia area (Tulcea County) and Rosia Poieni (Alba County) is possible.

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COMPARATIVE STUDY OF PHYSIOLOGICAL PARAMETERS AND PRODUCTIVITY OF TWO TOMATO VARIETIES AFTER APPLYING FUNGICIDE TREATMENTS AND ACETYLSALICYLIC ACID

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Abstract
In this paper were studied certain physiological parameters and productivity of three tomato groups in the field: treated with acetylsalicylic acid, systemic fungicides based on 60% mancozeb and 9% dimethomorph, and contact fungicides - $CuSO_4$ (Bordeaux) mixture, respectively. The acetylsalicylic acid was selected to replace the fungicides and to stimulate the plant defense system.
Physiologically there were no significant differences between treatments. The acetylsalicylic acid treated tomato group had a good resistance to Phytophthora infestans attack. The early production of tomatoes treated with sustainable fungicide - based on Cu - was significantly higher than that of tomatoes sprinkled with systemic fungicide (control group) and the total production did not present significant differences between treatments.

Keywords: acetylsalicylic acid, fungicides, *Lycopersicon esculentum,* plant physiology

Introduction

Modernization has led to an increase in the means and methods of superior valorization of vegetable production, as well as chemical disease control methods (Stoleru and Imre, 2008). Pesticides, more diversified year by year, influence vegetables quality by changing the biochemical composition and physiological parameters.

Fungicides have always been in the attention of scientists. Regardless of their origin, concentration or usage, they are toxic for the environment and human health, and they have been a lot of debate worldwide for years. He and Wetzstein (1994) cited by Tort et al (2005) showed that systemic fungicides application interferes with pollen development, causing delay in flourishing and leaf formation. In 2000, Pavlik and Jandurava cited by Tort et al (2005) reported that fungicides create a toxic effect on pollen germination and fruit formation. Similar results were obtained by Horotan and Oancea (2013) showing that tomato flowering was accelerated by the systemic fungicide solution, and fructification was lower compared to the control group (untreated) one. In addition, fungicides influenced the structure of mechanical tissues and fruits skin, which became breakable. Certain fungicides cause pollen grain structure abnormalities, as well as meiocites and bivalents abnormalities. The number of abnormalities increases proportionally with the fungicide dose used. The formation of sterile pollen leads to a decrease in tomato production (Öztürk Çali, 2009).

Copper fungicides applied foliary can affect the yield and plant growth of tomatoes. When applied to both leaves and soil, they reduce the total crop yield, fruits number and tomato growth (Sonmez et al, 2006). The fungicides with high concentrations of Cu can cause a total yield decrease of the crop, a decrease in the number of fruit and the height growth of tomato plants (Sonmez et al, 2006). Mazhoudi et al (1997) cited by Sonmez et al (2006), reported a decrease in the growth rate of tomato plants, at the application of 50 pM Cu treatment in a nutrient medium. The fungicide application, both systemic and contact, affect crop physiology by various processes, such as growth reduction, disturbances in the reproductive organs development, alteration of nitrogen and carbon metabolism leading to a decrease in the supply of plant nutrients. Some plants are more sensitive in critical moments of their development, like the seedling stage or reproduction (Petit et al, 2012). Pavlik and Jandurava (2000) cited by Tort et al (2005), showed that fungicides create a toxic effect on pollen germination and fruit formation. The formation of fruits may also be influenced by other factors, such as culture system and pedoclimatic factors. Creamer et al (1996) obtained a greater number of fruit and flowers in tomatoes cultivated in the conventional system compared to the organic farming, therefore tomato production was higher in conventional culture.

Within this paper we have studied various physiological parameters and productivity of three tomato groups in the field: treated with acetylsalicylic acid, systemic fungicides, and contact fungicides - $CuSO_4$ (Bordeaux) mixture, respectively. Acetylsalicylic acid was selected to replace the fungicides and to stimulate the plant defense system. Tomatoes present a large number of varieties. In the last years, older varieties have been replaced with new ones and hybrids with certain superior qualities, adapted to current environmental conditions, approved and listed in the "Official Catalogue of varieties of crop plants in Romania" (Indrea et al, 2012).

The "Buzău 50" variety was chosen because it is an appreciated Romanian product, it has a good reaction to temperature variations and good resistance to diseases (http://www.scdlbuzau.ro, http://www.rosiidingradina.ro).

Salicylic acid acts as a plant hormone that generates a positive effect on plant growth and photosynthesis, but mainly on the plant defense system. The acetyl group of salicylic acid does not change these characteristics of the plants (Horotan and Oancea, 2013).

Materials and methods

Two Romanian tomato varieties have been used: the **Sibiu** local (population) and **Buzău 50**. Three experimental tomato groups of each variety were formed: two groups treated with chemicals, i.e. systemic fungicides and contact fungicides and the third group treated with acetylsalicylic acid solution. From the two tomato varieties, 6 experimental variants located in 3 replicates have resulted. The chemicals used in this experiment are commonly used by farmers: the systemic fungicide based on mancozeb (60%) and dimethomorph (9%) - EU approved - considered as control group (TP), and contact fungicides based on $CuSO_4$ (Bordeaux mixture), a chemical treatment used in sustainable agriculture (TS). The treatment with acetylsalicylic acid (aspirin) (TA) is an original treatment proposed to replace other treatments. The chemical treatments were applied with the recommended dosage of 20 g/10 l water for systemic fungicide and 50 g/10 l water for Bordeaux mixture. The treatment with acetylsalicylic acid was obtained by mixing 500 mg aspirin with 1 l water (1.6 g/m^2). All treatments were administered at 10-day intervals.

The tomato crops were placed in subdivided plots, modeled in layers, 70 cm between rows and 30 cm between plants in the row. The experimental crop was placed in Şelimbăr, Sibiu county. The land has a southern exposition, middle texture soil (sandy-loam) and 3% humus content (Office of Pedological and Agrochemical Studies, Sibiu). Regular observations were made in the field, to the growth and development of vegetative organs, of inflorescences and fruit number of each variety and treatment. Quality and quantity production of tomatoes was monitored at harvest time, depending on the variety and the treatment used.

Growth parameters of vegetative organs - stem / leaf - were measured at intervals of 30 days, on specimens randomly selected from different groups of plants. Similar intervals were used for observation and determination of multiple physiological parameters: number of leaves, inflorescences and flowers in inflorescence, number of fruit.

Early harvesting began in August and was completed in October. Each harvest was weighed in kilograms (kg), production / lot / repetition.

Statistical analyses were made using one-way ANOVA test, parametric tests: k sample comparison of variances, t-Student test and nonparametric tests: Multiple pairwise comparisons using the Steel-Dwass-Critchlow-Fligner procedure, Friedman's test at $p<.5000$.

Results and discussion

For both varieties, 60 days after being planted in the ground, the tomatoes treated with acetylsalicylic acid (TA) and contact fungicide (Bordeaux mixture) (TS) recorded insignificant differences compared to the tomatoes treated with systemic fungicide (control group) (TP). The differences at 90 days after planting in the field were insignificant. (fig.1)

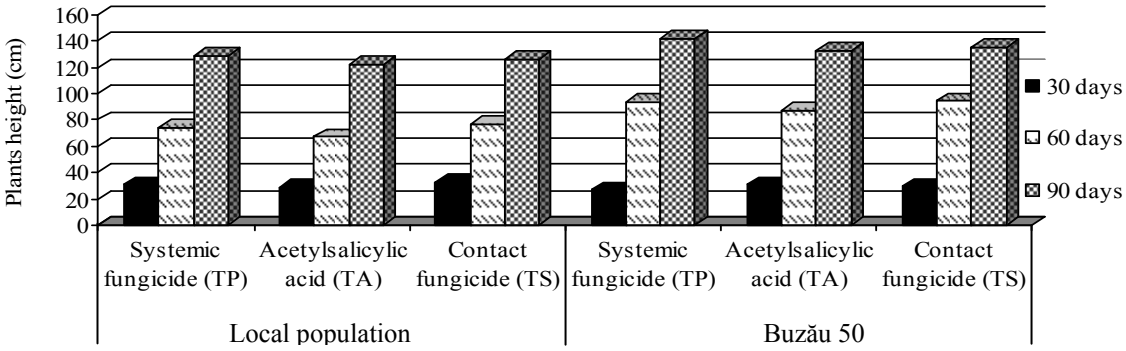

Fig.1. Tomatoes height growth (cm) of two experimental varieties treated with fungicides and acetylsalicylic acid (summer 2014)

At certain moments of their development, the plants are sensitive to some factors such as pesticides, that stress them. In response to these stressors, plants mobilize nutrients and metabolism products to develop a defense mechanism, to the detriment of growth (Petit et al, 2012). Plant growth can also be inhibited by the action of environmental factors such as light intensity and strong temperature fluctuations (F.W. Went, 1944). Creamer et all (1996) obtained insignificant differences regarding tomato growth in organic and conventional crop systems.

The average of tomato flowering showed insignificant differences compared to control for both varieties tested, both 60 and 90 days after planting in the ground. (fig.2)

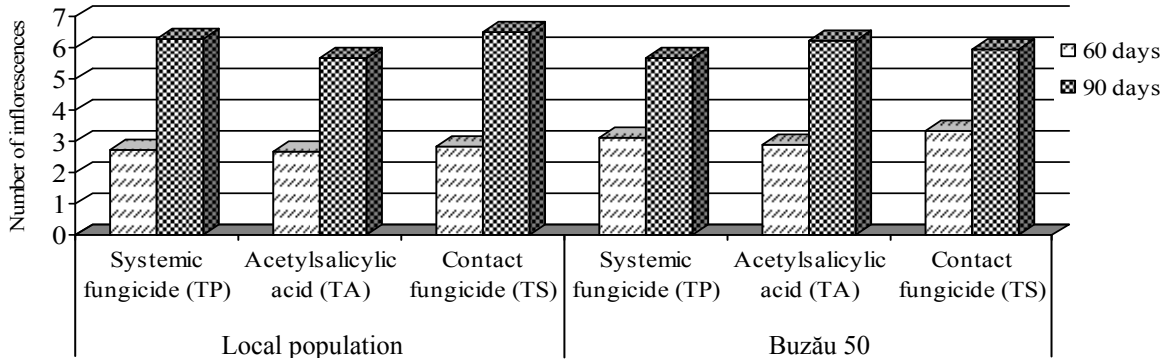

Fig.2. The inflorescence number of two experimental varieties treated with fungicides and acetylsalicylic acid (summer 2014)

Although the inflorescence number recorded no significant differences, the fruit number in August (60 days) has presented significant differences ($p<0.5000$) between treatments. The acetylsalicylic acid treated tomato (TA) had a significant increase in fruit number compared with the control group, the mean differences were by 1.33 fruits per plant in local variety and 2.5 fruits in Buzău 50, respectively. In the same period, the tomatoes treated with contact fungicide (TS) had very significant increase in the fruit number compared to the control (TP), the mean differences between treatments being 2.83 fruits in local variety and 4 fruits in Buzău 50. (fig. 3)

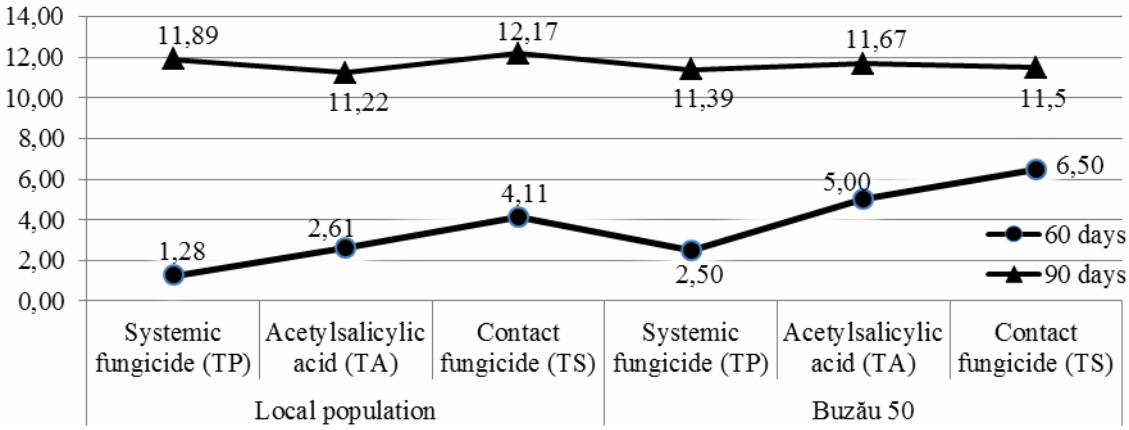

Fig.3. The fruits number of two experimental varieties treated with fungicides and acetylsalicylic acid (summer 2014)

At the beginning of September the *Pytophthora infestans* infection symptoms were obvious in all experimental variants. The treatments with systemic fungicide stopped plant infection, plants continuing to grow and form their fruit. The other variants – treated with acetylsalicylic acid and contact fungicide (Copper sulfate) slowed their growth and fruit formation, in order to form their defense response against the pathogen agent (Petit et al, 2012). After they managed to slow down even a *Phytophthora infestans* infection rate of over 80%, tomatoes treated in sustainable system continued fruit formation. Therefore, their differences in fruit number between treatments, after 90 days, were insignificant.

The tomato harvesting began in the first decade of August for acetylsalicylic acid (TA) and Bordeaux mixture (TS) variants, second decade of August for systemic fungicide (TP) variants respectively and continued until the second decade of October (Table 1).

Table 1. Dynamics of tomato production (kg/m^2), location Şelimbăr, August – October 2014

Variant		August					September					October		
Cultivar	**Treat.**	1	2	3	4	Tot.	1	2	3	4	Tot.	1	2	Tot.
Local Sibiu	**TP**	0.00	0.29	0.20	0.06	0.55	1.55	0.24	1.99	0.61	4.39	0.56	1.54	2.10
	TA	0.11	0.12	0.19	0.00	0.41	1.24	0.48	0.16	0.46	2.34	0.31	1.64	1.95
	TS	0.09	0.97	0.16	0.00	1.22	0.44	0.27	0.19	0.27	2.17	0.77	1.14	1.91
Buzău 50	**TP**	0.00	0.29	0.43	0.34	1.06	2.66	0.97	2.39	0.95	6.24	0.34	1.67	2.01
	TA	0.11	0.17	0.05	0.00	0.33	0.81	0.44	0.81	1.11	3.17	0.58	2.04	2.62
	TS	0.06	1.23	0.27	0.00	1.56	1.32	0.83	0.56	0.56	3.27	0.71	1.23	1.94

The largest fruit amounts have been harvested in the third decade of September and second decade of October, in all experimental variants, except for acetylsalicylic acid and contact fungicide treatment of local variety. Generally, the Buzau-50 variety had better yield dynamics than the local population of Sibiu. (fig.4).

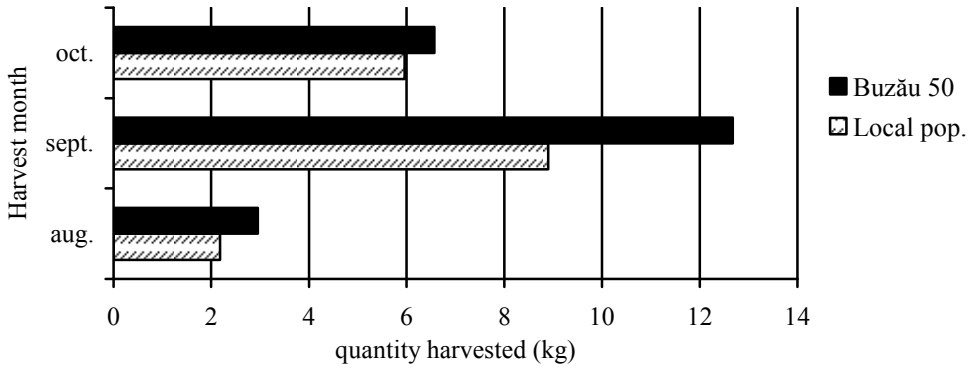

Fig.4. Tomato yield dynamics (kg/m^2) of two experimental varieties harvested during August 2014 – October 2014

The used treatments for disease control influenced the production level. The early production was between 0.38 kg/m^2 to acetylsalicylic acid treated variant (TA) and 1.39 kg/m^2 to Bordeaux mixture treated variant (TS). To the variant treated with acetylsalicylic acid the production had a decrease compared to the variant treated with systemic fungicide (TP), the difference being 0.42 kg/m^2. The variant treated with copper sulphate had an increase of 0.59 kg/m^2, compared to the control group. (fig.5)

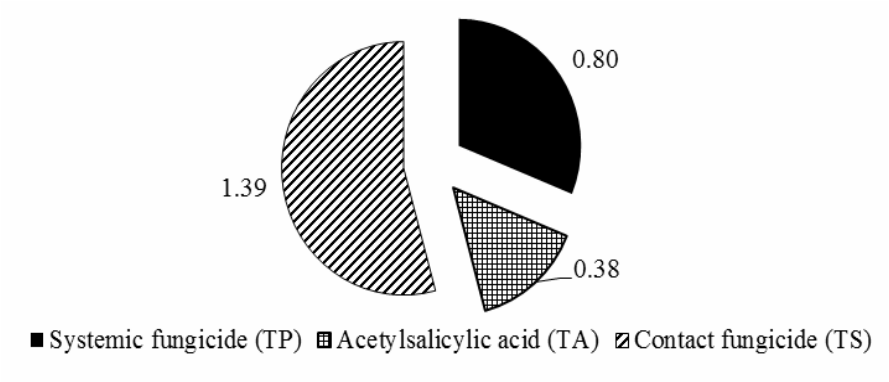

Fig.5. The treatment influence upon
early production of tomato, kg/m^2, August 2014

The total production was between 5.42 kg/m^2 for acetylsalicylic acid variant and 8.54 kg/m^2 for systemic fungicide variant (TP – control group). The production difference between those two variants was by 3.12 kg/m^2. For Bordeaux mixture treated variant (TS), the production difference was by 3.00 kg/m^2 compared to control (TP) (fig.6).

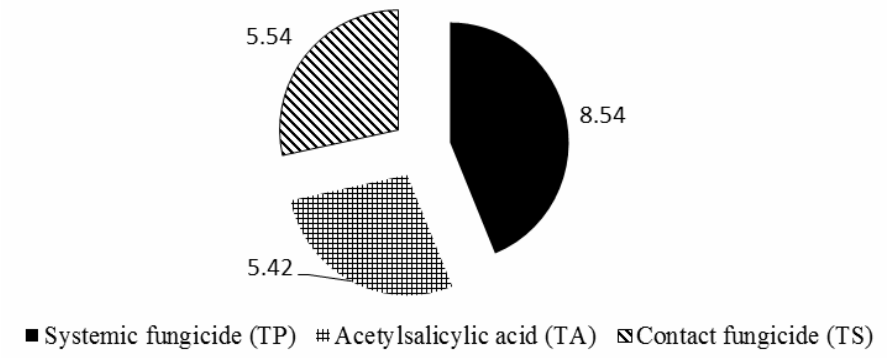

Fig.6. The treatment influence upon the total production of tomato,
kg/m^2, period of August - October 2014

Conclusions

There were no significant differences between experimental variants for the height growth and inflorescence number. The fruit number of Bordeaux mixture variant was significantly higher 60 days after planting in the ground. After 90 days, the differences were insignificant between variants.

The tomatoes treated with acetylsalicylic acid had a good resistance to *Phytophtora infestans* attack.

The Buzău 50 variety had a better yield dynamics compared to the Sibiu local variety.

Early production differences between acetylsalicylic acid and the systemic fungicide

treated variant were 0.42 kg/m^2 and 0.59 kg/m^2 between tomatoes treated with sustainable treatment and control group.

The total production differences between treatment were 3.12 kg/m^2 for acetylsalicylic acid tomatoes and 3.00 kg/m^2 for tomatoes treated with sustainable treatment compared to control.

These preliminary results enable us to pursue our research, in order to provide the farmers with another, better option for plant protection.

Acknowledgements

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HEALTHY ECOLOGICAL PRACTICES AND ECONOMICALLY ATTRACTIVE OF LEGUMINOUS PLANTS CROP FERTILIZATION

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Abstract
The strategy of current world agricultural development involves environmental practices that have to be sound and economically attractive. The use of biofertilizers in organic agriculture is a safe and cheap alternative to conventional chemical fertilizers. This consists of including in the agricultural microbiocenosis of population of symbiotic nitrogen fixers and / or associates, of the genus Rhizobium. The agro-ecological area Arad were tested with positive results a series of biofertilizers produced in Romania: Biotrofin, Ecoferil B., and Azotofertil.

Keywords: Biofertilizers, Organic Agriculture, Organic Practices.

Introduction:

Organic farming (organic, biological) through all its trends and it aims in updating the traditional methods, verified over the centuries through the evolution of human civilizations and combining them with modern methods, in order to ensure food security. The basic elements of technological links are supported by crop rotation, using green fertilizers for the fertilization, biological control of diseases, pests and weeds. Ecological agriculture involves healthy and attractive practices, while biological nitrogen fixation is a solution by which plants give up the use of chemical nitrogen as fertilizer and replacing it with nutrient solutions resulting from the bacterial activity. By stimulating of these activities it can introduce bacteria in the agricultural ecosystem of biological nitrogen an intake of between 50-100 kg / ha. Thus, biofertilizers can be an alternative solution to the use of chemical fertilizers.

Materials and method:

In 2014 there were studies conducted in the agro ecological area of Arad, this area is characteristic for vegetable growing in the west part of the country. We aimed at finding alternative solutions, reliable and inexpensive, to using chemical fertilizers which have nitrogen in the composition. There were verified several bio stimulators and products based bacteria with symbiotic capacity to fixing atmospheric nitrogen in the soil and making it available to plants in an acceptable form.

The experiment hook place during the period of 2011-2014 using the *Pisum sativum* L. Bőrdi variety and at the *Phaseolus vulgaris*, Valja variety, compliance each year of cultivation recommended technology for these species. The study comprised a single graduate factor (monofactorial), by the method of subdivided parcels, with variants in four repetitions

(witness without chemical fertilizers). The recovery of data was done by the method of analysis of variance of each year and a series of single factor the experiments was performed several years at the same location.

The biofertilizer Biotofin is a live bacteria that has two components, nitricphosphoric traps bacteria which secures the necessary nitrogen from the atmosphere, and *Bacillus megaterium,* which solubilized tricalcium phosphate making it accessible to plants. Ecoferil P product is a suspension of spores and vegetative cells of Bacillus megaterium, phosphaticum variety that insoluble products soluble like phosphorus, iron, magnesium, potassium and zinc. The Azotofertil product is a liquid culture of two nitrogen fixing bacteria, *Azotobacter chroococcum* and *Azospirilim lipoferum*, is a product that provides nitrogen, growth factors and phytohormones required in the plants development.

Pisum sativum L. and *Phaseolus vulgaris* L. are species of great economic value and because different types of chemical treatment applies both on seeds and vegetation, our research was conducted in order to evaluate the effects of induced unapproved strains of rhizobacteria (biofertilizers) on the growth and development of plants of peas and beans in the garden.

Results and Discussions :

The results opened after the research carried out during 2011-2014 at bush species of vegetables, were determined by the cumulative action of the factors studied as well as climatic conditions of the four years of research. *Phaseolus vulgaris* L. and *Pisum sativum* L. are species of vegetables that are influenced by the climatic factors in a positive or negative way.

After analyzing the data from the table 1.1 concerning the influence of the biofertilizers on the production of *Pisum sativum* L. in the ecological area of Arad, after the 4 years (2011-2014) of studies we have observed that there were significant differences. The difference between the biofertilizer applied at seed state, also just before germination, and in vegetation state, it is noted with significant results in all combinations compared with the untreated control witness MT1 (the traditional method without fertilizer application). The differences in production compared to the control specimen are between 606-481 kg of seed per hectare. Compared to the second control witness MT2 which represents the average of all the methods, significant result we have because at the combination of fertilizers (Biotrofin + Ecofertil P. and Biotrofin + Azotofertil) the difference in production was between 162-121 Kg of seeds on hectare.

At the interaction the variable x years, results indicate significant differences between the variants with combinations of fertilizers and between the variants in the years of the experiment, and in a possible repetitions of the experiment.

The influence of biofertilizers on the production of seed at the variety *Pharsalus vulgaris* L. in the ecological area of Arad it is presented in the table 1.2. The biofertilizers tested have outstanding effects of crop growth and yield, with very high statistical difference compared to the control witness MT1 (the traditional method without fertilizer application). The differences in production compared to this control witness over the four-year of studies, vary between 738 and 454 kg of seed per hectare, compared with the second witness of MT2, which represents the average of all the methods.

Very significant results stand out from the combination of the biofertilizers (Biotrofin + Ecofertil P. and Biotrofin + Azotofertil) production differences are between 205-169 kg seeds per hectare. Analyzing the interaction of x years, the results show very significant differences in the variants of combinations of biofertilizers and between these the variants both in the years of from the experiment and an eventual repeat of the experiment.

During the vegetation period there were made observations and determinations relating to the installation and product longevity and of the symbiosis of the two species leguminous. Therefore in the first 15 days of the sprouting phase (R2) there are formed nodosities on the roots, especially the main root, but they are functionally active in the percentage of (15-20%).

Table 1.1 The influence of biofertilization over the production at *Pisum sativum* L.in the agroecological area of Arad during (2011-2014)

V	Biofertilizers	Amount (l/ha)	The Application Method			t/ha	%	The Difference t/ha	Signification	
			Seed	Seedbed preparing Stage	In the vegetative stage				D^2_E	D^2_{VA}
1	The Classic Method	-	-	-	-	4.301	100	MT1	-	-
							90.6	-0.444	000	000
2	Biotrofin	10	Yes	-	-	4.782	111.2	0.481	xxx	xxx
							100.8	0.037		
3	Biotrofin	10	-	Yes	-	4.801	111.6	0.500	xxx	xxx
							101.2	0.056		
4	Ecofertil P	15	-	Yes	-	4.682	108.9	0.381	xxx	xxx
							98.7	-0.063	x	xx
5	Ecofertil P	15	-		Yes	4.703	109.3	0.402	xxx	xxx
							99.1	-0.042		0
6	Azotofertil	15	-	Yes	-	4.773	110.9	0.472	xxx	xxx
							100.6	0.028		
7	Azotofertil	15	-		Yes	4.760	110.7	0.459	xxx	xxx
							100.3	0.015		
8	Biotrofin Ecofertil P	10 15	-	Yes -	- Yes	4.878	113.4	0.577	xxx	xxx
							102.8	0.133	xxx	xxx
9	Biotrofin Azotofertil	10 15	-	Yes -	- Yes	4.907	114.1	0.606	xxx	xxx
							103.4	0.162	xxx	xxx
10	Azotofertil Ecofertil P	15 15	-	Yes -	- Yes	4.866	113.1	0.565	xxx	xxx
							102.6	0.121	xxx	Xxx
Average						4.745	-	-	-	-
							100	MT2	-	-
						DL5%	DL 1%		DL 0.1%	
						0.075	0.098		0.128	
						0.041	0.051		0.077	

Table 1.2 The influence of biofertilization over the production at *Phaseolus vulgaris* L. in the agroecological area of Arad during (2011-2014)

V	Biofertilizers	Amount (l/ha)	The Application Method			t/ha	%	The Difference t/ha	Signification	
			Seed	Seedbed preparing Stage	In the vegetative stage				D^2_E	D^2_{VA}
1	The Classic Method	-	-	-	-	2.199	100 80.5	MT1 -0.533	- 000	- 000
2	Biotrofin	10	Yes	-	-	2.739	124.6 100.3	0.540 0.007	xxx	xxx
3	Biotrofin	10	-	Yes	-	2.803	127.5 102.6	0.604 0.071	xxx	xxx
4	Ecofertil P	15	-	Yes	-	2.653	120.6 97.1	0.454 -0.079	xxx 0	xxx 0
5	Ecofertil P	15	-		Yes	2.711	123.3 99.2	0.512 -0.021	xxx	xxx
6	Azotofertil	15	-	Yes	-	2.748	124.9 100.6	0.549 0.016	xxx	xxx
7	Azotofertil	15	-		Yes	2.739	124.6 100.3	0.540 0.007	xxx	xxx
8	Biotrofin Ecofertil P	10 15	-	Yes -	- Yes	2.892	131.5 105.9	0.693 0.160	xxx	xxx
9	Biotrofin Azotofertil	10 15	-	Yes -	- Yes	2.937	133.6 107.5	0.738 0.205	xxx xxx	xxx xxx
10	Azotofertil Ecofertil P	15 15	-	Yes -	- Yes	2.901	131.9 106.2	0.702 0.169	xxx xxx	xxx Xxx
Average						2.732	-	-	-	-
							100	MT2	-	-
						DL5%	DL 1%		DL 0.1%	
						0.065	0.093		0.131	
						0.048	0.069		0.097	

Conclusions:

1. Effects of inoculation with nitrogen-fixing microorganisms becomes evident the extent to which to ensure the survival in full entirely on the seed or dispersed on the ground until the germination process starts, or when triggering the specific infectious process.
2. The use of biofertilizers such as Biotrofin, Ecofertil P. and Azotofertil, alone or associated with administration seed or at the seedbed and / or applied in the vegetation stage has positive effects on increasing the harvest for both of the tested species.
3. Biological considerations, technical, economical and sanitary attributes to its intense values in bacterial symbiosis (biofertilizers) a key role of compensation of the shortcomings of nitrogen at crops of *Phaseolus vulgaris* L. and *Pisum sativum* L.

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THE STUDY OF THE PREMATURE FLOWERING OF FENNEL (*FOENICULUM VULGARE, VAR. AZORICUM*) IN ENVIRONMENTAL CONDITIONS OF TRANSYLVANIAN PLATEAU

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Abstract
Heat is a very important factor for plants, and knowing the relationships between heat and plants has a paramount theoretical and practical importance. For protected crops, the occurrence of higher than optimal temperatures, generated by excessive solar radiation during the summer months, can be more frequent. Temperatures exceeding greatly the maximum level reduce the intensity of photosynthesis, enhance breathing and have an adverse effect on plants. When accompanied by drought, they cause plants wilting and premature occurrence of floriferous stems (as the case of green salad, spinach, fennel). In our experience, made in the Transylvanian Plateau, the experimental variants culture both in fields and in solarium showed high percentages of premature flowering.

