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Volume 44

Prathamesh Gorawala • Srushti Mandhatri
Editors

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Preface

This book provides the latest developments in agricultural research.

Chapter 1 - The phenolic complex of grapes, represented by a wide range of compounds, is characterized by powerful antioxidant properties, determining its multivariate biological effect on human body. The assortment of functional food products from grapes, including wines, as essential elements in human ration, is constantly increasing, especially in the areas with unfavorable environmental conditions. At the same time, the observed climate changes make adjustments to the phenology, agricultural biology, metabolism of grape plants, affecting biological value and crop quality, and necessitating a revision in classical technologies of viticulture and winemaking, including the selection of new areas for establishing vineyards. In this regard, the studies aimed at identifying quantitative relationships between territorial ampeloecological resources and the formation of a complex of grape phenolic compounds, as well as their transformations during crop processing, gain new relevance. In the authors' work, these issues are considered in relation to the areas of the Crimean Peninsula suitable for viticulture on the example of grapevine cultivars 'Cabernet-Sauvignon' and 'Kokur Belyi' (Vitis vinifera L.). Similarities and dissimilarities of agroecological resources of the vineyards located in various geographical objects are established using long-term data from the network of stationary weather stations of the Crimean Peninsula, digital terrain models SRTM-3 and ASTER GDEM, global climate model Worldclim ver. 2.0, mathematical modeling and statistical analysis. Asignificant factor of differentiating the territories, is the set of parameters of their heat supply. The HPLC method was used to determine the content of the most chemically labile monomeric and dimeric forms of phenolic antioxidants whith the highest bioavailability in the structural elements of grape berries and wines from various vineyards: the level of differences in the phenolic complex was assessed. Based on the identified correlations, it was shown that an increase in the heat supply of the territories, on one hand, was accompanied ($\alpha<0.05$) by accumulation of phenolic acids, flavonols, flavan-3-ols and procyanidins in grape seeds. On the other hand, it contributed to the oxidative polymerization of phenolic components during grape ripening and processing (also due to an increase in monophenol monooxygenase activity and must pH), followed by a decrease in the content of mono- and dimeric components in the skin and pulp of grapes, and flavan-3-ols in wines. This is naturally manifested in 'Kokur Belyi' cultivar. Moisture supply of vineyards adjusts the formation of a complex of phenolic antioxidants of' 'Cabernet-Sauvignon' grapes and wines. An increase in the heat supply of vineyards was accompanied by an increase in the proportion of malvidin-3-O-β-D-glucoside and its derivatives, and a decrease in the proportion of cyanidin and petunidin and their derivatives in the anthocyanin complex of berries. The territories, capable to obtain grapes and wines, enriched in phenolic antioxidants of various functional directions, were specified. This work is a part of comprehensive study carried out in the FSBSI Institute Magarach of the RAS on the zoning of ampeloecological resources of Crimea, including the identification and geoinformation modeling of multi-parametric relationships of spatial distribution of agroecological characteristics and metabolites of a grape plant (taking into account varietal specificity), and quality of crops and wine products.

Chapter 2 - For decades, chemical fertilizers have been used to boost agricultural crop yield. However, due to high procurement costs, environmental contamination, and the potential for land degradation from improper application, there is a current trend towards finding alternative solutions. The global demand for food that is both high-quality and produced sustainably, while preserving soil biodiversity, has led to a demand for premium organic ingredients. This can be achieved through utilizing organic waste in rural and urban areas by applying composting technology to produce organic fertilizers, thus reducing the use of harmful chemicals and protecting the environment. Bio-organic agriculture uses advanced biotechnologies, primarily relying on organic amendments such as nitrogen-fixing bacteria and humic substances, to produce crops. Therefore, utilizing bio-organic farming methods is a crucial step towards achieving agricultural sustainability. In this chapter, the authors highlight the benefits of four organic and biological amendments, including spent grain, vermicompost, PGPR, and humic substances, for enhancing soil fertility and health in different environmental conditions, ultimately promoting agricultural sustainability.

Chapter 3 - Humic substances (HSs) are typically found in nature in soil, plant, and natural water, and they are fundamentally created from organic matter decomposition. The main active components of HSs contain humic acid, humus, ulmic acid, fulvic acid, humin, and certain microelements. HSs are currently not only natural, but also harvested and engineered, and have been widely exploited in soil, plant, and animal productivity for the past 20 years. In the soil, HSs have the potential to improve a wide range of soil and play an essential role in the microorganism's activity in the environment. In addition, HSs are a part of soil humus and they represent the largest pool of recalcitrant organic carbon in the terrestrial environment. Also, these valuable compounds have bio-stimulative effects on plants, where they could boost the photosynthetic activity, and improve root system and plant growth, as well as reduce stress damages. The HSs also could affect specific metabolic pathways in plants leading to improved tolerance of abiotic and biotic stresses. Today, our soils are low on these HSs, and as a result, animals and chickens are not receiving adequate amounts in their regular diet. Many researches have indicated that when soil humus percentages fall below 2%, the soil cannot provide sufficient amounts of HSs materials into the crops grown for the quantities needed by livestock animals. Recently, HSs have been used as one of the alternative feed additives in animal husbandry to improve the economics and ecology of animal production by increasing the growth rate, improving feed efficiency and immunity, reducing the risk of disease, and increasing animal products quality. This book chapter discusses the functions that HSs play in soil, plant, and animals to keep agricultural sustainability. The authors assemble and describe the applications and role of such HSs in agricultural and environmental ecology.

Chapter 4 - Phenolic compounds are very important for grapes and wines quality since these compounds can take part in color, mouthfeel and wine ageing potential, and they are also related to the human healthy properties of moderate wine consumption. The content of phenolic compounds in grapes depends on different factors, such as variety, climatic and geographical factors, cultural practices, and the stage of grape ripeness. Foliar application of biostimulants has been studied in the last years in order to mitigate the effect of climatic change in grapes and to enhance their composition and quality. In this work, it was studied the effect of foliar application of methyl jasmonate plus urea (MeJ + Ur), in Tempranillo vineyard, on the content of phenolic compounds in grapes and wines, during two consecutive vintages. These compounds (anthocyanins, flavanols, flavonols, phenolic acids, and stilbenes) were analysed, in both grapes and wines, by high performance liquid chromatography (HPLC). The effect of foliar treatment was season dependent, probably due to the differences in pre-harvest rainfall recorded. In the first season, the MeJ + Ur treatment favored the biosynthesis of anthocyanins, increasing their content in grapes; however, this effect was not observed in the wines. In the second vintage, the MeJ + Ur foliar application did not enhance the anthocyanin content in grapes or in wines. With respect to the other phenolic compounds, the foliar treatment did not improve their content in grapes, except for total flavonols in the second vintage. Foliar application of MeJ + Ur seems to be a good tool in order to enhance the anthocyanin content in grapes, which it is very interesting due to anthocyanins are the main phenolic compounds in red varieties, and they are responsible for the wine red color. Nevertheless, further studies should be carried out to achieve a better knowledge of the plant response to this treatment according to the climatic conditions. Moreover, it is necessary to study how to transfer the improvement reached in the grapes anthocyanins content to the final wines.

Chapter 5 - Soil organic carbon (SOC) is a minor but critical component of most soils of the world, involved in practically all ecological services provided by soils, such as nutrient cycling and storage, biological diversity, water retention through favorable structure, and many others. In addition, SOC is one of the most important global carbon pools, containing more C than the atmosphere and biomass combined, and with a longer residence time. Thus, SOC has been proposed as a promising alternative for removal of atmospheric CO2, by means of plant biomass production and its natural incorporation within the soil mineral matrix. Such a strategy is very opportune because SOC sequestration always improves soil quality for agricultural and environmental functioning and would also achieve middle-term C sequestration with a potentially significant mitigation of global warming. In such context, identifying promising environments and situations for SOC sequestration throughout the world becomes important, and the humid tropics appear amongst the most feasible alternatives, offering favorable year-round temperatures, immense areas with adequate water availability, relatively low costs for agricultural and preservation/conservation land, huge biodiversity of both cultivated and spontaneous plants, and a typically abundant workforce. Notwithstanding, reaching such potential would require adequate scientific knowledge on factors and processes involved in SOC sequestration, which would ideally be specific for each of the various tropical environments and soils, and more critically, are often different from those in temperate regions. This chapter is not intended to be an exhaustive literature review, but rather to present novel and common-sense perspectives on the most important factors and practices affecting SOC retention and dynamics in the tropics, aiming to improve current and future management, policies and research initiatives.

Chapter 6 - Soils in the Cerrado, which covers 204 million hectares (24%) of Brazilian land area and is one of the world's largest agricultural frontiers, are characterized by low pH and, consequently, high Al3+ content and low P and Ca2+ contents. Soil pH (CaCl2) values below 4.4 increase the availability of aluminum and manganese, whereas a pH range of 5.4–6.4 ensures the availability of most nutrients essential for crops. Low Ca2+ content and high Al3+ content (Al toxicity) in the subsoil affect root growth, restricting the capacity of plants to absorb water and nutrients and limiting productivity. To contribute to the development of alternative methodologies for correcting acidity in the surface and subsurface layers of the soil, different methodologies for the surface application of limestone, gypsum, and hydrated lime, in no-till and agropastoral systems and their effects on soybean and maize yields were researched at the Advanced Research Center and Development for Rubber and Agroforestry Systems of the IAC of the APTA, which is located in the municipality of Votuporanga, SP, Brazil. This chapter presents the results of these researches.

Chapter 7 - Poverty reduction of the population below 10% was targeted by the government of Indonesia. The program called BEKERJA (Bedah Kemiskinan Rakyat Sejahtera) or poverty alleviation for peoples' prosperity. The program's target is to make poor farmers free from poverty through the maximized use of their yards. The executors were 4 (four) main institutions and 3 (three) supporting institutions under the Ministry of Agriculture in a mandate to achieve the farmers' welfare. This chapter presents the results of the programs' implementation, performance, and the obstacles faced to get an appropriate strategy for future poverty alleviation. The research locations were representative of the 4 (four) institutions, namely Banten, West Java, South Kalimantan, and East Java Provinces. Respondents were 15 related institutions and 30 farmers. Data was collected through group and individual interviews, as well as field observations which were then analyzed qualitatively. Results: program implementation is synergized with the main tasks of each institution. The average performance of the program was 30% and 10 aspects of obstacles were found as the result of a lack of coordination among the institutions. It was concluded that the program's implementation should be under the integration of all institutions as a team in a certain area that was approved by a responsible ministry. The policy's implication is that strategy on poverty alleviation should be tackled under inter-institution coordination.

Chapter 8 - The amount of waste oil produced by people in parallel with the level of life and development with the increase in population in the world has increased. As a result, the fact that the waste oils given to nature become threatening to the environment and life has caused the waste oil problem. All waste vegetable oil must be included in the waste oil collection system in order to prevent environmental pollution, protect the environment and human health. The users of this system are people producing waste oil. For this reason, people need to remove waste oil consciously. In this study, a survey has been conducted in Bursa with 384 participants in order to determine the level of knowledge about how people evaluate, collect and dispose of waste vegetable oil from their houses. The results of the survey have been statistically analyzed with the analysis of variance method. As a result of this survey, it was concluded that the people of Bursa do not have sufficient awareness about waste oil collection, are not aware of Waste Vegetable Oil Collection Centers or the number of these centers is not sufficient.

Chapter 1

Variability of Agroecological Resources of Crimea: Influence on the Formation of Complex Phenolic Antioxidants in Grapes and Wines

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Abstract

The phenolic complex of grapes, represented by a wide range of compounds, is characterized by powerful antioxidant properties, determining its multivariate biological effect on human body. The assortment of functional food products from grapes, including wines, as essential elements in human ration, is constantly increasing, especially in the areas with unfavorable environmental conditions. At the same time, the observed climate changes make adjustments to the phenology, agricultural biology, metabolism of grape plants, affecting biological value and crop quality, and necessitating a revision in classical

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technologies of viticulture and winemaking, including the selection of new areas for establishing vineyards. In this regard, the studies aimed at identifying quantitative relationships between territorial ampeloecological resources and the formation of a complex of grape phenolic compounds, as well as their transformations during crop processing, gain new relevance. In our work, these issues are considered in relation to the areas of the Crimean Peninsula suitable for viticulture on the example of grapevine cultivars 'Cabernet-Sauvignon' and 'Kokur Belyi' (*Vitis vinifera* L.). Similarities and dissimilarities of agroecological resources of the vineyards located in various geographical objects are established using long-term data from the network of stationary weather stations of the Crimean Peninsula, digital terrain models SRTM-3 and ASTER GDEM, global climate model Worldclim ver. 2.0, mathematical modeling and statistical analysis. Asignificant factor of differentiating the territories, is the set of parameters of their heat supply. The HPLC method was used to determine the content of the most chemically labile monomeric and dimeric forms of phenolic antioxidants whith the highest bioavailability in the structural elements of grape berries and wines from various vineyards: the level of differences in the phenolic complex was assessed. Based on the identified correlations, it was shown that an increase in the heat supply of the territories, on one hand, was accompanied ($\alpha<0.05$) by accumulation of phenolic acids, flavonols, flavan-3-ols and procyanidins in grape seeds. On the other hand, it contributed to the oxidative polymerization of phenolic components during grape ripening and processing (also due to an increase in monophenol monooxygenase activity and must pH), followed by a decrease in the content of mono- and dimeric components in the skin and pulp of grapes, and flavan-3-ols in wines. This is naturally manifested in 'Kokur Belyi' cultivar. Moisture supply of vineyards adjusts the formation of a complex of phenolic antioxidants of' 'Cabernet-Sauvignon' grapes and wines. An increase in the heat supply of vineyards was accompanied by an increase in the proportion of malvidin-3-O-β-D-glucoside and its derivatives, and a decrease in the proportion of cyanidin and petunidin and their derivatives in the anthocyanin complex of berries. The territories, capable to obtain grapes and wines, enriched in phenolic antioxidants of various functional directions, were specified. This work is a part of comprehensive study carried out in the FSBSI Institute Magarach of the RAS on the zoning of ampeloecological resources of Crimea, including the identification and geoinformation modeling of multi-parametric relationships of spatial distribution of agroecological characteristics and metabolites of a grape plant (taking into account varietal specificity), and quality of crops and wine products.

Keywords: vineyards, climatic parameters, grapes, wine, phenolic antioxidants

Introduction

Phenolic compounds are the most important antioxidants in grapes and grape products, including wines. Firstly, in terms of proportion, phenolic substances are the fourth (after water, carbohydrates and organic acids) component of grape berry. Secondly, the phenolic complex of grape berries is represented by a wide range of flavonoid compounds (flavan-3-ols and their polymeric compounds, anthocyanins, flavonols and their glycosides) and non-flavonoid structures (hydroxycinnamic and hydroxybenzoic acids and their derivatives, stilbenoids) (Donwey et al., 2006; Hanlin et al., 2011; Ostroukhova et al., 2018; Perestrelo et al., 2012; Peskova et al., 2017, Popov et al., 2017; Scollary, 2010; Zaitsev et al., 2014). Thirdly, phenolic substances exhibit antioxidant properties as antiradical agents that bind free radicals, and as chelating compounds that prevent the formation of radicals (Amić et al., 2007; Malesev et al., 2007; Pietta, 2000; Tarakhovsky et al., 2013). The catechol group (two hydroxyl groups in the B ring at position 3' and 4') makes the greatest contribution to radical-binding and chelating properties of the flavonoid molecule (Figure 1). At the same time, presence of 2,3-double bond flavonoids in the C ring in the molecule, conjugated with the C-4-oxo group, and C-3, A-5 hydroxy groups, significantly increases the antiradical effect of flavonoids, and replacementof the hydroxy group at C-3 position by sugars – reduces such effect (Amić et al., 2007; Soobrattee et al., 2005; Speisky et al., 2022). Epicatechin-gallates, myricetin and quercetin show the highest activity towards the superoxide-radical; kaempferol – towards radicals OH., N3, etc.; catechin – towards peroxide radicals (Bagchi et al., 2003; Braca et al., 2003; Tejero et al., 2007). Anthocyanins neutralize radical forms of oxygen and nitrogen in four times better effect than ascorbate and α-tocopherol do: cyanidin and delphinidin monoglycosides exhibit the highest antioxidant activity (Harvaux and Kloppstech., 2001; Kim and Lee, 2004). Phenolic acids are highly reactive towards peroxyl radicals, they are involved in terminating the oxidation chains of semiquinone radical (Choi et al., 2002; Ketsawatsakul et al., 2000).

The manifestation of one or another antioxidant activity in vivoby phenolic compounds depends not only on the presence of active centers in the molecule, but also on their ability to cross biological barriers (Han et al., 2012; Menschikova et al., 2012; Oteiza et al., 2005). Flavonoid monomers are easier than their dimeric and polymeric forms to penetrate the intestinal epithelial cells and beabsorbed by the body (Fernandes et al., 2012; Kocic et al., 2011). The bioavailability of stilbenoids is incredibly low: when 100 mg of resveratrol is uptaken, only 10 mg is absorbed (Tarakhovsky et al., 2013; Vesely et al., 2021). The multivariate biological effect of flavonoids and phenolic acids was experimentally proven: normalization of cellular metabolism and oxygen transportation, regulation of liver fat metabolism, strengthening of blood vessel walls, cardioprotective properties, anticarcinogenic, antitumor, antiphlogistic and anti-allergic activity, etc. (Bagchi et al., 2003; Dai and Mumper, 2010; Gengaihi et al., 2014; Mandal et al., 2009; Menschikova et al., 2012; Oh et al., 2021; Xia et al., 2010). At the same time, biological effect of plant antioxidants with phenolic nature on human body is characterized by the phenomenon of synergism (Rayalam et al., 2011). For this reason, the assortment of functional foods from grapes has increased in recent decades. Red wines are increasingly considered as an essential element of human diet, especially in areas with adverse environmental conditions (Avidzba et al., 2016; Chernousova et al., 2022; Zaitsev, 2022). It is shown that white wines also have a high biological potential due to a complex of phenolic antioxidants (Chernousova et al., 2022; Tkachenko et al., 2012).

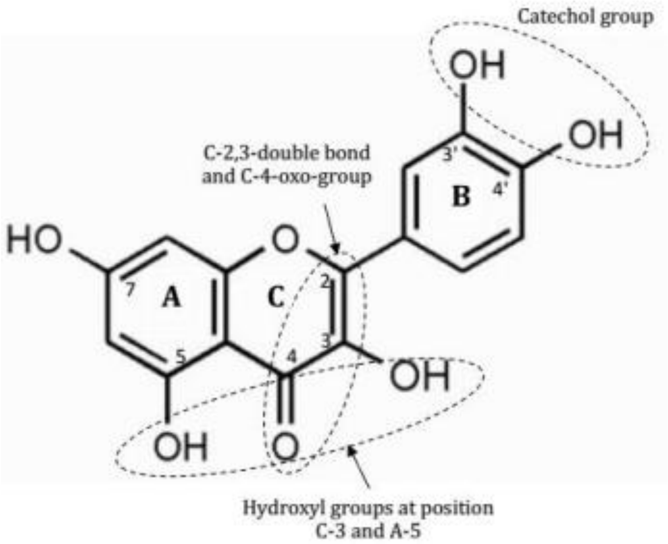


Figure 1. Quercetin molecule with groups responsible for binding of free radicals and metal ions.

In this regard, the work of scientists and experts is aimed at identifying factors of formation the phenolic complex of grapes and wines during harvesting and processing, as well as treatment, aging and storage of wines (Blancquaert et al., 2019; Chacón-Vozmediano et al., 2021; Fernandes de Oliveira et al., 2017; Levchenko et al., 2021a; 2021b; Merkytė et al., 2020; Rybalko et al., 2022a; Setford et al., 2017; Shah et al., 2021). Modern updating of research in this area is associated with the observed climate change (increase in the ambient temperature, in the difference between summer and winter temperatures, and in fresh water shortage), which makes the adjustment to phenology, agrobiology, metabolism of a grape plant, reflected in biological value and quality of yield and wines, up to the transformations in their style (Anesi et al., 2015; Beauchet et al., 2020; Merkytė et al., 2020; Jarvis et al., 2017; Levchenko, 2021c; Rienth et al., 2020; Rodrigues et al., 2022; Shah et al., 2022; van Leeuwen et al., 2019).

Possible negative consequences of climate change from the standpoint of biological value and quality of wine products necessitate a revision in classical technologies of viticulture and winemaking: from the selection of new territories for establishing vineyards, agricultural technologies, grape varieties, to the methods, regulations and conditions for grape processing and wine production. For an object-oriented assessment of ampeloecological conditions of grapegrowing on the territories, it is important to have the data on quantitative relationships between individual ampeloecological parameters and/or their complexes, as well as indicators that characterize grapevine development, vital activity of grape plants from the standpoint of transformations of basic and secondary metabolites and, as a result, the formation of quantitative and qualitative characteristics of grape yield and wine products.

Studies on the effect of individual orographic, edaphic and climatic factors on certain quality indicators of grape yield (including taking into account the genetic specificity of varieties) and wine products have a deep retrospective and cover almost all viticultural and winemaking regions in the world (Anesi et al., 2015; Beauchet et al., 2020; Donwey et al., 2006; Drappier et al., 2016; Lopez-Bustins et al., 2014; Markosov and Ageeva, 2009; Rienth et al., 2020; Rodrigues et al., 2022; Spayd et al., 2002; Ubalde et al., 2007; Yamane et al., 2006). However, when assessing territories (including in the prospect of climate change) for viticulture and winemaking, the content of sugars and titratable acids, potential and actual alcohol content and tasting assessment are traditionally considered as quality indicators of grapes and wines (Biasi et al., 2019; Bock A. et al., 2011; Ivanchenko et al., 2020; 2022;

Seguin B. and de Cortazar, 2005). Dependencies reflecting the relationship between ambient temperature, cropping capacity and sugar content in berries (Bock A. et al., 2013), as well as environmental effects (according to individual meteorological indicators during growing season) and “genotype-environment” effects are identified and mathematically described for a number of grapevine cultivars according to the following characteristics: productivity, mass concentration of sugars and titratable acids in grapes, tasting assessment of table wines (Guguchkina, 2010; Rybalko, 2022b).

At the same time, numerous studies show a significant variation in the phenolic profile of grapes depending on the natural and agrotechnical conditions of growth, which is associated with the protective function of phenolic compounds (Chacón-Vozmediano et al., 2021; Chowdhary et al., 2021; Blancquaert et al., 2019; Kumar et al. 2020). Under the influence of stress factors, the biosynthesis of phenolic compounds is stimulated or inhibited: a high level of plant insolation, a lack of moisture or nitrogen in the soil leads to an increase in the concentration of flavonoids in grapes; a lack of phosphates and low air temperatures - to the accumulation of anthocyanins; high temperatures - to the accumulation of flavonols, air temperature above 30°C inhibits the biosynthesis of anthocyanins (Chacón-Vozmediano et al., 2021; de Rosas et al., 2022; Gaiotti et al., 2018; He et al., 2010; Markosov, and Ageeva, 2008; Mori et al., 2007; Shah et al., 2021). In accordance with modern ideas and observations (Fournand et al., 2006; Teixeira et al., 2013), the biosynthesis of flavan-3-ols, procyanidins, phenolic acids in grapes are almost completedto the beginning of berry ripening, and then the oxidative polymerization occurs:as shown, the oxidase activity of grapes correlates with the annual temperature factor. The activity of oxidative and hydrolytic enzymes in grape berries is especially significant in the formation of phenolic complex and the resulted antioxidant potential of grape processing products, starting with wines (Fronk et al., 2017). The variability of the component composition and biochemical properties of grapes under the influence of natural and anthropogenic factors is determined by its species and varietal membership: adaptive characteristic of the variety, reaction norm of the genotype to environmental factors (Ghafoor et al. 2020; Margraf et al. 2016; Otto et al., 2022; Shimizu et al., 2022).

The problematic task of studying agroecological resources as factors that form basic and secondary metabolites of grapes is also to determine exact meso-climatic characteristics of the studied areas of grape growing, since they can significantly differ from the conditions at the nearest weather station, taken as a basis for obtaining climate information. Geoinformation and mathematical modeling of spatial variation of climatic indicators under the influence of orographical, hydrological and geographical parameters of the analyzed territoriesis a promising solution to this issue (Getahun 2012; Ostroukhova et al., 2021b; Ostroukhova et al., 2022; Rybalko et al., 2020; Rybalko and Baranova, 2022). The second important task is to select the most informative agroclimatic indices. In most cases, the development of a grape plant at temperatures below 10°C (biological zero) is impossible; and the temperature above 20°C is the most comfortable for growing of shoots, accumulation of sugars and a decrease in the concentration of titratable acids during grape ripening (Ferrer-Gallego et al., 2012). Moisture supply of the territory, like temperature and light conditions, occupies one of leading positions among the factors to determine growth and development of a grape plant. Lack of moisture delays the growth of shoots, bunches and berries, reduces cropping capacityin the current and next year. Modern science considers a wide range of climatic indices as factors in the metabolism of a grape plant. Among them: Winkler index, heliothermal Huglin index, sum of active temperatures, sum of biologically effective temperatures, Selyaninov hydrothermal coefficient, amount of precipitation per year, amount of precipitation per growing season, etc. (Bucur et al. 2019; Mihai et al. 2013; Monteiro et al., 2017; Karoglan et al., 2018). The more precise these characteristics are determined for a particular territory, the more effective its amplification is. For this purpose, various methods of data collecting and processing are used: remote sensing of the Earth, geoinformation and mathematical modeling of spatial variation of climatic indicators under the influence of orographic, hydrological and geographical parameters of analyzed territories (Egorov and Petrov, 2017; Irimia et al., 2014; Kryza et al., 2017; Naumova and Novikova, 2015; Schultze and Jones, 2010; Schultze and Sabbatini, 2019). For the Crimean Peninsula, which is characterized by a diverse relief, different distance of vineyards from the sea, indented coastline, taking into account the spatial variability of climatic parameters in assessing of ampeloecological resources is an essential condition for sustainable development of viticulture and grape processing industries of national economy.

Based on the above, the FSBSI Institute Magarach of the RAS conducts systemic researches on the zoning of ampeloecological resources, including identification and geoinformation modeling of multiparametric relationships in spatial distribution of agroecological characteristics and metabolites of a grape plant (taking into account varietal specificity), quality of yield and wine products (Levchenko et al., 2021a, 2021b, 2021c; Ostroukhova et al., 2018;

2021b; Rybalko, 2020; Rybalko et al., 2020, 2022a, 2022b). Present work aimed at studying agroecological resources of Crimean vineyards and their influence on the formation of a complex of phenolic antioxidants in grapes and wines is a part of these researches.

Variability of Agroecological Resources in the Vineyards of the Crimean Peninsula

This work presents the results of a study on the influence of agroecological parameters of territories on the formation of a complex of phenolic antioxidants in grapes and wines of the cultivars 'Cabernet-Sauvignon' and 'Kokur Belyi'. These grapevine cultivars were chosen in accordance with the following. In Crimea, 'Cabernet-Sauvignon', occupying about 2300 ha, is the second most common wine grape variety, which gives wines of high quality everywhere. Grapes and wines from this cultivar have powerful antioxidant properties due to their phenolic complex (Avidzba et al., 2016; Geçer et al., 2022; Jiang and Zhang, 2012). 'Kokur Belyi' (Vitis vinifera L.) is a Crimean autochthonous wine grape variety. According to morphological characteristics and biological properties, it belongs to the ecological and geographical group of grape varieties of Black Sea basin. ‘Kokur Belyi’ is the most common among white autochthonous varieties of Crimea, the area of its industrial plantings is constantly increasing (from 659 ha in 2016 to 911 ha in 2018), including outside the historical area of growing (Beybulatov et al., 2019; Ostroukhova et al., 2022; Rybalko et al., 2022). Compared to other common autochthonous white grapevine cultivars of Crimea (‘Shabash’ and ‘Sary Pandas’), ‘Kokur Belyi’ is more enriched in phenolic antioxidants (flavonols, hydroxybenzoic and hydroxycinnamic acids, flavan-3-ol monomers and B1-B8 procyanidins) and stilbenes at the stage of technical ripeness (Ostroukhova et al.2019; 2022).

The industrial vineyards of control, planted with grapes in various natural and climatic regions of Crimea, were selected for the research. Vineyards of ‘Cabernet-Sauvignon’ are located in the following geographical objects (Figure 2): Yalta city in the South Coast region; Privetnoe, Morskoe and Solnechnaya Dolina villages in the Mountain-Valley Coastal region; Vilino and Uglovoe villages in the Western Piedmont Coastal region. Control vineyards planted with ‘Kokur Belyi’ cultivar are located near Morskoe, Privetnoe, Solnechnaya Dolina and Vilino villages. Figure 2 shows the differences of vineyards by morphometric parameters of relief: absolute altitude above the sea level, steepness and exposure of a slope, relative excess over the talweg.

Determining of agroecological resources of grape growing areas was carried out as follows. The first stage was to determine the vineyard geographical coordinates, their orographic and hydrological parameters, and soil cover features. At the second stage, climatic parameters were calculated at the point of vineyard location using data from Crimean stationary weather stations for 2016-2021 years, digital elevation models SRTM-3, ASTER GDEM and global climate model World clim ver. 2.0. Generally accepted geoinformation and mathematical models, developed by the authors, were used to describe the patterns of spatial variation of climatic indicators under the influence of orographic, hydrological and geographical parameters of the analyzed territory (Ivanchenko et al., 2020; Rybalko, 2020; Rybalko et al., 2020).

The following parameters were defined for each vineyard:growing degree days above 10°C ($\sum T°C_{10}$), growing degree days above 20°C ($\sum T°C_{20}$), Huglin index, Winkler index, average growing season temperature ($t_{growing}$°C), average September temperature (t_{Sept}°C), total precipitation during the year (P_{year}), total precipitation during the growing season ($P_{growing}$), total precipitation during September (P_{Sept}), Selyaninov hydrothermal coefficient (HTC), which is the ratio of total precipitation during the growing season period increased by 10 times to growing degree days above 10°C (Ivanchenko et al., 2020; Rybalko, 2020; Rybalko et al., 2020).

Figure 2. Location of the analyzed vineyards of the Crimean Peninsula.

Experimental data on agroecological parameters of vineyards, component composition of the complex of phenolic antioxidants and biochemical properties of grapes and wines were processed by the variance (ANOVA), discriminant (Wilks statistics), and cluster (Euclidean distances - Ed) analyses (Statistica 10 programme). The comparison of quantitative characteristic values in independent subgroups was performed using Mann-Whitney U-test and F-test. Verification of statistical coefficients was carried out for the point of significance $\alpha<0.05$.

The results of determining climatic parameters of ‘Kokur Belyi’ vineyards in 6-year retrospective view using methods of geoinformation and mathematical modeling and their statistical processing are presented in Table 1, for ‘Cabernet-Sauvignon’ vineyards – in Table 2. The data of Tables 1 and 2 indicate that geographical location of vineyards determines the dispersion of most parameters of their heat supply in the study period at a significance level from $\alpha < 0.00001$ to $\alpha < 0.04$. The most significant difference between ‘Cabernet-Sauvignon’ and ‘Kokur Belyi’ vineyards was registered by the indices $\sum T°C_{10}$, Winkler index and $t_{growing}$. The highest values of growing degree days above 10°C, Winkler index and average growing season temperature were typical for vineyards in Morskoe village, the smallest – for vineyard of ‘Kokur Belyi’ cultivar in Vilino and ‘Cabernet-Sauvignon’ in Uglovoe. It should be noted that ‘Cabernet-Sauvignon’ vineyards, located in the Mountain-Valley Coastal region, were characterized by higher values of most heat supply parameters compared to ‘Kokur Belyi’ vineyards from these geographical objects.

Using total cumulative calculations of heat supply parameters, the vineyards of ‘Cabernet-Sauvignon’ grapevine cultivar are differentiated by heat resources with Wilks L.= 0.012 at $\alpha <0.00001$, ‘Kokur Belyi’ – Wilks L.= 0.00009 at $\alpha <0.00001$. The results of discriminant analysis of territorial heat supply parameters, reflecting the difference in vineyards in terms of heat resources are presented in Figure 2. The direction of arrows in the diagrams indicates an increase in values of agroecological parameters along the abscissae and ordinate axes. The diagram clearly demonstrates differencesin heat supply of ‘Cabernet-Sauvignon’ vineyards, located in different climatic regions of Crimea, and additionally allows assessing the difference in heat resources of grape growing areas in one regionusing the example of ‘Kokur Belyi’ cultivar. Compilation of the results of statistical data analysis (according to F-test and Euclidean distances) made it possible to distribute ‘Kokur Belyi’ vineyards by the increase in their heat supply in the series: Vilino <Privetnoe <Solnechnaya Dolina <Morskoe; and ‘Cabernet-

Sauvignon' vineyards – Uglovoe <Vilino <Yalta <Privetnoe, Solnechnaya Dolina <Morskoe.

Table 1. Values[1] of climatic parameters of 'Kokur Belyi' vineyards located in different geographical objects

Index	**Viticulture region/geographical location**				**α^2, less than**
	Western Piedmont Coastal region	**Mountain-Valley Coastal region**			
	Vilino	**Privetnoe**	**Morskoe**	**Solnechnaya Dolina**	
$\sum T^{\circ}C_{10}$	3845±191	3984±173	4372±123	4246±172	0.0003
$\sum T^{\circ}C_{20}$	2195±236	2400±186	2508±158	2434±186	0.04
Huglin index	2732±130	2513±169	2645±133	2782±165	0.005
Winkler index	1767±106	1930±156	2068±141	2052±156	0.004
t_{Sept}, °C	18.4±1.4	20.1±1.4	20.3±2.2	20.2±1.4	-
$t_{growing}$, °C	18.7±0.6	20.0±0.7	20.0±0.5	21.0±0.7	0.00002
HTC	0.67±0.15	0.77±0.29	0.71±0.37	0.71±0.29	-
P_{year}, mm	468±35	447±89	376±92	439±87	-
$P_{growing}$, mm	250±44	251±91	219±90	246±89	-
P_{Sept}, mm	33.2±28.4	23.6±16.2	27.0±10.4	24.8±17.1	-

[1] Arithmetic mean value ± standard deviation (SD).

[2] Significance level of the variance (F-test) of the indicator between vineyards.

A significant effect ($\alpha < 0.004$) of crop year during the study period on the indicators of heat supply for specific vineyards was revealed in relation to the Huglin and average growing season temperature indices. In general, a significant dispersion of heat resources, driven by the crop year meteorological conditions, was observed for 'Kokur Belyi' vineyards, located in Vilino, and for 'Cabernet-Sauvignon' vineyards fromYalta (Figures 3 and 4). The highest heat supply of the territories was recorded in 2018, the lowest - in 2012.

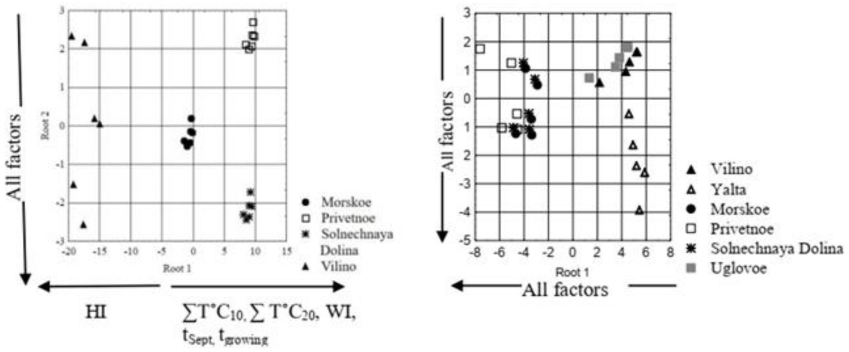
The vineyards of 'Cabernet-Sauvignon', located near Yalta city, had the highest amount of precipitation during the year, ranged from 435 mm in 2020 to 746 mm in 2018. Total precipitation amount in Yalta during the year was on average 44% higher ($\alpha < 0.04$) than in the vineyards of Mountain-Valley Coastal region, and 29% higher than in the Western Piedmont Coastal region. There was no significant difference in this parameter in the vineyards of both 'Cabernet-Sauvignon' and 'Kokur Belyi' grape cultivars of the same region.

Table 2. Values[1] of climatic parameters of ‘Cabernet-Sauvignon’ vineyards located in different geographical objects

Index	**Viticulture region/geographical location**						**α^2, less than**
	Western Piedmont Coastal region		**South Coast region**	**Mountain-Valley Coastal region**			
	Villino	**Uglovoe**	**Yalta**	**Privetnoe**	**Morskoe**	**Solnechnaya Dolina**	
$\sum T^{\circ}C_{10}$	3844±191	3763±191	4173±312	4310±172	4357±172	4321±172	0.00002
$\sum T^{\circ}C_{20}$	2246±236	2173±236	2414±327	2647±186	2661±186	2630±186	0.002
Huglin index	2724±130	2681±130	2845±107	2714±165	2753±165	2747±165	-
Winkler index	1767±106	1728±106	2083±126	2087±156	2108±156	2090±156	0.00001
t_{Sept}, °C	18.1±1.4	17.6±1.4	18.4±1.4	20.5±1.4	20.3±1.4	20.6±1.4	0.002
$t_{growing}$, °C	19.2±0.6	19.1±0.6	20.1±0.6	20.8±0.6	20.8±0.7	20.7±0.7	0.00001
HTC	0.67±0.17	0.69±0.17	0.77±0.17	0.57±0.20	0.55±0.20	0.56±0.20	-
P_{year}, mm	461±45	474±42	624±154	446±96	429±109	422±96	0.02
$P_{growing}$, mm	258±61	263±61	308±76	240±71	237±71	237±71	-
P_{Sept}, mm	32.4±29.4	33.2±29.4	45.1±51.6	23.6±16.2	22.6±16.2	26.6±16.2	-

[1] Arithmetic mean value ± standard deviation (SD).

[2] Significance level of the variance (F-test) of the indicator between vineyards.



‘Kokur Belyi’

'Cabernet Sauvignon'

Figure 3. The results of discriminant analysis of heat supply parameters, reflecting the difference in ‘Kokur Belyi’ vineyards by heat resources.

Interannual dispersion of total precipitation during the growing season, in September and Selyaninov hydrothermal coefficient were exceeding the territorial dispersion of indicators. Thus, in September 2017 in 'Cabernet-Sauvignon' vineyards of the Western Piedmont Coastal region there was no precipitation at all, but in 2018 the amount of precipitation was maximal in the region and amounted to 71-72 mm. In the vineyards of Yalta in the same years, this indicator shifted from 5.4 to 140.0 mm. The variation range of total precipitation in September in the vineyards of the Mountain-Valley Coastal region was from 3.6 to 48.0 mm. The minimum level of precipitation during the growing season in the vineyards of 'Cabernet-Sauvignon' cultivar in the Mountain-Valley Coastal region was observed in 2018 and amounted to 145-147 mm, in Yalta – in 2019 (204 mm), in the Western Piedmont Coastal region – in 2021 (192-197 mm). The maximum level of precipitation during the growing season in grape growing areas amounted to 322-396 mm, and was registered in 2016, 2021 and 2020, respectively.

Thus, the experimental data presented demonstrate a significant variability of agroecological resources of grape growing areas by natural climatic regions of the Crimean Peninsula, and in the case of heat supply parameters -by geographical objects within the boundaries of one region, and even vineyards with in the boundaries of one geographical object, due to the orographic and hydrological characteristics of specific vineyards. This is also evidenced by research results of 2015-2019. Using methods of geoinformation and mathematical modeling, we have determined agroclimatic parameters of 14 industrial 'Cabernet-Sauvignon' vineyards located in five viticulture regions of Crimea (Ostroukhova et al., 2021b). By similarity of 9 characteristics of heat and moisture supply, the vineyards were combined into 6 clusters, significantly different from each other. At the same time, vineyards located in different viticulture regions of Crimea were united in one cluster. Thus, cluster I combined vineyards from the Mountain-Valley (Alushta), Mountain-Valley Coastal (Veseloe, Morskoe) and Western Piedmont Coastal (Orlovka) regions. On the contrary, vineyards near Livadia village, depending on their distance from the sea and height above the sea level, were divided into different clusters, combining them, in one case, with the vineyards of Yalta, and in another – with the vineyards of Solnechnaya Dolina. At the same time, significant differences in the content of basic and secondary metabolites of grapes in the yield obtained from the territories of selected clusters were revealed, necessitating the accounting of agroecological resources of vineyard when assessing their influence on the formation of a complex of phenolic antioxidants in grapes and wines.

Phenolic Antioxidants, Carbohydrate-Acid Complex and Oxidase Activity of Grapes, Obtained in the Vineyards of Crimean Geographical Objects

The formation of a complex of phenolic antioxidants in grapes is associated with accumulation ofsugars, since their concentration regulates enzymatic activity involved in biosynthesis of phenolic compounds (Fernandez et al., 2007; Jacobo-Velázquez et al., 2015). The phenolic complex of grape berry begins its formation from germinating point, but essential accumulation and transformation of the components is observed during the period of berry growth and ripening (Ali et al., 2011; Bashir et al., 2018; Choi et al., 2020; Gianluca et al., 2021; Torres et al., 2020). According to most researchers, the biosynthesis of procyanidins, flavan-3-ols, phenolic acids in a berry is mainly completed by the beginning of its ripening, followed by their oxidative polymerization, and the synthesis of anthocyanins is carried out from the beginning of ripening to the onset of physiological ripeness (Bashir et al., 2018; Conde et al., 2007; Fournand et al., 2006; Teixeira et al., 2013). Anthocyanins and flavonols are mainly concentrated in the skin of berries; monomeric flavan-3-ols − in seeds; hydroxycinnamic acids and hydroxycinnamates − in mesocarp; hydroxybenzoates and hydroxybenzoic acids, procyanidins, tannins − both in the skin and seeds of berries (Colombo et al., 2021; Conde et al., 2007; Padilla-González et al., 2022; Šikuten et al., 2020; Xia et al. 2010).

About 63% of phenolic antioxidants are extracted from berries into the wine during the winemaking process. At the same time, it is obvious that the profiles of phenolic antioxidants of grapes and wine are not identical. The degree of transition of certain phenolic components from grapes to wine largely depends on the technical and phenolic ripeness of grapes. In this aspect, the development of phenolic complex in the process of grape ripening is manifested, on one hand, in accumulation of anthocyanins and tannins in the skin of berries, and in an increase in their extractability (due to hydrolysis of pectin and xyloglucan of skin cell walls by grape enzymes) in winemaking processes; and on the other hand, in an increase in the degree of polymerization of galloylated seed tannins, resulted in their extractability (predominant at the beginning of maturation) decrease (Bindon t al., 2014; Colombo et al. 2021; Rabot and Laurence, 2019; Quijada-Morín et al., 2015; Wang et al., 2022). Moreover, during grape processing, phenolic antioxidants, as follows from the diversity of their molecular structure, undergo oxidation induced by native oxidases to a varying degree (Danilewicz, 2021; Oliveira et al., 2011; Watrelot, 2021). Subsequently, the oxidation of phenolic substances passes into an autocatalytic stage, proceeding in accordance with a chain free radical mechanism, and involving almost all components of must and wine in the process of co-oxidation. Active acidity (pH) is the regulator of processes at all stages (Nguyen, 2021; Vivas, 2002).

From the foregoing it is obvious that when assessing the effect of heat and moisture supply of territories on the formation of a complex of phenolic antioxidants in grapes and, especially, wines, it is necessary to take into account not only their content in berries in a certain range of sugar accumulation, but also the prospects for their extraction from skins and seeds, and oxidative polymerization in winemaking processes.

Table 3. Physicochemical and biochemical parameters of grapevine cultivar of 2016-2021 crop years, from different vineyards

Parameters	**Values***			
	Vilino	**Privetnoe**	**Morskoe**	**Solnechnaya Dolina**
Sugar content, g L^{-1}	190±18 164-202	208±40 180-236	222±13 207-231	221±24 191-252
Titrated acids, g L^{-1}	5.8±1.2 4.3-7.1	6.0±0.04 5.9-6.0	5.7±0.9 4.8-6.5	5.3±0.9 4.2-6.5
pH	3.33±0.11 3.18-3.43	3.25±0.09 3.19-3.32	3.51±0.11 3.39-3.61	3.49±0.10 3.38-3.60
TRPh, mg L^{-1}	671±81 579-727	1105±334 869-1342	1104±395 688-1475	1393±568 1004-2371
Ph_0/TRPh, %	63±31 27-84	34±2 33-36	36±10 28-47	38±17 9-53
Ph_4/Ph_0, %	111±11 103-118	155±36 120-209	146±6.0 141-153	139±66 86-233
PPOx10^2, item	5.0±0.5 4.6-5.3	9.4±2.5 6.2-12.5	13.8±0.2 13.6-13.9	14.6±8.8 7.1-24.3

* Numerator – means±SD; denominator – range.

The analysis results of carbohydrate-acid, phenolic and oxidase complexes of grape yield from the studied vineyards and their statistical processing are presented in Tables 3 and 4. In the course of research we estimated: the content of sugars and titratable acids (in terms of the equivalent amount of tartaric acid) in grapes; the indicator of active acidity (pH); the concentration of phenolic components in the must with Folin-Ciocalteu reagent (OIV, 2020): immediately after crushing grapes (Ph_0), after 4-hour mush infusion at 20±2ºC (Ph_4) and after mush thermostating at 70ºC for 30 min (TRPh - technological reserve of phenolic substances) (Rybalko, 2020; Rybalko et al., 2020); the total content of anthocyanins in grape berries ($A_{pH1.0}$) and the amount of easily extractable anthocyanins ($A_{pH3.2}$) using Glories method (Rajha et al. 2017); the polyphenol oxidase activity (PPO, item) colorimetrically by the rate of pyrocatechol oxidation using freshly squeezed must.The degree of transition of phenolic components into the must during pressing whole berries (Ph_0/TRPh) relative to the technological reserve of components in grapes, and after 4-hour pulp infusion (Ph_4/TRPh and Ph_4/Ph_0), as well as the proportion of easily extractable anthocyanins ($Ea = A_{pH3.2}/A_{pH1.0}$) were calculated.

It was found that the yield of ‘Kokur Belyi’ from Vilino vineyards during the years of research differed (α <0.04) from that of the Mountain-Valley Coastal region location in a lower content of sugars (on average by 14%), and a higher proportion of phenolic antioxidants in the must from their technological reserve in grapes while pressing whole berries (by an average of 1.7 times). At the same time, total cumulative calculation of the following indicators − active acidity and polyphenol oxidase activity of the must, technological reserve of phenolic substances, the proportion of phenolic substances in the must relative to the technological reserve of components in grapes after crushing berries and after 4-hour mush infusion – differentiated the yield for all geographical objects, including the Mountain-Valley Coastal region with Wilks L.=0.0001 and α<0.006. The pH value was increasing in the series: Privetnoe (3.25±0.09) <Vilino (3.33±0.11) < Solnechnaya Dolina, Morskoe (3.49-3.51). The yield of ‘Kokur Belyi’ from Vilino was characterized by the smallest technological reserve of phenolic components (671±81 mg L^{-1}) and the lowest degree of their accumulation in the must during 4-hour mush infusion (111±11%). The highest indicator values were recorded, respectively, for grapes from Solnechnaya Dolina and Privetnoe villages. The PPO activity of the must was constantly increasing in the series: Vilino <Privetnoe <Morskoe <Solnechnaya Dolina from 0.050±0.005 units up to 0.146±0.088 units. The dispersion of the content of titratable acids in grapes was more determined by the crop year (α=0.017) than by the growing area.

Grape yield of ‘Cabernet-Sauvignon’ cultivar (Wilks L.=0.027 at α <0.005) was better differentiated by growing areas based on the total cumulative calculation of indicators, presented in Table 4, except for the content of titratable acids and easily extractable anthocyanins ($A_{pH3.2}$). At that, the highest significance level of inter-territorial dispersion was found for the content of sugars in berries (α <0.01) and Ea indicator (α <0.015). The greatest accumulation of sugars was observed in grapes from Yalta and Privetnoe, ranging from 218 to 298 g L^{-1}, which was on average 31% higher than in grapes from Vilino, and 17% higher − in grapes from other territories. Grapes from Yalta and Privetnoe were also characterized by the highest ratio (53±7% and 54±14%, respectively) of easily extractable anthocyanins in the anthocyanin complex. The lowest value of this indicator was in the yield from Uglovoe village (38±5%). In these conditions, grapes from Yalta had the lowest content of anthocyanin complex (400-1006 mg L^{-1}), and grapes from Privetnoe and Solnechnaya Dolina − the highest (1164±464 mg L^{-1} and 1199±152 mg L^{-1}, respectively). Grapes from Vilino were distinguished by the smallest ability to accumulate a complex of phenolic antioxidants (including anthocyanins) in the must after 4-hour mush infusion (Ph$_4$/Ph$_0$ ranged from 54% to 111%), while grapes from Morskoe village – by the greatest (87-190%). PPO activity was consistently increasing from 0.080±0.039 to 0.155±0.043 items in the series: Privetnoe <Uglovoe <Yalta, Vilino and Morskoe <Solnechnaya Dolina.

Table 4. Physicochemical and biochemical parameters of grapevine cultivar 'Cabernet-Sauvignon' of 2016-2021 crop years, from different vineyards

Parameters	**Values***					
	Vilino	**Uglovoe**	**Yalta**	**Privetnoe**	**Morskoe**	**Solnechnaya Dolina**
Sugar content, g L^{-1}	195±26 172-223	221±15 205-231	253±31 218-270	249±32 218-298	212±23 172-255	224±18 207-242
Titrated acids, g L^{-1}	6.6±0.3 6.3-6.9	6.8±1.7 5.6-8.0	6.7±0.8 5.9-7.4	4.8±1.0 3.5-5.8	5.9±1.6 3.7-8.6	6.6±1.1 5.6-7.7
pH	3.33±0.28 3.10-3.64	3.28±0.06 3.22-3.32	3.47±0.12 3.40-3.61	3.72±0.14 3.57-3.93	3.51±0.19 3.22-3.83	3.33±0.07 3.25-3.39
TRPh, mg L^{-1}	2077±301 1864-2290	1616±135 1481-1750	1709±723 1257-2543	2069±469 1681-2730	2105±431 1293-2880	1860±150 1704-2004
$A_{pH1.0}$, mg L^{-1}	1046±212 896-1196	1049±93 956-1141	637±324 400-1006	1164±464 677-1743	1054±218 679-1440	1199±152 1050-1411
$A_{pH3.2}$, mg L^{-1}	438±191 303-573	391±20 371-410	326±116 236-457	578±113 457-697	472±128 294-695	519±80 439-599
Ph$_0$/TRPh, %	25±15 14-36	20±6 16-24	19±9 9-25	21±2 18-24	17±5 11-32	29±17 17-54
Ph$_4$/Ph$_0$, %	82±41 54-111	134±41 105-164	107±13 96-121	113±26 81-140	146±35 87-190	117±13 104-131
Ea, %	41±10 34-48	38±5 33-43	53±7 45-59	54±14 34-67	45±7 29-56	43±2 42-45
PPO x10^2, item	11.6±4.0 8.7-14.4	10.0±4.9 5.1-14.8	11.1±2.7 8.0-12.9	8.0±3.9 4.0-12.5	12.0±6.2 5.7-22.7	15.5±4.3 10.7-18.8

* Numerator – means±SD; denominator – range.

