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Rector's Allocution

We have the special pleasure to let you know that the Review of our University, „Bulletin of Scientific Information", having ten years of consecutive issue, it achieved the recognition of the National Council for Scientific Research in Higher Education (NURC), being comprised in the category „National Reviews — C Category".

So, Bioterra University review „Bulletin of Scientific Information" works as a real platform for the information and exhibition of the most recent and valuable research in the agricultural field and connected sciences (food industry, agro-tourism, ecology, environment protection, agricultural economics etc).

This way, I express my gratitude to the contributors to our science magazine, to the authoritative academic and universitary personalities of whose studies are found in the selection done by the scientific board of our magazine with whom we have strong relations of partnerships in the development of jointed research projects.

I wish to our scientific science magazine many and consistent issues.

Prof. Floarea Nicolae, PhD
Rector of Bioterra University Bucharest

Editorial Board's Allocution

„Bulletin of Scientifc Information" was published at the initiative of several young researchers with the direct support of Bioterra University Board, having the first edition in 1998.

Years passed and this magazine has enriched continuously its scientific and didactic dowry becoming slowly but surely a veritable platform for academic information.

In 2008, this science magazine turned into a new more dinamic and attractive pattern, being published in special grafic features (full-color) and fully in English language. Also, since 2014, our science magazine benefits of a modern website: www.bsi.bioterra.ro.

Every year the editorial team has increased the number of members; nowadays it brings together numerous personalities of the scientific and academic world from different foreign countries, thus being a guarantor of a high scientific level.

Thanks to all our readers and collaborators that through their suggestions, criticisms and feedback contribute to the improving of our science magazine quality.

Prof. Petculescu Nicole Livia, PhD
Vice Rector of International Relations

SUMMARY

BACKGROUND OF THE LIFE ZEOWINE PROJECT: EFFECT OF ZEOLITE ON VINEYARD SOIL FERTILITY

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Abstract

Mediterranean vineyards are exposed to fertility decline due to continuous tillage and repeated applications of fertilizer and phytopharmaceutical products. Zeolites are considered to be one of the widely used natural inorganic soil conditioners to improve water holding capacity, infiltration rate and cation exchange capacity of agricultural soils, making the efficiency of fertilizers greater and the leaching losses lower.

In the present paper, the response of the chemical and biochemical indicators of soil fertility to different doses (0, 5 and 10 t ha^{-1}) of zeolite (clinoptilolite 0.2-1 mm Ø) has been evaluated in three vineyard soils after six month from zeolite application.

Generally, TOC and TN were not influenced by the zeolite treatments. Conversely, a significant increase of TK values was observed in the three experimental soils treated with zeolite at the highest dose (10 t ha^{-1}). This increase in nutrient content, accelerated SOC microbial mineralization process. In fact, even though TOC content was not affected, a decrease of humic substances (humic and fulvic acids), representing the more stable part of organic matter, in the vineyard soils treated with zeolite was found. The enhancement of microbial processes involved in soil organic matter degradation by applying zeolite was confirmed by the increase of dehydrogenase activity, an enzyme used as an indicator of general microbial metabolism, and by the decrease of organic matter stability in terms of its chemical-structural composition. In fact, the pyrolysis (Py-GC) results clearly showed a decrease of the more stable aromatic (pirrole, phenol and benzene) and an increase of labile aliphatic (furfural) compounds.

In view of this, other studies about zeolite application in combination with a source of organic matter are carrying out in the framework of the LIFE ZEOWINE project (LIFE17 ENV/IT/000427) in order to optimize the fertilizer use efficiency and preserve soil organic matter quantity and quality.

keywords: *soil conditioner, pyrolysis-gas chromatography, enzyme activity, soil quality*

Introduction

Due to the increasing pressure imposed to agricultural soils and to their consequent fertility decrease, the development of management strategies able to increase the productivity and quality of soils has become a common priority. In particular, Mediterranean vineyards are exposed to fertility decline due to continuous tillage to maintain bare soils, and to contamination due to repeated applications of copper, to fight against vine diseases such as mildew. Zeolites are crystalline, hydrated aluminosilicates (Rehakova et al., 2004), characterized by an ability to lose and gain water reversibly and to exchange their constituent elements without a major change of structure (Gholamhoseini et al. 2013). In view of this, the application of natural zeolite could be considered an effective solution for increasing water and nutrients efficiency in vineyard soils. The very high exchangeable cations capacity of zeolites ensure a stable and constant supply to plants for several vegetation seasons (Puschenreiter and Horak 2003).

In this paper the potential of zeolite for improving vine nutrition management has been studied with the aim of reducing dependence from mineral fertilizers in vineyard systems and increasing their fertility.

Experimental layout and Methods

The experimentation was set up in the San Miniato area (Pisa, Tuscany) in Central Italy. Two different vineyards (Trebbiano one year old, not in production yet, located in a flat area and Cabernet-Sauvignon 18 years old, located in an area with a 7% slope) were selected. Each vineyard was divided into three plots, where zeolite (natural clinoptilolite 85% with particles of 0.2-1 mm Ø, Zeocem) at the dose 0 t ha^{-1} (control), 5 t ha^{-1} and 10 t ha^{-1} was applied, respectively, and incorporated by ploughing to a depth of 30 cm. In each plot (135 m x 35 m with vine spacing of 2.5 m x 0.8 m), 3 composite soil samples were taken between rows from the 0–30 cm layer. In the vineyard Cabernet-Sauvignon, in order to evaluate the effect of soil slope, the soil samples were collected in two different vineyard zones, top (T) and bottom (B), and separately analyzed. The soil sampling were carried out immediately after the zeolite treatment and after six months. Chemical and biochemical parameters after six months from zeolite application are reported in this work. Electrical conductivity (EC) and pH were measured using selective electrodes. Total organic carbon (TOC) and Total Nitrogen (TN) content were measured with LECO, U.S.A. RC-412 Multiphase Carbon and FP-528 Protein/Nitrogen Determinators, respectively. Total phosphorus (TP) was measured using the method reported by Murphy and Riley (1962). Sodium pyrophosphate (0.1M, pH 11) at 60 °C for 24h under shaking was used to extract Total Humic Carbon (THC, humic and fulvic acids). Dehydrogenase activity was assayed using the method of Masciandaro et al. (2000). Pyrolysis-gas chromatography (Py–GC) was used to evaluate soil organic matter quality (CDS Pyroprobe 190 coupled to a Carlo Erba 600 GC) (Macci et al., 2012a). The following volatile pyrolytic fragments, corresponding to the major chromatographic peaks were considered: acetonitrile (E1), acetic acid (K), benzene (B), pyrrole (O), toluene (E3), furfural (N), and phenol (Y). Some ratios between relative abundances of some of the peaks were determined (Ceccanti et al., 1986): (i) O/N: mineralization index. The higher the ratio, the higher the mineralization of fresh organic matter. (ii) B/E3: humification index. The higher the ratio, the higher the humification of organic matter. (iii) AL/AR (Aliphatic/Aromatic compounds): index of "energetic reservoir" expresses the ratio between the sum of aliphatic products (K, N, and E1) and the sum of aromatic compounds (B, E3, O, and Y) (Ceccanti et al., 2007; Macci et al., 2012b).

Results and Discussion

The response of the chemical indicators of soil fertility to the different doses of zeolite is reported in table 1.

Table 1. Chemical parameters in the vineyard soils (T, Cabernet-Sauvignon Top; B, Cabernet-Sauvignon Bottom; Y, Trebbiano) after six months.

	treatment	pH	EC	TOC	TN	TP	TK
T	control	7.86b	0.23a	1.04a	0.09a	539b	640c
	5 t ha^{-1}	7.98a	0.19ab	0.98a	0.09a	548ab	726b
	10 t ha^{-1}	8.00a	0.16b	1.01a	0.10a	564a	773a
B	control	7.96a	0.21a	1.15a	0.10a	595a	608b
	5 t ha^{-1}	7.92a	0.20a	1.00a	0.08a	594a	649a
	10 t ha^{-1}	8.02a	0.15b	1.12a	0.10a	600a	678a
Y	control	8.34a	0.19a	0.94a	0.12a	654b	642b
	5 t ha^{-1}	8.26a	0.14b	0.94a	0.12a	657b	636b
	10 t ha^{-1}	8.27a	0.16ab	0.98a	0.15a	794a	775a

EC (dS m^{-1}), electrical conductivity; TOC (%), total organic carbon; TN (%), total nitrogen; TP (mgP kg^{-1}), total phosphorus; TK (mgK_2O kg^{-1}), total potassium.