Keywords: Fennel, premature flowering, climatic conditions

Introduction

Fennel is native of the area between the Mediterranean and the East Indies. As a medicinal and aromatic plant, it is grown both for its fruits, rich in volatile oils and for bulbs. Fennel plants are grown on fertile soils rich in nutrients. The main soils suitable for cultivation of fennel are chernozem and alluvial soils. Fennel is a plant that requires attention, because in summer, when is very hot, it tends to form floriferous stems and the bulbs are no longer developed. The premature flowering is closely related with growth factors, i.e. some vegetables grown in the summer, under long daytime, forms flowering shoots before they developing sufficient vegetative ones, and thus commercial production of leaves and roots is compromised.

A premature flowering may be favored by high temperatures, associated with dryness in the soil and air, as well as low nitrogen nutrition, which can be determined by a too tall plant (Indrea et al., 2012).

The beginning of the premature flowering can be associated with generic variety, environmental conditions in which the fennel was grown, and the growing period. In addition to the environmental conditions, premature flowering is also considered to be triggered by

other forms of stress, which are: drought, temperature, excessive soil moisture, nitrogen deficiency (Moore, 2005).

When the stress factor is drought, it causes wilting of plants, premature floriferous stems (in green salad, spinach, radishes, fennel), burns on leaves and fruits (tomatoes, peppers), spongy roots, and pollen viability loss. Temperatures between 3-17 °C determine the differentiation and formation of floriferous stems. These influences have implications in technological measures such as: avoiding preservation of bulbs at medium temperatures and the use of vernalization in order to reduce the risk of floriferous stems occurrence. The main goal is the study of agro technology and biological particularities related to fennel culture, in order to avoid premature flowering.

Material and method

The research conducted in this study was aimed to the establishment of some technology elements of fennel culture both in fields and in solarium, to ensure a higher and earlier level of production. The research was conducted during the years 2013-2014, at the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, as a bifactorial experience. Each variant had 3 repetitions, placed in accordance with the rules of experimental technique. The material used in the experiment is represented by **Orion F1** and **Rondo F1** hybrids, obtained by the Dutch seed company, Bejo. To obtain early production of fennel, both in fields and in solarium, the cultures are established through seedlings in nutrient cubes. The seeds were sown in nutrient cubes and were kept until they had 2-3 real leaves, after which they were planted in the field and in solarium respectively. Measurements were performed weekly, aiming the emergence of floriferous stem. The data thus recorded were interpreted statistically by analysis of variance method, and using the method of multiple comparisons (Duncan test), as well as by correlations between different characters analyzed.

Results and discussions.

The effects of premature flowering (Figure. 1) are complex and interrelated. Significant differences were observed in the vegetation period. At 6 weeks after transplantation, a very powerful flowering was observed on Rondo F1 hybrid, which was 21.67%, while for Orion F1 hybrid it was 13.33%. At harvest, the plants recorded a higher percentage of flowering, between 45 -55%.

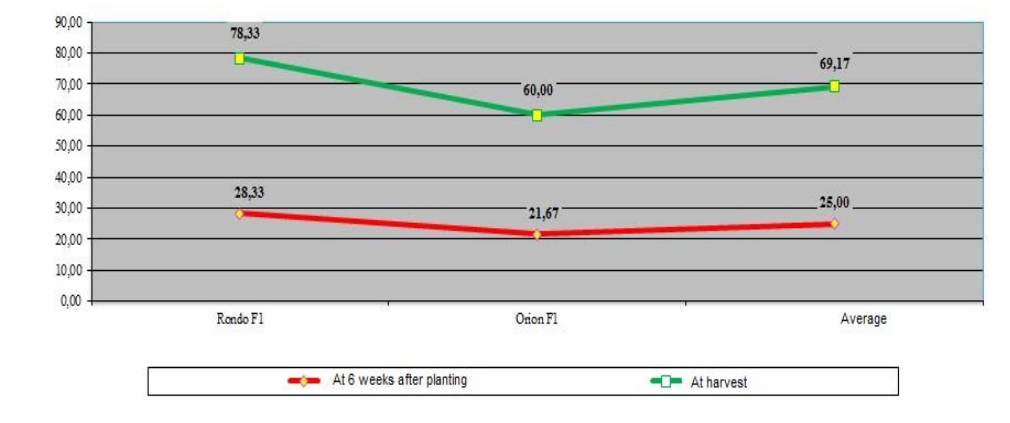

Figure 1. The percentage of flowering plants in fields in 2014

In this experience, performed in the Transylvanian Plateau area, some variants of the culture in fields showed very high percentage of premature flowering, which reached up to 90% (Figure 2). Culture founded in field recorded a very high percentage of premature flowering, premature floriferous stems occurred at all plants, average percentage of premature flowering reaching up to 78.33% at harvest.

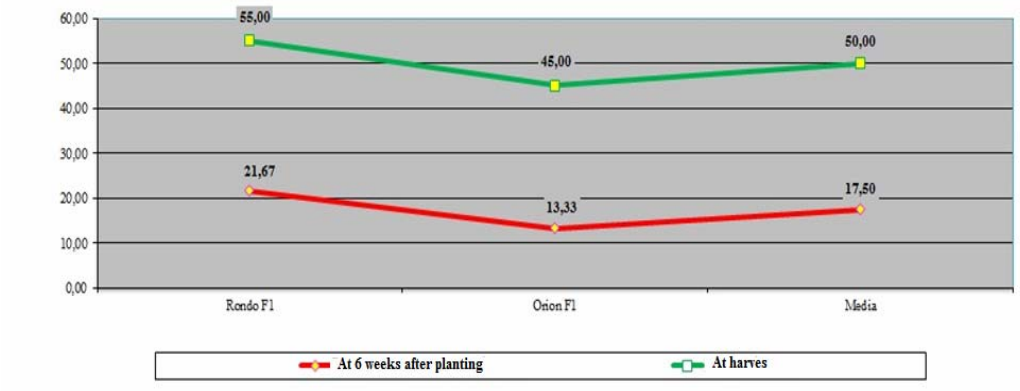

Figure 2. The percentage of flowering plants in solarium in 2014

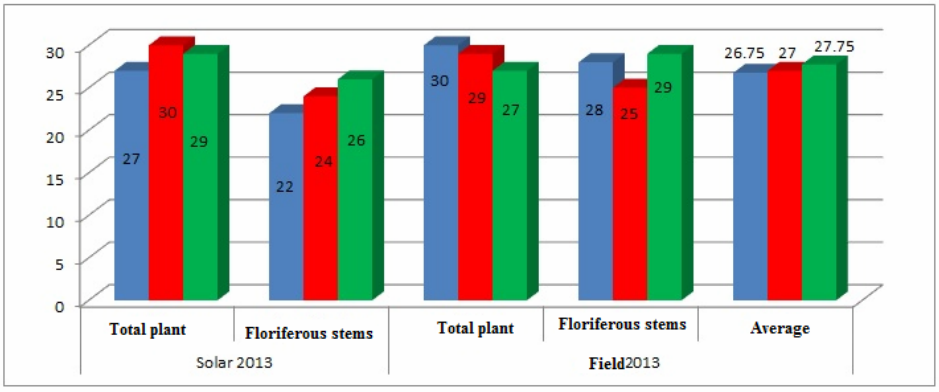

Figure 3. The flowering fennel premature to 2013

For the solarium cultures it has been noted that the fewest plants which issued floriferous stems were in the case of Orion F1 hybrid, and the highest percentage of premature flowering was reported on Rondo F1 hybrid (Figure 3).

From the point of view of place and culture period, significant differences are observed between yields in greenhouses and in the field. Due to bad weather conditions in field, on early culture, the average percentage of premature flowering had a value of 78.33%.

Table 1. The interaction the between epoch of planting and the number of floriferous stems

Epoch/hybrid	Rondo	Orion	Average hybrid
Epoch I	$6,1^{b}$	$5,8^{b}$	$1,8^{A}$
Epoch II	$11,8^{a}$	$13,8^{a}$	$3,9^{A}$
Average epoch	$9,0^{X}$	$9,8^{X}$	

* Differences between any variants followed by a common letter are not significant (Duncan test, $p \leq 0.05$)

In the second epoch of culture, there are no major differences between Orion hybrid and Rondo hybrid, in terms of premature flowering, but between epoch I and epoch II, there are significant differences in terms of the number of flowers, in both hybrids. Between the averages of varieties there are no significant differences, as well as between averages of planting epochs. Thus, based on the data analyzed in the above Table 1, we can say that the interaction between hybrids and planting epoch is important from statistically point of view and beyond.

Conclusions

When Orion F1 and Rondo F1 hybrids are grown under conditions of low temperature for a long time or prolonged drought, floriferous stems begin to emerge. Even if the plants bloom, some of the developed bulbs can be recovered. Also, leaves can be harvested too, since these are used for pharmaceutical purposes. Due to bad weather conditions in field, on early culture, the average percentage of premature flowering had a value of 78.33%, in solarium the percent being halved. Based on these results, we recommend cultivating the fennel in epoch I, to avoid premature flowering. Premature flowering process also causes a much smaller production, because the plants no longer develop normally when the stems begins floriferous and the bulbs do not reach the desired weight.

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PRODUCTION AND QUALITY EVALUATION OF TWO VARIETIES OF FENNEL (*FOENICULUM VULGARE* VAR. AZORICUM MILL) GROWN IN TRANSYLVANIAN PLATEAU

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Abstract.
In this experiment there were studied some agro technological particularities related to the growth of fennel, in order to enhance its cultivation. To achieve this goal, there were studied two hybrids of fennel (Rondo F1 and Orion F1), grown at different planting distances, as well as different planting ages, monitoring its quantitative and qualitative characteristics. The results proved that there are significant differences between these two hybrids of fennel. Generally, the highest values of all parameters were obtained on Orion F1 hybrid.

Keywords: Cultivar, density, ages, fennel

Introduction

The fennel belongs to the Apiaceae family. The species is found in the spontaneous flora of Mediterranean area, northern Africa, as well as in western Asia. Currently, fennel is grown as aromatic plant in many countries from Europe, Asia, North Africa and South America. In our country, fennel is less grown, only in south and south-west areas. The most important varieties of fennel belong to var. Azoricum. Because of compounds it contains, fennel is widely used as flavoring agent in food and drinks, in pharmaceutical and phototherapeutic industry, and also in confectionary (Blumenthal et al., 2000). It is used for flavors in many products, including soaps, perfumery and candles (Praditvarn and Samhandharaksa, 1990). Fennel seeds are used for pastries, meat and fish, ice cream, strong dinks and mixtures of herbs (Diaaz-Maroto et al., 2005). Fennel of Florence has a developed basal area, with a bulbous nature, which is eatable. Green vegetables like fennel, bring in alimentation a substantial contribution of vitamins and minerals, such as vitamins A and C, calcium and potassium (Indrea D et al., 2012).Fennel has culinary uses in many European countries. Both bulb and stems can be consumed fresh in salads, or cooked in different ways: braised, saute or in soups. The leaves are washed, cut into small pieces and prepared for salads, which are served with a juice sauce mixed with olive oil (Bianco et al., 2009). The research purpose is the study of two hybrids of fennel in environmental conditions of Transylvanian plateau, in order to encourage its high qualitative and quantitative production and to promote it among both consumers and producers.

Material and method

Quality and yield can be influenced by environmental conditions, age of seedlings and age of planting. For this study there were used two hybrids of sweet fennel (*Foeniculum vulgare*, Mill.), i.e. Rondo F1 and Orion F1, obtained from the company Bejo, Netherlands. Rondo F1 is a highly productive hybrid of fennel, suitable for summer and autumn growth. It has a fast rising, it is an early variety, with uniform and round bulbs and it is mature in about 75 days after transplantation. Orion F1 is a hybrid of fennel suitable for autumn growth, with round and white soft bulbs, providing high efficiency. The bulbs are mature in about 85 days after transplantation. Fennel seeds were planted in alveolar trays filled with peat. The seedling were transplanted in solarium when the fennel had 2-3 real leaves, at the distance of 20 cm between plants on a row and 40 cm between rows. The experimental plot size was 8 m^2.

Results and Discussion

The hybrid type has a very strong influence on growth and development of the fennel plants (Figures 1 and 2). According to the data from Figure 1, the plant height is between 9.3 cm for Rondo hybrid (at the moment of planting) and 61.4 cm for Orion hybrid (at 60 days after planting). As shown in Figure 1, the plant growth was constant, Orion hybrid plants were developed better then Rondo hybrid plants, throughout the experience.

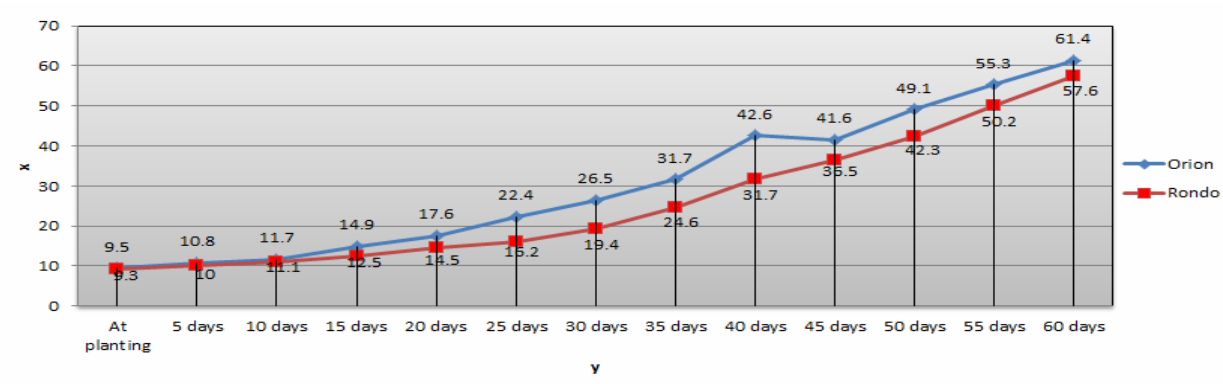

Figure 1 Height of fennel hybrids in time.

According to Figure 2, it results that the minimum number of leaves was registered at the moment of planting, when the seedling had 2 leaves, and the maximum number of leaves was 12 (at 60 days after planting). As shown in Figure 2, both Orion and Rondo hybrids had the same number of leaves at 60 days after planting. Considering Figure 1 and 2, we can say

that, the highest plants, with healthy leaves, important in terms of culinary and medicinal, are given by the Orion hybrid.

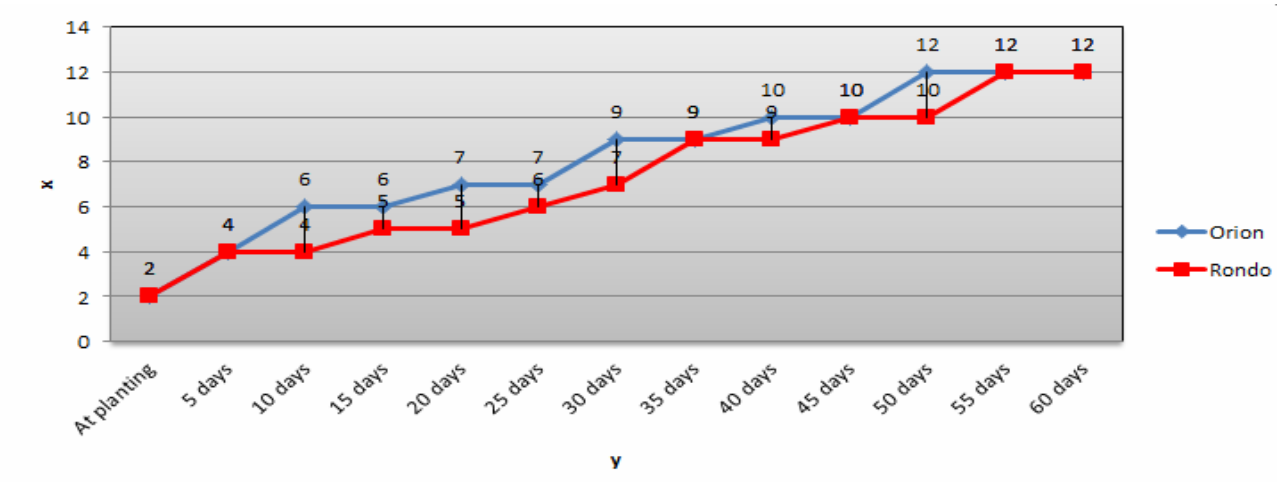

Figure. 2. Number of leaves per plant and hybrid.

Table 1. Results on the diameter of bulb (cm), diameter of plant (cm) and height of plant (cm)

Hybrid	Diameter of bulb		±d	Significance
	(cm)	%		
Orion	8,6	110,5	0,8	*
Rondo	7,0	89,5	-0,8	o
Average (Mt)	7,8	100	-	-
	Diameter of plant		±d	Significance
	(cm)	%		
Orion	63,8	106,1	3,7	**
Rondo	56,5	93,9	-3,7	oo
Average (Mt)	60,2	100	-	-
	Height of plant		±d	Significance
	(cm)	%		
Orion	86,2	107,2	5,8	*
Rondo	74,6	92,8	-5,8	o
Average (Mt)	80,4	100	-	-

*, **, ***/ $^{o, oo, ooo}$ Significant at P<0.05; 0.01 and 0.001 (*, **, *** positive $^{o, oo, ooo}$ negative)

According to the data from Table 1, diameter of bulb, diameter of plant and height are strongly influenced by cultivar. In order to observe the differences between the analyzed characters, DL test was used. The average of these characters has values between 7.8 cm

(diameter of bulb), 60.2 cm (diameter of plant) and 80.4 cm (height of plant). As shown in Table 1, the Orion hybrid expressed significant higher differences, in all three cases studied (diameter of bulb, diameter of plant and height of plant), in comparison with witness variant (average of experience). Beside the average of experience taken as witness, the Rondo hybrid has lower average values: diameter of bulb (7.0 cm), diameter of plant (56.5 cm) and height of plant (74.6 cm).

Table 2. Results on weight of fennel plants

Hybrid	Weight of bulb		±d	Significance
	(g)	%		
Orion	517,3	119,0	82,5	**
Rondo	352,3	81,0	-82,5	oo
Average (Mt)	434,8	100	-	-
	Weight of root		±d	Significance
	(g)	%		
Orion	69,1	117,0	10,1	**
Rondo	49,0	83,0	-10,1	oo
Average (Mt)	59,1	100	-	-
	Weight of leaves		d±	Significance
	(g)	%		
Orion	359,1	127,6	77,6	n.s
Rondo	203,9	72,4	-77,6	n.s
Average (Mt)	281,5	100	-	-

*, **, ***/ o, oo, ooo Significant at P<0.05; 0.01 and 0.001 (*, **, *** positive, o, oo, ooo negative)

According to Table 2, the weight of bulb, the weight of root and the weight of leaves are influenced by cultivar. The averages of experience are between 59.1 g (weight of root) and 434.8 g (weight of bulb). As shown in Table 2, the Orion hybrid has the weight of bulb (517.3 g) and a weight of root (69.1 g), higher then averages, taken as witness, differences between variants being significant distinct. Beside the average of experience, the Rondo hybrid has lower weight of bulb and weight of leaves. In both cases, the weight of leaves has the average values similar with average of experience, without significant differences. To be more clearly highlighted, differences between cultivar were calculated using the DL test.

The data presented in Table 3 highlights the influence of hybrid on production of fennel. As shown in Table 3, the Orion hybrid had a higher production (52.75 t/ha) then Rondo hybrid (49.35 t/ha), taken as witness. Differences in production of hybrids were very significant in favor of Orion hybrid, compared with Rondo hybrid, taken as witness. To

highlight the differences in production between two hybrids, the test DL was used. Beside average of experience (51.05 t/ha), taken as witness, the Orion hybrid had a higher production (52.75 t/ha), with a very significant difference. Compared with average of experience taken as witness, Rondo hybrid had a lower production. There was a very significantly lower difference between Rondo hybrid and average of experience.

Table 3. Results on total production of fennel

Hybrid	Total production		±d (t/ha)	Significance	±d (t/ha)	Significance
	(t/ha)	(%)				
Rondo (mt)	49.35	100.00	0.00	-	-1.70	ooo
Orion	52.75	106.89	3.4	***	1.7	***
Average (mt)	51.05	103.44	1.7	***	-	-

*, **, ***/ $^{o, oo, ooo}$ Significant at P<0.05; 0.01 and 0.001 (*, **, *** positive, $^{o, oo, ooo}$ negative)

Conclusions

From the real analysis of evolution of climatic factors, it results that Transylvanian Plateau territory offers good conditions for growth of fennel in protected areas. During the vegetation, the Orion F1 hybrid has had the best result concerning the growth and development of plant. The highest recorded production was 52.75 t/ha, on Orion F1 hybrid.

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PRELIMINARY RESEARCH REGARDING STORED MAIZE GRAINS CONTAMINATION LEVEL

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Abstract
Agricultural crops are vulnerable to infections by a wide spectrum of plant pathogens. In today's marketplace, the increasing complexity and wide distribution chain represent enormous challenges for food production. The increased fungal infection and cross-contamination hazards are associated with the globalization of cereal trade (Waage et al., 2006). Cereals are one of the most important sources of food (FAO, 2002), which have contributed to human nutrition for millennia. However, cereals are exposed to numerous biotic and abiotic stress factors, from cultivation and throughout their life cycle to processing.
The grain losses recorded during storage period on worldwide scale according to FAO estimations are between 5-10% of total production. In developing countries, due to reduced possibilities of implementing appropriate technologies, the reported damages during storage period may increase up to 30%.
In 2014, the maize grains which are to be stored on an indefinite period of time is affected by the presence of various pests specific to warehouse ecosystem.
The paper work presents a study regarding the occurrence and development of specific warehouse micromycetes during the first months of maize storage. Immediately after storage, it has been identified the specific micoflora for this period, respectively species *of Alternaria, Trichoderma, Cladosporium, Aurobasidium, Cephalosporium, Phytophtora, Aspegillus* and *Fusarium* genera. Crop safety and security can be achieved by maintaining climatic factors in stored spaces, thus limiting the populations level of contaminating microorganisms.

Key words: stored maize, phytopathogenic fungi, mycotoxin contamination.

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INFLUENCE OF SOME PESTICIDES ON USEFUL ORGANISMS

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Abstract
Pesticides are mostly synthetic chemicals that are used in agriculture to control pests. Pesticides include herbicides, insecticides, fungicides, nematocides and rodenticides. Excessive use of fertilizers and pesticides lead to environmental pollution. Soil desertification, wildlife deaths, reduced biodiversity and excessive use of freshwater resources were some of the results.
This work, and the application of agreed protocols for testing, is of crucial importance to the environmentally acceptable use of pesticides and to the further development of Integrated Pest Management systems.
Studies have been conducted to highlight the impact of plant protection products on the environment, in order to promote those with low toxicity, thus meeting the requirements of European legislation on sustainable use of pesticides.
This paper presents results regarding to the toxicity of pesticides belonging to the three major categories, namely, an herbicide, insecticide and fungicide, on three of the most important species, environmental components: *Apis mellifera*, *Eisenia foetida* and *Pseudokirschneriella subcapitata.*
New less toxic pesticides in relation to integrated pesticide management will reduce substantially the damage to environment.

Key words: pesticides, environment, Apis mellifera, Eisenia foetida, Pseudokirschneriella subcapitata

BUTTERFLY SPECIES COLLECTED FROM THE MOUNTAINS, EXISTING IN THE COLLECTION OF LUCIAN BLAGA UNIVERSITY OF SIBIU

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Abstract:
This paper represents a systematic list of species of Lepidoptera collected from the mountains of Romania and displayed in a collection at University "Lucian Blaga" of Sibiu. The collection comprises 1017 samples of which 660♂♂ and 357♀♀, belonging to 19 families, 334 genuses, and 572 species. The collection was acquired in 1998 from Lepidopterologist Levente Szekely from Brasov. The species collected from the mountains have systematic indication of each specimen collection, elevation and area designations and participation in geographic regions of Romania. Of great significance is the indication of the degree of endangerment for each species according to the IUCN. Systematic classification is updated according to the *Verzeichnis der Schmetterlinge Romäniens* (RÁKOSYet al., 2003).

Key words: butterflies, collection, mountains species.

Intorduction

Samples of the butterflies are kept in collections and such collections exist in the country and described in the following catalogues (POPESCU-GORJ 1964, NEMEŞ & VOICU 1973 KÖNIG, 1975 CIOCHIA & BARBU, 1980, CHOCOLATE & ROCK, 2000 MARCU & RAKOSY, 2002 SZÉKELY & CERNEA E. 2007), some of them becoming the only evidence of an unrecoverable past the biosphere, which is increasingly jeopardized. The publication of this paper, which includes data on species of butterflies collected from the mountains of Romania, may provide valuable information for specialists. The value of a collection of insects has been proven throughout history of zoology, but it is now increasing as the number of insect species sharply decreases nationally and globally, due to pollution and destruction of natural habitats and anthropogenic intervention.

Material and method

The material within this study was collected by lepidopterolog Levente Szekely between 1972-2000 from Romania (SZÉKELY, 1995, 1996, 1999a, b, 2003a, b, 2007, 2008, 2010, 2011) and we present specimens taken from the mountain area. The collection was performed using entomological nets for diurnal Lepidoptera and light source for noctuide species. The essential data for each specimen are noticed (day, month, year, sex, place of collection, geographical area and altitude). The material in entomological boxes is arranged in

systematic and represents the Lepidoptera collection of the "Lucian Blaga" University in Sibiu.

Results and discussions

In the Lepidoptera collection at "Lucian Blaga" University of Sibiu there are 1017 samples: 660♂♂ and 357♀♀., belonging to 19 families, 334 genuses, and 572 species. The collection was acquired in 1998 from the lepidopterolog Levente Szekely of Brasov. The entomological material is present within the headquarter of the Faculty of Agricultural Sciences, Food Industry and Environmental Protection. The collection is very important as teaching material in classes like: *Entomology* and *Pest populations control*.

In this study, the following abbreviations and meanings have been used:

CR - Critically endangered: it is estimated that the survival of these species in the next 10-20 years is unlikely if not eliminate the factors that have caused this situation and after analysis of quantitative cost a decay rate of the population with at least 50% in the last 10 years.

EN – Endangered: endangered taxa when there is a critical stage of threat, but shows high risk or threat extinction, critical in the immediate future, with an estimated probability of about 20% extinction in the next 20 years,

VU-Vulnerable: a taxon is vulnerable when it is not in the critical threat or endangered, but have a high risk of extinction or critical threat in the near future,

NT-Near threatened, includes taxa not included in CR, EN or VU but by worsening the degree of threat, it may take one or other of the three categories. This category largely replaces LR variant category IUCN 2000 Red List taxa common species (RÁKOSY, 2002; 2003; 2005).