The comparison of agro-climatic parameters of ‘Kokur Belyi’ and ‘Cabernet-Sauvignon’ vineyards, as well as yield indicators, has revealed the following relationships in the available database ($r=/0.53\text{-}0.87/$, at $\alpha<0.05$). The level of sugar accumulation in berries of ‘Cabernet-Sauvignon’ cultivar directly correlated with almost all parameters of heat supply of the territories (with the exception of t_{Sept}), in grapes of ‘Kokur Belyi’ – with Winkler index and $t_{growing}$; while an inverse correlation between sugar content and precipitation of vineyards in September (P_{Sept}) was revealed only for ‘Cabernet-Sauvignon’ grapes. During the years of research, the effect of heat and moisture supply of territories on the content of titratable acids in the yield of ‘Cabernet-Sauvignon’ was not registered. In ‘Kokur Belyi’ grapes, the content of titratable acids inversely correlated with Winkler and Huglin indices, and directly correlated with the amount of precipitation during the growing season. At the same time, the value of active acidity of grape must in both cultivars directly correlated with the Winkler index and $\sum T^{\circ}C_{10}$.

As far as ‘Kokur Belyi’ cultivar is concerned, technological reserve of phenolic antioxidants was positively associated with $\sum T^{\circ}C_{10}$, $\sum T^{\circ}C_{20}$ and $t_{growing}$; and negatively − with total precipitation during the year. The index Ph_0/TRPh correlated with the same heat supply parameters, but with a negative sign and higher coefficients. At the same time, the degree of accumulation of phenolic antioxidants in the must during 4-hour mush infusion directly correlated with $T^{\circ}C_{10}$, $\sum T^{\circ}C_{20}$. A direct correlation was found between PPO activity of the must and heat supply $\sum T^{\circ}C_{10}$, average temperature of September and of the growing season parameters. On the contrary, in the case of ‘Cabernet-Sauvignon’ grapevine cultivar, a significant influence of agroecological parameters of vineyards during the years of research on the technological reserve of phenolic antioxidants in berries (including the total amount of anthocyanins − $A_{pH1.0}$), Ph_0/TRPh and Ph_4/Ph_0 values, as well as PPO activity was not revealed. Moreover, an increase in the heat supply of vineyards in terms of $T^{\circ}C_{10}$, $\sum T^{\circ}C_{20}$, Winkler index, $t_{growing}$, and t_{Sept} to a lesser extent, was accompanied by the accumulation of easily extractable anthocyanins and an increase in their proportion in anthocyanin complex. An increase in total precipitation during the year had a negative effect on the accumulation of easily extractable anthocyanins.

Compilation of the above proves that the influence of heat and moisture supply of territories on the formation of carbohydrate-acid and phenolic complexes of grapes, firstly, depends on varietal specificity; secondly, it determines the similarities and/or dissimilarities in the yield from vineyards in a different distance from each other (Figure 4). Thus, the difference in carbohydrate-acid complex of 'Kokur Belyi' grapes grows up with an increase in the heat supply of vineyards (Figure 3), reaching the highest values in the yield from Vilino and Morskoe villages (Ed=32.0). As for the yield of 'Cabernet-Sauvignon' grapes, the effect of heat supply is not so obvious, incl. taking into account varietal susceptibility to the moisture supply of the territories. As a result, the yield from vineyards with the highest (Solnechnaya Dolina, Morskoe) and the lowest (Uglovoe) heat supply was characterized by a similar carbohydrate-acid complex (Ed=3.0-9.0); and the greatest differences (Ed=58.0) were between the yield from Vilino and Yalta, with growing territory distinguished by the increased precipitation during the years of research.

The indicators of the complex of phenolic antioxidants, differentiating the yield by growing areas, reflect not only the level of their accumulation in grapes (TRPh, $A_{pH1.0}$), but also the possibility of their transition into grape processing products, in particular, wine (Ph_0/TRPh, Ph_4/Ph_0, Ea). The indices Ph_0/TRPh, Ph_4/Ph_0 and Ea are determined by phenolic compounds of berry juice, as well as by the components of seeds and skins during grape processing and mush infusion. The extraction of components depends on polymerization level of seed procyanidins, permeability of cell walls in berry skin, and their accumulation in the must is largely conditional on the activity of grape oxidases, promoting oxidative polymerization, condensation and sedimentation of phenolic components. With respect to the cultivar 'Kokur Belyi', the increase in the heat supply of territories raises the content of phenolic antioxidants in grapes and their extractability during grape processing (crushing, pressing). But a parallel growth of PPO activity can lead to inhibiting component accumulation in the must during mush infusing. So, the yield of 'Kokur Belyi' from Privetnoe with the lowest Ph_0/TRPh values, but medium PPO activity (0.094±0.025 units), had the highest Ph_4/Ph_0 value: on average, by 17%, compared to grapes from other territories. The closest (Ed=9.0) to grapes from Privetnoe in terms of the content and properties of the complex of phenolic components was the yield from Morskoe village. The greatest difference (Ed=723.0) in the phenolic complex of 'Kokur Belyi' grapes was registered for vineyards of Vilino and Solnechnaya Dolina villages. Multidirectional influence of heat and moisture supply on the accumulation and properties of phenolic antioxidants of anthocyanin series in 'Cabernet-Sauvignon' grapes resulted as follows: the yield from Privetnoe and Yalta was similar in carbohydrate-acid complex (Ed=4.4), and significantly different in phenolic complex parameters (Ed=686.0); and the yield from

Vilino and Morskoe was characterized by similarcarbohydrate-acid (Ed=17.0) and phenolic (Ed=80.0) complexes.

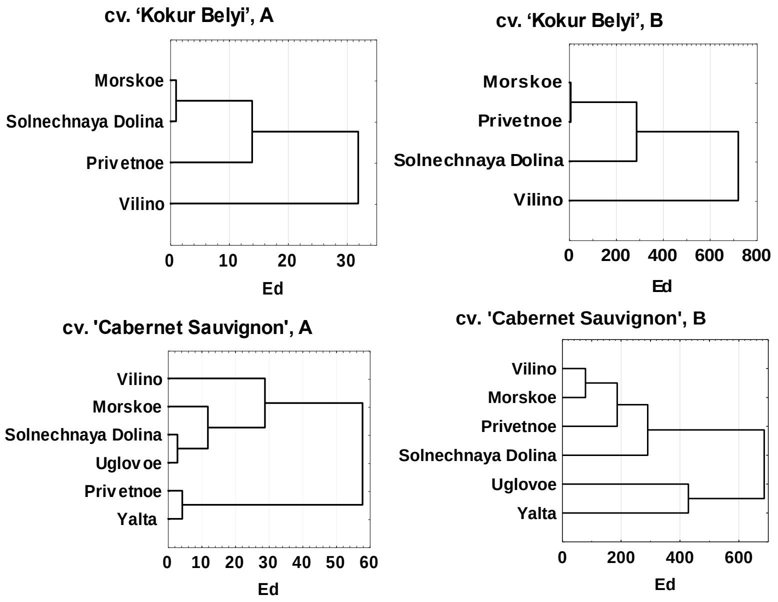


Figure 4. The results of hierarchical classification of yield parameters reflecting similarities and dissimilarities between carbohydrate-acid complex (A) and the complex of phenolic antioxidants (B) of grapes from different geographical areas.

All phenolic components of grape berry, including tannins, possess antioxidant activity to any extent, but monomeric and dimeric forms are more bioaccessible, its are more easily extracted into the must/wine from the solid parts of berries during winemaking processes (Allegro et al., 2021; Chen et al., 2015; 2020; Minatel et al., 2016; Platzer et al., 2021). Considering the above, we have analyzed the content of monomeric phenolic components and procyanidins in the structural parts of 'Kokur Belyi' and 'Cabernet-Sauvignon' berries, obtained from the studied vineyards using HPLC-method (Ostroukhova et al., 2021; Ostroukhova et al., 2022). Hydroxybenzoic and hydroxycinnamic acids, flavonols, flavan-3-ols, anthocyanins and B1-B4 procyanidins were identified.

Table 5. The content (arithmetic mean value*, mg kg^{-1}) of phenolic compounds in the skin, seeds and pulp of berries cv. 'Kokur Belyi' from different vineyards

Compounds	**Vilino**			**Privetnoe**			**Solnechnaya Dolina**			**Morskoe**		
	skin	**seed**	**pulp**	**skin**	**seed**	**pulp**	**skin**	**seed**	**pulp**	**skin**	**seed**	**pulp**
Hydroxybenzoic and hydroxycinnamic acids												
Gallic acid	12.7	16.8	6.9	5.1	28.1	3.3	9.3	44.6	4.4	0.8	28.9	0.5
Caftaric acid	22.5	4.7	14.2	18.1	8.6	7.3	11.8	9.7	4.1	16.1	9.6	8.2
Flavonols												
Quercetin	8.7	0.7	4.0	10.3	8.4	5.9	12.7	12.9	4.7	5.7	14.0	0.3
Quercetin -3-O-b-D-glycosides	23.3	1.1	2.0	3.7	11.3	4.2	28.5	12.2	4.4	0.1	13.6	0.9
Flavan-3-ols												
(+)-D-Catechine	10.9	27.8	2.0	10.3	153.1	1.1	7.5	141.2	1.4	10.2	229.8	2.5
(-)-Epicatechine	221.5	421.6	45.7	47.8	598.6	19.8	96.8	809.2	10.7	13.3	579.5	8.0
Procyanidins												
B1: epicatechine-4→8-catechine	16.6	23.9	5.1	9.3	53.0	3.5	11.2	77.2	2.2	15.5	49.5	4.8
B2: epicatechine-4→8-epicatechine	8.9	28.2	4.9	16.4	300.5	1.8	2.8	116.1	4.6	13.3	241.1	2.5
B3: catechine -4→8-catechine	25.3	94.2	1.8	17.0	217.8	2.0	8.6	251.1	1.1	13.0	220.0	2.9
B4: catechine -4→8-epicatechine	8.0	92.0	2.2	11.4	224.3	4.3	4.7	224.9	4.5	7.3	362.2	2.9

* SD-values were lower than 9% for all vineyards in each year of research. When taking into account the data of different years, SD-values were lower than 17%.

Table 6. The content (arithmetic mean value*, mg kg^{-1}) of phenolic compounds in the skin, seeds and pulp of berries cv. 'Cabernet Sauvignon' cultivar from different vineyards

Compounds	**Vilino**			**Uglovoe**			**Yalta**		
	skin	**seed**	**pulp**	**skin**	**seed**	**pulp**	**skin**	**seed**	**pulp**
Hydroxybenzoic and hydroxycinnamic acids									
Gallic acid	25.7	16.6	7.8	21.3	21.7	0.6	40.5	33.8	0.7
Caftaric acid	30.7	8.1	8.4	17.9	2.5	2.0	50.4	8.5	5.1
Flavonols									
Quercetin	30.0	1.3	0.9	35.1	0.8	0.9	102.1	7.2	1.0
Quercetin -3-O-b-D-glycosides	0.8	0.0	0.2	0.9	0.3	0.2	0.7	7.1	0.6
Flavan-3-ols									
(+)-D-Catechine	60.7	29.4	0.7	7.0	19.5	2.2	17.1	80.5	2.0
(-)-Epicatechine	1145.9	231.6	4.4	1450.5	190.9	3.8	2344.1	391.2	5.5
Procyanidins									
B1: epicatechine-4→8-catechine	32.4	27.3	1.1	29.2	35.1	6.8	21.7	50.3	2.7
B2: epicatechine-4→8-epicatechine	193.2	109.5	1.3	243.2	41.6	2.1	373.5	290.9	1.3
B3: catechine -4→8-catechine	25.4	55.9	1.2	6.7	99.0	0.7	15.7	42.9	1.4
B4: catechine -4→8-epicatechine	9.0	6.9	1.2	28.2	12.9	1.1	91.4	192.3	1.0
Anthocyanins	2841.4	1.1	3.0	3013.4	2.1	2.2	4576.0	3.1	1.8
	Privetnoe			**Morskoe**			**Solnechnaya Dolina**		
	skin	**seed**	**pulp**	**skin**	**seed**	**pulp**	**skin**	**seed**	**pulp**
Hydroxybenzoic and hydroxycinnamic acids									
Gallic acid	61.5	85.8	4.9	26.5	18.0	0.5	32.5	22.8	4.1
Caftaric acid	67.8	5.5	6.5	26.6	5.7	2.5	30.4	3.4	3.9
Flavonols									
Quercetin	100.9	1.3	0.5	46.3	2.0	1.0	42.8	0.8	3.1
Quercetin -3-O-b-D-glycosides	22.0	1.1	0.2	8.2	0.7	4.8	23.8	0.5	0.3

Compounds	Vilino			Uglovoe			Yalta		
	skin	**seed**	**pulp**	**skin**	**seed**	**pulp**	**skin**	**seed**	**pulp**
Flavan-3-ols									
(+)-D-Catechine	181.9	67.3	2.4	74.5	39.9	1.3	108.6	116.7	17.5
(-)-Epicatechine	2347.4	673.5	16.0	1303.6	485.3	5.4	1413.8	427.2	76.3
Procyanidins									
B1: epicatechine-4→8-catechine	19.9	38.8	3.4	25.8	88.3	1.0	15.5	58.6	9.2
B2: epicatechine-4→8-epicatechine	129.1	25.9	2.6	270.2	48.1	2.7	88.5	14.3	19.7
B3: catechine -4→8-catechine	29.0	118.8	1.3	49.1	63.4	0.2	14.2	152.9	11.1
B4: catechine -4→8-epicatechine	385.1	87.8	1.7	145.7	84.0	0.5	240.7	119.0	20.4
Anthocyanins	4701.4	4.5	12.7	2383.4	6.8	16.8	2251.1	9.9	48.7

*SD-values were lower than 10% for all vineyards in each year of research. When taking into account the data of different years, SD-values were lower than 20%.

As follows from Table 5, grape seeds of ‘Kokur Belyi’ are the structural berry elements, mostly enriched in monomeric and dimeric phenolic compounds. The content of components ranged from 711.0±113.8 mg kg^{-1} in grapes from Vilino up to 1748.2±201.4 mg kg^{-1} in grapes from Morskoe. The identified phenolic complex of seeds was dominated by flavan-3-ols and B1-B4 procyanidins: the components in grapes from Privetnoe and Morskoe were presented in parity proportion − 46-47% and 50%, respectively; the proportion of flavan-3-ols exceeded the proportion of B1-B4 procyanidins by 1.4 times in grapes from Solnechnaya Dolina, and by 1.9 times − from Vilino. The content of flavan-3-ols in grape seeds from Solnechnaya Dolina was 933.7-967.1 mg kg^{-1} and exceeded such ($\alpha=0.0002$) in grapes from Privetnoe and Morskoe by an average of 1.2 times, in grapes from Vilino − by 2.1 times. The content of B1-B4 procyanidins ingrape seeds was increasing by growing areas in the series: Vilino <Solnechnaya Dolina <Privetnoe <Morskoe. Seeds of grapes from Solnechnaya Dolina contained 1.7 times ($\alpha=0.000014$) more of phenolic acids − 52.7-55.4 mg kg^{-1}. Grapes from Vilino differed in the lower content of flavonols in seeds compared to grapes from other territories.

The total content of monomeric phenolic components in pulp (74.8±8.7 mg kg^{-1}) and skin (299.6±17.7 mg kg^{-1}) of ‘Kokur Belyi’ cultivar from Vilino was the largest and exceeded the indicator value in grapes from vineyards of the Mountain-Valley Coastal region by 1.9-3.8 and 1.8-6.5 times, respectively. The content of monomeric phenolic components in grape skin was decreasing by growing areas in the series: Vilino >Solnechnaya Dolina >Privetnoe >Morskoe, in pulp – Vilino >Privetnoe >Solnechnaya Dolina >Morskoe. The phenolic complex of grapes from Vilino was distinguished by the highest proportion of flavan-3-ols: in pulp − 54% (in other samples − 29-39%), in skin - 65% (in others − 25-54%). The content of flavan-3-ols, with significant predominance of (-)-epicatechine, in grape berries from Vilino exceeded such ($\alpha<0.05$) in grapes from other vineyards: in pulp − by 2.3-4.5 times, in skin – by 2.2-9.8 times. Grape yield from Morskoe was characterized by the lowest content of flavan-3-ols in pulp (10.5±1.6 mg kg^{-1}) and in skin (23.6±1.9 mg kg^{-1}). The content of flavonols in grape skin from Vilino and Solnechnaya Dolina (32.0-41.2 mg kg^{-1}) was on average 3.7 times higher than in berries from other territories. Morskoe grapes were characterized by the lowest content of flavonols in pulp (1.2±0.3 mg kg^{-1}). In other cases, the indicator values were from 6.0 to 10.1 mg kg^{-1} and did not differ by vineyards. At the same time, grapes from Solnechnaya Dolina were characterized by the highest proportion of flavonols in skin − 21% (in others − 6-9%), and grapes from Morskoe and Vilino − by the smallest proportion of the components in pulp − 3-7% (in others − 19-22%). The proportion of phenolic acids in the phenolic complex of grape pulp was 20-26%, in the phenolic complex of grape skin − 10-18%, with an average content in the yield from each vineyard of 8.8-21.1 mg kg^{-1} and 17.0-35.2 mg kg^{-1}, respectively. The highest content of phenolic acids in pulp and skin was recorded in grapes from Vilino. The content of dimeric flavanols − B1-B4 procyanidins − in grape pulp was 11.6-14.1 mg kg^{-1}. There was no significant difference in the indicator values by the areas of growing. Grapes from Solnechnaya Dolina were characterized by the lowest content of B1-B4 procyanidins in skin (27.3 mg kg^{-1}), being on average 2 times lower than indicator values in grapes from other vineyards. Grapes from Morskoe were distinguished by the highest proportion of procyanidins in the phenolic complex of pulp and skin – 39% and 52%, respectively.

In the case of ‘Cabernet-Sauvignon’ cultivar, the structural berry element, enriched the most in monomeric and dimeric phenolic antioxidants, was grape skin (Table 6). The content of components in grapes from Yalta and Privetnoe was 7633.0±915.3 and 8046.0±1126.4 mg kg^{-1}, which on average was 1.8 times higher ($\alpha<0.0002$) than in grapes from other territories. Moreover, the proportion of anthocyanins in the identified phenolic complex of skin varied from 53% (Solnechnaya Dolina) to 65% (Vilino), and did not differ by grape growing areas. The skin of grapes from Yalta and Privetnoe had the greatest quantity of anthocyanins - 4576.0-4701.4 mg kg^{-1}; the content of components in grape skin of berries from other vineyards was 2251.1-3013.4 mg kg^{-1}.

The closest precursors to anthocyanins are the flavonons, from which monoglucosides of cyanidin and delphinidin are formed at first instance. Further modification by adding a methyl group leads to the formation of peonidin monoglucosides, petunidin, malvidin, and their acylation with acetic or p-coumaric acid (Liu et al., 2018; Mannino et al., 2021). The entries in Table 7 demonstrate that the complex of anthocyanins is dominated by monoglucosides of anthocyanins, the proportion of which ranges from 59% in grapes from Yalta up to 72% in grapes from Uglovoe. The content of monoglucoside derivatives acylated by acetic acid is from 18% (Vilino) to 32% (Yalta), acylated by p-coumaric acid – from 9% (Uglovoe) to 16% (Morskoe). *Vitis vinifera* at the stage of ripeness is characterized by the predominance of malvidin-3-O-β-D-glucoside and its derivatives as final metabolites in transformation chaining of anthocyanin compounds (Narduzzi et al., 2015; Farida et al., 2016; Ostroukhova et al., 2019; Ostroukhova et al., 2012b Peskova et al., 2017). In the anthocyanin complex of ‘Cabernet-

Sauvignon’ berries from Solnechnaya Dolina village, the proportion of malvidin-3-O-β-D-glucoside and its derivatives is 51%; in grapes from other territories − 68-73% (Figure 5). There is a close inverse correlation (r = 0.90; α = 0.05) between the proportion of malvidin and petunidine monoglycosides (including their derivatives) in the complex of anthocyanins in berry skin, which is explained by the fact that during biosynthesis they are generated from the same precursor - delphinidin-3-O-β-D-glucoside (Liu et al., 2018; Mannino et al., 2021). The highest proportion of cyanidins (4%) and petunidins (15%) is observed in grapes from Solnechnaya Dolina village, the lowest (less than 2% and 10%, respectively) − from Yalta city. At that, in the anthocyanin complex of berry skin from grapes, growing in Solnechnaya Dolina, the proportion of delphinidin-3-O-β-D-glucoside and its derivatives (23%) remains high, exceeding such (α<0.003) in grapes from other territories by an average of 1.9 times. High concentration of delphinidin, petunidin and cyanidin monoglucosides in berries with a relatively low content of malvidin monoglucosides, indicates the incomplete formation of anthocyanin complex in grapes from Solnechnaya Dolina village.

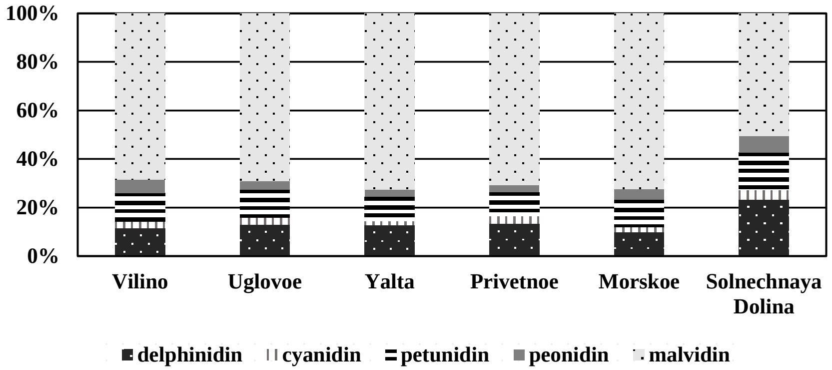


Figure 5. Composition (%) ofanthocyanin complex of monoglucosides and their derivatives in ‘Cabernet-Sauvignon’ skin of berries from different vineyards.

Phenolic antioxidants of berry skin of non-anthocyanin structures in most cases were represented by monomeric forms by 82-84% (Table 6), only in grapes from Morskoe the total proportion of phenolic acids, flavonols, flavan-3-ols averaged 74%. Caftaric and gallic acids in grape berry skin were in parity

Table 7. The content* (average, mg kg^{-1}) of anthocyanins in ‘Cabernet-Sauvignon’ skin of berries from different vineyards

Index	**Vilino**	**Uglovoe**	**Yalta**	**Privetnoe**	**Morskoe**	**Solnechnaya Dolina**
Delphinidin-3-O- β-D-glucoside	264.5	297.7	470.0	539.6	195.6	473.2
Cyanidin-3-O-β-D-glucoside	54.0	45.7	55.8	113.8	40.6	80.2
Petunidin-3-O-β-D-glucoside	225.9	247.0	302.9	319.3	168.8	262.4
Peonidin-3-O-β-D-glucoside	143.3	94.8	135.5	134.5	106.1	149.9
Malvidin-3-O-β-D-glucoside	1243.1	1473.1	1718.9	1863.4	1028.0	590.5
Delphinidin-3-O- β-D-glucoside-6'-O-acetate	63.1	91.3	112.9	92.6	39.0	49.1
Cyanidin-3-O-β-D-glucoside-6'-O-acetate	23.3	43.0	22.4	26.0	12.4	12.8
Petunidin-3-O-β-D-glucoside-6'-O-acetate	65.8	70.8	127.3	108.0	56.7	54.6
Peonidin-3-O-β-D-glucoside-6'-O-acetate	15.4	12.6	1.3	0.9	2.1	1.0
Malvidin-3-O-β-D-glucoside-6'-O-acetate	338.7	371.0	1192.3	1104.3	347.2	340.2
Petunidin-3-O-β-D-glucoside-6'-O-*p*-coumarate	42.4	28.0	30.8	35.8	38.9	30.4
Malvidin-3-O-β-D-glucoside-6'-O-*p*-coumarate	361.9	238.4	405.9	363.2	347.9	206.8

*SD-values were lower than 10%for all vineyards in each year of research. When taking into account the data of different years, SD-values were lower than 20%.

proportion (ratio 0.8 to 1.1), their total content varied from 39.2±4.1 mg kg^{-1} (Uglovoe) to 129.3±14.5 mg kg^{-1} (Privetnoe), which, in terms of percentage, did not exceed 2% in the complex of skin phenolic antioxidants. The interannual variance of the content of phenolic acids in the skin of berries exceeded such indicator variance by grape growing areas. The proportion of flavonols (quercetin and quercetin-3-O-b-D-glycosides) in the phenolic complex of grape skin from Vilino and Uglovoe vineyards was 2 times lower

(α <0.004) than in grapes from other territories and was less than 1%. Quantitative content of the components varied from 30.8±6.2 mg kg^{-1} (Vilino) to 122.3±19.5 mg kg^{-1} (Privetnoe). The proportion of flavan-3-ols in the phenolic complex of berry skin was 28-36%, and did not significantly differ between vineyards, ranging from 1206.6±116.7 mg kg^{-1} (Vilino) to 2529.3±471.6 mg kg^{-1} (Privetnoe). Unexceptionally, (-)-epicatechine was prevalent among all flavan-3-ols. The highest content of dimeric flavan-3-ols (B1-B4 procyanidins) was recorded in the skin of grapes from Yalta, Morskoe and Privetnoe villages, amounting to 502.2-563.1 mg kg^{-1} and exceeding such (α <0.003) in grapes from other territories by an average of 1.7 times. The proportion of B1-B4 procyanidins in the phenolic complex of berry skin from Morskoe reached 12%, and in grapes from other vineyards it was no more than 8%. Procyanidin B2 was prevailing in skin procyanidins, accounting for 74-79% in grapes from Yalta, Vilino, Uglovoe vineyards, and 23-52% − in grapes from the Mountain-Valley Coastal region; and procyanidin B4, the proportion of which in grapes from Privetnoe and Solnechnaya Dolina was reaching 67-68%.

The content of monomeric and dimeric phenolic antioxidants in grape seeds of ‘Cabernet-Sauvignon’ ranged from 426.3 to 1137.4 mg kg^{-1} and was 1.4-2.1 times less than that in ‘Kokur Belyi’ from similar geographical objects. The number of components in ‘Cabernet-Sauvignon’ grape seeds during the years of research by growing areas was increasing in the following series: Uglovoe, Vilino <Morskoe, Solnechnaya Dolina <Yalta, Privetnoe. Flavan-3-ols and B1-B4 procyanidins accounted for 91-96% of the complex of identified phenolic compounds in grape seeds. At the same time, monomeric flavan-3-ols prevailed in ‘Cabernet-Sauvignon’ grapes from the Mountain-Valley Coastal region, reaching 59-65% in the phenolic complex; flavan-3-ols and B1-B4 procyanidins were represented in parity proportion: 43-54% and 41-52%, respectively, in grapes from Uglovoe, Vilino and Yalta. The highest content of flavan-3-ols in seeds was in grapes from Privetnoe: 740.9±138.3 mg kg^{-1}, exceeding such in grapes from Morskoe, Solnechnaya Dolina, on average, in 1.4 times, from Yalta – 1.6 times, from Uglovoe, Vilino – 3.1 times. The content of B1-B4 procyanidins in seeds varied from 199.5 mg kg^{-1} to 576.4 mg kg^{-1} and was increasing by the areas of grape growing in the following series: Uglovoe, Vilino <Morskoe, Privetnoe <Solnechnaya Dolina <Yalta. Seeds of grapes from Uglovoe, Vilino, Morskoe and Solnechnaya Dolina contained 23.7-26.3 mg kg^{-1} of phenolic acids; in grapes from Yalta this indicator was 1.7 times higher (α<0.0008), in grapes from Privetnoe – 3.7 times higher. Grapes of ‘Cabernet-Sauvignon’ cultivar from Yalta were characterized by a higher content of flavonols in seeds compared to grapes from other territories.

In ‘Cabernet-Sauvignon’ grape pulp the total amount of identified phenolic antioxidants ranged from 22.5 to 53.9 mg kg^{-1}. The exception was the grapes from Solnechnaya Dolina, in pulp of which the content of components was at the level of 214.3±26.3 mg kg^{-1}: monomeric flavan-3-ols accounted for 44%, B1-B4 procyanidins − 28%. A close to this ratio of monomeric and dimeric flavan-3-ols (1.6-2.0) was determined in grape pulp from Morskoe and Privetnoe. In grapes from other territories, this ratio ranged from 0.6 (Uglovoe) to 1.2 (Yalta). Grapes from Vilino and Privetnoe differed in the highest content of phenolic acids (16.1±4.0 mg kg^{-1} and 11.4±2.2 mg kg^{-1}): the proportion of components reached 53% in grapes from Vilino; the content of phenolic acids in grape pulp from other territories was in the amount of 2.6-8.0 mg kg^{-1}. The highest content of flavanols in pulp in both quantitative (5.8±1.1 mg kg^{-1}) and fractional (16%) terms was distinguished by grapes from Morskoe.

A number of relationships between the content of monomeric and dimeric phenolic antioxidants in structural elements of berries and agroclimatic parameters of vineyards were revealed: correlation coefficients, significant at $\alpha<0.05$, amounted to/0.67-0.98/. It is shown that component accumulation in seeds of ‘Kokur Belyi’ and ‘Cabernet-Sauvignon’ grapes is in direct correlation with heat supply parameters (growing degree days $\sum T^{\circ}C_{10}$, $\sum T^{\circ}C_{20}$, Winkler index, average growing season temperature and September temperature), and in inverse correlation with moisture supply parameters (total precipitation during the growing season and during September). It was found that, on the contrary, the content of components in skin and, especially, in pulp of ‘Kokur Belyi’ grapevine cultivar is negatively correlated with the most of heat supply parameters, and positively - with their moisture supply. In the case of ‘Cabernet-Sauvignon’ cultivar, there was no correlation between the total accumulation of monomeric and dimeric phenolic substances in skin and pulp, on one hand, and agroclimatic resources of territories, on the other hand.

A detailed data analysis shows that the relationships between the content of phenolic acids in structural elements of ‘Kokur Belyi’ and ‘Cabernet-Sauvignon’, and the parameters of heat and moisture supply of vineyards correspond to the patterns presented above. At the same time, quantitative content of gallic acid in berry elements of ‘Kokur Belyi’ cultivar depends more on agroclimatic resources of vineyards than on the content of caftaric acid. A positive correlation between heat supply parameters of grape growing areas (for ‘Kokur Belyi’ − with all parameters except Huglin index; for ‘Cabernet-

Sauvignon’ − with Huglin index), and the content of flavonols in grape seeds (the closest − with quercetin) was revealed. On the contrary, there was no correlation in the case of skin and pulp. The data available show a direct correlation between the amount of (+)-D-catechine (‘Kokur Belyi’), (-)-epicatechine (‘Cabernet-Sauvignon’), B2 procyanidins (epicatechine-4→8-epicatechine − for ‘Cabernet-Sauvignon’), B4 procyanidins (catechine -4→8-epicatechine) in grape seeds, and the parameters of Huglin index (only for ‘Cabernet-Sauvignon’), growing degree days $\sum T^{\circ}C_{20}$, $\sum T^{\circ}C_{10}$, Winkler index, average September temperature. For ‘Cabernet-Sauvignon’ grapes, a negative correlation was revealed between the accumulation of (-)-epicatechine in seeds and moisture supply of vineyards. An inverse relationship between the content of (-)-epicatechine, B3 procyanidins (catechine -4→8-catechine) and most of heat supply parameters in skin and pulp of ‘Kokur Belyi’ cultivar was revealed. The content of B2 procyanidins in the skin of ‘Cabernet-Sauvignon’ berries, on the contrary, directly correlated with the Huglin index of vineyards. There was no correlation between the accumulation of anthocyanin complex in the skin of ‘Cabernet-Sauvignon’ berries and agroclimatic resources of growing areas in the available database. But direct correlation was identified between the rate of monoglucosides of malvidin and its derivatives in anthocyanin complex of ‘Cabernet-Sauvignon’ berry skin and the parameters of growing degree days above 10 °C, growing degree days above 20 °C, Winkler index, average growing season temperature, and inverse – with the parameters of total precipitation during the year and growing season typical for vineyard territories. On the contrary, the proportion of cyanidin and petunidin, and their derivatives is inversely correlated with the parameters of heat supply in vineyards: $\sum T^{\circ}C_{10}$, Winkler index, average growing season temperature. It was found that the higher the proportion of delphinidin monoglycoside and its derivatives in the anthocyanins complexis, the lower total precipitation during the growing season and Selyaninov hydrothermal coefficient are. The proportion of monoglucosides of malvidin and its derivatives is directly correlated with these moisture supply indicators.

The relationships presented can be commented on as follows. High level of heat supply in vineyards was determining the accumulation of monomeric and dimeric phenolic antioxidants in grape berries, especially in seeds. It was shown in the works (Adams et al., 2006; Levchenko et al., 2021; Gianluca et al. 2021) that the formation of flavan-3-ol, phenolic acids and procyanidins in a berry is mainly completed by the beginning of its ripening, and followed by the oxidative polymerization; biosynthesis of anthocyanins and their interconversion are carried out from the beginning of ripening to the ripeness onset (Gianluca et al. 2021). Our earlier studies (Ostroukhova, et al., 2022) show that Crimean autochthonous varieties, in particular 'Kokur Belyi', compared to classic varieties, are characterized by more intensive dynamics of flavan-3-ol, phenolic acids and procyanidins during grape ripening. The results of these studies on the example of 'Kokur Belyi' cultivar demonstrate the effect of heat supply resources of grape growing area on the intensity of transformation process of phenolic components in berries during ripening. Greater heat supply of vineyards gives more active enzymatic transformation of monomeric and dimeric forms of components, leading to their polymerization, and lower quantitative content of phenolic acids, quercetin, (-)-epicatechine, B3 procyanidins in grape skin and pulp of 'Kokur Belyi' cultivar by the time of technical ripeness of the yield. Multidirections of heat supply on the formation of phenolic complex of berries resulted in the lowest content of monomeric phenolic components in skin and pulp of grapes from Morskoe, as the most heat supplied in the years of observation, and the highest content of procyanidins in seeds compared to grapes from other territories. On the contrary, grapes from Vilino, as the least heat supplied area, were characterized by the lowest content of procyanidins in seeds and the highest content of monomeric components in skin and pulp. In the case of 'Cabernet-Sauvignon', the presented data allow us to make a conclusion only about intensifying the processes of interconversion in the components of anthocyanin series in grape skin with an increase in the heat supply of vineyards. In this respect, the fact of slowing down the formation of anthocyanin complex during grape ripening in Solnechnaya Dolina, reflected in a low proportion of malvidin-3-O-β-D-glucoside and its derivatives, requires special attention, compared to other territories, even if their climatic parameters are close to each other. Perhaps, this is due to peculiarities of agrotechnology system, low relative air humidity over past 3 years, soil composition (Rybalko et al., 2022; Ostroukhova et al., 2021a; Ostroukhova et al., 2021b). In general, during the period of observation, it was noted that the formation of a complex of monomeric and dimeric phenolic antioxidants in 'Cabernet-Sauvignon' grapes, especially in pulp and skin, is more dependent on the moisture supply of vineyards than that of 'Kokur Belyi' grapevine cultivar.

The results of hierarchical classification of parameters of the complex of monomeric and dimeric phenolic components in the structural parts of berries are an indirect proof of our conclusions (Figure 6). Grapes of 'Kokur Belyi' from Morskoe and Privetnoe are the closest in terms of the complex of phenolic antioxidants of seeds and skin. The greatest difference in the complex of components in grape seeds is revealed for the vineyards of Vilino and Solnechnaya Dolina, in grape skin and pulp – for Vilino and Morskoe villages. By the complex of dimeric and monomeric phenolic components in the skin of ‘Cabernet-Sauvignon’ cultivar, grapes from Vilino are the closest to grapes grown in the village Morskoe, and grapes from Yalta – to grapes from Privetnoe. Grapes from Privetnoe and Solnechnaya Dolina had the greatest difference. Similarity of componental complex in grape seeds of ‘Cabernet-Sauvignon’ was registered for growing territories in Uglovoe and Vilino; Solnechnaya Dolina and Morskoe. Grapes from Uglovoe and Privetnoe were the most different in qualitative composition and the content of phenolic antioxidants in seeds. The complex of components in the pulp of ‘Cabernet-Sauvignon’ berries was the closest to each other in grapes from Yalta and Uglovoe.

The Complex of Phenolic Antioxidants of Wines from Grapes of ‘Kokur Belyi’ and ‘Cabernet-Sauvignon’ Cultivars Obtained in Vineyards of Crimean Geographical Objects

In the process of winemaking (grape processing, must clarification, must/mush fermentation), monomeric and dimeric fractions of phenolic compounds easily pass from solid elements of berries into the must and wine. The derivatives of hydroxycinnamic acids, flavan-3-ols, procyanidins are initiators and agents of redox processes, occurring at first stages of wine preparation enzymatically, and then by the mechanism of free-radicals (Speisky et al., 2022; Oliveira et al., 2011; Danilewicz, 2021).

In order to assess whether the identified differences were preserved in the composition and content of monomeric and dimeric phenolic antioxidants of wines, obtained from different vineyards of Crimea, experimental samples were prepared in the conditions of micro-winemaking. Samples of wines from ‘Kokur Belyi’ were produced according to the following scheme: crushing grapes using roll crusher → pressing mush on a basket press → sulfiting (75-80 mg SO_2 L^{-1}) and settling must at a temperature of 10±2ºC → must fermentation on the yeast culture I-128 from the Magarach Collection of Winemaking Microorganisms with limited air access at a temperature of 22 ± 2ºC until the sugars are completely utilized → self-clarification, decantation and analysis of wines. When making wines from ‘Cabernet-Sauvignon’ cultivar, mush was fermented on the yeast culture I-652 until comprehensive sugar utilization.

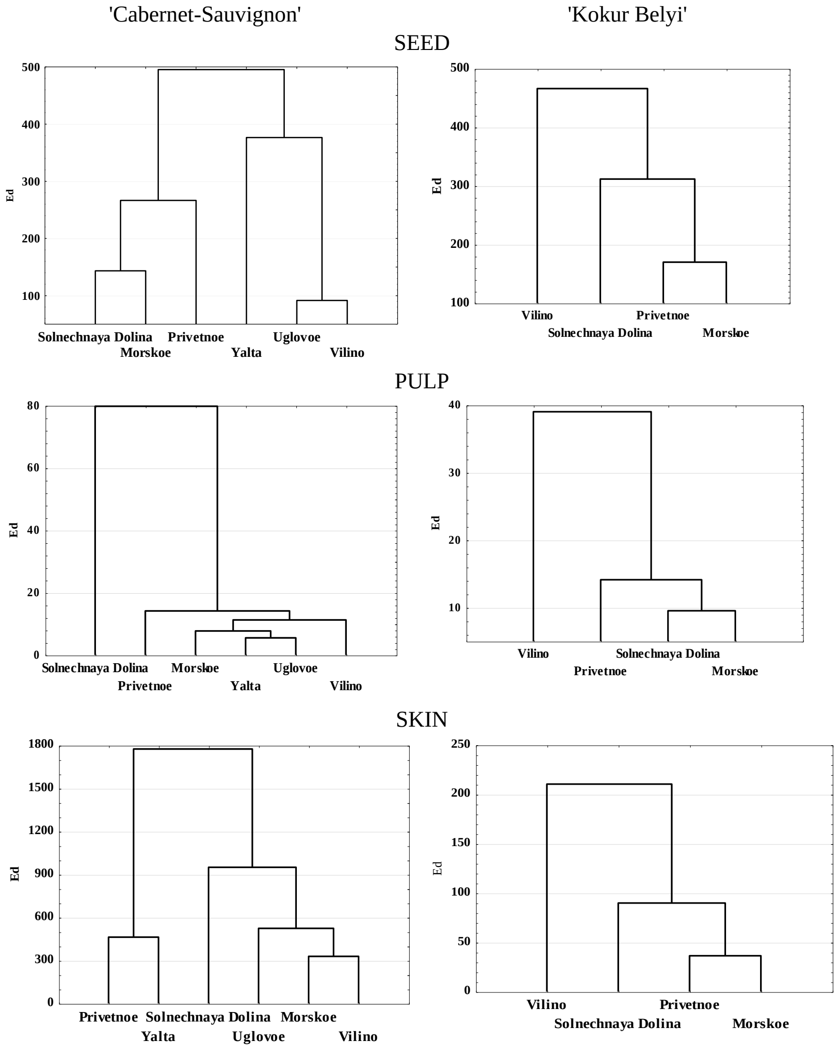


Figure 6. The results of hierarchical classification of grapes from different geographical objects by the complex of monomeric and dimeric phenolic antioxidants in structural parts of berries.

The content of ethyl alcohol in wine samples was 9.2-14.3% vol.; pH in wines from ‘Kokur Belyi’ was from 3.14 to 3.36, in wines from ‘Cabernet-Sauvignon’ - 3.19-3.82. The concentration of titratable acids in ‘Cabernet-Sauvignon’ wines from Morskoe was 5.6±0.3 g L^{-1}, in other red wines it averaged 7.4±0.9 g L^{-1}. Wines of ‘Kokur Belyi’ grapes from Solnechnaya Dolina differed from other wines ($\alpha<0.05$) in 1.2 times lower content of titratable acids (6.0±0.8 g L^{-1}).

The results of HPLC analysis of the complex of monomeric and dimeric phenolic antioxidants of ‘Kokur Belyi’ wines from the Mountain-Valley Coastal region are presented in Table 8. These data show the lowest (178.2±19.9 mg L^{-1}) content of components in wines from Morskoe village with the highest proportion (39±3%) of B1-B4 procyanidins among the analyzed wines, indicating the intensity of oxidative polymerization of phenolic compounds even at the stage of grape ripening. The concentration of mono- and dimeric phenolic components in wines from Privetnoe and Solnechnaya Dolina was on average 2 times higher. Phenolic acids (mainly gallic acid) − 44-67%, were predominant in the complex of phenolic antioxidants in all wines from ‘Kokur Belyi’ cultivar. The concentration of phenolic acids in wines from Solnechnaya Dolina was reaching 241.3±16.4 mg L^{-1}, exceeded such in wines from Privetnoe and Morskoe villages in 1.4 and 3.1 times. This is probably a consequence of the relatively high content of components in grape seeds from Solnechnaya Dolina. The highest content of flavan-3-ols, both in percentage (26±5%) and quantity (91.7±17.6 mg L^{-1}), was observed in wines from Privetnoe. Their concentration exceeded such in other wines from ‘Kokur Belyi’ cultivar by an average of 2.9 times. One of factors to accumulate flavan-3-ols in wines from Privetnoe is a relatively low PPO activity in grapes. On the contrary, a low level of flavan-3-ols (36.1±3.8 mg L^{-1}) in wines from Solnechnaya Dolina, containing the greatest quantity of components in seeds and skins, can be explained by a high PPO activity of grapes, followed by oxidative polymerization of the most labile fraction of phenolic compounds during processing.

The ‘Cabernet-Sauvignon’ wine from Yalta and Privetnoe turned out to be the closest (Ed=79) in terms of the complex of phenolic antioxidants due to its quantitative content (1362.1-1377.0 mg L^{-1}), the ratio of monomeric (on average, 88%) and dimeric components, (-)-epicatechine and (+) -D-catechine (8.3-9.6), the proportion of malvidin-3-O-β-D-glucoside and its derivatives in anthocyanin complex (on average, 85%) (Table 9, Figure 7). Similarities in the complex of mono- and dimeric phenolic components of wines from Yalta and Privetnoe are a consequence of close complex of components in grape skin from these territories (Figure 6). At that, technological stock of phenolic components, including anthocyanins, in grapes from Privetnoe was 1.2 and 1.8 times higher than in grapes from Yalta (Table 4), suggesting the enrichment of phenolic complex of grapes and wines from Privetnoe with highly polymerized tannins and anthocyanin-tannin complexes. These differences in the phenolic complex of grapes and wines from the studied vineyards are interconnected with a higher heat supply of Privetnoe and moisture supply – of Yalta. A complex of mono- and dimeric phenolic antioxidants in wines of ‘Cabernet-Sauvignon’ cultivar from Vilino is close (Ed=91) to such in wines from Privetnoe in terms of componental percentage, but exceeds its quantity by an average of 5%, reaching 1446.9 mg L^{-1}. This is explained by the higher degree of component extractability from solid parts of pulp into the must in grapes from Vilino village (Table 4). The complex of identified phenolic antioxidants in wines of ‘Cabernet-Sauvignon’ from Morskoe is similar (Ed=118) to such in wines from Solnechnaya Dolina.

Table 8. The content*, mg L^{-1} of phenoliccomponents in wines of ‘Kokur Belyi’ grapevine cultivar from different vineyards

Compounds	**Morskoe**	**Privetnoe**	**Solnechnaya Dolina**
Hydroxybenzoic and hydroxycinnamic acids			
Gallic acid	4.1	6.3	43.9
Caftaric acid	74.7	161.8	197.4
Flavonols			
Quercetin	2.1	2.9	3.2
Quercetin -3-O-b-D-glycosides	0.4	0.9	1.9
Flavan-3-ols			
(+)-D-Catechine	7.8	11.3	16.0
(-)-Epicatechine	20.1	80.4	20.1
Procyanidins			
B1: epicatechine-4→8-catechine	6.2	19.9	11.0
B2: epicatechine-4→8-epicatechine	46.9	28.8	22.5
B3: catechine -4→8-catechine	9.8	17.6	22.4
B4: catechine -4→8-epicatechine	6.2	21.1	24.6

*SD-values were lower than 9%for all vineyards in each year of research. When taking into account the data of different years, SD-values were lower than 17%.

At the same time, wines from Morskoe and Solnechnaya Dolina differ from wines from Yalta, Privetnoe and Vilino in a lower (1.6 times) concentration of components (787.6-981.8 mg L^{-1}), lower (1.5 times) ratio of (-)-epicatechine and (+)-D-catechine (5.5-7.4), lower proportion of malvidin-3-O-β-D-glucoside and its derivatives in the anthocyanin complex (79-80%)

and a higher proportion of delphinidin-3-O-β-D-glucoside, petunidin-3-O-β-D-glucoside and their derivatives (15-17%). The complex of mono- and dimeric components of wines from Morskoe and Solnechnaya Dolina is similar with the complex in grape seeds from these territories, with this background, the reduced concentration level of components in wines can be explained by oxidative polymerization of components both at the stage of grape ripening and during grape processing and pulp fermentation, which is associated with a relatively high PPO activity of grapes (Table 4). Among the studied areas of ‘Cabernet-Sauvignon’ growth, the vineyards of Morskoe and Solnechnaya Dolina villages are characterized by the highest level of heat supply, leading to intensification of polymerization processes of labile fractions of phenolic compounds in both cases. Wines from Uglovoe, amongst other wines prepared from ‘Cabernet-Sauvignon’ cultivar, are characterized by the lowest content of mono- and dimeric phenolic antioxidants (627.8±109.1 mg L-^{1}), the lowest proportion of anthocyanins (32±2%) and flavan-3-ols (22±3%), the highest proportion of B1-B4 procyanidins (25±3%) and phenolic acids (17±2%). The distance degree of phenolic complex of both wines and grapes (Table 4 and Figure 5) of Uglovoe village from samples of other territories is the highest, which, considering the above relationships, can be explained by the lowest heat supply of vineyard.

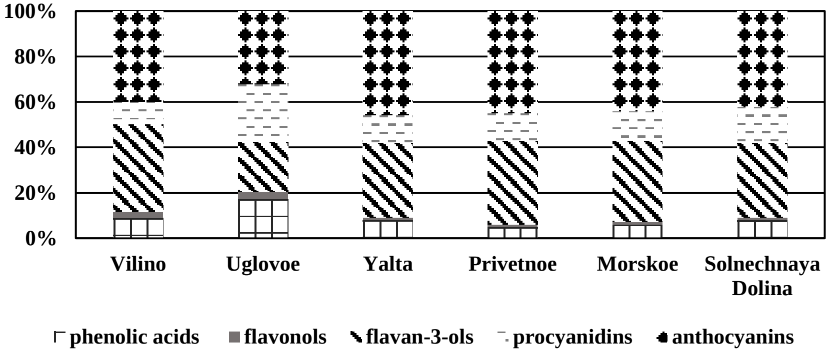


Figure 7. Composition (%) of the complex of phenolic antioxidants in ‘Cabernet-Sauvignon’ wines from different vineyards.

Table 9. The content*, mg L^{-1} of phenolic components in wines of ‘Cabernet-Sauvignon’ grapevine cultivar from different vineyards

Compounds	**Vilino**	**Uglovoe**	**Yalta**	**Privetnoe**	**Morskoe**	**Solnechnaya Dolina**
Hydroxybenzoic and hydroxycinnamic acids						
Gallic acid	37.5	21.8	20.5	32.4	38.1	20.1
Caftaric acid	87.7	85.7	89.4	38.9	20.0	39.4
Flavonols						
Quercetin	33.9	11.7	10.8	11.1	8.3	6.1
Quercetin -3-O-b-D-glycosides	3.3	8.6	6.4	3.4	3.0	2.8
Flavan-3-ols						
(+)-D-Catechine	45.6	38.3	42.7	54.2	42.1	40.3
(-)-Epicatechine	515.1	99.5	408.5	452.4	310.7	222.1
Procyanidins						
B1: epicatechine-4→8-catechine	33.7	23.0	15.6	37.3	20.6	32.4
B2: epicatechine-4→8-epicatechine	58.5	84.8	86.5	57.2	50.2	57.2
B3: catechine -4→8-catechine	17.7	20.7	17.8	22.9	22.8	14.6
B4: catechine -4→8-epicatechine	41.7	31.2	40.8	42.4	30.2	21.2
Anthocyanins						
Delphinidin-3-O- β-D-glucoside	20.1	8.3	19.4	22.0	18.4	14.3
Cyanidin-3-O-β-D-glucoside	1.8	0.6	1.2	3.4	1.1	1.1
Petunidin-3-O-β-D-glucoside	22.1	8.0	29.4	28.1	22.3	16.1
Peonidin-3-O-β-D-glucoside	10.9	3.6	15.5	12.1	11.5	8.7
Malvidin-3-O-β-D-glucoside	350.7	87.6	353.2	351.6	232.8	170.2
Delphinidin-3-O- β-D-glucoside-6’-O-acetate	8.1	18.0	6.9	8.4	10.6	11.9
Cyanidin-3-O-β-D-glucoside-6’-O-acetate	10.8	19.8	4.5	6.5	2.4	6.1
Petunidin-3-O-β-D-glucoside-6’-O-acetate	4.5	7.5	14.9	10.4	15.6	8.6
Peonidin-3-O-β-D-glucoside-6’-O-acetate	2.3	2.4	0.0	0.6	1.7	1.9
Malvidin-3-O-β-D-glucoside-6’-O-acetate	116.6	38.6	147.8	148.6	97.1	66.8
Petunidin-3-O-β-D-glucoside-6’-O-*p*-coumarate	1.5	1.0	0.8	0.7	1.3	1.9
Malvidin-3-O-β-D- glucoside-6’-O-*p*-coumarate	22.7	7.2	29.7	32.5	20.8	23.5

*SD-values were lower than 10%for all vineyards in each year of research. When taking into account the data of different years, SD-values were lower than 20%.