Generally, TOC and TN did not significantly changed after six months from the zeolite application. Conversely, the contents of TP and TK were slightly higher in zeolite treated soils in comparison with control soils. In particular, a significant increase of TK values was observed in the three experimental sites treated with zeolite at the highest dose (10 t ha^{-1}). Even though TOC content was not affected by the zeolite treatments, a decrease in total humic carbon (Fig.1(a)) was found in the vineyard soils treated with zeolite. This decline can be explained by the fact that zeolite is able to improve the nutrients and water availability and thus, the microbial soil conditions, with consequent stimulation of the microbial processes of soil organic matter degradation. Beneficial effects of applying zeolite on soil microbial functionality are confirmed by the trend of dehydrogenase activity (Fig. 1(b)). This enzyme, which is considered a direct measure of the microbial population growth and activity (Garcia et al., 1997), showed the highest values in the vineyard soils treated with the highest dose of zeolite.

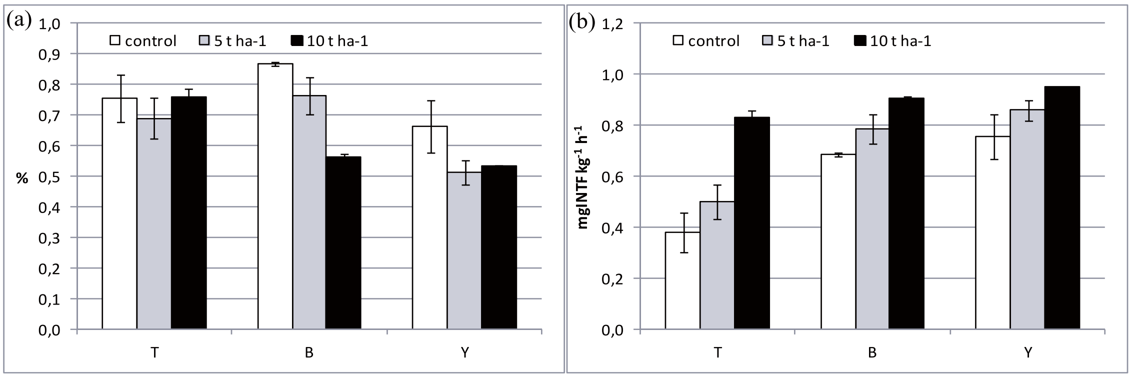

Figure 1. (a) Total Humic Carbon (THC) and (b) Dehydrogenase activity in the vineyard soils (T, Cabernet-Sauvignon Top; B, Cabernet-Sauvignon Bottom; Y, Trebbiano) with different doses of zeolite (0, 5 and 10 t ha^{-1}) after six months.

In the present paper, the effect of zeolite application on organic matter quality in terms of its chemical-structural composition was evaluated by using the pyrolysis technique (Table 2). The lower values of pyrrole/furfural (O/N) index in the zeolite treated soils with respect to control soils (Table 2), can be explained by the significant decrease in O pyrolytic fragment, thus indicating the decomposition of more stable SOM, since substances releasing O (pyrrole) are chemically and microbiologically more stable than those producing N (Masciandaro et al., 1998; Masciandaro and Ceccanti, 1999).

The humification index B/E3 (benzene/toluene), represents the structural condition of the condensed aromatic nucleus of SOM, that is the degree of condensation of aromatic rings.

B/E3 was lower in zeolite treated soils (Table 2), confirming that, the activation of mineralization processes in such microbial enriched soils, determined a significant degradation of condensed aromatic structures (B) and an increase of less condensed humic substances producing E3 with respect to the respective control soils.

Table 2. Pyrolytic fragments and indices after six months from the beginning of the experimentation. For each site, different letters indicate statistically different values among the treatments (P< 0.05).

	treatment	E1 %	K %	B %	E3 %	Y %	O %	N %	B/E3	O/N	O/Y	AL/AR
	control	25.3c	5.5ab	21.1a	17.1b	14.7a	10.6a	5.6a	1.23a	1.88a	0.72b	0.57c
T	5 t ha^{-1}	33.0b	5.0b	17.4b	16.8b	13.5a	9.5a	5.0ab	1.03b	1.90a	0.71b	0.75b
	10 t ha^{-1}	38.1a	5.8a	15.4b	19.0a	9.4b	7.6b	4.7b	0.81c	1.62b	0.81a	0.95a
	control	32.2b	5.5	19.3b	15.1b	15.1a	9.1a	3.7a	1.27a	2.46a	0.60b	0.63c
B	5 t ha^{-1}	33.3b	4.8	23.1a	18.2a	9.6b	7.8b	3.3a	1.28a	2.36a	0.81a	0.80b
	10 t ha^{-1}	39.0a	5.4	18.3b	18.3a	8.4b	7.0b	3.5a	1.00b	2.00b	0.83a	0.92a
	control	29.8b	6.6	24.1a	18.2b	9.4a	7.6a	4.3a	1.32a	1.77a	0.81a	0.69b
Y	5 t ha^{-1}	34.8a	5.7	22.2a	20.1a	7.6b	5.7b	3.9a	1.10b	1.46b	0.75a	0.80a
	10 t ha^{-1}	34.6a	6.0	22.9a	19.4ab	7.6b	5.7b	3.8a	1.18b	1.50b	0.75a	0.80a

Finally, AL/AR (aliphatic/aromatic compounds) expresses the ratio between the sum of aliphatic (acetic acid, furfural and acetonitrile) and aromatic (benzene, toluene, phenol and pyrrole) products. The soils amended with zeolite showed an increase in this pyrolytic ratio. Zeolite addition significantly increased the acetonitrile (E1), mostly derived from aliphatic compound and decreased the pyrrole (O), phenol (B) and benzene (B), mostly derived from aromatic compounds (Table 2).

Conclusions

The application of zeolite to the vineyard soils, especially at the higher dose (10 t ha-1) improved their cations exchange capacity and thus their ability to retain nutrients. The increase of available N and K in the three experimental sites (T, B and Y) treated with zeolite significantly promoted microbial metabolic activity (dehydrogenase activity) and utilization of soil organic matter, with the consequent change in soil organic matter composition. In particular zeolite application, especially at the higher dose, contrasted the accumulation of soil organic carbon pool. This is well evident considering the reduction of humic and fulvic acids and the alteration of the structural composition of soil organic matter. Py-GC clearly showed a decrease of the more stable aromatic (pirrole, phenol and benzene) and an increase of labile aliphatic (furfural) compounds. In view of this, other studies about zeolite application in combination with a source of organic matter are carrying out in the framework of the LIFE ZEOWINE project (LIFE17 ENV/IT/000427) in order to optimize vineyard nutrition, improve fertilizer use efficiency and, at the same time, preserve the pool of soil organic matter.

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BENEFITS OF SOIL ECOLOGICAL CULTIVATION

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Abstract

Ecological cultivation of the soil corresponds to the healthy and high quality foods requests. Also, it provides the protection of natural resources on long term for the benefit of future generations. In order to practice the system of soil ecological cultivation is important to reduce the environment pollution due to the agricultural works. The organic soil is the focus, therefore, maintain and increase soil fertility is very important. Base soil fertility is life in the soil. Soil fertility, soil productivity that is a decisive attribute, whose level in using drops. A general principle applies to organic farming is that land should be as unobtrusive, but conditions in our country is hardly give up plowing. Minimum and direct sowing systems requires appropriate equipment, heavy investment and trained.

keywords: *soil ecological cultivation, organic soil, organic farming*

Introduction

The organic soil is the focus, therefore, maintain and increase soil fertility is very important. Base soil fertility is life in the soil. Soil fertility, soil productivity that is a decisive attribute, whose level in using drops. It therefore needs to recover first from fattening, ensuring a chain of specific effects of organic farming: healthy soil - healthy plants - healthy animals - healthy people.

Work done correctly makes life in the soil and provide needed space for plant roots, contributing to: improve soil structure, raising and reducing compaction, incorporating crop residues and organic fertilizers, weed control, preparing the germinative bed.

Ground work to ensure a stable structure with a maturity and an appropriate resilience to neutralize some restrictive factors: excessive rainfall, heavy rains, the settle by agricultural machines, washing the fine soil and nutrients, formation of crust, siltation.

Discussions

A general principle applies to organic farming is that land should be as unobtrusive, but full of weeds conditions in our country is hardly give up plowing. Minimum and direct sowing systems requires appropriate equipment, heavy investment and trained.