SYSTEMATIC LIST OF THE MACROLEPIDOPTERA (INSECTA: LEPIDOPTERA) COLLECTED FROM THE MOUNTAINS

No.	No Ro*	No. K.&R.**	Taxon	Place and date of collection	Area of Romania	Degree of endangerment
1	3327 Ro	K. & R. 6763	*Dendrolimus pini montana* Staudinger, 1871	1♂, Muntele Roşu, 20.VII.1993, 1300 m alt.	BT, TR, MM, MT, MD; DB	
2	3331 Ro	K. & R. 6769	*Cosmotriche lunigera* Esper, 1784 (sin. *C. lobulina* Denis& Schiffermüller, 1775)	2♂♂, Muntele Roşu (Masivul Ciucaş), 20.VII.1983, 1300 m alt., Cheile Bicazului, 4.VIII.1996	BT, TR, MM, OT, MT, MD	
3	3481 Ro	K. & R. 7001	*Pieris bryoniae wolensky* Berger, 1925	1♀, Valea Jepilor, Masivul Mucegi, 25.VII.1991, 1800 m alt.	TR, MM, MT, MD	VU
4	3596 Ro	K. & R. 7205	*Argynnis addipe addipe* Denis& Schiffermüller, 1775	1♂, Cheile Bicazului, 5.VIII.1986, 1100 m alt.	BT, CR, TR, MM, OT, MT, MD; DB	NT
5	3628 Ro	K. & R. 7257	*Nymphalis antiopa antiopa* Linnaeus,1758	2♂♂, Cheile Bicazului, 11, 12.VIII.1985, 1100 m alt.	BT, CR, TR, MM, OT, MT, MD; DB	VU
6	3690 Ro	K. & R. 7361	*Erebia euryale syrmia* Fruhstorfer, 1909	1♂, Piatra Arsă, Munţii Bucegi, 21.V.1993, 2100 m alt. 1♀, Piatra Craiului Munţii Bucegi, 24.VIII.1995, 1600 m alt.	BT, CR, TR, MM, OT, MT, MD	NT
7	3691 Ro	K. & R. 7361	*Erebia manto trajanus* Hormuzachi, 1895	1♀, Valea Jepilor, Masivul Mucegi, 11.VIII.1994, 1800 m alt.	BT, TR, MM, OT, MT, MD	VU
8	3692 Ro	K. & R. 7366	*Erebia epiphron transsylvaniza* Rebel, 1908	1♂, Babele (Masivul Bucegi), 11.VIII.1984 1♀, Muntele Zăgan Masivul Ciucaş, 20.VII.1993, 1700 m alt.	BT, TR, MM, OT, MT, MD	NT
9	3695 Ro	K. & R. 7372	*Erebia aethiopus* Esper, 1777	1♂, Cheile Bicazului, 2.VII.1996, 1100 m alt. 1♀, Întregalde, Munţii Apuseni,	BT, CR, TR, MM, OT, MT, MD; DB	NT

				27.VII.1997		
10	3697 Ro	K. & R. 7384	*Erebia gorge* Hübner, 1804	1♂, Lacul Bâlea, Munţii Făgăraş, 29.VII.1994, 2200 m alt.	BT, TR, OT, MT, MD	VU
11	3699 Ro	K. & R. 7396	*Erebia pronöe regalis* Hormuzachi, 1937	1♂, Caraiman, Masivul Bucegi, 1.VIII.1990, 2100 m alt.	TR, MT	NT
12	3701 Ro	K. & R. 7404	*Erebia melas carpathicola* Popescu Gorj&Alexinschi, 1959	1♂, Munţii Rodnei, 23.VII.1985	TR	VU
13	3779 Ro	K. & R. 7542	*Macaria liturta* Clerck, 1759	1♀, Muntele Roşu, 20.VII.1993, 1300 m alt.	BT, TR, MM, MT, MD; DB	
14	3977 Ro	K. & R. 7910	*Glacies coracina* dioszeghyi Schmidt, 1930	2♂♂, Vârful Omu (Masivul Bucegi), 1.VIII.1993, 2400 m alt., Piatra Arsă (Masivul Bucegi), 31.VII.1993, 2100 m alt.	TR, MM, OT, MT	
15	4337 Ro	K. & R. 8538	*Eupithecia icterata icterata* Villers, 1789	1♂, Muntele Roşu, 21.VII.1993, 1300 m alt.	BT, CR, TR, MM, OT, MT, MD; DB	
16	4008 Ro	K. & R. 7969	*Geometra papilionaria* Linnaeus, 1758	1♂, Cheile Bicazului (Harghita), 2.VIII.1996, 1100 m alt.,	BT, CR, TR, MM, OT, MT, MD; DB	
17	4105 Ro	K. & R. 8184	*Idaea aversata aversata* Linnaeus, 1758 (sin. *I. remulata* Linnaeus, 1758)	1♀, Săcele (Bv.), 8.VII.1998, 650 m alt., Cheile Bicazului, 5.VIII.1996, 1100 m alt.	BT, CR, TR, MM, OT, MT, MD; DB	
18	4165 Ro	K. & R. 8297	*Entephria cyanata* Hübner, 1809	1♂, Cheile Tişiţei (M-ţii Vrancei),3.VI.1995, 900 m alt.	BT, TR, MM, OT, MT, MD	NT
19	4201 Ro	K. & R. 8343	*Chloroclysta citrata* Linnaeus, 1761	1♂, Cheile Bicazului-Harita, 2.VIII.1996, 1100 m alt.	BT, CR, TR, MM, MT, MD; DB	NT
20	4226 Ro	K. & R. 8385	*Colostygia pectinataria* Knoch, 1781	1♂, Cheile Bicazului-Harghita, 4.VIII.1996, 1100 m alt.	BT, CR, TR, MM, OT, MT, MD	
21	4228 Ro	K. & R. 8391	*Hydriomena furcata* Thunberg, 1784	1♂, Cheile Bicazului-Harghita, 5.VIII.1996, 1100 m alt.	BT, CR, TR, MM, OT, MT, MD	
22	4286 Ro	K. & R. 8470	*Perizoma verberata* Scopoli, 1763	Valea Jepilor (Masivul Bucegi), 1♂, 14.VIII.2000, 1700 m alt. 2♀♀, Valea Jepilor	TR, MM, OT, MT, MD	

				(Masivul Bucegi), 2.VIII.1998, 14.VIII.2000, 1700 m alt.		
23	4375 Ro	K. & R. 8604	*Rhinoprora chloerata* Mabille, 1870	2♂♂, Masivul Piatra Mare, 10.VI.1990, 1000 m alt., Săcele (Bv.), 5.VI.1998, 650 m alt.	BT, CR, TR, MM, MT, MD; DB	
24	4337 Ro	K. & R. 8538	*Eupithecia icterata icterata* Villers, 1789	1♂, Muntele Roşu, 21.VII.1993, 1300 m alt.	BT, CR, TR, MM, OT, MT, MD; DB	
25	4389 Ro	K. & R. 8625	*Aplocera simpliciata* Treitschke, 1835	1♂, Lacul Bâlea (M-ţii Făgăraş), 27.VII.1994, 2100 m alt. 2♀♀, Valea Jepilor (Masivul Bucegi), 6.VIII.1993, 1800 m alt., Caraiman (Masivul Bucegi), 5.VII.2000, 2100 m alt.	BT, TR, OT, MT	NT
26	4473 Ro	K. & R. 8738	*Ptilodon capucina* Linnaeus,1758	1♀, Muntele Roşu, 1.VIII.1995, 1300 m alt.	BT, CR, TR, MM, OT, MT, MD; DB	
27	4948 Ro	K. & R. 9748	*Apamea monoglypha* Hufnagel, 1766 (sin. *polyodon* Linnaeus, 1761, nec. Clerck, 1759)	1♀, Valea jepilor (Masivul Bucegi), 25.VI.1994, 1400 m alt.	BT, CR, TR, MM, OT, MT, MD; DB	
28	5302 Ro	K. & R. 10084	*Ochropleura flammatra flammatra* Denis&Schiffermüller, 1775	1♂, Caraiman (M-ţii Bucegi), 26.IX.1994	BT, CR, TR, MM, MT, MD; DB	
29	5307 Ro	K. & R. 10089	*Diarsia mendica mendica* Fabricius, 1775	1♂, Lacul Roşu-Cupaş, 17.VII.1993, 1300 m alt.	BT, CR, TR, MM, OT, MT, MD	
30	5369 Ro	K. & R. 10209	*Xestia ochreago* Hübner, 1790	1♂, Valea Jepii (M-ţii Bucegi), 11.VIII.1994, 1600 m alt.	TR,MT	EN
31	5381 Ro	K. & R. 10232	*Anaplectoides prasina* Denis&Schiffermüller, 1775	1♀, Lacul Roşu-Cupaş, 28.VII.1978, 1300 m alt.	BT, CR, TR, MM, OT, MT, MD	
32	5441 Ro	K. & R. 10375	*Limantria monacha* Linnaeus,1758	1♀, Cheile Bicazului (Harghita), 2.VIII.1996, 1100 m alt.	BT, CR, TR, MM, OT, MT, MD	

*Corresponding serial number from the catalog of butterfly species Romania (Rákosy L., Goia M., Kovács Z., 2003)

** Classification and nomenclature proposed by (Karsholt O., Razowski J., 1996)

Concusions

This paper contributes to the study of Lepidoptera fauna collected from mountains in Romania during 1983-2000, found in the collection of the University "Lucian Blaga". Of the 572 existing species in this study are presented only 32 species belonging to 7 families (*Lasiocampidae, Saturniidae, Lemoniidae, Sphingidae, Thyatirinae, Drepanidae, Geometridae*). Species are updated after Romania's catalogue and there are given the geographical areas where these species were collected. Of great importance is the presentation of the IUCN degree of endangerment for each species. The documentary and scientific value of Lepidoptera Collection of the Lucian Blaga University provide to those interested a valuable documentary material that can be used for systematic studies, faunal and zoogeographical.

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TECHNICAL AND ECOLOGICAL SOLUTIONS IN STIMULATING PRODUCTIVITY BY USING BIOSTIMULATORS FOR THE BITTER CUCUMBER (MOMORDICA CHARANTIA L.)

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Abstract
In practice shows us that the use of biofertilizers in organic agriculture is a safe and cheap alternative compared to conventional chemical fertilizers. It consists in the inclusion in the microbiocenosis of population of symbiotic nitrogen fixers and / or associates of the genus Rhizobium, species with atmospheric nitrogen fixation at a rate of up to 70% of the nitrogen need. For this purpose in the area of agro-ecological Arad, for the species Momordica Charantia L. (The Bitter Cucumber) with tropical origins they were tested several biofertilizers produced in Romania: Biotrofin, Ecoferil B., Azotofertil.

Keywords: Biofertilizers, Agro-ecological area Arad, Chemical Fertilizer, *Rhizobium*

Introduction

Momordica charantia L. (Bitter Cucumber), with tropical origins, has many therapeutic uses as an important source of active ingredients in fighting bad health conditions. The acclimatization of the species *Momordica charantia* L. in the western part of Romania started in 2001 in the agro-ecological area Arad. The extract obtained from the fruit of the bitter cucumber and / or from all the plant showed a hypoglycemic action remarkable in the treatment of diabetes, the active substance with such properties being "Charantia", a sterol glycoside [1, 2]

With a poorly developed root system in depth and a tendency toward growth and branching on the surface of the soil, the studied cultivar offers optimum development especially on light soils, well-structured and rich in hummus with neutral reaction and sandy loam texture. Sandy soils determine earlier and higher yields because it favors the necessary warming and the accumulation of a number of degrees, closed to the optimum conditions required by the species [3].

Materials and the method

The studies was conducted in the agro-ecological area of Arad (West Romania), trying to find methods for improving the cultivation technology for the species *Momordica charantia* L. Knowing that this species is unisex (flowers on the same plant are male and female) and the ratio of male flowers and female flowers is in favor of male ones (1: 25-28),

the research carried out in the years 2011-2014 was aimed to reduce this ratio, using biofertilizers and operations performed in green (pruning and shoot bending) with notable effects on yield.

The use of biofertilizers in field and also in protected crops (greenhouses) is motivated by the benefits they confer, beside the yield increase being the harmlessness to human and animal health.

In the performed studies there were used the following biofertilizers: **Atonik** (biochemical stimulator), **Revital** (biological product that causes intensification of plant metabolism), **Florone** (growth regulator obtained by hydrolysis of proteins), **Raykat** (specially formulated to induce and promote plant growth and development), **Razormin** (increases energy in cells), **Nemagold** (marigold and natural product made from seaweed).

Along with the use of growth regulators application, alone or in various combinations, there have been implemented three control systems for *Momordica charantia* L., i.e. pruning and bending of the shoots (Factor B), removal of the first shoots of the first 8 -10 knots of the main strain and bending the shoots at a height of 2 meters - height of the trellis – (Factor B_1); and only the bending of the shoots at a height of 2 meters (Factor B_2), respectively.

The experiment was structured in two randomized blocks with four repetitions. The value of the data was determined considering the method applied to the experiments variation in the randomized blocks over several years in the same location. With some exceptions, the technology is similar with that for cucumber grown on high trellis. During the vegetation period we have determined the morpho-functional parameters and the yield on each variant.

Results

Analyzing the results obtained in the 4 years of research (2011-2014) on the first factor, we note that that the biofertilizers use both individually and in various combinations cause positive effects on *Momordica carantia* L.

The best results were obtained by the combination Nemagold 10 L when preparing the seedbed + Florone 0.1% - 3 treatments in vegetation + Azotfertil – 15 L / ha, 2 treatments in vegetation, leading to a yield of 24.6 t / ha, and Revital 0 1% administered before planting the seedlings + Raykat 0.5%, 3 treatments in vegetation + P Ecofertil -15L / ha, 3 treatments in vegetation leading to a yield of 4.3 t / ha.

The factors represented by plant control activities (pruning and bending the shoots) also influences yields, i.e. pruning and removal of the first shoots of the first 8 -10 knots of the main strain and bending the shoots at a height of 2 meters, gives the best results.

In the experiment in which both biofertilizers and plant control works done in green have been tested, the best combination in the agro-ecological area of Arad is obtained by the use of bio-stimulators consisting of Nemagold 10 L/ha when preparing the seedbed + Florone 0.1% - 3 treatments in the state of vegetation every week + Azotfertil – 15L / ha, 2 treatments in vegetation, combined with pruning and removal of the first shoots at the first 8 -10 knots of the main strain and bending the shoots at a height of 2 meters.

The main results are presented in Table 1.

Conclusions

1. The therapeutic importance of this species and the optimal conditions for ecological agriculture in the area Arad justify the research carried out on the species *Momordica carantia* L.
2. The use of biofertilizers at the species Momordica carantia L. it is a cheap and safe alternative to conventional chemical fertilizers, alternative threat includes in the agricultural microbiocenosis, symbiotic nitrogen-fixing microorganisms populations and / or the combination of both.
3. Biofertilizer use in this species with unisexual flowers, with a low ratio in male and female flowers (1: 22-28) is a new concept for stimulating the growth and development of the vegetative mass and ultimately increase the quantity and quality of production.
4. The products tested in the study and the results after biotechnologies processes are most suitable, they are harmless to humans and the environment, and are accepted by ecological agriculture.

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Table 1. The influence of biofertilizers and of operation done in green over the production *Momordica charantia* L.

V		Dose % L/ha	Seed	Seedling	Preparing the seedbed	In vegetation	B_1				B_2				A- Biofertilizers			
							t/ha	D (t/ha)	%	S	t/ha	D (t/ha)	%	S	t/ha	D (t/ha)	%	S
1	The Classic Method	-	-	-	-	-	19,6	Mt	100	-	17,1	Mt	100	-	18,4	Mt	100	-
2	Atonik Revital Atonik	1:2000 0,1 0,005	Yes - -	- Yes -	- - -	- - Yes (3)	21,0	1,4	107,1			2,1	112,3		20,1	1,7	109,2	
3	Atonik Revital Revital	1:2000 0,1 0,1	Yes - -	- Yes -	- - -	- - Yes (3)	22,1	2,5	112,8	X		2,2	111,7		20,6	2,2	112,0	
4	Revital Florone	0,1 0,1	- -	Yes -	- -	- Yes (3)	24,1	4,5	122,9	Xxx		2,3	125,7	X	22,8	4,4	123,9	X
5	Revital Reykat	0,1 0,5	- -	Yes -	- -	- Yes(3)	23,7	4,1	120,9	Xxx		2,5	114,6	X	21,7	3,3	120,1	
6	Razormin Florone	0,5 0,1	- -	Yes -	- -	- Yes(3)	23,3	3,7	118,9	Xxx		3,7	121,6	Xxx	22,1	3,7	122,8	Xx
7	Nemogold Nemogold Florone	10 10 0,1	- - -	Yes - -	Yes - -	- - Yes(3)	23,6	4,0	120,4	Xxx		4,5	126,3	Xxx	22,6	4,2	122,2	Xx
8	Nemogold Nemogold Reykat	10 10 0,5	- - -	Yes - -	Yes - -	Yes(3) - -	24,2	4,6	123,5	Xxx		3,8	122,2	Xxx	2,5	4,1	133,7	X
9	Nemogold Florone Azotfertil	10 0,1 15	- - -	Yes - -	Yes - -	Yes(3) - -	26,3	6,7	134,2	Xxx		5,8	133,9	Xxx	24,6	6,2	133,7	Xxx
10	Revital Raykat Ecofertil,P	0,1 0,5 15	- - -	- - -	- - -	- - -	26,1	6,5	133,2	Xxx		5,4	131,6	Xxx	24,3	5,9	132,1	Xxx
11	Razormin Florone Azotofertil	0,5 0,1 15	- - -	- - -	- - -	- - -	25,6	6,3	132,1	xxx		5,1	129,8	Xxx	24,1	5,7	131,0	Xxx
	B- In vegetation							23,6				20,7		Dl	A	B	B*A	A*B
	The differences(t/ha)							2,9				Mt		5%	2,19	2,57	3,28	3,96
	%							114,0				100		1%	2,63	3,11	4,27	4,72
	Significance							x						0,1%	2,96	3,58	4,73	5,96

PLANTSCAPING – THE LINK BETWEEN NATURE AND WORK ENVIRONMENT

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Abstract
Since the 1960s, plantscaping was spread first in Europe work environments, and later in USA. The many psychosocial, aesthetic and physical benefits of plants at work are historically dated and supported by different researchers of last century. Improving employees' creativity, productivity growth, reducing stress, when plants are integrated part of space working, are presented and documented by contemporary research. Besides beautiful plant growth, plantscaping also accentuate architectural details and interior space and can mask undesirable features. The need to consider the corresponding distance, light, containers, colors, sculptural elements and the environment is crucial for indoor landscaping. Even if is desired a traditional, classic, elegant or contemporary decor, indoor plants are a must. Integrating nature indoors can be an effective method to increase nature insertion in a world largely urbanized.

Keywords: indoor landscape, ornamental plants, physical, psychosocial benefits, work environment

Introduction

In the last 20 years, worries associated with quality of indoor office environment alerts working places worldwide, especially when contemporary design of green buildings are becoming standards for new constructions.

An important part of an employee's life it is represented by the workplace. Workplace is defined as the entire physical working environment (Briner, 2011). Nowadays employees spend between 6 to 9 hours per day at work. In general a person is operating in interior spaces in a proportion of 80-90% (Setten *et al.,* 2013). Given the unrelenting amount of time spent in the working environment, is common sense that physical conditions in the workplace have a role in comfort, satisfaction, wellbeing and effectiveness (Dravigne *et al*., 2008, Li, 2012), and even in mental health of employees (Shibata and Suzuki, 2004). Therefore, the physical environment in offices should be carefully planned, designed and managed (Bodin and Bodin, 2009). Bringslimark (2009) asserts that wellbeing intensifies in contact with an environment with plants that are aesthetically pleasing.

The design of the office has a direct correlation with optimum performance at work, and is competitive advantage for the company. Businesses that ignore the design and layout of interior spaces fail to optimize the value of "human capital" (Beautyman, 2006).

Good design at work can make a big difference in satisfaction, attraction and motivation of employees, also decreasing stress (Li *et al*., 2012; Han, 2010). It can also affect the level of knowledge and skills of employees, how innovative and creative they are (Shibata

and Suzuki, 2004) and how they respond to business and technological changes. A bad design at work, by contrast, is related to business with low performance and a high level of stress experienced by employees (Amble, 2005).

In the EU and US, it was started a real awareness campaign, which includes scientific studies on plant benefits at work and adding aesthetic striking values. This campaign was targeted on the need to create a workplace that can be familiar and warm. Therefore, in developed countries, plantscaping is a practice widely used not only in large companies but also in small ones.

In Romania, the use of ornamental plants at work proves to be a cultural feature, but the interior design of plants is not a priority for management. Decorating the interior space is not achieved by plantscaping principles such as plant species that are suitable for a certain environment, depending on height, diameter, color, design fundamentals, which are focal points, or to cover unaesthetic elements, or provide privacy. Plant integration in the work environment in Romania is achieved by the aesthetic sense of management team or employees, and not by a specialist in the field. However, even on small scale yet, multinationals take a step forward and employ specialized companies to decorate the interior space with plants.

This trend among employers led to increased recognition of the importance of designing a working environment that meets the physical and emotional needs of workers, so they can be more productive and involved in their job demands.

Bodin and Bodin (2008) points out that an efficient working environment should provide positive sensorial stimulation, through the proper use of color, light, flavor, space and furniture. These items are considered as critical for effective work activities and jobs, and this leads to increased employee productivity, and in the current context plants aligns these considerations.

Today's community of designers turns to use organic components as part of the office design. The focus is to create healthier conditions for employees using environmentally friendly materials and finishes that do not contribute to poor indoor air quality or adversely affects the health of employees (Luparello, 2004).

Incorporating plants or representations of nature in interior design can play a vital role in creating an environment that supports not only their destination, and to promote mental and physical well-being of their occupants (Raanaas *et al*., 2011).

Discussion

Office landscape from origin to contemporan

Eradication of nature or appearance of nature, in architecture and interior design trends in the twentieth century has led to houses and office buildings that had no restorative and regenerative value (Joye, 2007). To understand the problems and needs of present development of offices, it is necessary a briefly review of the historical context.

According to Walker (1992) and Snyder (1995) in the late 1950s and early '60s office landscape or "Burolandschaft", i.e. office planning approach, was developed and implemented widely in Germany and then in America. The Americans received this type of design at first with hesitation, because that was different from office models prevailing at that time.

Pile (1978) states that the concept of Burolandschaft was developed by Eberhard and Wolfgang Schnelle, Quickborner team, a group of management consultants and designers specialized in organizing offices. The team found that the physical design office environment plays a significant role in work operations, and employee productivity and efficiency could be magnified by improving communication between them. Developers of Burolandschaft found that every employee should become an active participant. Among their primary goals, the main one was to create a pleasant work place that provides visual and physical comfort by incorporating ornamental plants (Pearson-Mims and Lohr, 2000).

Burolandschaft type design offices have noted improved employee morale, efficiency, job satisfaction, and reducing absenteeism when plants were incorporated compared to conventional offices (Pile, 1978; Pearson-Mims and Lohr, 2000).

In the next period, design offices start to diversify severe from the original Burolandschaft design. According to Lohr (2000) placement, quantity and quality of plants and furniture began to differentiate levels of employees. In particular, the incorporation of large ornamental plants became a significant indicator of status (Snyder, 1995). Managers were given large groups of plant floor for screening in their areas of work, while employees have been given smaller table plants that offered less screening (Pearson-Mims and Lohr, 2000).

Due to the frequent use of indoor plants in work environments were introduced and initiated a number of industrial activities, including larger scale production of ornamental plants and companies specialized in interior space planning (Manaker, 1997).

Between 70's and 80's many tests, of office landscape and their benefits for employees productivity some with rudimentary methods were undertaken, but with multiple and mixed results. Observations and affirmation of growth and improve employees productivity in the working environment remained since 1970s, despite limitations of the studies from this period (Snyder, 1995).

It is believed that plants in particular were responsible for the term of office landscape (Duffy, 1969 Rodgers, 1968). Contemporary research studies using controlled conditions, nevertheless documents the positive effects on behavior and attitudes of employees working in indoor environments where ornamental plants are present (Bringslimark *et al.,* 2009; Dravigne *et al*., 2008; Li *et al*., 2012; Raanaas *et al*., 2011; Shibata and Suzuki, 2004).

For a more balanced environment and also sustainable, is essential to knowledge the elements and the principles of designing an indoor landscape (Ingels, 2009). These principles can be linked to some laws of architectural composition. The design process begins by determining user needs and desires and space conditions. Design principles are important in organizing elements for a visually pleasing landscape. The design has different view plans. Top plan provides height control and suggests environmental control, the vertical plane defines / divides space, the basic plan, floor, based on the patterns of the plants and the pots are placed (Fig. 1.).

People feel more comfortable psychologically in a landscape that includes the order and repetition (DelPrince, 2013). Psychological comfort is also affected by feelings of pleasure that a viewer perceives from a unique and harmonious landscape (Fig. 2).

Fig. 1. Organized items for a pleasant landscape

Fig. 2. Harmonious landscape

(Original photos)

A harmonious composition can be achieved through the principles of proportion, order, repetition, and unit (Ingels, 2009). All the principles are interrelated, and applying a

principle helps achieve the other. Psychological and physical comforts are two important concepts in design, which are obtained by using these principles.

The concept of interior space planning, respectively offices, is generally limited in terms of plant species and the desired size range. Generally ornamental plants used have a functional and decorative purpose (DelPrince, 2013). In open offices at the ends of the partitioning screens, ornamental plants that have between 125 cm and 145 cm can be placed with a general spread of 550 cm to 795 cm, depending on available space and the overall effect desired. Usually, an architect or specialized designer will decide the areas of plants placement, offering various options (Conklin, 1978; Ingels, 2009). Usually open offices have a good interior lighting, ranging between 1345 lx and 1615 lx generally filled with natural light available windowed areas (Ingels, 2009).

Spaces with special purposes such as the lobby, coffee shops, reception areas, elevators and hallways can receive different treatments with a more varied in terms of height and placement of plants. It can be used various plant species with high rise to create interesting effects (Conklin, 1978; Ingels, 2009; DelPrince, 2013).

Different plants serve various purposes and functions in interior design, such as emotional and symbolic: a person maintains contact with nature; architectural: offers privacy, new perspectives, define and divide space; engineering: traffic control, brightness reduction, noise control; aesthetic: Background, sculpture, lines, mitigation of architecture elements.

Types of designs

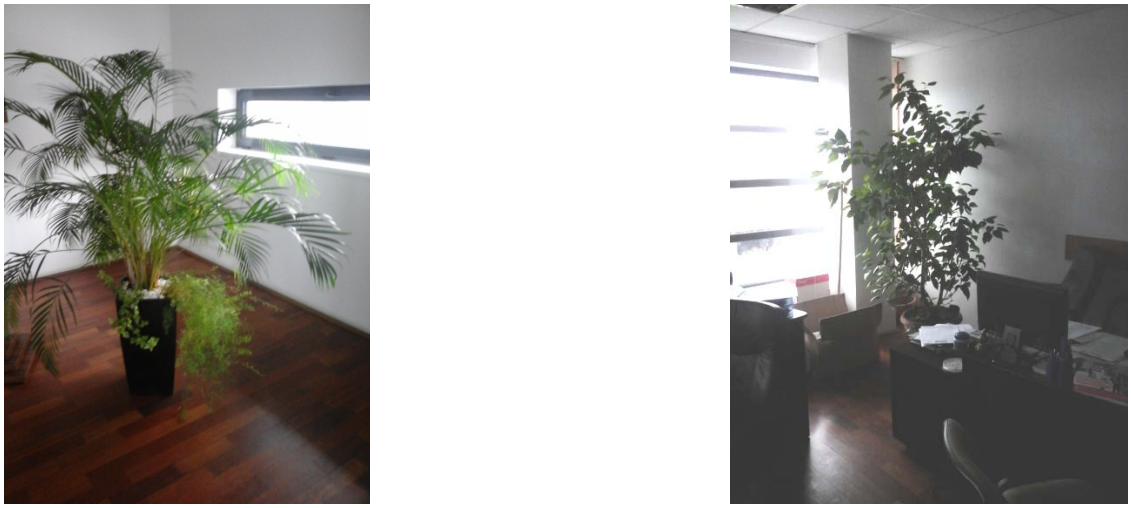
In the interior decor, it is necessary that the plant to fit in the harmony of space. The shape and direction of the plant species lead the eye towards the landscape (Hansen, 2010). Ornamental plants should contribute, together with other elements of the indoor space, to achieve a harmonious ensemble. No matter how beautiful it would be an arrangement or a potted plant, they lose their decorative value if have wrong location (DelPrince, 2013).

Formal design: This contains straight lines, rectangles, the same elements on both sides, and balance due the same elements on each side of the axis (Hansen. 2010). Philosophy of the design: vegetation dominates people.

Informal or naturalist design: this type of design includes curves, soft forms, round, natural plant formations, different elements on either side of the axis, balance due amount of attraction on each side of the axis (Hansen, 2010). Philosophy: humans are part of nature.

Besides of beautiful plant growth, plantscaping also handles to accentuate architectural details and interior space and can mask undesirable features. Angles and horizons can be

mitigated, shaped, or amended by adding ornamental plants. Large plants will be put in pots or tall vases and placed directly on the floor in a corner or in front of an item of furniture or a table to be viewed from above, throughout their length (Arteca, 2014). Higher plants are placed in the back of a wall or in the center (Fig. 3 a,b)



a.) centered **b.) in the corner**

Fig. 3 Types to place large plants indoors (Original photos)

Medium-sized flowers can be placed on the table into a corner or on any piece of furniture if they are arranged by height table. If their layout in the setting arrangement is made horizontal, it can be placed at any point of the table or other piece of furniture that allows viewing (Appell, 2000). Small flowers can be placed on any piece of furniture or table height to a maximum height of 2/3 of the average height of a person, to allow viewing of the whole composition in all directions.

Between plants and pots right balance improves beauty and draw attention to the desired destination. It is recommended to use color container similar to the plant to add a sense of peace and harmony in the interior space, or contrasting colors to provide a dramatic note, as plant stands out (Appell, 2000).

It is necessary to respect not only the rules regarding compliance with the harmony of the interior, but as well the appropriate choice of assortment according to the requirements of the plants to environmental factors (Arteca, 2014).

Selection of indoor plants, depending on their ornamental characteristics

The quality of the plants decorative by flowers and ornamental plants in containers is usually defined by visual appearance, which depends on the dimension, shape, color, number of flowers and leaves available. Plants in containers include a large number of species with different specific ornamental features that make them very attractive. For decorative potted

plants with flowers the main quality parameters are represented by color, and number of flowers while parameters of quality for indoor plants are leaf shape, size and greenness intensity (Wang et al., 2005).

Plants that are usually grown indoors are divided into two categories:

• Decorative plants with flowers: *Anthurium, Spathiphyllum, Orchyds*

• Decorative plants with leaves: *Aglaonema, Dieffenbachia, Ficus benjamina, Dracaena, Monstera.*

In the decor of indoor spaces, green leafy plants are the dominant element because they create a soothing sensation (Wang et al., 2005). The effect of these ornamental plants is given by the size, shape, position, and in many cases, the color of the leaves: *Ficus, Sansevieria, Monstera, Dracaena, Cordyline, Diffenbachia,* palms, ferns., *Hedera helix, Philodendron, Chamaedorea elegans, Chlorophytum comosum, Dieffenbachia, Epipremnum aureum, Ficus spp., Schefflera*.

A very important quality for any plant is that it should be evergreen to maintain permanent decorative character, with possible exceptions, such as bonsai, which is quite attractive even without leaves. Green leafy species are also very attractive, especially if the shape is unusual or interesting (such as *Monstera deliciosa, Sansevieria trifasciata*), but some with different color than green are considered to be more exciting. At some plants leaf are naturally colored in different colors as if Begonia Rex, while at the others with forms colored in green gives a special touch and a raised aesthetic value: *Ficus sp., Chamaedorea, Dracaena*. The leaves should be beautiful by their shape and color. Another quality of indoor plant iscompacted growth habit, because space is a limiting factor in any interior space in a crowded city (Randhawa and Mukhopadhyay, 2010).

Conclusions

Recognizing the benefits of nature has been done for centuries. The ability to adapt what surrounds us, especially indoor environments, provides a means of integrating nature or elements of nature in places where we live and can be a way to use the built environment to enhance the health and well-being. An interior space is more attractive and pleasant when ornamental plants are an integral part of the setting. Offices are based on indoor plants to humanize the workplace and improve employee productivity. Ornamental plants have always been undoubtedly, and will continue be an important component of the work environment. Intensive research started documenting scientific ornamental plants welfare benefits, improve

productivity, reduce stress, increase creativity and human comfort (Shibata and Suzuki, 2004, Bringlismark *et al*., 2009),

Besides these psychosocial benefits so important for the wellbeing of employees, are also other major functions of indoor plants. These functions include dividing the space into separate areas or annexation, the management or control of pedestrian traffic, reducing glare and reflection glare and screening to block or to create and provide privacy. Aesthetic value of ornamental plants is consistent and depends on the choice of plants suitable for every situation given environment (Hansen, 2010). Any space and area have a primary goal and this should be the starting point for any landscape design.