In general, the research results allow to conclude that heat supply of grape growing areas largely determines the accumulation of phenolic antioxidants in structural elements of berries, and the intensity of their transformations both during grape ripening, processing, and wine production, leading to significant differences in the phenolic complex of young wines from different vineyards. This is naturally manifested in the case of ‘Kokur Belyi’ cultivar. Moisture supply of vineyards adjusts the formation of a complex of phenolic antioxidants in grapes and wines of ‘Cabernet-Sauvignon’ grapevine cultivar.

The antioxidant activity of grapes and wines is determined not only by the quantity of certain components, but also by their ability to bind free radicals, which depends on the structure of substances (Chen et al., 2020; Chen et al., 2015; Platzer et al., 2021; Farkas et al., 2004; Minatel et al., 2016). As a result of studies conducted at the Scientific Research Institute of Nutrition of the Russian Academy of Medical Sciences, adequate and upper tolerable levels of consumption of biologically active components or their groups were established (Table 10). Under the adequate level of consumption, daily demand of healthy person in nutritive and biologically active substances and its consumption is meant, under the upper tolerable level of consumption - the highest level of daily consumption of biologically active substances without adverse effect to a person. The information presented in Table 10 allows us to assess the significance of differences in the phenolic complex of wines obtained from grapes of different growing areas from the standpoint of antioxidant activity of the components and the adequacy of their quantitative content to the demands of healthy person.

Table 10. Adequate and upper tolerable levels of consumption of phenolicantioxidants*

Substance, a group of substances	**Adequate consumption level, mg per day**	**Upper tolerable consumption level, mg per day**
Hydroxycinnamic acids (including caffeic)	10	20
Gallic acid	100	300
Flavonols	30	100
Flavan-3-ols	50	100
Procyanidins	50	500
Anthocyanins	50	150

*Recommended levels of consumption of nutritive and biologically active substances: Guidelines. MR 2.3.1.1915-04. M.: Federal Center of the State Sanitary and Epidemiological Supervision of the Ministry of Health of Russia, 2004. 46 p.

Wines from ‘Kokur Belyi’ grapes, growing in the Mountain-Valley Coastal region of Crimea, and wines from ‘Cabernet-Sauvignon’ grapes, from the Western Piedmont-Coastal region, can be an additional source of caftaric acid in human nutrition: 100-150 ml of wine containthe quantity of components, which satisfy daily demand. Above all, biological effect of hydroxycinnamic acids is associated with inhibition of a low density of lipoprotein oxidation (Menschikova et al., 2012). Regardless of the grapevine cultivar and its growing area, gallic acid in wines is present in biologically insignificant amounts. The concentration of flavonols in all wines from the standpoint of daily human demand was extremely low: component amount in only 1 L of wine from Vilino corresponded to an adequate level of consumption. Flavonols improve the elasticity and permeability of blood vessel walls and coronary circulation (Aron et al., 2008; Osakabe et al., 2013). The amount of monomeric flavan-3-ols and anthocyanins, corresponding to an adequate level of consumption, was contained in 100-110 ml of wines of ‘Cabernet-Sauvignon’ grapevine cultivar from Privetnoe, Yalta and Vilino; in 150-190 ml of wines from Morskoe and Solnechnaya Dolina; in 250-360 ml of wines from Uglovoe. The flavan-3-ols in biologically significant amount were present only in wines of ‘Kokur Belyi’ from Privetnoe village: the quantity of components close to the adequate level of consumption was contained in 540-560 ml of wines. According to the studies (Sato et al., 2001; Bagchi, et al., 2003), monomeric flavan-3-ols have a number of health-improving effects on human body. They inhibit prostaglandin biosynthesis, catalyzed by cyclooxygenase-2 and leading to the suppression of inflammatory processes, as well as induce apoptosis of tumor cells, etc. Anthocyanins also have multi-dimension positive effect on human body, but most often they are used to improve the elasticity of blood vessel walls and vision acuity (Mozos et al., 2021; Nomi et al., 2019). The amount of procyanidins in wines, from the point of corresponding to an adequate level of consumption by the areas of grape growing, did not differ significantly in the case of ‘Cabernet-Sauvignon’ cultivar, and was contained in 320-400 ml of wine, for ‘Kokur Belyi’ – in 600-650 ml of wine.

It should be noted that the combination of differences in phenol, oxidase and acid complexes of grape cultivars ‘Kokur Belyi’ and ‘Cabernet-Sauvignon’, obtained from different vineyards, has led to dissimilarities in sensory characteristics of young wines. Wines from grapes of ‘Kokur Belyi’ were characterized by a varietal aroma and fruity flavor with hints of odoriferous herbs and honey. At the same time, wines from Privetnoe were distinguished by light straw color, light flavor, mild aroma; wines from

Solnechnaya Dolina − as a rule, by dark straw color, dense structure, full-bodied flavor with light oxidation tones. Wines from grapes of ‘Cabernet-Sauvignon’ cultivar had a ruby or dark ruby color, varietal berry aroma. Wines of ‘Cabernet-Sauvignon’ from Vilino, Yalta and Uglovoe were distinguished by the presence of nightshade and odoriferous herbal tones in aroma, mild full-bodied and tannic flavor. Wines from Privetnoe, Morskoe and Solnechnaya Dolina were characterized by a complex berry-spicy aroma with hints of ripe cherries, dried fruits, milk cream, and soft and velvety flavor.

Thus, the research results indicate the possibility of obtaining dry wines of different styles, enriched in mono- and dimeric phenolic antioxidants in biologically significant amounts from ‘Kokur Belyi’ and ‘Cabernet-Sauvignon’ cultivars, depending on agroclimatic resources of growing areas.

Conclusion

One of factors to protect the population from adverse environmental conditions, associated with the pollution, is the enrichment of food products with natural antioxidants, among which the phenolic complex of grapes, distinguished by its multivariate biological activity. At the same time, global climate change makes adjustments to the metabolism of grape plants, which is reflected, among other things, in the content of phenolic components in grapes.

In this regard, we assessed the influence of agroecological conditions of ‘Kokur Belyi’ and ‘Cabernet-Sauvignon’ grape growing on the formation of a complex of mono- and dimeric phenolic antioxidants in berries and wines. Basicset of the research (2016 - 2021) included the vineyards located in three natural regions of the Crimean Peninsula. Using methods of geoinformation and mathematical modeling, HPLC, Statistica 10, the following was established.

The vineyards of ‘Kokur Belyi’ grape cultivar differed (α <0.00001) in terms of heat resources in the series: Vilino <Privetnoe <Solnechnaya Dolina <Morskoe; ‘Cabernet-Sauvignon’ – in the series: Uglovoe <Vilino <Yalta <Privetnoe, Solnechnaya Dolina <Morskoe. The vineyards of ‘Cabernet-Sauvignon’ cultivar located near Yalta city were characterized by higher precipitation degree.

The increase in heat supply of territories was accompanied (r=/0.67-0.98/, α <0.05), on one hand, by the accumulation of phenolic acids, flavonols, flavan-3-ols and procyanidins in grape seeds; on the other hand, it contributed to oxidative polymerization of phenolic components during grape ripening and processing (including due to an increase in monophenol monooxygenase activity and must pH), leading to a decrease in the content of mono- and dimeric components in grape skin and pulp, flavan-3-ols in wines. This naturally manifested itself in the case of ‘Kokur Belyi’ grapes. Moisture supply of vineyards adjusts the formation of a complex of phenolic antioxidants in ‘Cabernet-Sauvignon’ grapes and wines. An increase in the heat supply of vineyards was accompanied by an increase in the proportion of malvidin-3-O-β-D-glucoside and its derivatives, and a decrease in the proportion of cyanidin and petunidin and their derivatives in the anthocyanin complex of berries.

From the standpoint of component antioxidant activity and the adequacy of its quantitative content to the needs of a healthy person, the territories to obtain wines enriched in phenolic antioxidants of various functional direction were determined as follows: caftaric acid - vineyards in the Mountain-Valley Coastal region of Crimea (‘Kokur Belyi’), and Western Piedmont-Coastal region (‘Cabernet-Sauvignon’); flavan-3-ols and anthocyanins - Privetnoe, Yalta and Vilino (‘Cabernet-Sauvignon’).

The difference in the sensory characteristics of wines depending on the territory of grape cultivation is shown.

The research results are significant for an object-oriented assessment of climatic conditions of grape growing areas, in particular, in terms of the formation of a complex of phenolic antioxidants in wines, as well as their sensory style in the requested direction.

Disclaimer

None

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Chapter 2

Towards a Green Economy and Agricultural Sustainability Using Environmental Friendly By-Products

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Abstract

For decades, chemical fertilizers have been used to boost agricultural crop yield. However, due to high procurement costs, environmental contamination, and the potential for land degradation from improper application, there is a current trend towards finding alternative solutions. The global demand for food that is both high-quality and produced sustainably, while preserving soil biodiversity, has led to a demand for premium organic ingredients. This can be achieved through utilizing organic waste in rural and urban areas by applying composting technology to produce organic fertilizers, thus reducing the use of

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harmful chemicals and protecting the environment. Bio-organic agriculture uses advanced biotechnologies, primarily relying on organic amendments such as nitrogen-fixing bacteria and humic substances, to produce crops. Therefore, utilizing bio-organic farming methods is a crucial step towards achieving agricultural sustainability. In this chapter, the authors highlight the benefits of four organic and biological amendments, including spent grain, vermicompost, PGPR, and humic substances, for enhancing soil fertility and health in different environmental conditions, ultimately promoting agricultural sustainability.

Keywords: sustainability, humic substances, PGPR, saline soil, environmental-friendly

Introduction

Egypt climate is dry, hot, and is dominated by desert. It has a mild winter season with rain falling along coastal areas and a hot and dry summer season (May to September). Daytime temperatures vary by season and change with the prevailing winds [1]. In the coastal regions, temperatures range between average winter minimums of 14°C (November to April) and average summer maximums of 30°C (May to October) [2, 3]. Temperatures vary widely in the inland desert areas, especially during the summer, where they range from 7°C at night to 43°C during the day [4-6]. During winter, temperatures in the desert fluctuate less dramatically but can reach 0°C at night and as high as 18°C during the day [6, 7]. Egypt also experiences hot wind storms, known as “khamsin,” which carry sand and dust and sweep across the northern coast of Africa [8-10]. These khamsin storms typically occur between March and May, increase the temperature by 20°C in two hours, and last for several days [11-13].

Egypt is a highly arid country and it receives very little annual precipitation [14, 15]. The majority of rain falls along the coast, with the highest rainfall received in Alexandria, approximately 200 mm of precipitation per year. Precipitation decreases southward, and Cairo receives a little more than 10 mm of precipitation each year, although it experiences humidity during the summer months. Areas south of Cairo receive only traces of rainfall yet can suddenly experience extreme precipitation events resulting in flash floods [6, 45].

According to the United Nations Environment Programme (UNEP), utilizing environmental friendly by-products in agriculture can help to create a more sustainable and eco-friendly economy [17-19]. For example, the conversion of plant waste into biofuels and organic fertilizers can reduce the amount of waste sent to landfills and improve soil health (UNEP, 2011). Additionally, sustainable agricultural practices, such as crop rotation and reduced chemical use, have been shown to have positive impacts on the environment, such as reducing soil erosion and improving water quality [64]. Overall, by utilizing environmentally friendly by-products and sustainable agricultural practices, we can move towards a green economy and agricultural sustainability [20-28].

The agriculture challenge in Egypt by this time and after that is to produce food, at least sufficient for the growing population of above 2.6% per annum [29-31]. Mineral fertilizers seem to be the quickest way of boosting crop production; however, their costs, environmental problems, and other constraints frequently deter the farmers from using these in recommended quantities and balanced proportions [32-36]. As a consequence of these and other constraints, there seems no option but to exploit the potential alternative sources of plant nutrients fully. So, increasing attention should be focused on the efficient utilization of organic fertilizers and the use of such low-cost nutrient sources as spent grain and other organic wastes to reduce the cost of production and sustain and maintain soil health and productivity [37]. Developing cheap production technology may reduce dependence on non-renewable energy sources; it is imperative to exploit renewable biological sources [38-40]. These sources include recycling plant nutrients within agricultural production systems and various organic systems waste outside agriculture [41-44]. In this connection, our book chapter about organic and bio-organic ameliorants for increasing the yield of wheat and corn by improving the chemical and biological properties of saline-sodic and calcareous soils are reviewed here as under the following headings which will be discussed further: Saline-sodic properties; calcareous soil properties; characterization of soil ameliorants; biological correction of the plant and soil systems; humic substances to enhance the land degradation and plant productivity:

1. Saline-Sodic Soil Properties

Globally salt-affected soils account for approximately 46 million hectares (20%) of agricultural land [46]. More than 60% of salt-affected soils classified as saline-sodic soils, the saline-sodic soils possess EC > 4 dS m^{-1}, pH > 8.2 and exchangeable sodium percentage (ESP) >15% [47]. In Egypt, the total area of agricultural land is 3.36 million acres, which is 3.8% of the country's entire territory. One of the main obstacles to agricultural production in Egypt is soil salinization, which leads to land degradation [48]. The factors that led to the soil salinization and degradation effects are the key to environmental impediments that severely influence agricultural productivity and sustainability in arid and semi-arid regions [49, 50].

Soil salinization is defined as an excessive accumulation of salts within the soil profile to the extent that it decreases plant growth. It has been one of the major environmental problems that have threatened agricultural productivity since ancient times [31, 32]. Salt affected soils are in general classified as; saline, sodic, or saline-sodic, based on their respective electrical conductivity (EC) and sodium adsorption ratio (SAR) of the saturated paste extracts or the sodium on the exchange sites (exchangeable sodium percentage, ESP) [52]. Saline soils are characterized by having high EC values (> 4 dS m^{-1}), while saline-sodic soils have both high EC (> 4 dS m^{-1}) and SAR (>13) of the saturation extract and an ESP > 15. Sodic soils are those which have low EC (< 4 dS m^{-1}) but have high SAR’s (>13) or ESP > 15 [51, 52]. Saline-sodic soils can be considered highly degraded and least productive due to their simultaneous salinity and sodicity on soil physical, chemical, and biological properties. High salinity retards plant growth by creating osmotic imbalances and specific ion toxicities. In addition to physical effects, chemical, biological, and biochemical property deteriorations have been well reported in the literature for saline and sodic soils [31, 32, 33, 35].

It is important to understand how the salinity affected the soil and plant growth rates to identify appropriate organic soil fertilization strategies in salt spaces (Figure 1.1). The salinity can be defined as the dissolved mineral salts accumulated in soil solution and rhizosphere [54]. The natural effects of primary salinization or secondary salinization effects may be the leading causes of soil salinity mineral deposition in natural salts and sediment. The atmospheric deposition from sea salts the intrusion of seawater into the ground-water of coastal areas is another form of soil salt. Water overuse can significantly decrease the standard water-table [54]. Salt may rise due to soil-evaporation and soil-plant evapotranspiration under high water table conditions. Secondary salinity may be caused by irrigation sometimes carried out on salinity soils with salty water.

This use of wastewater helps to face the current scarcity of water resources for farming, which is determined by the competition with different human and industrial uses. However, attention must be paid to the quality of the water used and the fact that seasonal/temporary salinization can be partially controlled by fulfilling appropriate leaching requirements [37, 38].

Besides, repeated soil additives of organic materials to crop-land may be considered an anthropogenic salts source. Therefore, appropriate organic materials management strategies, such as controlled bio-degradation processes (i.e., composting), are crucial to minimizing the potentially negative environmental impact of waste applications before their use in agriculture [54]. The soluble salts distribution in the soil profile is influenced by leaching and evaporation from the soil surface. Certain specific ions accumulated, such as chloride (Cl), can be directly toxic depending on plant-specific tolerances and may induce physiological disturbances. Water deficits or osmotic effects are among the major factors that bring a decline in cell division and reduce plant growth (Figure 1), thus limiting crop production [8, 9, 10]. Also, the salts excess may adversely influence biological, physical, and chemical soil properties. Soil sodicity (i.e., excess of Na^+ in the rhizosphere) is a secondary consequence of soil salinity effects, typical for clay soils and affects their soil properties. In these soils, the exchangeable Na^+ is bound to the negative clay charges, causing the clay particles' de-flocculation. As [57, 58] found, the high exch-Na^+ percentage can lead to clay swelling and dispersion of clay, as well as soil aggregates breaking. As a consequence, both the water-holding capacity and water infiltration rate could be reduced by this process. Saline soils are more comfortable to be reclaimed than sodic ones because, generally, the former requires leaching of soluble salts, while the latter also requires a Ca^{2+} source to replace the excess Na^+ [11, 12].

Soil chemical properties such as pH, cation exchange capacity (CEC), exchangeable sodium percentage (ESP), soil organic carbon are also affected by salinity, and the osmotic and matric potential of soil solution is altered [57, 59]. Most soils affected by salt are deficient in various nutrients; more fertilizer applications may be required. The deficiency of micro-nutrients appears to be a side effect of salinization and may result from soil alkalinization and ion competition [47]. Moreover, [35] showed an increase in soil salinity inhibits several soil enzymatic activities, such as alkaline phosphatase, dehydrogenase, urease, and β-glucosidase. Simultaneously, [61] indicated the effects of salinity on soil microbial biomass carbon and crop yield. The *Azospirillum* bacteria part of the plant growth-promoting rhizobacteria was strongly reduced in saline-sodic soils [62]. Therefore, organic and biological tools to improve soil properties in salt-affected soils are crucial to guarantee farmers' income, particularly in arid world regions.

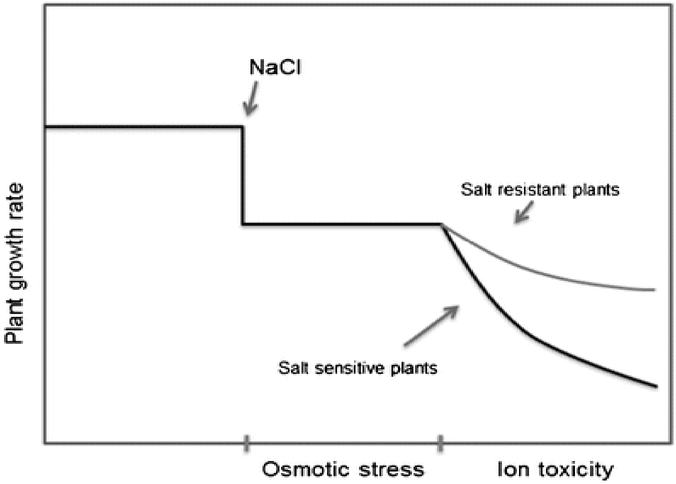

Figure 1. The relationship between the vegetation density and osmotic stress.

2. Calcareous Soil Properties

The calcareous soils identified by the presence of $CaCO_3$ in the parent material can be more than 15 percent calcium carbonate equivalent in calcium horizons the layer of secondary carbonate build-up usually Ca^{2+} or Mg^{2+} carbonates and 5 percent more carbonate than a base layer [8, 43]. Calcareous soils in the soil have $CaCO_3$ that can occur in different ways, such as powdery, nodules, crusts, etc. Their chemical and physical parameters vary-the critical differences in water solubility and hydrolysis among these types. Soils with high $CaCO_3$ belong to calcisols and similar subsets of other soils with calcium horizons; they are relatively popular in the soil's drier areas. Although $CaCO_3$ is a significant factor controlling the properties of the systems in calcareous soil systems, it is also affected by other soil characteristics, such as the pH of $CaCO_3$ water suspensions, the soil organic matter, and the aeration.

In semi-arid and arid regions, calcareous soils are common and extend to 30% of arable soils worldwide [64]. The total area of calcareous soil, almost all in the arid, semi-arid tropics and sub-tropics of both hemispheres, could well amount to some billion hectares. In Egypt, the cultivation of calcareous soils faces several difficulties, such as surface crusting, and silt was the textural fraction with the highest effect in inducing surface crusting and cracking, high pH (7.5 to 8.5) and loss of N fertilizers, low availability of nutrients significantly P and micro-nutrients (Zn^{2+}, Fe^{2+}, Mn^{2+}, and Cu^{2+}) and nutritional imbalance between some elements (K^+ and Mg^{2+}) and calcium. Under such severe conditions, desired yield levels are challenging to attain [65, 66]. One of the primary explanations why P is not readily available to the plant? P is high reactivity results in precipitation or adsorption of between 75 and 90 percent of the plant P available in these soils with individual metal complexes such as calcium in calcareous soils and iron in acidic soils [67].

Often, dicalcium phosphate ($CaHPO_4$) and tricalcium phosphate $Ca_3 (PO_4)_2$ are P fixed form on soils with high pH. Solubility is very low in these root zones of tricalcium phosphate. It is fixed to alkaline soils in three shapes.

1) The phosphorus fixation was considered identical to the following chemical equation in calcareous soil with pH > 7.5.
2) $Ca(H_2PO_4)_2 + 2Ca^{+2} \leftrightarrow Ca_3(PO_4)_2 + 4H^+$
3) The phosphorus precipitation on the surface of $CaCO_3$ is fixed by reacting with Ca as in the chemical equation.
4) $Ca(H_2PO_4)_2 + 2CaCO_3 \leftrightarrow Ca_3(PO_4)_2 + 2CO_2 + 2H_2O$
5) In calcareous alkaline soils, phosphorus fixation is also high. Clays saturated with Ca^{2+}, a divalent cation, fix more phosphorus than a valence ion saturated clay. Clay] - Ca^{+2} - H_2PO_4

In this way, the fixation due to clay surfaces in the soil around pH = 7 [68].

The concentration of Zn^{2+}, Fe^{2+}, Mn^{2+}, and Cu^{2+} deficiencies is standard in the Egyptian soils with a high $CaCO_3$ due to reduced solubility at alkaline pH values. The CEC of the calcareous soils depends on their clay and organic matter type and content. In Egypt's highly calcareous areas, west of the Nile Delta, the CEC is about 15 meq100 g^{-1} soils [20, 48]. Because the nutrient elements in the exchangeable form are considered available to plants, the greater the CEC of the base-saturated soil. Temperature also has a significant effect on dissolving $CaCO_3$ under hot climates. Since the Egyptian soil is classified as arid or hyper-arid, the dissolving of calcium carbonate is probably.

3. Characterization of Soil Ameliorants That Are Applicable in Arid Soils

Reclamation of saline-sodic and calcareous soils requires that calcium ions replace part or most enhanced soil chemical and biological properties, exchangeable sodium at least in the root zone. The reclamation processes of saline-sodic soils are reasonably well understood, and the addition of a variety of organic and biological ameliorants is widely recognized and practiced. Some reclamation processes that have been practiced in the past include gypsum and organic matter [8, 31]. The reclamation of saline-sodic and calcareous soils can be accomplished in many ways, the best being dictated by local conditions, available resources, and the kinds of crops to be grown during reclamation. For quick results, cropping must be preceded by applying a biological and organic ameliorant followed by water irrigation to organic decomposition matter and soluble salts and other reaction products of the amendment.

3.1. Spent Grain

Spent grain (SG) is the major by-product of the beer industry, representing around 85% of the total by-products generated [69]. The SG constitutes approximately 25.4, 21.8, 11.9, 24.0, 10.6, and 2.4% cellulose, arabinoxylan, lignin, protein, lipid, and ash. Minerals, vitamins, and amino acids are also found in SG. The percentage of protein is high, which indicates nitrogen content in the SG. This high protein encourages using SG in composting for agriculture use. The mineral elements include calcium, cobalt, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium, and sulfur, all in concentrations lower than 0.5% [17, 69, 70, 72]. There is report [21] that: (1) spent grain is considered as a source of nitrogen to the compost pile because its C: N ratios range from 17:1 to 12:1; (2) spent grain from the microbreweries had about 1 pound of nitrogen in every 100 pounds of the product while the spent grain from the large brewery contributed about 4 pounds of nitrogen per 100 pounds of product, (3) the spent grain from the microbreweries is quite wet because its water content range is 66-77% and, (4) the concentration of macronutrients of P, K, Ca, and Mg ranges are highly in spent grain organic materials.

The spent grain has several uses, among other uses, such as food ingredient for animal and human, energy production, charcoal production, as a brick component, paper manufacture, a substrate for cultivation of microorganisms, substrate for enzyme production, and organic waste for agriculture use and an adsorbent for heavy metals (bioremediation). A broader area of the application currently under more extensive investigation is the potential for composting or direct addition to agricultural soils. Composting offers opportunities to dispose of waste and produce a sold grain commodity that is difficult to compost alone because of its high humidity content. However, mixtures with wastewater sludge and bulking agents have been successful [20]. Compared to commercial compost, such products supported less growth than commercially available composts. This research indicates that a further rise in waste to generate multipurpose compost may be overcome [7]. An additional SG opportunity is a bioremediation replacement, whereby seeds may provide nutrients to facilitate microbial pollutant degradation and spoilage content or mineral pollution adjustment. This result may involve matching the materials according to demand and growing grains, mixed with sterile soils containing 32 percent crude oil and no degrading oil microbial consortium like bio-factions with specialist microorganisms adapted for digestion of different pollutants. More rapid degradation of the oil fractions was observed in the presence of SG at 7 and 21 days of incubation, particularly for higher molecular weight hydrocarbons.

3.2. Spent Grain Vermicomposting

Organic materials such as spent grain are a major source of environmental pollution, causing serious waste disposal problems, emitting odor and ammonia to the air, contaminating the soil, and posing health risks. Spent grain vermicomposting, the decomposing process of organic waste into a valuable organic fertilizer product and soil conditioner using earthworms, can resolve this problem and new ideas on this organic waste in Egypt.

3.2.a. Vermicomposting Technology

Vermicomposting is generally defined by exploiting earthworms' best biological activity and microorganisms as the solid phase decomposition of organic residues in aerobic conditions [73]. Vermicomposting is usually defined as the solid phase decomposition of organic residues in the aerobic environment by exploiting earthworms and microorganisms' optimum biological activity [74]. Vermicomposting is an environmentally friendly process of bio-oxidation and stabilization of organic materials such as paper residues [75], animal dungs, industrial materials [76], municipal sewage sludges [77]. Nutrient recycling through vermicomposting involves the joint action of earthworms and microorganisms [78]. Recently, vermicomposting has been defined as "the bi-oxidation and stabilization of the organic material involved in the joint action of earthworms and mesophilic microorganisms." The vermicompost materials obtained from earthworm activity have been rich in enzymes such as proteins and vitamin growth hormones that test amylases such as cellulose, chitinase, and immobilized microflora useful for plants [79]. Vermicomposting involves composting organic waste by activity with the earthworms. This has been proven effective in the processing of wastewater sewage sludge and solids, brewing products, paper waste, industrial residues, food, and animal waste, as well as horticultural residues from processed potatoes, dead plants, and the champignon industry [73]. Earthworms act as mechanical blenders. By comminuting the organic matter, they change its physical and chemical status, slowly reducing its C: N ratio, raising the surface area exposed to microorganisms, and making it much more suitable for microbial activity and further decomposition. They transfer fragments and are high in bacteria, thus significantly homogenizing the organic content during their movement through the earthworm intestines. The end product or vermicompost is a finely dispersed peat-like, porous substance with a high-water capacity and contains most nutrients in plant-friendly forms. These earthworm casts are rich in organic matter and have high mineralization rates, meaning nutrient such as nitrogen (particularly ammonium and/or nitrate) are available much more freely on plants [55, 58, 59]. The various vermicomposting stages are the following [73]: (1) Initial pre-composting phase: Organic waste is pre-composted for about 15 days before being fed to earthworms. Compounds are readily decomposed, and possible hazardous compounds harmful to earthworms are removed throughout this process; (2) Mesophilic phase: during this step, earthworms combine it with mineral particles and enhance microbial activities and provide organic waste with a condition for organic manure production through the characteristic functions of organic matter breakdown; (3) The process of stabilization and maturation vermicompost.

Vermicomposting, a biochemical method consisting of earthworm consortia and microorganisms for the degradation/breakdown of organic materials, is recognized as a plausible solution for improving crop yields and soil fertility maintenance/improvement with no harm to the environment.

Earthworms, known as the plowman of nature, are excellent soil fertility indicators that simultaneously enhance the physical, chemical, and biological composition of host soil. Most of the earthworm-digested soil is discharged into the soil atmosphere in the form of fine mucus-covered granular aggregates, which are rich sources of NPK, micronutrients, and beneficial microbes. The earthworm excreta called 'vermicomposting' is very useful in combating soil salinity and is anti-pathogenic, decreases pesticide use on crops [26, 60]. Earthworms function as essential motors of vermicomposting while increasing aerobic microbial activity, speeding the enzyme production, and breaking up complex organic compounds into simpler compounds to be further degraded by microorganisms [83].

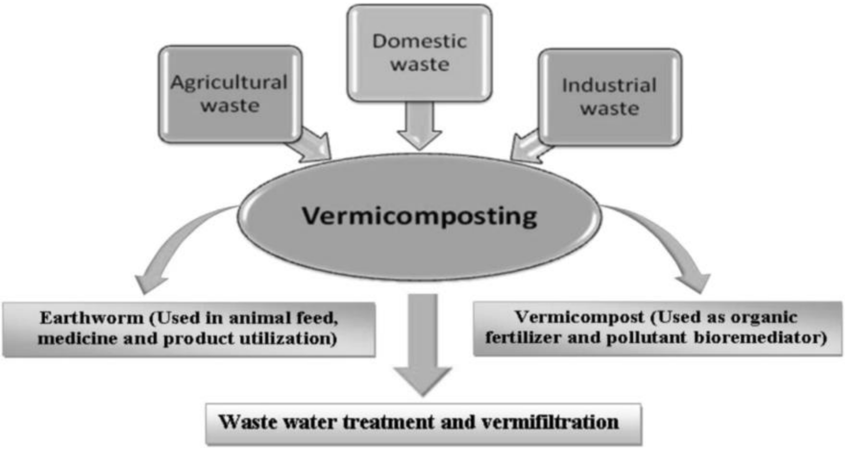

Figure 2. Vermicomposting technology from different organic materials and its application [85].

Vermicomposting is one of the zoo-composting method which are a quick, efficient, easy to control, and cost-effective composting method that can produce a valuable product [11]. It is particularly suited for disposing of dispersed, urban organic waste at the site where the organic waste is made. Previous studies have shown that vermicomposting can turn organic waste into useful compost. Vermicomposting is the biochemical oxidation and processing of organic matter through aerobic and room temperature contact between earthworms and microorganisms [79–81]. The earthworm's behaviors and microorganisms on the decomposition of organic matter for lignocellulose degradation and other degradation resistant substances abounding in green waste; mushrooms are particularly essential [30, 79, 84].

It follows that our understanding of vermicomposting can increase by studying the effects of earthworm activity, quantity, and community structure of the microorganism. The vermicomposting of crop waste and cow dung, has observed that microbial numbers are decreased, but the passage increases microbial diversity through the intestinal earthworms [27, 85]. Although previous studies have recorded microbial communities in some final vermicompost materials, microbial communities' improvements during vermicomposting of organic waste still require further research [85]. In this process, two useful products are obtained: vermicompost and earthworm (Figure 2).

3.2.b. History of Vermicomposting

An earthworm has captured the attention of thinkers such as Pascal and Thoreau [73]. Civilizations like Greece and Egypt respected the positive role of earthworms in the soil fertility. The earthworm's benefit was recognized by the ancient Egyptians first. "Earthworms are sacred" the Egyptian Pharaoh of Cleopatra (690-630 B.C.) recognized the significant part of the Nile Valley fertilizing worms after annual floods. Death was punished by extracting earthworms from Egypt. In fear of offending the God of fertility, Egyptian farmers were not allowed to touch an earthworm. The ancient Greeks found the earthworm to have a significant role to play in improving soil quality. The Greek philosopher Aristotle (384–322 B.C.) referred to worms as the soil's intestines. The earthworms are fully supporting the conviction and fulfillment of the visions of Sir Charles Darwin, who called them unheralded warriors of humanity and farmers' mates and said that there could be no other organism in the universe who played such a significant part in the evolution of life on earth [86].

3.3. Plant Growth Promoting Rhizobacteria (PGPR)

Plant growth-promoting rhizobacteria (PGPR) are bacteria that colonize the rhizosphere and stimulate plant growth through various mechanisms, such as nitrogen fixation and the conversion of phosphates into plant-accessible forms (Figure 3). The use of PGPR in agriculture will replace chemicals, industrial fertilizers, pesticides, etc. It can be said that the method of infecting soil with bacteria that promote plant growth was proposed to remove defects and add life to soil under maize plant growth [87, 88]. Of course, it was only after applying microscopy that it became clear that there was a scientific method behind it. [89] was recommend to use of PGPR with legumes on arable land. Studies of root colonization of the rhizosphere of grasses have confirmed that soil bacteria can convert atmospheric nitrogen into plants that they can use [90]. Rhizobacteria can cause both positive and negative and neutral effects on wheat plants [91].

In general, about 2-5% of rhizosphere bacteria are stimulating plant growth [1]. Rhizobacteria, which stimulate plant growth, are important tools for the sustainable and future development of agriculture. One of the mechanisms of absorption of bacteria by soil particles is ion exchange. The soil then has a high natural fertility when soil organisms secrete inorganic nutrients at a sufficient rate to maintain rapid plant growth. The use of PGPR in agriculture is the most promising approach to increasing plants' productivity and yield on soils subject to salinization and to relieve stress [2, 92]. These PGPR relieve a wide range of salt stress and encourage plants to resist salinization due to changes in hydraulic conductivity, osmotic pressure, inactivation of toxic sodium and photosynthesis activity (Figure 3) [91].

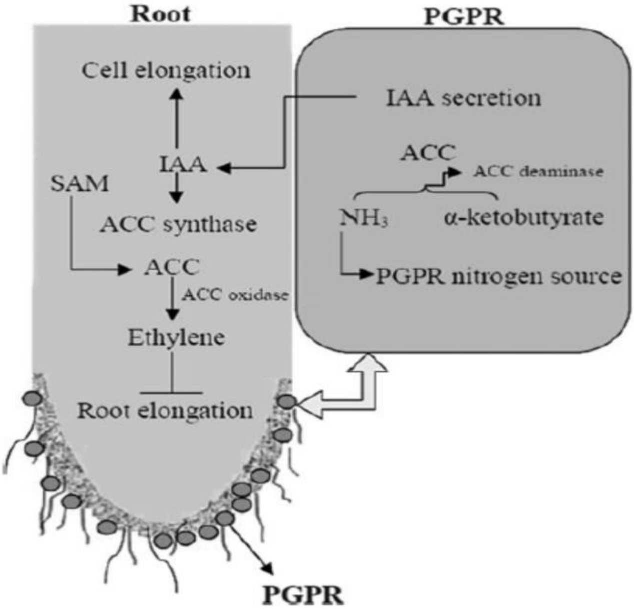

Figure 3. Plant growth-promoting rhizobacteria colonizing the rhizosphere for facilitating plant growth.

3.3.a. The Mechanisms of Nitrogen Fixation by Plant Growth-Promoting Rhizobacteria

The direct mechanism of PGPR on the biological nitrogen fixation by bacteria nitrogen stabilizers is classified according to plant properties (as a bio-fertilizer). The bacteria associated with the root/legumes are characteristic and attack the roots to produce nodules such as rhizobium from family rhizobacteria of alfa-protobacteria class (Figure 4). Simultaneously, the free-living nitrogen fixers, namely, *Azospirillum* and *Paenibacillus*, don't possess specificity to plant [91]. The free-living nitrogen fixations bacteria do not reach the plants' tissue; they continue to close to the roots. The atmospheric nitrogen of the bacteria is not used to support them, but rather that the plant takes it on, and that nitrogen will become better accessible [2].

The term of PGPR was coined over three decades ago as a strongly root-colonizing bacterium on the surface, and one or more mechanisms increase the plant yield [87]. Plant growth-promoting rhizobacteria can affect plant growth by different direct and indirect mechanisms [2]. PGPR directly influences plant growth by fixing atmospheric nitrogen, availability of insoluble phosphates, secreting hormones such as IAA, GAs, and Kinetins, besides ACC deaminase development regulate ethylene [2, 91, 93]. The mechanisms that indirectly support plant growth include systemic resistance to deleterious bacteria, nutrient competitions, parasitism, and toxic metabolites. According to [61, 94], numerous soil bacteria species that thrive in the plant rhizosphere and can develop in, on, or around plant tissues and stimulate plant growth through a plethora of mechanisms. Soil bacteria are extremely important in biogeochemical cycles and have been used for decades of crop production. The determinants of plant health and soil fertility are the plant bacterial interactions in the rhizosphere [94, 95].

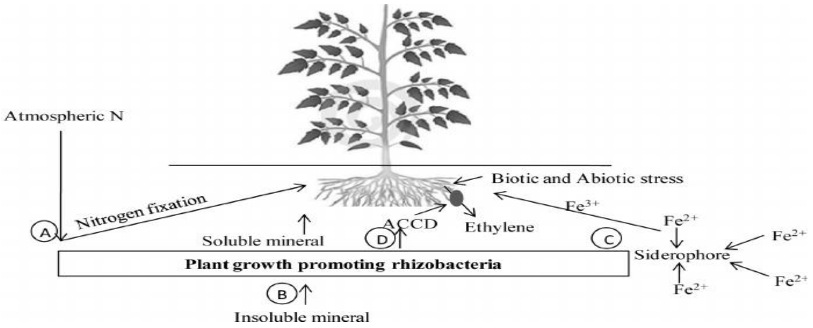

Figure 4. The main way for biological nitrogen fixation through rhizobacteria.

A PGPR formulation, including *Azospirillum lipoferum, Azotobacter chroococcum, Pseudomonas fluorescens,* and *Bacillus megaterium* significantly enhanced germination rate, vigour index, and chlorophyll content of Catharanthus roseus [88, 95]. Bacteria from extreme environments are known to be adaptive to the surrounding environmental conditions. Bacteria from saline conditions can tolerate high salt concentrations, from water-deficit areas can survive in high temperature and low moisture content. Bacteria undergo various morphological, biochemical, and physiological adaptations to survive in the changing environmental conditions shown (Figure 1.5). These bacteria can act as a potential source of PGPR. They can survive and establish the roots of plants growing in the harsh environment, thereby exerting a beneficial effect on plant growth and disease control under abiotic stress [88]. The direct and indirect mechanisms used by PGPR to enhance plant growth shown (Figure 5).

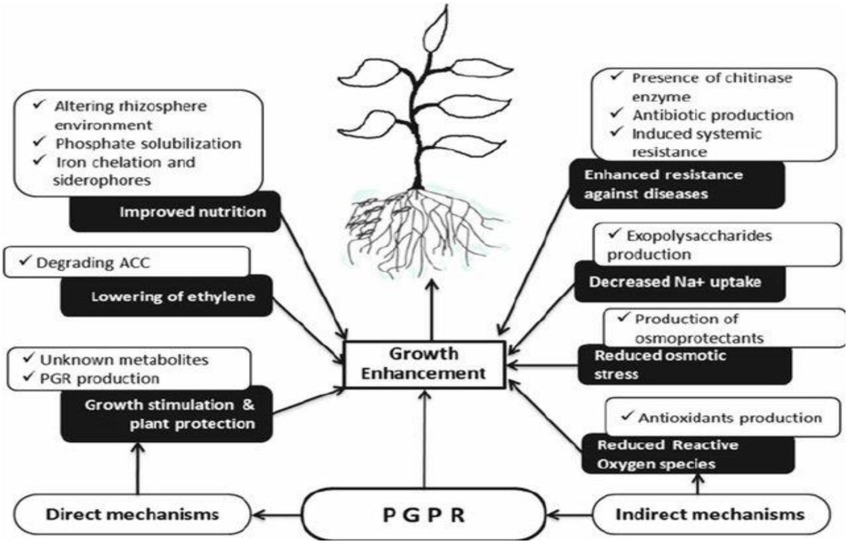

Figure 5. The direct and indirect mechanisms used by PGPR to enhance plant growth [88].

Over-reliance on chemical fertilizers led to disturbance hazards, secondary outbreaks of pests usually kept under test by natural enemies, insecticide resistance, pollution of the environment, and biodiversity declines [96]. The growing costs of chemical fertilizers and their harmful consequences involve natural methods for crop safety and growth. This includes the use of animal manure, crop residues, microbial inoculum, and composts. *Azotobacter* bacteria culture have been used as a bio-fertilizers to decrease negative effect for chemical fertilizers [97–99]. They develop biodiversity and soil biology, sustain physical soil properties, and enhance environmental health.

Bio-fertilizers are living organisms made of probiotic microorganisms that can colonize the rhizosphere or interior plants and enhance root growth when applied to adjacent soil seeds or plant tissue. PGPR was used to enhance seeds plant germination as bio-fertilizers to sowing materials [3, 4, 5]. Bacteria related to PGPR will colonize the root system and/or even intercellular surface plant areas [97]. Bio-fertilizers is mainly attributed to improvements in the different soil properties of rhizospheric, such as soil pH, soil water retention and partial O_2 consumption, which can influence the plant rhizo-oxidation capacity of PGPR strains to colonize the rhizosphere [100, 101].

3.3.b. The Use of PGPR to Protect Plants from Biotic and Abiotic Stresses

The influence of PGPR in the alleviation of harmful effects caused by abiotic stresses has been reported [39], such as drought [102], waterlogging [103], oxidative stress, and salinity [104]. PGPR improved plant growth under salinity stresses [104, 105]. The tolerance of plants against abiotic stress due to physical and chemical changes induced by PGPR is termed as "induced systemic tolerance" (IST) see (Figure 6). The resistance of plants to abiotic stresses caused by changes in soils' physical and chemical properties caused by the combined action of PGPR is IST [39].

Native rhizobacteria from plants under drought conditions like in arid regions are more competent in enhancing tolerance against water stress [106]. The application of PGPR has been effective in reducing the harmful effects of drought [107]. In various climatic conditions, the treatment of crops with PGPR crops contributed to an increase in crop yields due to an increase in root biomass and, as a result, better assimilation of water and mineral nutrition elements.

Crops treated with PGPR such as *Azospirillum*, *Klebsiella*, and *Paenibacillus* under varied agro-climatic conditions have shown improved growth and yield with extensive root growth, facilitating better uptake of water and minerals [107]. Similar effects of *Az. brasilense* were recorded against salinity in wheat seedlings with relatively higher water content [16, 108], which could be due to various physiological changes induced by the colonizing bacteria. [109] Showed a higher water status and elastic adjustment in *Azospirillum*-inoculated wheat, leading to higher grain yield with better mineral quality. Various mechanisms involved in the mitigation of abiotic stress include an increase in proline levels [110], a decrease in excessive ethylene through ACC deaminase [111], reduction in uptake of Na ions through exopolysaccharide production. Exopolysaccharide production could restrict Na^+ influx into the roots. Proline and glutamate accumulation could act as osmoprotectants to reduce the negative effects of salinity, and water stress in *Azospirillum* treated plants [86, 87]. More than one mechanism seems to be involved in mitigating abiotic stress by PGPR. The PGPR strains are useful for improving germination and plant growth under salinity stress and enhancing plant growth, toxic metals production, and drought stress development [91, 112]. There are also reports of *Azospirillum* helping in the mitigation of abiotic stresses, such as salinity and drought [39] (Figure 6).

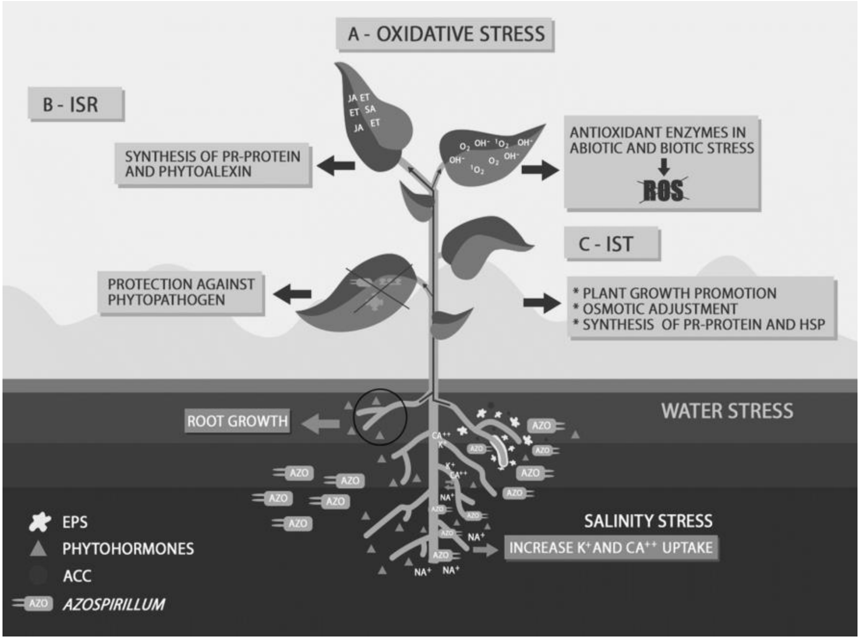

Figure 6. Mechanisms of tolerance of biotic and abiotic stresses induced by *Azospirillum* in plants [39].

In Figure 6 where: (1)- ISR, induced systemic resistance; (2)- ACC, is 1-aminocyclopropane-1-carboxylate Phytohormones mediated by increased levels of phytohormones (3)- JA, is jasmonic acid; ET is ethylene; (4)- SA is salicylic acid; and SAR, is systemic acquired resistance—a mechanism previously studied with phytopathogens—controlled by intermediate levels of SA; IST is tolerance of abiotic stresses, named as induced systemic tolerance.

3.4. Using of Humic Substances as Natural Organic Amendments to Soils and Plants

Humic substances (HS) are dark brown or dark natural organic formations, widely distributed in various natural objects: in soils and peat, in coals and shales, in marine and lake sediments, in the waters of rivers and lakes [113]. Humic substances are part of the organic matter of these biocosal bodies, being its main component. So, for example, in mineral soils, the share of HS is up to 80–90% - the total content of the organic element [114]; in bottom sediments, the proportion of HS is 9–60%, in peat - up to 50, in earthy brown coals - up to 60, in weathered brown and hard coals - from zero to 100, in marine water - up to 20, and in the water of rivers and lakes - 60–85% [114].

Humic substances are the most natural and thermodynamically stable form of conservation of organic substances in the biosphere. HS also includes melanin (pro-humic or para-humic) substances synthesized by fungi and bacteria [104, 114, 115]. Also, humic substances were isolated from brown algae, obtained from the mussel, from lignin-containing material, and even from treatment facilities' sediments [114]. In terms of environmental importance, the hepatitis HS is central to the composition of the organic matter of soils, Sapropels, etc., since HS perform several essential functions: accumulative, transport, tread, and physiological, as well as [116]. This is one of the main links in the functioning of ecological systems. In the process of the biological cycle of compounds of biophilic elements, a significant role is played by the rotation of organic molecules, which are structural blocks of biological macromolecules and are repeatedly used at various trophic levels in ecological systems [117–119].

Humic substances also affect the formation of various plant tissues [114]. As top dressing established by humic acids (HA) effectively influenced parenchyma's development, weaker - collenchyma, and least of all - sclerenchyma. Moreover, the palisade parenchyma's development and a more robust structure of the conducting bundles were observed primarily in HA and a mixture of humic acids. Humic substances significantly accelerate the growth and development of plant buds, positively affect all phases of the mitotic cycle of cells and increase the mitotic index values by 1.5 times [114].

Various humic substances have different effects on the shape, size, and number of maize root systems [120]. So, among humic substances, the weakest cytological influence was exerted by himatomelanic acid (HMA). Humic acids contributed to a significant elongation and narrowing of the cells of the root systems. At the same time, a decrease in the number of layers was noted in the root bark. Fulvic acids lengthened and expanded root cells more evenly; the root's central cylinder developed more powerfully. The mixture of humic acids exhibited a heterogeneous effect: HA exerted a predominant influence on tomatoes and fulvic acid (FA) on sugar beets and wheat.

Different HS groups have other effects on the differentiation of wheat and maize plant growth [121]. So, in the greenhouse conditions in spring and autumn, the differentiation of the growth cone was best accelerated by HA and FA [114]. Humic substances can also affect the structure of leaf blades, and petioles of corn and wheat plants [121, 122]. The biological activity of HS biological activity, as a rule, increases in the following order: HA → HMA → FA, also, the beneficial biological effect of HS increases with an increase in the duration of humification [123, 124]. Foliar treatment of corn and wheat plants influenced the indices of germination energy and seeds germination at a certain germination temperature. So, the biological activity of HS was a critical reflection of the properties of these compounds. Understanding the biochemical pathways of interaction between the plant and the soil, in which HS plays a leading role, will allow the development of effective technologies for influencing crops under stress by foliar treatment of plants with HS solutions. The wheat seeds treatments that encouraged plant growth and increased grain yield of HS treatment by 65 percent and increased [37]. The use of HS in suspension wheat seedlings to enhance seeds germination and plant growth [37, 97]. The folk application of leonhardite. extracts stimulated on shoot growth and promoted the accumulation of K, B, Mg, Ca and Fe in leaves under field conditions for olive (*Olea europaea L.*) [125]. Humic substances enhanced the nutrient uptake by the plants and increased the permeability of root. Wheat production increased irrespective of urea doses in plots with the application of humic substances with 50 mg L^{-1} and HS isolated from vermicompost. And at higher nitrogen concentrations, the effect of using a solution of HS was not observed. Application of plant growth promoting bacteria with humic substances on corn (*Zea mays L.*), sugar cane (*Saccharum officinarum L.*), Tomatoes (*Solanum lycopersicum L.*), common beans (*Vicia faba L.*) and pineapples (*Ananas comosus L.*) led to decrease in both biotic and abiotic stresses [94]. In our opinion, this important agroecological effect is practically not taken into account and should be considered as one of strategies for plant productivity management.

4. Biological Correction of the Plant-Soil System by Bio-Organic Ameliorants

4.1. Influence of Organic and Biological Ameliorants on Soil Chemical Properties

Organic materials recycling it is known that several organic materials, such as farmyard manure, agro-industrial by-products (spent grain), and composts, can be used as amendments to enhance and sustain the overall soil fertility [10, 23, 45]. The same amendments could likely be considered for soil remediation in the salt-affected areas due to their high organic matter content. Organic matter has several beneficial effects on agricultural fields, such as: the slow release of nutrients, soil improvement, and soil protection against erosion [23, 24, 25]. The addition of spent grain and compost in the soil at two rates of 1% and 2% compared with NPK chemical fertilizers was more effective on chemical properties under different aerobic and anaerobic conditions and plant growth in calcareous soils in the western desert of Egypt [19, 126].