Given these conditions of differentiation, the specific objectives of tillage may be:
- Adjustment of physical characteristics, chemical and biological, whiles creating

optimal conditions for seed incorporation, their germination and the subsequent growth of plants;

- Maintaining and enhancing soil fertility restoration and regularly show raising layer soil incorporation of crop residues left after harvesting the plants, manure, green fertilizer, natural deposits of mineral fertilizers, amendments, etc.;
- Weed control and some diseases and pests that development cycles in relation to the ground;
- Enhancement effect of other technological elements, plant vigor and efficiency of fertilizers, irrigation water, crop rotation is closely related to how prepared the soil, root system of young plants grow more easily in a loose layer, than in one compact, and so work is more intense micro-worked soil.

Economic efficiency of a culture is closely linked to how they are executed and why is the work of soil quality. Ground work is the technological component through rationalization leads to a substantial reduction in fuel consumption as soil preparation requires 35-65% of the total energy consumed in a culture technology.

Organic farming is a different model from the conventional intensive agriculture, but also too traditional of subsistence agriculture. Organic farming is creative, instructive, scientific and advanced, allowing correction of serious environmental problems and social care, and resolve imbalances facing the current agriculture and farmers in general.

Most important characteristics of plants are encouraging organic cultivation, maintenance and reproduction of natural functions of soil. Organic cultivation differs significantly from the conventional and intensive methods and effects. Ground work is mainly intended immediate effect a series of positive role from the very ground work objectives: basic work, preparatory work for the bed germinative maintenance of fields. Often, however, when the work is inadequate, the effects of ground work can be immediate negative or lasting effect, remaining.

Reduce traffic on the ground and making works best when the soil (in the technological and humidity) are important condition for more efficient cultivation in environmental system.

Soil as part of the ecosystem, a biological system is open and lively from the presence of micro-organisms and is dynamically located in an exchange of energy and substance with the environment, but the main repository of organic matter. Accumulated in soil organic matter has a role on the development of most processes and soil properties. Also, soil organic matter, is one of the most important reservoirs of carbon (organic or mineral), which in turn is transformed under certain conditions, with a certain intensity, atmospheric carbon dioxide, a potential source for accelerating greenhouse effect. This important resource soil is in constant interaction with human activities in the agricultural and applied technology. Carbon stored in soil through photosynthesis may be released as CO_2 by mineralization or decomposition.

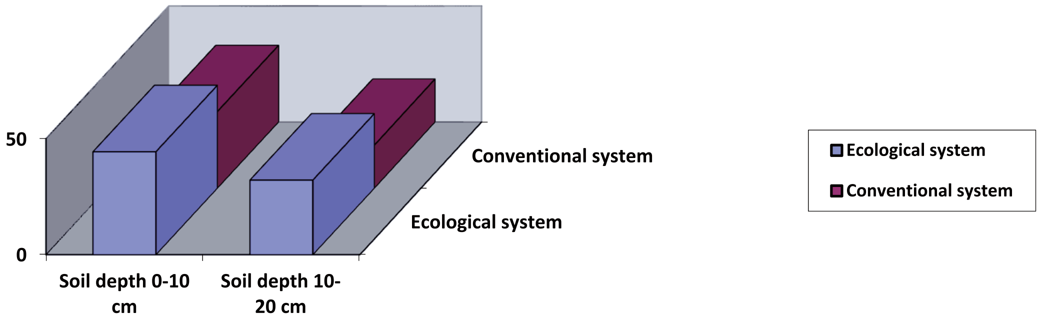
The transformation of matter is defined by a complex biological process: humification - mineralization. Much of the loss is due to anthropogenic carbon, for example through the work and soil erosion. The relationship between agriculture and the loss of carbon is complex, but it is clear that there is a link between agriculture and climate change. Raising physical layer through the work of arable soil, removing the main production and replace it with fertilizers help to change the system. Annual removal of biomass crops is a removal of carbon and nutrients in agricultural ecosystems. After repeated cycles of removal of biomass from the system, the soil is poor in nutrients and organic elements.

Soil conservation practices are those which not only reduce soil erosion but be required to increase soil carbon content. Best management practices for sequestration are related to cultural remnants, such as conservation work directly sowing, minimal work, mulch soil, appropriate rotations, cover crops, eliminating grubbing summer, application of organic fertilizers and compost, optimizing fertilization soils.

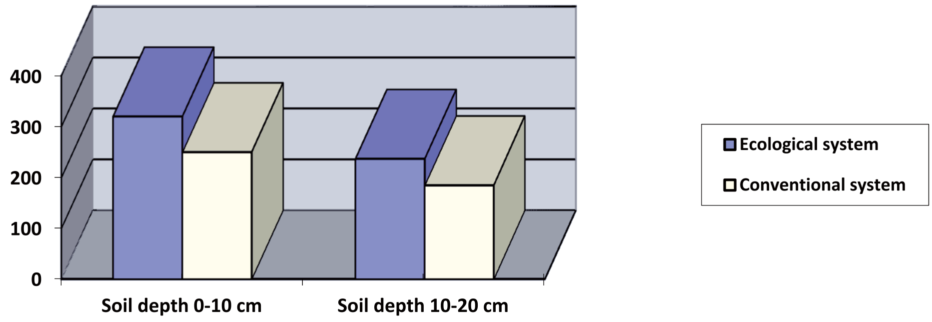
Plant debris left on the soil surface or incorporated surface where soil conservation systems, contribute to increased biological activity and is an important source of CO_2. It restores soil structure and improves the overall drainage of the soil, allowing faster water infiltration into soil. The result is a more productive soil, better protected against wind and water erosion and requires less fuel to prepare the bed germinative.

The advantage of organic cultivation of the soil is to achieve a balance between crop production and environmental protection, which can be guaranteed to prevent environmental imbalances, with possible negative influence on the results of production even further.

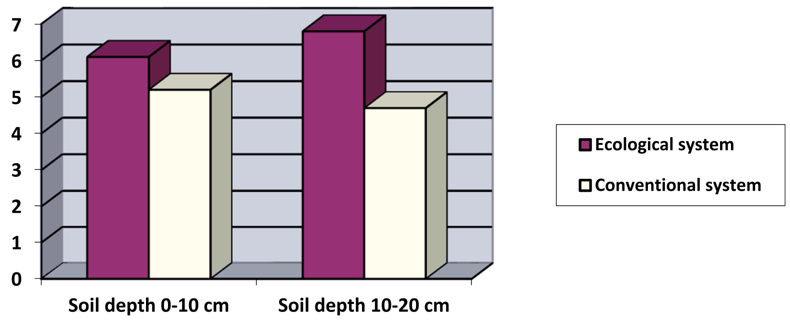
Favorable effects of soil induced by environmental work are extending the optimal range of humidity, a workability better structure and better soil drainage.



Basal respiration ($\mu g\ CO_2$/ g/ 24h) on soil depth



Microbial biomass (µg C/g) on soil depth



Metabolic quotient ($qCO_2\ 10^3$) on soil depth

Conclusions and outlook

The objectives of soil protection in Europe adopted a work can be applied to organic soil and our soil, whether that effect is applied correctly all necessary measures:

• reducing tillage intensity and aggression, respect for the right soil to be covered with vegetation all year, stopping capillarity to the soil surface (dependent crops by mechanical work to maintain soil structure and layers of vegetable soil protection) anti-erosion systems of work soil, land suitable slope;

• use of agricultural machines with bodies which raise and small soil by tapping and selling of land on the line of least resistance, not by cutting, compression and compaction (specific conventional system), reducing road traffic and performance at a crossing in many technological operations;

• increasing the capacity of absorption and accumulation of water (to avoid soil erosion, stabilization of pore continuity, avoid the settle soil humus content optimization, preserving structural stability);

• diversification of agricultural practices (crop rotation with winter summer, those with annual multi-annual).