Interior design adds more color, vibration, space and aesthetic value to all offices. Plantscaping provides indoor art that emphasizes adding their unique identity and corporate image.

Urbanized lifestyle means that people spend about 80% to 90% of their time in indoors. This accentuates the importance of indoor air quality and a good layout of the office design. An improved work environment created by interiorscaping will contribute to a better quality of employees' life in a space that they spend more than 8 hours a day.

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ORNAMENTAL PLANTS USED AS BIOINDICATORS FOR AIR POLLUTION

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Abstract
One of the serious and severe problems facing people around the world is air pollution, due to its cross-border dispersion of pollutants throughout the world. Urbanization also has a negative impact on the environment, mainly by producing different kinds of pollution, changing physical and chemical properties of the atmosphere, and land use challenges. Plants have the ability to absorb gaseous pollutants and particulate matter through leaf stomata in particular; they tend to show certain symptoms depending on their level of sensitivity to exposure to pollutants. Plant responses to air pollutants are assessed using Air pollution tolerance index (APTI). The index is also used to select the species of plants that are tolerant to air pollution. Four physiological and biochemical parameters such as pH leaf extract, ascorbic acid, total chlorophyll and relative water content are combined in a formulation which means air pollution tolerance index of ornamental plants.

Keywords: pollutants, environment, plants, biomonitoring, pollution parameters

Introduction

In developing countries in the world, and not only, urban air pollution has become a crucial issue for environment and for human health. High concentrations of air pollutants in most urban areas of the world today come from different sources such as traffic, vehicles, residential heating, power generation and industry (Lopez *et al*., 2005). Sources in turn resulted from rapid economic growth, industrialization, urbanization with all her items, especially with increasing energy demand (Madhavi and Badarinath, 2005).

The main pollutants that contribute to poor urban air quality include: carbon monoxide (CO2), lead (Pb), sulfur oxides (SOx), nitrogen oxides (NOx), particulate matter (PM), photochemical oxidation such as ozone (O_3) and ozone precursors such as volatile organic compounds and hydrocarbons (Costa, 2001).

Current scientific research document that people worldwide are exposed to these pollutants every day. Urban air pollution causes a range of adverse health effects. Recent evidence suggests that public health impact can be considerable (WHO).

Air pollution is not only a threat to human health but also to the urban environment, and can contribute to serious problems at regional and global level (Enete *et al*., 2013).

Ensuring an adequate quality of the environment, protecting it - a necessity for survival and progress - is a matter of current interest and certain social evolution. In this respect, is important the preservation of the environment, reducing negative effects of human

activity and its implications. The purpose of air quality assessment by using plants is to identify and quantify potential consequences of the impact of air pollutants on the environment and human health.

As Bamniya et al. (2011) suggest, ornamental plant responses to ambient pollutants may provide a simple method of monitoring these pollutants, and a method to reduce pollution. Tolerant plant species if used can have a remarkable effect on various aspects of urban environmental quality and cleanliness of life in a city.

Mechanisms of plants for uptaking air pollutants

To mitigate urban pollution so severe, there are various ways and means that can be adopted. Many attempts were made to reduce ambient pollutants but unfortunately by synthetic methods. The common feature of all methods used is that of all the waste material and toxic chemical nature. Thus, people have a great need for such a natural process where should not be waste and toxicity (Verma and Gupta, 2013).

In the monitoring and maintenance of ecological balance, plants play an important role by actively participating in the cycle of gas and nutrients, like carbon dioxide or oxygen. Plants have leaves area that play a major role in the absorption and accumulation of air pollutants to reduce the level of ambient air pollution and also for noise pollution abatement (Escobedo *et al*., 2008, Harju *et al.,* 2002).

Plants have the property to change the structure and physiology of leaves, even if air pollution cause environmental stress (Verma and Gupta, 2013).These pollutants can alter leaf epidermis (Enete *et al.,* 2013) and may affect stomata conductance. Many plants when exposed to ambient pollutants, conducts physiological changes before submitting visible damage to the leaves (Dohmen *et al*., 1990). Analysis of the factors that determine resistance and susceptibility is used to understand the physiological and biochemical responses of plants to air pollution (Harju *et al.,* 2002).

The mechanism by which plants reduce or eliminate ambient pollutants is done mainly by taking leaf stomata and once they have penetrated the leaf, the gases are distributed in the intercellular spaces and are absorbed by water films. By measuring changes in plants is possible to estimate the general effect of pollutants (Agbaire and Esiefarienrhe, 2009).

Various studies have documented the effects of air pollutants on the sensitivity and tolerance of plants that varies with the change in the relative water content, the content of

ascorbic acid (Hoque *et al*., 2007), pH leaf extract (Klumpp *et al*., 2000) and chlorophyll content (Flowers *et al*., 2007).

Importance of pH in changing SO_2 toxicity has been demonstrated by different studies. Researches made by Verma and Gupta (2013) put in evidence that plants with pH around 7 are more tolerant, while plants with lower pH are more sensitive.

Conklin et al., (2000) suggest that another parameter for determine the tolerance of plants to air pollution is ascorbic acid, a natural antioxidant that has the ability to control cell division and cell expansion, and can activate defense mechanism or in stress condition has the ability to take over certain processes. The essential element for life process of photosynthesis in green plants is chlorophyll. Changes in chlorophyll content of plants can serve as relative indicators of environmental quality (Verma and Gupta, 2013).

Air pollution tolerance index

APTI is an effective and safe method for screening a large number of plants on their sensitivity to pollutants. Determination with APTI is a very simple method and applied in all ambient contexts without requiring any expensive environmental monitoring device. As bioindicators are used particularly sensitive species while tolerant species can be used as a sink for atmospheric pollutants (Krishnaveni *et al*., 2013). Parameters used for air pollution tolerance index are: Relative Water Content- a crucial prerequisite for plant life. Relative Water Content of a leaf represents the water present in it relative to its full turgidity (Mahecha *et al.,* 2013).

Leaf extract pH- The change in leaf extract pH might influence the stomatal sensitivity due to air pollution. Total Chlorophyll - Chlorophyll is an index of productivity of plant (Raza and Murthy, 1988). Chlorophyll content of plants varies from species to species; age of leaf and also with the pollution level as well as with other biotic and abiotic condition (Katiyar and Dubey 2001). Ascorbic acid is a strong reductant (Mahecha *et al*., 2013). Plants that are exposed to a polluted environment often show significant changes in their morphology, physiology and biochemistry.

According to Agarwal et al., (1991), plants are classified into three categories in terms of sensitivity: APTI < 10 are sensitive, APTI 10- 16, intermediate and APTI ≥ 16 tolerant. Landscape Architects use APTI method to select the species tolerant to air pollution (Agbaire and Esiefarenrhe, 2009). Plants with a good score on APTI are: *Ficus spp.* (Mahecha *et al*.,

2013), *Sansevieria spp., Heliconia, Codiaeum, Canna indica, Cordyline terminalis* (Nugrahani *et al.,* 2012), *Red rose,* (Otuu *et al*., 2014) etc.

The equation proposed by Singh and Rao (1983) is recommended for calculating air pollution tolerance index:

$$APTI = \frac{A(T+P)+R}{10}$$

Where:

A= ascorbic acid (mg g^{-1} FW),

T= total chlorophyll (mg g^{-1} FW),

P = leaf extract pH and

R= relative water content of the leaves.

In urban areas plants are recommended as cost effective and efficient ways to reduce pollution. Plants also can add and improve aesthetic value. Plants that are sensitive to pollution are also recommended to indicate pollution as an early warning system to protect human health. Particularly sensitive plants help in indicating air pollution and the tolerance ones are an aid in reducing air pollution (Mahecha *et al.,* 2013).

Conclusions

To provide ecosystem functions, such as air filtration, improved temperature, filtering and storing water, urban environments appeals to vegetation. This way of greening, that uses vegetation to eliminate or reduce atmospheric pollutants, is a useful and green tool for cleaning the environment. This method of selecting species that are resistant and tolerant to high levels of air pollution is very welcome, given the rapid urbanization that brings so many problems. An approach that can be used in the selection process of urban biodiversity is a simple method, known as APTI- Air pollution tolerance index.

It is suggested that the combination of four parameters of index, is the best method to identify levels of sensitivity of plants. According to research conducted by Agarwal *et al.,* (1991) the plants are grouped into three categories according to their sensitivity to air pollutants. An APTI value equal to or less than 10 is considered sensitive, while APTI value greater than 10 is considered tolerant and able to withstand significant amount of air pollution.

Extremely relevant for the science of environmental pollution, is biomonitoring of air pollution and its impact on biochemical parameters.

The urban environment offers a unique opportunity to merge environmental management with a landscape design to provide a variety of goods and social services to people.

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RESEARCH CONCERNING THE PLANT DENSITY ON THE YIELD OF DIRECTLY SOWING ONION (*ALLIUM CEPA* L.)

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Abstract
The main reason for the research is to obtain high yields in onion bulbs for fresh consumption, contributing to the improvement of the culture technology by direct sowing in the Gherla area of two onion varieties. In this research, two onion cultivars were used as biological material: Density 4 and Ramata Rossa di Milano. The general recommendations on plant density for onion are very different, depending by the author and experimental conditions. Comparing the yields obtained by the variants, it can be observed that Ramata Rossa di Milano, at a higher density, registered the highest production.

Keywords: direct sowing, onion variety, onion-yield, plant density

Introduction

The onion varieties and hybrids are specialized by crop systems and destination of bulbs (Ciofu et al., 2004). Choosing the best onion cultivars has an overwhelming importance in increasing the economic efficiency of this crop (Popandron, 2002). The general recommendations on plant density for onion are very different, depending by the author and experimental conditions. Some authors recommend that a minimal density of plants, to ensure normal production, i.e. between 35-40 plants/m^2 (Bălaşa, 1973). Krug et al. (1986), Chaux and Foury (1995), recommend densities up to 150 plants/m^2 for common varieties and 80-100 plants/m^2 for hybrids, respectively.

Aims and objectives

The main reason for the research is to obtain high yields in onion bulbs for fresh consumption, contributing to the improvement of the culture technology by direct sowing in the Gherla area of two onion varieties.

Materials and methods

In this research, two onion cultivars were used as biological material: **Density 4** and **Ramata Rossa di Milano**. In order to achieve the research objectives, polyfactorial comparative experiments were conducted. The factors considered included: variety, sowing time and plant density, each with two grades.

a)  b) 

Fig. 1. Variety bulb: a) Density 4; b) Ramata Rossa di Milano (source: Căprariu, 2013, Gherla)

Results and discussion

Comparing the yields obtained by the variants, it can be observed that Ramata Rossa di Milano, at a higher density, registered the highest production.

Table 1

The combined influence of the experimental factors on the obtained yield at Gherla, 2013

Experimental Variant			Average yield		Differences t/ha	Significance	Relative yield (%)	Differences t/ha	Significance
Variety	Sowing time	Density (million pl/ha)	t/ha	%					
Density 4	Spring	1 (Mt.)	54.12	100.0	-	-	102.9	1.53	ns
Ramata Rossa di Milano	Spring	1	51.07	94.4	-3.05	o	97.1	-1.53	ns
Average (Mt.)			52.59	-	-	-	100.0	-	-
Density 4	Spring	2 (Mt.)	51.31	100.0	-	-	93.5	-3.54	oo
Ramata Rossa di Milano	Spring	2	58.38	113.8	7.08	**	106.5	3.54	**
Average (Mt.)			54.85	-	-	-	100.0	-	-
Density 4	Autumn	1 (Mt.)	19.77	100.0	-	-	90.4	-2.11	o
Ramata Rossa di Milano	Autumn	1	24.00	121.4	4.33	**	109.6	2.11	*
Average (Mt.)			21.89	-	-	-	100.0	-	-
Density 4	Autumn	2 (Mt.)	17.06	100.0	-	-	78.6	-4.66	oo
Ramata Rossa di Milano	Autumn	2	26.38	154.6	9.32	***	121.4	4.66	**
Average (Mt.)			21.72	-	-	-	100.0	-	-
DL/*LSD* (p 5%)					1.63			1.63	
DL/ *LSD* (p 1%)					3.11			3.11	
DL/ *LSD* (p 0,1%)					7.97			7.97	

Conclusion

Plants obtained by direct sowing in spring in both varieties and sowing densities were normally developed, yielding an average of 54.12 t/ha for variety Density 4, to a density of one million pl/ha, and 51.31 t/ha at a density of two million plants/ha. The variety Ramata

Rossa di Milano obtained an average yield of 51.07 t/ha at a density of one million plants/ha and 58.38 t/ha at a density of two million plants/ha.

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THE INFLUENCE OF THE SOWING TIME ON THE QUANTITY AND QUALITY OF THE DIRECT SOWING ONION YIELD (*ALLIUM CEPA* L.)

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Abstract
This paper presents the results obtained by the researches performed within the period of 2013-2014 in the north-western part of Romania in what concerns the direct sowing onion. Two cultivars were used: Density 4 and Ramata Rossa di Milano, sowed in spring and autumn time, at two densities. The cultivar Density 4, sowed in the spring resulted in a total yield consisting of 54.74 t / ha at the density of one million plants/ha and 51.53 t / ha at the density of two million plants/ha. Ramata Rossa di Milano, sowed in the same period, presented a total yield of 50.43 t / ha at the density of one million plants/ha and 58.22 t / ha at the density of two million plants/ha. In autumn a much lower yield was achieved in comparison with the spring. The best yield quality was obtained at the cultivar Density 4 cultivated in spring, at the density of one million plants/ha with a high percentage of 97.49 % from total.

Keywords: onion, direct sowing, yield, sowing.

Introduction

One of the human most important activities was plants growing, so the close relation between the environmental conditions and plant development is well-known since the oldest times and affects the yield, its quality and nutrient contents (Ciofu, 2004).

The desire to obtain higher yields led to the improvement and development of agro-technical measures that can modify and influence environmental conditions in order to provide the optimal conditions for plant growth. It is mandatory for the farmer to know the environmental natural conditions of the area where they are living (Popandron, 2012).

Continuous population growth requests obtaining greater and higher quality yields. In the measures that may lead to increased agricultural production, particularly important are those which refers to introduction aside unproductive land and increase production per a surface unit. (Blaga *et al.*, 2005).

The share of vegetable consumption in human nutrition has been growing and healthy eating has to include vegetables and fruits. Recently, the standard of living begins to be measured by the amount of vegetables that somebody consumes (Bayer CropScience, 2014).

Onion is a profitable crop, if normal production levels are achieved. Growing profitable onion crops requires a certain level of yield, estimated at 15-20 t/ha for direct sown onion and 20-25 t/ha for seedling onion (Apahidean, 1996, 2000, 2004; Dina, 1979; Ciofu, 2003).

It is generally estimated that direct sowing reduces onion production costs by 32% compared other cultivation methods (Butnariu *et al*., 1992).

The yield obtained in the southern part of Italy by Caruso *et al*. in 2010-2011 was between 44.3 t/ha and 60.0 t/ha. The research factors were sowing time and plant density.

The sowing time has a great impact on yield. The technical literature recommends for Romania the sowing of onion in late autumn or early spring, using seed quantities of 8-10 kg/ha in autumn and 5-6 kg/ha in spring, at a depth of 2-3 cm in autumn and 1-2 cm in spring (Popescu and Zăvoianu, 2011).

Opinions differ with regard to the optimal sowing time. Popandron (2012) recommends sowing from August 25 - September 5 for autumn sowing. If sown earlier, the onion plants will grow too much, favoring bolting in spring. If sown after September 5, the plants risk freezing, resulting in damages to or the complete loss of the crop.

For spring sowing, Popandron (2002) recommends the first decade of March or the last decade of February. Any delay would have a negative impact on the yield.

The yield of a crop sown on March 20 was 15% higher compared to another sown on April 5 (Negru, 1974).

Materials and methods

In this research, two onion cultivars were used as biological material: **Density 4** and **Ramata Rossa di Milano**. Density 4 is a tardy variety with good resistance to drought and storage. The bulb is large, round, slightly elongated, tapered to the top, golden coloured, sweet and slightly spicy. Storability and resistance to mildew (*Peronospora destructor*) are also very good. It is suitable for fresh consumption, storage, food industry processing and preparation of spices (Figure 1).

Fig.1.Density 4 variety bulb.
(source: Căprariu, 2013, Gherla)

Fig.2.Ramata Rossa di Milano variety bulb.
(source: Căprariu, 2013, Gherla)

Ramata Rossa di Milano is a highly productive, tardy variety, with vigorous growth, large, elongated bulbs tapering backwards, of crimson red colour both outside and inside. Storability is very good and it is suitable for fresh consumption and storage (Figure 2).

In order to achieve the research objectives, polyfactorial comparative experiments were conducted. The factors considered included: variety, sowing time and plant density, each with two grades.

- **Factor A – variety**

  a1 – Density 4;

  a2 – Ramata Rossa di Milano;

- **Factor B – sowing time**

  b1 – Spring (February - March);

  b2 – Autumn (August - September);

- **Factor C – plant density**

  c1 – One million plants./ha;

  c2 – Two million plants./ha.

The combination of experimental factors resulted in eight experimental variants, divided into blocs, each variant in three repetitions (Table 1).

The total area of the experimental plots is 14 m^2 - 10 m in length and 1.4 m in width, and the whole experiment was conducted on an area of 336 m^2.

The experiment was conducted in Gherla, in the northwest of the Transylvanian Plateau, on Someşul Mic River, at the contact area of the Transylvanian Plain with Someş Plateau, at approx. 47° N and 24° NE.

Table 1

Experiment variant at Gherla, 2013-2015

Variant	Variety	Sowing time	Density (million plants/ha)
V1-a1b1c1	Density 4	Spring	1
V2-a1b1c2	Density 4	Spring	2
V3-a1b2c1	Density 4	Autumn	1
V4-a1b2c2	Density 4	Autumn	2
V5-a2b1c1	Ramata Rossa di Milano	Spring	1
V6-a2b1c2	Ramata Rossa di Milano	Spring	2
V7-a2b2c1	Ramata Rossa di Milano	Autumn	1
V8-a2b2c2	Ramata Rossa di Milano	Autumn	2

The soil in the area where experiments were conducted is composed of clay loam with high humus content and low alkaline reaction, very suitable for vegetable crops.

The average annual temperature is around 8°C. Early frosts were reported in mid-September (September 17), with late frosts at the end of April (April 22). Average duration without freezing temperatures is 180 days.

We have used 4 kg/ha of seeds for a density of one million plants/ha and 8 kg/ha for a density of two million plants/ha.

The desired density was achieved by using different quantities of seeds and by thinning two-three weeks after springing up.

We have made observations concerning the influence of variety, sowing time and plant density on bulb yield.

The commercial quality of the bulbs and the yield were determined for each repetition, after harvesting, by sorting them into two quality groups (STAS 1424).

The results were statistically processed using the ANOVA software, by analysis of variance and Duncan's test, and the connections between certain indicators were determined by correlation.

Results and discussion

In the case of the autumn-sowed crops, for all experimental variants, the yield was much lower as compared to the spring-sowed crop.

On overall average, the spring-sowed onion crop gave a much bigger yield (53.73 t/ha) than the autumn-sowed crop (Table 2).

The very high percentage of plants that developed floriferous stems was the main cause for a decrease in production.

The autumn-sowed crop is virtually compromised with a very significant difference of 33.71 t/ha.

Table 2

Unilateral influence of the sowing time on the obtained yield, Gherla, 2013-2014

Variant	Total yield		Differences t/ha	Significance	Relative yield (%)	Differences t/ha	Significance
Sowing time	t/ha	%					
Spring (Mt.)	53.73	100.0	-	-	145.7	16.86	***
Autumn	20.02	37.3	-33.71	ooo	54.3	-16.86	ooo
Average (Mt.)	36.87	-	-	-	100.0	-	-
DL/*LSD* (p 5%)			0.28			0.28	
DL/ *LSD* (p 1%)			0.47			0.47	
DL/ *LSD* (p 0,1%)			0.88			0.88	

The combined influence of the cultivar and the sowing time in the case of the bulbs yield (Table 3) shows that the yield's highest value, 54.32 t/ha, was recorded for the spring sowed

Ramata rossa di Milano cultivar. At Density 4, the largest yield was obtained by spring sowing, i.e. 53.13 t/ha, with a difference of 1.19 t/ha, compared to Ramata rossa di Milano.

In autumn sowing, Ramata rossa di Milano achieved 7.84 t/ha more than the control because the percentage of plants that developed floriferous stems was lower (58 %).

Table 3

The combined influence of the variety and the sowing time on the obtained yield, Gherla, 2013-2014

Experimental Variant		Total yield		Differences t/ha	Significance	Relative yield (%)	Differences t/ha	Significance
Varity	Sowing time	t/ha	%					
Density 4	Spring (Mt.)	53.13	100.0	-	-	98.9	-0.60	o
Ramata rossa di Milano	Spring	54.32	102.2	1.19	**	101.1	0.60	*
Media/Average (Mt.)		53.73	-	-	-	100.0	-	-
Density 4	Autumn (Mt.)	16.10	100.0	-	-	80.4	-3.92	ooo
Ramata rossa di Milano	Autumn	23.94	148.7	7.84	***	119.6	3.92	***
Average (Mt.)		20.02	-	-	-	100,0	-	-
DL/*LSD* (p 5%)				0.56			0.56	
DL/ *LSD* (p 1%)				1.18			1.18	
DL/ *LSD* (p 0,1%)				3,35			3,35	

The plants density influences the yield only in the spring is presented in Table 4.

Table 4

The combined influence of the plant density and the sowing time on the obtained yield Gherla, 2013-2014

Experimental Variant		Total yield		Differences t/ha	Significance	Relative yield (%)	Differences t/ha	Significance
Plant density (mil pl./ha)	Sowing time	t/ha	%					
1	Spring (Mt.)	52.58	100.0	-	-	97.9	-1.15	ooo
2	Spring	54.87	104.4	2.29	***	102.1	1.15	***
Media/Average (Mt.)		53.73	-	-	-	100.0	-	-
1	Autumn (Mt.)	29.19	100.0	-	-	100.8	0.17	n.s.
2	Autumn	19.85	98.3	-0,34	n.s.	99.2	-0.17	n.s.
Average (Mt.)		20.02	-	-	-	100.0	-	-
DL/*LSD* (p 5%)				0.49			0.49	
DL/ *LSD* (p 1%)				0.71			0.71	
DL/ *LSD* (p 0,1%)				1.06			1.06	

Table 5 presents the combined influence of the three experimental factors regarding the yield of onion bulbs. The highest yield level of 58.22 t/ha was obtained for Ramata rossa di Milano, sown in spring at a density of two million plants/ha. In the case of Ramata rossa di Milano a difference of 6.69 t/ha was achieved, as compared with Density 4, in the same period and at the same density. The difference is positive in a quite significant way. For the density of one million plants/ha, Ramata rossa di Milano presented a difference of - 4.31 t/ha as compared with Density 4, which is significantly negative. In the case of autumn-sowing, for all experimental variants, the yield was much lower than in spring, and Ramata rossa di Milano registered a very significant yield increase, for all densities as compared with Density 4.

Table 5

The combined influence of the experimental factors on the obtained yield, Gherla, 2013-2014

Experimental Variant			Total yield		Differences t/ha	Significance	Relative yield (%)	Differences t/ha	Significance
Variety	Sowing time	Density (mil pl./ha)	t/ha	%					
Density 4	Spring	1(Mt.)	54.74	100.0	-	-	104.1	2.15	**
Ramata rossa di Milano	Spring	1	50.43	92.1	-4.31	ooo	95.9	-2.15	oo
Media/*Average* (Mt.)			52.58	-	-	-	100.0	-	-
Density 4	Spring	2(Mt.)	51.53	100.0	-	-	93.9	-3.35	ooo
Ramata rossa di Milano	Spring	2	58.22	113.0	6.69	***	106.1	3.35	***
Media/*Average* (Mt.)			54.87	-	-	-	100.0	-	-
Density 4	Autumn	1 (Mt.)	17.67	100.0	-	-	87.5	-2.52	oo
Ramata rossa di Milano	Autumn	1	22.71	128.5	5.04	***	112.5	2.52	**
Media/*Average* (Mt.)			20.19	-	-	-	100.0	-	-
Density 4	Autumn	2 (Mt.)	14.52	100.0	-	-	73.2	-5.32	ooo
Ramata rossa di Milano	Autumn	2	25.17	173.3	10.65	***	126.8	5.32	***
Media/*Average* (Mt.)			19.85	-	-	-	100.0	-	-
DL/*LSD* (p 5%)					0.73			0.73	
DL/ *LSD* (p 1%)					1.27			1.27	
DL/ *LSD* (p 0,1%)					2.84			2.84	

The best commercial quality for the bulbs was obtained for the spring sowed ones. The cultivar Density 4 registered 54.74 t/ha of first quality yield, at the density of one million plants/ha, and 51.53 t/ha at the density of two million plants/ha, with high percentages: 97.49% from the total in the first case and 96.75 % in the second one (Table 6).

Table 6

Commercial quality of the onion bulbs, Gherla, 2013-2014

Variety	Sowing time	Density (million plants/ha)	Yield (t/ha)			Percentage 1st quality	
			Total	1st quality	2nd quality	Of total	Compared to 2nd
Density 4	Spring	1	54.74 g	53.36 g	1.38 b	97.49 d	97.44 g
Density 4	Spring	2	51.53 f	49.85 f	1.68 b	96.75 d	91.01 f
Density 4	Autumn	1	17.67 b	17.22 b	0.44 a	97.41 d	31.52 b
Density 4	Autumn	2	14.52 a	13.12 a	1.40 bc	88.89 c	24.05 a
Ramata Rossa di Milano	Spring	1	50.43 e	43.82 e	6.61 d	86.87 b	80.07 d
Ramata Rossa di Milano	Spring	2	58.22 h	46.51 e	11.71 e	79.87 a	85.08 c
Ramata Rossa di Milano	Autumn	1	22,71 c	19.73 d	2.98 c	86.87 b	36.06 c
Ramata Rossa di Milano	Autumn	2	25.17 d	19.98 c	5.19 d	79.01 a	36.70 c
DS 5%			0.92 – 1.01	1.37 – 1.50	0.85 – 0.94	1.87-2,06	2.08-2.29

Conclusions

In the case of the autumn-sowed crops, for all experimental variants, the yield was considerably lower as compared to spring-sowed crops, the main cause for a decrease in production being the high percentage of plants that developed floriferous stems.

The autumn-sowed crops are practically compromised in what regards the bulbs yield, the difference, a very significant one, being of 33.71 t/ha as compared to the yield of the spring sowed crops.

On overall average, the spring-sowed crop gave a larger yield (53.73 t/ha) than the yield for the autumn-sowed crop (20.02 t/ha).

The plants resulted from the spring-sowed crops for both cultivars and sowing densities developed normally with an average yield of 54.74 t / ha for Density 4 at the density of one million plants/ha and 51.53 t / ha at the density of two million plants/ha. Ramata Rossa di Milano registered an average yield of 50.43 t / ha at the density of one million plants/ha and 58.22 t / ha at the density of two million plants/ha.

The best commercial quality of bulbs was obtained in the spring-sowed ones. The cultivar Density 4 registered 54.74 t/ha first quality yield, at the density of one million plants/ha, and 51.53 t/ha at the density of two million plants/ha with high percentages of 97.49 % from total in the first case and 96.75 % in the second one.

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CONVERSION OF LACTOSE INTO MICROBIAL BIOMASS, OPTION TO REDUCE ORGANIC POLLUTION LOAD OF WHEY

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Abstract
Whey is a by-product from cheese-making process containing almost all of the total lactose and salts found in milk and because of its high organic content, when is discharged to the environment, causes serious polluting problems. Lactose is the most abundant component in whey (ca. 5% w/v) and represents an important environmental problem. The aim of this study is the investigation of liquid sour cheese whey suitability as substrates for the development of a yeast strain which is capable of metabolizing lactose, thus reducing the soluble chemical oxygen demand (COD).
In this study, aerobic fermentation *of* deproteinized cheese whey (whey permeate) with Kluyveromyces marxianus *NRRLY 1195* was carried out at 35^0C, pH=5.5 and 200rpm to examine simultaneous yeast biomass production and COD removal, reducing the pollution potential of whey. Batch culture in 300 mL flasks was carried out. The lactose concentration and COD modified corresponding to the phases of yeast growth. About 95.81% of lactose (91.56% of soluble COD) was utilized after 24 h. The biomass concentration increased from 1.27 g/L to 15.15 g/L.
The use of whey for the production of yeast biomass is an advantageous process as the pollutant load is significantly reduced and whey lactose is converted into raw yeast biomass, with application in the production of foods, feeds, bio-chemicals.

Keywords: *Kluyveromyces marxianus*, whey, biomass, batch culture, lactose, COD, pollution

Introduction

Cheese whey is a greenish-yellow liquid product from the cheese-making process and contains water and most of the milk water-soluble components (approximately 5% lactose, 0.9% nitrogen compounds, 0.8% minerals and small vitamin amounts) (Ghaly, 2004).

The world whey production is estimated to be over 10^8 tones per year, showing a 1–2% annual growth rate (Smithers GW, 2008). Cheese whey represents an important environmental problem because of the high volumes produced (to make 1 kg of cheese about 10 L of whey are generated) and its high organic matter content (Grba S., 2002). However, since it contains low concentration of solids (6-7 % (w/v)), cheese whey usually is considered a liquid waste. Cheese whey is usually considered as a concentrated wastewater, lactose being largely responsible for the high biochemical oxygen demand (BOD), of approximately 30–50 g L^{-1} and the chemical oxygen demand (COD), of approximately 60–80 g L^{-1} (Siso M, 1996, Zafar S, 2005), as opposed to 200 ppm (0.2 g/L) in case of sewage (Mawson A.J., 1994). Its disposal as waste poses serious pollution problems for the environment because the high BOD value of whey can disrupt the biological processes of wastewater treatment plants. In addition, the continuous discharge of whey onto land can endanger the chemical and

physical structure of the soil, resulting in a decrease in crop yield and when released into water bodies, reduces aquatic life by depleting the dissolved oxygen, thus making it a threat to human and animal health (Siso G., 1996, Panesar P.S, 2007) For this reason, industrially developed countries have specific legislation governing the disposal of this pollutant effluent of the dairy industry. Thus, whey poses a major threat to environmental and human health, for which an effective and permanent solution is urgently needed.