As [127] highlighted, soil amended with organic matter showed the highest P concentrations, and P availability increased with the application of all amendments except sand, which had no significant effect. Conversely, organic amendments releases macronutrients during the mineralization process, converting soil phosphates into available forms, improving release from hardly soluble rock minerals due to high total acidity [127, 128]. Additionally, under saline soils, the available potassium (K_2o) can increase through the increase of CEC linked to organic matter content in the saline soil. In particular, the application of municipal solid waste amendment can improve soil fertility and finally contributing to productivity of salt-affected soils [129]. Moreover, K^+ is likewise essential to maintain the turgor pressure of plants under drought and salinity stress. In a recent study, a mixture of spent grain and compost residue 1% and 2% significantly reduced Na^+ content and improved CEC and the contents of available N, P, and K in soils [44]. Therefore, proper selection of organic fertilizers as nutrient sources, timing, and the method of their application to soil can be considered equally important [44]. Applications of two slag doses affected positively on the exchangeable sodium percentage level compared to the initial was increased, ESP was reduced by 87% and 90% respectively, [52]. Moreover, the high electrolyte concentration in saline-sodic soil increases the soil solution's osmotic pressure and hinders ions' uptake by the plant root systems; this phenomenon is one of the major causes of reduced fertility in saline and sodic soils [41]. Soil organic matter is generally derived from the remains of plant roots, leaves, stubble, animal manures, by transformation of soil bacteria, fungi, and earthworms. This mechanism is a source of plant nutrients, providing energy for soil biota and biochemical processes to hold the soil nutrients.

In addition to its role in soil chemical reactions, organic matter influences some physical characteristics, as it helps to create friable consistency, improves soil structure, and increases soil water holding capacity [41]. The small farmers do not know various types and techniques of recycling organic materials efficiently and even the composting scientific methods and philosophy. They spoil a lot of valuable organic material in the form of fuel, home plaster, and cattle manure [130]. Despite all these, organic materials such as animal manure, green manure, and crop residues are traditionally used by the farmers and remain the undisputed key to soil productivity by supplying plant nutrients, especially nitrogen. During the organic amendments, there has been a renewed interest in using organic sources to reduce and enhanced bioremediation of heavy metal soil productivity [131].

The influence of different fertilizations (mineral fertilizer, compost, and spent grain) on soil properties were studied during 30 days of cultivation. The pH of organic fertilized pots decreased from 8.00 to 7.50, while the increase was by mineral fertilization. The total nitrogen in the soil was not influenced by different fertilization. P was increased by organic application and stayed constant on the other fertilized pots but decreased on the mineral fertilized pots [70].

The organic amendments improved soil structure and increases the water-holding capacity of the soil [44, 132, 133]. Chemically, it increases the soil's capacity to buffer changes in pH, increases CEC, reduces phosphate fixation, and serves as a reservoir of secondary nutrients and micronutrients. Biologically, organic matter is the energy source for soil microorganisms. Organic matter in soils exists as partially decomposed plant and animal residues, living and dead organisms, and humidified organic matter or humus. [134] reported that organic matter was the basic resource of several nutrient elements. Almost 95% of nitrogen and sulphur reside in organic matter, and phosphorus availability was increased with organic matter. [130] Concluded that more than 95% of the total nitrogen and sulphur and up to 75% of the phosphorus in surface soils were in organic forms. [63] It was estimated that 20-75% of total soil phosphorus was of organic origin, and ammonia volatilization decreased in the presence of organic matter.

It provides nutrients to the soil, improves its water holding capacity, and helps the soil maintain good tilth and better aeration for germination of seeds and plant root development [135]. [136] Reported that incorporating biochar organic amendments into soil under aerobic rice system returns most of the nutrients and helps conserve soil nutrient reserves in the long term and improve grain yield significantly. Also observed that the organic matter, phosphorus, and potassium content of the soil were increased. Similarly, initiated a field study to compare the long-term effectiveness of digested municipal sewage sludge, cotton straw decreased the soil salinity with increase soil depth, amended soil experienced a gradual pH increase over the study period, with the topsoil amendment with cotton straw amendments generally maintained the highest soil C, P, N concentrations, while the sewage sludge had the highest EC concentrations, in the 0 to 10 cm soil horizon [127].

The application of vermicompost helps the microorganisms in soil to produce polysaccharides, which build up better soil structure [73]. Nitrogen fixation and availability of essential plant nutrients are also increased due to improved microbiological activities in organic matter amended soil [101]. [34, 133] studied organic matter mineralization in soils previously amended with organic materials. They found that the nitrogen contents in calcareous soil were increased significantly over control. A relatively large amount of nitrogen was released from the soil due to exchangeable NH_4^+ in the soil. Similarly, [60, 98] observed the application of PGPR decrease in soil pH after the wheat crop harvest and enhancing the soil basic cations such as Ca^{2+}, $Mg^{2+,}$ and organic acids produced during decomposition of incorporated PGPR had resulted in such a low pH. The PGPR bio-ameliorants, increase the total N, P and maize plant productivity had either maintained or increased compared to the initial status due to the inculcation of *Mycorrhizae* and *Azospirillum brasilence* as a PGPR [101, 102]. Despite crop removal, the available N status had increased in PGPR treatments, whereas it had decreased in control treatments. Due to a higher level of organic compounds in soil and the subsequent release of N through mineralization, the increase was due to a higher organic matter level.

Applications of organic amendments and *Azospirillum* to soils also improves soil fertility by enhancing nutrient retention by chelating micronutrients to keep them available and buffering pH against rapid changes. It is a matter of interest that the growth of green manuring crop on droughty soil to supply N for succeeding crops may lower the yield due to soil moisture depletion [107, 138]. N, animal materials can substantially contribute to enhance the total N and available K, and other essential nutrients.

Similarly, [138] studied the effect of integrated use of farm manure and synthetic nitrogen fertilizer improves nitrogen use efficiency, yield, and grain quality in wheat plant. They concluded that of continuous cropping under various ameliorants, the differences in organic carbon, total N, available K were significantly influenced. The organic farming and total hydrolyzed N status-declined with fertilizer N alone increased with conjunctive use of fertilizer N and organic agriculture. The continuous use of organic agriculture increased the total N in soil. The combined application of organic and inorganic N sustained productivity even at a lower level of application [128]. The results indicated that without N fertilization, depletion of N might affect the sustainability of corn cropping system. The soil pH was decreased with the application of plant growth-promoting rhizobacteria [97]. He also observed that the use of inorganic fertilizers with and without organic and plant growth-promoting rhizobacteria significantly enhanced the available N, P, K, and organic carbon content of the soil. [20] also found that the application of organic amendments in the case where spent grain at the rate of 23,8 t ha^{-1} with *Az. braselince* inculcation were better than application with compost and mineral fertilizers. He reasoned that this improvement due to the acceleration of the decomposition rate of bio-organic materials after fertilizer application.

4.2. Influence of Organic and Biological Ameliorants on Soil Biological Properties

Biological soil properties are very reactive to small changes occurring in management practices. Therefore, it is possible to use them for evaluating the effects of the application of organic matter and PGPR on soil biological characteristics [4, 139]. The salinization may much disturb a large variety of microbial mediated processes and biological properties in soil [47]. Demonstrated that in different sites affected by saline liquid residues, microbial biomass C, biomass N, and fungal ergosterol had the highest values at the low-saline site and the lowest values at the high-saline site. Exogenous organic matter applications to cropland are known to improve soil biological functions, also showing positive effects in the salt-affected soils. [42] showed that, applied the beer industry organic wastes with *Azospirillum* bacteria under greenhouse experiments at 10 and 20 g kg^{-1} soil significantly increased urease and dehydrogenase activities compared to N, P, K chemical fertilizers under growing corn plants in calcareous soil in arid regions.

Similarly, the low concentrations of salts had a stimulating effect on carbon mineralization, but they can become toxic to microorganisms with increasing concentrations [52]. The rate of the organic matter mineralization (as carbon and nitrogen) could depend on the type of organic material incorporated into the soil and response patterns to salinity stress [17]. In particular, soil amendments application affected the bacterial community structure and enhanced enzyme activities and microbial activity in salt-affected soil than mineral amendments in soil [53]. This result confirms that the incorporation of organic waste can be a practical low-input agro-technological approach to minimize toxicity conditions induced by salinization. Also, it has been demonstrated that manure and compost application to saline soil in dryland conditions can reduce ESP by 50% than unamended soil while significantly increasing different enzyme activities (urease, alkaline phosphatase, and dehydrogenase) [7, 8]. Amendment incorporation under high soil salinity or sodicity may also provide a buffer of pH in saline and alkaline soils and positive influence on microorganisms' activity [47].

Moreover, [54] found that organic sources (farmyard manures, different agro-industrial by-products, and composts) improved microbial activity in saline and sodic soil showing an increase in urease activity after amendment addition. While cropping generally brings the best economic return in the short term, pastures and organic manures can also play an essential role in the reclamation of saline and sodic soils [1]. Many studies have compared the effectiveness of inorganic and organic amendments, and results have depended on the situation. However, the combinations of inorganic and organic amendments may be the most efficient means of reclamation to sustainable agriculture.

Th combinations desulfurization gypsum ameliorants with the organic sources have been further found to reduce sodic soils dispersion and was better choice for improving the quality of saline-sodic [140]. Investigations of [44] assessed the feasibility of utilizing spent grain, *Azospirillum*, compost, and NPK ameliorants on soil reclamation. They claimed from these studies that a decreasing trend in soil pH was recorded in the spent grain treated pots as compared to control and compost. Organic matter percentage was increased in almost all the mature/raw organic materials applied ameliorants when compared with control. They also observed a decrease in EC values after using various organic materials and *Azospirillum* in the pot experiment. In contrast, the values of EC in other ameliorants and control were found non-significant statistically. Concluded from these studies that the use of plant growth promoting rhizobacteria enhanced wheat plant growth and soil biological and chemical parameters compared with nitrogen fertilizer [141].

The content of organic matter in most soils of Egypt is small (usually < 1.00%), which is due to the climatic features of the arid zone: high temperatures and low rainfall, as well as sparse vegetation of flat areas [1]. Therefore, at present, the replacement of mineral fertilizers with organic ones is a very important task, not only from the standpoint of providing agricultural crops with main nutrients (NPK), but also from the standpoint of economics and greening of crop production. Although in the beginning, farmers appreciated chemical fertilizers for their rapid effects and relative ease of handling, but only after some time, they began to recognize the limitations behind their use [142].

Organic fertilizers increase the use of ash elements and nitrogen by crops, which are part of organic fertilizers, and reduce the loss of mineral nutrition elements of plants by improving the water-holding capacity of soils [142, 143]. Soils in which organic fertilizers and/or ameliorates are regularly applied are populated with many prokaryotes, fungi, etc. Because together with organic fertilizers and/or ameliorants, millions of various microorganisms and fungi are introduced into the soil, as well as newly formed humic substances which stimulate the growth and development of plants. A long-term field experiments were conducted by [144] to determine the effect of compost, sewage sludge and farmyard manure application rate additions on organic carbon, microbial biomass, dehydrogenase activity and soil respiration were detected both in variants with high doses of sewage sludge increased, and in variants with high doses of compost and cattle manure.

Conclusion

Highlighting the use of four organic and biological by-products, spent grain, vermicompost, PGPR, and humic substances, led to the collection of a huge amount of information that help the reader form a clear view of the improved organic and biological materials that enhance the fertility of saline and calcareous soils under different environmental conditions. Where the importance of organic wastes in increasing and improving the fertility of saline and calcareous soils was discussed in depth. The authors recommend that these organic materials should be added to improve soil fertility and soil health under deferent environmental conditions.

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Chapter 3

Humic Substances and Their Potential to Enhance Soil, Plants, and Animals' Productivity: A New Concept for Sustainable Agriculture

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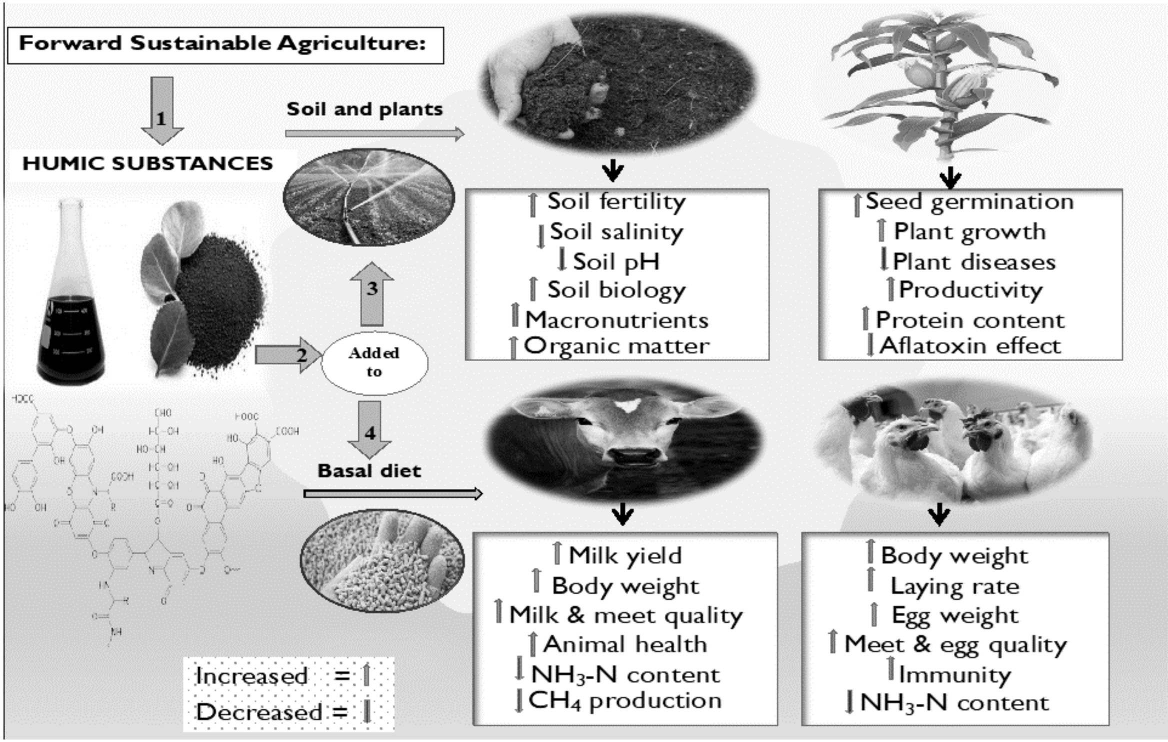
Abstract

Humic substances (HSs) are typically found in nature in soil, plant, and natural water, and they are fundamentally created from organic matter decomposition. The main active components of HSs contain humic acid, humus, ulmic acid, fulvic acid, humin, and certain microelements. HSs are currently not only natural, but also harvested and engineered, and have been widely exploited in soil, plant, and animal productivity for the past 20 years. In the soil, HSs have the potential to improve a wide range of soil and play an essential role in the microorganism's activity in the environment. In addition, HSs are a part of soil humus and they represent the largest pool of recalcitrant organic carbon in the terrestrial environment. Also, these valuable compounds have bio-stimulative effects on plants, where they could boost the photosynthetic activity, and improve root system and plant growth, as well as reduce stress damages. The HSs also could affect specific metabolic pathways in plants leading to improved tolerance of abiotic and biotic stresses. Today, our soils are low on these HSs, and as a result, animals and chickens are not receiving adequate amounts in their regular diet. Many researches have indicated that when soil humus percentages fall below 2%, the soil cannot provide sufficient amounts of HSs materials into the crops grown for the quantities needed by livestock animals. Recently, HSs have been used as one of the alternative feed additives in animal husbandry to improve the economics and ecology of animal production by increasing the growth rate, improving feed efficiency and immunity, reducing the risk of disease, and increasing animal products quality. This book chapter discusses the functions that HSs play in soil, plant, and animals to keep agricultural sustainability. We assemble and describe the applications and role of such HSs in agricultural and environmental ecology.

Keywords: soil, plant growth, animal, sustainable agriculture, humic substances

Graphical Abstract



1. Introduction

Sustainable agriculture is an agricultural system that uses locally accessible organic ingredients without depleting natural resources. The agriculture sector needs to improve soil, plant, and animal production to prevent its degradation while maintaining a higher quality of products. Humic substances (HSs) are a widely accessible raw material that can be used to improve agriculture by increasing soil, plant, and animal productivity and quality. The chemical composition of the HSs has been well analyzed and evaluated (IHSS, 2019). The content of various components (C, H, N, O) in the IHSS standard and reference fulvic and humic acids varied from 50 to 60%, N from 0.7 to 5.1 percent, H from 3.5 to 4.8 percent, and O from 31.6 to 45.5 percent (Ritchie and Perdue, 2008).

HSs have the potential to improve a wide range of soil and plant properties to achieve agricultural sustainability (Hafez et al., 2020). HSs have a complex organic matter structure, which decreases the toxicity of herbicides, heavy metals, and soil-polluting radionuclides (Volkov and Polyakov, 2020). Furthermore, it has been demonstrated that HS transmits micronutrients, particularly iron, from soil to plants and increases soil microbial populations (Maruf and Rasul, 2019). HSs, notably humic acids and fulvic acids, have piqued the interest of scientists for a variety of reasons, one of these is to decrease soil degradation and salinity effects (Rashad et al., 2020). HSs have generally been used to boost soil and plant development (van Rensburg, 2015), but they have also been successfully employed in poultry feeding in a variety of forms and concentrations (Hudák et al., 2021).

The chemical composition of HSs is directly connected to their effect on the biological stimulation of plant development (Garca et al., 2019). The influence of these chemicals on plant development is determined by the way of administration of HSs to the plants, the concentration of bioactive constituents the source, molecular weight, and dosage of the humic fraction, and the plant species (Nardi et al., 2021). HSs stimulate the lateral roots and root hairs number and length in the root system. HSs amendment to soils has been reported as an effective method for suppressing soil-borne diseases and supporting plant health (Kätterer et al., 2014; Maji et al., 2017). Wei et al. (2020a) found that different forms of humic compounds can affect the abiotic and biotic characteristics of the soil, leading to the control of soil-borne pathogens and indirectly aiding plant development.

On the other hand, global production of poultry meat, particularly broiler chicken meat, has been of increasing trend since the mid-twentieth century and is expected to increase at a faster rate in the future. Also, the global poultry sector is characterized by faster growth in consumption and trade than any other major agricultural sector (FAO, 2008). Antibiotics are widely used to improve the growth of animals (Dibner and Buttin, 2002). However, it is observed that antibiotics have a negative effect because their residual effect in poultry products causes many problems related to human health (Donoghue, 2003). So, the European Union has banned the supplementation of antibiotic growth promoters in the poultry feed industry since 2006 (El-Hack et al., 2016) and this is followed by many countries in the world. Therefore, poultry nutritionists have conducted many research studies to find natural products such as HSs that could cause improvements with poultry and to obtain other agents against the emergence of infectious diseases. Currently, studies have documented the importance of HSs additives as alternatives for antibiotics in different avian species and have shown positive effects (Ashour et al., 2014; Saeed et al., 2015; El-Hack et al., 2016; Khalifa et al., 2021).

In recent years, there has been an increase in interest in the use of humic compounds in animal diets (Yüca & Gül, 2021). Humic compounds (including humic and fulvic acids) are recognized as safe and natural feed additives that improve animal welfare and product quality. Humic compounds, which include humic acids, fulvic acids, and humins, are natural organic chemicals present in the soil that are generated through the humification of dead organic matter. Oxyhumolite (oxidized brown coal) is a good source of these chemicals (Šamudovská and Demeterová, 2010). The primary component of these compounds is humic acids. This fraction is insoluble in acidic solutions (pH 2) but soluble in alkaline solutions. These acids have a high molecular weight ranging between 5000 and 10,000 Da (Islam et al., 2005). They feature a wide range of physical, chemical, and biological qualities that make them excellent for animal husbandry. They have antioxidant and anti-inflammatory properties, and they help animals' gastrointestinal tracts operate properly, allowing them to grow faster while also increasing immunity and reproductive (Huculak et al., 2018).

Piglet diets containing humic acids have been found to promote their health and weight gain (Wang et al., 2020). Existing research on the impact of humic chemicals in bovine diets has been inconsistent (Terry et al., 2018). Little research has been conducted on the utilization of humic acids in dairy cow diets (Zigo et al., 2020). The study's findings suggest that using humic compounds as a feed supplement can have a positive influence on milk production attributes due to their potential to modify rumen fermentation processes. Assuming that the inclusion of humic acids in cattle diets will improve nutrient utilization from feed and digestive system functioning, particularly rumen metabolism, we anticipate that humic acid will be a promising addition.

After reviewing more scientific articles that proved the effectiveness of humic materials in increasing the productivity of plants, soil, and animals and increasing their quality to reach a sustainable agricultural.

From this, it is clear that sustainability in agriculture does not start only with the product, but starts from the beginning of the cultivation of the seed in the soil and its passage through the stages of growth, to the feeding of animals and the production of milk and meat to reach the highest quality produced. Therefore, sustainability has achieved its goals.

The main objectives of this book chapter were to 1) describe the applications and role of such HSs in agricultural and environmental ecology, 2) physical and chemical roles especially enhancement soil, plant, and animal production to achieve sustainable agriculture, and 3) collect more information about HSs mechanism in our food from growing plants in the soil to consuming meat products to keep sustainability in agriculture.

2. Role of Humic Substances and Their Forms in the Soil

2.1. Composition and Hypothesis Theory of the Formation of Humic Substances

Some constituents of plant tissues, such as lignin components, relatively resistant to the action of exoenzymes of microorganisms and fungi, accumulate in bioinert bodies, changing only externally, and together are their organic part (Nardi et al., 2017). It is emphasized that the initial plant material's nature determines HSs composition and properties.

The aromatic composition of HSs can be used to predict its resistance to chemical and biological degradation (Calderín et al., 2014). The creation of a complex and heterogeneous molecular network that provides recalcitrance appears to be linked to the stability of HSs in particular (Carvalho, 2015). In this context, HSs stability occurs by adsorption of functional groups on clay mineral surfaces and physical protection within the pores of soil clay particles, limiting microbe and enzyme accessibility (Lipczynska-Kochany, 2018).

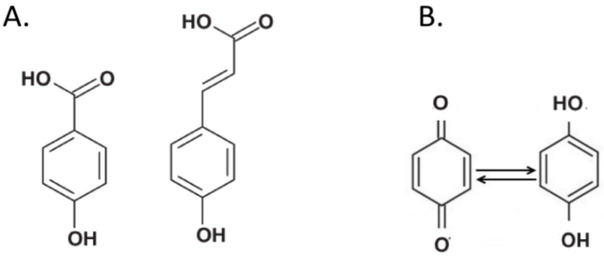


Figure 1. The phenolic acids compounds are considered major components of soil humic substances. (A) Typical chemical structure of phenolic acids. (B) Quinones (left) are groups that accept electrons and are reduced to hydroquinones (right). According to (Nardi et al., 2021).

Conventional phenolic compounds have been thought of as the “building blocks” of humic substances (Stevenson, 1994; Chen et al., 2018). Phenolic acids, i.e., chemical substances having an aromatic core and phenolic and carboxylic functionalities (Figure 2), are evaluated at up to 35% in HSs (Lehtonen et al., 2004). The involvement of dihydroxy-aromatic acids as structural building blocks in metal complexation has been verified by HSs research (Borges et al., 2005). The reducing capabilities or electron-donating capacities (EDCs) of phenols are one of their distinguishing characteristics (Aeschbacher et al., 2012).

During the first stage of humification, high molecular weight humic acids (HAs) and humans are formed. They are then transformed into FA and ultimately degrade to carbon dioxide (cited from (Popov and Chertov 2000; Popov and Shishova 2001).

The degradation hypothesis of humification was initiated by the studies of (Drachev, 1927, Rashad et al., 2022). This author showed that humus (more precisely, HSs) - dark-colored ligno-protein compounds - is formed from plant residues and the cellular matter of microorganisms (Figure 2). The interaction of lignin with protein is carried out as a result of chemical condensation like Schiff bases. There are no humic acids as specific products of humification in the soil.

This hypothesis of humification was further developed in the works of (Rashad et al., 2022). Following the point of view of this author (Hafez et al., 2020), at the first stage of humification, the leading role belongs to the process of carboxylation decomposition products of humic substances (Figure 3), i.e., the new formation of humic acids, accompanied by the fractionation of the

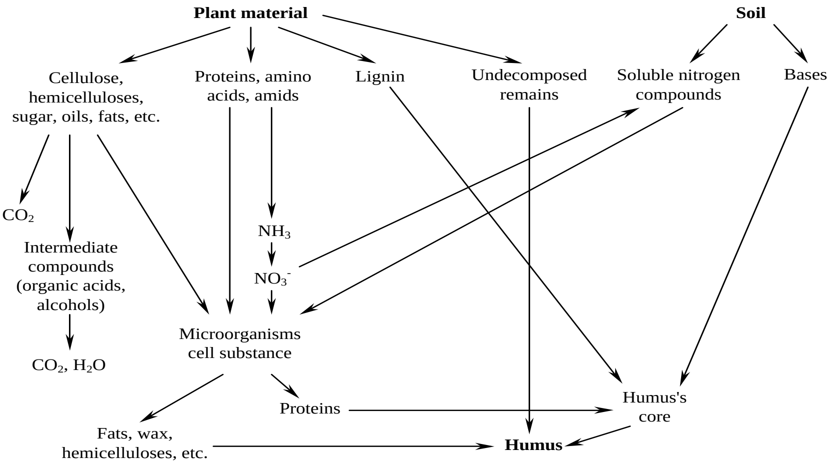


Figure 2. Schematic representation of humus formation mechanism during plant residues decomposition in soil (Popov I.A., 2020).

system into HA and FA, as well as the formation of the nitrogenous part of the molecule. In this case, the process of oxidative acid formation undergoes not monomers but high-molecular decomposition products of plant residues (Maris Klavins & Purmalis, 2010). The formed HAs from the first stages of their existence are high-molecular compounds. Fractionation occurs due to plant residues with compounds that make up plant ash and the mineral part of the soil; organo-mineral derivatives of varying degrees of solubility are formed. The chemical composition of plant residues significantly affects the formed HA's main parameters and their yield (Hafez et al., 2021). At the second stage of humification, the further transformation of the formed HAs molecules occurs - aromatization, accompanied by a decrease in molecular weight and a decrease in nitrogen. There is a gradual destruction of humic acids to carbon dioxide, water, ammonia, or nitrogen oxides (Figure 3).

As (Aleksandrova, 1981) believed, the main elementary links of humification include oxidative acid formation, the formation of the nitrogenous part of the molecule, fractionation, and further transformation of newly formed humic acids (their further aromatization and hydrolytic decomposition, sorption, condensation), as well as the processes of interaction with the mineral part of the soil.

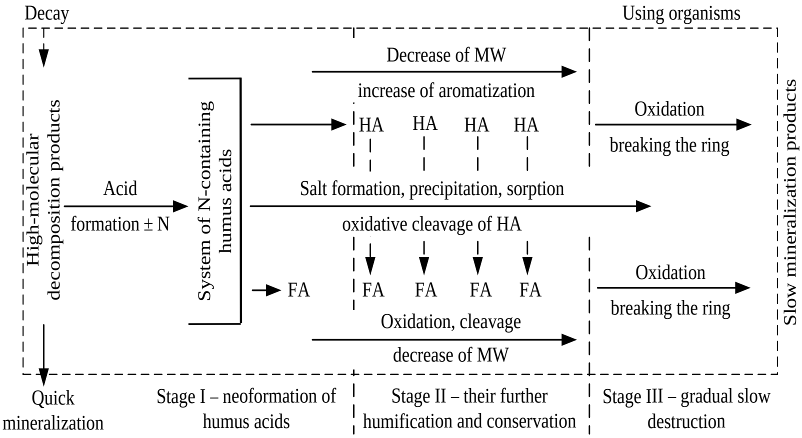


Figure 3. Scheme of humification and further transformation of humus substances in soils.

According to the views of (Popov and Chertov 2000; Popov and Shishova 2001), the degradation processes of plant biopolymers and some small molecules included in the form of monomers in these polymers lead first to the progressive development of humins, then to the appearance of soluble HAs, and finally, the most soluble FA (Figure 4). Nevertheless, these authors consider it probable (albeit minor) to form HA from FA, as well as humin from HA (Figure 4, dashed arrows).

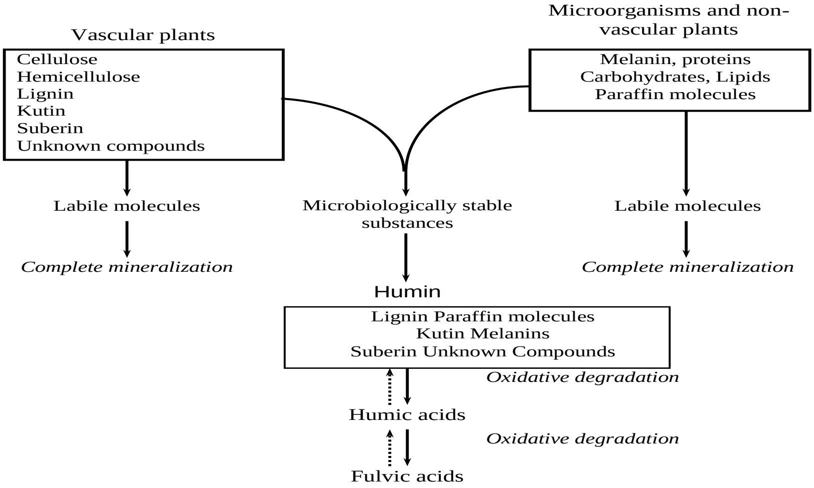


Figure 4. Degradation path of formation of humic substances (Hafez et al., 2020).

3. Humic Substances to Enhancement Soil Fertility and Plant Healthy

3.1. Relationship between Humic Substances Soil Microorganisms

Beneficial soil organisms can get a lot of energy from humic substances such as humic acid extracted from green compost to soil microbes and plant growth (Figure 5). Humic substances and organic components provide energy and several mineral requirements to soil (Popov I.A., 2020). Beneficial soil organisms lack the physiological functions that allow them to absorb sunlight and must depend on carbon-containing materials on the soil to survive. The energy held inside the carbon bonds is used to power numerous metabolic reactions in these organisms (Hafez et al., 2020).

Beneficial soil microorganisms (algae, bacteria, fungus, nematodes) have a variety of roles in soil fertility and plant health. Bacteria, for example, produce a large amount of organic acids that aid in the solubility of mineral nutrients bound in soil (Hayat et al., 2010). Bacteria also produce polysaccharides such as sugar-based chemicals that aid in the formation of soil aggregates. (Luciano P Canellas & Olivares, 2014). The soluble materials from HSs are released into the soil by other helpful soil microbes such as Actinomyces. The HSs enhancement of Antibiotics which are taken up by the plant to protect it from pests. Antibiotics also help to maintain a healthy ecosystem of soil organisms on the root surface and in the soil around the roots (Primo et al., 2015). Fungi also play an important role in soil health. Mycorrhizae, for example, help plant roots absorb water and trace nutrients. Other fungi degrade plant materials and crop residues, releasing stored nutrients for other creatures. Fungi release a wide range of organic components that aid in the formation of humus and soil particles (H. Li et al., 2019).

Soil animals that are beneficial to the soil form tunnel-like canals in the soil. The soil can breathe and exchange gases with the atmosphere through these pathways. Soil animals also help to generate humus and keep the concentration of soil microbes in check (Alkorta et al., 2003). To support the billions of living-microorganisms forms essential for fertile soil and a healthy plant, a fertile soil must have enough carbon-containing components. Fertile, healthy soil is the one that is living microscopic living (Luciano Pasqualoto Canellas et al., 2013; Hayat et al., 2010).

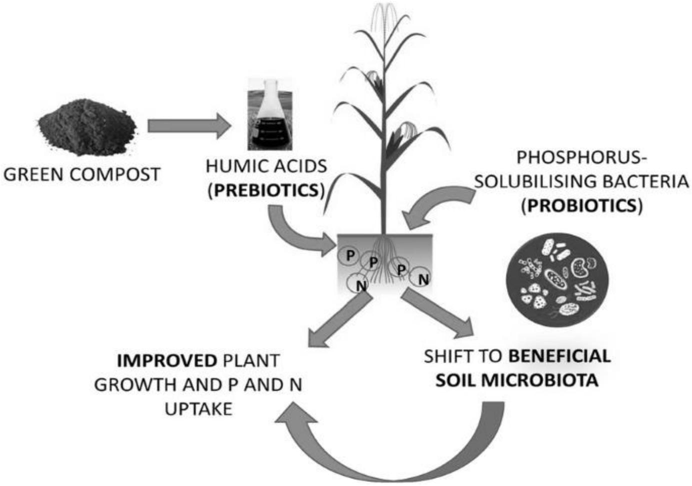


Figure 5. Extracted Humic acid from green compost to improve soil microbiota and plant growth.

3.2. Humic Substances to Enhance Water Holding Capacity

Humic substances improve the soil water-holding capacity. The ability to hold water is the most significant function of humic compounds in the soil (Yang et al., 2021). In terms of quantity, water is the most important substance transported by plants from the soil. Humic materials promote the formation of a soil properties structure that allows water to infiltrate and stay within the root zone (Figure 6). Humic compounds act as water sponges due to their enormous surface area and intrinsic electrical charges. These sponge-like compounds can store seven times their volume in water, which makes them more water-resistant than sod clays (Yang et al., 2021). When needed, water retained in the topsoil acts as a carrying medium for nutrients required by soil micro-organisms and soil-plant roots (Hafez et al., 2021).

The most vital component of fertile soil is, without a doubt, available water. During periods of drought, soils with high humic material concentrations store water for crop use (Popov, 2004). This is why gardeners who use HSs fertilizers and incorporate production strategies that conserve humic compounds may often harvest a crop during dry periods.

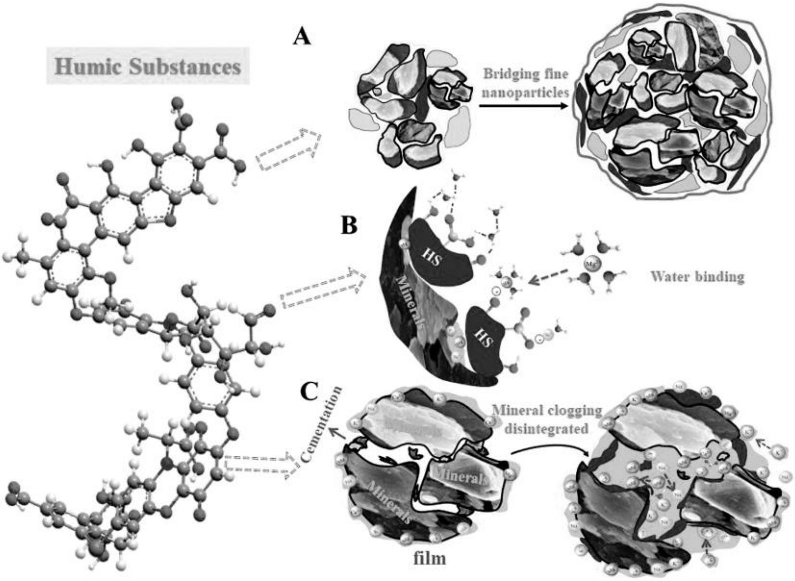


Figure 6. Diagram of the influence of humic substances on the soil physical and chemical properties (A): Soil structure; (B) Water holding capacity; (C) Soil cation exchange (Yang et al., 2021).

HSs are essential to the construction of friable (loose) soil. Soil fragments include a variety of carbon-containing HSs (aggregates) (Popov, 2004). Bacterial-produced complex carbohydrates and HSs combine with clay, sand, and silt to form soil aggregates. As humic components become intimately connected with the mineral portion of the soil, colloidal complexes of humus-clay and humus silt aggregates form (Hafez et al., 2021; Rashad et al., 2022). Electrical processes increase the cohesive forces that cause extremely small soil particles and clay components to attract each other, resulting in the formation of these aggregates. These aggregates, once formed, aid in the production of a desirable aggregate structure in the topsoil, allowing it to become more friable (Yang et al., 2021). Improved tilth and more porous apertures are found in soils with a good crumb structure. These soil pores allow for gaseous exchange with the atmosphere as well as increased water infiltration (Shaddad et al., 2020).

3.3. The Practices to Preserve the Humic Substances in the Soil for the Longest Period

With different HSs, the average residence periods of these organo-mineral complex aggregates vary (Ertani et al., 2013; Neidig et al., 2007). Based on radiocarbon dating of extracts from non-disturbed soils, the mean residence period of HSs within these aggregates is 1140 years, 1235 years, and 870 years for humin, humic, and fulvic acids, respectively (Popov, 2004). Excess fertilization and tilling procedures that promote excessive weathering of sods have decreased the residence duration of HSs in the environment. Soils misused by anhydrous ammonia treatments and other harmful techniques (those that remove humic material) can reduce residence periods by hundreds of years. Under ideal circumstances, the annual turnover time of organic carbon contributed from plant and animal leftovers is around 30 years. Humic chemicals must be kept within the body for it to function properly. Growers must use agricultural strategies that prevent HSs from decomposing to keep them in the soil. Growers must devise methods for extending the residence time of humic compounds (Nardi et al., 2021). Avoiding damaging fertilizing procedures, rotating crops, minimizing pesticide use, deep plowing, and mixing crop residues in the topsoil with minimum tillage practices are all crucial. Soils with sufficient HSs have improved tilth, workability and can thus be maintained more efficiently for plant productivity.

HSs stabilize and inactivate soil enzymes. Soil enzymes (complex proteins) are stabilized by covalent interaction with HSs in the soil (Nardi et al., 2021). These enzymes are less susceptible to microbial destruction after stabilization. The enzyme activity is considerably diminished or fails to operate once it has been stabilized and bonded to the humic material (Scherer et al., 2011). However, because many of these connections are weak, these enzymes can be released during pH changes in the soil. When some humic substance components react with soil enzymes, they become more closely bonded. Phenolic enzyme complexes, for example, are typically linked to clays, which further stabilize the enzymes. Potential plant pathogens' activity is limited by these enzyme stabilizing mechanisms (M. Klavins & Purmalis, 2014; Nardi et al., 2021). The prospective plant pathogen's enzymes bind to humic compounds as they release enzymes meant to tear down the plant's defenses. The pathogens are unable to infect potential host plants as a result.

3.4. Plant Growth and Nutrients Development

HSs have an indirect and direct effect on plant development. Many different scientific journals have shown positive relationships between soil humus levels, plant yields, and product quality. The factors that provide energy of beneficial micro-organisms in the soil; affect the soil water holding capacity, impact the soil structure, release, and increase of plant nutrients from soft minerals, increased trace minerals availability in the soil, and overall soil fertility improved are referred to as indirect effects. The changes in plants' metabolism that occur as a result of the uptake of organic macromolecules such as humic acids (HA) and fulvic acids (FA) are examples of direct impacts. When these chemicals enter plant cells, they cause biochemical changes in membranes and other cytoplasmic components. Some of the biochemical improvements in root plant metabolism as influenced by HSs, are summarized in Figure 4.

3.4.1. Nutrient Uptake

Humic substances play a role in the uptake of important plant nutrients (Luciano P Canellas & Olivares, 2014; Liu et al., 2019; Nardi et al., 2017). Increased uptake of important plant nutrients such as total nitrogen (TN), available phosphorus (A.P), and available potassium (A.K) is one of the stimulative effects of humic compounds on plant growth (K) (Hafez et al., 2021a; Hafez et al., 2021b; Rashad et al., 2022). The need for (N P K) fertilizers applications is reduced when sufficient humic compounds are present in the soil. As the amount of humic compounds in soils decreases, a false demand for higher N P K concentrations emerges (Maris Klavins & Purmalis, 2010). To preserve crop production, several growers have reported increased demand for soluble acid fertilizers in recent years (Luciano Pasqualoto Canellas et al., 2013). Increased nitrate fertilizer ingredient seeping into groundwater is also a sign of impending difficulties. The use of either ry or liquid HSs in soils improves fertilizer efficiency considerably. When plants are irrigated with liquid suspensions of HAs or fulvic acids, other studies have documented higher calcium (Ca) and magnesium (Mg) (S. Li et al., 2020). A reduction in the toxicity and leaching of nitrogen compounds into subsoil water is another fundamental mechanism that maximizes fertilizer efficiency and is related to a function of humic substances. These major plant nutrients are held in a molecular form by humic compounds, which lowers their solubility in water (Elgharably, 2008; Hopkins & Ellsworth, 2005; Maris

Klavins & Purmalis, 2010). These binding activities assist avoid nitrogen volatilization into the atmosphere by reducing leaching into the subsoil.

3.4.2. Soil-Root Crosstalk

The term soil and root crosstalk was introduced by (Nardi et al., 2021) to describe and discuss the relationship between soil-plant biological activity and rhizosphere in the soil. The discharge of root exudates and the release of root border cell into the soil rhizosphere are examples of root biological activity. Ions, low-high molecular weight molecules with the ability to change soil properties are found in root exudates.

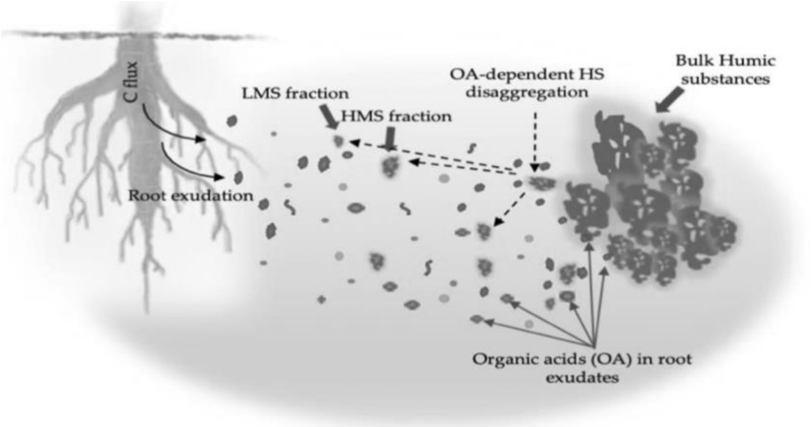


Figure 7. Root exudates include chemicals, particularly low molecular weight organic acids (OA), that may impact the solubility of soil HSs (bulk HSs) by promoting their disaggregation to create LMS and HMS fractions (Nardi et al., 2021).

Organic acids, for example, have long been recognized as important components in soil evolution and formation. Organic acids have the ability to change the mineral weathering conditions by altering soil complex capacity, soil pH, and mineral element contents (Nardi et al., 2021). They can change the macrostructure of the HSs, allowing tiny portions to be released. (Figure 7). These fractions can bind to cell receptors on the root's surface or penetrate root cells, causing biological activity by promoting their disaggregation to create LMS and HMS fractions.

4. Humic Substances as Plant Biostimulants

The ability of HSs to activate distinct metabolic pathways has the biggest influence on HSs functioning in plants. Photosynthesis is considered the primary metabolic process behind the generation of organic matter and O_2 on our planet, research into the role of HSs in photosynthesis control is critical (Berbara and Garca, 2014). Yang et al., (2004) discovered that HSs enhanced chlorophyllases (A and B) activities in Pachira macrocarpa plants. The effects of HSs on photosynthesis were also indicated in research of Humic Acid use on lettuce plants. Humic acid was discovered to boost photosynthetic activity and increase chlorophyll concentration (Haghighi et al., 2012). HSs have been shown to improve root system and plant growth as well as to reduce stress damage, their effects also extend to soil characteristics and structure of microbial community (Canellas and Olivares, 2014).

The chemical composition of HSs is directly connected to their effect on the biological stimulation of plant development (Garca et al., 2019). The influence of these chemicals on plant development is determined by the way of administration of humic substances to the plants, the concentration of bioactive constituents the source, molecular weight, and dosage of the humic fraction, and the plant species (Nardi et al., 2021). HSs stimulate the lateral roots and root hairs number and length in the root system.

HSs are known to have a variety of effects on plant metabolism. The biostimulation activity of HSs has been directed for stress reduction. It has been shown that in hydroponic maize seedlings, humic compounds with higher molecular mass increase the rate of phenylpropanoid metabolism, allowing maize seedlings to adapt to varied stresses and HSs modification (Schiavon et al., 2010). Humic acid application increased bulb yield, clove diameter, and bulb weight loss, thereby improving post-harvest quality in garlic (Abdel-Razzak and ElSharkawy, 2013).

HSs promote plant growth in the laboratory and the field, as assessed by an increase in shoots and roots length, and in their fresh and dry weights. Higher leaf chlorophyll concentrations, more lateral root initiation, enhanced micro-, and macronutrient absorption, and a variety of other biological benefits are also produced by HSs (Nardi et al., 2002). These impacts have been related in part to the complexing characteristics of HSs, which improve the availability of micronutrients from almost soluble hydroxides, and to the retention of optimum Fe and Zn contents in solution (Cesco et al. 2000).

Enzymes related to the plasma membrane (PM) are another target of HSs on plant roots (Canellas et al., 2002). The effects of HSs on the expression of certain genes, such as the two H+-ATPase isoforms Mha1 and Mha2, have been established at the molecular level (Quaggiotti et al., 2004). Muscolo et al., (2007b) showed that HSs, especially those with a low molecular mass, are taken up by plant cells and could influence plant metabolism. In this regard, different humic fractions have been suggested to have hormone-like activity (Zandonadi et al., 2007), whose biological action appears to resemble the reactions caused by gibberellic and indole-3-acetic acid (IAA). Organic matter that has been humified promotes plant development and increases crop yields (Eyheraguibel et al., 2008). High molecular weight HSs can also enhance root development via auxin-mediated mechanisms (Trevisan et al., 2009). HSs may have agricultural benefits as a source of novel organic-mineral fertilizers and as plant stress inhibitors (Kaufmann et al., 2007; Schiavon et al., 2008). HSs stimulate carbon and nitrogen metabolism by raising the activity of glycolysis, Krebs cycle, and N absorption enzymes (Muscolo et al., 2007a).

5. Humic Substances as a Plant Protection Agent

The behavior of HSs on plant-pathogen protection has been documented, and study results showed that humic substances can augment the plant defense mechanisms against phytopathogens, either directly inhibiting these microbes or enhancing the growth of antagonistic microorganisms, allowing for increased plant protection (Jindo et al., 2020; Pereira et al., 2021). Humic compounds are resistant to microbial action and can function as a carrier for these microbes. Furthermore, HSs have the effect of promoting the release of organic acids from plant roots, and these complexes serve as a source of nutrients for Plant Growth-Promoting Bacteria, which can improve plant root growth and colonization by these microorganisms, resulting in several benefits for plants and microorganisms (Maji et al., 2017; Olivares et al., 2017; Nardi et al., 2021).

5.1. Effect of Humic Substances on Abiotic Stresses

Abiotic factors, for instance, temperature, moisture, mineral nutrients, and pollutants cause disease in plants when they occur at levels that are too high or too low for the plants to tolerate (Agrios, 2005). The use of HSs improves plant health, increases germination, and promotes plant defense mechanisms against abiotic and biotic stressors (Giovanardi et al., 2016; Olivares et al., 2017). The main key mechanisms targeted by humic substances-based biostimulants are shown in Figure 8.

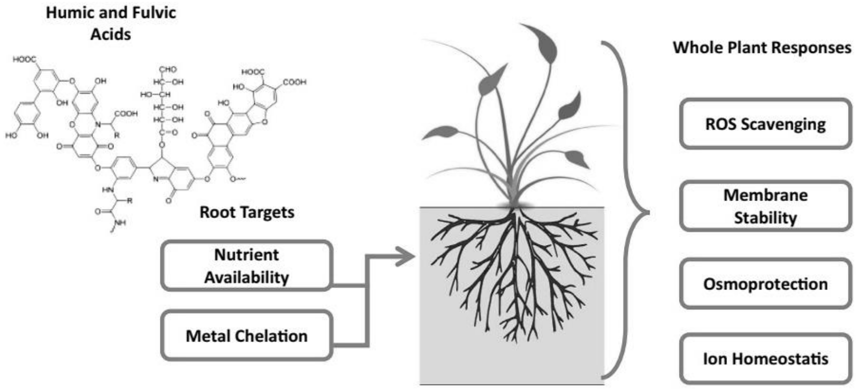


Figure 8. The main targeted mechanisms by humic substances-based biostimulants (Van Oosten et al., 2017).

Under abiotic stress such as drought, the authors reported an increase in reactive oxygen species (ROS) production in the roots and leaves of rice and wheat caused by HSs treatment (Hernández et al. 2012; Yuksel, 2022). ROS production, on the other hand, is an essential plant defense signal for all types of pathogen responses (González-Bosch, 2018). Humic acid also improves plant tolerance to salt stress. HA mostly down-regulated genes involved in salt stress and downstream metabolic processes. Furthermore, HA up-regulated genes encoding transcription factors (TFs) are important in plant growth as well as salt stress resistance, while down-regulating TF genes are involved in secondary metabolic activities (Cha et al. 2021).

The poisoning of heavy metals and their accumulation in food chains are major environmental and health issues in modern societies (Rizwan et al. 2016). HSs can increase plant tolerance to copper toxicity in citrus fruits (Mahmoudi and Zoghalchi, 2015), and increase watermelon yield (Salman et al. 2005).

5.2. Effect of Humic Substances on Biotic Stresses

Humic substances amendment to soils has been reported as an effective method for suppressing soil-borne diseases and supporting plant health (Kätterer et al., 2014; Maji et al., 2017). Wei et al., (2020a) found that different forms of humic compounds can affect the abiotic and biotic characteristics of the soil, leading to the control of soil-borne pathogens and indirectly aiding plant development. Afifi et al., (2017) stated that humic substances had reduced Fusarium wilt disease severity by (84.7%) on cucumber, improved the plant vegetative growth, and increased activities of dehydrogenase enzyme, it also augmented the total number of soil microorganisms.

6. Humic Substances as an Organic Additive for Sustainable Animal Production

6.1. Animal Performance

Humic substances have been demonstrated to have a favorable influence on average daily milk yield. The daily milk production increased by 18–20 percent while metabolic energy and crude protein of dry matter intake decreased by 13.5–14.5 percent, compared with the control group (Mikityuk et al., 2010). Similarly, Naumova et al., (2010) found that the experimental group’s milk yield increased by 6.4%. Furthermore, the milk production per cow increased by 103.5 kg, which is greater than the control group. HSs improved milk production in Saanen goats but did not affect milk composition, or the total number of somatic cells and bacteria Degirmencioglu and Ozbilgin (2013). On the other side, they enhance dairy cow milk production and the milk fat-to-protein ratio Tomassen and Faust (2018). (Yüca & Gül, 2021) found that the addition of humate (75 g) to the diet had a beneficial impact on colostrum-specific gravity, milk production, and milk composition. Also, the fat yield in the experimental group was enhanced by 7.8%. Potůčková and Kouřimská, (2017) reported that HA maintains the gut flora and therefore promote the consumption of nutrients from animal feed, affecting the composition of raw milk from dairy cows and goats.

The inclusion of humate at 20 or 40 g daily to lactating cows enhanced nutrient digestibility, ruminal fermentation, and milk yield. Furthermore, humate supplementation enhanced milk fat content and improved the amounts of CLA and UFA in the milk fatty acids profile. (El-Zaiat et al., 2018) found that HA enhanced milk production and quality, as well as the growth rate of their offspring, with no negative impact on goat health. Moreover, there are many studies on the effect of HSs on growth performance. McGlone (et al., 2006) reported that HSs supplementation to the diet enhanced pig's growth performance and reduced ammonia excretion from manure. Similarly, Mikityuk et al., (2010) found that the addition of (4 g/head per day) of HSs increased the bodyweight of newborn calves by 22.4 compared to the reference group. The supplementation of HA to the diet of broiler chickens at a rate of 0.5 mL L^{-1} of drinking water has a significant effect on body weight gains and yields of edible carcass parts. Also, Abu Hafsa et al., (2021) illustrated that the addition of 10 g HA/kg of diet enhanced the growth performance, nutrient digestibility. On the other side (Zigo et al., 2020) found that the supplementation of HA at (0.5% and 1.0%) did not show an enhancement in quails' final body weight and slaughter characteristics. The addition of HSs at 0.5% to laying hen feed had a good impact on laying rate, daily egg production, egg weight, feed conversion, and eggshell quality, according to (Mikityuk et al., 2010).