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ENVIRONMENTAL IMPACT CAUSED BY WILD UNGULATES IN PROTECTED AREAS

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Abstract

Protected areas play a crucial role for conservation of natural habitats and ecosystems. Protecting biodiversity means maintaining in good condition ecological processes in order to ensure a good state of harmony between natural resources and environmental conservation. It is known that one of the major threats of the Conservation is the presence of alien or invasive species, especially if they are introduced into protected areas. The International Union for Conservation of Nature (IUCN) writes in the list of the 100 invasive species the wild boar (Sus scrofa) and the goat (Capra hircus). These ungulates represent a serious threat for natural ecosystems because they are able to change natural habitats reducing biodiversity. The primary aim of this study is to evaluate some aspects connected with environmental relationships mainly between flora and fauna in protected areas, focusing on wild boars and feral goats. Secondly, it is analysed the "Human dimension" aspect caused by the presence and damage of wild animals linked to damage to crops and to dry stone wall supporting the typical cultivated terraces. To reach our goal we use as a case study the Portofino Natural Park in North-West Italy, because it houses one of the largest biodiversity concentration in the Mediterranean area. In this protected area the wild boar is the most invasive between the two studied animal species, because of soil damages that influence the hydro-geological balance of the territory. Finally, this species can quickly increase its population thanks to its high prolificacy and low biological and predator mortality.

keywords: *environmental impact, plant biodiversity, protected areas, feral goat, wild boar*

Introduction

In the last decades, wild ungulates, especially the wild boar, have further increased their distribution and consistence in almost all the Italian territory [3; 13]. It is truly remarkable that in the last 30 years in Italy wild boar has quintupled its distribution area [1; 5] and the latest estimates suggest the population of wild boar in Italy have reached nearly one million animals, although there are no precise data about it. The main reasons for such a large population explosion are: high reproductive potential, easy adaptability to different habitats, abandonment of rural areas by man and therefore new wilderness areas and low predator mortality [4]. These are also some of the reasons why the International Union for Conservation of Nature (IUCN) writes in the list of the 100 invasive species some of these ungulates [9]. Nowadays the wild boar (*Sus scrofa* L.) is distributed in 83.5% of Italian provinces, followed by the roe deer (*Capreolus capreolus* L.) with 64.1%, the fallow deer (*Dama dama* L.) with 52.4%, the deer (*Cervus elaphus* L.) with 44.7%, the mouflon (*Ovis orientalis musimon* Pallas) with 32.0% and the chamois (*Rupicapra rupicapra* L.) 21.4% and the ibex (*Capra hibex* L.) with 14.6% and finally the Apennine chamois (*Rupicapra pyrenaica ornate* Bonaparte) with 3.9% [3]. The wild goat (*Capra hircus* L.) was the chief ancestral stock from which the various breeds of domestic goats have been derived and feral goat is descendent of domesticated animal that have reverted to the wild [22]. By definition, feral animals have gone through a prolonged period of domestication in the past and now exist in a wild and free-roaming state with self-sustaining population [22]. The wild boar is without any doubt the ungulate that causes the most problems ever. The problems are many and different from each other: from car accidents to the transmission of diseases to other animals, from damages to crops to effects on natural ecosystems and biodiversity. Many studies have highlighted the concern for the alteration and damage to habitat conservation caused by wild ungulates [2; 7; 8; 19; 21] and it is recognized that wild ungulate species have a profound effect on the environment and this may often cause conflict with human land-use objectives [16; 18].

In this study we follow the approach suggested by some Authors [16; 17] and we use the term "effect" to describe the impact on the environment caused by wild ungulate activities and "damage" when the impact is recognised to be over the damage threshold or when we generally talk about negative events related to the agro-ecosystem caused by wild ungulate [18; 17]. We made this choice because effect could be positive or negative, whereas damage is always negative in term of extreme consequence or conflict with human interest or management objectives, but in fact from the very outset the presence of herbivores has many effects on the whole structure and the ecological functioning of an ecosystem. Generally speaking, the effects of ungulates on plant species may result as it follows: a decrease or an increase in plant diversity by selective pressure; changes in plant structure without change in diversity or abundance; or no ascertainable influence.

Materials and methods

This research was carried out in Portofino natural Park, one of the most important protected areas in North-West cost in Italy. This area is characterised by a presence of different ecosystems that house one of the largest biodiversity concentration in the Mediterranean area. This study took place in all the Park area and focus on the presence of two different ungulate species: wild boar (*Sus scrofa* L.) and feral goat (*Capra hircus* L.). At the moment, they live in two different environments: feral goat occupies the South area of the Park and wild boar spreads in the remaining territory. We started by investigating the effects on vegetation making in Portofino's Park area. Basically, the southern coastal area was evaluated for the presence of feral goat whereas the remaining part is mainly occupied by wild boar. For these reasons, we use two different methods to study the behaviour and the effects produced by these animals on the environment, especially on vegetation. In fact, we know that the main environment impact produced by wild boar is caused by rooting activities, whereas the effects produced by feral goat depend on grazing activities [8; 12; 15]. The survey has been developed since 2013 and it has consisted of several site inspections to study directly the environmental situation in its different habitat: olive grove, mixed woodland, ilex woodland and maquis shrubland. During the site inspections, we have completed some photographic surveys and several detailed descriptions considering entity, type and frequency of damage caused by two different ungulate species that have been studied. The penetrometer survey has been used to evaluate soil density caused by trampling and rooting activities. Moreover, it has been analysed and quantified the damage entity caused by wild boar to dry stone wall. The amount of small landslides has been compared with the previous amount recorded three years before in similar environmental sites.

Results and discussions

The results point out that the two observed wild ungulates cause in Portofino protected area different types of environmental impact on plant biodiversity and on soil structure, especially depending on eating features and behaviour habits. Wild boars transfer often following the same path so that they give birth to trails without vegetation and the soil becomes very hard and compact, as shown in Table 1.

Table 1 - Soil density difference caused by wild boar trampling activity.

	CONTROL AVERAGE Kg/cm^2	TESTER AVERAGE Kg/cm^2	VARIATION %
DRY SOIL	2.05	3.40	66
WET SOIL	1.17	2.70	131

Table 1 illustrates results from several penetrometer experiments in different soil conditions and shows how significant is the penetration resistant difference from dry to wet soil (66% *vs* 131%) [20]. The compact soil areas refer to all those areas mostly used by wild animals. The trampling effect not only concerns the birth of animal paths, but also could interest whole sectors of the territory.

Soil alteration in structure caused by the presence of wild boars, as from Table 2, has interested all the ecosystems we have studied, even though with different impact and effects. The most important changes in soil density are recorded in maquis shrubland, that is to say the preferred ecosystem chosen by wild boars because it is a safe shelter during the day. In particular, the rooting activity tills the soil and reduces its density, as shown in Table 2 [20]. Both the compact soil and rooting activity are two key factors as regards the environment because they foster erosion, soil loss and hydro-geological unbalance.

Table 2 - Soil density variation caused by wild boar trampling activity and by rooting activity in different ecosystems.

	TRAMPLING ACTIVITY			ROOTING ACTIVITY		
ECOSYSTEM	**CONTROL Kg/cm^2**	**TESTER Kg/cm^2**	**VARIATION %**	**CONTROL Kg/cm^2**	**TESTER Kg/cm^2**	**VARIATION %**
OLIVE GROVE	1.75	3.17	81	1.75	1.40	-35
MIXED WOODLAND	1.80	2.80	56	1.80	0.71	-30
ILEX WOODLAND	1.97	2.70	37	1.97	0.80	-60
MAQUIS SHRUBLAND	1.60	3.70	130	1.60	0.40	-75

The main reasons of these negative effects are: 1) the reduction in coverage herbaceous vegetation and shrubs; 2) rooted soil is easier to be transported by rain; 3) compact soil is less permeable and promote water runoff.

Moreover, it is clear that hydro-geological problems are exasperated by the destruction of dry stone wall, basic structures to keep soil stable, especially in a slope context. In Table 3 we have considered that the percentage in damages to dry stone wall increased more than 11% (236 m) during a three years study period. For this reason and for all the soil problems deterioration we noted, it has been also recorded a significant increase in landslide events in Portofino protected area [20].

Table 3 - Damage to dry stone walls and number of landslides recorded from 2011 to 2014 in Portofino protected area.

	2011	2014	DIFFERENCE	DIFFERENCE %
DAMAGE TO THE DRY STONE WALLS (M)	2090	2326	236	11.29
NUMBER OF LANDSLIDES	38	91	53	130

In recent years, researches on the *Human dimension* aspect, focusing particularly on protected areas, have become very popular because the wild animals conflict is becoming more and more serious and perhaps more important than the material direct damage. This kind of conflict is much more clear in areas cultivated that are into protected areas territory because often it increases social conflicts between rural areas inhabitants and protected areas managers, generating several side effects, such as the increase in the dropout rate from rural areas because of a negative psychological state [13].

This study, furthermore, demonstrates that both feral goats and wild boars influence plant structures: the feral goat produces more effects because it can stand on hind legs and reach also the upper plants, the wild boar instead produces more effects and damages on grassland due to rooting activity. Woody plants are damaged by both ungulate species because of barking activity: the wild boar uses body friction on plants bark to remove ectoparasites, whereas the feral goat eats plants bark because of it is able to eat tannin compounds [6]. For instance, some experiments demonstrate that feral goats eat almost the Mediterranean plants not minding chemical or physical-mechanical defence systems generally present in some plant species [6], as can be found in Table 4.