Biological wastewater treatment technologies can be useful in safer disposal of whey within environmental specifications, but these are expensive. To overcome this problem, a better alternative is subjecting whey to processes through which value added products can be manufactured, and which may reduce, totally or partially, the disposal costs. Availability of the lactose carbohydrate in whey and the presence of other essential nutrients for the growth of microorganisms make whey a potent raw material for the production of different value added products through biotechnological means (Panesar P.S, 2007).

Therefore huge amounts of whey considered as "waste" have the potential use in obtaining various value added products like animal feeds, biofuels, enzymes, chemicals. Bioconversion of whey into single cell protein, (biomass), or ethanol has already been performed in several countries (Mawson A.J., 1994).

Yeast biomass could be produced from whey by yeasts *Kluyveromyces*, *Candida*, and *Trichosporon* capable of metabolizing lactose (Braga A.R.C, 2012).

The use of whey for the production of yeast biomass has the advantages that it is a simple treatment process, and the final discharge of the whey is facilitated since the pollutant load is significantly reduced and the whey lactose is converted into yeast biomass (Moeine H, 2004)

The aim of this study is to investigate the liquid sour whey permeate (without proteins) suitability as substrates for the development of *Kluyveromyces marxianus* NRRLY 1195 yeast strain, which is capable of metabolizing lactose, the major whey constituent, with biomass accumulation and soluble COD reduction.

Materials and methods

The fresh sour cheese whey was obtained from dairy producing farms in Alba and preserved by freezing at -20 ^{0}C until use, to prevent lactose hydrolysis by *Lactobacillus*. Lactose concentration in the whey was 4.25% (w/w).

Microorganism

Kluyveromyces marxianus NRRLY 1195 yeast strain was obtained from ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL. The yeast strains stock cultures were maintained on YM agar slants medium at 3^0C for future use. *Kluyveromyces* sp is yeast characterized by its ability to hydrolyze and assimilate lactose (Parrondo, 2000). This characteristic is because of its enzymatic system, capable of synthesizing galactose permease and b-galactosidase, enzymes responsible for the transport and degradation of lactose to glucose and galactose (Barberis, 2002).

Inoculum preparation

Inocula developments were carried out in more phases. A loopful of *Kluyveromyces marxianus* grown on YM agar plates was used to inoculate 100 mL Erlenmeyer flasks containing 50 mL of sterile liquid media ATCC 200. The flasks were incubated in thermostat, aerobically, at 150 rpm and 28^0C for 24 h. In the phase two the actively grown cells of each 24 h old inoculum from the flasks were used as pre-culture to inoculate aseptically 300 mL Erlenmeyer flasks containing 100 mL of pasteurized whey permeate and incubated under conditions as described above. Finally, 10% (v/v) of starter culture was inoculated in sterile whey permeate media and then 15 mL of 6 h old suspension was directly inoculated in 300 mL Erlenmeyer flasks containing 150 mL medium.

Culture conditions

Batch fermentation was performed in 300 mL Erlenmeyer flasks, in aerobic conditions (shaking at 200 rpm), pH=4-5, at 35^0C, for 24 h. Fresh cheese whey permeate was sterilized in autoclave at 121^0C, for 15 minutes and after cooling was transferred to flasks. The initial COD value was 50526 mg/L.

Analytical methods

The samples were withdrawn at indicated time intervals and analyzed for yeast biomass, reducing sugar (lactose), and COD. Yeast biomass was determined on the basis of weight of dry biomass. 5 mL of the culture broth was centrifuged at 5500 rpm for 15 min. After two washing cycles with distilled water and centrifugations, the resulting pellets were dried at 105^0C, for 4 h. Cell-free supernatant was use for lactose and COD measurement.

Reducing sugar (lactose) concentration was measured by using Munson-Walker method.

The COD of centrifuged culture medium was determined with a Hanna photometer using a colorimetric method. All the analyses were carried out in triplicate and the average values are presented (with standard error less than 5 % of the mean).

Results and discussions

In this study, the batch fermentation of *Kluyveromyces marxianus* NRRLY 1195 was conducted in sour whey permeate growth medium (protein free) without any supplementation of nitrogen/carbon source, for 24 h.

The inoculum (10%) of *K. marxianus* NRRLY 1195 was cultivated in media containing 4.25% initial lactose concentration and the cell dry weight obtained was 15.15 g/L. There is a direct relationship between β-galactosidase (lactase) activity and biomass production. This enzyme uses lactose as energy source, hydrolyzing lactose into glucose and galactose which are more soluble. Anvari M.(2011) evaluated β -galactosidase activity in five lactose fermentative yeasts strains and showed that strain of *Kluyveromyces marxianus* had highest enzyme activity (up to 9012 EU/mL).

Yeasts lactose metabolism was studied by several authors (Guimaraes P.M., 2010, Kebbouche-Gana Salima, 2014). During *K. marxianus* NRRLY 1195 fermentation, a progressive fall of lactose concentration was observed, lactose concentration starting to diminish progressively right from the beginning of the fermentation. In this experiment, lactose utilization started at the end of lag phase of this yeast strain, after 6 h. The yeast biomass concentration increased exponentially during the logarithmic growth phase reaching 14.6 g/l and at the end of this phase, which was reached after 20 h from the beginning of the fermentation, lactose concentration was only 0.06%. The stationary growth phase reached after 20 h and a final lactose reduction of 95.81% was been achieved. There is obvious from the results obtained that a direct relationship between β -galactosidase activity and biomass production in whey permeate exist. Biomass accumulation and lactose consumption during the batch growth of yeast strain on whey permeate are presented in figure 1.

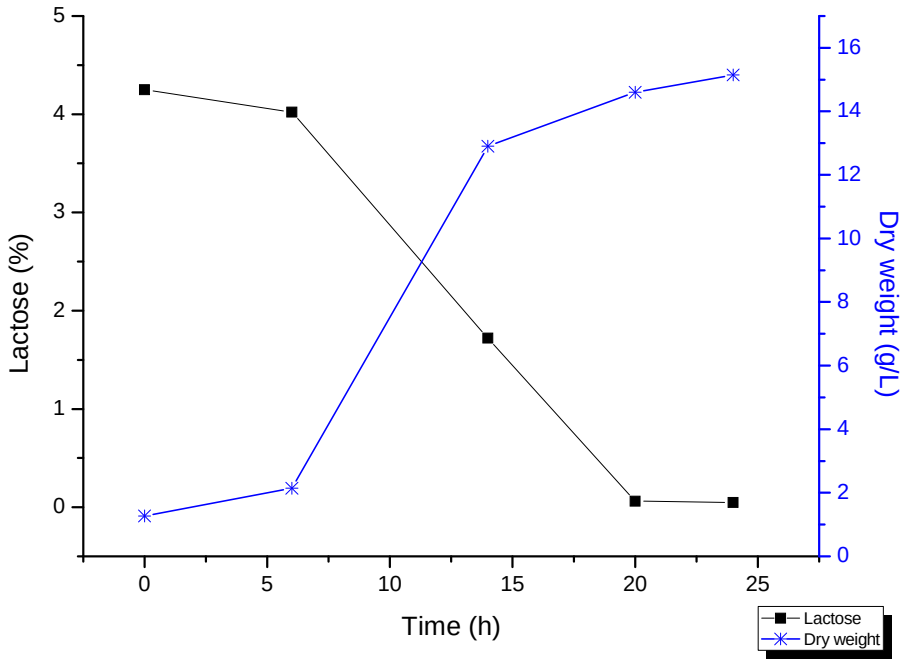

Figure 1 Lactose consumption and biomass concentration during the growth of *K. marxianus* on whey permeate

The parameter COD decreased rapidly up to 20h of fermentation due to a rapid increase of biomass concentration, most of large biodegradable lactose being consumed by yeast during the first 20h. COD continues to decrease until the end of 24 h fermentation. The final residual lactose concentration was less than 0.05% corresponding to a COD of 4263 mg/L. The residual COD in the fermented supernatant of whey permeate is likely due to other residual soluble organic compounds present in whey or produced during fermentation. Using yeast culture of *K. marxianus* initial COD decreased from 50526 to 4263 mg/L corresponding to a 91.56% total reduction of COD.

The lactose concentration and soluble COD modified corresponding to the principal phases (lag, exponential and stationary) of the yeast growth under batch conditions. In figure 2 is illustrated lactose consumption and COD reduction.

The reduction of COD was fairly linear in permeate after fermentation and followed lactose utilization by *Kluyveromyces* marxianus NRRLY 1195 yeast strain during the growth phase. The slopes of the COD reduction and lactose utilization were similar in whey permeate experiments. Another explanation for residual COD at the end of the fermentation may be residual lactose existing in whey permeate because this yeast strain can't metabolize all

lactose or it was possible that some inhibitory compound was present in the medium which prevented further COD reduction.

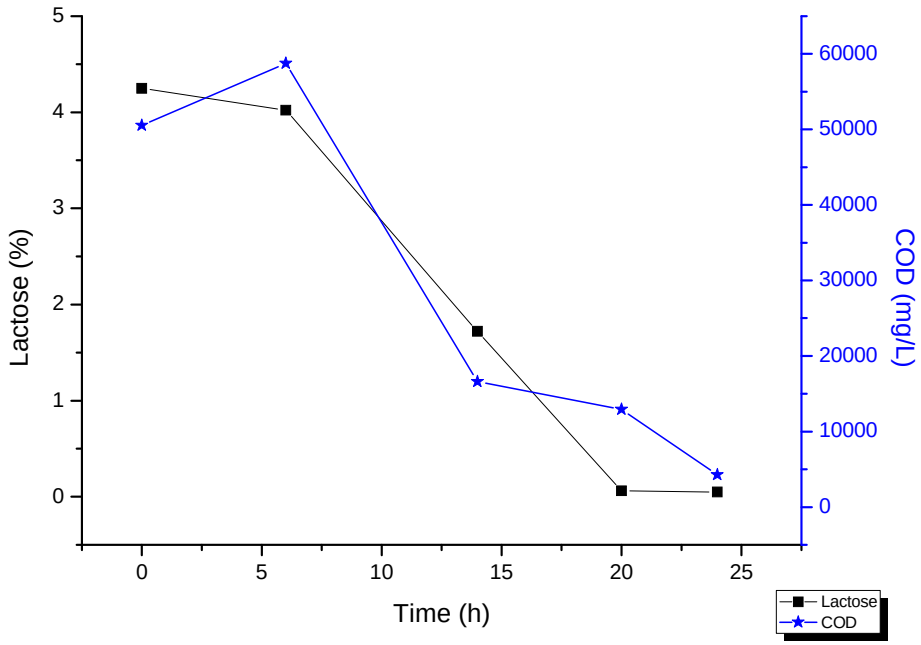

Figure 2 Variation of lactose and COD during whey permeate fermentation

In this study 95.81% of the lactose was utilized by the yeast strain. The total COD abatement was of 91.56%, which means a proportional economic saving when the effluent was treated to reduce contamination to legal levels. The COD reduction shows that lactose was converted to yeast cells and is in agreement with various authors. The COD removal efficiency in Yadav batch fermentation (2014) was 78-79 %, in Anvari experiment (2011) with *K.marxianus* was 88.49% of original value, and 42.98% in Ghaly study (2004), respectively, using *Kluyveromyces fragilis*. Recovering the yeast biomass reduced the total COD by 91.56% of its initial value in the whey permeate and reduced pollution potential before disposal in the environment.

Conclusion

Whey is a valuable by-product of dairy industry. Lactose is the most abundant component in whey (5% w/v) and represents an important environmental problem. Rapid cell

growth, lactose utilization, and biomass production resulted in rapid reduction of COD. The total COD reduction in our experiment was 91.56% of initial value.

The results of this study suggest that submerged cultivation of yeast strains *Kluyveromyces marxianus* NRRLY 1195 on whey permeate without any supplementation could be an attractive alternative and a viable candidate for valuable yeast biomass production and simultaneous removal of pollutants from whey. With adequate reduction of chemical oxygen demand, disposal of the spent whey is no longer a serious problem in the dairy industry.

From industrial point of view, the yeast biomass is an important raw material with application in the production of foods, feeds, bio-chemicals.

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EFFECT OF NITROGEN AND PHOSPHORUS ON THE BIOACCUMULATION OF HEAVY METALS FROM A CONTAMINATED SOIL

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Abstract
We performed a bivariate pot experiment looking for the influence of nitrogen and phosphorus on the uptake of heavy metals from a contaminated soil. Results have shown a strong effect of N and P on the available fractions of heavy metals in soil at the end of the experiments, on the accumulation factors from soil to roots and on the transfer factors from roots to aboveground parts. N and P also significantly influenced the oxidative stress variables in plants. We conclude that in order to predict the bioaccumulation of heavy metals in the field one needs information not only on metals' concentrations and soil variables influencing their chemical mobility, but also on the distribution of plant major nutrients influencing their growth.

Key words: heavy metals, bioaccumulation, oxidative stress, nitrogen, phosphorus

Introduction

Inoculating the soil with mycorrhizal fungi and bacteria has a heterogeneous effect on the bioaccumulation of heavy metals in plants, sometimes decreasing it, other times increasing it (Neagoe et al. 2009, 2013, 2014, Nicoară et al. 2014). This may hypothetically due to the fact the microorganisms stimulate also the uptake of nutrients, especially of phosphorus, as pointed out and documented in the mentioned articles. In this context our objective was to investigate the effect of nitrogen and phosphorus on the uptake of heavy metals from plants and on their effect on the oxidative stress variables. We present here only a short summary of the results, which will be published *in extenso* later.

Methods

The contaminated soil originated from the Zlatna area and had general properties as presented in table 1. The model plant was *Agrostis capillaris*. The experimental design is presented in table 2. For analytical methods we refer to Neagoe et al. (2009, 2013, 2014). The data processing procedure is presented in figure 1.

Table 1 General characterization of the experimental soil.

Variable	pH (H_2O)	Moisture %	EC µS/cm	Soil resp. $mgCO_2/g$ d.w	N-NH_4^+ mg/kg d.w.	N-NO_3^- mg/kg d.w.	P-PO_4^{3-} mg/kg d.w.
average	3.2	9.75	25.1	17.84	9.11	5.51	21.86
SD	0.13	1.03	14.75	3.96	5.17	3.62	7.1
n=13 Elements	As	Cu	Fe	K	Mn	Pb	Zn
	mg/kg d.w.						
average	81.75	314.9	25162	11852	502.1	532.3	248.6
SD (n=13)	52.07	106.9	3721	2598	179	268.7	59.4

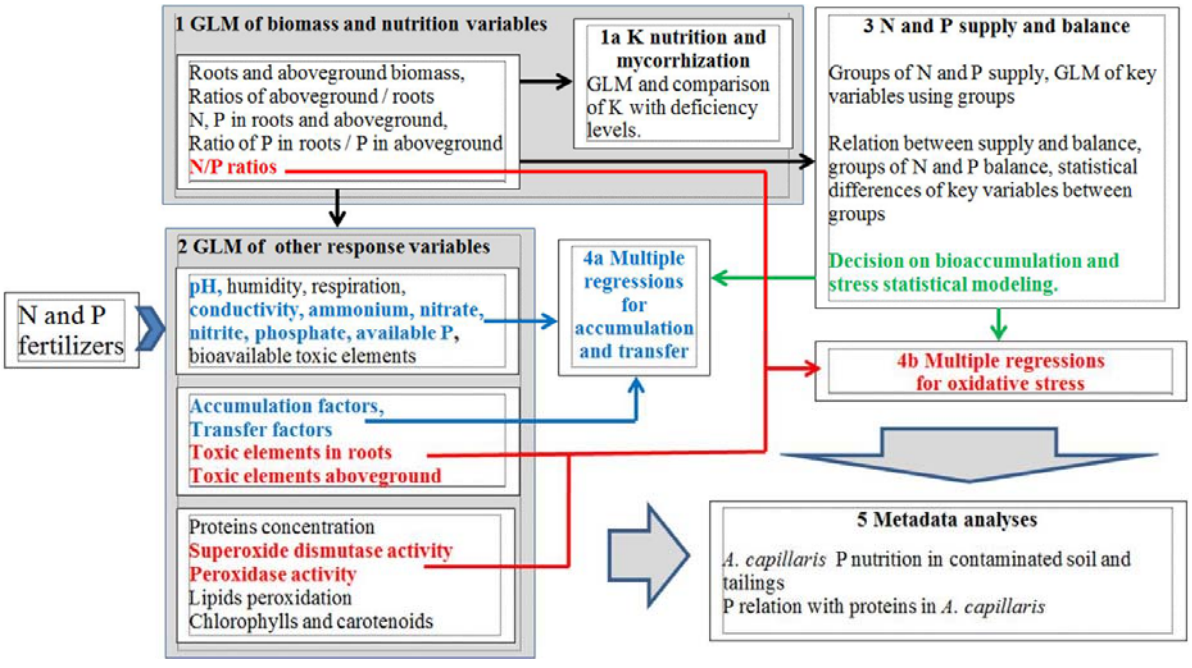
Table 2 Experimental design.

No. treatment	Lab code	Experimental treatment	No. pots
1	**N(-), P(-)**	450 g polluted soil + 60 ml water + 22.5 g $CaCO_3$ + 0.3g seeds	5
2	**N(+), P(-)**	450 g polluted soil + 10ml **sol. 1 N** + 50 ml dist.water + 2 2.5g $CaCO_3$ + 0.3g seeds	5
3	**N(-), P(+)**	450 g polluted soil + 10 ml **sol. 1 P** + 50 ml dist.water + 22.5 g $CaCO_3$+ 0.3g seeds	5
4	**N(+), P(+)**	450 g polluted soil + 5ml **sol. 1 N** + 5ml **sol. 1 P** + 50 ml dist.water + 22.5 g CaCO3 + 0.3g seeds	5
5	**N(++), P(-)**	450 g polluted soil + 10 ml **sol. 2 N** + 50 ml dist.water + 22.5 g $CaCO_3$ + 0.3g seeds	5
6	**N(-), P(++)**	450 g polluted soil + 10 ml **sol. 2 P** + 50 ml dist.water + 22.5 g $CaCO_3$ + 0.3g seeds	5
7	**N(++), P(++)**	450 g polluted soil + 5 ml **sol. 2N** + 5 ml **sol. 2 P** + 50 ml dist.water + 22.5 g $CaCO_3$ + 0.3g seeds	5
8	**N(+), P(++)**	450 g polluted soil + 5 ml **sol. 1 N** + 5 ml **sol. 2 P** + 50 ml dist.water + 22.5 $CaCO_3$ + 0.3g seeds	5
9	**N(++), P(+)**	450 g polluted soil + 5 ml **sol. 2 N** + **5 ml sol .1 P** +50 ml dist.water + 22.5 g $CaCO_3$ + 0.3g seeds	5
10	**N(+++), P(-)**	450 g polluted soil + 10 ml **sol. 3 N** + 50 ml dist.water + 22.5 g $CaCO_3$ + 0.3g seeds	5
11	**N(-), P(+++)**	450 g polluted soil + 10 ml **sol. 3 P** + 50 ml dist.water + 22.5 g $CaCO_3$ + 0.3g seeds	5
12	**N(+++), P(+++)**	450 g polluted soil + 5 ml **sol. 3 N** + 5 ml **sol. 3 P** +50 ml dist.water + 22.5 g $CaCO_3$ + 0.3g seeds	5
13	**N(+++), P(+)**	450 g polluted soil + 5 ml **sol. 3 N** + 5 ml **sol. 1P** + 50 ml dist.water + 22.5 g $CaCO_3$ + 0.3g seeds	5
14	**N(+), P(+++)**	450 gpolluted soil + 5 ml **sol 1 N** + 5 ml **sol. 3 P** + 50 ml dist.water + 22.5 g $CaCO_3$ + 0.3g seeds	5
15	**N(+++), P(++)**	450 g polluted soil + 5ml **sol 3 N** + 5 ml **sol. 2 P** + 50 ml dist.water + 22.5 g $CaCO_3$ + 0.3g seeds	5
16	**N(++), P(+++)**	450 g polluted soil + 5 ml **sol. 2 N** + 5 ml **sol. 3 P** + 50 ml dist.water + 22.5 $CaCO_3$ + 0.3g seeds	5
			Total pots = 80

1N = echiv. of 15
2N = echiv. of 30
3N = echiv. of 60
} kg N/ha as NH_4NO_3

1P = echiv. of 15
2P = echiv. of 30
3P = echiv. of 60
} kg P/ha as K_2HPO_4

Figure 1 Data processing procedure.

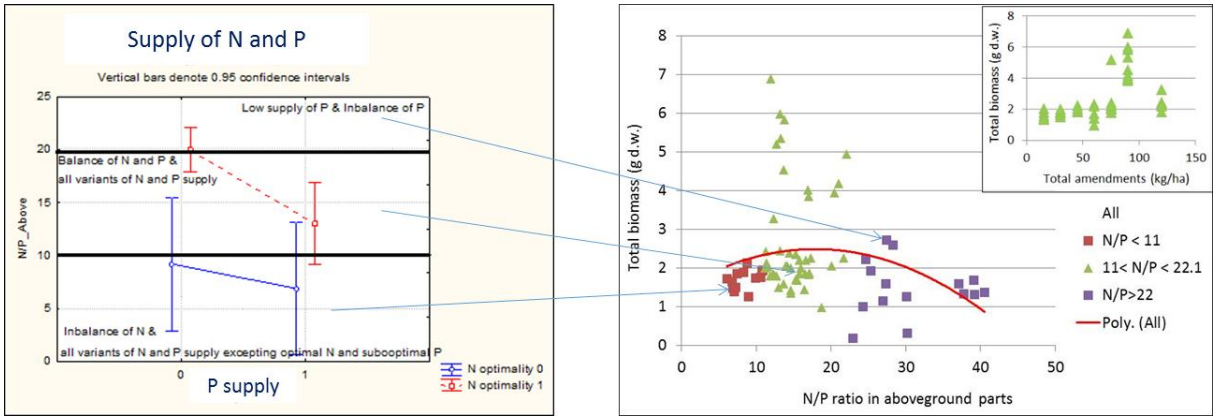


Legend: GLM = Generalized linear model method

Results

The plant development was controlled both by the supply of N and P and by their balance. The original soil had large P deficiency, and as a result N addition determined larger biomass only in the variants where enough P was supplied. Potassium was at optimal levels and did not have an influence on the variants. The overall pattern is presented in figure 2.

Figure 2 The effect of N and P supply and balance on biomass.



Legend: Codes 0 and 1 in left graph refer to classes of supply (suboptimal and optimal)

The accumulation factor of Cu, Pb and Zn in plants was computed in two variants: with respect to total concentrations in soil, and with respect to the phytoavailable concentrations. In both variant it could be predicted in function of soil variables using multiple regression (table 3). The transfer factors from roots to shoots could also be predicted in a similar way in function of N and P soil variables (results not shown here).

Table 3 Multiple regression for the prediction of accumulation factors from soil to roots.

	Cu AF total Coef.	p-level	R^2		Pb AF total Coef.	p-level	R^2		Zn AF total Coef.	p-level	R^2
Intercept		0.000	0.409	Intercept		0.000	0.358	Intercept		0.000	0.202
Log $N\text{-}NO_3^-$	0.416	0.000		Lg EC	0.365	0.002		pH	-0.436	0.000	
pH	0.279	0.005		pH	-0.304	0.010		Lg P Sol	-0.213	0.047	
Log $N\text{-}NO_2^-$	0.243	0.011		Lg$N\text{-}NO_3^-$	0.292	0.011					

	Lg Cu AF bioav. Coef.	p-level	R^2		Lg Pb AF bioav. Coef.	p-level	R^2		Lg Zn AF bioav. Coef.	p-level	R^2
Intercept		0.38	0.781	Intercept		0.223	0.594	Intercept		0.000	0.52
Lg P soil	-0.49	0.000		Lg P soil	-0.32	0.002		Lg P soil	-0.61	0.000	
Lg $P\text{-}PO_4^{3-}$	0.503	0.000		Lg $P\text{-}PO_4^{3-}$	0.449	0.000		pH	-0.42	0.000	
Lg $N\text{-}NO_3^-$	0.223	0.003		Soil moist.	0.335	0.000					
Lg EC	0.354	0.000		Lg $N\text{-}NO_2^-$	-0.24	0.019					
pH	0.198	0.011		Lg $N\text{-}NO_3^-$	0.188	0.040					

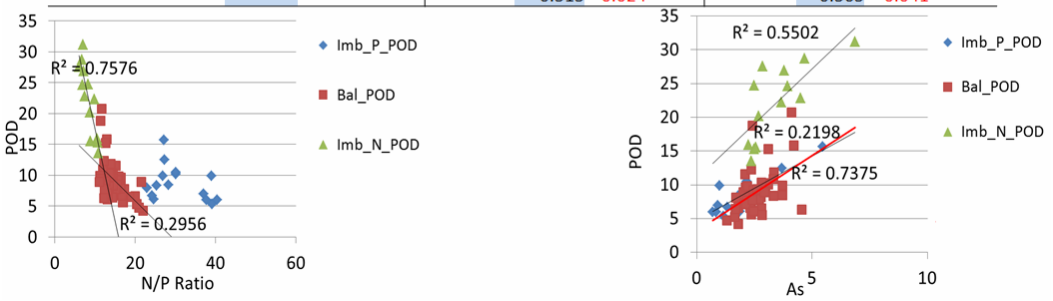
Legend: EC = electrical conductivity, P sol = total P in soil.

Superoxide dismutase (SOD) and peroxidase activities (POD) could be predicted by multiple regression differently in function of the N/P balance. When the plants nutrition was balanced or had low N/P ratio (N imbalance) the increase of N/P ratio decreased the oxidative stress, while the increase of metals increased the oxidative stress (figure 3). At very large N/P ratios (P imbalance) only metals in plants positively correlated with the oxidative stress variables.

Figure 3 Prediction of oxidative stress variables by multiple regression and visual representations of POD activity in function of N/P ration and As.

Regression summary for dependent variables

		P imbalanced, n = 16			Balanced P and N, n=43			N Imbalanced, n=14		
		Coef.	p-level	R^2	Coef.	p-level	R^2	Coef.	p-level	R^2
SOD above	Intercept		0.410	**0.762**	Intercept	0.000	**0.474**	Intercept	0.000	**0.784**
	As	0.604	0.000		N/P above	-0.561	0.000	N/P above	-0.883	0.000
	Zn	0.391	0.030		Cu	0.284	0.023			
		Coef.	p-level	R^2	Coef.	p-level	R^2	Coef.	p-level	R^2
POD above	Intercept		0.921	**0.773**	Intercept	0.000	**0.362**	Intercept	0.000	**0.854**
	Cr	0.671	0.000		N/P above	-0.462	0.000	N/P above	-0.661	0.000
	Zn	0.352	0.032		Pb	0.313	0.024	As	0.363	0.041



Legend: SOD and POD are in units / mg protein, toxic elements are in mg/kg d.w.

Conclusion

There is a strong and complex effect of N and P on metals bioaccumulation by *Agrostis capillaris* from Zlatna polluted soil, and on oxidative stress variables of plants.

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FUNCTIONAL FOODS IN REALTION TO HUMAN HEALTH: A REVIEW

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Abstract
In this modern and industrialized world, significantly changes have occurred in the concepts of nutrition. Former emphasis has been given on survival through hunger satisfaction and food safety to prevent the development of risk of ailments but more recently food sciences now aim at developing foods to health while at the same time reducing the risk of some major and minor diseases. Within the last decades, the term functional foods gained more attention throughout the world. That is because it has additional physiological benefits beyond that of meeting basic nutritional needs which is already supported by scientific data show that food with nutritive and non-nutritive components have the potential to modulate target functions in the body which are relevant to human health and reduction of disease risk. The present study overviewed the concept of functional food in relation to plant sources with strength of evidence supporting their functionality, safety considerations and recommendations intakes.

Keywords: Functional foods, Human health, Phytochemicals, Plant resources

Introduction

All foods are functional and provide the therapeutic benefits clearly not a new concept. The primary role of food is to provide taste, aroma and sufficient nutritive value to meet the health requirements of an individual. Hippocrates the father of medicine nearly 2,500 years ago adopted that *"Let food be thy medicine and medicine be thy food,"* is gaining renewed interest in health enhancing role of specific foods (Haslaer, 1998). From the perspective of healthy living, it is obligatory for all to eat healthy food. It is pointed in this context about knowledge of nutritional composition of food including quality and quantity of foods with recommended values beneficial for a healthy life. However, it is concluded there are no 'good' or 'bad' foods only 'bad' diets may develop the risk of diseases. Although overall message for health promotion is that which types of food to include or avoid achieving a balanced diet for which extensive guidance is available (Lupton, 1996) (Conveney, 2000).

a) Concept of functional foods

Today, concept of functional foods derives from the observation that some foods and beverages have beneficial physiological effects on human health that are not explained by their nutritional content. There is now trend has developed, nutrition science moved on from the classical concepts of avoiding nutrient deficiencies and to support the idea of "positive" or "optimal" nutrition. A comprehensive lifestyle is mandatory for good health which includes

not only balanced diet but also regular exercise, stress avoidance and other positive health practices. This could only be possible to maximize health and reduce disease risk when all of these issues are addressed along with concept of functional foods (Toma & Pokrotnieks, 2006).