6.2. Blood Biochemical Parameters

The evaluation of the physiology or health condition of living organisms requires the use of serum biochemistry and oxidative status indicators. Stepchenko, (2006) found that the cows fed with the humic had a 7.7% increase in total protein in their blood serum. Albumin and gamma globulin concentrations also increased by 8.3% and 14.2%, respectively, throughout this period, contributing to enhanced defensive responses in the experimental group's animals. Similarly, El-Zaiat et al., (2018) illustrated that the goats given HA had higher total protein, globulin, and glucose levels in their blood, as well as lower urea nitrogen, cholesterol, non-esterified free fatty acids, and ketone levels (Figure 9). Furthermore, the serum cholesterol and low-density lipoprotein concentrations except that 0.75 g HA reduced and high-density lipoprotein enhanced along with increasing HA level (Arif et al., 2018).

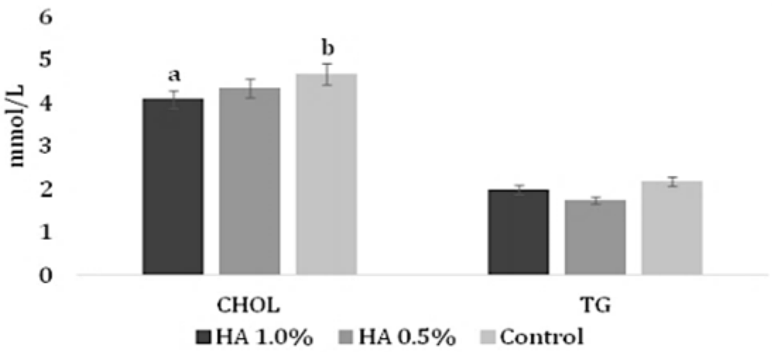


Figure 9. The impact of humic acid supplementation to diet on the levels of cholesterol and triglycerides.

Kovacik et al., (2020) reported that the supplementation of HA to the diet increased the endogenous antioxidants (TAC) concentrations. Furthermore, raising the HA content in the diet reduced the production of reactive oxygen species (ROS) and, as a result, the synthesis of malondialdehyde in the animal's blood was greatly reduced. Blood cholesterol concentration can enhance with the supplementation of 10 g HA/kg of diet, and modify the caecal fermentation activity without any harmful effects on the health of growing rabbits (Abu Hafsa et al., 2021). Moreover, serum Ca, albumin, and serum NEFA and blood Beta-hydroxybutyric acid concentration were enhanced with the supplementation of 75 g of humate (Yüca & Gül, 2021).

6.3. Animal Health

Probiotics, organic acids, and humates have been used as feed supplements due to they do not adversely affect animal health, are environmentally friendly and improve the quality and amount of the products after the prevention of the use of antibiotics as feed supplements (Kutlu and Serbester 2014). Furthermore, Because of the European Union's ban on the use of antibiotics as growth promoters, the search for alternative feed additives in animal production has increased (Hassan et al., 2010). The addition of HA improves laying hen egg production and the biological features of hatchable eggs, as well as having an immunostimulating impact (Figure 10), shown in enhanced phagocyte activity and B cell response (Kocabagl et al., 2002). Similarly, HSs have been shown to increase weight gain and improve immune system function (Hassan et al., 2010). Furthermore, HA supplementation had an inhibitory impact on toxins and infections as well as defense mechanisms against pathogens (Dabovich et al., 2003).

Galip et al., (2010) reported that because of the many binding sites contained in their structure, HA is considered adsorbent. Humic acids are considered to be able to reduce bacterial endotoxin absorption and systemic availability, which might be immensely helpful to animal and human health. In another study, Nagaraju et al., (2014) found that broiler performance and immunological condition can be improved by adding HA up to 0.1% to low nutrient density antibiotic-free diets without influencing carcass properties. Furthermore, humic acids have been linked to a variety of favorable impacts on animal performance and health. They reduce the levels of mycotoxins while inhibiting the growth of dangerous bacteria and moulds, potentially leading to improved gut health (Hudák et al., 2021). In lactating crossbred Slovak Pied cattle and × Red Holstein cows, (Zigo et al., 2020) studied the effects of humic acid supplementation (100 g/d) on milk yield, somatic cell count (SCC), and mastitis.

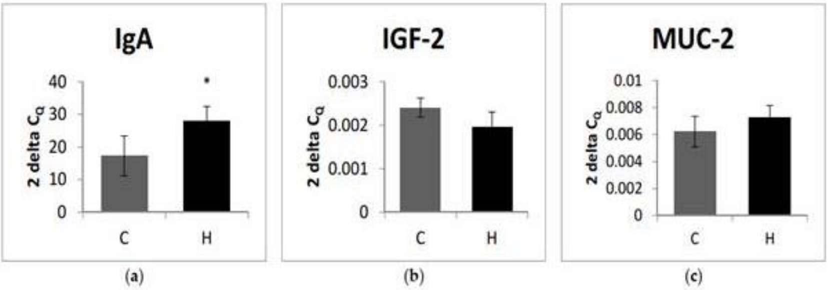


Figure 10. Effect of addition HA with 0.5% on laying hen's relative expression of IgA (**a**), IGF-2 (**b**), and MUC-2 (**c**) genes in the cecum. Columns marked with stars are significantly different from control: * $p < 0.05$.

They reported that HA addition reduced milk urea content and SCC without changing other milk ingredients. Furthermore, using organic acids in broiler and laying hen production improves their immunity and productivity by enhancing nutrient use. Anti-inflammatory, adsorptive, and antibacterial properties of humic substances make them beneficial in a wide range of animal husbandry applications (Nagaraju et al., 2014). The supplementation of 75 g of humate to the diet improved postpartum problems and pregnancy (Mehmet, 2021). In addition, adding HA to broiler chicken diets in combination with organic acids improved antibody response against Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) on day 16 without affecting blood biochemical contents (Akaichi et al., 2022). Therefore, disease control using HSs is the most important factor that affects animal production, improving immunity, and maintaining animal health.

6.4. Animal Products Quality

Meat and meat products with higher levels of minerals, polyunsaturated fatty acids (PUFAs), and antioxidants are popular and highly regarded all over the world. In livestock, ecological additives have received increased attention as an alternative to antibiotics. In addition, customers prefer organic ingredients. Wang et al., (2008) reported that HSs addition to chicken and pork diets has a considerable influence on meat quality during storage and improve meat colour, due to faster myoglobin production. Furthermore, humic acid was linked to increased fat marbling values and lower back fat thickness in pork, most likely due to its effects on protein and lipid distribution. Ozturk et al., (2010) also reported that the supplementation of humic acids to the diet improves growth, meat quality, carcass characteristics as well as, blood and gastrointestinal tract parameters. Similarly, Nagaraju et al., (2014) found that the inclusion of HA in broiler diets enhances meat quality. The addition of enzymes (Axtra XAP) and potassium humate to canola-based broiler diets improved carcass and meat quality parameters such as breast weights, WHC, and colour coordinates, as well as increasing the proportion of PUFAs, n-6 and n-3 fatty acids, and the PUFA/SFA ratio which are important factors of the nutritional value of meat. Moreover, Skalická et al., (2021) reported that the use of 0.7% and 0.3% Humac Natur Mycosorb as a feed additive resulted in an increase in Ca and Mg content in broiler breast and thigh muscles, Mutual interactions were related to changes in element concentrations seen in chicken muscle after the addition of humates used in this research. Hudák et al., (2021) found that the addition of 0.7 percent HSs to broiler feed in both natural and acidified forms had a significant impact on the composition and quality of breast meat.

On the other hand, the addition of humic minerals to the diet of cows can be improved the suitability of milk (Figure 11), for cheese production (Teter et al., 2021). To sum up, To sum up, Humic is a good organic feed supplement that can improve the nutrient quality of animal products.

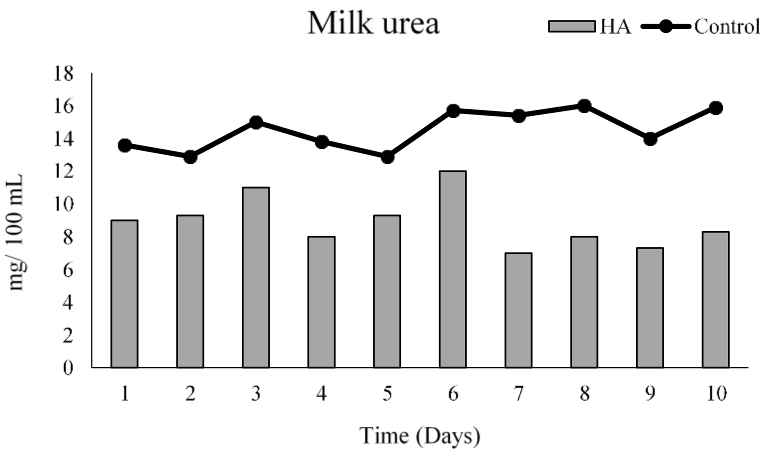


Figure 11. Milk urea content with or without HA supplementation to diet on the 10th day of lactation. Edited from (Zigo et al., 2020).

6.5. Environmental Pollution

Ruminants are necessary for food security, but there is growing concern about the impact of cattle on the environment, especially in terms of greenhouse gas emissions. (Terry et al., 2018). Beef cattle are expected to emit around 50 kg NH_3 per animal per year in greenhouse gases (GHGs). Ammonia converts to nitrogen oxides (NOx) in the atmosphere, leading to the nitrogen cycle's instability. and, as a result, a rise in the planet's temperature (Gronwald et al., 2018). The global warming effect of NH_3 is thought to be 265 times larger than that of CO_2 because it is a precursor of both the greenhouse effect of the ozone layer-depleting gas and nitrous oxide (N_2O). Water pollution or eutrophication, odor nuisance, soil contamination, and acidification are some of the other problems linked with ammonia (Reis-de Souza et al., 2019).

Váradyová et al., (2009) observed that the supplementation of HA at 10 g/kg DM to a forage-based diet, that NH_3-N production was decreased linearly by 24.4%. Moreover, McMurphy et al., (2011) reported that HSs have a strong nitrogen binding, which has been hypothesized to promote rumen microbial production while reducing N excretion and CH_4 emissions into the environment. Humic compounds (HCs), which are complex organic compounds comprising diverse functional (carboxyl, hydroxyl, phenolic) groups, have lately been examined for their ability to suppress the methanogenic activity of anaerobic consortia (Carvalho, 2015; de Melo et al., 2016). Similarly, Khadem et al., (2017) observed that HCs affected the activity of anaerobic sludge: Except Methanospirillum hungatei, 75% suppression of methane buildup may be achieved by adding 1 g/L HC to a medium containing hydrogenotrophic methane-forming bacteria present in anaerobic sludge.

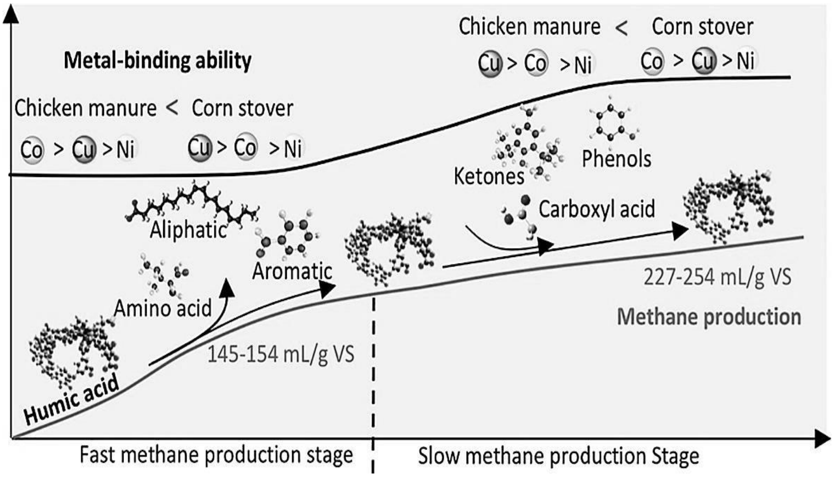


Figure 12. Effect of HA supplementation on heavy metal remediation and methane reduction.

Also, HA's nitrogen binding properties benefited in reducing ammonia nitrogen (NH_3-N) emissions from cattle feedlots. Furthermore, adding HSs to the diet of beef calves resulted in a significant increase in N retention. (Terry et al., 2018). The addition of HSs to batch incubations at 60 mg/liter of total solution with 40 g of wet soils reduced CH_4 emission in anoxic conditions (Figure 12). They found that CH_4 suppression was up to 40% in anoxic environments. Sheng et al., (2018) tested HSs in an *in vitro* batch culture and reported that it consistently suppressed CH_4 generation when added at up to 3.6 mg/mL of inoculum throughout a 48-hour incubation period using rumen fluid. In another study of multiple methanogenic consortia, partial suppression of methanogenesis was only detected at an HC concentration of 10 g/L, whereas total inhibition was observed at an HC concentration of 20 g/L. (Yap et al., 2018).

Similarly, Sheng et al. (2019) and Terry et al. (2018) observed that when HSs were added at up to 3.6 mg/mL of incubation fluid from cattle, CH_4 was reduced by 12.8% after 48 hours of incubation. Previous studies indicate that humic acid plays an important role as an organic additive in reducing harmful gases that pollute the environment and cause global warming. Therefore, humic acid is considered one of the most promising additives in implementing a sustainable and environmentally friendly agricultural production strategy.

Conclusion

Humic substances represent the organic material mainly widespread in nature. Application of humic substances (HSs) natural or harvested and engineered have been widely exploited in different agriculture aspects to improve soil characteristics, plant, and animal productivity. It is essential to add HSs fertilizers to the soil to achieve soil sustainability. They improve soil structure and fertility by influencing nutrient uptake and root architecture. Besides, HSs have positive effects on plant physiology. The biochemical and molecular mechanisms underlying these events are only partially known. Additionally, the use of HSs products such as humic acid as a feed additive to livestock animals can provide beneficial effects on growth and milk production traits due to their ability to modify rumen fermentation patterns. In addition, enhance animal health and the quality of their products. Previous studies indicate that HSs play an important role as an organic additive in reducing harmful gases that pollute the environment and cause global warming. Therefore, HSs is considering one of the most promising additives in implementing a sustainable and environmentally friendly agricultural production strategy. Finally, we elaborated the numerous roles of these organic materials as well as physical-chemical roles especially enhancement of soil-plant and animal production to achieve sustainable agriculture.

Conflict of Interest

The author declares there are no actual or potential competing interests, including any financial, personal, or other relationships with other people or organizations.

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Chapter 4

Methyl Jasmonate Plus Urea Foliar Applications: Effects on Tempranillo Grapes and Wine Phenolic Composition

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Abstract

Phenolic compounds are very important for grapes and wines quality since these compounds can take part in color, mouthfeel and wine ageing potential, and they are also related to the human healthy properties of moderate wine consumption. The content of phenolic compounds in grapes depends on different factors, such as variety, climatic and geographical factors, cultural practices, and the stage of grape ripeness. Foliar application of biostimulants has been studied in the last years in order to mitigate the effect of climatic change in grapes and to enhance

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their composition and quality. In this work, it was studied the effect of foliar application of methyl jasmonate plus urea (MeJ + Ur), in Tempranillo vineyard, on the content of phenolic compounds in grapes and wines, during two consecutive vintages. These compounds (anthocyanins, flavanols, flavonols, phenolic acids, and stilbenes) were analysed, in both grapes and wines, by high performance liquid chromatography (HPLC). The effect of foliar treatment was season dependent, probably due to the differences in pre-harvest rainfall recorded. In the first season, the MeJ + Ur treatment favored the biosynthesis of anthocyanins, increasing their content in grapes; however, this effect was not observed in the wines. In the second vintage, the MeJ + Ur foliar application did not enhance the anthocyanin content in grapes or in wines. With respect to the other phenolic compounds, the foliar treatment did not improve their content in grapes, except for total flavonols in the second vintage. Foliar application of MeJ + Ur seems to be a good tool in order to enhance the anthocyanin content in grapes, which it is very interesting due to anthocyanins are the main phenolic compounds in red varieties, and they are responsible for the wine red color. Nevertheless, further studies should be carried out to achieve a better knowledge of the plant response to this treatment according to the climatic conditions. Moreover, it is necessary to study how to transfer the improvement reached in the grapes anthocyanins content to the final wines.

Keywords: phenolic compounds, urea, methyl jasmonate, foliar application, grape, wine

Introduction

Phenolic compounds are very important secondary metabolites, which are related with grapes and wines quality, since are responsible for some organoleptic attributes, such as color, flavor, bitterness, astringency and wine ageing potential (Garrido and Borges, 2013; Santos-Buelga and Freitas, 2009). In addition, phenolic compounds are characterized by antioxidant, antimicrobial, antiinflammatory and cardioprotective properties, which can help to improve the human health and prevent various diseases with a moderate consumption of red wine (Nemzer et al., 2022; Vejarano and Luján-Corro, 2022). The content of phenolic compounds in grapes depends on several factors such as grape variety, climatic and geographical factors, cultural practices, and the degree of ripeness of grapes (Gil et al., 2012; Hornedo-Ortega et al., 2020; Meng et al., 2012). Pre-fermentative practices in the cellar and some operations carried out during the winemaking can also influence the levels of phenolic compounds in the final wines (Garrido and Borges, 2013; Nemzer et al., 2022; Vejarano and Luján-Corro, 2022). These compounds can be classified as flavonoid (anthocyanins, flavanols, and flavonols) and non-flavonoid compounds (phenolic acids, and stilbenes) (Cosme et al., 2018; Merkyte et al., 2020). Flavonoid compounds belong to a chemical family characterized by a structure of 15 carbon atoms, which comprise two aromatic rings bound through a 3 carbon chain (C6-C3-C6). Flavonoids can occur both in free and conjugated form (Garrido and Borges, 2013; Merkyte et al., 2020). Anthocyanins are glycosides of anthoycy-anidins, which means that are made up of an anthocyanidin molecule, the aglycone, to which a sugar is attached by a β-glycoside bond. In red grapes, six anthocyanidins have been described: cyanidin, peonidin, delphinidin, pelargonidin, petunidin, and malvidin, being the last the most representative in *V. vinifera* grapes. Pelargonidin is not present in wines (Heras-Roger et al., 2017). Anthocyanins can be found as mono-glycosylated, but also can be esterified as acetyl-glucosides, *p*-coumaroyl-glucosides, and caffeoyl-glucosides (Castillo-Muñoz et al., 2010; He et al., 2010). Anthocyanins are mostly located in the grape skins and are the phenolic compounds responsible for the red color of wines. Also, anthocyanins participate in the copigmentation reactions (Boulton, 2001). Flavonols are located in the grape skin and its biosynthesis depends on the degree and intensity of grape illumination (Heras-Roger et al., 2017). In grapes, the following aglycones have been identified: quercetin, kaempferol, isorhamnetin, myricetin, laricitrin, and syringetin, that can be bound to different sugars (Garrido and Borges, 2013). Flavonols are important compounds for the copigmentation process (Boulton, 2001; de Freitas et al., 2017; Heras-Roger et al., 2016) and for the astringency and bitterness (Hufnagel and Hofmann, 2008; Preys et al., 2006). Flavanols comprise catechins and tannins, which can be found in grape skins, seeds or stems (Boso et al., 2019). These compounds contribute to astringency sensations due to the precipitation of the salivary proteins, and also, participate in the bitterness of wines (Smith et al., 2015).

Non-flavonoid compounds include phenolic acids and stilbenes. Phenolic acids are subclassified as hydroxybenzoic and hydroxycinnamic acids. These compounds can be found in free form or esterified and therefore, its origin can be from the hydrolysis of other phenolic compounds. Hydroxybenzoic acids are commonly found esterified in grapes and as free acids in wines. The main hydroxybenzoic acid is the gallic acid (Heras-Roger et al., 2017). The hydroxycinnamic acids are the predominant phenolic acids found in both grapes and wines. The main hydroxycinnamic acids are caftaric, *p*-coutaric, and fertaric acids (Garrido and Borges, 2013). Due to the presence of a double bond in the lateral side, there are two isomeric forms: *cis* and *trans*. In grapes, caftaric and fertaric acids are mainly found in the *trans* form. These compounds are related to the astringent properties of both grapes and wines (Garrido and Borges, 2013). Stilbenes are compounds based on a two aromatics rings linked by an ethene bridge. These compounds are considered phytoalexins, which means that are produced in the vineyard due to adverse external conditions (Garrido and Borges, 2013; Heras-Roger et al., 2017). The content of stilbenes in wines is low, but these compounds are very important because of its beneficial health properties. Among them, *trans*-resveratrol has been described as the main stilbene in wine (Guerrero et al., 2009; Nemzer et al., 2022).

In last years, the global warming is affecting to the wine sector. The raising of temperature disrupts vine phenology, vegetative cycles and grape quality (Mira de Orduña, 2010). A mismatch between the technological maturity and the phenolic maturity of grapes has been observed. In this way, the greater water stress and higher temperatures conditions, resulting from climate change, make that grapes achieved early the technological maturity, whereas the phenolic maturity is not reached when grapes are ready for harvest, which also affects to the final wines, producing wines with a high alcoholic degree and low acidity (Garde-Cerdán et al., 2023; Mira de Orduña, 2010). To mitigate the climate change effects on grape phenolic composition, different approaches are being studied. The foliar application of elicitors is one of them since, there are so many studies about the effect of foliar application of methyl jasmonate (MeJ) to the vineyard in order to improve grape phenolic composition (Gil-Muñoz et al., 2017). Elicitors are compounds able to trigger a defense response in plants, which undergone an enhance in the production of secondary metabolites, such as phenolic compounds (Ruiz-García and Gómez Plaza, 2013). More deeply, foliar application of MeJ to vineyard increase the biosynthesis of some phenolic compounds, since the enzymes involved in their synthesis undergone an activation, mainly the enzymes related to stilbenes and anthocyanins biosynthesis (Garde-Cerdán et al., 2023). Portu et al. (2015c) described an increase, in both grapes and wines, of anthocyanins and stilbenes content, besides improving wine flavonols concentration. Similar effects have been showed by Portu et al. (2016, 2018). Furthermore, the effect of foliar application of urea to vineyard also has been studied, since involves an efficient and fast assimilation of urea. Urea is a nitrogen source widely employed by its characteristics, such as, small molecular size, higher water solubility, and low cost (Lasa et al., 2012; Pérez-Álvarez et al., 2021). The effect of this foliar application is not clear. Previous works describe that its foliar application did not exert a large impact on grape phenolic composition (Portu et al., 2017), whereas other authors observed an increase on the content of several flavonols and anthocyanins in grapes (Portu et al., 2015b), or an increase in the stilbenes content in must and wines, because urea foliar application favored the resveratrol and piceid synthesis (Garde-Cerdán et al., 2015). Moreover, foliar application of urea on grape phenolic composition during two consecutive vintages showed an increase in some flavanols in the second year of the study, while no effect was observed in the first season (Portu et al., 2017).

As above-mentioned, the foliar application of these compounds, MeJ and urea (Ur), separately improves the biosynthesis of some phenolic compounds, for this reason, seems interesting to study the effect of MeJ and Ur combined foliar application on grapes and wines phenolic composition. Therefore, the aim of this work was to study how the foliar application of MeJ + Ur to vineyard affect to the phenolic composition of grapes and wines over two consecutive vintages.

Materials and Methods

Vineyard Site, Grapevine Treatments and Vinifications

For this trial, red grapes from Tempranillo (*Vitis vinifera* L.) variety were employed. The vineyard was located in Finca La Grajera, Logroño, La Rioja, Spain (Lat: 42º26’25.36” North; Long: 2º30’56.41” West; 456 meters above sea level). Vines were planted in 1997, were grafted onto a R-110 rootstock and trained to a VSP (vertical shoot positioned) trellis system with a vine spacing of 2.80 m x 1.25 m. In this study, two different applications were carried out to vineyard of: i) control (sprayed with water solution of Tween 80 alone), and ii) methyl jasmonate plus urea (MeJ + Ur). To apply the treatment, aqueous solutions were prepared with a concentration of 10 mM of methyl jasmonate (MeJ) plus urea in a total dose of 6 kg N/ha (MeJ + Ur) according to exposed by Garde-Cerdán et al. (2016) and Pérez-Álvarez et al. (2021); Tween 80 (1 mL/L) was used as wetting agent and control plants were sprayed with a water solution of Tween 80 alone. The applications were performed to vineyard twice, at veraison and one week later, and, for each application, 200 mL/plant was sprayed over leaves. These applications were carried out in triplicate and were arranged in a complete randomized block design along the vineyard, with 10 vines for each replication and treatment, the study was performed during two consecutive seasons, 2019 and 2020.

Grapes were harvested at their optimum technological maturity, i.e., when the probable alcohol reached 13º, and the weight of 100 berries remained constant. Then, a random set of 50 berries, per replicate and treatment, was collected and frozen at -20°C in order to subsequent determination of the grape phenolic composition. At the winery, the clusters were destemmed and crushed, each treatment and replicate were elaborated distinctly. The resulted pomace was elaborated in one 30 L-tank for each one. Therefore, 6 elaborations were carried out (2 treatments × 3 repetitions/treatment). 50 mg SO_2/kg of grapes was added to protect the paste–must and the alcoholic fermentation was induced by inoculation (at a dosage of 20 g/hL) with a commercial *Saccharomyces cerevisiae* strain (Safoeno SC22, Fermentis, Marcq-en-Barœul, France), and it was carried out at a controlled temperature (at 20 +/− 2°C). Once the alcoholic fermentation finished (the content of residual sugars were below than 2.5 g/L), a comercial *Oenococcus oeni* strain (Viniflora CiNe, CHR Hansen, Hørsholm, Denmark) at 1 g/hL was inoculated into the wines, to perform the malolactic fermentation (MLF) (at 17 +/− 1°C). Once the MLF was finished, for each wine, aliquot samples were frozen and stored at −20°C until their analysis.

Analysis of Grape Phenolic Compounds by HPLC-DAD

Extraction of Grape Phenolic Compounds

About 50 g of frozen grapes from each sample were extracted following the methodology described by Portu et al. (2015b). In summary, grapes were immersed into 50 mL methanol/water/formic acid (50:48.5:1.5, v/v/v) and then, this mixture was homogenized by Ultra-Turrax T-18 (IKA, Staufen, Germany) at high speed (18,000 rpm) for 1 min. Samples were maintained in an ultrasonic bath (ARGO LAB, Carpi, Italy) for 10 min and were centrifuged at 5000 rpm at 10°C for 10 min. A second extraction was carried out with the pellet. The supernatants were combined and stored at -20°C until analysis.

Sample Preparation for the Analysis of Non-Anthocyanin Phenolic Compounds

Anthocyanins are compounds that can cause interferences in the chromatographic separation and identification of other phenolic compounds. For this reason, it is necessary to perform a separation step. PCX SPE cartridges (500 mg, 6 mL; Bond Elut Plexa, Agilent) were used following the method described by Portu et al. (2015b). The non-anthocyanin phenolic compounds fraction was dried in a centrifugal evaporator (miVac, Genevac Ltd., Suffolk, UK) at 35°C and re-dissolved in 1.5 mL 20% (v/v) methanol aqueous solution. The anthocyanin-free fraction was used to analyze non-anthocyanin phenolic compounds (flavonols, flavanols hydroxybenzoic and hydroxycinnamic acids and stilbenes).

Analysis of Grape Phenolic Compounds by HPLC-DAD

An Agilent 1260 Infinity II chromatograph, equipped with a diode array detector (DAD) was used to analyze the phenolic compounds. The methodology described by Portu et al. (2015b) was employed, a flow rate of 0.630 mL/min was established. Samples were filtered and injected on a Licrospher® 100 RP-18 reversed-phase column (250 x 4.0 mm; 5 µm packing; Agilent) with pre-column Licrospher® 100 RP-18 (4 x 4 mm; 5 µm packing; Agilent), both thermostated at 40ºC.

For the analysis of anthocyanins, 10 µL of grape extract were injected and the eluents used were (A) acetonitrile/water/formic acid (3:88.5:8.5, v/v/v), and (B) acetonitrile/water/formic acid (50:41.5:8.5, v/v/v).

For the non-anthocyanin phenolic compounds fractions, the injection volume was 20 µL. Eluents were (A) acetonitrile/water/formic acid (3:88.5:8.5, v/v/v), (B) acetonitrile/water/formic acid (50:41.5:8.5, v/v/v), and (C) methanol/water/formic acid (90:1.5:8.5, v/v/v).

The identification of phenolic compounds was carried out according to the retention times of available pure compounds and the UV-Vis data obtained from authentic standards and/or published in previous studies (Castillo-Muñoz et al., 2009). Quantification was performed with the extraction of DAD chromatograms, at 520 nm (anthocyanins), 360 nm (flavonols), 320 nm (hydroxycinnamic acids and stilbenes), and 280 nm (gallic acid and flavanols) and the calibration graphs of the respective standards ($R^2 > 0.99$) were employed. If a standard was not available, quantification was made according to the calibration graph of the most similar compound. Hence, malvidin-3-O-glucoside was used for anthocyanins, quercetin-3-O-glucoside was used for flavonols, *trans*-caftaric acid was used for free hydroxycinnamic acids and the corresponding tartaric esters, catechin was used for procyanidins B1 and B2, epicatechin was used for epigallocatechin, and *trans*-piceid and *trans*-resveratrol were used for their respective *cis* isomers.

Concentrations were expressed as mg/kg of fresh weight. The results for phenolic compounds are calculated as the average of the analyses of three samples (3 field replicates, n = 3).

Analysis of Wine Phenolic Compounds by HPLC-DAD

Sample Preparation for the Determination of Non-Anthocyanin Phenolic Compounds

Again, to avoid interferences, an extraction of non-anthocyanin phenolic compounds was performed, according to Portu et al. (2015b). 3 mL of each wine sample was diluted with 3 mL of 0.1 N HCl, then was passed through the PCX SPE cartridges (500 mg, 6 mL; Bond Elut Plexa, Agilent, Palo Alto, CA), which previously has been conditioned (5 mL of methanol and 5 mL of water). The non-anthocyanin phenolic compounds fraction was eluted with 2 × 3 mL of ethanol, dried in a centrifugal evaporator (miVac, Genevac Ltd.) at 35°C and re-solved in 1.5 mL of 20% (v/v) methanol aqueous solution.

Analysis of Wine Phenolic Compounds by HPLC-DAD

The methodology employed to analyze the phenolic compounds in wines was the same described previously for grapes.

Statistically Analysis

The statistical elaboration of the data was performed using SPSS Version 21.0 statistical package for Windows (SPSS, Chicago, IL, USA). Phenolic compounds data in grapes and wines were processed using a variance analysis (ANOVA) ($p \leq 0.05$). The differences between means were compared using the Duncan test ($p \leq 0.05$).

Results and Discussion

Effect of MeJ+Ur Foliar Application on Grape Phenolic Compounds

Table 1 shows the anthocyanin composition of Tempranillo grapes from control vines and vines foliarly treated with methyl jasmonate plus urea (MeJ+Ur) treatment, in the 2019 and the 2020 seasons. In 2019, all individual non-acylated anthocyanins and therefore, total non-acylated anthocyanins, presented a higher content in MeJ+Ur grapes than in control grapes. Among acylated anthocyanins, an increase on the content of peonidin-3-acglc, delphinidin-3-cmglc, cyanidin-3-cmglc, petunidin-3-cmglc, peonidin-3-cmglc, and malvidin-3-*trans*-cmglc was observed in MeJ+Ur treated grapes, in comparison with control grapes. In this first year of the study, also MeJ+Ur grapes undergone an increase in total acylated and total anthocyanins content with respect to the control ones (Table 1). Anthocyanins are the phenolic compounds responsible for the red color in grapes and wines. In 2020, the content of anthocyanins in the grapes was less affected by the MeJ+Ur foliar treatment than in 2019. MeJ+Ur grapes only showed a higher content of cyanidin-3-glc and peonidin-3-acglc, and a lower content of malvidin-3-*trans*-cmgl than control grapes (Table 1). Control grapes showed a higher total content of acylated anthocyanins in comparison with MeJ+Ur grapes. However, differences on total anthocyanin content between control and MeJ+Ur grapes was not detected. It is noticeable the different effect of foliar treatment among seasons. Hence, these results support the idea that the response to foliar application of MeJ depends on seasonal conditions (González-Lázaro et al., 2022; Paladines-Quezada et al., 2019) and, according with these results, MeJ+Ur foliar treatment also showed a seasonal dependence (Table 1). Among seasons, there were differences between the accumulated rainfall recorded, in 2019 (519.7 mm); this value was higher than in 2020 (497.60 mm).

In 2019, acylated anthocyanins accounted for around 19% of total anthocyanins, whereas in 2020 accounted for around 29%. The main anthocyanin in both seasons was malvidin-3-glc. Previous studies about the effect of foliar treatment of urea or MeJ, applied separately to vineyard, have described an increase on the synthesis of anthocyanins and therefore, a higher content of anthocyanins in treated grapes than in control ones (Portu et al., 2015b, 2015c, 2016, 2018), in agreement with the observed results about MeJ+Ur foliar treatment, especially in 2019 season (Table 1). In fact, MeJ foliar application induces the activation of enzymes that participate in the biosynthesis of phenolic compounds (Delaunois et al., 2014; Ruiz-García and Gómez-Plaza, 2013). The enzyme phenylalanine ammonia-lyase (PAL) stands out since its activity is necessary for the accumulation of phenolic compounds (Portu et al., 2016).

Table 1. Anthocyanin content (mg/kg) referred to fresh weight in grapes from control and MeJ + Urea (MeJ+Ur) treatment, in 2019 and 2020 seasons

	2019		**2020**	
	Control	**MeJ+Ur**	**Control**	**MeJ+Ur**
Delphinidin-3-glc	123.77 ± 12.34 a	171.99 ± 7.88 b	50.58 ± 1.69	56.08 ± 10.13
Cyanidin-3-glc	25.83 ± 3.66 a	45.65 ± 2.93 b	8.44 ± 0.34 a	10.80 ± 1.30 b
Petunidin-3-glc	85.84 ± 6.28 a	117.78 ± 6.68 b	47.93 ± 1.59	52.61 ± 8.93
Peonidin-3-glc	45.73 ± 3.95 a	76.50 ± 6.57 b	19.77 ± 0.91	24.61 ± 3.94
Malvidin-3-glc	215.76 ± 8.13 a	267.93 ± 19.97 b	169.84 ± 0.91	167.32 ± 23.70
Total non-acylated	**496.94 ± 32.57 a**	**679.85 ± 41.73 b**	**296.57 ± 7.58**	**311.43 ± 47.42**
Delphinidin-3-acglc	10.42 ± 0.75	11.45 ± 0.48	6.66 ± 0.12	6.94 ± 0.38
Cyanidin-3-acglc	3.84 ± 0.02	3.79 ± 0.10	3.60 ± 0.02	3.58 ± 0.01
Petunidin-3-acglc	6.86 ± 0.27	7.40 ± 0.33	5.57 ± 0.11	5.57 ± 0.24
Peonidin-3-acglc	4.48 ± 0.08 a	5.09 ± 0.05 b	3.85 ± 0.04 a	4.08 ± 0.06 b
Malvidin-3-acglc	11.71 ± 0.26	12.45 ± 0.95	10.53 ± 0.42	9.82 ± 0.58
Delphinidin-3-cmglc	16.28 ± 0.68 a	22.30 ± 1.00 b	14.31 ± 0.38	13.83 ± 1.16
Cyanidin-3-cmglc	6.21 ± 0.28 a	8.59 ± 0.24 b	5.38 ± 0.17	5.88 ± 0.30
Petunidin-3-cmglc	12.97 ± 0.26 a	16.73 ± 0.81 b	12.47 ± 0.25	11.37 ± 0.84
Peonidin-3-cmglc	8.27 ± 0.06 a	11.36 ± 0.52 b	7.42 ± 0.07	7.33 ± 0.34
Malvidin-3-*cis*-cmglc	4.55 ± 0.08	4.64 ± 0.13	4.66 ± 0.21	4.32 ± 0.16
Malvidin-3-*trans*-cmglc	36.74 ± 2.11 a	44.04 ± 2.89 b	51.03 ± 0.75 b	39.29 ± 2.08 a
Malvidin-3-cfglc	4.21 ± 0.02	4.24 ± 0.13	10.90 ± 1.42	10.80 ± 1.06
Total acylated	**126.54 ± 1.10 a**	**152.08 ± 6.68 b**	**136.37 ± 1.96 b**	**122.53 ± 6.99 a**
Total anthocyanins	***623.48 ± 32.23 a***	***831.93 ± 45.64 b***	***432.94 ± 9.42***	***433.95 ± 53.95***

Nomenclature abbreviations: glc, glucoside; acglc, acetylglucoside; cmglc, *trans*-*p*-coumaroylglucoside; cfglc, caffeoylglucoside.

All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$), the absence of letters means no significant differences ($p > 0.05$).

Table 2 shows the phenolic composition of Tempranillo grapes from control vines and vines foliarly treated with control and methyl jasmonate plus urea (MeJ+Ur) treatments, in the 2019 and the 2020 seasons. In 2019, MeJ+Ur treatment did not increase the individual or total flavonols content in grapes. Moreover, control grapes showed a higher content of quercetin-3-glcU, kaempferol-3-gal and total flavonols when compared with MeJ+Ur grapes. Again, the effect of MeJ+Ur foliar treatment was different in 2020. In the second year of the study, only two individual flavonols were affected by

MeJ+Ur foliar treatment, kaempferol-3-glcU + 3-glc and isorhamnetin-3-glc, which showed a higher concentration than in control grapes (Table 2). In this season, no significant differences were observed in the content of total flavonols between control and MeJ+Ur grapes. Flavonols are compounds of importance in the color stability of red wines due to their participation in copigmentation reactions with anthocyanins (Boulton, 2001). In addition, these compounds contribute to the astringency and bitterness sensations (Gonzalo-Diago et al., 2014; Preys et al., 2006). The predominant flavonol in both seasons was myricetin-3-glc (Table 2). With respect to the effect of foliar application of MeJ and urea, separately, to vineyard, there are studied which describes different effects. Portu et al. (2015c) did not observe a big effect on grapes flavonol content after foliar application of MeJ, likewise Ruiz-Garcia et al., (2013) showed that flavonol content was normally unaffected by MeJ treatment. Portu et al. (2017) described no significant differences in grape flavonol content between control and grapes from vines treated with urea. However, Portu et al. (2015b) observed a certain effect of urea foliar treatment on grape flavonols content. Therefore, foliar application of MeJ and urea, separately, did not exert a consistent effect to improve grapes flavonol content and their combined application neither.

Regarding flavanols, in 2019, MeJ+Ur and control grapes only showed differences on the content of epicatechin-3-gallate and epigallocatechin (Table 2). Control grapes presented a higher content of these flavanols when compared with the content of MeJ+Ur grapes. Despite it, control and treated grapes did not show differences on grapes total flavanols content. In 2020, MeJ+Ur grapes presented a higher content of catechin and epicatechin in comparison with control grapes (Table 2). MeJ+Ur grapes showed a significant higher content of total flavanols than control grapes. Flavanols contribute to the astringency and to the colour of red wines through the interaction with anthocyanins (Ferrer-Gallego et al., 2010). In the first year of the study, catechin was the major flavanol in grapes, followed by epicatechin, similarity to those found in 2020 (Table 2). Previous studies about the effect of foliar application of MeJ and urea separately on grape flavanol content have been carried out. Portu et al. (2015c) described that MeJ foliar application to vineyard did not affect flavanol synthesis, since MeJ grape samples presented a similar flavanol levels than control grape samples. Portu et al. (2017) studied the effect of foliar application of urea and phenylalanine; these authors described that season had a marked influence on flavanols.

Table 2. Flavonols, flavanols, phenolic acids and stilbenes content (mg/kg) referred to fresh weight in grapes from control and MeJ + Urea (MeJ+Ur) treatments, in 2019 and 2020 seasons

	2019		**2020**	
	Control	**MeJ+Ur**	**Control**	**MeJ+Ur**
Flavonols				
Myricetin-3-glcU	27.15 ± 2.47	23.38 ± 3.03	16.26 ± 0.70	15.16 ± 2.70
Myricetin-3-gal	35.08 ± 3.48	37.78 ± 3.32	22.16 ± 1.58	21.46 ± 4.08
Myricetin-3-glc	181.66 ± 15.36	183.44 ± 4.32	82.27 ± 4.81	80.40 ± 15.70
Quercetin-3-glcU	164.18 ± 15.66 b	110.84 ± 6.94 a	24.60 ± 1.67	32.50 ± 15.70
Quercetin-3-glc	172.29 ± 14.90	144.07 ± 27.94	32.82 ± 0.70	37.40 ± 4.05
Laricitrin-3-glc	33.29 ± 3.44	30.76 ± 1.28	30.31 ± 1.31	29.19 ± 3.62
Kaempferol-3-gal	2.48 ± 0.23 b	1.76 ± 0.20 a	0.46 ± 0.04	0.48 ± 0.06
Kaempferol-3-glcU+3-glc	15.99 ± 1.83	14.71 ± 1.87	2.17 ± 0.35 a	3.66 ± 0.21 b
Isorhamnetin-3-glc	12.17 ± 1.18	10.44 ± 1.15	3.54 ± 0.21 a	4.96 ± 0.81 b
Syringetin-3-glc	21.88 ± 1.52	21.14 ± 0.82	12.03 ± 0.94	15.04 ± 2.24
Total flavonols	***666.15 ± 33.09 b***	***578.33 ± 24.51 a***	***226.61 ± 5.16***	***240.25 ± 37.79***
Flavanols				
Catechin	63.31 ± 3.37 b	52.13 ± 6.94 a	11.06 ± 0.31 a	13.85 ± 1.45 b
Epicatechin	39.10 ± 3.85	34.75 ± 5.90	11.09 ± 0.43 a	19.76 ± 2.47 b
Epicatechin-3-gallate	14.12 ± 2.12 b	10.34 ± 0.98 a	8.24 ± 0.76	8.21 ± 0.85
Epigallocatechin	4.42 ± 0.32 b	2.64 ± 0.23 a	8.25 ± 0.91	9.53 ± 1.01
Procyanidin B1	28.28 ± 3.57	31.99 ± 1.43	10.26 ± 1.10	10.82 ± 0.78
Procyanidin B2	n.d.	n.d.	n.d.	n.d.
Total flavanols	*149.24 ± 9.65*	*131.84 ± 13.63*	*48.90 ± 1.71 a*	*62.17 ± 2.77 b*
Hydroxybenzoic acid				
Gallic acid	6.00 ± 0.80	5.83 ± 0.71	5.20 ± 0.57	6.12 ± 0.23
Hydroxycinnamic acids (HCAs)				
trans-Caftaric acid	6.54 ± 0.09	7.17 ± 0.65	1.51 ± 0.07	1.76 ± 0.23
trans+*cis*-Coutaric acids	4.62 ± 0.40 b	3.30 ± 0.47 a	0.17 ± 0.03 a	0.94 ± 0.06 b
trans-Fertaric acid	1.78 ± 0.19	1.62 ± 0.30	1.34 ± 0.23	0.98 ± 0.10
Caffeic acid	0.43 ± 0.05 b	0.25 ± 0.02 a	0.26 ± 0.03	0.31 ± 0.03
p-Coumaric acid	0.36 ± 0.09	0.27 ± 0.05	0.14 ± 0.01	0.14 ± 0.02
Ferulic acid	2.27 ± 0.15 b	1.88 ± 0.10 a	10.56 ± 1.65	9.58 ± 0.13
Total HCAs	***15.99 ± 0.86***	***14.49 ± 1.46***	***13.98 ± 1.36***	***13.71 ± 0.42***
Stilbenes				
trans-Piceid	12.75 ± 1.06	12.76 ± 0.33	5.37 ± 0.38	5.70 ± 1.02
cis-Piceid	1.70 ± 0.24	1.64 ± 0.04	1.13 ± 0.09 a	2.32 ± 0.21 b
trans-Resveratrol	0.63 ± 0.05 b	0.34 ± 0.04 a	0.11 ± 0.02 b	0.04 ± 0.01 a
cis-Resveratrol	0.35 ± 0.03	0.35 ± 0.03	0.20 ± 0.02 a	0.39 ± 0.04 b
Total stilbenes	***15.43 ± 1.30***	***15.09 ± 0.28***	***6.82 ± 0.46***	***8.45 ± 1.26***

Nomenclature abbreviations: glcU, glucuronide; gal, galactoside; glc, glucoside.

All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$), the absence of letters means no significant differences ($p > 0.05$). n.d.: not detected.

Also, they observed certain trend to increase the total amount of these compounds for Ur foliar application, although it was not significant. In addition, Portu et al. (2015b) observed that the low dose of urea tested (0.9 kg N/ha) had the major impact in the grape's composition, increasing the content of epicatechin-3-gallate and total flavanols content with respect to control grapes. Flavonols and flavanols are related to anthocyanins since these compounds share a big part of their biosynthetic pathway (Garde-Cerdán et al., 2023). However, the fact that in 2019, the anthocyanin content increased when MeJ+Ur was applied to vineyard but the flavonols and flavanols content decreased or not were affected could indicate that the foliar application of this elicitor plus urea induced primarily the activation of enzymes related to anthocyanin synthesis and not those enzymes related to flavonols and flavanols synthesis. This idea was postulated for MeJ foliar application previously (Portu et al., 2015c). The different effect of MeJ+Ur foliar treatment observed between seasons could be explained, apart from the different climatological conditions, by the fact that in 2019 season, one month took place between the first application and the date of harvest, whereas in 2020, this period was longer, 1 month and 20 days. This could produce a decrease on the effect of the foliar application on the grape phenolic composition (Garde-Cerdán et al., 2023).

Phenolic acids are compounds mostly colorless, odorless and tasteless, but are precursors of volatile phenols (Gonzalo-Diago et al., 2017; Heras-Roger et al., 2017). Gallic acid was the only hydroxybenzoic acid found in grapes (Table 2). Differences on its content among control and MeJ+Ur samples were not found in both seasons studied. Regarding hydroxycinnamic acids (HCAs), in 2019, control grapes were characterized by a higher content of *trans*+*cis*-coutaric acids and caffeic and ferulic acids in comparison with MeJ+Ur grapes (Table 2). However, the total concentration of HCAs did not show differences among control and MeJ+Ur samples. In 2020, grapes from grapevines foliar treated with MeJ+Ur presented a higher content of *trans*+*cis*-coutaric acids when compared with control grapes. Hydroxycinnamics acids can be decarboxylated and contribute to form ethylphenols, undesirable compounds to wine quality, during the wine ageing in oak barrels (Garde-Cerdán et al., 2010). It is noteworthy that the distribution of HCAs in grapes from both seasons was quite different. Surprisingly, the content of esterified HCAs in grapes in 2020 season was very low (Table 2). In 2019, the main HCAs was *trans*-caftaric acid, whereas in 2020 was ferulic acid. Previous reports (Portu et al., 2015c, 2016, 2018) observed that the content of hydroxybenzoic and hydroxycinnamic acids in grapes was independent of MeJ foliar application. Likewise, Portu et al. (2015b) described non effect of urea foliar treatment on HCAs grapes content.

For stilbenes, as has been observed with other families of phenolic compounds, the effects of MeJ+Ur foliar treatment on their content in grapes were not the same in both seasons (Table 2). In 2019, a slight significant difference was found among control and MeJ+Ur grapes. Control grapes showed a higher content of *trans*-resveratrol. In 2020, the effect of foliar treatment on grape stilbenes content was different. MeJ+Ur grapes were characterized by a higher content of *cis*-piceid and *cis*-resveratrol and a lower content of *trans*-resveratrol when compared with control grapes (Table 2). The main stilbene in both seasons was *trans*-piceid. Grapes and wines are an interesting source of stilbenes for the human diet (Garde-Cerdán et al., 2015). These compounds present antioxidant activity, antifungal and antibacterial properties, and cardioprotective and anticancer attributes, which are considered beneficial for human health (Benbouguerra et al., 2021; Gil-Muñoz et al., 2017; Guerrero et al., 2009). These results, where a non or slight effect on grapes stilbene content was observed, contrast with those described in bibliography. Portu et al. (2015c, 2018) showed that stilbenes were greatly increased by the foliar application of MeJ. Although the report of Portu et al. (2016) was more like the results of the present study, since *trans*-resveratrol was the only affected by the MeJ foliar treatment. Furthermore, Portu et al. (2015b) showed not differences on stilbenes compounds among control and MeJ grapes.

Effect of MeJ+Ur Foliar Application on Wine Phenolic Compounds

Table 3 shows the anthocyanin composition of Tempranillo wines from control and MeJ+Ur treatment, in 2019 and 2020 seasons. Regarding anthocyanins content, in 2019, the big effect of MeJ+Ur foliar treatment described in grapes (Table 1) was not observed in wines (Table 3). Probably due to a lower release of these compounds during the process of vinification. This fact has been previously observed (Portu et al., 2018) and the explanation could be that foliar application of MeJ increases the protein content in the skin cell wall of grapes, which produces a more rigid cell wall structure (Paladines-Quezada et al., 2019). This effect on the skin cell wall could make difficult the release of phenolic compounds during winemaking (Ortega-Regules et al., 2006).