Table 4 – Example of some effects on Mediterranean plant species by feral goat activities in Portofino protected area.

PLANT SPECIES	BARKING/BITING EFFECTS	GRAZING/BROWSING EFFECTS
Ampelodesmos mauritanicus Poiret		X
Arbutus unedo L.	X	X
Asparagus sp.		
Calycotome spinosa L.		X
Castanea sativa Miller	X	X
Cistus spp.		X
Erica arborea L.	X	X
Euphorbia spp.		X
Myrtus communis L.		X
Olea europaea L.	X	X
Pinus spp.		
Pistacia spp.	X	X
Quercus ilex L.	X	X
Rubus ulmifolius L.		X

As regards biodiversity effects due to ungulates presence experimental surveys show that these animals produce different effects: the wild boar usually has more selective pressure on plant biodiversity rejecting most of the plant species examined, although the total indirect damage is howsoever high, whereas feral goat usually eats almost all the plant species in the Park territory. It is also known that this animal is able to eat a wide range of plant species thanks to some morphological and physiological features of the digestive tract. For this reason, it eats indifferently plants with physical defences, like thorns, or containing toxic or antinutritional compounds, such as essential oils and tannins, because it has particular neutralizer enzymes in salivary gland (e.g. proline) and in gastric juices [14].

Conclusions

Our results point out that the presence of wild ungulates in the Portofino protected area causes very complex ecological and environmental impact difficult to solve. The main concern is about long term damages which cannot be seen directly, for instance those connected with the hydro-geological balance of the territory or the plant asset and its diversity. The focus considering the environmental problem, generally peculiar in protected areas, concerns difficulties in managing livestock in a correct way, that is to say maintaining the ungulate density population in harmony with the ecosystem. For this reason, the wild boar is the ungulate species that gives important troubles for future conservation, especially because it is an animal which can quickly spread all over the territory thanks to its high prolificacy, peculiar of this species, to low biological and predatory mortality and to its high adaptation to different habitats. All these effects sharpen the environmental problems and represent a serious threat to this protected area. However, some Authors [15; 16; 17] proved that a sustainable wild boars density may have beneficial impacts on soil and on the ecosystem in general because lightweight rooting action helps the soil aeration and the shuffling of organic matter.

This experimental study has the aim to show that feral goat population in Portofino protected area, at present, does not cause irreversible environmental problems, also because it eats indiscriminately almost all plant species, so that it does not generate alarming impact on plant asset and the soil structure damages are lightweight. This research argues that feral goat population living in Portofino protected area has a constant numeric quantity due to high female mortality by mastitis disease [6; 10]. The reason of this disease is that goat is a domestic species and, even if the feral goat is a rewilded animal, it conserves domestic features and it has lost some aspects typical of wild animals [22]. Finally, considering this environmental problem from a global point of view and considering real and future issues, we suggest to adopt some solutions to prevent future possible negative effects.

The best way to solve the feral goat environmental problem is the complete eradication of this species from protected areas. Removing feral goat population is a powerful conservation tool to restore ecosystems [7]. The reasons of this choice are: 1) introduced mammals are major drivers of biodiversity loss and ecosystem degradation; 2) the Portofino feral goats population came from an illegal introduction, illegally realized in the 1990s; 3) the Portofino feral goats do not belong to a significant genotype, as previous research has documented [10]; 4) feral goat is actually a rewilded domestic animal [22]. According to some Authors [7], we point out that goats have been eradicated successfully from 120 islands worldwide, and Portofino is a such promontory that could be assimilated to an island ecosystem [6]. Regarding wild boars it is thought that a number reduction would be enough in order to have an animal density in harmony with the ecosystem, but it is also important to keep this number below a threshold control [1; 4; 11; 13].

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CO-COMPOSTING AS A NEW VALORISATION STRATEGY FOR PHYTOREMEDIATED SEDIMENTS AND *POSIDONIA OCEANICA* RESIDUES

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Abstract

Sediment recycling through co-composting process to obtain techno-soils can be considered as a significant example of circular economy application concept in sediment management. Techno-soil arising from decontaminated sediments has been successfully used as constituent of peat-free substrates for growing ornamental plants.

Posidonia oceanica L., (Delile) is one of the main endemic marine plants, which grows all along the coastline of the Mediterranean Sea. Removal and disposal of plant residues on the coast poses several economic and environmental problems, given that the dead materials are generally treated as waste. However, Posidonia and other seaweed residues can be used for compost preparation. Posidonia-based compost has been proposed as a promising nursery growth for plant production.

The main objective of this paper was to co-composting *Posidonia oceanica* residues and decontaminated sediments to recycle two wastes into a peat-free growth substrate. The co-composting process was monitored during the time following physical, chemical, biochemical, and toxicological parameters. Posidonia-based composts responded to the main characteristics for the preparation of growth substrates, in terms of electrical conductivity, organic carbon content and germination index.

The mineral characteristics of decontaminated sediments were organically enriched during the co-composting process with *P. oceanica* residues, especially in terms of humic carbon and enzymatic activities (butyrate esterase).

keywords: *composting process; peat-free growth substrate; Posidonia oceanica L.; sediment; techno-soil*

Introduction

Phytoremediation can represent an effective technology for decontaminating marine dredged sediments and converting them into a techno-soil (Masciandaro et al., 2014; Doni et al., 2015). Techno-soil arising from decontaminated sediments has been successfully used as constituent of peat-free substrates for growing ornamental plants (Mattei et al., 2018). Moreover, the physical and chemical characteristics of the techno-soil can be considerably improved and valorised through a process of co-composting of decontaminated sediments and green wastes (Mattei et al., 2016) or with sewage sludges and wood chips (Macía et al., 2014). It is noteworthy that sediment recycling through co-composting process to obtain techno-soils has been reported as a significant example of circular economy application concept in sediment management (Lord, 2017). Bearing in mind that hundreds of millions of cubic meters of sediments are dredged every year to keep waterways and harbours navigable (Netzband and Adnitt, 2009), it is worth recalling that, in a vision of sustainability and of circular economy, the proper management of such sediments plays a vital role.

Posidonia oceanica L., (Delile) is one of the main endemic marine plants, which grows all along the coastline of the Mediterranean Sea. Removal and disposal of plant residues on the coast poses several economic and environmental problems, given that the dead materials are generally treated as waste. However, composting preparation can be realistic alternatives to landfill disposal (Cocozza et al., 2011). Moreover, Posidonia and other seaweed residues can be used for compost preparation in Italy at 20% (w/w) of initial mixture (Italian Regulation 75/2010). The main objective of this paper was to co-composting *Posidonia oceanica* residues and decontaminated sediments to recycle two wastes into a growth substrate.

Materials and methods

Two different types of compost based on *Posidonia oceanica* residues were obtained by composting, mixing (w/w): 20% residues of *P. oceanica* with 80% green residues, (Mixture 1, M1), and 20% residues of *P. oceanica* with 60% green residues and 20% already decontaminated sediments (Mixture 2, M2). Each heap was about 3 m^3. All the materials presented suitable characteristics for compost preparation, according to Italian legislation (Reorganization and review of the fertilizer regulations 75/2010) (Table 1). *P. oceanica* residues have been recovered on the coast (43° 19’ 18.2” N, 10° 27’ 27.5” E) and were subjected to periodic washing to reduce salinity (< 2.5 dS/m) and remove sand residues. Decontaminated marine sediments through phytoremediation and landfarming come from two previous research projects (Masciandaro et al., 2014; Doni et al., 2105). The composting activity lasted approximately 6 months (June 2017 - December 2017).

Volatile solids (VS) were measured at 550°C as loss on ignition (EN 13039). Electrical conductivity (EC) was estimated on water extract (1:5, v/v) following EN 13038 protocol. Total organic carbon (TOC) and total nitrogen (TN) were assessed by RC-412 multiphase carbon and FP-528 protein/nitrogen (LECO Corporation). Humic substances were determined according to the method of Ciavatta et al., (1990). The germination test was carried out following Hoekstra et al. procedure (2002), on water extract (1:5, v:v), with *Lepidium sativum* seeds. Butyrate esterase (EC 3.1.1.1) activity was measured according to Marx et al. (2001) and Vepsäläinen et al. (2001) protocols. Analysis of variance (ANOVA) was used to evaluate the differences (p < 0.05%) between times and mixtures.