There is increasing scientific evidence that nutrition plays crucial role in the prevention of chronic diseases like diabetes, cardiovascular diseases, obesity and cancer most of them are diet related. The food not only necessary for living but also as a source of mental and physical well-being contributing to promote health and reduction of risk of a pathologic process. Generally, those foods are considered to be part of normal diet that contains biologically active components, which offer the potential of enhanced health or reduced risk of disease (Hasler, 2002).

b) History of uses

Food and nutrition have become challenging issues in modern societies and functional food is now very popular term in this regard. It was first introduced in Japan in the mid-1980s and refers to foods containing ingredients that significantly affects on bodily functions in addition to being nutritious. Functional foods have no universally accepted definition but intensive research efforts in this field made more prominent growing food category which is recognized as "functional food" at global level. Because of growing interest in functional foods, market of these products has been emerging rapidly. To date, the biggest functional food markets are in Japan is the only country that has formulated a specific regulatory approval process for functional foods. It is known as Foods for Specified Health Use (FOSHU), these foods are eligible to bear a seal of approval from the Japanese Ministry of Health and Welfare. Currently, 100 products are licensed as FOSHU foods in Japan. Now functional food market is flourishing in USA and Europe (Swinbanks & O'Brien, 1993, Bech-Larsen & Scholderer, 2007).

Estimates, however, of the magnitude of this market vary significantly, as there is no consensus on what constitutes a functional food. It depends on the consumption and awareness of functional foods which vary from country to country. It is estimated by (Waltham, 1998) the market value of functional foods reached at $28.9 billion. More significant, perhaps, is the potential of functional foods to mitigate disease, promote health, and reduce health care costs.

Impacts on human health with scientific evidences

Regarding the concept of functional food in relation to health has increased substantially and consumers take interest in components of foods with added health benefits. Many scientific organizations and academic are actively working on functional foods in order to establish the scientific basis to support claims for functional components. The desire for an improved quality of life, consumers must have a clear understanding of potential health benefits of functional foods and a strong confidence in the light of scientific evidence that are used to document health effects and claims. Moreover, changing trends in population demographics and socio-economic also indication of urgent need for foods with additional health benefits. A number of studies show that socio-demographic characteristics also play an important role (Hasler, 2002, Holm, 2003).

Research on functional food link to health is assumed to be the main motivation behind the use of functional foods (Burges et al, 2009). Although monitoring taste was also found to be a critical factor for the future acceptance of functional foods (Verbeke, 2006). An increase in life expectancy as well as improvements in the costs of health care have stimulated researchers, health professionals and the food industry to investigate how these changes can be managed more effectively. Recently a massive body of research on functional foods has been done in different countries to address cognitive, motivational and attitudinal determinants of the public acceptance (Urala & Lähteenmäki, 2003, Verbeke, 2005). Any claims about health benefits attributed to functional foods based on sound scientific evidence but too often only so-called "emerging evidence" is the basis for marketing some functional foods.

In Table 1, scientific evidences and the recommended intake levels are summarized in order to support the functionality and variety of functional foods components.

Table 1: Strength of evidence for functional foods currently on the U.S. market*

	Functional food	Bioactive component	Health benefit	Type of evidence	Strength of evidence	Recomme-nded amount or frequency of intake	Regulatory status
1	Fortified margarines	Plant sterol and stanol esters	Reduce total and LDL cholesterol	Clinical trials	Very strong	1.3 g/d for sterols 1.7 g/d for stanols	Health claim
2	Psyllium	Soluble fiber	Reduce total and LDL cholesterol	Clinical trials	Very strong	1 g/d	Health claim
3	Soy	Protein	Reduce total	Clinical trials	Very	25 g/d	Health claim

			and LDL cholesterol		strong		
4	Whole oat products	β-Glucan	Reduce total and LDL cholesterol	Clinical trials	Very strong	3 g/d	Health claim
5	Fatty fish	(n-3) Fatty acids	Reduce TG, reduce heart disease cardiac deaths and fatal and non-fatal myocardial infarction	Clinical trials; epidemiological studies	strong	2/wk	Qualified health claim for dietary supplements
6	Garlic	Organosulfu-r compounds	Reduce total and LDL cholesterol	Clinical trials	moderate	600–900 mg/d	Conventional food or dietary supplement
7	Green tea	Catechins	Reduce risk of certain types of cancer	Epidemiological	Weak to moderate	Unknown	Conventional food
8	Tomatoes and processed tomato products	Lycopene	Reduce risk prostate cancer	Epidemiological	Weak to moderate	Daily	Conventional food
9	Cranberry juice	Proanthocya-nidins	Reduce urinary tract infections	Small number of clinical trials	Moderate	300 mL/d	Conventional food

Source ((Hasler, 2002)
*Foods that have an FDA-approved health claim (sterol/stanol esters, oats, psyllium, soy) generally are supported by two dozen or more well-designed published clinical trials. For example, the soy health claim petition contained _40 clinical trials, while there are only a few clinical trials on cranberry juice and urinary tract infections. TG, triglycerides; CLA, conjugated linoleic acid; GI, gastrointestinal

Plants as a source of functional foods

The use of functional foods for promoting health and specific dietary recommendations for treating or preventing various types of illness is as old as practice of medicine, have been documented in Hippocratic and Vedic texts and the canons of traditional Chinese medicine. Herbs and spices are added to food to enhance flavor. The foods with culinary and non-culinary herbs to produce healing mixtures were the part of Traditional Chinese remedies.

Many of these have documented medicinal uses that render them functional foods. For instance, Peppermint (*Mentha piperita*) tea, Ginger (*Zingiber officinale* root), cinnamon (*Cinnamomum spp* bark) and licorice (*Glycyrrhiza glabra* root) has a long history of use in Chinese herbal tonics for digestive complaints and disorders. Peppermint oil contains spasmolytic components that block calcium channels in smooth muscle. Thyme (*Lamiaceae spp*) was used as a vermifuge in ancient Egypt and its oils possess potent antimicrobial properties. Cranberry (*Vaccinium macrocarpon*) juice has traditionally use for

treatment of urinary infection (http://www.mdheal.org/articles/word2/functionalfoods2.htm). An overview of specific phytochemicals from edible plants associated with health promotion and disease prevention are described in Table 2.

The most studied food sources of these phytonutrients are soy beans (*Glycine max*) and tea (*Camellia sinensis* leaves), but tomatoes (*Lycopersicon esculentum*), broccoli (*Brassica oleracea*), garlic (*Allium sativum*), turmeric (*Curcuma longa*), tart cherries (*Prunus cerasus)* and various types of berries are also receiving considerable attention as functional food candidates (http://www.nutriwatch.org/04Foods/ff.html). Numerous clinical data indicates that plant based diet with physiologically active ingredients known as phytochemicals can reduce the risk of chronic diseases. Unfortunately, only a small number of these plants have had substantive clinical documentation along with significant scientific agreement" required by the FDA for authorization of a health claim which include oat soluble (β-glucan) fiber (U. S. Food and Drug Administration, 1997), soluble fiber from psyllium seed husk (U. S. Food and Drug Administration, 1998), soy protein (U. S. Food and Drug Administration, 1999) and sterol- and stanol-ester–fortified margarine (U. S. Food and Drug Administration, 2000). Research on some other plant resources for health benefits are discussed briefly below:

*a) Garlic (*Allium sativum*)*

It has a long history of uses along with a wide variety of medicinal purposes. Garlic has been shown to have a modest blood pressure lowering effect in clinical studies due to the presence of numerous physiologically active organo-sulfur components (Silagy & Neil, 1994).

Table 2: Phytochemicals Associated with Health Promotion and Disease Prevention

	Group	Biological activities	Food sources
1	Carotenoids	Quench singlet and triplet oxygen, increase cell-cell communication	Vegetables, margarine, egg yolk, butter fat, red orange fruits
2	Glucosinolates,	Increase xenobiotic	Cruciferous vegetables
3	Isothiocyanates	metabolism, alter estrogen metabolism	horseradish
4	Inositol phosphates	Stimulate natural killer Cell function, chelate, divalent cations	Bran and soy foods
5	Isoflavones	Estrogen agonist and antagonist, induce apoptosis	Soy foods, kudzu
6	Lignans	Estrogen agonists antagonists, inhibit tyrosine kinase	Flax seed, rye
7	Phenolic acids	Antioxidant, enhance xenobiotic metabolism	Diverse fruits, vegetables

8	Phytoallexins	Antioxidant, platelet inhibition, induce apoptosis	Red wine, grape seed
9	Polyphenols	Antioxidant, enhance xenobiotic metabolism inhibit numerous enzymes	Diverse fruits, vegetables, red wine, tea
10	Saponins	Antimicrobial, immune boosting, cytotoxic to cancer cells	Legumes, nuts, herbs
11	Sterols	Bind cholesterol, decrease colonic cell proliferation, stimulate T-helper-1 cells	Nuts, seeds, legumes, cereal grains
12	Sulfides	Antimicrobial, antioxidant	Garlic, onions

Source (http://www.mdheal.org/articles/word2/functionalfoods2.htm)

*b) Tomatoes (*Lycopersicon esculentum*)*

Tomatoes and tomato products are also being investigated for their unique properties such as their role in cancer chemoprevention and most significant dietary source of lycopene, a non-provitamin A carotenoid that is also a potent antioxidant (Clinton, 1998).

c) Others

Clinical trials, which have specifically examined the effect of almonds on blood lipids, have found that these tree nuts significantly reduced total cholesterol by 4–12% and low density lipoprotein (LDL) cholesterol by 6–15%. Although, traditionally foods high in fat are not considered heart-healthy except for fatty fish but evidence is accumulating on the cardiovascular benefits of a variety of nuts, when they are part of a diet that is low in saturated fat and cholesterol (Spiller, *et al.*, 1998).

On the other hand, studies in animals consistently show that consumption of green tea reduces the risk of various types of cancers (Yang, Chung, Yang, & Chhabra, 2000). Furthermore, food that is a source of polyphenolics and is just beginning to be investigated for its potential benefits to heart health is chocolate (Bruinsma & Taren, 1999).

Safety Issues and considerations

Although there is evidence that some or certain functional foods or ingredients can play a role in disease prevention and health promotion, safety considerations is critical. Safety concerns and issues have recently been raised which is relevant to herbs are complex. The Food and Nutrition Board of the National Academy of Sciences has devoted considerable attention to the upper safe limit for intake of essential nutrients (Milner, 1999). For the safety of public health, an expert panel stated in the IFT "Expert Report on Functional Foods that components already considered through pre-established programs as generally recognized as safe (GRAS) substances and approved food additives should be accepted for functional foods

(Institute of Food Technologists, 2005). Also in 2000, the General Accounting Office (GAO) released a report that raised concerns about the safety of certain functional foods (U. S. General Accounting Office, 2000). In June of 2001, the FDA issued warning letters to the food industry concerning the use of "novel ingredients" such as St. John's wort in conventional food due to absence of such safety information poses a significant safety risk to some consumers (Greeson et al, 2001).

Conclusions

In this industrialized world, significantly changes have occurred in the concepts of nutrition but more recently food sciences is developing foods with additional health benefits widely known as functional foods while at the same time reducing the risk of some major and minor diseases develop another concept in food sciences as nutraceutical (combined term from nutrition and pharmaceuticals). Within the last decades, the term functional foods gained more attention throughout the world. Extensive research is currently directed toward increasing our understanding of "functional foods." Academic, government and private research institutes around the globe are devoting substantial efforts to identifying how functional foods and food ingredients both from plant and animals (phytochemicals and zoochemicals) might help prevent chronic disease or optimize health, thereby reducing healthcare costs and improving the quality of life for many consumers.

On the other hand, consumers must realize that foods are not good and bad, only dietary pattern may be good or bad. Any health benefits attributed to functional foods should be based on sound and accurate scientific criteria and evidence including rigorous studies of safety and efficacy. Further researches are necessary to find out reasons behind the differences and understand consumers' needs for functional foods in order to meet the greatest challenges to nutritional health are chronic diseases including obesity, heart diseases, diabetes and cancer. When functional foods do provide genuine public health solutions, they contribute immensely to the public welfare. They help to provide the very conditions for life; they help us to increase our capacities, to exercise our rights, and to live well together then use of functional foods under these circumstances is permissible with health safety considerations.

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DAILY OPERATION AND ADAPTABILITY WITHIN MODERN WASTEWATER TREATMENT PLANTS

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Abstract
Operating a wastewater treatment plant poses many challenges, due to the influent variability, manifested in flow, temperature, loading a.s.o. Considering the static building characteristics of such a plant, the operating regime must be changed frequently, in order to allow the activated sludge to perform its duty, i.e. bringing the water into the required discharge parameters. In case of high volumes of organic loaded water, corroborated with low temperatures, addition of $FeCl_3$ is an affordable solution to prevent sludge bulking and to comply with the national discharge requirements.

Keywords: activated sludge, SVI, ferric chloride.

Introduction

When designing a wastewater treatment plant (WWTP), besides the population equivalent inhabitants (considered as the organic biodegradable load having a five-day biochemical oxygen demand - BOD_5 - of 60 g of oxygen per day, as defined by the Urban Waste Water Treatment Directive 91/271/EEC of 21 May 1991), it should be considered the specificity of each place, including the industry, pluvial regime and climatic conditions. All these are required because the "cocktail" forming the activated sludge has a certain inertia, and changes in the influent composition and flow materialize in hindering the bacterial activity, with detrimental effects on the effluent characteristics.

Materials and method

In our case we have considered a WWTP for more than 15,000 population equivalent, situated in Sibiu County. The WWTP, commissioned two years ago is one of the most modern ones in Romania, having the possibility of N and P removal from wastewater, in order to fully comply with Romanian and European standards.

The WWTP was provided by the manufacturer with an activated sludge, able to cope with the wastewater to be treated, consisting of a bacterial mixture of *Achromobacter, Alcaligenes, Arthrobacter, Citromonas, Flavobacterium, Pseudomonas* and *Zooglea* (Jenkins, 1993), as well as *Nitrobacter* and *Nitrosomonas* (Water Environment Society, 1987). The sludge comprised also fungi, protozoa, algae, with a definite role in the sustainability of the

micro-ecosystem. Even if not with a positive role in water treatment, ciliates from *Vorticella* genus can also be found in such an activated sludge, their proportion being an indicator of the treatment conditions and efficiency (Simionescu, 2009). If the oxygen is less than required, or the organic loading is too high, the generated sludge contains larger amounts of such ciliates that decrease its sedimentation capacity, with the sub-sequent sludge swelling and missing the desired effluent characteristics. Besides oxygen and load, reduced temperatures can determine an increase of ciliates proportion.

The WWTP is equipped with input (influent) sensors for flow, temperature, pH, BOD, COD and N, and effluent parameters (flow, temperature, pH, BOD, COD, N, P) are also automatically measured. The whole plant is managed by means of a SCADA (Supervisory control and data acquisition) system that provides real-time information on these parameters, this allowing the operators to take immediate action in case of the plant good functioning is jeopardized.

The performance of the studied WWTP is assessed by the compliance of the main parameters (BOD, COD, TSS, Ntot and Ptot) with the Romanian and European standards (discharge requirements), but the removal efficiency is also very important.

In this respect, daily analyses are made for the discharge requirements, according to the Romanian and European analytical standards and norms, but also for Mohlman or Sludge Volume Index (SVI). This index provides valuable information on the sludge sedimentation capability and is calculated with the formula:

$$SVI\ (mL/g) = \frac{SV(mL/L)}{SU(g/L)}$$

Where:

SV = sludge volume, after 30 minutes of settling

SU = dry matter content of the sludge

If SVI has values between 50 and 100 mL/g, the sludge sedimentation process develops well, and its bulking or foaming are prevented. If the values are above 200 mL/g the sludge will not settle and it will be lost in the secondary sedimentation tank, with detrimental consequences for the effluent parameters. Among the causes of the reduction of sedimentation capability is the increased presence of filamentous bacteria, which are favored by large amounts of organic matter in the influent, corroborated with low temperatures.

Because is quite impossible to raise the temperature of such a input water volume, WWTP operators try continuously to find other solutions to prevent sludge bulking, one of it being the addition of $FeCl_3$.

The paper presents an example of correlation between the influent characteristics and discharge parameters, as well as an action taken to improve these parameters.

Results and discussion

The delay between wastewater entrance in the plant and biological/tertiary treatment being 3-4 hours, the functional parameters of activated sludge reactors can be adjusted accordingly. This "tuning" is very dependant on the local conditions, and among practitioners it is considered more "art" than science…

The table below (1) presents the results of the plant in two "normal" operating days (25.09.2014 and 09.10.2014) and the situation of another, cooler day with a similar organic influent load (29.01.2015), but with the danger of sludge foaming. The possibility of foaming was proven by the microbiological analysis which resulted in massive presence of filamentous bacteria. To remediate the situation, the operators have supplemented the air inflow and added a lager amount of $FeCl_3$ that has lead to the compliance with the Romanian standards.

Table 1. Influent and effluent characteristics at the studied WWTP.

Day	INFLUENT				SL.	$FeCl_3$	EFFLUENT				
	CCO-Cr	CBO5	N-total	P total	SVI		CCO-Cr	CBO5	NH4	total-N	P total
	mg O2/l	mg O2/l	mg/l	mg/l	mL/g	Kg	mg/l	mg/l	mg/l	mg/l	mg/l
Prescribed values	500	300	-	5	-	-	125	25	2	10	1
25.09.2014	510	230	44.03	3.8	85.83	85	9.6	5	0.11	9	0.9
09.10.2014	365	232.3	51	4.5	81.96	58	19.2	6	0.01	9.5	0.85
29.01.2015	326	220	49.38	3.8	129.9	228	19.3	6	1.15	9	0.9

As it may be seen from this table, the values are within the admitted limits, this proving that the addition of $FeCl_3$ improves the sludge quality and sedimentation capacity.

Conclusion

The addition of $FeCl_3$, when water temperature is low and the organic load is high is beneficial for the settling capacity of the sludge and helps complying with the national norms concerning water discharge after treatment, as the case of the studied treatment plant.

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BIOACCUMULATION OF HEAVY METALS IN MAIZE FROM COPŞA MICĂ AREA

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Abstract.
The risk assessment of pollution with heavy metals involves in the exposure assessment phase the application of bioaccumulation models. Here we report a comparison between general models from the literature applied in the case of Copşa Mică area and a statistical model developed for a single species with local data. There are important differences in the values predicted, and as a result between the distances from the contamination source included in different risk categories.

Keywords. Heavy metals, bioaccumulation, maize

Introduction

A large area of agriculture land near Copşa Mică is cultivated with maize, both in the floodplain and on the hills of the nearby valleys. This provided the opportunity to extensively sample a single species along contamination and geomorphologic gradients. The objective was to build a statistical bioaccumulation model for this species in the area and to compare its performance with other. We present a summary of the results, their extended form is under preparation for a journal.

Methods

The research program and the range of measured concentration have been presented some time ago at a conference held in Sibiu (Iordache et al. 2010). In short, the sampling was organized by 12 transects crossing laterally the floodplain at different distances, and other transects located on Valea Viilor slopes (details are on-line in the scientific reports of the funding project (at http://metagro.cesec.ro/index.php?option=com_content&task=view&id=13&Itemid=27). The data set was used to build statistical models of bioaccumulation and the results were compared with several models from the literature (table 1). Another statistical model was built for the prediction of metals in soil in function of the distance from pollution source, and the predictions were used as input in the bioaccumulation model in order to calculate the distance at which the metals in plants decrease below the legal threshold.

Table 1 Statistical models of bioaccumulation in plants selected from the literature.

		lg **Cd** în frunze (ax + b)			lg **Cu** (ax + b)		lg **Pb** (ax+b)		lg **Zn** (ax+b)		
		a	b		a	b	a	b	a	b	
Bechtel- Jacobs (1998, cited by Efroymson et al. 2001)	x= lg concentration in soil	0.546	-0.207		0.394	0.291	0.561	-0.577	0.555	0.684	
Vrânceanu et al. (2010) Field data from various plant species, Copșa Mică	x= lg concentration in soil	0.7344	0.3086		0.4503	0.414	0.8393	0.3183	0.653	0.7291	
Vrânceanu și colab. (2010) Experimental data, maize leaves, spiked Copșa Mică soil	x= lg concentration in soil	1.334	-1.116						0.549	0.653	
		lg **Cd** în frunze (ax+by+c)			lg **Cu** (ax+b)		lg **Pb** (ax+b)		lg **Zn** (ax+by+c)		
		a	b	c	a	b	a	b	a	b	c
Efroymson și colab. (2004) Field data from various plant species	x= lg concentration in soil y= pH	0.53		-0.3	0.47	0.57	0.67	-1.09	0.33		1.89
Efroymson și colab. (2001) Field data from various plant species,	x= lg concentration in soil y=pH	0.564	-0.27	1.152	0.394	0.669	0.561	-1.328	0.64	-0.77	2.362

Results

Only for Pb from the the floodplain there was a good correlation between the total concentrations in soil and those in the leaves of plants (table 2). The heterogeneity of soil in Valea Viilor area was much larger leading to not significant correlations. Consequently we take the case of Pb and the floodplain. Figure 1 show the decrease of Pb concentrations in soil with the distance from the source, and figure 2 presents the selected correlation.

Table 2 Results of the regressions for the bioaccumulation of polluting metals in maize leaves. All concentrations are log transformed for normalisation.

y = ax + b		Târnava floodplain					Valea Viilor				
Dependent variable (y)	Independent variable (x)	r	p	a	b	N	r	p	a	b	N
Metal in leaves	Metal in soil										
Cd	Cd	0.347	0.044	0.399	-0.017	34	-0.085	NS	-0.118	0.425	6
Cu	Cu	0.057	NS	0.068	0.989	56	0.006	NS	0.007	1.026	39
Pb	Pb	0.667	0.000	0.782	-1.015	56	0.153	NS	0.123	0.311	33
Zn	Zn	0.319	0.016	0.286	1.431	56	0.187	NS	0.112	1.628	39

Figure 1 The decrease of Pb in the floodplain soil with the distance from the source.

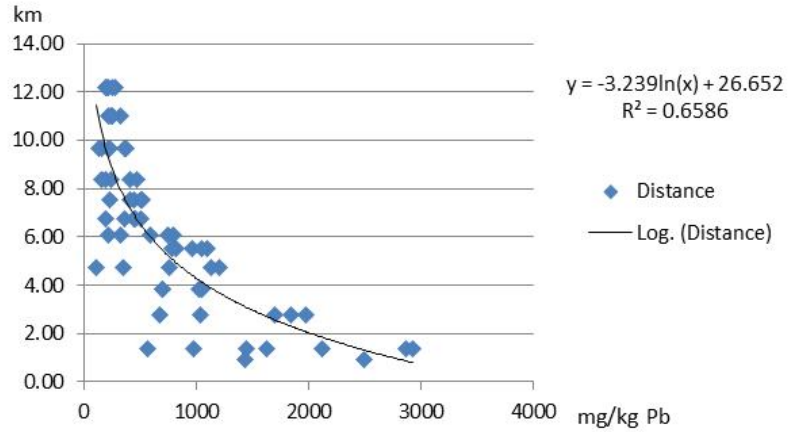
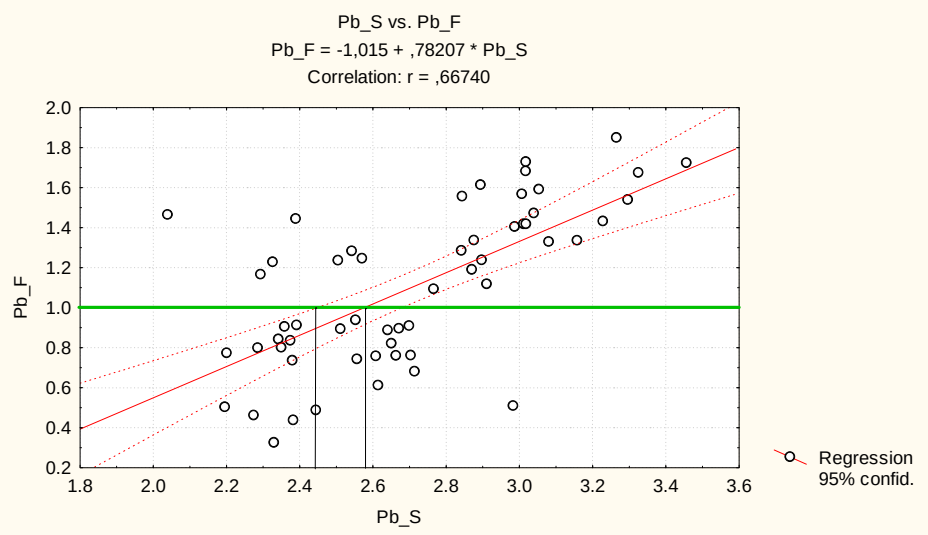


Figure 2 Correlation between Pb in leaves and in soil. One can notice that due to the large confidence interval (95%) the acceptable limit of Pb in leaves is reached at 276 mg/kg Pb in soil instead of the value of 380 mg/kg calculated with the linear equation.



Legend: Pb_F is lg (Pb in leaves), Pb_S is lg (Pb in soil).

The acceptable limit of Pb in soil resulted from our model and from the models presented in table 1 were introduced in the equation of figure 1 in order to calculate the distance from the source at which this soil contamination is smaller than this threshold. Results as summarized in table 3. One can see that there are important differences between the distances. The fact the the model was developed in Copșa Mică area (Vrânceanu et al. 2010) did not improve the situation because it was not based on maize samples, but on a mixture of species.

Conclusions

Statistical model from the literature are not appropriate for the risk assessment when they are developed in other sites or are based on samples from many species. For one species and one area a specific statistical model is needed for the exposure assessment.

Table 3 **Comparison between the critical distance from pollution source calculated with models from literature and with our model.**

	lg Pb in soil, threshold	mg/kg Pb in soil, threshold	Critical distance
Bechtel Jacobs (1998)	2.811	647	5.69
Vrânceanu and colab. (2010)	0.812	6.490	20.59
Efroymson and colab. (2004)	3.119	1316	3.39
Efroymson and colab. (2001)	4.150	14117	negative value
Cojoc and Iordache (2011)	2.576	377	7.44

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SEA BUCKTHORN (*Hippophae rhamnoides* L.), BASIC COMPONENT OF SPECIAL PLANTATIONS IN MOUNTAIN AND SUB-MOUNTAIN AREA

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Abstract
Special plantations may be established with blueberry, raspberry, sea buckthorn, hazel etc., belonging to the spontaneous flora of mountain and sub-mountain area in our country, but which may be cultivated also in plantations, by using improved species with higher nutritional qualities and rich productions. One of the most valuable fruits of this category is the sea buckthorn (Hippophae rhamnoides L.), and one of the products obtained from sea buckthorn fruits is the fresh pressed juice. The main issue consists in the possibility to extend the shelf life of sea buckthorn fruits in order to obtain the fresh juice at the time of producing derivative preparations. Low temperatures created by means of artificial cold may extend the shelf life of sea buckthorn fruits. Artificial cooling is used for keeping fruits both for a short period (days) and for a longer period (months).

Keywords: sea buckthorn, preservation, freezing, quality, sea buckthorn juice

Introduction

For our country, where the mountain area extends on 74,000 km², with huge reserves of biodiversity and important agro-economic and cultural traditions, particularly in rural environment, the need to develop a modern and efficient agriculture in the mountain and sub-mountain area is a must (Rey, 2014).

Romanian mountain area, with a surface of over 3.2 million hectares of poor quality agricultural lands (3^{rd} or 4^{th} quality class), through the 850,000 small and medium farms, obtains top quality products providing the basic food for approximately 3 million inhabitants of the rural and urban area, production which may be doubled by a better management (Rey, 2014).

Special plantations are various cultures of trees or bushes established by the improvement of some spontaneous plant species. Population increase and food products diversification led to the need to obtain larger and larger productions.

In many world areas, special plantations develop on large extent, representing an alternative to the ordinary monocultures of classical agriculture. The cultivation of these special plantations is usually a family business, on smaller areas, but which provides a reasonable income for the entire family.

One of the plants cultivated in special plantations is sea buckthorn (*Hippophae rhamnoides* L.). Due to the high perishability and low preservation time of its fresh fruits, for obtaining top quality preparations during the entire year, it is necessary to store fruits for a longer time period.

The refrigeration procedure is used for the short-time fruits preservation, where fruits are preserved at temperatures closer to their freezing point (0^0 to 5^0 C), without water freezing (Radu, 1973).

The freezing procedure is used for fruits preservation on a longer time period. By this procedure, fruits are subject to certain very low temperatures (down to -20^0 C), so as the greatest part of water in fruits content is transformed into fine ice crystals.

Material and method

Among berries, sea buckthorn fruits are ones of the richest in nutrients and vitamins, fact that determined the study of their behavior under conditions of storage and preservation by freezing (Beveridge, T. et al., 1999).

The very rich content of bioactive elements of berries, from the spontaneous and culture flora, results in their increased perishability, which has lead to the finding of some methods for fruits' storage and preservation on a long period, in order to obtain processed products with nutritional values close to those of fresh fruits.

For our study, sea buckthorn cultivars of Şerpeni, Şerbăneşti and Sf. Gheorghe were used, cultivated in a plantation established in 2006 on a 7.50 ha area, in Roşia village, Sibiu County, belonging to NP PROD S.R.L. trading company.

Fruits were mixed from all cultivated species, in approximately equal ratios, their harvest being made in August, September and October.

The quality of sea buckthorn fruits stored and preserved by freezing is given by the value of the ratio between acidity level and contained sugar value. This work deals with the variation of this ratio under the conditions of sea buckthorn fruits storage and preservation by freezing, determining the acidity and sugar content from the juice obtained by pressing sea buckthorn fruits.

After harvesting at full maturity of top quality, fresh, healthy sea buckthorn fruits, certain treatments were applied, such as: sorting, cleaning and refrigeration at temperatures ranging between 0^0 and 6^0 C. were used.

Cleaning consisted in the removal of foreign bodies, leaves and branches. Sorting was made by removing unhealthy, damaged fruits not having the specific color of the species. Packaging was made in plastic bags dosed in equal quantities, then they were stored in refrigerated rooms (0^0-6^0 C), for the storage period.

Fruits freezing have been performed within 2 – 3 days after harvest, any extension of the preservation term leading to the product quality damage.

The study's main objectives have been the correlation of optimal freezing temperature and the acceptable preservation term according to the fruits behavior at freezing and the constant preservation of the value for the ratio between acidity and sugar quantity of the juice obtained from frozen fruits.