Table 3. Anthocyanins content (mg/L) in wines from control and MeJ+Urea (MeJ+Ur) treatments, in 2019 and 2020 seasons

	2019		**2020**	
	Control	**MeJ+Ur**	**Control**	**MeJ+Ur**
Delphinidin-3-glc	14.67 ± 2.72	15.68 ± 1.44	6.48 ± 0.67 a	10.20 ± 1.88 b
Cyanidin-3-glc	2.21 ± 0.06	2.54 ± 0.33	1.57 ± 0.07 a	1.88 ± 0.13 b
Petunidin-3-glc	20.48 ± 3.40	22.68 ± 1.06	13.81 ± 2.37	17.45 ± 2.27
Peonidin-3-glc	6.38 ± 0.60 a	8.70 ± 1.07 b	2.83 ± 0.56 a	4.33 ± 0.90 b
Malvidin-3-glc	89.68 ± 8.97	99.17 ± 3.46	82.84 ± 8.04	89.45 ± 8.15
Total non-acylated	***133.42 ± 15.69***	***148.77 ± 4.45***	***107.53 ± 11.53***	***123.29 ± 11.78***
Delphinidin-3-acglc	2.51 ± 0.24	2.66 ± 0.07	2.39 ± 0.19	2.64 ± 0.16
Cyanidin-3-acglc	1.35 ± 0.00 a	1.36 ± 0.01 b	1.36 ± 0.01	1.38 ± 0.01
Petunidin-3-acglc	2.61 ± 0.20	2.66 ± 0.04	2.59 ± 0.23	2.77 ± 0.19
Peonidin-3-acglc	2.12 ± 0.07 a	2.41 ± 0.02 b	1.74 ± 0.10	1.90 ± 0.09
Malvidin-3-acglc	5.93 ± 0.46	5.95 ± 0.19	6.73 ± 0.44	6.49 ± 0.30
Delphinidin-3-cmglc	3.76 ± 0.35	4.09 ± 0.13	3.81 ± 0.57	4.53 ± 0.58
Cyanidin-3-cmglc	1.79 ± 0.09 a	2.11 ± 0.09 b	1.79 ± 0.11	2.04 ± 0.15
Petunidin-3-cmglc	2.90 ± 0.19	3.11 ± 0.02	2.86 ± 0.35	3.19 ± 0.38
Peonidin-3-cmglc	2.37 ± 0.11 a	2.82 ± 0.18 b	2.28 ± 0.20	2.66 ± 0.29
Malvidin-3-*cis*-cmglc	1.71 ± 0.03	1.70 ± 0.05	1.82 ± 0.02 b	1.68 ± 0.06 a
Malvidin-3-*trans*-cmglc	9.33 ± 0.46	9.52 ± 0.33	9.84 ± 1.52	11.10 ± 2.30
Malvidin-3-cfglc	1.99 ± 0.09	1.88 ± 0.17	1.59 ± 0.06	1.63 ± 0.08
Total acylated	***38.37 ± 2.22***	***40.28 ± 0.45***	***38.80 ± 3.65***	***42.01 ± 4.35***
Total anthocyanins	***171.80 ± 17.75***	***189.04 ± 4.81***	***146.33 ± 15.18***	***165.30 ± 16.01***
Vitisin A	2.00 ± 0.16 b	1.68 ± 0.03 a	1.51 ± 0.02	1.55 ± 0.04
Vitisin B	1.97 ± 0.12 a	2.19 ± 0.05 b	1.78 ± 0.05 a	1.99 ± 0.08 b

Nomenclature abbreviations: glc, glucoside; acglc, acetylglucoside; cmglc, *trans*-*p*-coumaroylglucoside; cfglc, caffeoylglucoside.

All parameters are listed with their standard deviation ($n = 3$). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$), the absence of letters means no significant differences ($p > 0.05$).

Within non-acylated anthocyanins, only the content of peonidin-3-glc increased in wines elaborated from MeJ+Ur grapes (Table 3). With respect to the rest of anthocyanins, MeJ+Ur wines presented a higher content of cyanidin-3-acglc, peonidin-3-acglc, cyanidin-3-cmglc, peonidin-3-cmglc and vitisin B when compared with control wines. Furthermore, in 2020, the effect of foliar MeJ+Ur treatment on the anthocyanins content of wines was slight (Table 3). MeJ+Ur wines were characterized by a higher content of delphinidin-3-glc, cyanidin-3-glc, peonidin-3-glc, and vitisin B and a lower content of malvidin-*cis*-cmglc. Regarding total anthocyanins content, MeJ+Ur wines, from both years of study, did not show differences with control wines (Table 3). Malvidin-3-glc was the main anthocyanin in control and MeJ+Ur wines. Unlike the results described above, Portu et al. (2016) did not observe differences on the vitisins (A and B) content of MeJ wines in comparison with control wines, whereas MeJ wines showed a higher content of total anthocyanins than control ones. However, Portu et al. (2015a) also observed an increase on vitisin B, and total anthocyanins content in MeJ wines in comparison with control ones. Vitisins are compounds formed during the alcoholic fermentation by reaction of malvidin-3-O-glucoside with pyruvic acid (A) or with acetaldehyde (B) (Bakker and Timberlake, 1997). Furthermore, Portu et al. (2015a) observed that the low dose of urea employed in that study increased the total anthocyanins content, several individual anthocyanins and vitisin B; however, with a high dose urea, anthocyanins content in wines did not improve. The dose of our study (6 kg N/ha) was higher than the two doses tested (0.9 and 1.5 kg N/ha) by Portu et al. (2015a).

Table 4 shows the phenolic composition of Tempranillo wines from control and MeJ+Ur treatment, in 2019 and 2020 seasons. In the family of flavonols, in 2019, a decrease in the concentration of some compounds was observed in MeJ+Ur wines (Table 4). MeJ+Ur wines showed a lower content of myricetin-3-gal, quercetin-3-glcU, free-quercetin, free-kaempferol and free-laricitrin than control wines. However, the total flavonols content in wines did not show differences among control and MeJ+Ur wines (Table 4). In 2020, a different effect of MeJ+Ur foliar application was observed, with respect to 2019. MeJ+Ur wines were characterized by a higher content of myricetin-3-gal, myricetin-3-glc, quercetin-3-glc, laricitin-3-glc, kaempferol-3-glcU+3-glc, isorhamnetin-3-glc, syringetin-3-glc, free quercetin, free-kaempferol and free-laricitrin in comparison with control wines (Table 4). In this season, the total flavonols content in MeJ+Ur wines was significantly higher than in control ones. The foliar application of urea (individually) also increased the total flavonols content in wines (Portu et al., 2015a). Furthermore, Portu et al. (2016, 2018), in their studies about the effect of MeJ foliar application on wine phenolic content, observed absence of differences on flavonols content among control and MeJ wines. The increase on flavonols content described in MeJ+Ur wines could have a positive effect, because of these compounds are related to the color stability of wines (González-Lázaro et al., 2022).

Regarding flavanols, in 2019, MeJ+Ur wines presented a higher concentration of epicatechin, procyanidin B1 and procyanidin B2, but the total flavanols content was unaffected by the MeJ+Ur foliar treatment (Table 4). In 2020, MeJ+Ur wines showed a higher content of epigallocatechin and procyanidin B1 than control wines, and once again the total flavanola content in wines was unaffected by the foliar application of MeJ+Ur to vineyard (Table 4). This absence of effect, on wine flavanols content, agrees with the observed for the individual foliar application of MeJ (Portu et al., 2015c, 2016) and for the urea foliar application effect (Portu et al., 2015a).

Table 4. Flavonols, flavanols, phenolic acids and stilbenes content (mg/L) in wines from control and MeJ+Urea (MeJ+Ur) treatments, in 2019 and 2020 seasons

	2019		**2020**	
	Control	**MeJ+Ur**	**Control**	**MeJ+Ur**
Flavonols				
Myricetin-3-glcU	12.16 ± 1.20	12.29 ± 1.28	6.64 ± 0.39	7.94 ± 1.24
Myricetin-3-gal	15.56 ± 0.34 b	13.81 ± 0.85 a	8.14 ± 1.05 a	13.33 ± 1.72 b
Myricetin-3-glc	110.56 ± 6.68	119.18 ± 6.25	31.94 ± 6.38 a	65.18 ± 11.97 b
Quercetin-3-glcU	85.40 ± 11.76 b	53.31 ± 9.82 a	11.35 ± 1.11	14.89 ± 2.64
Quercetin-3-glc	94.97 ± 11.20	75.28 ± 8.84	57.77 ± 6.23 a	83.88 ± 10.62 b
Laricitrin-3-glc	17.50 ± 1.22	17.03 ± 0.62	10.79 ± 0.37 a	15.16 ± 2.29 b
Kaempferol-3-gal	1.58 ± 0.23	1.12 ± 0.19	0.16 ± 0.01	0.18 ± 0.02
Kaempferol-3-glcU+3-glc	7.24 ± 1.14	5.09 ± 0.88	0.70 ± 0.10 a	0.99 ± 0.10 b
Isorhamnetin-3-glc	1.73 ± 0.24	1.76 ± 0.13	0.23 ± 0.04 a	0.60 ± 0.06 b
Syringetin-3-glc	11.25 ± 1.06	11.46 ± 0.51	8.92 ± 0.59	12.92 ± 2.90
Free-myricetin	12.56 ± 0.46	13.38 ± 1.24	18.61 ± 3.15	25.61 ± 3.85
Free-quercetin	18.85 ± 1.69 b	11.57 ± 2.19 a	14.36 ± 1.39 a	19.22 ± 2.32 b
Free-kaempferol	10.09 ± 0.69 b	7.56 ± 0.60 a	3.95 ± 0.32 a	4.70 ± 0.38 b
Free-laricitrin	2.34 ± 0.06 b	2.08 ± 0.11 a	4.70 ± 0.29 a	5.67 ± 0.51 b
Free-isorhamnetin+syringetin	0.54 ± 0.05	0.48 ± 0.08	0.38 ± 0.03	0.47 ± 0.10
Total flavonols	***402.34±29.87***	***361.02±38.48***	***178.57±6.30a***	***277.97±53.04b***
Flavanols				
Catechin	16.62 ± 1.12	20.49 ± 3.04	8.18 ± 1.57	8.90 ± 1.07
Epicatechin	19.02 ± 1.22 a	23.16 ± 0.58 b	10.07 ± 1.46	11.76 ± 1.44
Epicatechin-3-gallate	17.24 ± 1.84	18.78 ± 3.05	n.d.	n.d.
Epigallocatechin	1.50 ± 0.23	1.98 ± 0.32	6.14 ± 0.93 a	10.44 ± 1.82 b
Procyanidin B1	7.47 ± 0.96 a	12.23 ± 1.36 b	2.64 ± 0.42 a	5.03 ± 1.00 b
Procyanidin B2	16.34 ± 1.50 a	24.58 ± 3.75 b	n.d.	n.d.
Total flavanols	*81.99 ± 2.40*	*96.98 ± 9.19*	*26.13 ± 4.77*	*36.91 ± 6.16*
Hydroxybenzoic acid				
Gallic acid	29.84 ± 4.11	29.69 ± 5.74	14.46 ± 1.04	18.10 ± 3.73
Hydroxycinnamic acids (HCAs)				
trans-Caftaric acid	4.42 ± 0.53 a	7.48 ± 0.46 b	9.19 ± 1.00	9.88 ± 1.52
trans+*cis*-Coutaric acids	2.65 ± 0.29 a	4.70 ± 0.22 b	7.07 ± 0.71	7.04 ± 1.36
trans-Fertaric acid	1.12 ± 0.10	1.07 ± 0.09	1.48 ± 0.04 a	2.06 ± 0.21 b
Caffeic acid	30.43 ± 0.71	30.02 ± 0.61	12.11 ± 2.28	15.42 ± 3.01
p-Coumaric acid	10.52 ± 0.98	12.38 ± 2.07	7.30 ± 1.46 a	11.45 ± 2.08 b
Ferulic acid	2.31 ± 0.29 b	1.71 ± 0.16 a	2.08 ± 0.37 a	3.14 ± 0.40 b
Total HCAs	***52.19 ± 3.53***	***54.01 ± 7.90***	***39.24 ± 2.48 a***	***50.09 ± 6.85 b***
Stilbenes				
trans-Piceid	3.55 ± 0.22	3.83 ± 0.20	0.87 ± 0.08 a	2.10 ± 0.41 b
cis-Piceid	0.24 ± 0.04 a	0.41 ± 0.03 b	0.95 ± 0.13 a	1.35 ± 0.16 b
trans-Resveratrol	0.58 ± 0.02 b	0.47 ± 0.02 a	1.87 ± 0.07 a	3.16 ± 0.38 b
cis-Resveratrol	0.63 ± 0.10 a	0.83 ± 0.02 b	0.50 ± 0.04 a	0.77 ± 0.16 b
Total stilbenes	***5.15 ± 0.43***	***5.67 ± 0.37***	***4.28 ± 0.37 a***	***7.65 ± 1.43 b***

Nomenclature abbreviations: glcU, glucuronide; gal, galactoside; glc, glucoside.

All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$), the absence of letters means no significant differences ($p > 0.05$). n.d.: not detected.

The gallic acid content did not show differences among MeJ+Ur and control wines in none of the studied vintages (Table 4).

With regard to hydroxycinnamic acids, in 2019, the wines elaborated from MeJ+Ur grapes showed an increase in *trans*-caftaric acid, and *trans*+*cis*-coutaric acid and a lower content of ferulic acid than control ones (Table 4). In 2020, the foliar application of MeJ+Ur produced wines with a higher content of *trans*-fertaric acid, *p*-coumaric acid and ferulic acid. It is noteworthy that in this season, the total HCAs content was significantly higher in MeJ+Ur wines than in control wines (Table 4). Previous studies described that the individual foliar application of urea did not affect the total HCAs content in wines (Portu et al., 2015a), and neither the MeJ foliar application affected the HCAs content in wines (Portu et al., 2015c, 2016).

Finally, the individual stilbenes also have been affected by the foliar treatment studied (Table 4). In 2019, MeJ+Ur wines presented a higher content of *cis*-piced and *cis*-resveratrol and a lower content of *trans*-resveratrol in comparison with the control wines. Differences on total stilbenes content were not described (Table 4). In 2020, all individual stilbenes increased their content in wines elaborated with MeJ+Ur grapes, and therefore, the total stilbenes concentration was higher in MeJ+Ur wines than in control ones. Portu et al. (2016) did not describe an increase on total stilbene content in MeJ wines in comparison with control wines, whereas in other study, Portu et al. (2015c) showed an increase on total stilbene content in MeJ wines. Furthermore, urea foliar application neither affected the total stilbenes content in wines respect to the control ones (Portu et al., 2015a).

Multifactor Analysis of Variance of Phenolic Compounds

Table 5 shows the results from the multifactor analysis of variance of grape phenolic compounds. Foliar application of MeJ+Ur significantly affected the grapes anthocyanin content. All individual and total non-acylated anthocyanins content was improved by MeJ+Ur treatment. MeJ+Ur also affected the content of peonidin-3-acglc, delphinidin-3-cmglc, cyanidin-3-cmglc, petunidin-3-cmglc and peonidin-3-cmglc content in grapes in comparison with control ones (Table 5). Regarding to the season effect, most anthocyanin presented a higher content in 2019 than in 2020 season, except for malvidin-3-*cis*-cmglc, malvidin-3-*trans*-cmglc and malvidin-3-cfglc. This can be explained by a dilution effect produced by the differences on the meteorological conditions among the two seasons studied, as has been above mentioned. In addition, the different effect of foliar treatment observed between seasons can be explained by the fact that there was a difference on the days passed among the date of the first application of treatments and the date of harvest, which was shorter in 2019 than in 2020 vintage. The longer time passed in 2020 could affect to the effectiveness of foliar treatment.

Treatment and season interaction was significant for all anthocyanins and total anthocyanins content, except for delphinidin-3-acglc, cyanidin-3-acglc, petunidin-3-acglc, malvidin-3-acglc and total acylated anthocyanins (Table 5). The effect of MeJ+Ur foliar treatment on grape flavonols content was low. MeJ+Ur foliar application only affected quercetin-3-glcU and kaempferol-3-gal, decreasing their content on grapes in comparison with control grapes (Table 5). The effect of season on this family of phenolic compounds was very similar to those observed for anthocyanins. In 2019, the flavonols grape content was higher than in 2020, except for laricitrin-3-glc. The effect of season can be explained by the same reasons explained above. Treatment and season interaction was only significant in quercetin-3-glcU, kaempferol-3-gal, isorhamnetin-3-glc and total flavonols (Table 5).

Regarding flavanols, MeJ+Ur treatment only affected the epicatechin-3-gallate content in grapes (Table 5), compound which undergone a decrease regarding control grapes. For this family of phenolic compounds, season also affected all individual and total flavanols content in grapes, except for epigallocatechin. Once again, in 2019, the flavanols content was higher than 2020 season (Table 5). Treatment and season interaction was significant for all individual flavanols and total flavanols content, except for procyanidin B1. Flavonols and flavanols share some part of the biosynthesis pathway with anthocyanins. MeJ+Ur foliar application seems to induce the anthocyanins biosynthesis. However, this effect for flavonols and flavanols was not observed. This result support the idea above-mentioned about MeJ+Ur treatment could induce the activation of enzymes related with anthocyanins synthesis instead of those enzymes related to flavonols and flavanols synthesis.

Treatment, season and their interaction did not affect the gallic acid content of grapes (Table 5).

MeJ+Ur foliar treatment only affected the caffeic acid content, decreasing it. Season affected all individual HCAs content (Table 5). Once again, their content was higher in 2019 season than in 2020, except for ferulic acid, which content was higher in 2020. Treatment and season interaction only affected *trans*+*cis*-coutaric acids and caffeic acid content in grapes (Table 5).

Finally, treatment affected the stilbenes grape content. MeJ+Ur foliar application increased the *cis*-piceid and *cis*-resveratrol content, whereas decreased the *trans*-resveratrol content in grapes (Table 5). Season affected all individual and total stilbenes content, except for *cis*-piceid. In 2019, their content was higher than in 2020 season. Treatment and season interaction affected *cis*-piceid, *trans*-resveratrol and *cis*-resveratrol content in grapes (Table 5).

Table 5. Multifactor analysis of variance of grape phenolic compounds (expressed as mg/kg) referred to fresh weight

	Treatment (T)		**Season (S)**		**Interaction (T x S)**
	Control	**MeJ+Ur**	**2019**	**2020**	
Anthocyanins					
Delphinidin-3-glc	87.18 a	114.03 b	147.88 b	53.33 a	**
Cyanidin-3-glc	17.14 a	28.22 b	35.74 b	9.62 a	***
Petunidin-3-glc	66.89 a	85.20 b	101.81 b	50.27 a	**
Peonidin-3-glc	32.75 a	50.56 b	61.12 b	22.19 a	***
Malvidin-3-glc	192.80 a	217.63 b	241.84 b	168.58 a	*
Total non-acylated	396.75 a	495.64 b	588.39 b	304.00 a	**
Delphinidin-3-acglc	8.54	9.04	10.93 b	6.65 a	N.S
Cyanidin-3-acglc	3.72	3.69	3.82 b	3.59 a	N.S.
Petunidin-3-acglc	6.21	6.49	7.13 b	5.57 a	N.S:
Peonidin-3-acglc	4.16 a	4.59 b	4.78 b	4.00 a	***
Malvidin-3-acglc	11.12	11.13	12.08 b	10.17 a	N.S.
Delphinidin-3-cmglc	15.29 a	18.06 b	19.29 b	14.07 a	***
Cyanidin-3-cmglc	5.79 a	7.24 b	7.40 b	5.63 a	***
Petunidin-3-cmglc	12.72 a	14.06 b	14.85 b	11.93 a	***
Peonidin-3-cmglc	7.85 a	9.35 b	9.82 b	7.38 a	***
Malvidin-3-*cis*-cmglc	4.61	4.48	4.60	4.49	*
Malvidin-3-*trans*-cmglc	43.89	41.67	40.39 a	45.16 b	***
Malvidin-3-cfglc	7.55	7.52	4.22 a	10.85 b	N.S.
Total acylated	131.46	137.31	139.31 b	129.45 a	***
Total anthocyanins	***528.21 a***	***632.943 b***	***727.71 b***	***433.45 a***	*****
Flavonols					
Myricetin-3-glcU	21.70	19.27	25.26 b	15.71 a	N.S.
Myricetin-3-gal	28.62	29.62	36.43 b	21.81 a	N.S.
Myricetin-3-glc	131.97	131.92	182.55 b	81.33 a	N.S.
Quercetin-3-glcU	94.39 b	71.67 a	137.51 b	28.55 a	***
Quercetin-3-glc	102.55	90.73	158.18 b	35.11 a	N.S.
Laricitrin-3-glc	31.80	29.98	32.02	29.75	N.S.
Kaempferol-3-gal	1.47 b	1.12 a	2.12 b	0.47 a	**
Kaempferol-3-glcU+3-glc	9.08	9.19	15.35 b	2.91 a	N.S.
Isorhamnetin-3-glc	7.85	7.70	11.30 b	4.25 a	*
Syringetin-3-glc	16.95	18.09	21.51 b	13.53 a	N.S.
Total flavonols	***446.38***	***409.29***	***622.24 b***	***233.43 a***	***
Flavanols					
Catechin	37.19	32.99	57.72 b	12.46 a	*
Epicatechin	25.10	27.26	36.93 b	15.43 a	*
Epicatechin-3-gallate	11.18 b	9.27 a	12.23 b	8.22 a	*
Epigallocatechin	6.34	6.09	3.53 a	8.89 b	**
Procyanidin B1	19.27	21.40	30.13 b	10.54 a	N.S.
Total flavanols	***99.07***	***97.01***	***140.54 b***	***55.54 a***	***

	Treatment (T)		**Season (S)**		**Interaction (T x S)**
	Control	**MeJ+Ur**	**2019**	**2020**	
Hydroxybenzoic acid					
Gallic acid	5.60	5.97	5.92	5.66	N.S.
Hydroxycinnamic acids (HCAs)					
trans-Caftaric acid	4.02	4.47	6.86 b	1.63 a	N.S.
trans+*cis*-Coutaric acids	2.39	2.12	3.96 b	0.55 a	***
trans-Fertaric acid	1.56	1.30	1.70 b	1.16 a	N.S.
Caffeic acid	0.35 b	0.28 a	0.34 b	0.28 a	***
p-Coumaric acid	0.25	0.21	0.31 b	0.14 a	N.S.
Ferulic acid	6.42	5.73	2.07 a	10.07 b	N.S.
Total HCAs	***14.99***	***14.10***	***15.24***	***13.85***	**N.S**
Stilbenes					
trans-Piceid	9.06	9.23	12.77 b	5.54 a	N.S.
cis-Piceid	1.42 a	1.98 b	1.67	1.72	***
trans-Resveratrol	0.37 b	0.19 a	0.48 b	0.08 a	***
cis-Resveratrol	0.28 a	0.37 b	0.35 b	0.29 a	***
Total stilbenes	***11.12***	***11.77***	***15.26 b***	***7.63 a***	**N.S.**

Nomenclature abbreviations: glc, glucoside; acglc, acetylglucoside; cmglc, *trans*-*p*-coumaroylglucoside; cfglc, caffeoylglucoside; glcU, glucuronide; gal, galactoside. For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$), the absence of letters means no significant differences ($p > 0.05$). Interaction: *, $p \leq 0.05$, **, $p \leq 0.01$, ***, $p \leq 0.001$, and N.S., not significant ($p > 0.05$).

Table 6. Multifactor analysis of variance of wine phenolic compounds

	Treatment		**Season**		**Interaction (T x S)**
	Control	**MeJ+Ur**	**2019**	**2020**	
Anthocyanins					
Delphinidin-3-glc	10.58	12.94	15.18 b	8.34 a	N.S.
Cyanidin-3-glc	1.89 a	2.21 b	2.37 b	1.72 a	N.S.
Petunidin-3-glc	17.14	20.06	21.58 b	15.63 a	N.S.
Peonidin-3-glc	4.60 a	6.51 b	7.54 b	3.58 a	N.S.
Malvidin-3-glc	86.26	94.31	94.43	86.14	N.S.
Total non-acylated	**120.47 a**	**136.03 b**	**141.10 b**	**115.41 a**	N.S.
Delphinidin-3-acglc	2.45	2.65	2.59	2.52	N.S
Cyanidin-3-acglc	1.36 a	1.37 b	1.36 a	1.37 b	N.S.
Petunidin-3-acglc	2.60	2.71	2.63	2.68	N.S:
Peonidin-3-acglc	1.93 a	2.15 b	2.27 b	1.82 a	N.S.
Malvidin-3-acglc	6.33	6.22	5.94 a	6.61 b	N.S.
Delphinidin-3-cmglc	3.78	4.31	3.93	4.17	N.S.
Cyanidin-3-cmglc	1.79 a	2.07 b	1.95	1.91	N.S.
Petunidin-3-cmglc	2.88	3.15	3.01	3.03	N.S.
Peonidin-3-cmglc	2.32 a	2.74 b	2.59	2.47	N.S.
Malvidin-3-*cis*-cmglc	1.76 b	1.69 a	1.71	1.75	*
Malvidin-3-*trans*-cmglc	9.58	10.31	9.42	10.47	N.S.
Malvidin-3-cfglc	1.79	1.76	1.93 b	1.61 a	N.S.
Total acylated	**38.59**	**41.14**	**39.32**	**40.40**	N.S.
Total anthocyanins	***159.06***	***177.17***	***180.42 b***	***155.82 a***	N.S.
Vitisin A	1.76 b	1.61 a	1.84 b	1.53 a	**
Vitisin B	1.88 a	2.09 b	2.08 b	1.88 a	N.S.

Table 6. (Continued)

	Treatment		Season		Interaction (T x S)
	Control	**MeJ+Ur**	**2019**	**2020**	
Flavonols					
Myricetin-3-glcU	9.40	10.12	12.23 b	7.92 a	N.S.
Myricetin-3-gal	11.85 a	13.57 b	14.69 b	10.73 a	***
Myricetin-3-glc	71.25 a	92.18 b	114.87 b	48.56 a	*
Myricetin free	15.59 a	19.50 b	12.97 a	22.11 b	N.S.
Quercetin-3-glcU	48.38 b	34.10 a	69.36 b	13.12 a	**
Quercetin-3-glc	76.37	79.58	85.12 b	70.82 a	**
Quercetin free	16.60	15.40	15.21	16.79	***
Laricitrin-3-glc	14.14 a	16.10 b	17.26 b	12.98 a	*
Laricitin free	3.52	3.88	2.21 a	5.18 b	**
Kaempferol-3-gal	0.87 b	0.65 a	1.35 b	0.17 a	*
Kaempferol-3-glcU+3-glc	3.97	3.04	6.16 b	0.85 a	*
Kaempferol free	7.20 b	6.13 a	8.23 b	4.32 a	***
Isorhamnetin-3-glc	0.98 a	1.18 b	1.74 b	0.42 a	N.S.
Syringetin-3-glc	10.09	12.19	11.36	10.92	N.S.
Isorhamnetin + Syringetin	0.46	0.47	0.51	0.42	N.S.
Total flavonols	***290.45***	***319.50***	***381.68 b***	***228.27 a***	**
Total flavanols					
Catechin	12.40	14.70	18.55 b	8.54 a	N.S.
Epicatechin	14.55 a	17.46 b	21.09 b	10.92 a	N.S.
Epicatechin-3-gallate	17.24	18.78	18.01		
Epigallocatechin	3.82 a	6.21 b	1.74 a	8.29 b	*
Procyanidin B1	5.06 a	8.63 b	9.85 b	3.84 a	N.S..
Procyanidin B2	16.34 a	24.58 b	20.46		
Total flavanols	***54.06 a***	***66.95 b***	***89.49 b***	***31.52 a***	N.S.
Hydroxybenzoic acid					
Gallic acid	22.15	23.89	29.76 b	16.28 a	N.S.
Hydroxycinnamic acids (HCAs)					
trans-Caftaric acid	6.81 a	8.68 b	5.95 a	9.53 b	N.S.
trans+cis-Coutaric acids	4.86	5.88	3.67 a	7.06 b	N.S.
trans-Fertaric acid	1.30 a	1.56 b	1.10 a	1.77 b	**
Caffeic acid	21.27	22.72	30.23 b	13.76 a	N.S.
p-Coumaric acid	8.91 a	11.91 b	11.45	9.38	N.S.
Ferulic acid	2.20	2.42	2.01 a	2.61 b	**
Total HCAs	***45.71***	***52.05***	***53.10 b***	***44.66 a***	N.S.
Stilbenes					
trans-Piceid	2.21 a	2.97 b	3.69 b	1.49 a	*
cis-Piceid	0.60 a	0.88 b	0.33 a	1.15 b	N.S.
trans-Resveratrol	1.23 a	1.81 b	0.52 a	2.52 b	***
cis-Resveratrol	0.57 a	0.80 b	0.73	0.64	N.S.
Total stilbenes	***4.72 a***	***6.66 b***	***5.41***	***5.96***	*

Nomenclature abbreviations: glc, glucoside; acglc, acetylglucoside; cmglc, *trans-p*-coumaroylglucoside; cfglc, caffeoylglucoside; glcU, glucuronide; gal, galactoside. For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$), the absence of letters means no significant differences ($p > 0.05$). Interaction: *, $p \leq 0.05$, **, $p \leq 0.01$, ***, $p \leq 0.001$, and N.S., not significant ($p > 0.05$).

Table 6 shows the results from the multifactor analysis of variance of wine phenolic compounds.

The effects observed in grapes were slightly different in wines. MeJ+Ur treatment significantly increased the wine content of cyanidin-3-glc, peonidin-3-glc, total non-acylated anthocyanins, cyanidin-3-acglc, peonidin-3-acglc, cyanidin-3-cmglc, peonidin-3-cmglc and vitisin B, whereas decreased the malvidin-3-*cis*-cmglc and vitisin A content in comparison with control wines. The effect of season factor also was minor in wines than in grapes, being higher the content of all individual and total non-acylated anthocyanins, except for malvidin-3-glc, in 2019 than in 2020 season (Table 6). In 2019, also was higher the content of peonidin-3-acglc, malvidin-3-cfglc and vitisins A and B than in 2020, while in 2020, cyanidin-3-acglc, malvidin-3-acglc and total anthocyanins content was higher than in 2019. Treatment and season interaction only was significant for malvidin-3-*cis*-cmglc and vitisin A wines content (Table 6).

Regarding flavonols, MeJ+Ur treatment increased myricetin-3-gal, myricetin-3-glc, myricetin free, laricitrin-3-glc and isorhamnetin-3-glc content whereas decreased quercetin-3-glcU, kaempferol-3-gal and kaempferol free content in wines in comparison to control wines (Table 6). Season affected most of flavonols (except for quercetin free, syringetin-3-glc and isorhamnetin+syringetin). All individual and total flavonols presented a higher content in wines from 2019 season than in 2020. Except for myricetin free and laricitrin free, which showed a higher content in wines from 2020 vintage (Table 6). Treatment and season interaction was significant for myricetin-3-gal, myricetin-3-glc, quercetin-3-glcU, quercetin-3-glc, quercetin free, laricitrin-3-glc, laricitrin free, kaempferol-3-gal, kaempferol-3-glcU+-3-glc, kaempferol free and total flavonols wine content.

Treatment affected most of flavanols. MeJ+Ur wines presented a higher content of all individuals and total flavanols content than in control wines, except for catechin and epigallocatechin (Table 6). Season affected all individual and total flavonols content, being higher in 2019 than in 2020 season except for epigallocatechin, which showed a higher content in 2020. Treatment and season interaction was only significant for epigallocatechin content.

The gallic acid content in wines was only affected by season factor and was higher in 2019 (Table 6).

MeJ+Ur treatment affected the *trans*-caftaric acid, *trans*-fertaric acid and *p*-coumaric acid, increasing their concentration in wines (Table 6). Season affected the HCAs content in wines, but this factor showed an opposite effect.

For this family of phenolic compounds, the content was higher in wines from 2020 season, except for caffeic acid concentration, that was higher in 2019 and for *p*-coumaric acid content, that did not show differences among seasons (Table 6). Treatment and season interaction was only significant for *trans*-fertaric acid and ferulic acid content.

Treatment affected all individuals and total stilbenes content so, MeJ+Ur foliar application could induce the synthesis of stilbenes. Fact previously observed for the MeJ foliar application (Portu et al., 2015c, 2018). Season affected the stilbenes content in wines in different ways. In 2019 season, a higher content of *trans*-piceid was observed, whereas the content of *cis*-piceid and *trans*-resveratrol was higher in 2020. Treatment and season interaction was significant for the content of *trans*-piceid, *trans*-resveratrol and total stilbenes in wines.

Conclusion

This work studies the effect of MeJ+Ur foliar application to vineyard on grape and wine phenolic composition over two consecutive vintages. MeJ+Ur foliar treatment seems to induce the synthesis of anthocyanins in grapes. However, the effect of MeJ+Ur foliar treatment was season dependent, probably due to different rainfalls recorded among seasons, and the different quantity of days that taken place between the data of the first foliar application of MeJ+Ur, and the harvest day, which was quite different between vintages.

With respect to the other phenolic compounds, foliar treatment did not improve their content in grapes, except for total flavonols in the second vintage.

Even if in grapes an improvement on the biosynthesis of anthocyanins has been observed, in wines, probably due to the reinforcement that skin cell walls undergone after elicitor foliar application, the extraction of anthocyanins during the fermentation process was hindered. And for this reason, the effect of foliar treatment observed in grapes was quite different from those described in wines. It should be noted that in none of the vintages studied MeJ+Ur foliar application showed a negative effect on the phenolic composition of both grapes and wines.

MeJ+Ur foliar application seems to be a good tool in order to enhance the anthocyanins content in grapes, but further studies should be performed to understand the seasonal dependence of the effect of this treatment on grape phenolic composition. In addition, it must be study how achieve transfer better the phenolic compounds of grapes to the final wines. To achieve this purpose maybe a longer maceration process should be carried out.

Disclaimer

None

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Chapter 5

Soil Organic Carbon in the Humid Tropics: Properties, Retention and Management

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Abstract

Soil organic carbon (SOC) is a minor but critical component of most soils of the world, involved in practically all ecological services provided by soils, such as nutrient cycling and storage, biological diversity, water retention through favorable structure, and many others. In addition, SOC is one of the most important global carbon pools, containing more C than the atmosphere and biomass combined, and with a longer residence time. Thus, SOC has been proposed as a promising alternative for removal of atmospheric CO_2, by means of plant biomass production and its natural incorporation within the soil mineral matrix. Such a strategy is very opportune because SOC sequestration always improves soil quality for agricultural and environmental functioning and would also achieve middle-term C sequestration with a potentially significant mitigation of global warming. In such context, identifying promising environments and situations for SOC sequestration throughout the world becomes important, and the humid tropics appear amongst the most feasible alternatives, offering favorable year-round temperatures, immense areas with adequate water availability, relatively low costs for agricultural and preservation/conservation land, huge biodiversity of both cultivated and spontaneous plants, and a typically abundant workforce. Notwithstanding, reaching such potential would require adequate scientific knowledge on factors and processes involved in SOC

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sequestration, which would ideally be specific for each of the various tropical environments and soils, and more critically, are often different from those in temperate regions. This chapter is not intended to be an exhaustive literature review, but rather to present novel and common-sense perspectives on the most important factors and practices affecting SOC retention and dynamics in the tropics, aiming to improve current and future management, policies and research initiatives.

Keywords: soil organic matter, oxisols, ultisols, soil management

1. Introduction to Tropical Environments and Soils

The initial step needed in this essay is a working definition of the tropics and tropical environments. Most people, layman or academic, often think of the tropics as those areas of the globe where temperatures are always warm, or at least where winters are mild. In fact, although these expectations are met for a large area of the tropics, actually there are several definitions for the tropical zone, and for this reason, the initial parts of now-classic texts on tropical soils or landscapes (Sanchez, 1976; Van Wambeke, 1992; Thomas, 1994) deal precisely with those explanations. To our purposes, the tropics can be defined as:

1) all lands and water masses located between the latitudes of ca. 23.4° N and S, i.e., the tropics of Cancer and Capricorn, respectively (Figure 1). These are the only locations where the sun can be at a 90° angle upon a flat land surface, and where sunshine hours vary little around 12 h, approximately between 10 h 30 min. and 13 h 30 min.;

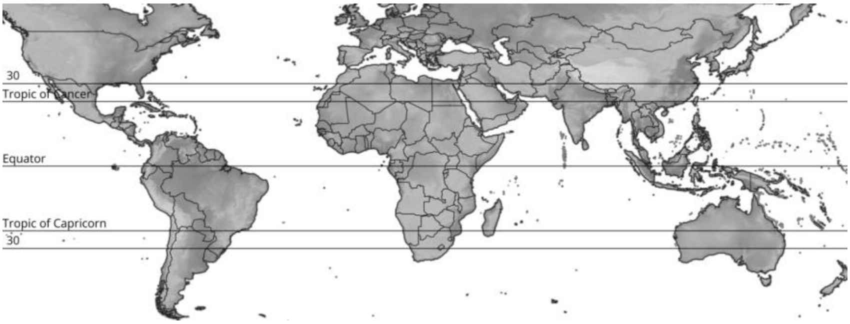

Figure 1. The tropics of Cancer and Capricorn, and the 30°paralells, arbitrarily chosen as the extent of the subtropics. Lighter shades of gray represent higher altitudes. Map prepared by Welton Rocha Jr., Univ. Fed. Lavras.

2) land surfaces where the differences in mean temperatures throughout the day are typically greater than differences between mean temperatures of the coolest and warmest months. As van Wambeke (1992) stated, in the tropics there are no seasons defined by marked changes in air temperature, and especially in soil temperature;
3) land surfaces where most soils are marked by an *iso* qualifier for soil temperature regimes, as defined in Soil Taxonomy (Soil Survey Staff, 2014). This implies that winter and summer mean soil temperatures differ by less than 6°C to a depth of 50 cm. Van Wambeke (1992) stressed that, in fact, soil climate is the *only* attribute shared by all tropical soils, and what differentiates them from temperate soils.

It can perhaps be summarized from the previous definitions that the tropics are the areas marked by more or less constant temperatures throughout the year. A major part of the tropical land surface is indeed warm year-round, but this is true only for the lowlands, where air temperatures are mostly >20°C and soil temperatures even warmer, often reaching 28°C in subsoils, where most weathering of minerals and rocks occurs (Thomas, 1994). However, site altitude plays a major role in defining air and soil temperatures, and some mountain areas are actually cool or cold every month, most importantly in the Andean cordillera and plateau. The late Frank D. Calhoun used to say in his Tropical Soils class at The Ohio State University that “in the tropics, where it is warm, it is warm the whole year, every year; but where it is cold, it is cold the whole year, every year”, stressing the remarkable temperature stability of these areas.

In this essay, we shall include in our working definition of tropics those significant highlands of moderate altitudes (e.g., 900-1,400 m a.s.l., Sanchez, 1976) with latitudes ca. 20°, which present more pronounced differences between the warmest and coolest months, that technically can be categorized as “warm temperate” in the Köppen climatic classification. In these areas, temperatures are never cold enough to restrict plant growth, except on some rocky mountain tops. Finally, significant land masses are located in latitudes >23.4°, in parts even near 30° (Figure 1), and where altitudes are low (e.g., <200 m a.s.l.), they are marked by relatively high temperatures in most months, such as in southern Brazil. These areas are often termed “subtropical”, which is a helpful concept implying that there are many climatic, vegetational and soil similarities with tropical areas *per se*. In the subtropics, mean temperatures are typically ca. 18°C and only briefly fall below 15°C (Thomas, 1994), which is still very favorable to mineral weathering, and thus can be more fairly treated aside with the tropical zone rather than with the temperate areas. However, our broad working definition does not include soil moisture or precipitation, which are better explained by their effect on vegetation, as discussed next.

1.1. Climatic-Vegetation Provinces in the Tropics

Another frequent simplification, even among technical workers and scientists of tropical countries, is that tropical areas are mostly humid, i.e., that precipitations are typically high and widely exceed evaporation, resulting in positive water balances. Although again this expectation is often met, the tropics actually include zones varying widely from arid to perhumid, depending on a series of geographical settings, just as temperate areas.

The most obvious climate-vegetation province meeting the expectations above comprises the rainforests around the Equator, most notably the Amazon in South America and the Congo basin in Africa, insular or continental southern Asia and northern Oceania, and the smaller extensions in Central America and the Caribbean, often termed as “jungle” in older, common language. Such areas are marked by year-round warm or hot temperatures, short (<3 months) or inexistent dry seasons, and high precipitation (>1,200 mm yr^{-1}) and humidity, to the point that plant growth in some mountains and other places is limited by constant cloudiness (van Wambeke, 1992). Some areas marked by monsoonal rains are even wetter (>1,700 mm yr^{-1}), especially in the Asian coastlines, where tropical cyclones are common (Thomas, 1994). In addition, many rainforest areas occur along coastlines where warm currents favor precipitation, including the Atlantic Rainforests in eastern Brazil and eastern Madagascar. Especially along the Equator, the lack of a marked dry season has historically acted as a hindrance to extensive deforestation and land reclamation, since establishment of annual crops was made difficult by excessive moisture during harvest season. Thus, such areas have been instead converted to extensive grazing, even if only temporarily, and where short dry seasons occurs, forests have been replaced by croplands or pastures, as historically in southeastern Brazil, and more recently in the southeast Amazon basin.

Tropical savannas are also very extensive provinces, marked by a dry season that typically is 3-5 months long, which precludes full tree coverage on the area, resulting in a variable combination of grasses, shrubs and trees, interspersed by riparian forests and, in the ecotonal transitions to the rainforest biomes discussed above isolated forests in mosaics. Natural wild fires are quite common in the dry season (Bloom, 1998). In South America, these provinces comprise the Brazilian *Cerrados* and the *Llanos* of Venezuela, respectively south and north of the Amazon rainforests (Zinn & Lal, 2013), occupying plateaus of low to moderate altitudes (mostly 200-1,500 m). In Africa, savannas also occupy immense and botanically very diverse areas to the North, East and South of the Congo rainforest, which varying widely in climate and vegetation with the Zambezian woodlands and *miombos*, among others, and are occupied by the most part of the population of that continent (Geldenhuys & Golding, 2008). Approximately 25% of Australia is occupied by tropical and subtropical northern savannas, historically occupied by extensive livestock grazing and low populations (Gordon, 2008). Other much smaller tropical savannic formations occur in western Central America. Unlike the Equatorial rainforests, the precipitation patterns of savanna provinces favor at least one annual rainfed crop per year, and where irrigation is used, even three crops a year. Precipitation is often considerable, exceeding 1,000 mm in large parts of the *Cerrados*, and a gently rolling topography favors mechanization, and thus these areas can be highly productive for a broad range of crops, where liming and fertilization are properly done (see 2.2).

Considerable areas in the tropics are marked by semi-arid to arid climates, where dry seasons can extend for more than five or six months, and strong insolation results in wide water deficits, with climax native vegetations that offer limited biomass accretion and soil coverage, surviving with little or no foliage for many months. In these conditions, annual crops are very risky or nearly impossible without irrigation, but surface- and groundwaters are not always of proper quality, carrying dissolved salts that preclude their use. Important areas with these features occur in the Brazilian Northeast *Caatinga*, and in the African *Kalahari-Highveld* and *Sahel* regions (Geldenhuys & Golding, 2008). Where ground or surface waters of adequate quality are not available for irrigation, crop productivity is generally low, and in many or most years there is actually the risk of harvesting less than what was sown. This is the case in many areas of the Brazilian *Caatinga*, where population density was historically much higher than in the *Cerrados*, and frequent, multi-year droughts have created massive migration to moister areas to the south and large cities.

Much less significant but still considerably large climate-vegetational provinces are defined by specific geographical features, including, in South America, the Andean and Guianan plateaus, the former marked by cold and/or arid conditions, and the warm *Pantanal* and *Chaco* wetlands, which will not be treated here.

1.2. Tropical Landscapes and Soils

The main geomorphological processes – i.e., the creation and erosion of land masses and surfaces – that occur in the tropics are broadly similar to those operating in non-glaciated temperate zones. The buildup of major mountain ranges comes from tectonic forces which obviously are not affected by climate, although land denudation through erosion (and thus the ensuing terrain isostatic uplift) are proportional to precipitation and temperature (Thomas, 1994; Bloom, 1998). In this sense, a most important feature of the tropics is that rock weathering and sediment transport are relatively intense, due to the steadily warm temperatures and intense summer precipitations. In his authoritative review of tropical geomorphology, Thomas (1994) stresses that the high rainfall intensity is perhaps the most distinctive feature that tropical climates impart to land surfaces, since daily precipitations reaching or exceeding 400 mm, sometimes 1,500 mm in 3-6 days, are not unusual in the monsoonal tropics.

In consequence of accelerated physical and chemical weathering, in many of areas of the humid tropics, crystalline and other bare rock outcrops present a rounded form resulting from spheroid exfoliation, as exemplified by the world-famous *Pão-de-Açúcar* (sugarloaf) and *Corcovado* dome-like mountains in coastal Rio de Janeiro, Brazil. Although these *bornhardt* hills, often used as a poster images for academic texts on weathering, are typical of the humid rainforest provinces, they are also widespread in the savanna and other drier areas (Bloom, 1998). In addition, very steep slopes under rainforests are common where orogenic rains occur in mountain ranges along coastlines, and despite the thick soil coverage, are subject to landslides and other mass wasting processes during the summer. However, this is not the case for the main rainforest provinces, such as the Amazon and Congo basins, which occupy mostly lowlands and are marked by low reliefs, and thus catastrophic mass wasting events are much less frequent. In addition, most of the savanna and dryland biomes, despite also subject to intense rainfalls, tend to present gently rolling to nearly flat topographies, dissected along extensive, long-term denudation or erosion surfaces (King, 1956; Silva, 2009). Another major difference between temperate and tropical zones is that land surface lowering by long-term denudation is thought to occur actively on both the soil surface *and* the weathering front, which can occur tens- to hundreds of meters below the surface, as reviewed by Thomas (1994) and Bloom (1998).

Features due to intense precipitations are widely reflected on regional hydrography. Most importantly in terms of areal extension, large drainage basins such as those of the Amazon and Congo (Figure 1) are comparable in area to the Mississipi and other major basins in temperate areas, although with relatively lower reliefs, and typically much higher discharges. Other major tropical river basins, such as La Plata and São Francisco in South America, and Zambeze in Africa, also present very large discharges, often greater than those of main rivers in temperate zones, and typically involve higher reliefs than those in the Amazon and Congo, currently dissecting crystalline shields or more recent (but still very old) mountain ranges. We refer the reader to Thomas (1994) for the most comprehensive work on tropical geomorphology.

Tropical soils are as variable as the climate-vegetational provinces and landscapes summarized above. However, a general rule of thumb is that, in lowland forest and savanna biomes, most soils developed under high temperatures and precipitations during long periods and on old land surfaces (e.g., Silva, 2009), which has resulted in advanced to extreme weathering stages. Although weathering processes are outside our scope, it is important to recall the Van't Hoff or Q10 rule (van Wambeke, 1992), i.e., that chemical reactions rates typically double or triple for an increment in temperature of 10°C, which is the basic reason why weathering in the humid tropics is faster than in the temperate zone, where mean annual temperatures are typically 10ºC lower. Where weatherable minerals abound in the soil parent material (as well as on clayey rocks and sediments), clay contents are typically high (Thomas, 1994), mostly >40% and often >60%. Thus, soils on lowland forests and savannas, and many others on humid areas with altitudes up to 1,300 m, are very deep, with B horizons often extending beyond 2 m, and in many Oxisols, going to 5-10 m (e.g., Zinn & Miranda, 2021, Figure 2a) or more. Furthermore, such soils are also highly-weathered in chemical and mineral composition, with clay mineral suites marked by low activity clays and oxides of Fe and Al. However, in the cooler and drier tropics, and on steep slopes along mountain ranges, soils and saproliths are much shallower and less developed. In addition, most if not all tropical humid soils are subject to intense bioturbation by very active and numerous insects typical of the tropics, i.e., termites and leaf-cutting ants, which feed on fresh or dead plant residues and thus accelerate total organic matter decomposition (van Wambeke, 1992).

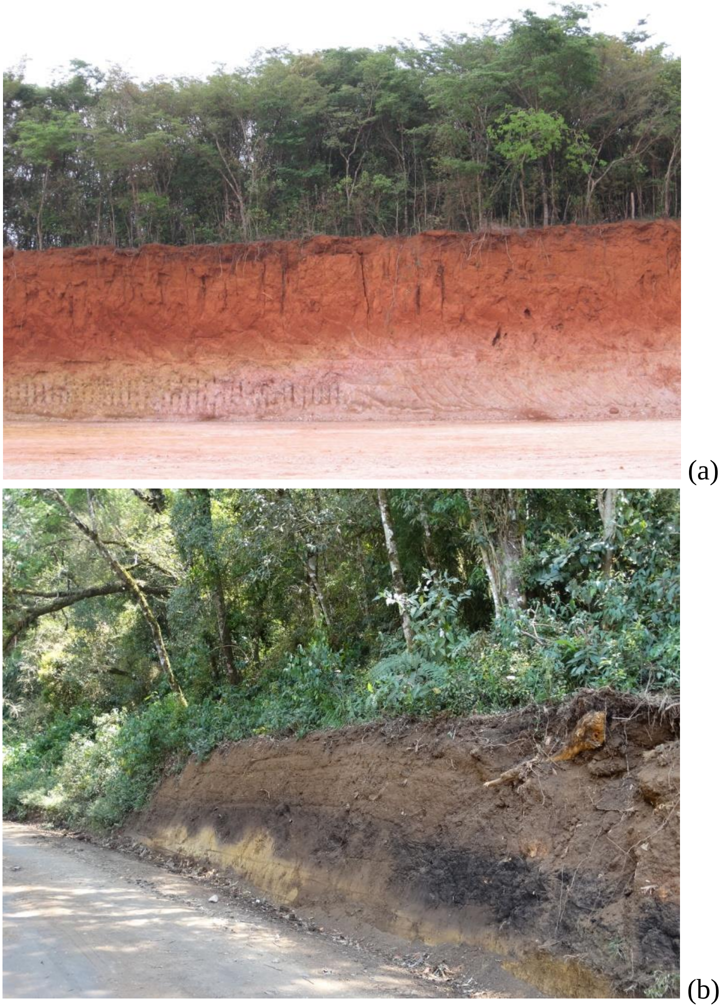

Figure 2. Tropical humid soils with high SOC stocks, Minas Gerais, Brazil: (a) Thick (A+B horizons reaching 7 m depth), gibbsitic Oxisol developed from a Paleosol after limestone (Miranda & Zinn, 2021), 890 m elevation; (b) kaolinitic Ultisol, 1.5 m thick with sombric (SOC-rich, dark subsoil) horizon, developed from gneiss, 1,300 m elevation.

All soil orders, with the likely exception of Gelisols, occur in the broad tropics and subtropics. The most relevant soil orders, in terms of area and economic importance, are briefly described below, focusing on properties more related with the scope of this chapter, i.e., soil organic carbon. No attempts were made to present the areal occurrences of these main soils in the tropics, due to the paucity of current, coarse-scale mapping for the tropical zone, and the limited correlation between main soil classification systems such as FAO-WRB (IUSS, 2015) and US Soil Taxonomy (Soil Survey Staff, 2014). The reader is referred to global distribution and areal estimates provided in the FAO-WRB publication or at www.nrcs.usda.gov.

Oxisols are thought to be the main soil order in the forest and savanna biomes in South America and Africa (see maps in www.nrcs.usda.gov), although they can also be found widely through the humid subtropics and even in some drier areas as relicts from a moister past climate (e.g., van Wambeke, 1992). In fact, Oxisols are the archetypical soils of the humid tropics, and are not known to develop under temperate climates. These soils are so extensive because they develop from nearly all soil parent materials, except quartzite and sandstones (Zinn & Lal, 2013), and represent an utmost stage of soil development and weathering which can only be achieved by long-term exposure to high temperatures and precipitations, and thus advanced leaching of bases and silica in freely drained terrains of near-level topography or with most slopes <8%. Such extreme leaching results in low or very low nutrient (esp. Ca, Mg, K, P) availability, acid reaction and Al^{3+} toxicity, and notably the accumulation of resistant minerals in both coarse particles (dominated by quartz, with minor rutile, zircon, hematite, magnetite) and clays, dominated by kaolinite and Al- and Fe-(hydr)oxides. Clay contents are very variable (ranging from 15 to >80%), and in some Oxisols, low-activity 2:1 and interlayer phyllosilicates are minor clay components (e.g., Lima et al., 2021; Fruett et al., 2022), whereas sand and silt particles are in many cases partly composed by clays cemented by Fe/Al oxides, resulting from the dismantling of laterites and ferricrete layers (Thomas, 1994). Oxisols probably comprise the deepest of all soil profiles (Figure 2a), typically showing strong yellow to red colors due to the contents and proportion of fine goethite and hematite, and most are well-drained, although drainage depends much more on soil structure patterns than on texture. Where Oxisols have granular structure (typically associated with wide predominance of Fe and Al (hydr)oxides over kaolinite in clays), they present limited water availability even with high clay contents, since the large packing void porosity results in very low bulk densities compared to other soil orders (Pádua et al., 2015), and very efficient drainage and root growth (Figure 3a). However, where kaolinite prevails over Fe and Al oxides, a blocky structure typically develops (Fig. 3b), resulting in somewhat but slower drainage and often higher water availability. The main suborders of Oxisols, those of ustic and udic soil moisture regimes (Ustox and

Udox), have been very successfully converted to high-productivity annual and perennial crops in Brazil, and to a much lesser extent in other countries, provided that liming and fertilization are used adequately and frequently. Fast-growth tree plantations and pastures require much less of such inputs to become highly productive.