Table 1. Main physical-chemical characteristics of materials

	Posidonia oceanica **residues**	**Green wastes residues**	**Decontaminated sediments**	**Italian Regulation 75/2010**
Volatile solids (% ds)	83.2	80.2	4.11	-
Electrical conductivity (dS/m)	3.15	0.242	0.282	-
Cd (mg/kg ds)	0.252	<0.1	<0.1	< 1.5
Cu (mg/kg ds)	6.95	10.6	51.7	< 230
Ni (mg/kg ds)	10.2	35.6	44.3	< 100
Pb (mg/kg ds)	7.21	8.2	46.1	< 140
Zn (mg/kg ds)	25.2	31.2	23.7	< 500

Results and discussion

The bio-oxidative phase was reached for M1 mixture in the first 120 days of composting (temperature range 32-65°C), while M2 mixture remained in the mesophilic stage (temperature range 25-40°C). These results are consistent with other authors findings: Saidi et al. (2009) reported a thermophilic peak during composting of *Posidonia oceanica* residues mixed with vegetables wastes, while Mattei et al. (2016) stated a mesophilic phase up to 35°C throughout the treatment period for sediment and green waste composting process. Physical-chemical and biochemical characteristics of the mixtures during co-composting process are reported in Table 2. VS represented an amount of organic matter available for microbial degradation; it significantly diminished during the bio-oxidative phase (120-150 d). In M1, the decrease continued also in the cooling and maturating phases (200 d), as a consequence of mineralization process. On the other hand, in M2 the VS slightly increased during the last phase of composting process, probably due to the activities of colonizing microbial population and earthworms, and growth of fungi and actinomycetes. A similar trend can be noticed for TOC, which reached final values above the minimum threshold value fixed at 4% for plant growth substrate (Italian Regulation 75/2010). TN significantly increased, probably as a consequence of a concentration effect due to the weight reduction of the pile (Paredes et al., 2000). Gusain et al. (2018) reported an overall nitrogen gain in composting process of aquatic weed as a consequence of the different rates of ammonification and nitrification in the piles. Notwithstanding the marine origin of *P. oceanica* residues, the initial electrical conductivity (EC) values ranged between 0.388 and 0.613 dS/m and decreased during the composting process. This decrease could be due to the combined effect of release of organic acids and soluble ions leaching (Mattei et al., 2016). Moreover, the EC values were also below the threshold of 0.7 dS/m for plant growth substrate (Italian Regulation 75/2010). Butyrate esterase activity, a non-specific esterase involved in the cycling of carbon related to live biomass content (Wittmann et al. 2004), represented an indirect measure of overall microbial activity. It significantly decreased after the thermophilic stage in M1 mixture, underlining the successful stabilization of fresh organic matter during composting process. On the other hand, in M2, where sediment was present, the level of butyrate activity slightly increased over time, especially in the maturation phase. It is noteworthy that also in M2 was reached comparable values of butyrate esterase to M1, thus meaning the enrichment effect in microbial activity of *Posidonia* residues and green wastes in sediment co-composting.

Table 2. Physical-chemical and biochemical characteristics of the composts (mean, n=3). Volatile solids, (VS, % ds); pH; Electrical conductivity (EC, dS/m); Total Organic Carbon (TOC, %C ds); Total Nitrogen (TN, %N ds); Butyrate esterase (mmol MUB/ kg ds). Different lowercase letters mean values significantly different (HSD Tukey - $p<0.05$) over time within treatment. Different capital letters mean values significantly different (HSD Tukey - $p<0.05$) between treatment within time.

	days	**VS**	**EC**	**TOC**	**TN**	**Butyrate esterase**
M1	**0**	89.6 aA	0.613 aA	41.6 aA	0.938 aA	15608 bA
	30	78.1 abA	0.370 bA	35.6 abA	1.301 bA	17536 aA
	80	75.3 bA	0.394 bA	30.2 abA	1.378 bA	12353 cA
	150	72.0 bcA	0.427 bA	28.5 abA	1.300 bA	10917 cA
	200	62.9 cA	0.294 cA	27.9 bA	1.275 bA	12237 cA
M2	**0**	18.5 bB	0.388 aB	7.20 abC	0.201 aC	8172 aB
	30	16.2 bB	0.403 aA	7.33 ab C	0.338 bcB	9265 aB
	80	15.4 cB	0.405 aA	6.34 bB	0.300 bB	7734 aB
	150	22.6 aB	0.210 bB	8.47 aB	0.361 cC	9493 abA
	200	20.9 abB	0.147 cB	6.62 abB	0.321 bC	10728 bA

The transformations of organic matter, with special reference to the formation of humic substances (humification), have a key role in valorising sediments in co-composting process. All results are reported in Table 3. As expected, M1 mixture presented the highest values of humic (HC) and non-humic carbon (NHC); during the co-composting process, the stabilization phase had involved both these fractions, thus resulting with lower values with respect to the initial ones. Conversely, in M2 there was a stabilization of both humic and non-humic fractions. The results about humic substances confirmed Mattei et al. (2016) findings about sediment co-composting with green waste: a better preservation of humic substances of the mixture with decontaminated sediments (M2) due probably to the adsorption of humic matter onto sediment solid phase and formation of organo-mineral complexes. The germination index (GI) is an important measure to assess the maturity and evaluate phytotoxicity of different matrices, like compost. The level of germination tests (Table 3) found was considered not phytotoxic for seed germinations: in fact, all values were higher than 50%, the threshold value expressing phytotoxicity (Zucconi and De Bertoldi, 1987).

Table 3. Humic carbon and germination index of the composts (mean, n=3). Humic carbon, (HC, %C ds); Non-humic carbon, (NHC, %C ds); and germination index (GI, %). Different lowercase letters mean values significantly different (HSD Tukey - $p<0.05$) over time within treatment. Different capital letters mean values significantly different (HSD Tukey - $p<0.05$) between treatment within time.

	days	**HC**	**NHC**	**GI**
M1	**0**	9.85 aA	6.73 aA	150 aA
	200	7.93 bA	4.79 bA	158 aA
M2	**0**	4.00 aB	1.97 aB	116 aB
	200	5.32 aB	2.51 aB	139 bA

Conclusions

The co-composting process resulted effective in valorising chemical, physical and biochemical properties of *P. oceanica* residues and decontaminated sediments. The mineral characteristics of decontaminated sediments were organically enriched during the co-composting process with *P. oceanica* residues, especially in terms of humic carbon and biological activity.

Both *P. oceanica*-based composts responded to the main characteristics for the preparation of growth substrates, thus allowing to obtain a satisfactory peat-free growth substrate.

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POTENTIAL ANTHRAX LETHAL FACTOR (LF) INHIBITORS. SELECTION AND PROTECTION STRATEGIES FOR THE ENVIRONMENT, FOOD AND THE HUMAN BODY

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Abstract

Current research has identified a series of compounds containing the rhodanine ring that can effectively inhibit lethal factor (LF), the basic component of anthrax. The Molecular Docking technique has once again found its usefulness in selecting those potential inhibitors of the lethal factor of anthrax and finding the most effective strategies for protecting the environment, food and the human body.

keywords: *lethal factor (LF), anthrax, molecular docking, rhodanine compounds*

Anthrax is an infectious disease caused by the bacterium Bacillus anthracis [1]. This bacterium infects the human and animal body, attacking the respiratory system, the skin or the digestive tract. Depending on the way of entering the body, anthrax may or may not be deadly because the skin anthrax is rarely deadly, while inhaled anthrax, a potential bioterrorism weapon, is very dangerous and fatal for both the human and animal body [2]. After anthrax spores are inhaled, they adhere to alveolar macrophages where they germinate. Then the bacteria migrate into the lymph nodes where it multiplies very quickly [3] and secretes an exotoxin composed of three proteins.

The three Bacillus anthracis secreted exotoxin constitutive proteins are: protective antigen (PA, 83 kDa), lethal factor (LF, $Zn2+$ -metalloprotease, 90 kDa), edema factor (EF, 89 kDa) [3-5].

PA is a protein consisting of 4 domains [6] that bind to the surface of the receptor cell through the terminal carboxyl groups. After proteolytic activation by furin-protease, PA releases an N-terminal fragment (PA20, 20 kDa) and a C-terminal fragment (PA63, 63 kDa) [6]. PA63 heptamerizes and is capable of binding both LF and EF. After endocytosis (membrane transport) of the resulting complex, the LF and EF molecules are released and exert their toxic action [7].

In the literature, it has been shown that LF represents an essential target for therapeutic agents that can inhibit its catalytic activity or block association with PA [8, 9]. In this respect, our research will be directed only to this component (LF) of Bacillus anthracis.