The temperature range of -18^0 up to -20^0 C was used for the freezing of sea buckthorn fruits for different preservation terms up to 360 days.

For defining the fruits behavior, determinations were periodically made on sugar and on the acidity of sea buckthorn juice obtained by the fruits storage after having been defrosted at time intervals of 90, 120, 270 and 360 days.

The analysis of the two components of sea buckthorn fruits juice was carried out according to the following methods:

- The determination of directly reducing sugar was made by means of Luff-Schorl iodometric method by titration in the presence of starch as indicator with sodium thiosulphate solution 0,1N, factor = 0.9524, using filtered sea buckthorn juice;
- The determination of sea buckthorn juice acidity was made by titration with NaOH solution 0,1N, factor = 1.0201, in the presence of phenolphthalein (filtered sea buckthorn juice was used thereto);

The analyses on sea buckthorn juice were performed in Larex laboratories in Sibiu.

Results and discussions

For defining the fruits behavior, analytical determinations were periodically made on the juice of fresh fruits, as well as frozen fruits stored for 60, 120, 180, 240, 300 and 360 days.

The sugar content decreases at the end of the shelf life by a ratio of 5 – 7 refractometric degrees. (fig.1)

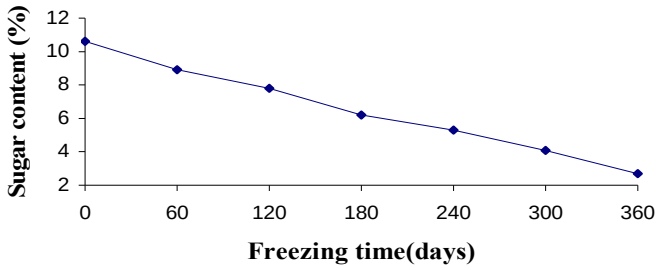

Figure 1. Variation of sugar content

The acidity of sea buckthorn fruits frozen and stored for 360 days decreases compared to fresh fruits acidity by 25%, having a slight increase at the middle of the shelf life.

Even if acidity decreases during preservation time, it does not reach limits influencing the product quality such as to lead at its damage. (fig. 2)

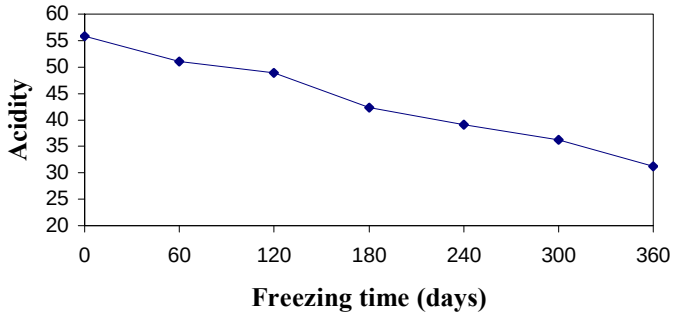

Figure 2. Variation of acidity content

Conclusions

1. The exploitation of fresh fruits is hindered by the fact that they are not consumed directly as fruit, only under the form of preparations or derived products. The sea buckthorn fruits storage and preservation by freezing offers the possibility to consume fresh products without preservatives.

2. The preliminary treatments applied in the freezing process (fruits' cleaning, sorting, their packaging in plastic bags, as well as their prior preservation in refrigerating rooms at temperatures ranging between 0^0 and 6^0 C) provided appropriate conditions for the freezing operation.

3. In order to obtain top quality sea buckthorn products, fruits are recommended to be frozen within 48 hours as of their harvest, to be stored and preserved at a freezing temperature of -18^0 C and for an acceptable preservation period of 360 days.

4. The value of the ratio between sugars and acidity remains approximately constant for a preservation period for frozen fruits of maximum 360 days, then a major increase of acidity value is recorded, leading to a major depreciation of sea buckthorn fruits.

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DISTRIBUTION OF HEAVY METALS AS RELATED TO SOIL PHYSICO-CHEMICAL CHARACTERISTICS

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Abstract
The main purpose of the present study was to determine the influence of soil physico-chemical characteristics on the mobility and distribution of certain heavy metals in contaminated soils. 30 soil samples from Copşa Mică area were collected on different depths and were analyzed by AAS for Pb, Zn, Cd, Ni, and Cu. The main physico-chemical characteristics believed to affect the heavy metals mobility in soil of the study area were examined such as: pH, texture DOC, CEC and electrical conductivity. Pearson's correlation coefficient was used in order to measure the degree of correlation between the logarithms of the metal data and the main properties of soils. The results of the study indicate that all of the studied heavy metals are concentrated on the surface soil and generally decreasing in the lower part of the soil, this due to their mobility and physical properties of soil.

Keywords: heavy metals, pollution, soil physico-chemical properties

Introduction:

Soil contamination by heavy metals is a spatial phenomenon, for this reason, in a site-specific management strategy, it is essential to understand the spatial distribution and the speciation and mobility of contaminants within the area. Determination of the total heavy metal content in soils is the main indicator of the contamination degree, but it is not sufficient for assessing all potential hazards [1-3].

The study of the heavy metals behaviour in the soils affected by the metallurgical industry has aimed the following: investigation of the distribution of the heavy metals in the soil profiles, contamination rate and heavy metals mobility in correlation with the physico-chemical characteristics of soils.

Material and methods:

The study area

For our experiments we have chosen 30 soil samples collected from 3 sites (plots) on different depths, near Copşa Mică (Figure 1), situated eastwards from the pollution source (SOMETRA S.A.) as presented in Table 1.

Figure1. Location map of the investigated area (Google Earth capture)

All plots are located near the National Road 335 on the right side of the road going from Sibiu to Medias.

Table 1. Experimental sites description

Site	Plots	GPS coordinates	Altitude (m)	Plots surface (m^2)	Distance from the pollution source (Km)
1	**P1**	46°6'45.73"N 24°14'12.54"E	301.7	2248	aprox. 1
2	**P2**	46°06'40.31"N 24°14'59.66"E	327.9	25416	aprox.1.5
3	**P3**	46°07'11.37"N 24°16'46.88"E	281.1	4873	aprox. 4

In order to identify the plots position on the map we have use the GPS coordinates collected in the field conditions from each plot using a mobile device (Trimble R6 GPS System), based on GPS technology. After establishing the delimitation points, the recorded surface perimeter was calculated for each plot separately (Table 1). Data collected from the field experiments were then transferred into computer using a specialized software in order to obtain ortho-photo superimposed cadastral plans, scale 1: 2000.

Studied plots are located within the administrative territorial unit of Copşa Mică town, Sibiu county, being identified with the cadastral numbers: 541 (Plot 1), 563/2 (Plot 2) and 831 (Plot 3).

From the point of view of the agricultural classes of use, these lands are classified as follows: Plot 1 and Plot 2 as arable lands and Plot 3 as pasture.

Figure 2. Cadastral map at the scale of 1:2000 (Plot 1)

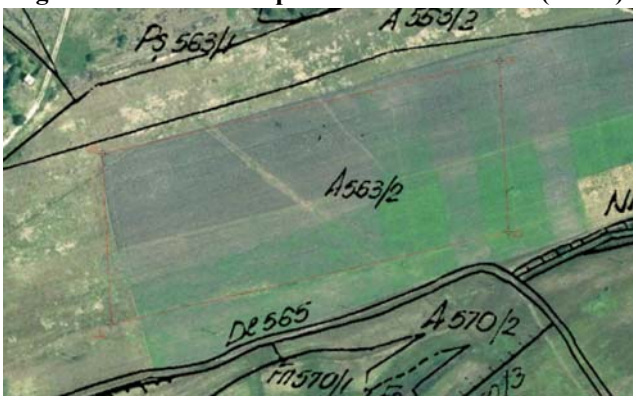

Figure 3. Cadastral map at the scale of 1:2000 (Plot 2)

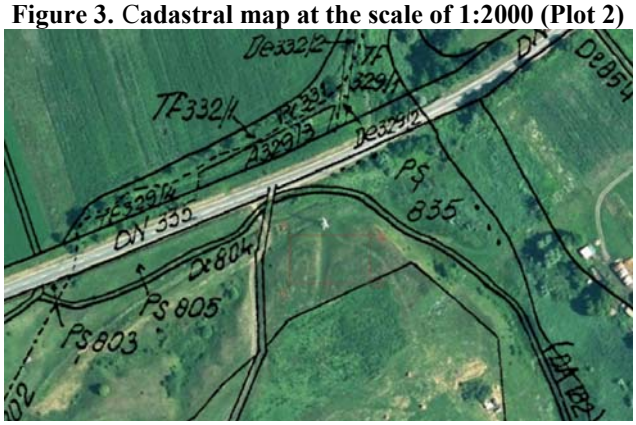

Figure 4. Cadastral map at the scale of 1:2000 (Plot 3)

Soil sampling and analysis

Soil samples collected form all experimental plots on different depths (0-20, 20-40 and 40-60 cm) were air dried and passed through a 2-mm stainless sieve and then the soil texture was determined by the pipette method. The pH and the EC were determined in water with a 1:5 (soil:water) ratio. Soil organic carbon and humus content were analyzed by using Walkley–Black procedure. Mobile forms of P and K were carried out by spectrophotometry

and flame spectrometry, in calcium acetate lactate (CAL) solution. The value of CEC was obtained by calculation from the exchangeable cations contents determined in 0.1 M $BaCl_2$ extraction (w/v= 1:20). Soil solution was obtained after centrifugation of soils which had been equilibrated with deionized water (100% WHC) for 24 h at 20 °C and analyzed for dissolved organic carbon (DOC) concentration using a Multi N/C analyzer. The total metal concentrations in soil sample were analyzed by AAS after digestion in aqua regia [4]. For quality assurance, replicate samples, blanks and standard soils were included in all analyses [5-6].

Results

The main physico-chemical characteristics of soil and the total content of heavy metals, calculated as the average of 3 individual determinations, are given in Table 2. In order to identify the degree of soils pollution, the total concentrations of Cd, Zn, Pb, Ni and Cu were compared with the references values in accordance Romanian Ordinance Ministry No. 756/1997 (Table 3).

Table 2. General soil characteristics and total metal concentrations of research sites

Soil Parameter	Unit	Plot 1			Plot 2			Plot 3		
		Depth (cm)								
		0-20	20-40	40-60	20-40	40-60	40-60	0-20	20-40	40-60
pH_{H20}	-	5.25	5.85	6.60	5.01	5.84	6.52	5.19	6.17	6.62
EC	µS/cm	72.30	59.00	86.70	81.20	58.60	58.70	76.60	44.20	36.10
Organic carbon	%	0.81	0.46	0.06	0.46	0.23	0.12	1.35	0.87	0.58
Humus	%	1.40	0.79	0.10	0.79	0.40	0.21	2.33	1.50	1.00
P_{-CAL}	mg kg	30.90	19.03	18.24	33.52	14.27	12.65	54.23	56.04	75.63
K_{-CAL}	mg/kg	68.63	115.20	103.28	89.48	121.23	84.82	84.81	143.96	105.31
DOC	mg/L	200.69	107.84	40.65	174.49	80.43	64.39	406.23	329.21	154.62
CEC	meq/ 100g	37.09	60.52	64.47	28.63	36.64	42.77	28.05	28.33	35.67
Zn	mg/kg	622.70	543.11	75.34	764.26	421.93	82.79	316.13	272.52	94.05
Cd	mg/kg	11.89	6.42	0.25	9.85	4.05	0.45	4.65	3.55	0.35
Pb	mg/kg	615.36	118.64	13.32	349.29	26.87	20.82	177.63	93.13	14.57
Ni	mg/kg	50.85	57.45	41.77	41.17	43.51	37.74	26.40	30.06	34.90
Cu	mg/kg	48.64	37.99	21.73	49.75	20.61	18.24	33.18	24.87	22.49

Table 3. References values for some heavy metals (total forms) concentration in soil (Romanian Ordinance Ministry No. 756/1997) [7].

Trace element (ppm)	Normal content	Alert threshold		Intervention threshold	
		Sensitive utilizations	Less sensitive utilizations type	Sensitive utilizations	Less sensitive utilizations type
Cd	1	3	5	5	10
Zn	100	300	700	600	1500
Pb	20	50	250	100	1000
Ni	20	75	200	150	500
Cu	20	100	250	200	500

The soil texture in all plots is a sandy loam, with clay contents ranging from 110 to 187 g/kg. The pH values are ranging from 5 up to 7, which suggests acidic to neutral conditions for all the soil samples. Also, high electrical conductivity values were in the found in Plot 1 and Plot 2, close to the former smelter with decreasing values in Plot 3. Cation exchange capacity (CEC) showed a variation, from 20 to 70 meq/100 g, reflecting a high buffer capacity of the soils. The highest values of organic matter were found in soil samples collected from Plot 3, at approximately 4 km eastwards from the pollution source of the investigated area, which reflects the former use of Plot 3 as a pasture.

The highest amounts of heavy metals were found in the upper horizons of soil profiles. The recorded total concentration range of each metal of concern (in the surface soil) was as follows: 4.65–11.89 mg kg^{-1} for Cd, 316.13–764.26 mg kg^{-1} for Zn, 177.63 –615.36 mg kg^{-1} for Pb, 26.4-50.85 mg kg^{-1} for Ni and 33.18-49.75 mg kg^{-1} for Cu. The total contents of Pb, Cd and Zn in the upper horizon are significantly higher than the Romanian limit values for soils (Table 3) in all 3 plots. The total content of Cu and Ni in the upper horizon is under the maximum allowable limit of 100 ppm for Cu and 75 ppm for Ni, but over the normal values (Figure 5), according to Romanian norms.

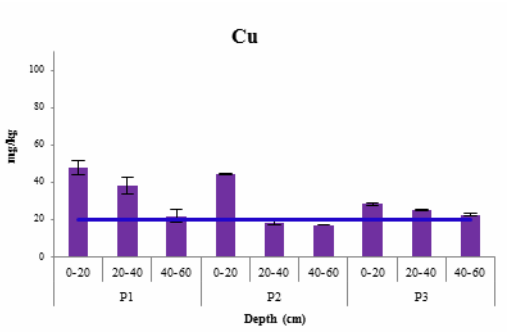

Figure 5. Cu variation in soil profile

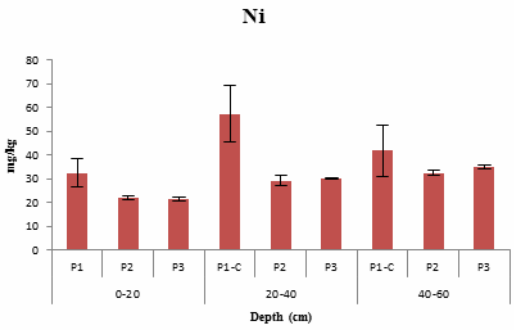

Figure 6. Ni variation in soil profile

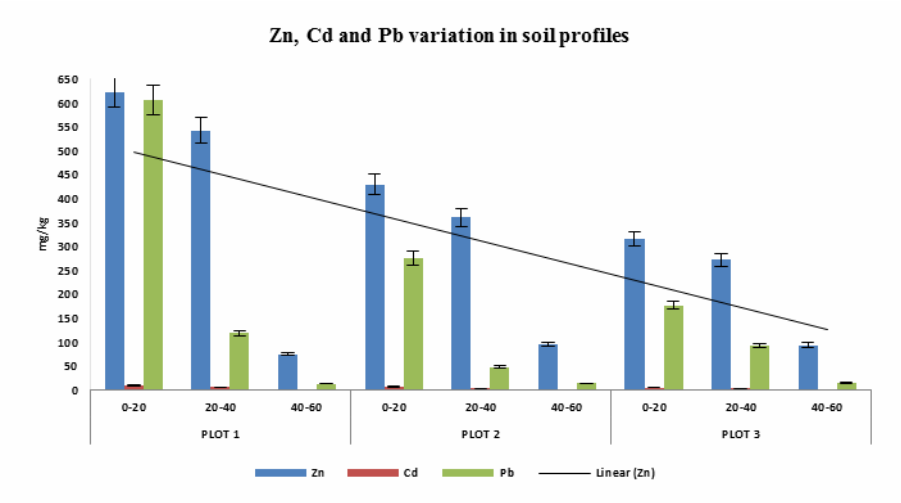

Figure 7. Variation of total content of Cd, Zn and Pb in soil profile (soil depth 0-20, 20-40, 40-60 cm)

Distribution of the analyzed metals in the area is influenced by the soils properties and generally decreases with depth. With increasing distance from the industrial source the contamination decreases and the concentration of metals are below the threshold for intervention but higher than background values, especially for Pb, Cd and Zn (Figure 7).

The relationships between the trace element concentrations in soils and soil physico-chemical properties were described using Pearson's correlation matrix (Table 4).

Table 4. Pearson's correlation calculated between metal concentration in the analyzed soils and soils physico-chemical characteristics (pH, humus and clay content). (n=37).

Pearson's correlation	Ni (ppm)	Cu (ppm)	Zn (ppm)	Cd (ppm)	Pb (ppm)	Clay %	pH	Humus %
Ni (ppm)	**1**							
Cu (ppm)	-.121	**1**						
Zn (ppm)	-.138	**.883****	**1**					
Cd (ppm)	-.291	**.907****	**.974****	**1**				
Pb (ppm)	**-.456****	**.881****	**.819****	**.905****	**1**			
Clay %	**.645****	-.098	-.280	**-.325***	-.310	**1**		
pH	**.726****	-.241	**-.349***	-.408*	-.330*	**.510****	**1**	
Humus %	**.721****	**.845****	**.833****	**.867****	**.856****	**-.435****	-.413*	**1**

* Significant at 0.05; ** Significant at 0.01

By calculating the Pearson's correlation coefficients it was found positive correlations ($p < 0.01$) between Zn, Cd, Pb and Cu and between these heavy metals and humus content in the soil. Moreover, it was observed that between the heavy metal content in soil and the

content of clay and soil pH exist negative correlations with a medium strength and less significant ($p < 0.05$).

Discussions

In terms of agrochemical characteristics it can be concluded that all studied soils are characterized by a sandy-loam texture, with an average of 72% sand, 12% clay and 16 % silt. Soil reaction varies from moderate to weak acid in the upper horizon and moderately acid to neutral in lower horizons. Total capacity of cation exchange is medium to high, in all analyzed soil profiles, reflecting a high buffer capacity of the soils. No measurable amounts of carbonates were found and organic carbon and humus contents are very low. Moreover, the degree of soil supply with nutrients (N, P and K) is low to medium

Copşa Mică soils are characterized by excessive pollution with heavy metals, especially with Cd, Zn and Pb which are to be found in the upper horizons of soil profiles. Cd, Zn and Pb total concentration in the surface horizons exceeds the intervention limits, which are, according to the Regulation No. 756/1997, 1 mg kg^{-1} for Cd, 100mg kg^{-1} for Zn and 20 mg kg^{-1} for Pb, respectively. The results obtained for Cu and Ni illustrate that these heavy metals are to be found under the maximum allowable limit, but over the normal values. Distribution of Cd, Zn, Pb, Cu and Ni in the studied area is significant ($p < 0.01$) influenced by the humus content and the less influenced by the pH or clay content, except for Ni.

Increasing the distance from the industrial source the pollution, intensity decreases and the concentration of metals are below the threshold for intervention but higher than normal values. The total content of the studied heavy metals generally decreases with depth.

Conclusions

The laboratory studies conducted to find the soil characteristics which are correlated with the distribution of heavy metals in soil emphasized the differences between the behavior of the heavy metals found in the upper horizons compared to the heavy metals found in the lower horizons. Correlation coefficients have shown that exist a positive correlation between Zn, Cd, Pb and Cu content in soil profile and between these heavy metals and humus content of the soil.

Pearson's correlation coefficients which have been used in this study are statistical tools which indicates the degree of association or link between metals and soil physico-chemical properties without indicating the cause, more soils analyses should be conducted in order to understand better the heavy metals behaviour in soil.

Plots 1 and 2, situated closer to the former smelter, are most affected by the historical contamination caused by the industrial activities in the area, Plot 3, situated at approximately 4 km from the source, being less affected by heavy metal pollution. The results confirm our previous studies [8], which highlighted the fact that, as the distance from the pollution source grow, the metal concentration in the soil decreases.

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INTRASPECIFIC COMPETITION AND ITS IMPLICATIONS FOR PLANT BREEDING PROGRAMME

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Abstract
Intraspecific competition effects in a diploid red clover synthetic cultivar were analysed based on interference phenomena among component populations, expressed by the value of quantitative characters.
Significant changes due to intraspecific competition were noticed at population level caused by the following possible situations: reciprocal or unilateral shortcomings; reciprocal or unilateral advantages; indifference reaction.When breeding synthetics it is necessary to know the general and specific reaction of populations to the competition effects occurring at the level of single or combined populations in order to obtain higher yield.

Key words: red clover, variability, phenophases

Introduction

The researches conducted by DEMARLY et al. (1964) and GUY (1965) on alfalfa, TAYLOR and KENDALL (1965), SAVATTI jr. (1983), SAVATTI et al. (2004) on red clover, and HORIUKI YAMADA (1960) on barley, SAKAY (1963) on rice, SIPOS et al. (1981) on various agricultural plants, showed that considering various populations, similarity of necessities seems to represent an essential competition factor, but depending on population density, percentage of perishing plants correlates with it. Regarding the populations where the density has an appropriate level, this constitutes a positive element for the population as a whole, in this case cooperation reactions occurring between biotypes components.

In the context of the above mentioned, in plant breeding it is extremely important to know the intraspecific competition, which may take place in a synthetic variety, the manifestation of interference phenomena between component populations, expressed by the value of quantitative characters.

Material and Methods

The studied biological material consisted in four populations, components of a synthetic variety of diploid red clover, denoted by A, B, C, D. The four populations vary both in their origin and in the degree of congeniality.

The working method consisted in associating the A, B, C, D populations in all possible combinations in pairs, so that they can track the effects of the intraspecific competition, compared to that shown in the single population.

Our 16 variant experiment; 12 in mixed populations and four on single populations, repeated four times, with a 1 m^2 of harvestable area for each parcel, was conducted based on the assumption of SAKAY (1957), according to which "the competitive pressure is constant when the distance between plants is constant". Row spacing was 25 cm. As the used populations do not display any genetic markers, it was very difficult to determine the populations to which belonged each individual, during its vegetative and generative development. In order to individualize the plants pertaining to a certain population a 1m long wooden perch, marked every two centimetres, was used. In a given combination, the seeds of a population, water impregnated to ensure a homogenous germination and emergence, were disposed between the perch marks, while the seeds of the other population were arranged between signs. Thus, each population is recognized throughout the phenophases of their development. On single plots, the seeds of a population were all sown at 1 cm distance.

During the growing season, the first year of growing, the following quantitative characters were monitored:

- number of seedlings in the first true leaf stage;
- total number of leaves / plant, 40 days from sowing;
- plant height in the florescence phase;
- total number of stems / plant;
- total number of blossoming stems / plant;
- the number of inflorescences / plant;
- number of flowers for a medium inflorescence;
- total amount of dry matter / plant
- number of seeds / plant.

Each of the studied characters includes numerical series from the 16 seeded variants, resulting from combining two populations, including individual populations.

For example: the mixture of A_B populations shows the value of population A character in the presence of the B population. A is considered as the population generator while B as its competitor. B_A indicates the reverse situation of the above shown situation.

The statistical interpretation of quantitative characters values was done according to the method described by CHALBI (1967).

Results and discussion

The particular analysis of every specified quantitative character was carried out. The average comparison method, applied to various versions, emphasized significant differences between the average values of a given population, upon the changing of its competitor.

All observed characters highlight the emergence of significant changes due to the competition effect. It should be noted, however, that for a given population not all characters are necessarily affected and nor with the same intensity.

The behaviour of the changes occurred in a given population for various quantitative characters show that, on average, the competition modifies morphological characters in the same direction.

The data in Table 1 illustrates the situation for the various studied characters, by the correlation coefficients occurring between morphologic characters.

Table 1

Correlations among different morphological characters of red clover populations (A, B, C, D)

Morphological characters	Height	Dry matter	Stems	Total blossoming stems	Inflorescences
Height	-	+0,959	+0,465	+0,880	+0,810
Dry matter		-	+0,805	+0,975	+0,940
Total number of stems			-	+0,865	+0,789
Total number of blossoming stems				-	+0,975
Inflorescences					-

DL 5% = 0,495

In order to emphasize the intraspecific competition aspects, the number of inflorescences / plant dynamics will be shown. We focused on this character, considering that this phenophase displays, at least theoretically, the specific stage, regarding both the accomplishment of the vegetative phase as well as the beginning of the generative one.

The results for each singular population are presented in Figure 1.

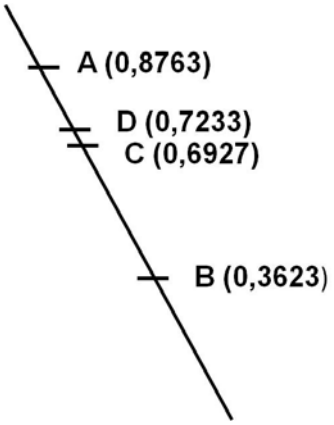

Fig.1. Log_{10} (number of inflorescences + I of single populations)

As mentioned, the four populations differ in their genetic origin and structure. It is therefore normal to find differences between them, presumably due to the inequality in the action of the genes controlling the blossoming and precocity ability.

Figure 2 shows the interaction of populations and the changes caused by various competitors. We mention that the smallest significant difference for DL 5% is 0.1719.

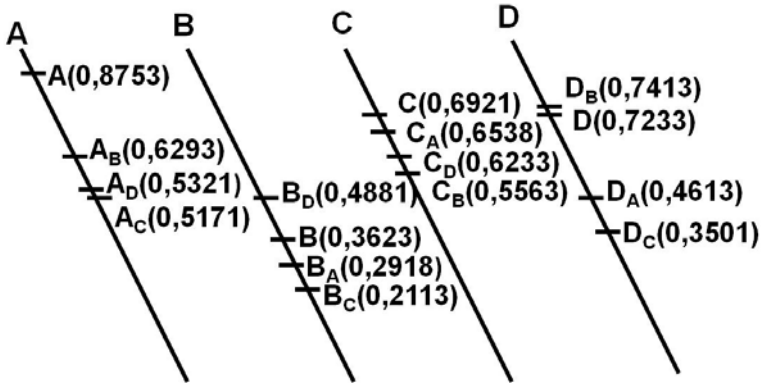

Fig.2. Number of inflorescences for each population changed into Log_{10} (number of inflorescences + I)

The presented results highlight some particularly revealing aspects as result of the competition between populations, changes whose importance and nature depend on the competitor structure.

The competition triggered changes may be statistically classified according to three possible situations for the paired populations:

a) *mutual or unilateral disadvantage*: A population in the presence of D population and D population in the presence of the A population, respectively displays changes in the inflorescence number, due to mutual competition, in relation to the number of inflorescences displayed by the singular A and D populations; the A_C combination manifests a unilaterally disadvantage reaction;

b) *the mutual or unilateral advantage*: B_D and D_B display higher values of the inflorescence number / plant that the singular B and D populations. In this situation, mutual benefit is less prominent as compared to those occurred in other quantitative characters, which will be shown below; the C_A manifests a unilateral advantage reaction;

c) *the indifference reaction*: the C population presents the same reaction in all cases, for all competition combinations.

In order to illustrate these three aspects, table 2 presents the data demonstrating the above regarding the three characters: number of plants after 40 days, plant height and inflorescence number.

The mutual disadvantage phenomenon is observed for the A_B and B_A combinations as well as unilaterally for the A_C.

The unilateral advantage phenomenon occurs for the C_A combination and of slightly pronounced indifference for the B_C combination.

Table 2

Relative changes for the A, B, C, D populations reported to single population

	A_B	A_C	A_D	B_A	B_C	B_D	C_A	C_B	C_D	D_A	D_B	D_C
No of leaves after 40 days	-0.12	0.15	-0.04	-0.22	-0.14	-0.14	-0.14	-0.05	-0.05	-0.12	-0.11	-0.12
Plants height	-0.15	-0.11	-0.14	-0.08	-0.03	-0.15	-0.04	-0.10	-0.05	-0.04	-0.06	-0.05
No of inflorescences	-0.63	-0.54	-0.65	-0.48	+0.12	-0.59	+0.67	-0.25	-0.50	-0.02	-0.14	-0.29

Conclusions

One could hypothesize that the comparison of a population in a competition is reflected in the adaptation of its own genetic formulas to the competition phenomenon acting on different phenophases of the development.

An already established fact is that the "variability reserve" in an allogamous population ensures a better adaptability to the intra- and inter population competition conditions. These are genetic formulas most able to withstand the effect of the selection pressure to reach reproductive age, while less adapted populations will be eliminated. Theoretically, it is likely that the composition of a red clover genetic population, in a particular situation, to be preserved or to evolve due to a complex system of advantages and disadvantages regarding the competition response types.

Plants escaping the elimination action, due to intraspecific competition in the early growth stages undergo significant changes, as highlighted in the presented results. It is obvious that a population losing, due to competition, 50% of the potential floral organs, it shows outside any competition, has undeniably, a diminished role, in the same proportion during reproduction.

The adaptive capacity due to the diversity of genetic formulas composing a red clover population, on the one hand, and the harmony or contradiction that may result from the association of two populations must be explained by biological interactions occurring within each population. Knowledge of these phenomena has a particular value regarding improvement, as to achieve superior agronomic progress by creating a variety knowing the general and specific capacities of each constituent in the competition process occurring within singular or combined populations.

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BIOSAFETY ISSUES FOR ROMANIA - A REVIEW

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Abstract
The scope of this article is to review biosafety capacity for Romania facing the accession into the European Union. The article is revealing important procedures development and measures implementation for ensuring that the GM soybean is no longer cultivated in Romania before the 1st of January 2007 as this is the date of Romania accession into the European Union. Romania developed a competent model for capacity building in the domain of inspection and control for ensuring the traceability of the GM soybean which today is continuing to function for the successful implementation of the European biosafety framework.