Tropical Ultisols are widespread in South America, especially in Brazil, and can occur near or associated with Oxisols, with similar clay mineralogy (and colors), but usually with a lower degree of leaching of silica and bases. In many cases, these soils occupy portions of the landscape with more pronounced slopes than Oxisols (Zinn & Lal, 2013, Figure 2b), or where parent materials favor coarser textures, as in basalt/sandstones strata in the *Paraná* basin. Tropical Alfisols have so many similarities with Ultisols, namely an argillic or kandic B horizons (i.e., higher clay contents in subsoil), that van Wambeke (1992) treats them simultaneously in the same chapter of his book. The author notes "that the differentiating characteristics between the two orders, base saturation, is not an efficient discriminator in soils that have low cation exchange capacities", mostly because in such a case a high base saturation does not necessarily imply a high nutrient status if cation exchange capacity is too low. That being said, there are however a few important differences between these orders in the tropics: Alfisols tend to develop under savanna or drier climates, from more nutrient-rich parent materials such as basalt and limestone, and to contain many coarse rock fragments, even when associated with Oxisols (Lima et al., 2022). Important Alfisol areas occur in tropical India and West Africa, whereas these soils are not as common in tropical South America. However, both Ultisols and especially Alfisols can be prone to compaction or "hard-setting" of their coarse-textured surface horizon, further aggravating their overall higher bulk densities, compared to Oxisols.

Dystrophic Inceptisols and Entisols also occur commonly associated with Oxisols and Ultisols, on steeper slopes (Figure 3b, 4c) or near rock outcrops. A very important area in the tropics, humid or arid, is occupied by sandy Entisols (Psamments and Quartzipsaments). Inceptisols and Entisols are much more limited in land use than Ultisols and Alfisols, in special Quartzipsamments and shallow, stony Entisols, although Fluvents are often used for horticulture due to good fertility and water availability. Major areas of tropical Aridisols and associated Entisols/Inceptisols occur in both North Africa and Australia, as well as Southern Arabia and parts of the Andean plateau and the Brazilian Northeastern hinterlands.

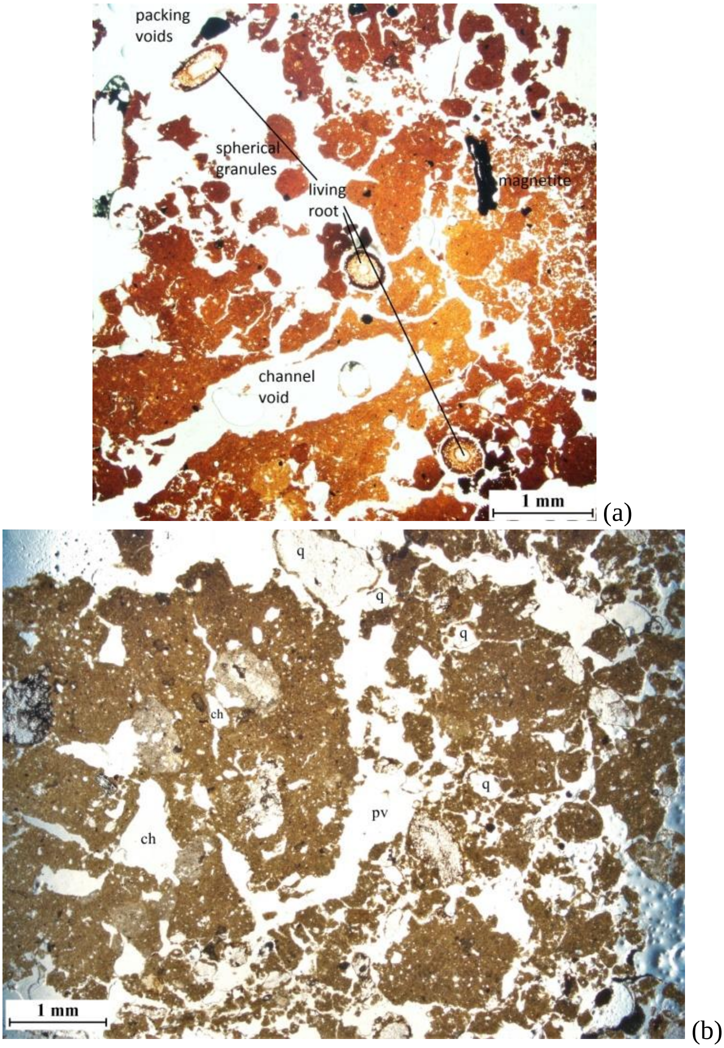

Figure 3. Soil thin sections of two tropical humid soils: (a) large macropore space (packing voids and faunal channels) of a gibbsitic-hematitic Oxisol with granular structure, developed on mafic rock, and (b) subangular blocky structure, marked by fissures (planar voids-pv) and higher bulk density, in a kaolinitic Inceptisol developed on pellitic rocks. Plane polarized light, near Lavras, Brazil. Photos by Marla Araujo (a) and Eduane Pádua (b), Univ. Fed. Lavras. Obs. ch –chamber voids, q-quartz.

Vertisols are relatively rare in tropical South America, but occupy very important areas in India, Australia and Sudan, typically developing on poorly drained lowlands (van Wambeke, 1992). According to this author, these soils often have good fertility and organic matter contents, but their seasonal dimensional instability can pose major limitations of their use, and in fact, only in India tropical Vertisols support intensive agriculture, due to more favorable tillage properties. Tropical Andisols are mostly restricted to recent volcanic areas in Andean and Central America, occupying smaller areas in southeast Asia and Africa. Despite their limited occurrence, due to their generally high fertility and easy cultivation, they often comprise highly populated areas. The amorphous composition of their clays can result in very high retention of soluble and colloidal organic matter and dark colors, although P fixation can limit production, and physical instability can pose problems to structures and mass wasting during intensive rains or snowmelt. Tropical Spodosols typically occupy coastal plains and other sandy deposits under forests, and due to their low fertility are mostly cultivated with silviculture, pastures and other low-input uses.

2. Soil Organic Carbon (SOC) in Tropical Soils

The first scientists and explorers that pioneered in the humid tropics were struck by the strong red to yellow soil colors, due to variable but considerably high contents of clay-sized hematite (Fe_2O_3) and goethite (FeOOH), respectively. Although these colors are more notable in Oxisols, they also occur in tropical Ultisols, Alfisols, Inceptisols and other soil orders. Most of these pioneers were accustomed to dark colors of surface layers of SOC-rich Mollisols, Histosols, Spodosols and other major soils under temperate climates, and logically assumed that these tropical soils had low or very low SOC contents (Greenland et al., 1992). Such perceptions also resulted from the fact that high temperatures favor biological activity and thus organic decomposition, as demonstrated by the negative correlation between soil organic matter contents and mean annual temperatures in the USA, in the seminal work by Jenny (1941). However, when enough data on tropical soils became available, in most cases their SOC concentrations were found to be unexpectedly similar, and sometimes higher, than those in SOC-rich temperate soils (Sanchez & Logan, 1992). It has been estimated that SOC stocks in the tropical zone comprise ca. 32% of SOC stocks in the world (Eswaran et al., 1993), and although the world tropics comprise ca. 36% of the global land area, that is a significant C pool since a large part of the tropical zone is under arid or semi-arid climate, mostly in Africa, Arabia and Australia (Figure 1), with low or even null SOC contents. However, even this and other gross estimates are probably conservative, since deep soil layers (mostly absent in temperate areas) with considerable SOC concentrations are seldom accounted for (see below, also Figure 2a).

Another unexpected dimension of the SOC dynamics under tropical climates refer to the effects of agricultural practices, especially those involving conventional soil tillage, which typically decreases SOC concentrations and stocks by soil aggregate disruption and enhanced aeration. In the tropics, average SOC losses after conversion of native vegetation to croplands are relatively low in comparison to those under temperate climates, as demonstrated by independent meta-analyses. For the case of Brazil, a mostly tropical and subtropical humid area, average SOC losses in topsoils varied from 10-14% (Zinn et al., 2005, 2018). Such losses reached up to 30% in the global tropics (Don et al., 2011), and both figures are low compared to global average SOC losses of 30 to 58% considering all climates (e.g., Mann, 1986; Guo & Gifford, 2002). The reasons for both high steady-state SOC concentrations and low SOC losses upon cultivation in the humid tropics are treated in more detail in the next section.

2.1. SOC Retention in the Humid Tropics

Soil organic matter (SOM) affects most soil properties involved in plant productivity (Murphy, 2015). SOM is not a simple or homogenous substance, but actually a combination of innumerous organic substances which exist in a *continuum* (e.g., de Nobili et al., 2020) of particle-sizes (from coarse, fresh organic residues to small molecules), chemical composition (highly variable contents of its main components, mostly C, H, N and O, the latter proportional to decomposition degree), and chemical function (e.g., complex to simple carbohydrates, long-chain and aromatic compounds, acids etc.), all interacting to determine its alteration or “humification” status. SOM is a critical component for the proper agricultural or environmental function of any soil, since it is a considerable nutrient pool (for mostly N, P and S, but also micronutrients), a C and energy source to the biota, and has important effects on soil aggregation, aeration and water retention at low suctions.

SOC is the C component of SOM, and its most widely used indicator in soil tests, since SOM cannot be accurately determined due to its widely variable composition across different soils, and along different depths of the same soil. SOC is mostly expressed in concentration (% weight or g C kg^{-1} soil) or as a stock (Mg C ha^{-1} or kg m^{-2}, to a specific depth). Basically, SOC concentrations in any given soil are the steady-state result of a balance between the inputs of organic residues (or SOC-rich sediments, in some depositional areas), and the respective losses by biological decomposition and soil respiration (Luo & Zhou, 2006), leaching or erosion. SOC concentrations are generally higher in surface layers, where inputs are more abundant and frequent, as the result of aboveground litterfall and belowground growth and turnover of shallow root systems. Biological diversity and decomposing activity are also higher at surface layers, which demonstrates that the effect of higher inputs at top soils clearly overtakes that of also higher decomposition rates. Thus, a commonly exponential decrease in SOC concentrations with increasing depth occurs in most soils under all climate types, although some forested mountain areas can present SOC-rich subsoils due to leaching (Figure 2b). However, in the humid, lowland tropics, as noted before, temperatures are seldom or never cold enough to prevent photosynthetic activity, so biomass accretion takes place for most of the year if water is available, unlike under temperate or colder areas, or under arid climates. This *enhanced and frequent organic input* is a critical feature of the humid tropics that must be kept in mind when discussing both their high SOC retention and relatively low SOC losses upon cultivation (although not for dryland tropics).

There are also two other critical factors increasing SOC retention in the humid tropics. In these areas, intense and continuous faunal bioturbation (mostly by termites, leaf-cutting ants and earthworms) results in vertical homogenization and thus in more distributed of SOC throughout deeper layers than under temperate climates. Sanchez (1976) keenly noted that, despite Oxisols and Mollisols having similar SOC contents in the top 1 m, Mollisols tend to have 20% more SOC in the 0-15 cm layer, which is another reason why relatively more SOC is depleted when surface layers of such soils are cultivated. In addition to this deeper distribution in the top 1 m, SOC storage can be considerable at deeper layers of many tropical humid soils: Some Oxisols can have SOC concentrations of ca. 0.66% at a 5 m depth (e.g., Zinn & Miranda, 2021, Figure 2a). Since biological activity at these depths is typically very low, such a scenario implies huge but mostly unknown SOC stocks, and is probably much more common than reported in the literature on the humid tropics, due to the difficulty in sampling these deep layers by most soil scientists. In addition, physical, chemical and mineralogical properties of tropical humid soils also help explain their pronounced ability to retain SOC. There is considerable evidence that in tropical savannas, SOC concentrations are proportional to clay (or clay + silt) contents, since more clay allows for increased sorption of colloidal SOC compounds, and thus stronger SOC stabilization (e.g., Zinn et al., 2007a; Barthès et al., 2008, Fujisaki et al., 2018). Although this sorptive mechanism also works for most temperate soils, it is likely even more effective in the tropics: the considerably high contents of Al and Fe oxides, so common in tropical humid soils, have been for decades thought to be involved in the stabilization of colloidal and soluble SOC forms (e.g., Greenland et al., 1992). In fact, further research showed that SOC concentrations were actually more correlated with clay-sized Fe and Al oxides than clay or kaolinite contents, to a 1-m depth, in Brazilian savannas (Zinn et al., 2007a).

In addition, slightly-altered organic residues present in soils, commonly occurring in coarse particle sizes (mostly sand, i.e., 2.0-0.05 mm in diameter), are generally more susceptible to biological decomposition than colloidal SOC. This so-called *particulate organic matter* (POM) exists either outside or occluded *within* soil aggregates, which in the latter case results in slower attack by fauna and thus in reduced decomposition, compared to aggregate-free POM (Zinn et al., 2007b). However, the overall warm temperatures in the tropics result in that most SOC occurs in a highly altered (humified), colloidal form, which tends to be more stable and resistant than coarser, fresher POM, with important consequences to SOC changes after cultivation. In Brazil, POM typically comprises ca. 25-40% in savanna lowlands (Zinn et al. 2007a) and only ca. 10% in forested highlands, where more humified SOC is stabilized by sorption (Araujo et al., 2017). It has been also shown that POM occlusion tends to be lower in sandy soils (Zinn et al. 2007b) compared to those where there is abundant clay to form aggregates

The knowledge of such correlations between SOC retention with soil properties is relatively recent and not investigated in most of the world's humid tropics, and there were later reports that these conclusions were valid only for lowlands, i.e., at elevations up to 600 m. At altitudes >900 m, the overall lower air and soil temperatures often result in even higher SOC concentrations, but these are nearly homogenous among many contrasting soils, so that soil texture and clay mineralogy do not correlate significantly with SOC concentrations (Araujo et al. 2017). Although these data refer mostly to Brazilian soils, similar trends can be broadly expected for the tropical humid zone, where high soil clay and oxide contents interact with year-round, intense photosynthetic and biological activity, which must be investigated in other tropical areas. However, these mechanisms are not dominant in tropical Spodosols and Andisols, where most SOC is stored by very different processes, respectively as a precipitated Fe/Al organic complex or by sorption to amorphous, recent volcanic materials (e.g., Buol et al., 2014). Finally, in tropical Histosols, large amounts of SOC are stored by paludization in saturated, anoxic conditions along drainage ways and floodplains (Leng el al, 2019), or by aggregate formation through SOC preservation or cementation in cold, high mountains, in both cases with little or no interaction with soil mineral particles.

2.2. SOC Management in the Humid Tropics

As commented before, soil properties and SOC retention mechanisms in the humid tropics present many important contrasts with those under temperate climates, where most soil research was historically developed. These critical differences have certainly delayed a proper understanding of how tropical humid soils function, and the conception of specific knowledge and practices for adequate soil management in tropical agriculture. In fact, only after the mid-1970's decade, crop, pasture and silviculture production in immense tropical savanna areas became economically and environmentally more sustainable (Zinn & Lal, 2013). These improvements came as direct consequence of the Green Revolution spurred by the late Norman Borlaug, later awarded the 1970 Nobel Peace Award, and today a highly technological, globally competitive agriculture is well established and profitable in Brazil (Figure 4a), India and other tropical countries, although much remains to be done in many other nations. As a consequence of this relatively recent surge in agricultural importance, food production and security increased exponentially, and simultaneously, basic and applied scientific research and technological development on all agricultural and environmental fields were also spurred in these tropical nations. This includes Soil Science and, more specifically to our purposes, on how SOC affects other soil properties critical to agriculture, and how SOC is itself affected by land use change and soil management.

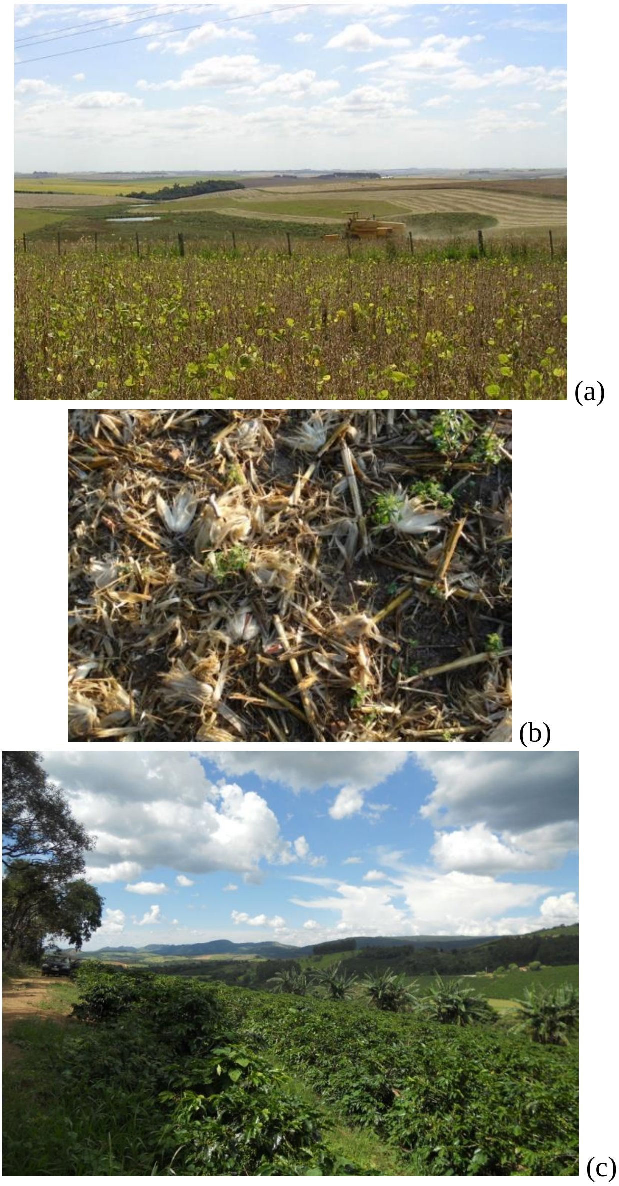

Figure 4. Some examples of agricultural management favorable to SOC sequestration in Brazil: (a) no-till crop rotations on subtropical clayey Oxisols developed from basalt, Rio Grande do Sul; (b) soil cover with corn harvest residues in a small farm plot on an Oxisol in Minas Gerais, and (c) short-variety coffee crops with brachiaria grasses between tree rows.

Basically, in soils under climax native vegetations, be it either forest, savanna grassland or any other, SOC concentrations (in both surface and subsurface layers) are mostly at or near a steady-state level, that varies only slightly across time, due to seasonal, annual or decadal climate variation. Deforestation, or change of native non-forest vegetation to agriculture, typically decreases SOC concentration and stocks, mostly due to the ensuing lower organic inputs under the new crop, but especially by frequent tillage. Tillage intentionally disrupts soil structure and decreases the size of large aggregates, aiming to offer a softer seedbed, also enhancing soil aeration. This practice started pre-historically with the development of the moldboard plow, at least 10,000 years ago. This technological revolution also aimed to *accelerate* SOM decomposition, in order to mineralize nutrients contained therein to the crops (Lal, 2007). However, as a consequence of continuous, long-term use of plows, disks and harrows, soil degradation, erosion and SOC depletion typically ensues in most areas (e.g., Mann, 1986; Guo & Gifford, 2002; Don et al., 2011). This general trend does not necessarily apply where low-biomass natural grasslands or scrublands are replaced with plantation forests or other high biomass crops, especially when tillage is not frequent or if no-till is used (e.g., Fruett et al., 2022). In these cases, SOC can actually increase compared to native vegetations, and although these situations are relatively rare in the humid tropics, can be more likely in the drier tropics, especially if irrigation is feasible.

In any type of climate and location, adequate soil management for agricultural or environmental purposes must include provisions to conserve current SOC stocks, or ideally to increase them. The increase in SOC concentration and stocks in soils, i.e., SOC sequestration, is a win-win strategy (Lal, 2006) that both enhances soil quality for agriculture and environmental purposes, at the same time it removes CO_2, the most important greenhouse gas, from the atmosphere, through photosynthesis, residue alteration and mid- to long term stabilization as soil humus. The quantitative potential for SOC sequestration in different ecosystems and managements has been extensively reviewed (Lal et al., 2018, among many others), to which the reader is referred, and this topic will focus on particular soil factors affecting it in the humid tropics. Basically, the most viable and cost-effective strategies for SOC sequestration or preservation involve: 1) decreasing SOM losses through decomposition, leaching and erosion; and 2) increasing C inputs to soil. These strategies are independent, but for better results they ideally must be simultaneous, and can be achieved by multiple activities, as discussed next.

2.2.1. Reducing SOC Depletion

Due to the warm soil temperatures and their seasonal stability that define the humid tropics, SOM decomposition is indeed accelerated compared to temperate and cold climates, and thus SOC can be easily depleted with agricultural cultivation if organic input addition and humification rates are outpaced. Thus, decreasing SOC decomposition rate is a critical first step to efforts for SOC conservation and sequestration in the tropics, which can be achieved by more than a few practices. Fire in tropical areas has been noted for decades as a factor resulting in SOC and N losses in surface soils, but not in subsurface layers, although aboveground biomass is obviously most depleted (Jiang et al., 2020). Thus, fire is to be avoided when possible, but basic soil conservation practices are generally required at least for annual croplands, since sheet and rill erosion mostly affect soil surface layers richer in SOC, which is thus preferentially carried away and lost (Polyakov & Lal, 2008). Such SOC losses through erosion are especially intensive after rainforests are converted to annual crops (Thomas, 1994). However, perhaps the most critical practice is to reduce soil disturbance during tillage prior to planting, wherever this is feasible. For annual crops, this often involves replacing annual tillage with no-till or conservation tillage, which has become very widespread in the humid tropics of Brazil and elsewhere, even more than under temperate climates, where seasonal freezing may not favor no-till. For perennial and tree crops, tillage is much less frequent, and often done only after long periods of time (Ledo et al., 2021), as several decades for coffee stands. However, reducing SOC losses in these areas can also involve eliminating mechanical soil cultivation in order to control weeds, especially using highly-disruptive rotavators (e.g., Cogo et al., 2013) and opting for post-emergence cutting or herbicides.

Another recommendation, which however is not always feasible, is to avoid intensive tillage in soils more prone to rapid SOC losses, and give preference to other soils and areas where such losses are less likely, either by edaphic or climatic factors. In Quartzipsamments and other sandy soils, where soil structure and humic adsorption are very restricted due to inherently low clay contents, SOC depletion and leaching occur typically faster than in finer-textured soils (e.g., Zinn et al., 2005). In addition, as noted earlier, site altitude is inversely correlated with air and soil temperatures, and most of the area of the humid tropics are marked by relatively low altitudes (<500 m, Figure 1), thus presenting high temperatures favoring rapid organic decomposition. This is the general cause why even in rainforest zones, SOC stocks are lower at warmer sites (Das et al., 2022), as demonstrated also by lower SOC in the

Amazon, compared with the Atlantic rainforest in Brazil (Bernoux et al., 2002). Conversely, tropical mountain ranges and highlands present much lower temperatures and thus lower decomposition rates, which results in higher SOC retention (Araujo et al., 2017, Figure 2b) and deserves further research. In southeastern Brazil, recent research (França et al., 2023) reported that coffee cultivation at higher elevations (ca. 1,260 m, Figure 4c) can not only preserve but actually promote considerable SOC sequestration even in shallow, nutrient-poor soils, if implemented with judicious practices such as organic fertilizers and planting grasses between coffee tree rows (see next).

2.2.2. Increasing C Inputs to Soils

This strategy can be achieved by either increasing *in situ* biomass production, or application of organic residues or other C sources from outside the land plot, or a combination of both. The application of manure to soils has been done for millennia as the most traditional fertilization practice, i.e., aiming to add nutrients required by crops, but has also the great advantage in adding organic C, and if done continuously, increases SOC and also biological activity and overall soil quality. However, in any climate, only ca. 8% of the added C input will effectively be incorporated as humus SOC into soils (Fujisaki et al., 2018), and this percent depends on residue properties and the decomposition rate, which is high in the global tropics, regardless of soil moisture. A global meta-analysis has demonstrated that SOC gains with manure applications are higher for non-tropical (30%) than for subtropical climates (24%), probably because of higher temperatures and decomposition in the latter, but more research is still required to have a better picture for tropical soils (Gross & Glaser, 2021).

A relatively recent option to add more stable C sources to soils is *biochar*, composed by organic residues treated at high temperatures under limited O_2 availability, which is marked by become highly C-rich and porous. Biochar application to soils has many benefits similar to those of conventional residues and composts, such as improving soil physical properties, sorption of nutrients and contaminants, and has an advantage in which can be produced with mineral nutrient sources as biochar-base fertilizers (e.g., Carneiro et al., 2018). Global data have shown that biochar additions can increase SOC in an average 84% of the cases, although tropical conditions showed much lower figures of ca. 40% (Chagas et al., 2022), which again reflects faster decomposition through higher temperatures. More specifically under tropical conditions, biochar application has been noted to provide higher SOC sequestration (25%) than conservation tillage (18%) and cover crops (16%) (Das et al., 2022), among other practices. However, biochar properties, its permanence and effects in soils all depend strongly on the nature of the organic residue use and temperature of production, among also other factors, not to mention cost and transport logistics, which vary widely within countries and regions. Thus, and also due to the relatively high costs and low volumes of organic fertilizers and biochar, they have mostly been applied onto small areas used for horticulture and high-value crops, such as coffee. For the much larger areas of annual crops, plantation forests and pastures, the only feasible enhancement of C inputs involves producing more biomass directly *in situ*, which can also be achieved by many different practices.

For annual croplands, the most basic step to increase SOC inputs is the elimination of fallowing, still used in many parts of the tropics, as a traditional (and sometimes even religious) way to allow the land to "rest". However, most often fallowing results in lower biomass accretion, which aside with the continuous decomposition of SOM and residues from the last crop, can result in SOC loss, especially where the land is kept nearly bare and erosion-prone. Another obvious set of practices are the judicious use of liming, fertilizers, pest control and, where needed and feasible, irrigation, which combined will also increase crop biomass and thus SOC sequestration. The choice of species and varieties producing high biomass is also critical, either as monoculture, or at least in rotations of crops with low and high biomass (e.g., soybean/corn). The functional composition of crop residues is also relevant, as there is great variation in the resistance to decomposition: herbaceous N-fixing leguminous residues, such as those in soybeans and peanuts, decompose swiftly and even mineralize N that thus favor decomposition of antecedent SOC, whereas grassy cereals such as corn (Figure 4b), rice or wheat are more recalcitrant and are more easily stabilized in soils. Agricultural intensification in the form of continuous use of the same tract of land with two, or even three cultivations throughout the year, is the most unique potential of the tropics to increase SOC sequestration, especially where irrigation is feasible. Of course, all these practices can be coupled to conservation or minimal tillage as mentioned in 2.2.1, to optimize SOC sequestration.

Perennial crops, including fruit and other food trees, fast-growth trees for wood, intensively managed pastures and other non-tree crops such as sugar cane have been demonstrated to be a more viable alternative for potential SOC sequestration (Ledo et al., 2020). This potential is reinforced by lower SOC losses after conversion from native vegetation, due to the typically low intensity of soil preparations for perennial crops. Another meta-analysis for Brazil (Fialho & Zinn, 2014) suggested that eucalypt plantations can both increase or deplete SOC previously existent under native vegetation, depending on soil mechanic preparation among other factors, but average SOC changes were not significant. Since such plantation forests sequester much C in aboveground biomass and wood, this offsets the fact that average SOC changes are null, and suggests that soil management under fast-growth tree plantations can still be improved. Coffee crops, which are a main export crop in tropical countries, have been noted to conserve SOC stocks in levels similar to those under >20 yrs-old secondary forests on clayey Oxisols, provided that weeds are allowed to grow between coffee tree rows (Cogo et al., 2013, Figure 4c). Although there are only very few published research on the specific effect of altitude on SOC changes under agriculture in the tropics (*and* in temperate areas), there is already some evidence that lower temperatures at mountain ranges can preserve or increase SOC contents under perennial crops. For instance, considerable SOC sequestration (ca. 30 Mg C ha^{-1}, to a 40 cm depth) was reported at altitudes of 1,260 m (but not 940 m) under 3-yr old coffee plantations planted on shallow, stony Inceptisols (França et al., 2023), which should motivate further research on other tropical humid highlands.

Grasslands and extensive pastures, in all climate zones, are marked by low or null soil preparation and other cultural practices, and often sustain considerable animal and human populations. Grasslands, when managed with controlled grazing with high animal density per area, have a potential to conserve or sequester SOC sequestration (Phukubye et al., 2022), especially in the savannas, due to their active and fasciculate root systems. Active pastures in the rainforest areas of Panama have similar SOC stocks to those under 15-yr secondary forests, although 100-yr old secondary forest presents much higher stocks (Neumann-Cosel et al., 2010). However, many of the pastures in the humid tropics are actually degraded by excessive grazing and erosion, thus representing considerable SOC losses (Damian et al., 2023). In order to reach their SOC sequestration potential, intensification in the form of fertilization and consortia with other plants is required. In fact, a relatively recent development that can potentially enhance SOC sequestration and improvement of degraded lands in the tropics are crop-livestock systems, which have shown remarkable results in South-American savannas (Ayarza et al., 2022), and must be investigated in other parts of the tropics.

Conclusion

In this essay, the main characteristics of the tropical environments, landscapes and soils were briefly reviewed, aiming to provide the reader with the most important factors affecting SOC retention and dynamics in this region critical for global climate, agriculture and population. This was never meant to be an extensive literature review on SOM and SOC, or not even on SOC in the tropics, but rather a collection of texts varying from classical books to recent meta-analyses, selected to substantiate a particular perception gained after more than 20 years studying this topic. The central focus on the humid rather than on the general tropics was less due to personal experience than to the more abundant literature on the former areas, where agriculture is currently more important (even if more recent), since these are the places where most agricultural and soil research efforts are stronger. It is my hope that it can be useful for those interested on SOC in the tropics, regardless if that involves its agricultural, environmental, scientific or policy aspects, and this is the reason why no particular issue was extensively elaborated on.

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Chapter 6

Methodologies for the Surface Application of Limestone, Gypsum and Hydrated Lime in No-Till and Agropastoral Systems

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Abstract

Soils in the Cerrado, which covers 204 million hectares (24%) of Brazilian land area and is one of the world's largest agricultural frontiers, are characterized by low pH and, consequently, high Al^{3+} content and

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low P and Ca^{2+} contents. Soil pH ($CaCl_2$) values below 4.4 increase the availability of aluminum and manganese, whereas a pH range of 5.4–6.4 ensures the availability of most nutrients essential for crops. Low Ca^{2+} content and high Al^{3+} content (Al toxicity) in the subsoil affect root growth, restricting the capacity of plants to absorb water and nutrients and limiting productivity. To contribute to the development of alternative methodologies for correcting acidity in the surface and subsurface layers of the soil, different methodologies for the surface application of limestone, gypsum, and hydrated lime, in no-till and agropastoral systems and their effects on soybean and maize yields were researched at the Advanced Research Center and Development for Rubber and Agroforestry Systems of the IAC of the APTA, which is located in the municipality of Votuporanga, SP, Brazil. This chapter presents the results of these researches.

Keywords: *Glycine max*, *Zea mays*, no-till, agropastoral system

Introduction

The inadequate use of soil preparation equipment is the main factor of soil degradation and depending on the degree of alteration of the physical properties, these can produce conditions limiting the development of the crops and, consequently, affect their yield (Silva, 2000). Spera et al. (2005) emphasize that soils managed under conventional tillage present serious compaction problems, such as the development of a hardened subsurface layer. By contrast, no-till system, due to the small mobilization of the soil, preserves the aggregates and their covering properties (Bertol et al., 2004). No-till system is considered the most sustainable and efficient management for soil conservation due to all the benefits promoted by its chemical, physical and biological characteristics, such as: reduction of the direct impact of the rain drops, increased infiltration of water infiltration, decrease in surface runoff, reduction of thermal amplitude and moisture management, reduction of weed infestation, increase of organic matter content, expansion of the "sowing window," reduction of fuel consumption and nutrient cycling, among other advantages (Borges, 2015). Due to all these advantages, no-till system is recommended whenever possible, as it is an example of a sustainable production system with environmental, social and economic benefits to all society. Produces less greenhouse gases, accumulates carbon in the soil and reduces CO_2 emissions, since it requires less agricultural processes when compared to the conventional no-till system, improves water quality, reduces soil loss, increases crop yield and product diversification, and improves property profitability, encouraging farmer management in the field (Borges, 2012). However, to obtain these results, it is necessary to obey three basic principles required by no-till system: absence of preparation (no soil disturbance), crop rotation and permanent soil cover (straw) (Borges et al., 2015).

Other soil conservation management system is the agropastoral system, where the pasture uses the soil correction and residual fertilization applied in the field, that benefits from the physical soil condition and of the straw provided by the pasture (Vilela et al., 2003). In agropastoral systems, grain crops and livestock are produced simultaneously. These systems typically combine soybean and/or maize cultivation with beef and/or dairy cattle production on the same site and have been adopted in several biomes in Brazil (Santos et al., 2011). The tropical forages used in these systems, like those of the genus *Urochloa*, produce large amounts of dry matter and have vigorous, deep root systems. These forages increase drought tolerance and nutrient cycling (Garcia et al., 2008; Calonego and Rosolem, 2010; Almeida et al., 2018), improve physical soil attributes, reduce weed incidence, and protect against wind and water erosion (Allen et al., 2007; Sarto et al., 2020). In Brazil, most agropastoral systems are in the Cerrado biome (the Brazilian savanna), which covers 204 million hectares (24%) of Brazilian land area and is one of the world's largest agricultural frontiers, and soils are characterized by low pH and, consequently, low P and Ca^{2+} contents and high Al^{3+} content (Al toxicity) (Araujo et al., 2012; Mendes et al., 2012; Souza et al., 2016). Soil pH ($CaCl_2$) values below 4.4 increases the availability of Al and Mn, whereas a pH range of 5.4–6.4 ensure the availability of most essential nutrients for crops (Malavolta, 2006). Al toxicity associated with low Ca^{2+} content in the subsoil affects root system growth, limiting soil exploration in the surface layer. This restricts the capacity of Al-sensitive plant species to access water and nutrients (Carvalho and van Raij, 1997; Rampim et al., 2011; Pauletti et al., 2014) and limits crop yields (Pavan et al., 1982; Ritchey et al., 1982), especially under water deficit conditions (Zandoná et al., 2015).

One option for improving the conditions for root development in these systems is the use of limestone and gypsum. Liming (application of limestone) is the most efficient means of increasing pH, Ca^{2+} and Mg^{2+} contents and base saturation and reduces exchangeable Al in the soil. However, the reaction of limestone is generally limited to the site of soil application, and it does not reduce subsoil acidity quickly (Caires et al., 2004). Moreover, when limestone is applied to the surface, the low mobility of its products of dissolution restricts the efficiency of this soil conditioner in reducing acidity in subsurface layers of the soil with variable loads, which depends on the leaching of organic and/or inorganic salts throughout the soil profile (Caires et al., 2006).

The limitations of surface application of limestone have important implications for systems without soil tillage, such as no-till systems and agropastoral systems under no-till. In these systems, soil acidity correction is usually carried out by applying limestone on the surface without incorporation into the soil. This results in chemical stratification, including pH stratification, with high pH levels in the upper few centimeters of the soil profile (Caires et al., 2005). In addition, crop yield responses to surface liming remain clear (Martins et al., 2014), as there is no direct relationship between soil acidity attributes and crop responses to liming under no-till conditions, especially under normal rainfall (Caires et al., 2001a; Tissi et al., 2004).

In agropastoral systems, the dynamics associated with grazing may further influence the responses of soils and crops to surface liming compared with no-till systems that are only cultivated with cash and cover crops (non-grazed systems) (Martins et al., 2014). Caires et al. (2011a) reported that grain yields of maize and soybean were not influenced by liming applied to the surface or incorporated into topsoil. Magalhaes et al. (2017) verified that the dry matter production of *Urochloa* hybrid ‘Mulatto II’ was also not influenced by the form of limestone application. Similarly, Caires et al. (2001b) reported that liming (whether surface applied or incorporated into the soil) did not influence barley grain yields, which were limited by prolonged water deficit during the flowering stage. In a global meta-analysis of the effects of liming on soil pH and crop yield, Li et al. (2019) concluded that liming increased yield regardless of environmental and experimental conditions but that the improvement depended greatly on crop species, liming material, liming application method, and soil texture.

By contrast, gypsum, a byproduct of the phosphoric acid industry (Caires and Guimarães, 2018), moves down the soil profile during drainage after application to the soil surface, increasing the Ca^{2+} supply and reducing toxic levels of Al^{3+} (Sumner, 1995). As a result, surface application of gypsum improves root growth and the absorption of water and nutrients by plant roots (Ritchey et al., 1980; Carvalho and van Raij, 1997; Caires et al., 2016). Gypsum improves the root environment at greater depths, decreasing Al toxicity, and supplies Ca and $S\text{-}SO_4$ to the soil (Souza et al., 2012), resulting in favorable conditions for root system growth and water and nutrient absorption by plants (Zandoná et al., 2015).

The responses of grain yields to gypsum application are also variable. Caires et al. (2011a), Rampim et al. (2011), Dalla Nora and Amado (2013), Dalla Nora et al. (2014), Pauletti et al. (2014), and Zandoná et al. (2015) observed increased grain yields, whereas Carvalho and Nascente (2014) reported that the application of gypsum did not improve plant development conditions and did not significantly affect soybean grain yield. Increases in the grain yields of maize and barley (*Hordeum vulgare*) were reported by Caires et al. (2001b) and Michalovicz et al. (2014). Caires et al. (2011b) verified that gypsum application increased maize yield but did not affect soybean yield. Dalla Nora and Amado (2013) further confirmed the greater impact of gypsum application on the grain yield of maize compared with soybean. Dalla Nora et al. (2017) found that applying gypsum increased the yields of maize (8.2%) and wheat (8.7%) compared with the corresponding controls, whereas the increase in soybean yield was smaller (6.6%). They attributed the greater responses of maize and wheat to gypsum to the fact that the roots of *Poaceae* have a lower cation exchange capacity than those of *Fabaceae*.

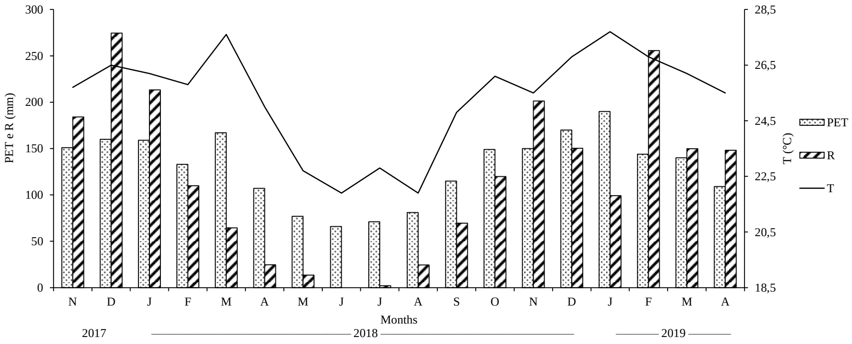
The limitations of surface application of limestone noted above have spurred interest in new sources of Ca inputs and application methodologies that improve the development of the crop root system throughout the soil profile. One interesting option is hydrated lime, which is obtained industrially by the hydration of virgin lime (Primavesi and Primavesi, 2004). Hydrated lime is a fine powder composed of calcium hydroxide ($Ca(OH)_2$) and magnesium hydroxide ($Mg(OH)_2$) (Alcarde, 2005). However, the application of hydrated lime in grain crop production systems is a relatively new practice, and little information is available on the methodology of its application and its effects on crop yield, particularly in no-till and agropastoral systems.

To contribute to the development of alternative methodologies for correcting acidity in the surface and subsurface layers of the soil in no-till and agropastoral systems and their effects on soybean and maize yields, two researches were developed, one with surface application of limestone and gypsum and another with surface application of hydrated lime. The researches were developed at the Advanced Research Center and Development for Rubber and Agroforestry Systems of the IAC of the APTA. This chapter presents the results of these researches, extracted from Borges et al. (2018a), Borges et al. (2019a), Borges et al. (2018b) and Borges et al. (2019b).

Description of Site, Soil and Climate

The Advanced Research Center and Development for Rubber and Agroforestry Systems is located in the municipality of Votuporanga, São Paulo State, Brazil (20º20’S, 49º58’W and 510 m altitude). The soil in the experimental area is classified as Arenic Hapludult (Soil Survey Staff, 2014), hereafter referred to as Ultisol, sandy texture. The plot size was 5 m long and 5 m wide, totaling 25 m^2. The climate in the region is tropical with dry winters (Aw-type according to Köppen’s classification) with average annual maximum, minimum, and mean temperatures of 31.2°C, 17.4°C and 24°C, respectively, and annual average rainfall of 1328.6 mm.

Monthly data on potential evapotranspiration (PET), rainfall (R), and average temperature (T) in Votuporanga from 1 November 2017 to 30 April 2019 are shown in Figure 1.



Source: CIIAGRO (2020)

Figure 1. Data on PET, R, and T in Votuporanga, São Paulo State, Brazil, from November 2017 to April 2019.

Description of Crop Systems

The no-till system was implemented in an area that was intended for grain production in a conventional soil tillage system. In the 2009/10 season, the no-till system was adopted. The crops used in this system were: soybean, maize, sunn hemp (*Crotalaria juncea*), grain sorghum (*Sorghum bicolor*) and sorghum-sudangrass hybrid (*S. bicolor* × *S. sudanense*) intercropped with Congo grass (*Urochloa ruziziensis* syn. *Brachiaria ruziziensis*). In the 2016/17 season, soybean were grown in the Spring-summer season and sunn hemp in the Fall-winter season. The crop rotation used in the no-till system from September 2009 to August 2017 is presented in Table 1, and the quantity of nutrients used in the period from September from 2011 to August 2017 is shown in Table 2.

Table 1. Crop rotation used in the period from September (Sep) from 2009 to August (Aug) 2017

Set/Mar	**Apr*/Aug**	**Sep/Mar****	**Abr/Ago**	**Set/Mar**	**Abr/Ago**
2009/10		**2010/11**		**2011/12**	
Soybean	Sunn hemp	Maize	Sunn hemp	Soybean	Sorghum-sudangrass hybrid
2012/13		**2013/14**		**2014/15**	
Soybean	Grain sorghum	Soybean	Sunn hemp	Maize	Sorghum-sudangrass hybrid +
					Congo grass
2015/16		**2016/17**			
Soybean	Sorghum-sudangrass hybrid +	Soybean	Sunn hemp		
	Congo grass				

* March. ** April.

Table 2. Quantities of nutrients used from 2009/10 to 2016/17 seasons

N	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**
---------- kg ha^{-1} ----------								
2009/10			**2010/11**			**2011/12**		
12	60	60	112	67	86	20	88	76
2012/13			**2013/14**			**2014/15**		
12	60	60	14	70	70	144	168	142
2015/16			**2016/17**					
42	174	138	12	60	60			

The agropastoral system was implemented in an area that was also used for grain production in a conventional soil tillage system. In the 2010/11 season, the no-till system was adopted. The crop rotation used in this system is: soybean (Spring-summer season), sunn hemp (after soybean) (Fall-winter season), and maize intercropped with palisade grass (*U. brizantha* syn. *B. brizantha* cultivar Marandu) (after sunn hemp) (Spring-summer season). In this system, newly weaned beef cattle are introduced sixty days after harvest of maize intercropped with palisade grass. The animals stayed in the area for fourteen months and then the area is closed for thirty days, and the grass is desiccated with glyphosate (a broad-spectrum systemic herbicide). Next, soybean is sown in no-till system on palisade grass straw. The grazing system used in agropastoral system is continuous and the stocking rate varied between 2-4 animal unities ha^{-1} according to the forage offered. The animals allowed to move freely about the pasture. Different generations of animals pastured on the same area. The crop rotation used in the agropastoral system from September 2011 to August 2017 is presented in Table 3, and the quantity of nutrients used in the period from September from 2011 to August 2017 is shown in Table 4.

Table 3. Crop rotation used in the period from September (Sep) from 2011 to August (Aug) 2017

Sep/Mar	**Apr*/Aug**	**Sep/Mar****	**Apr/Aug**	**Sep/Mar**	**Apr/Aug**
2011/12		**2012/13**		**2013/14**	
Maize + Palisade grass	Palisade grass	Palisade grass	Palisade grass	Soybean	Sunn hemp
2014/15		**2015/16**		**2016/17**	
Maize + Palisade grass	Palisade grass	Palisade grass	Palisade grass	Soybean	Sunn hemp

* March. ** April.

Table 4. Quantities of nutrients used from 2011/12 to 2016/17 seasons

N	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**
---------- kg ha^{-1} ----------								
2011/12			**2012/13**			**2013/14**		
112	100	48	-			14	70	70
2014/15			**2015/16**			**2016/17**		
120	102	94	-			12	60	60

Crop Management

Soil samples were collected from the 0.0–0.2 and 0.2–0.4 m layers before the application of the treatments to determine the initial soil characteristics and fertility (van Raij et al., 2001). The samples were collected at ten random points in each crop system in the 0.0–0.2, and 0.2–0.4 m layers; thus, ten subsamples were collected from each layer of crop system. The ten subsamples were homogenized and pooled to form a composite sample of each layer of each crop system. Soil was sampled with a metal probe and air-dried before analysis. The results are shown in Table 5.

Table 5. Chemical characterization of the soil in the 0.0-0.2 and 0.2-0.4 m layers, 2017

	P (resin)	**S-SO_4**	**Organic matter**	**pH**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**Al^{3+}**	**Total acidity**	**Base saturation**
	mg dm^{-3}		**g dm^{-3}**		**---------------- $cmol_c$ dm^{-3} ----------------**					**%**
	---------- 0.0–0.2 m ----------									
NTS[*]	34	3	4.3	4.3	0.28	1.0	0.6	0.4	3.1	38
APS[**]	22	4	4.5	4.5	0.21	0.7	0.7	0.2	2.5	39
	---------- 0.2–0.4 m ----------									
NTS	9	4	4.1	4.1	0.27	0.8	0.4	0.5	2.8	34
APS	15	5	4.1	4.1	0.25	0.5	0.4	0.6	2.8	29

[*] No-till system. [**] Agropastoral system.

The treatment schedule in the 2017/18 season with details of soil and crop management in both researches is shown in Table 6.

Table 6. Treatment schedule in the 2017/18 season

Date	**Activity**
10/30/2017	soil characterization
11/03/2017	straw sampling
11/22/2017	surface application of limestone and gypsum/hydrated lime
11/24/2017	maize sowing
12/11/2017	first topdressing fertilization of maize
12/14/2017	palisade grass sowing
12/18/2017	second topdressing fertilization of maize
03/27/2018	maize harvest
04/16/2018	soil sampling

pH (1:2.5 soil/0.01 M $CaCl_2$ suspension) and total acidity pH 7.0 (H^+ + Al^{3+}) were determined. S-SO_4 content was determined by the calcium phosphate ($Ca_3(PO_4)_2$) method, and the levels of P, K^+, Ca^{2+}, and Mg^{2+} in the soil were determined by extraction with ion-exchange resin (van Raij et al., 2001). These results were used to calculate the values of base saturation through the relationship between the content of exchangeable bases in the soil (Ca^{2+}, Mg^{2+}, and K^+).

The maize hybrid Dow AgroSciences 2B587 Power Core™ was sown mechanically on the straw of sunn hemp under no-till at a row spacing of 0.8

m and density of 6.0 seeds m^{-1} (0.0167 m plant spacing). Basic fertilization was performed at sowing at a dose of 315 kg ha^{-1} of 08-28-16 fertilizer (8% N, 28% P_2O_5, and 16% K_2O), with 1.7% Ca and 3.6% $S\text{-}SO_4$. The first topdressing fertilization of maize was carried out using 20-00-20 fertilizer (20% N and 20% K_2O) at a dose of 270 kg ha^{-1}. The second topdressing fertilization of maize was carried out using ammonium sulfate (20% N and 22% $S\text{-}SO_4$) at a dose of 250 kg ha^{-1}. Maize on no-till system and agropastoral system is shown in the Figures 2 and 3.

Figure 2. Maize on no-till system, 12/13/2017.

Figure 3. Maize on agropastoral system, 12/13/2017.

Palisade grass was sown mechanically in no-till system and agropastoral system using 10 kg ha^{-1} of forage seeds with a cultural value of 50% mixed with simple superphosphate fertilizer (18% P_2O_5, 16% Ca, and 8% S-SO_4); common practice) at a dose of 60 kg ha^{-1}. Two rows were sown between rows of the maize crop. The cultural value of seed indicates seed quality and is calculated according to the following formula: Cultural Value = (% germination × % purity)/100.

On April 16, 2018, new soil samples were collected for chemical analysis, determination of fertility (van Raij et al., 2001), and calculation of the doses of limestone, gypsum and hidrated lime to be reapplied according to the treatments. The samples were collected at five random points in each plot in the 0.0–0.2, and 0.2–0.4 m layers; thus, five subsamples were collected from each layer of each plot. The five subsamples were homogenized and pooled to form a composite sample of each layer of each plot. Soil was sampled with a metal probe and air-dried before analysis.

The treatment schedule in the 2018/19 season with details of soil and crop management is shown in Table 7.

Table 7. Treatment schedule in the 2018/19 season

Date	**Activity**
08/30/2018	surface application of limestone and gypsum/hydrated lime
11/27/2018	palisade grass mowing
12/03/2018	soybean sowing
04/17/2019	soybean harvest
04/24/2019	soil sampling
04/29/2019	sunn hemp sowing

Soybean sowing was carried out mechanically under no-till on the palisade grass straw using the soybean cultivar 74HO112TP IPRO Paranaiba. Seeds were sown at a row spacing of 0.5 m and density of 14 seeds m^{-1} (0.0714 m plant spacing). Fertilization was applied at sowing at a dose of 300 kg ha^{-1} of 04-20-20 fertilizer (4% N, 20% P_2O_5, and 20% K_2O) with 4.9% Ca, 3.6% S-SO_4, 0.05% B, 0.06% Mn and 0.27% Zn. Soybean sowing on no-till system is shown in the Figure 4.