The Lethal Factor (LF)

As shown, lethal factor (LF) is a metalloprotease capable of joining six members of the MAPKK protein family (the only known cellular substrate of LF) via the N-terminal [10]. The LF action blocks the signal of MAPKK proteins to other immune cells to fight infection [11]. LF consists of four structural domains. Domain I is the overlap of LF PA binding. Domain II resembles the toxin-enzyme VIP2 secreted by Bacillus cereus although it does not exhibit a catalytic activity. Domain III is inserted into field II and appeared as a result of duplication of structural elements of domain II. Domain IV, which resembles field I, is the catalytic center of the LF [12]. Domains II, III and IV together form a 40Å groove in which a peptide of 16 amino acids represents the N-terminus of binding its natural MAPKK-2 substrate.

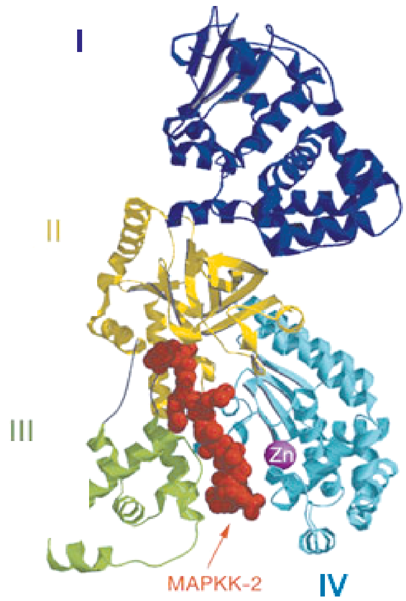


Figure 1. The structural domains of LF [12] (PDB code 1J7N) [13]

The application of computational methods in studying the formation of intermolecular complexes is the subject of intense research in recent years. One of these methods called "Molecular Docking" can be used to predict the favorable orientations of a molecule (ligand) towards a second molecule (receptor) so that binding of the two molecules will form a stable complex [14]. Knowing the preferred guidelines can be used to predicting the binding affinity between two molecules using scoring functions. This method is also commonly used in predicting the binding affinity of small molecules (drugs) for target proteins or the biological activities of these molecules (drugs) [15].

The "Molecular Docking" technique can be regarded as a "key locking" problem (Figure 2), well finding that relatively correct "key" (small molecule - drug) orientation that will open the "the lock".

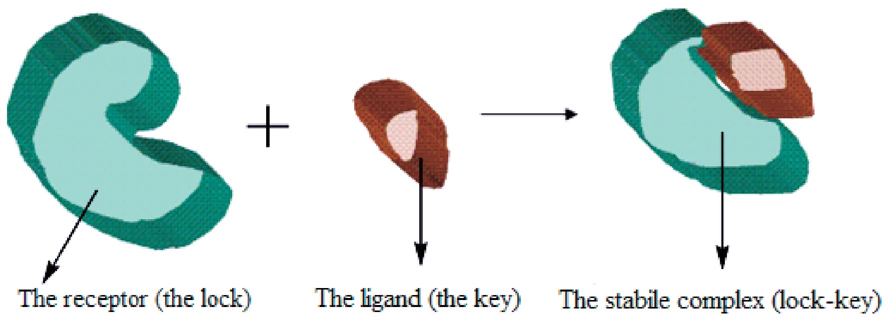


Figure 2. Forming of the key-lock complex

The simulation of the "docking" process is much more complicated because the protein and the ligand are separated by a certain physical distance, and to find a favorable orientation of the ligand it performs a certain number of spatial or conformational movements. These movements involve translation or rotation movements as well as internal changes in the structure of the ligand, that is to say the torsion angles [16]. All these conformational spatial movements induce an energy "cost" of the system so that after each movement the total energy of the system can be calculated. The total energy of the system can be estimated using the scoring function. Most scoring functions are based on the field of molecular mechanics forces. A low negative energy indicates the stability of the system.

Results and discussions

Both the structure of the structural domains of LF [12] (PDB code 1J7N) [13] and the structures of the 4 compounds containing the rhodanine ring were prepared for the "molecular docking" process using the Hex 5.0 program [17], being modeled using 3D parametric functions encoding both surface form, electrostatic charge and the potential distribution. These parametric functions are based on basic spherical or polar orthogonal functions.

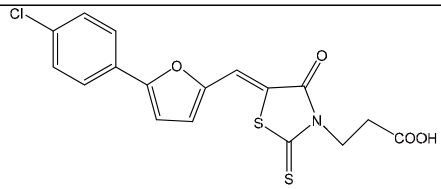
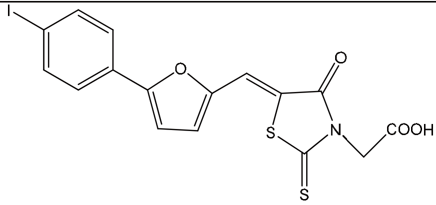
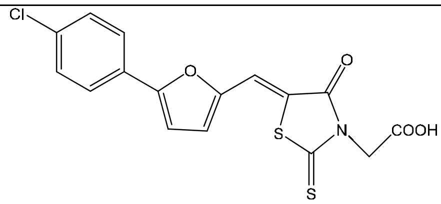
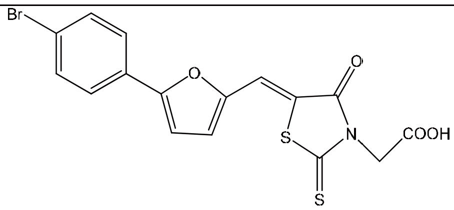
Compounds	Structure
1	
2	
3	
4	

Table 1. The structures of the 4 compounds involved in our research

In order to obtain a more efficient "docking", the Fourier algorithm was used to accelerate search for the most favorable docking orientations in the ligand translation molecule, and on the other hand the polar approximation spherical, which also allows the translation and rotation of the ligand to generate and evaluate optimal orientations.

Since the translation and rotation of the ligand, the docking process is more complete Table 2 shows the values of the binding energies (kJ / mol) (E_{tot}) of the rhodanine compounds-LF.

Binding energies E_{tot} (kJ / mol)	Rhodanine compounds			
	1-LF	2-LF	3-LF	4-LF
	-287.70	-289.19	-278.64	-283.38

Table 2. Values of binding energies (affinities) of rhodanine compounds-LF complexes

From the data presented in Tables 1 and 2 it can be concluded that Cl-rhodanine compound (structure 3 in Table 1)-LF, having the lowest energetic values, represent potential inhibitor of LF.

Figure 3 shows the structure of Cl-rhodanine-compounds-LF complex with the lowest energy binding (kJ / mol).

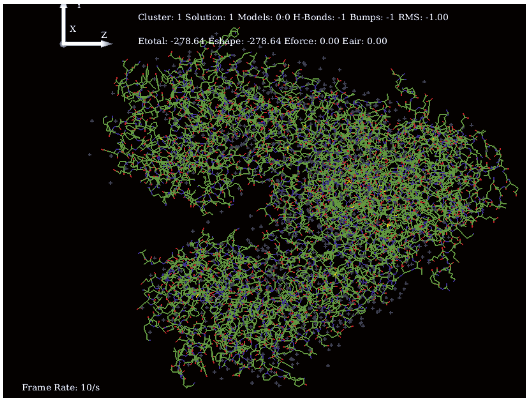


Figure 3. The structure of Cl-rhodanine-compounds-LF complex

Conclusion

In this article, the "Molecular docking" technique was applied to select those rhodanine components with possible inhibitory activity for the lethal factor of anthrax (LF). From the data presented in Table 2, it can be concluded that the highest affinity for LF is compound 3 from Table 1. The data presented are in agreement with the experimental data. This technique has once again found its usefulness in selecting those potential inhibitors of the lethal factor of anthrax and finding the most effective strategies for protecting the environment, food and the human body.