Keywords: Biosafety, GM soybean, capacity building, Romania

The Ministry of Environment in Romania is designated as the competent authority for issuing import permits and approvals regarding deliberately release into environment and placing on the market of GMOs for cultivation/commercialization and also for field testing activities, first time complying with the Law 214/2002 which was transposing the draft form of the Directive 2001/18/EC, for the year 2006. By now this regulatory framework is repealed and the same procedures are set out by the law 247/2009 for approving the Governmental Emergency Ordinance 43/2007 for fully transposing the Directive 2001/18/EC (Antofie and Baz, 2006).

In 2006 Romania approved the import of 1449,746 t *seeds* of genetically modified soybean –GM soybean (9 cultivars) for placing on the market and approved the placing on the market of other *2,336 t seeds* of GM soybean certified in Romania (6 cultivars). Officially, Romania placed on the market a total GM soybean quantity of 3,785.746 t according to official records. Based on these figures Romania was really interested in cultivating GM soybean (Brooks and Barfoot 2012). For 2006 also, the Ministry of Environment approved the import of 0.548 t of GM soybean seeds for field testing according to the procedures laydown in the Directive 18/2001. In this regard three international private companies were applying notifications for this scope.

Before 2007 Romania was in the position to remove from the market place the entire GM soybean for cultivation or as seeds because in the European Union this GMP was not

approved yet. Thus, the Ministry of Environment in collaboration with other authorities started procedures to be sure that all GM soybean produced during 2006 will be removed from the market until 1 January 2007, when Romania will no longer cultivate GM soybean cultivars.

Current situation of GM soybean cultivation and farmers

According to the Romanian officials the entire surface cultivated in Romania with GM soybean recorded for 1 July 2006 was 118,000.00 ha and were registered 1080 farmers (Otiman et al 2008). Following the inspection and control activities of all involved authorities, developed during the summer of 2006, it was considered that in Romania it was cultivated a surface area of 127,945.00 ha and have been registered 1153 farmers. The inspection and control activities based on a specific collaboration Protocol among all involved authorities revealed that there existed at least 151 unauthorized farmers which are cultivating 4,000.00 ha. In the latest case these farmers were under monitoring and traceability procedures, in order to ensure traceability until harvesting and further for storing and also for ensuring that in 2007 on the Romanian territory will be no longer GM soybean saved seed for cultivation.

Romanian integrated activity for inspection and control regarding GM soybean

Biosafety inspection and control activities, and particularly regarding GM crops, were accomplished by the direct involvement of four authorities and also today. In order to avoid any overlaps regarding responsibilities, it was decided that an inter-institutional Protocol no 3357 between: [1] National Environment Guard (belonging to the Ministry of Environment), [2] Ministry of Agriculture, [3] National Sanitary Veterinary and Food Safety Authority and [4] National Consumer Protection Authority. This Protocol is also today grounding especially responsibilities including the collaboration procedures for inspection and control activities. Each authority will design its own inspection and control plan, according to the requirement stated into the Protocol, the communication and exchange of information will be on a permanent basis and revised when it is the case.

Moreover the National Environment Guard is collaborating with the Biosafety Inspection Body belonging to the Ministry of Agriculture, as well as with the National Sanitary Veterinary and Food Safety Authority Regarding for control and inspection activities related to GMP. This collaboration was set up in 2006 until at the county level for data transfer regarding the GM soybean yield, delivered quantities to the beneficiaries, storing in

warehouses (yield undelivered for processing) until their complete disposal. Also, according to the Protocol, there were realized joint inspections in order to check the culture authorization situation for GM soybean and also for track infringements regarding the authorization. Today the same Protocol is applied for inspecting the current GMP already approved for being placed on the market or for field tests.

Contributions of the National Environment Guard (NEG)

The National Environment Guard (NEG) is the competent authority in charge with inspection and control activities under the Ministry of Environment. For the entire year 2006, NEG accomplished multiple actions of inspection and control of farmers cultivating GM soybean, in collaboration with the Inspection Body of the Ministry of Agriculture. Their task was to verify the conformity of documents, waste management and applied biosafety measures, in accordance to the Directive 18/2001.

At that moment, Ministry of Environment holds the centralized situation of GM soybean transmitted by the Ministry of Agriculture, which received the situation from the county level. These records includes surface area, locations, contact addresses and farmer list and is grounding the data base of the National Register regarding the GM soybean farmers from Romania, which is managed by the Ministry of Agriculture.

Contributions of the Ministry of Agriculture

The Ministry of Agriculture was the competent authority for establishing the National Register of GM soybean farmers, which was public open to all involved authorities into inspection and control activities. Under this occasion the Ministry of Agriculture established the Biosafety Inspection Body which participated and traced the entire GM soybean assessment activity, harvesting, transport, yield storage and delivery. In order to develop an uniform and complying activity, this inspection body was trained for this special purpose in 2006. The Biosafety Inspection Body established files for each production unit which are comprising: cadastral maps, authorization documents, minutes of the controls, evaluation forms for yields and harvesting, locations for warehouses, etc. The Institute for Cultivar Testing and Registering, was and it is responsible with cultivar registration for Romania, and was in charge for erasing all GM soybean cultivars which were no longer accepted for the UE, starting with the 1st of January 2007. Still, no conservation measures were in place for continuing the maintenance of the GM soybean in the public research.

Labeling GMO or products containing GMO on the food chain from the producers to the retailers (excepting the retailers) started in 2006, complying with the Governmental Decision no 137/2006, and during the public meetings organized by the Ministry of Agriculture for the GM soybean case, the farmers were informed about that Government decision of forbidding further GM soybean cultivation in Romania after 1 January 2007. For infringements situations the Ministry of Agriculture are asking NEG for applying the law.

Contributions of the National Sanitary-Veterinary and Food Safety Authority

The National Sanitary-Veterinary and Food Safety Authority is the competent authority for inspection and control activities belonging to the entire food/feed chain from the producers to the retailers excepting primary producers and retailers.

Between 1 January and 31 August 2006, the National Sanitary Veterinary and Food Safety organized over one thousands control actions regarding the GM soybean traceability. Starting with July 2006 the first control action was developed and it referred labeling and traceability for food/ feed. For the same period almost 300 control actions took place and they applied the sampling procedures at the county level.

The National Sanitary Veterinary and Food Safety Authority organized training sessions for their own specialized personal, focused on EU legislation and practical aspects such as GMO sampling and detection methods.

The National Sanitary Veterinary and Food Safety Authority established the Molecular Biology and Genetically Modified Organism Unit within the Institute of Diagnostic and Animal Health designated as the GMO Laboratory. This unit followed a serial of procedures required for the ISO 17035 accreditation (kits- purchasing analyze kits and references materials, it was prepared the required documentation, there were accomplished all steps for method validation by using 200 samples from the territory and it was finalized RENAR audit). This laboratory will be fully operational during October 2006 and will serve all control activities. For infringements situations NSVFSA are asking NEG for applying the law.

Contributions of the National Consumer Protection Authority

The National Consumer Protection Authority is the competent authority in charge with retailers control, regarding the proper labeling and for ensuring the last ring of the food chain regarding traceability: from the producers to the consumers. It will elaborate for the forth trimester of this year their own inspection and control plan of the retailers regarding GM

soybean. For environment infringement situations NSVFSA are asking NEG for applying the law.

Public participation in decision making process

The Ministry of Environment has published all notifications regarding GM soybean on the website www.mmediu.ro and through the Ministerial Press Office were published articles into the mass media according to procedures presented in the Directive 18/2001. The Ministry of Agriculture has initiated information campaigns of the GM soybean farmers by sending them personalized letters and by posting announcements in all local official places in order to make sure that all citizens are aware that for 2007 GM soybean cultivation is banned in Romania and on the other hand they informed farmers that governmental subsidiaries of 200 Euros/ha will be handed to farmers if they will cultivate conventional soybean.

Future soybean cultivation in Romania

Starting with 1 January 2007 the GM soybean cultivation is banned for the Romanian territory up to the moment when at the EU level the GM soybean will be granted for cultivation.

Perspectives

The Ministry of Environment will develop further the legislative and capacity building, in order to comply with the EU requirements.

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STATISTICAL METHOD USED IN MORPHOLOGY DIFFERENCES OF *CENTAUREA STOEBE* SPECIES

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Abstract
Centaurea stoebe specie is known in Europe with two ploidy levels (2x and 4x) but the differences between these two cytotypes are hardly recognizable in wild flora. In order to achieve the goal, regarding the identification and promotion of Centaurea species from spontaneous flora of Transylvania, the aims of this study were related the taxa identification based on statistical packages offered by current statistical methods. Morphological differences of these species are well differentiated by statistical methods and the accuracy of the results is completed by alternative using of ordination and classification methods. Highlighting of the discriminative characters through statistical tests facilitates the recognition work of both cytotypes. Combining the morphological differences, geographical distribution and European studies could make a recognizing diagnosis of two ploidy levels. So the two types are different in this research, after inflorescence characters (length-IL and width-IW). For convenience to determine C. stoebe cytotypes was adopted a name for each ploidy levels, namely C. stoebe subsp stoebe. (2x) and C. stoebe subsp. micranthos (4x), according to European studies too.

Key words. PAST-software, classification and ordination methods, *Centaurea stoebe* cytotypes, diploid, tetraploid.

Introduction

Studying the species of *Centaurea* genus became a necessity in systematically and economic recovery terms. The majority of taxonomies issues found in this genus were caused by unclear morphological differences, lack of a correlation between morphological variation and geographical distribution, respectively. At a national level, there are few morphological studies on this genus, most of them were made by Prodan and Nyárády (1964) [10, 11]. The results were taken by the researchers, perpetuating the information regarding some peculiar characters [17].

The discussed topic presents a great importance for biodiversity because these native species are the genetic resource for taxonomy, medicine and floriculture. Besides the promoting of native species to enrich the cultivated assortment present an importance for ecological balance insides of habitats. Another important aspect for economical valorization is the production, transportation and storage costs, which are lower for native species. Furthermore, native species present a high adaptability for environmental changes and greater resistance to pests and diseases.

The attention of this topic is headed at species with polymorphic individuals due of natural hybridisation or different number of chromosomes, the reason of determination problems [14, 15, 16, 17].

In wild flora there are two cytotypes of *C. stoebe* (diploid 2x and tetraploid 4x), which are similar from morphological point of view. Although the both ploidy levels were studied at European level, in Romania the inquiries on this species are reduced. In Romania, the C. stoebe has two varieties: stoebe şi coziensis (Nyar.) Soó and the other species from this subgenus (C. micranthos Gmel.) are treated as independent taxa [2, 13].

Material and method

*Centaurea stoeb*e (2x, 4x) was monitored from morphological point of view in different population: Porumbacu Valley (SB), Scărişoara (AB) and Turzii Gorge(CJ) but also on herbarium vouchers [17,18] (Foto 1).

Photo 1. *Centaurea stoebe* from Porumbacu Valley population

For each, sample were made different measurements which were grouped in quantitative traits 16 characters) based on length-L and width-W of measured organs and 4 ratio recorded on 32 individuals. The characters were named after an initial letter of measured plant side (I-inflorescence, M-middle bracts, B-bracts, L-leaf, S-steam, A-achene and P-pappus). Thereby were tracked the morphological traits of steam (ST, SB), inflorescence (IN, IW, IL, ILW), leaf (LW, LL, LLW), middle bracts (MW, ML, MLW, BW, BL, FL, FN) and achene (AL, AW, PL, ALW).

All measurements were made on fully development plants with an undamaged terminal flower. The most measurements were made on the bracts, appendices and achenes (Foto 2). The observations and measurements were made on the field and in the laboratory. For statistical processed of morphological data were computed with ordination and

classification methods in PAST software-Paleontological Statistics. 2.15 [4, 5]. Furthermore, for comparative observations were used the vouchers herbarium from Natural History Museum Sibiu with some interventions of redetermination [17].

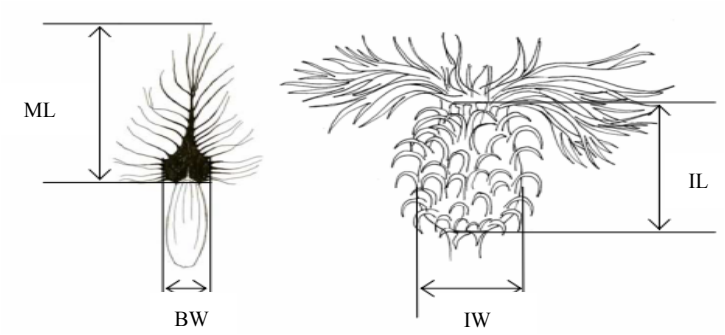

Photo 2. Morphological chracters of *Centaurea* measured on the midlle involucral bract (ML, BW) and inflorescence (IW, IL)

Results and discussions

It is known that the Romanian diagnoses of C. stoebe şi C. micranthos, make it impossible to distinguish them, such being the case the morphological characters were statistical analyzed, to assess any morphological differences. For each sample there were computed with PAST package the univariate indices (mean, standard error, deviation, median percentile and skewness distribution) [1, 4, 5, 12, 17].

The normal distribution of each character from data set was tested with Shapiro-Wilk (W) test (Tab. 1). For this test, the null hypothesis (H_0)- the sample was taken from a population with a normal distribution. If the given (p) is less than alpha level (0.05), then the null hypothesis can be rejected. The deviated value from normal distribution was transformed with logarithm function. For characters: SB, IN, LW, MW, ML, BL, AW, PL, the p-value were less than 0.05 and were transformed with PAST package.

Before the statistical analysis of morphological characters was computed the correlation matrix *Pearson* (r_{xy}) for each pair. The algorithm of the correlation matrix used the correlation index - *Pearson* and the signification were calculated with *t test* for n-2 freedoms degrees. From linear correlation table can see that there is not any significant correlation of morphological characters, all values are under threshold by ±0.95 (Tab. 2).

The highest value of correlation matrix (r-0.87) is recorded between MW and MLW because the second character (ratio between length and width of middle bracts) is derived from the first (width of middle bracts). After testing the correlation of morphological pairs, all characters were included in further analysis.

Table 1. Normal distribution matrix of *C. stoebe* individuals computed by Shapiro-Wilk test

	Morphological characters studied																		
Normal distribution	ST	SB	IN	IW	IL	ILW	LW	LL	LLW	MW	ML	MLW	BW	BL	FL	AW	AL	ALW	PL
Shapiro-Wilk W	0.96	**0.71**	**0.87**	0.97	0.97	0.98	**0.64**	0.97	0.97	0.92	**0.95**	**0.89**	0.97	**0.83**	0.97	**0.93**	0.95	0.98	**0.88**
P (normal)	0.40	0.00	0.01	0.77	0.71	0.88	0.00	0.75	0.80	0.08	0.25	0.02	0.66	0.00	0.73	0.10	0.37	0.86	0.01

Table 2. Linear correlation table (r≥0.95) of morphological characters of the *C. stoebe* species

	Morphological character analysed																		
	ST	SB	IN	IW	IL	ILW	LW	LL	LLW	MW	ML	MLW	BW	BL	FL	AW	AL	ALW	PL
ST	0.00																		
SB	0.02	0.00																	
IN	0.44	0.11	0.00																
IW	0.08	0.21	0.41	0.00															
IL	0.40	0.23	0.44	0.46	0.00														
ILW	0.10	-0.11	-0.18	-0.75	0.18	0.00													
LW	0.17	-0.16	0.09	0.07	0.07	-0.06	0.00												
LL	-0.25	-0.40	-0.34	-0.29	-0.30	0.22	0.20	0.00											
LLW	-0.31	-0.13	-0.31	-0.20	-0.24	0.16	-0.75	0.50	0.00										
MW	0.06	0.45	0.03	0.49	0.18	-0.48	0.18	-0.31	-0.35	0.00									
ML	0.16	0.01	-0.22	0.30	0.46	0.06	0.11	-0.04	-0.08	0.29	0.00								
MLW	0.05	-0.44	-0.20	-0.43	0.01	0.59	-0.17	0.33	0.38	-0.87	0.20	0.00							
BW	-0.01	0.41	0.16	0.22	-0.07	-0.43	-0.26	-0.38	-0.05	0.43	-0.41	-0.65	0.00						
BL	-0.31	-0.30	-0.20	-0.38	-0.55	-0.01	-0.04	0.12	0.09	-0.11	-0.50	-0.10	0.16	0.00					
FL	-0.12	0.14	0.12	0.11	-0.18	-0.31	0.33	0.23	-0.14	-0.07	-0.34	-0.14	-0.02	-0.08	0.00				
AW	0.08	0.09	0.06	0.09	-0.28	-0.42	-0.01	-0.33	-0.21	0.45	-0.30	-0.60	0.44	0.34	-0.05	0.00			
AL	0.29	-0.17	0.27	0.30	-0.04	-0.42	0.20	-0.34	-0.42	0.19	-0.12	-0.27	0.15	0.15	0.19	0.13	0.00		
ALW	0.14	-0.21	0.16	0.19	0.21	0.01	0.14	0.05	-0.09	-0.22	0.15	0.29	-0.24	-0.20	0.21	-0.72	0.59	0.00	
PL	-0.14	-0.26	-0.25	-0.09	-0.33	-0.11	-0.22	0.13	0.28	0.00	-0.36	-0.14	0.11	0.36	-0.03	0.43	0.20	-0.20	0.00

The PCA Analysis - *Principal components analysis* is another ordination method used when analyzed individuals are not very well determined or there are doubt in their determination. Is the best method described by the Person in 1901 and consist of covariance analysis computed with original data [9]. The plot can be described as multidimensional points agglomeration and expressing the data in such way as to highlight their similarities and differences. The PCA method finds an *eigenvalue* which is a percentage variance in correlation with *eigenvectors* for all combinations (components). If the computed variance, for the first and second components has a high percent, it can be said that the PCA analysis is significant.

This significance is given by *Jolliffe cut-off* indices and it show how many principal components are significance [4, 5].

Analyzing the entire data set according principal components (PCA) it can see that only two morphological characters are significant different (IL – inflorescence length and IW – inflorescence width), becoming discriminant in our analysis (Tab. 3).

Table 3. Morphological characters values of *C. stoebe* computed with PCA

Axis	*Morphological characters*									
	ST	**SB**	**IN**	**IW**	**IL**	**ILW**	**LW**	**LL**	**LLW**	**MW**
1	0.02	0.01	0.03	0.47	0.88	-0.01	0.00	-0.02	-0.03	0.01
2	-0.02	0.02	0.01	0.84	-0.46	-0.22	0.00	-0.01	-0.01	0.03
	ML	**MLW**	**BW**	**BL**	**FL**	**AW**	**AL**	**ALW**	**PL**	-
1	0.01	-0.02	0.00	-0.02	-0.01	0.00	0.01	0.03	-0.02	-
2	0.00	-0.12	0.08	0.00	0.04	0.01	0.07	0.01	0.02	-

The plots of the PCA analysis show that two characters (IL, IW) are positive correlated with principal component (OX axis), which are encountered at 70.60 % from total of individuals (Fig. 1). Comparing with the second axis, the correlation is encountered at 23.17 % from a total of individuals, it can be said that both characters (IL, IW) are discriminant in differences between individuals. The width of inflorescence-IW for a percentage by 23.17 % from analyzed individuals is negative proportional with the length of inflorescence-IL. In conclusion, roughly 95 % of *C. stoebe* individuals are differentiated with IW and IL.

Because the morphological differences in the wild flora of both ploidy levels are impossible, the PCA analysis highlighted the existence of two discriminant characters, but the analysis were computed further with other methods.

The plant sample can be differentiated with classification methods too, namely: hierarchical methods (dendrogram-UPGMA), non-hierarchical methods (KmC), discriminant analysis, similarity analysis etc.

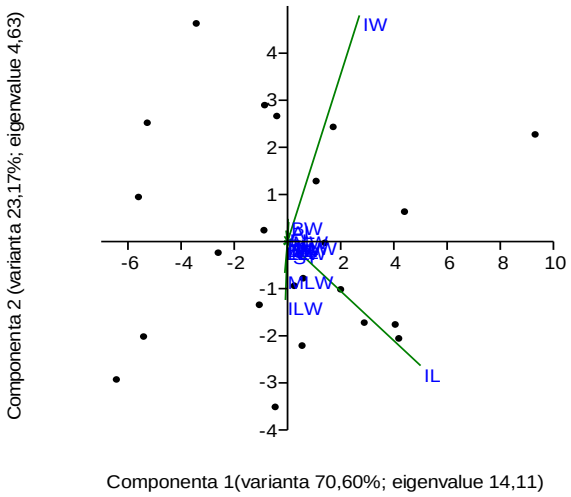

Figure 1. PCA plot of *C. stoebe* computed on var-covar matrix (Jolliffe cut-of semnificative indice=0.73)

The hierarchical methods were used for an overview of morphological differences of *Centaurea* species. This method can be achieved by two algorithms: *Unweighted pair-group average method* (UPGMA) based on de distance mean between individuals of two groups followed by *Ward's method* (WM). This method grouped the species based on minimal variances within a group.

The UPGMA divided the analyzed individuals by morphological similarity with *Wards method* algorithm. Although in a moment of collected samples it was not known if were any morphological differences between individuals, it seems that UPGMA grouped in three clusters. One of them (group I) is better clustered from a morphological point of view and the other 2 groups (group II and III) are polymorphic (Fig. 2).

Group I is homogeneous and includes similar individuals, collected from different places, especially places with weeds. Group II includes individuals collected from the meadows of Turda Gorges.

Unfortunately this analysis is not always very conclusive, especially when we have a cophenetic correlation by 0.66. This coefficient shows that the dendrogram does not totally reflect the similarity between original data. However it is certain that there are morphological differences and they are confirmed by European researchers.

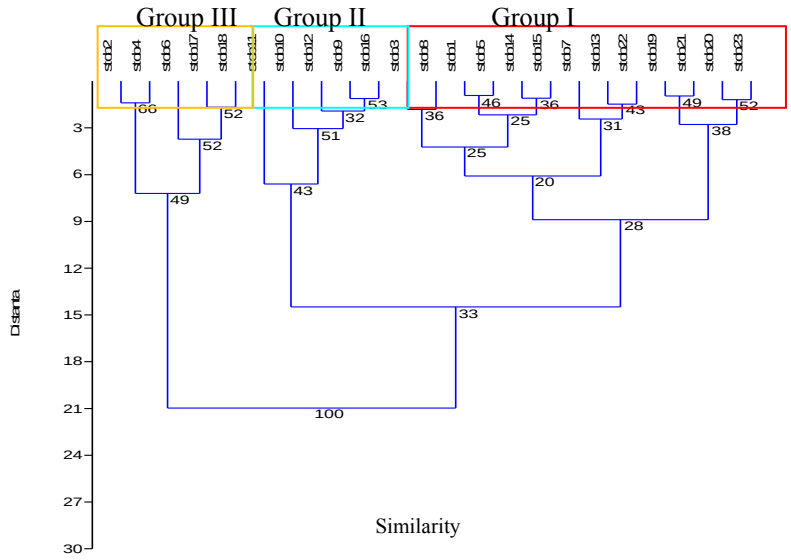

Figure 2. UPGMA cluster analysis (cophenetic correlation 0.66)

Another method used for differences between individuals is non-hierarchical analysis-*K-means Clustering-KmC*. This method divides the individuals into desired number groups and it is designed to minimize the variability within the group and maximize the variability between groups. For each group is computed a mean of differences and compared with ANOVA test.

This test gives for each group an F value and a significance prague-*p*. This significance value shall not be construed as type I error because the selected groups maximize differences between them. In this case, *p* -value shows that a group of *C. stoebe* individuals differ from other groups. In this case, individuals were divided in two groups and compared with dendrogram results. The result of UPGMA dendrogram overlaps with the KmC outcome divided the individuals in two groups (Fig. 3).

For each individual was calculated discriminant score with *Hotelling's* classification which maximize the differences between two sets of multivariate data. The classification is represented with a histogram and the equality of means is tested with *Hotelling's T-squared.* The discriminant analysis can be used to accepted or rejected the hypothesis that the individuals group are different from the morphological point of view. This analysis calculates the classification percent and the middle point of the discriminant score are 0 value. In this

study, the discriminant analysis *Hotelling t2* confirmed the existence of morphological in two clusters with 100% percentage of classification.

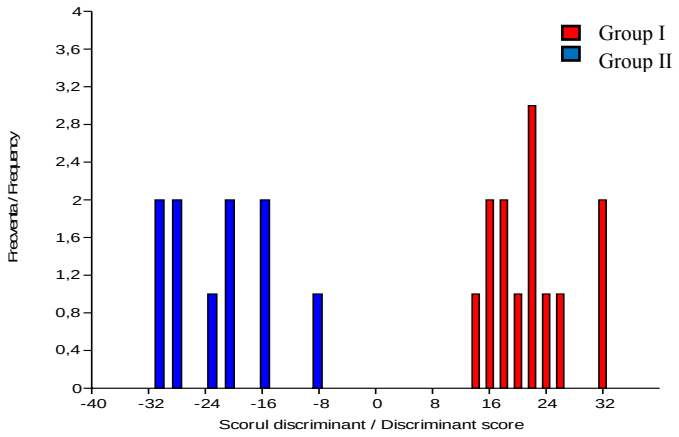

Fig. 3. Discriminant score of two individuals groups of *C. stoebe* (F 1.95; p 0.32)

The differences between clusters were computed with Euclidian distance, which were converted into ranks. The test was performed with 9999 combination and $p < 0.05$, has computed an average rank within the group- 93.59 and the average between groups- 158.6, giving a dissimilarity coefficient R (0.51) at significance level *p* 0.0001. This coefficient computed showed sub unitary value, which indicates that this group of individuals belonging to the same species and there existing a difference between them (Fig. 4).

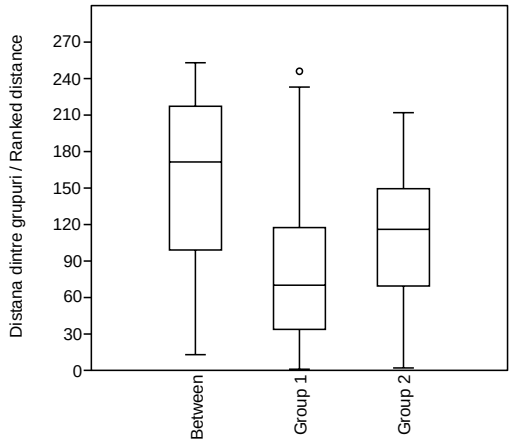

Fig. 4. Ranked distance between groups and within groups of *C. stoebe* individuals, computed with ANOSIM analysis

The two morphological groups highlighted into *C. stoebe* species were further analyzed from the perspective of discriminant characters (IL and IW) (Fig. 5).

Plots of morphological characters were compared with univariate value and both have an overlap. The width of inflorescence- IW is impossible to use into wild flora for recognition the both groups because the group I is included into group II. From the length of inflorescence- IL point of view, there is a very clear boundary because the group I has a higher value than group II.

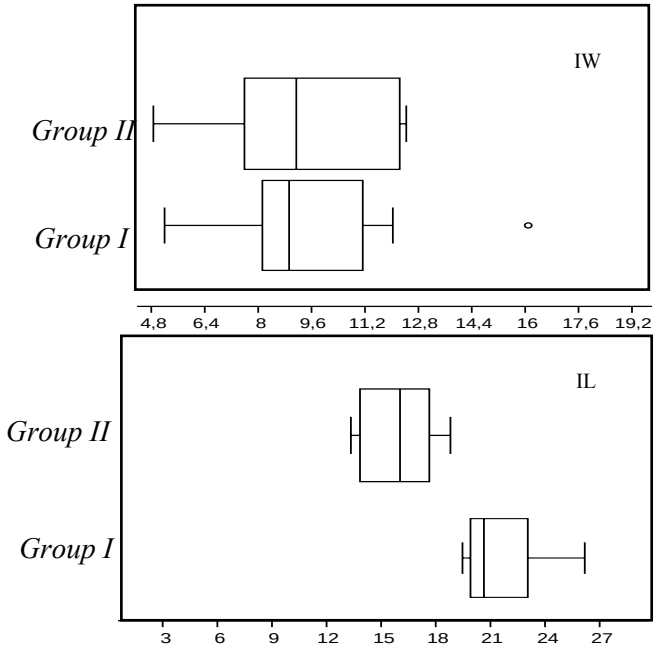

Fig. 5. Discriminant morphological characters variation of *C. stoebe* individuals

When comparing the morphology of two *C. stoebe* groups with European study, it can be concluded that in Romania grows both cytotypes (2x şi 4x) and these are differentiated by length of inflorescence [6, 7]. From these comparisons, it can be concluded that the second group (group II) is the diploid taxon (2x) and the first group is the tetraploid taxon of (4x) of *C. stoebe.*

Conclusions

According to European studies the diploid was noted as *C. stoebe* subsp. *stoebe* (2n = 18) and has the following characteristics: length of inflorescence 13.5–17 mm, 6-10 fimbriae on one side, whitish colored on distal part and length of pappus is half from achene length. The tetraploid was noted as *C. stoebe* subsp. *micranthos* (2n = 36) and has the following

traits: length of inflorescence is 19.5–23 mm, 4 - 7 fimbriae on one side, brownish color, with pappus length about 1/3 from achene length.

According to the classification analysis but also to geographical distribution, it has been observed that tetraploids are found in anthropogenic areas with plenty of weeds. The diploid has been observed in opened fields with fewer weeds.

As a decorative plant *C. stoebe* is suitable for sunny places arrangement, on rocky-sandy substrate and roundabouts. It can be considered an ornamental plant in bouquets and floral arrangements because it has numerous small flowers but also because the green-gray leaves with a silver appearance.

From the geographical point of view, there is not a clear demarcation between cytotypes. *Centaurea stoebe* subsp. s*toebe* is a widespread species from the Rhine to the Eastern Russia and in Transylvania has been mentioned on hills, meadows, railways, plowing, preferably with stony or sandy places [3, 8, 10, 18]. *Centaurea stoebe* subsp. *micranthos*, is a Ponto-Pannonian-Balkan element, often in arid grassland in Eastern Asia, Eastern and Central Europe. In Transylvania, it is very common, found on the arid hills and meadows [3, 8, 10, 18].

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