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ASSESSMENT OF NUTRACEUTICAL FEATURES OF DIFFERENT FOODS FROM CONVENTIONAL AND MYCORRHIZED FARMING

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Abstract

In the present study the total antioxidant capacity produced from conventional farming and farming with arbuscular mycorrhizal fungi, bacteria and Streptomices derived from rhizosphere was evaluated using ORAC assays. Total phenolic content was also analyzed in order to evaluate the influence of farming on bioactive compounds such as polyphenols. The obtained results showed that mycorrhized farming, compared with conventional one, improved the quality of foods increasing their antioxidant activity and the total phenolic content. The treatment increased significantly the antioxidant activity in all the products examined: 27 % in wheat, 22% in corn, 18% in potato, 12 % in milk, 24 % in lettuce, 35% in pear and 8% in apricot. A significant increase of polyphenolic content was also seen in wheat, corn, potato, milk but not in lattuce, pear and apricot. In conclusion, the use of this consortium of fungi and bacteria represents an ecologically and economically relevant solution in cultivations to get crops with improved nutritional and nutraceutical value.

keywords: *antioxidant activity, mycorrhizal crops, nutraceutical, polyphenols*

Introduction

In recent years, an ever greater interest has been directed to sustainable agricultural production and in this direction numerous research has been carried out to develop new technologies that would allow reducing chemical inputs, fertilizers and pesticides, thereby diminishing the production costs and contributing to the safeguard the environment (Zijlstra et al., 2011). Particular attention has been paid to the study of those rhizospheric microorganisms providers of key agro-ecological services, and has been at the forefront of generating and promoting sustainable agricultural production technologies. These microorganisms, often referred to as "bioenhancers", "biofertilizers", and/or "bioprotectors" play a primary role in improving the growth, nutrition and health of plants with which they interact (Giovannetti et al., 2013). The most important suppliers of these agro-ecological benefits are arbuscular mycorrhizal fungi (AMF), which can form symbiotic associations with mutual beneficial effects with the roots of most terrestrial plants. Several studies have clearly demonstrated the fundamental role played by these fungi at the soil-root interface, thereby improving the multi-trophic and protective interactions affecting the productivity, competitiveness and survival of the majority of plants species in both natural and cultivated ecosystems (Fouad et al., 2014).

On the basis of these premises it is clear that agricultural productions using performing AMF consortia may have a lower environmental impact, as they need less fertilizers, less irrigation, less plant protection treatments for the defense of the products, in particular of herbicidal products (Daisog et al., 2012). Recent studies have also showed that the correct use of these microorganisms induces in the plant higher production of antioxidants and can increase the nutraceutical value of its products (Giovannetti et al., 2012; Ceccarelli et al., 2010). There is increasing evidence that several plant secondary metabolites (phenolics, carotenoids, lutein, β-carotene, lycopene, zeaxanthin etc) can exert protective functions in the human body, and thus they are starting to be recognized as important integrands of a nutritious diet that promotes long-term health. In fact, some plant secondary metabolites can act as antioxidants by quenching free radicals thus reducing the risk of health problems related to the production of free-radicals, such as cancer and atherosclerosis (Rao & Rao 2007; Simopoulos 1998). Improving the quality of food by utilizing ecologically sustainable farming practices is therefore a priority that should be supported and encouraged, and which goes hand in hand with the acquisition of healthier and more secure eating habits at the same time. Some data regarding the evaluation of AMF effect on a crop are available but the ability of AMF to improve nutritional quality need further and more global investigations. The aim of the present work was to evaluate the impact of a mixture containing selected mycorrhizal fungi, bacteria and Streptomices derived from rhizosphere on total antioxidant activity of different crops. The results were compared with the same crops untreated with mixture.

Material and methods

The studied foods came from different parts of Italy and the milk is derived from cows fed with mycorrhized feed. All standards and reagents were of analytical grade.

Inoculum preparation

The commercial inoculum “Mycosat F” from Centro Colture

Sperimentali (Aosta, Italy) was a mixture of mycorrhizal fungi, bacteria such as Streptomices derived from rhizosphere. The soil was inoculated at sowing time per hectare with 2-6 kg of fungi and 1-5 Kg of bacteria at the concentration of 10^5 units forming colonies.

Samples extraction

Samples extraction was performed according to the Bacchiocca method (Bacchiocca et al., 2006), with some modifications. Each sample was weighted (10g) and suspended (1:2 w/v) in acetone (70:30 v/v) with 5% of perchloric acid (v/v), shaken for 2 h in the dark at 4°C, then centrifuged at 5000 *g* for 20 min. The extraction was repeated twice and the supernatants were collected and used for the assays.

Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay followed the original method (Cao et al., 1997) with minor modifications (Ninfali et al., 2005). Briefly, 100µl of each extract was used to prepare dilution series (1:10, 1:100: 1:1000) and added to a final reaction mixture (final volume 1 ml) containing 800 µl sodium phosphate buffer (75 mM, pH 7.0) with fluorescein sodium salt (0.05 mM) plus a 100 µl solution of 2,20-Azobis (2-methylpropionamide) dihydrochloride (400 mM). As for standard solution, 100 µl of 50 mM 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) was added instead of sample; the control consisted of sodium phosphate buffer (75 mM, pH 7.0). Fluorescence was recorded every 5 min at 37°C at 485 nm excitation, 520 nm emission for 60 cycles using a Perkin-Elmer Victor™ X^3 apparatus (Waltham, MA). The ORAC was expressed in micromoles of Trolox equivalents per 100 g of fresh weight (TE mmol/100g); the value derived from the following formula: (As-Ab/At-Ab)*KAH. As, area subtended by the curve (AUC) of fluorescein in the sample (calculated by the program Perkin Elmer 2030 Work Station). At and Ab, Trolox and control AUCs, respectively. K, dilution factor; A, Trolox concentration (moles L^{-1}); H, ratio between the extract and the product (volume/weight, L g^{-1}).

Total Phenolic content

Total phenolic content (TPC) of extracts was determined according to Folin-Ciocalteu's colorimetric method described by Singleton (Singleton et al., 1999) with some modifications. Gallic acid was used as the standard and total phenolic content was expressed as mg of gallic acid equivalents (GAE) / 100 g of fresh weight.

Statistical analysis

Results of ORAC, TPC assays are the average (± standard deviation) of three independent experiments, and each experiment was performed in triplicate. Results were statistically analyzed using the Student's t test. Differences were considered significant for $p \leq 0.05$.

Results

The antioxidant capacity of extracts was evaluated with the ORAC assay and the results are presented in Table 1. The unit of ORAC increased significantly in mychorrhizal products compared to those non mychorrhizal. The increase of total antioxidant capacity was of 27 % in wheat, 22% in corn, 18% in potato, 12 % in milk, 24 % in lettuce, 35% in pear and 8% in apricot.

Table 1. Total antioxidant activity in non mycorrhizal (NM) and mycorrhizal products (M) evaluated by ORAC test. Data are the mean values ± SD. Within each product values with asterisk are significantly different ($p \leq 0.05$).

Sample	Type	ORAC (µmol TE/100 g Fresh weight)
	M	3365 ± 50*
Corn	NM	1519 ± 28
	M	1857± 30 *
Potato	NM	506 ± 10
	M	600 ± 17 *
Milk	NM	515 ± 9
	M	577 ± 8*
Lettuce	NM	1320 ± 160
	M	1640 ± 80*
Pear	NM	550 ± 50
	M	745 ± 60*
Apricot	NM	487 ± 11
	M	528 ± 10*

The total polyphenol content (TPC) evaluated by Folin-Ciocalteu's assay is shown in table 2. The total polyphenol content was significantly higher in mycorrhizal wheat, corn, potato and milk respect to the non mycorrhizal products. In particular in wheat the content of polyphenols increased by 40%. No significantly difference has been found in pear, apricot and lettuce.

Table 2. Total content of the polyphenols in non mycorrhizal (NM) and mycorrhizal products (M). Data are the mean values ± SD. Within each product values with asterisk are significantly different ($p \leq 0.05$).

Sample	Type	TPC (mg of GAE/100gr Fresh weight)
Wheat	NM	166 ± 2
	M	233 ± 18*
Corn	NM	101 ± 2.5
	M	117.4 ± 3*
Potato	NM	35.4 ± 1,0
	M	40.8 ± 0,0 *
Milk	NM	56.5± 1.5
	M	61.7 ± 0.5 *
Lettuce	NM	39± 10
	M	44± 8
Pear	NM	27 ± 4
	M	34 ± 3
Apricot	NM	23.9± 2.6
	M	22 ± 2.5

Conclusions

In this study we evaluated the effect of selected mycorrhizal fungi and bacteria of rhizosphere on total antioxidant capacity of some foods. The results show that arbuscular mychorizzal fungi and bacteria consortia improve the antioxidant and nutraceutical values of the analyzed products by increasing their content of bioactive molecules. These results are in agreement with a recently published study (Raiola et al., 2015) where it has been reported the ability of the same consortia of mycorrhizal fungi and bacteria to improve nutritional quality of foodstuffs. It was also reported that the use of a similar microbiological consortium in vineyard cultivation increased the antioxidant properties of *Sangiovese* wines with respect to wines from conventional agriculture (Gabriele et al. 2016). The data from the scientific bibliography agree to give great importance to the antioxidants substances contained in foods as the main responsible for reducing oxidative stress and preventing metabolic, cardiovascular and tumor pathologies (La Marca et al. 2012; Longo et al. 2011; Conklin 2000). So are needed further investigations in order to establish the effect of these products on human health.

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