Figure 4. Soybean sowing on no-till system, 03/12/2018.

Sunn hemp was sown on the soybean straw in the no-till system at a row spacing of 0.5 m and density of 25 seeds m^{-1} (0.04 m plant spacing) under no-till. Sunn hemp on no-till system, at flowering, is shown in the Figure 5.

Figure 5. Sunn hemp on no-till system, 06/07/2019.

Surface Application of Limestone and Gypsum

In this research three methodology of surface application of limestone and gypsum were evaluated:

T1 - ensure that Ca^{+2} occupied 70% of cation exchange capacity (CEC) in the 0.0–0.2 m layer and ensure that Ca^{+2} occupied 60% of effective cation exchange capacity (ECEC) at a depth of 0.2–0.4 m;

T2 - ensure that Ca^{+2} occupied 60% of CEC in the 0.0–0.2 m layer and ensure that Ca^{+2} occupied 50% of ECEC at a depth of 0.2–0.4 m;

T3 - ensure that Ca^{+2} occupied 50% of CEC in the 0.0–0.2 m layer and ensure that Ca^{+2} occupied 40% of ECEC at a depth of 0.2–0.4 m.

In T4, the control treatment, limestone and gypsum was not applied. In T1, T2 and T3 the limestone and gypsum was applied in a broadcast system (manually) on the soil surface (superficial application) on November 22, 2017 (Spring), on August 30, 2018 (Winter), and on September 12, 2019 (Winter) in T1, T2, and T3 treatments, in no-till and agropastoral systems.

The limestone contained 31% CaO and 21% MgO, and the gypsum contained 17% CaO and 14% S (S-SO_4). To calculate the required amount of limestone, the following equation was used according to the methodology of Borges et al. (2021):

limestone amount (Mg ha^{-1}) = Ca^{2+} saturation in CEC − exchangeable Ca^{2+} content in $cmol_c$ dm^{-3} at a depth of 0.0–0.2 m CEC was calculated as the sum of the contents of exchangeable cations:

CEC = $Ca^{2+} + Mg^{2+} + K^+$ + total acidity ($H^+ + Al^{3+}$) at a depth of 0.0 – 0.2 m

Ca^{2+} saturation (70, 60, and 50%) was calculated as:

100 × Ca^{2+}/CEC at a depth of 0.0 – 0.2 m

To calculate the required amount of gypsum, the following equation was used according to the methodology of Caires and Guimarães (2018):

gypsum amount (Mg ha^{-1}) = Ca^{2+} saturation in ECEC − exchangeable Ca^{2+} content in $cmol_c$ dm^{-3} at a depth of 0.2 – 0.4 m × 6.4

ECEC was calculated as the sum of the contents of exchangeable cations:

$ECEC = Al^{3+} + Ca^{2+} + Mg^{2+} + K^{+}$ at a depth of 0.2 – 0.4 m

Ca^{2+} saturation (60, 50, and 40%) was calculated as:

$100 \times Ca^{2+}/ECEC$ at a depth of 0.2 – 0.4 m

The agronomic characteristics and grain yield of maize in no-till system in the 2017/18 season are demonstrated in Table 8. It is found that treatments differed from each other ($P < 0.05$) only in relation to plant height being control treatment showed higher plant height than T2. Although several studies with superficial application of limestone demonstrate the efficiency of this practice in maize grain yield in no-till system (Miranda and Miranda, 2000; Miranda et al., 2005; Caires et al., 2015), in this research this was not observed, probably because there was no enough time for limestone to react to the ground, since the application of limestone and gypsum occurred only fourteen days before maize sowing. According to Ciotta et al. (2002), surface application of limestone has its reaction reduced by lower contact between soil particles and limestone, consequently delaying its depth action in the profile.

Table 8. Agronomic characteristics and grain yield of maize in no-till system in the 2017/18 season

Treatments	**Insertion height***	**Plant height**		**Final stand**	**Number of cobs**	**One -grain mass**	**Grain yield**
	-------- **m** --------			-------- **ha^{-1}** --------		**g**	**kg ha^{-1}**
T1	1.12	2.00	ab**	66146	48958	35.85	5451
T2	1.09	1.88	b	69271	49479	33.99	5121
T3	1.06	1.96	ab	69792	49479	34.26	5460
T4	1.05	2.04	a	67188	61458	34.66	7410

* Insertion height of first cob. ** Within columns, means followed by the same letter are not significantly different according to least significant difference (0.05).

Source: Borges et al. (2018a).

The water deficit from 01/15/2018 to 02/25/2018 provided a reduction in the number of cobs ha^{-1}, which reflected in low grain yield. According to Santana et al. (2018), on the occasion of flowering, average temperatures greater than 26ºC accelerate the development of this phase, and less than 15.5ºC retarding it and, each degree above the average temperature of 21.1ºC, in the early sixty days after sowing, it can accelerate flowering in two to three days, and 24ºC higher night temperatures, provide increased breathing, causing decrease in the redistribution rate of photoasimilated and consequent reduction in grain yield.

The agronomic characteristics and grain yield of maize in agropastoral system in the 2017/18 season are demonstrated in Table 9. It was found that the treatments did not differ from each other ($P < 0.05$) in relation to the agronomic characteristics and grain yield. As in the no-till system, there was not enough time for limestone reaction in the soil, as surface application of limestone creates a front of the acidity of the soil in depth, proportional to the dose and time (Caires et al., 2000; Rheninheimer et al., 2000).

Table 9. Agronomic characteristics and grain yield of maize in agropastoral system in the 2017/18 season

Treatments	**Insertion height***	**Plant height**	**Final stand**	**Number of cobs**	**One -grain mass**	**Grain yield**
	-------- m --------		**-------- ha^{-1} --------**		**g**	**kg ha^{-1}**
T1	1.03	1.91	69792	63542	32.31	7643
T2	1.02	1.88	64583	64583	33.45	7111
T3	0.99	1.89	70833	60417	31.57	6727
T4	0.98	1.94	71354	65104	35.06	8362

* Insertion height of first cob.

Source: Borges et al. (2018a).

The agronomic characteristics and grain yield of soybean in no-till system in the 2018/19 season are demonstrated in Table 10. It is found that treatments differed from each other ($P < 0.05$) only in relation to one -grain mass being control treatment showed higher one -grain mass than T1. Caires et al. (2003) also did not verify difference in relation to soybean grain yield between control treatment (without limestone and gypsum) and treatments with limestone and gypsum in no-till system in three years of soybean cultivation.

Table 10. Agronomic characteristics and grain yield of soybean in no-till system in the 2018/19 season

Treatments	**Insertion height***	**Plant height**	**Final stand**	**One -grain mass**		**Grain yield**
	-------- m --------		**ha^{-1}**	**g**		**kg ha^{-1}**
T1	0.05	0.79	132500	13.03	b*	4100
T2	0.06	0.84	117778	13.57	ab	3514
T3	0.04	0.76	108889	13.18	ab	4190
T4	0.04	0.78	139167	14.27	a	4735

* Insertion height of first pod. ** Within columns, means followed by the same letter are not significantly different according to least significant difference (0.05).

Source: Borges et al. (2019a).

The absence of soybean response to gypsum application may be related to the fact that soybean root system growth, in the absence of water deficit, is not influenced by the reduction of Al in subsoil (Caires et al., 2001a) and, lack of soybean response to limestone application may be due to surface application of limestone has its reaction reduced by lower contact between soil particles and limestone, consequently delaying its depth action in the profile (Ciotta et al., 2002).

Surface Application of Hydrated Lime

In this research three methodology of surface application of hydrated lime were evaluated:

T1 - ensure that Ca^{+2} occupied 70% of CEC in the 0.0–0.2 m layer;
T2 - ensure that Ca^{+2} occupied 60% CEC in the 0.0–0.2 m layer;
T3 - ensure that Ca^{+2} occupied 50% CEC in the 0.0–0.2 m layer.

In T4, the control treatment, hydrated lime was not applied. In T1, T2 and T3 the hydrated lime was applied in a broadcast system (manually) on the soil surface (superficial application) on November 22, 2017 (Spring), on August 30, 2018 (Winter), and on September 12, 2019 (Winter) in T1, T2, and T3 treatments, in no-till and agropastoral systems.

The hydrated lime contained 40% CaO and 27% MgO. To calculate the required amount of hydrated lime, the following equation was used according to the methodology of Borges et al. (2022):

hydrated lime amount ($Mg\ ha^{-1}$) = Ca^{2+} saturation in CEC − exchangeable Ca^{2+} content in $cmol_c\ dm^{-3}$ at a depth of 0.0 – 0.2 m

CEC was calculated as the sum of the contents of exchangeable cations:

$CEC = Ca^{2+} + Mg^{2+} + K^{+}$ + total acidity ($H^{+} + Al^{3+}$) at a depth of 0.0 – 0.2 m

Ca^{2+} saturation (70, 60, and 50%) was calculated as:

$100 \times Ca^{2+}/CEC$ at a depth of 0.0 – 0.2 m

The agronomic characteristics and maize yield in no-till system in the 2017/18 season are demonstrated in Table 11. It is found that treatments differed from each other ($P < 0.05$) only in relation to plant height being T2 showed lower plant height.

Table 11. Agronomic characteristics and grain yield of maize in no-till system in the 2017/18 season

Treatments	**Insertion height**[*]	**Plant height**		**Final stand**	**Number of cobs**	**One - grain mass**	**Grain yield**
	-------- m --------			**-------- ha^{-1} --------**		**g**	**kg ha^{-1}**
T1	1.01	2.00	a[**]	68750	64063	32.78	6416
T2	0.96	1.84	b	67708	51389	32.77	4172
T3	1.00	2.00	a	67188	64063	34.54	6754
T4	1.05	2.04	a	67188	61458	34.66	7410

[*] Insertion height of first cob. [**] Within columns, means followed by the same letter are not significantly different according to least significant difference (0.05).

Source: Borges et al. (2018b).

The agronomic characteristics and maize yield in agropastoral system in the 2017/18 season are demonstrated in Table 12. It was found that the treatments did not differ from each other ($P < 0.05$) in relation to the agronomic characteristics and grain yield. It is emphasized that the effect of hydrated lime was not probably observed because there was no enough time for the hydrated lime to react to the soil, since the application of hydrated lime occurred only two days before maize sowing.

Table 12. Agronomic characteristics and grain yield of maize in agropastoral system in the 2017/18 season

Treatments	**Insertion height**[*]	**Plant height**	**Final stand**	**Number of cobs**	**One-grain mass**	**Grain yield**
	-------- m --------		**-------- ha^{-1} --------**		**g**	**kg ha^{-1}**
T1	0.97	1.98	69271	65104	34.05	7886
T2	1.00	1.96	65104	59896	32.93	6693
T3	1.04	1.98	66667	64063	34.36	8313
T4	0.98	1.94	71354	65104	35.06	8362

[*] Insertion height of first cob.

Source: Borges et al. (2018b).

The agronomic characteristics and grain yield of soybean in no-till system in the 2018/19 season are demonstrated in Table 13. It is found that treatments differed from each other ($P < 0.05$) only in relation to insertion height of first pod being T1 showed lower insertion height of first pod than T2. All treatments provided plant height greater than 0.65 m. According to Bonetti (1983), the minimum plant height recommended for mechanical harvesting is 0.65 m, considering that lower plants tend to produce equally low pods and therefore difficult to be harvested mechanically (Lazarini, 1995).

Table 13. Agronomic characteristics and grain yield of soybean in no-till system in the 2018/19 season

Treatments	**Insertion height***		**Plant height**	**Final stand**	**One -grain mass**	**Grain yield**
	-------- m --------			**ha^{-1}**	**g**	**kg ha^{-1}**
T1	0.04	b**	0.78	139167	14.27	4735
T2	0.07	a	0.80	133333	15.16	5043
T3	0.05	ab	0.78	133333	14.50	4639
T4	0.05	ab	0.90	131667	14.91	4818

* Insertion height of first pod. ** Within columns, means followed by the same letter are not significantly different according to least significant difference (0.05).

Source: Borges et al. (2019b).

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Chapter 7

Farmers' Poverty Alleviation Strategy Through Poultry Farming: The Importance of Integrated Institutions

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Abstract

Poverty reduction of the population below 10% was targeted by the government of Indonesia. The program called *BEKERJA (Bedah Kemiskinan Rakyat Sejahtera)* or poverty alleviation for peoples' prosperity. The program's target is to make poor farmers free from poverty through the maximized use of their yards. The executors were 4 (four) main institutions and 3 (three) supporting institutions under the Ministry of Agriculture in a mandate to achieve the farmers' welfare.

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This chapter presents the results of the programs' implementation, performance, and the obstacles faced to get an appropriate strategy for future poverty alleviation. The research locations were representative of the 4 (four) institutions, namely Banten, West Java, South Kalimantan, and East Java Provinces. Respondents were 15 related institutions and 30 farmers. Data was collected through group and individual interviews, as well as field observations which were then analyzed qualitatively. Results: program implementation is synergized with the main tasks of each institution. The average performance of the program was 30% and 10 aspects of obstacles were found as the result of a lack of coordination among the institutions. It was concluded that the program's implementation should be under the integration of all institutions as a team in a certain area that was approved by a responsible ministry. The policy's implication is that strategy on poverty alleviation should be tackled under inter-institution coordination.

Keywords: *BEKERJA*, farmer, institution, poverty alleviation

Introduction

Efforts to reduce poverty levels continue to be carried out by the government of Indonesia. It was targeted that the reduction of poverty will be in the range of 8.5-9.0% by 2022 (Said, 2021), down from 10.14% (27.54 million poor people) in March 2021 (BPS, 2021). The national poverty reduction program was reported as being more effective and equitable when it is prioritized in rural areas (Tarigan et al., 2019), therefore in 2018 a poverty alleviation program directed at poor farmers was launched. The program called *Bedah Kemiskinan Rakyat Sejahtera (BEKERJA)* or poverty alleviation for peoples' prosperity. The programs' design is based on Minister of Agriculture regulation No. 27/PERMENTAN/RC.120/5/2018 which states that: 1) The target is to make poor farmer households free from poverty by maximizing the use of their yard. 2) Poor family beneficiaries are those who are recorded in available data from the Ministry of Social Affairs and then approved by the Ministry of Agriculture. 3) Yard farming packages consist of 50 healthy poultry (chickens or ducks), feed, barn, and health services accompanied by the socialization of poultry farming as well as facilitators or assistants (Kementan, 2018). A special package of vegetables and annual crops was distributed to those who own land. The delivery of the program's package should be managed by an institution responsible for the program.

There are 4 (four) main institutions who responsible or *Penanggungjawab* (PJ) for the program's execution. They are: 1) *Badan Penyuluhan dan Pengembangan Sumberdaya manusia Pertanian (BPPSDMP*) or Extension and Human Resources Agricultural Agency; 2) *Badan Penelitian dan Pengembangan Pertanian (BALITBANGTAN)* or Agency for Agricultural Reseach and Development; 3) *Direktorat Jenderal Peternakan dan Kesehatan Hewan (PKH)* or Directorate General for Animal Husbandry and Veterinary Services and 4) *Badan Ketahanan Pangan* (BPK) or Agency for Food Security. Each institution has responsibility for a certain location that is targeted for poverty alleviation programs, namely the Province of Banten, West Java, South Kalimantan, and East Java for institutions number 1 to 4 respectively. Besides the four main agencies, there are three other institutions responsible for supporting the availability of the barns, namely *Direktorat Jenderal Sarana dan Prasarana Pertanian (PSP)* or Directorate General of Agricultural Facilities and Infrastructure*, Direktorat Jenderal Hortikultura*, or Directorate General of Horticulture for supporting the horticulture and *Direktorat Jenderal Perkebunan or* Directorate General of Plantation who supports the annual plant.

The implementation of the poverty alleviation program through various institutions that have certain responsibility related to agriculture development is expected to perform the maximum goal of the program which is to maintain "farmers' welfare." Such efforts need to be studied to obtain an appropriate poverty alleviation strategy for farmers in the future. This paper illustrates how the four institutions executed the program, the performance, and the obstacles that influence its performance.

Methodology

Locations

Research locations were decided based on the results of discussions with the 4 (four) main institutions responsible for the program (Table 1). Each province is represented by one district that has received the complete program package and the poultry are already in the production phase. From each district, at least 2 (two) villages that had representatives of good, medium, and low-performance farmers were selected through snowball sampling (Parker et al., 2019).

Data

Primary and secondary data were obtained from 7 (seven) national and provincial 4 (four) level institutions. A special focus group discussion (FGD) was conducted at 4 (four) districts attended by 15 participants consisting of representatives from the district, sub-district, village staffs, and farmers. In-depth interviews based on the structured questionaires were conducted with individual farmers from 10 villages, with 3 farmers from each village representing the best, medium, and lowest performances. The main questions consisted of the program's execution at the beneficiaries' level, the performance, and the obstacles encountered. Then the data were analyzed qualitatively (Evers, 2015).

Table 1. Research locations for each responsible institutions' program in 2018

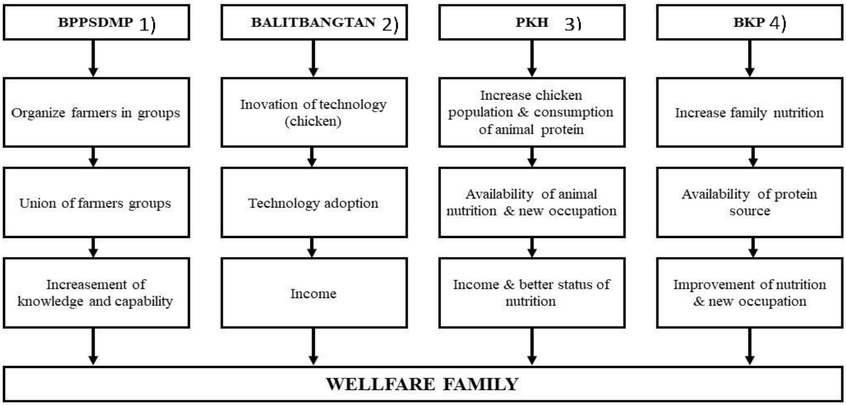
No	**Locations**	**Responsible Institutions**			
		BPPSDMP	**Balitbangtan**	**PKH**	**BKP**
1	Province	Banten	West Java	South Kalimantan	East Java
2	District	Pandeglang	Tasik Malaya	Hulu Sungai Utara	Jember
3	Sub-district	Saketi Keroncong Cipeucang	Salopa Condong hilir	Amuntai Selatan Amuntai Tengah	Gumuk Mas Ledokombo Kalisat
4	Villages	1.Parigi 2.Sukajaya 3.Pasir mae	1.Tanjungsari 2.Sepat Manunggal	1.Kayakah 2.Tapus	1.Karang rejo 2.Sukogiri 3.Sumber katempa

Source: Primary Data.

Results and Discussions

The Design and Implementation of the Program's Goals

To achieve the goals of the program, each institution has its technical guideline (Figure 1) as the basis for the program's implementation. Since there were no details or steps for the implementation, each institution adjusted the implementation of the BEKERJA program to reflect their main responsibility or as part of the supporting responsibility. All institutions try to increase farmers' welfare through a certain method that could be synchronized with their main tasks and functions to get efficient resources such as funding and human resources (assistance staff) during the program's implementation.



Source: 1) BPPSDMP (2018). Technical guidance for the implementation of BEKERJA Program; 2) BALITBANGTAN (2018) Technical guidance for the implementation of BEKERJA Program; 3) DGLS (2018) Technical guidance for the implementation of BEKERJA Program; 4) BKP (2018) Technical guidance for the implementation of BEKERJA Program.

Figure 1. Poverty Allevation Approach of Each Institution.

The figure illustrated that each institution adopted certain indicators of farmers' welfare namely: BPPSDMP which increases the knowledge and capability of the farmers through the farmers' group union as a part of their education; BADANLITBANG focuses on the technology adoption by introducing innovation about chicken technology and the existence of new types of chickens, which actually could also be classified as part of education. These two different ways are part of education which is one of 24 poverty alleviations' single indicators stated by BPS (2021). Increasing or improving education has had a remarkable result in poverty alleviation as reported by Xue Eryong and Zhou Xiuping (2018) who stressed that poverty eradication must rely on intellectual support. A case in Indonesia reported by Islami and Anis (2019) also emphasized that poverty could be overcome through education.

Another indicator of farmers' welfare in Indonesia is the level or status of nutrition (BPS, 2021). PKH and BKP have been adopted to increase the farmers' welfare. How poverty and malnutrition are deeply interrelated was reported by Siddiqui et al. (2020). Therefore, tackling poverty alleviation should be done in synergy with nutritional status because of both elements being a cause and consequence of each other. This finding is also supported by Larsen et al., (2014) who reported that improving food security for poverty alleviation has a strong positive effect.

Besides single indicators, there are multiple indicators used to measure welfare such as the Human Development Index (HDI), measuring the dimensions of healthy life (nutrition is an important component). Knowledge is a component of education, and a decent life (income is part of the indicator). If the goals of each institution are packed into one output such as HDI, it is believed that the program will be meaningful. Consequently, the program must be implemented in one location (not in different locations as with BEKERJA). By selecting one location for the implementation of the program, then all institutions can be integrated into a team to execute the program starting from increasing the knowledge of the farmers in raising poultry (BPPSDMP), introducing the technology of raising new breeds of poultry that have high production (BALITBANGTAN). The higher production of poultry will provide a better source of animal protein (PHK) intake as well as increasing family income.

The Desgin and Implementation of Farmers' Beneficiaries

The steps for the beneficiary's selection are presented in Table 2 as 1) Being included in the Ministry of Social's version of the list of poor families; 2) Meet the criteria as a poor household after going through verification by the verifier team; 3) Determined by letter and ratified by the Commitment Officer. The verifier team includes the central team (4 main institutions) together with the relevant district offices, the District Social Workforce (TKSK), and village officials. The results of the verification are submitted to the provincial supervisors to be submitted to Echelon 1 of the Ministry of Agriculture; 4) Verification of the village of beneficiaries is carried out in two stages, namely, i) on-desk verification by TKSK and village officials, and ii) verification of sampling in the field as well as in direct interviews with PFH. Validation of PFH data set through *Kepmensos* No. 57 of 2017 which has been selected according to the criteria for the agricultural sector. If there is information that is out-of-date, such as the PFH having died with no heirs, the PFH has moved residence, the PFH has increased their welfare, the PFH does not have electricity or a yard according to the criteria, it is excluded from the list of recipients. Furthermore, there is verification of sampling in the field by the verifier to see and interview PFH who questions the information. PFH sampling was selected around 7.5 – 10% of the total number of PFH in the beneficiary villages.

Table 2. The design and implementation of farmers' beneficiaries based on the responsible institutions

No	**The design of the beneficiary's selection**	**The implementation of beneficiary's selection**			
		BPPSDMP	**BALIT BANGTAN**	**PKH**	**BKP**
1	The PHF main occupation is as farmer	Yes	Yes/no	Yes	Yes
2	Registered as PFH by the Ministry of Social (Integrated Data No. 57 – 2017	Yes/no	Yes/no	Yes/no	Yes/ no
3	has been set in the list of poor sub-districts based on MOA No 27 PERMENTAN/RC.120/5/2018	Yes/no	Yes/no	Yes	Yes
4	The verification should be carried out at the village level	Yes -	Yes-	Yes-	Yes +
5	Own land/space for the barn	Yes/no	Yes/no	Yes/no	Yes
6	Beneficiaries in the productive age	Yes/No	Yes/no	Yes/no	Yes/ no
7	Total beneficiaries 7.5 – 10% of the total number of PFH in the beneficiary villages	No	No	No	No

Source: result from the analysis of primary data.

Unfortunately, the design was not easy to be fully implemented, namely, all district institutions reported that the database from the Social Ministry (2017) was out of date which makes it difficult to identify. The solution is to have up-to-date data at the village level (2019) which was used as the reference, and new beneficiaries were proposed. This fact impacts the data from the Ministry of Agriculture such that it also cannot be used as a complete guideline. The verification that should be carried out at the village level was also found to be not as the design because during the field survey some beneficiaries were found who had big permanent houses. When the team asked the program assistant for an explanation of the findings, it was explained that "the determination of this beneficiary of the program was chosen intentionally to prove that if the chickens are raised by non-poor families, success could be guaranteed and used as an example or demonstration for the beneficiaries at the village." The intention of choosing beneficiaries from non-poor farmers was also in anticipation of poor farmers selling the chickens while still under the growing or production phase which is contrary to the rules of the program.

A special beneficiary's selection at the village level was found in East Java (BKP), in which the verification and validation data were done by a special team from the district to village levels consisting of 90 people under decree No.188.45/470/1.12/2018 (The Regional Planning Agency, 2018). The team consisted of the steering committee from the district level, such as the district head, the head of the prosecutor's office, the head of the police station, and chaired by the district secretary. At the sub-district level, all the sub-district heads were involved while at the village level were all village heads, the household organization or Rukun Warga, and the head of neighbourhood or Ketua rukun tetangga. This special team decided on the beneficiaries based on 4 social indicators and 5 technical indicators, and the results of this selection came out with 19% rejected beneficiaries which is only 12,698 from 15,606 PFH (Wahyuni and Tarigan, 2020).

The requirement of available land for the barn is also difficult to implement considering that poor farmers have very narrow and limited space for houses, therefore it is almost impossible to have space for the chicken barn. This fact can easily be found by the research team during the survey. The chicken barn from the program is lined up in front of their homes, and beneficiaries or those who had no land to put the barn just placed it across the road in front of their house. Information about the loss or disappearance of the barn with all the chickens inside was also reported by the beneficiaries because the barn was close to the main road that was very easily transported by a pickup truck.

Additional criteria were added according to the needs of the institution. The BPPSDMP, PKH, and BKP have certain criteria that prefer beneficiaries to be members of Kawasan Rumahtangga Pangan Lestari (KRPL) or the Sustainable Food Household Zone. Instead, BALITBANGTAN prefers farmers who have not joined the KRPL or any other group. This requirement was an emphasis where each institution could implement the program effectively in synergy with their main responsible activities. Regarding the use of farmer groups as an effective forum for program implementation, there have been many studies and recommendations (Rahmadanih et al, 2018; Othman et al, 2020). The importance of the farmers' group as a learning class, a place of cooperation, and a production unit was also emphasized by Kiptot and Franzel (2019).

Another difference and difficulty for the village officials in beneficiary selection was that only around 7.5 – 10% of the total number of PFH in the beneficiary villages were eligible, considering that the majority of the population in the village are poor. This regulation created a particular problem such as social jealousy in the village before the program was implemented. It is suggested that the next poverty alleviation program should be allocated at certain locations so that there would be greater eligibility at all village levels.

The Design and Implementation of the Package of the Program

The BEKERJA program was a design package consisting of 50 head of poultry (chicken or duck) per poor farmer household, a ready barn, feed, and complete vaccine and assistance or facilitation without detailed criteria on the breed, the sex ratio, and age of the poultry. Based on the 7 item package design, only the number of chickens was same design item. All other designs were different in the implementation (Table 3).

These differences in packages have consequences in the way of raising or managing the poultry, since all packages included new technologies for the farmer. Raising a new breed of poultry was difficult because farmers are accustomed to raisng chickens in small numbers, with no vaccine, being fed with kitchen waste, all of which do not require special time but as a side activity and without a barn. For farmers who receive Day Old Chicks (DOC) which need special care in feeding and health care (vaccination), this requires new knowledge. With the differences in design and implementation, the District Volunteer Workers in charge of assisting 50 PDF, had problems with the program's performance. Therefore, even though the beneficiaries had received the socialization of the raising system it is still all new and not easy to implement. This is especially true since the majority of farmers (85%) who are responsible for raising the poultry were the wives (Tarigan and Wahyuni, 2020).

Table 3. The Design and Implementation of the Beneficiaries Package Based on the Responsible Institutions

No	**Package design**	**Implementation**			
		BPPSDMP	**Balitbangtan**	**PKH**	**BPP**
1	Supplier of chicken	Office of Artificial insemination centre- Lembang	Livestock research centres and private companies	Government breeding centre BPTU- HPT *Pelaihari*	Private companies PT Sumber Unggas
2	Breed of poultry	Crossbreed chicken	Superior native chicken (*KUB*)	Native Duck *(Alabio)*	Crossbreed chicken
3	Number of Chicken (tails)	50 mix (male + female)	50 unsexing	50 (male+ female)	50 (male + female)
4	Age	2 months	DOC	1 month	1-2 months
5	Vaccine	ND and AI	Mareks and ND-IB	ND and AI	ND and AI
6	Feed	Grower-layer	Starter – grower-layer	Grower - layer	Grower-layer
7	Ready Barn	Ready barn	Brooder n other facilities	IDR 500.000	Ready barn and IDR.500.000

Source: Primary data.

The results of another study about this program in the Sukabumi district reported by Karyadara et al., (2022) is that the implementation of the work program has not run optimally because socialization has not fully touched Poor Households (RTM), and data collection uses old data. The inhibiting factor is that the number of companions is not proportional to the number of RTM, and the amount of feed given is not sufficient until the harvest. However, the supporting factor is the existence of guidelines for the implementation of work programs.

The Performance and Obstacles of the Program

To be free from poverty, a household should have income more than the poverty line or *Garis Kemiskinan* (GK) in Indonesia as IDR.401,220 / capita/month or IDR.1.84 million/household / month (BPS, 2019). Based on the Minister of Agriculture regulations No. 27 / PERMENTAN/ RC.120/5/2018, the expected farmers' additional income from the Program

BEKERJA is IDR.2 to 2.5 million per month (Reisha, 2019). The income is calculated from the development of chicken production which is monitored by an assistant called *Tenaga Kerja Sukarela Kecamatan* (TKSD) or District Volunteer Workers PDF for BADANLITBANG location while a finance management unit activity or *Unit Pengelola Keuangan Kegiatan* (UPPK), based on certain indicators for BKP. However, monitoring activities could not run as expected due to the limited number of TKSD, a TKSD in charge of assisting 50 while *a UPPK* must be responsible for 100 and there is even a UPKK that has to handle 600 beneficiaries. Therefore, not all beneficiaries could be well monitored. The results of the up-to-date data during the survey were from the UPPK estimation on the number of beneficiaries who still keep raising the poultry. Furthermore, information on the performance is collected based on UPPK's guidance to meet the beneficiaries who had best, moderate, and low performance to be interviewed. The results as presented in Table 4 is without mention of income since the beneficiaries have no special performance record for the program.

Table 4. The performance of the existing poultry based on the responsible institutions in 2019

No	Performance of the poultry	Responsible Institutions				Average
		BPPSDMP	Balitbangtan	PKH	BKP	
1	Best performance (Layers)	9	18	> 20-25	7	16
2	Moderate performance (Layers)	3	11	> 15-20	3	11
3	Low performance (Layers)	1	7	10--< 15	0	6
4	The Existing Poultry (%)	13	26	66	20	30

Source: Primary data.

BPPSDMP

It was reported that the majority of the poultry were dead, caused by attacks of disease. Early symptoms were becoming limp and finally the chicken dies (about 60%). The first attacks happen one month after the chickens were delivered and when the second attack happened, farmers decided to slaughter the limp chickens for consumption, only leaving about 10 chickens.

The best beneficiaries' performance had 9 layers in various phases; 2 are incubating, 2 are laying eggs and 5 are hatching. The beneficiaries are reported to have increased consumption and income from the sale of chicken eggs but we have no detailed record. The moderate beneficiaries had 3 layers, each producing 8, 9, and 11 eggs which hatched into 2, 5, and 6 chickens while the lowest beneficiary's performance had 1 layer and 2 chickens left.

BALITBANGTAN

The implementation of the BEKERJA Program in Tasikmalaya Regency found a delay in dropping the DOC so that only 70.86% were sent. At the same time, it was reported that mortality reached 88%, mainly due to the lack of feed. The PFH cannot afford to buy factory feed for DOC when the feed delivery was too late. The delay in feed is mainly because the funding in the 2018 fiscal year was no longer available, while the funding at the beginning of the year is not available yet.

The beneficiaries that still raise poultry is around 26%, in which the best, moderate, and lowest beneficiaries still have 18, 11, 7 layers respectively and they also had sold the male chickens to buy bran and for consumption. Farmers who are successful in maintaining adequate chicken feed are famers who can buy bran and have leaves such as cassava from their yard. The bran and leaves were used as a substitution for chicken feed.

PKH

The BEKERJA program package in South Kalimantan included *Alabio* ducks that were specifically requested by the head of the Province Agriculture offices, especially to preserve the development of local ducks, which are already sustainable in the location as 90% of the area is swamp and farmers are familiar with the raising system and management. Farmers already know about duck farming and it is also supported by the existence of duck breeding institutions, egg marketing, duck hatcheries, and cooperatives for marketing the main products (DOD and broiler ducks) as well by-products such as duck feathers and duck bone meal. Genetically, ducks are more disease resistant than chickens therefore the performance of the duck is good. From the total beneficiaries of 1,588 PFH with a total number of ducks reaching 79,400 tails, during the study there were still 51,660 tails (65.75%). From this finding, a lesson learned could be how important is the existing natural and cultural resources in a program? The target location should be taken into consideration as the source or tipping point of development. A similar finding was also reported by (Jordaan et al., 2014) that stakeholders tend to neglect the influence of the existing structure and farmers' behavior in the development process.

The best beneficiaries' performance had > 20-25 layers, the moderate beneficiaries had > 15-20 layers and the lowest beneficiary's performance had 10-< 15 layers. This success was achieved through management such as when the female ducks start laying eggs at the age of 6 months the eggs are collected first then hatched about one week later. The cost of a hatching reaches IDR.2,000/egg. Every 125 eggs that are hatched could produce 30 DOD which can be directly sold for up to IDR.15,000/head, while unhatched eggs are usually consumed or sold as salty eggs. The income from selling the DOD and eggs was prioritized to buy ducks' feed, the rest is to meet the family food needs and even help pay for childrens' school fees as well as buy clothes for "Hari Raya." This outcome could definitely alleviate PFH from poverty and provide a new source of occupation. The success story reported by the farmers shows that the duck farming industry has been able to eradicate poverty. Industrial development as a means of poverty alleviation is an integral part of the "Five-pronged Poverty Alleviation Measures" which had a significantly positive effect on the livelihood of farmers (Liu et al., 2021).

In the case of the BEKERJA program, the package provided uses free business capital so it is hoped that it will give maximum results. However, we refer to the finding of Mhlanga et al., 2020 who emphasized that to tackle poverty, especially among the smallholder farmers, encourages financial inclusion such as to provide easy access capital instead of the free package as the better strategy.

BKP

The BEKERJA program in Kabupaten Jember started in May 2018 but the package was only delivered at the beginning of rainy season to September, therefore, it was reported to cause quite a high mortality and over time the mortality increased until only 20% of chickens were left. The delay of the package delivery was caused by the verification of the beneficiaries as described in the design and implementation of the beneficiaries' selection.

The best beneficiaries' performance has 7 layers, the moderate has 2-5 layers and the lowest had an empty barn left. The existing beneficiaries reported that the achievement was supported by the wives of the PFH who took care of raising the chickens since they spent most of their time around the house. Most of the existing beneficiaries used to sell the female chickens on purposed and only spare one cock as parent stock because 50 chickens are too big a number for them. Besides taking quite a lot of time, it also requires a large amount of capital for the feed. It is recognized that raising superior chickens is not as easy as native chickens, especially in large quantities at once. To solve this case Lalljee et al., (2019) reported that small ruminant farming generated income in most instances. The way it is practiced creates opportunities for simple changes in husbandry and management that could make it more efficient in poverty alleviation.

Conclusion and Recommendation

Conclusion

1) The implementation of the BEKERJA program was synergized with the main tasks of each institution. Each institution has a different output of a single poverty indicator such as education, nutrition status, and income which are part of multiple poverty indicators called the Human Development Index (HDI). To get the HDI value, all institutions should execute the program in integration and at the same location.
2) The design of beneficiaries was difficult to be implemented especially because the data was out of date and the limited number of beneficiaries can create social jealousy, therefore, implementing the program for all poor farmers in a certain village would be worthwhile.
3) The design of the packages was not in the appropriate timing, season, quantity, and quality, therefore, an integrated institution as a working team is needed.
4) The average performance of the program was low (33%) as the effect of 10 obstacles such as disease, new poultry breeds, new rearing systems, shortage of feed, shortage of monitoring personnel, the

scattered locations of beneficiaries, too many chickens, the need for special attention and time. Farmers also need to be given a special, simple card to record livestock development and poultry raising. A demonstration plot is needed at each program location.

5) To avoid all these obstacles, a success story of beneficiaries in South Kalimantan should be added.

Policy Implication

To get an appropriate strategy for the poverty alleviation of farmers needs, integration of certain institutions as a team under legal authorizations of the Ministry of Agriculture is needed. The team should execute the program in a certain location with multiple indicators of welfare such as HDI. The sources of the program packages should come from the existing natural sources at the program location, complete with supporting institutions.

Disclaimer

None

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Chapter 8

Consumer Aspects on the Disposal of Waste Vegetable Oil

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Abstract

The amount of waste oil produced by people in parallel with the level of life and development with the increase in population in the world has increased. As a result, the fact that the waste oils given to nature become threatening to the environment and life has caused the waste oil problem. All waste vegetable oil must be included in the waste oil collection system in order to prevent environmental pollution, protect the environment and human health. The users of this system are people producing waste oil. For this reason, people need to remove waste oil consciously. In this study, a survey has been conducted in Bursa with 384 participants in order to determine the level of knowledge about how people evaluate, collect and dispose of waste vegetable oil from their houses. The results of the survey have been statistically analyzed with the analysis of variance method. As a result of this survey, it was concluded that the people of Bursa do not have sufficient awareness about waste oil collection, are not aware of Waste Vegetable Oil Collection Centers or the number of these centers is not sufficient.

Keywords: Bursa, environmental pollution, statistical analysis, survey, waste vegetable oil

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Introduction

The increased consumption with industrialization, population growth, and high living conditions causes the depletion of natural resources. To protect natural resources and prevent environmental pollution, it is necessary to reduce the amount of waste, expand recycling, and save energy and water. By bringing valuable waste into the economy, environmental pollution is prevented, and resource use is reduced (Gökalp et al., 2018).

Vegetable oil consumption depends on food demand by population and income growth in the world (FAO, 2020). While vegetable oil production was 203 million tons worldwide in 2019, consumption was 200 million tons (URL-1, 2022). In Turkey, 21 kg of vegetable oil is consumed per person per year, and the amount of vegetable oil used in the kitchen is up to 1.7 million tons (Yalili Kilic and Kilic, 2017). Vegetable oils used for frying in kitchens lose their physical and chemical properties after a while and become waste oil that has a carcinogenic effect on human health (Chen et al., 2014). Waste vegetable oils constitute 25% of wastewater pollution and harm birds, fish, and other species when reaching the receiving environment. Mixing waste vegetable oils into the garbage is prohibited. Waste vegetable oils spilled into garbage cause fire in solid waste landfills. Also, waste vegetable oils cause groundwater pollution (Anonymous, 2010). For these reasons, it is significant that waste vegetable oils are disposed of safely or recycled in a way that is not harmful to humans and the environment.

Some countries carry out various applications in collecting waste vegetable oils from homes, restaurants, and industrial establishments. It aims to collect 2.4 kg of waste per person in the European Union, but 1.2 kg of waste vegetable oil can be ordered (URL-2, 2022). Some examples from various countries show how to collect waste vegetable oil. In Belgium, the collection of waste oil in homes is overseen by local governments. For this purpose, there are collectors that visit door to door in the neighborhoods, but there are also regional waste collection centers. Waste vegetable oils are collected in 50-200 liter plastic and metal containers by collecting companies in Germany between 1 week and two months. To collect waste vegetable oil produced in houses in Austria, 3-liter containers are distributed to households, and when these containers are filled, they are requested to be emptied into heated tanks in waste collection centers established in certain areas in the neighborhoods. The National Consortium for Mandatory Used Oil Collection was established in 1998 to coordinate member businesses and find solutions for the collection, transportation, storage, and processing of waste oils throughout Italy. The consortium works with local and government officials on collecting and evaluating waste oils and conducting training activities to raise public awareness (Çanakcı, 2011).

In this study, a survey was conducted to determine the people's awareness about waste vegetable oil collection, and the results were evaluated. The study participants were selected voluntarily, and the questions were asked to people face to face in three main districts of Bursa city, Turkey. It is aimed to determine people's knowledge level about how to remove waste vegetable oils, waste vegetable oil collection systems, the usability of waste vegetable oils in different sectors, and the environmental effects of waste vegetable oils in this survey.

Materials and Methods

In this study, a survey was conducted to determine the current waste vegetable oil collection situation in Bursa, Turkey. For this purpose, the survey questions were asked to people living in Nilufer, Osmangazi, and Yildirim, three significant districts of Bursa city. The survey, which consists of personal information and evaluation of waste vegetable oils, was applied to 384 volunteer people who were randomly selected. The sampling error was accepted as 5% according to the simple random sampling method as described by Yazıcıoğlu and Erdoğan (2004).

A confidence analysis was conducted for internal consistency. Kolmogorov-Smirnow and Shapiro-Wilk normality tests were used to test the normality of all questions (Drezner et al., 2010; Hanusz and Tarasinska, 2015). SPSS 23 (IBM, USA) was used to analyze the data, and the frequency and factor analyses have been carried out (Gustafsson, 1992; Bartholomew, 1995; Dolan, 1997; Düzgüneş et al., 2015). In addition, whether the differences between the demographic characteristics of the participants were statistically significant for the answers to the questions were determined by analysis of variance.

Results

In this survey, 59% of the participants are female and 41% are men due to the application of the survey at daytime. 40% of them are university graduates and

50% are in the 26-45 age range, which indicates that the survey is conducted with a more realistic approach by people with a high level of awareness. 62% of the participants have a household size of 3-4 and 65% of them are married. 28% of the participants work in a private sector and salary of 61% of the participants is above the minimum wage (Table 1).

Table 1. Demographic characteristics of the participants

Demographic characteristics	**Frequency**	**Mean**	**Standard deviation**	**Demographic characteristics**	**Frequency**	**Mean**	**Standard deviation**
Gender	**(%)**			**Education status**	**(%)**		
Female	59		0.49	Primary school	25		0.92
Male	41			High school	29		
Age				University	40		
18-25	22	28.5	0.83	Graduate Education	6		
26-45	50			**Job status**			
46-59	22			Unemployed	22		1.57
> 60	6			Worker	19		
Marital status				Officer	10		
Married	65		0.47	Private sector	28		
Single	35			Student	15		
Household size				Retired	6		
< 2	17	3.5	0.62	**Income status**			
3-4	62			Below minimum wage	17		0.77
5-7	21			Minimum wage	22		
> 8	-			Above minimum wage	61		

Reliability analysis was conducted to test the reliability of the questionnaire data used in the study. In this analysis, Cronbach's alpha question group are one of the most used tests to determine the reliability of the data. According to this test, if the Cronbach alpha value is above 0.70, it can be said that the data used in the study has reliability. The Cronbach's alpha value obtained as a result of the Cronbach's alpha test applied to the data used in the study is 0.723 and it can be said that the data used in this study are reliable. In addition, Kolmogorov-Smirnow and Shapiro-Wilk normality tests were also applied to the data. As a result of these tests, the study data did not show a normal distribution. For this reason, non-parametric tests were used on the data.

The answers given to the waste oil questions according to the education level are given in Table 2. According to Table 2, it has been determined that 39% of the university graduates take the waste oil to a collection center of the municipality, 16% pour it into the sink and 37% throw it in the trash. In addition, 32% of primary school graduates and 27% of secondary school graduates stated that they take the waste oil to a collection center in the

Table 2. Distribution of responses to question about waste oil according to educational level

Questions	**Primary-Secondary School**		**High School**		**University**		**MSc and above**		**Total**	
	F	**P (%)**	**F**	**P (%)**	**F**	**P (%)**	**F**	**P (%)**	**F**	**P (%)**
How do you remove the vegetable waste oil from your home?										
I pour it down the sink	12	12.5	29	26.4	25	16.3	6	25	72	18.8
I'm trashing	43	44.8	31	28.2	57	37.3	7	29.2	138	36.0
I'm saving and taking it to the collection center	31	32.3	30	27.3	59	38.6	6	25	126	32.9
No idea	10	10.4	20	18.2	12	7.8	5	20.8	47	12.3
In your opinion, how can waste vegetable oils be evaluated?										
Recycleable	50	52.1	62	56.4	77	50.3	15	62.5	204	53.4
It can be used as raw material	19	19.8	25	22.7	43	28.1	7	29.2	94	24.6
Cannot be recycled or used as raw material	3	3.1	4	3.6	13	8.5	0	0	20	5.2
No idea	24	25	19	17.3	19	12.4	2	8.3	64	16.8
What do you think will happen if the waste vegetable oils are not collected?										
Clogs sewer pipes	26	27.1	18	16.4	25	16.3	7	29.2	76	19.8
Causes great damage to the environment	35	36.5	50	45.5	66	43.1	9	37.5	160	41.8
Transforms into a poisonous form and harms living things.	23	24	34	30.9	51	33.3	5	20.8	113	29.5
No idea	12	12	8	7.3	11	7.2	3	12.5	34	8.9
Do you know that 1 liter of waste oil pollutes 1 million liters of clean water?										
Yes	47	49	48	43.6	83	54.2	10	41.7	188	49.09
No	49	51	62	56.4	70	45.8	14	58.3	195	50.91

F: Frequency, P: Percent.

Table 3. Distribution of response to question about waste oil evaluation and collection according to educational level

Questions	Primary-Secondary School		High School		University		MSc and above		Total	
	F	P (%)	F	P (%)	F	P (%)	F	P (%)	F	P (%)
What would you do if you saw waste vegetable oils spilled into the environment?										
I get very angry	39	40.6	18	16.4	32	20.9	4	16.7	93	24.3
I try to warn those concerned	49	51	70	63.6	95	62.1	14	58.3	228	59.5
I take legal action	2	2.1	7	6.4	2	1.3	1	4.2	12	3.1
I don't care much	6	6.3	15	13.6	24	15.7	5	20.8	50	3.1
What do you think should be done to make the public more conscious about collecting vegetable waste oil?										
There should be more broadcasts on this subject on television and radio.	23	24	22	20	50	32.7	1	1	96	29.7
This issue should be studied in detail in schools	5	5.2	0	0	5	3.3	0	0	10	3.1
The society should be warned with various posters, flyers and announcements	19	19.8	25	22.7	23	15	0	0	67	20.7
Fines should be applied	3	3.1	1	0.9	8	5.2	0	0	12	3.7
All	46	47.9	62	56.4	67	43.8	0	0	175	54
Which of the following do you think would be more beneficial if it was produced from vegetable waste oils?										
Fuel	30	31.3	33	30	56	36.6	7	29.2	126	39
Biogas	5	5.2	4	3.6	12	7.8	5	20.8	26	8
Biodiesel	10	10.4	15	13.6	26	17	5	20.8	56	17.3
Chemical product	11	11.5	8	7.3	18	11.8	2	8.3	39	12.1
No idea	40	41.7	50	45.5	41	26.8	5	20.8	136	42.1
Which of the economic and ecological choices is more important for you in the evaluation of waste vegetable oils?										
Economic development	6	3	10	9.1	8	5.2	1	4.2	25	7.7
Nature protection	53	55.2	58	52.7	59	38.6	10	41.7	180	55.7
Economic development with ecologically balanced	24	25	32	29.1	80	52.3	13	54.2	149	46.1
No idea	13	13.5	10	9.1	6	3.9	0	0	29	8.9

F: Frequency, P: Percent

municipality. On the other hand, to the question about what will happen if waste oil is not collected, 36.5% of primary school graduates, 45.5% of secondary school graduates, 43.1% of university graduate,s and 37.5% of graduate and higher graduates answered that they would harm the environment.

Table 3 shows the variation of the answers given to the questions about the collection and evaluation of waste oil according to the education level of the participants. To the question of what should be done to increase the participation of the public for the collection of waste vegetable oils, the answer was given that all options should be applied at most for all educational situations. In addition, 37% of university graduates suggested using waste oil as fuel, while 27% stated that they had no idea. To the question of which is the most important economic and ecological option in the evaluation of waste vegetable oils, university and master's graduates gave the most answers to ecology and balanced economic development, while primary and secondary school graduates gave the most answer to nature protection.

Table 4. Factor analysis of public awareness level in waste vegetable oil collection

Factor	**Factor 1**	**Factor 2**	**Factor 3**	**Communalities (hi)**
In your opinion, how can waste vegetable oils be evaluated?	0.691			0.526
Do you know that 1 liter of waste oil pollutes 1 million liters of clean water?	0.618			0.482
Which of the following do you think would be more beneficial if it was produced from vegetable waste oils?	0.614			0.667
How do you remove the vegetable waste oil from your home?		0.586		0.463
What do you think should be done to make the public more conscious about collecting vegetable waste oil?		0.547		0.356
Which of the economic and ecological choices is more important for you in the evaluation of waste vegetable oils?		0.513		0.305
What would you do if you saw waste vegetable oils spilled into the environment?			0.734	0.578
What do you think will happen if the waste vegetable oils are not collected?			0.544	0.436

Table 4 shows the results of factor analysis regarding people's awareness of waste oil collection. All the factors affecting the answers given by the survey participants to the questions were gathered under three main factor groups. According to the results of the factor analysis, the question most affected by the factors defining the participants what was would you do if you saw waste vegetable oils spilled into the environment emerged. The least affected question is which of the economic and ecological choices is more important for you in the evaluation of waste vegetable oils has been.

The results of the analysis of variance regarding whether the differences between the questions asked in the study and the answers given to the questions of 8 characteristics such as gender, education level, township etc., of the participants are statistically significant are given in Table 5. According to the results of the analysis, the differences between gender, education level, the district where the participants lived, and the ages of the participants were found to be statistically significant ($P < 0.05$). The differences between the participants' occupation, income status and other characteristics such as the number of people in the family are not statistically significant in terms of the answers given to the questions ($P > 0.05$). The differences between the genders of the participants are statistically significant for the answers given to the first survey question. The differences in the answers between the genders of the participants in the question about what is made of waste vegetable oils are important. This shows that genders have different approaches to the evaluation of waste oil. As for the education level of the participants, the differences between the 15th question and the district they live in are statistically significant for the answers to the 3rd question. In addition, gender-age, gender-district, education level-district and gender-education level-district iterations were also found to be statistically significant in terms of different questions ($P < 0.05$).

Table 5. The results of the analysis of variance among factors in the survey

Factor	**Question**	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Gender	1	3.702	1	3.702	4.485	0.035
Educational level	15	33.918	4	8.480	3.050	0.017
Town	3	5.103	2	2.552	3.192	0.042
Gender*Age	1	10.135	3	3.378	4.093	0.007
Gender*Town	1	6.518	2	3.259	3.948	0.020
Educational Level*Town	2	18.154	6	3.026	2.558	0.020
Gender*Educational Level*Town	3	12.265	6	2.044	2.557	0.020
	5	10.878	6	1.813	2.429	0.026

Conclusion

In this study, the people's awareness about waste vegetable oil collection was examined. For this purpose, a survey was applied to randomly selected people living in Bursa. In addition, the data were statistically analyzed.

The data were obtained from various age groups, household size, marital status, job area, education, and income levels. The female and male participants of the survey were nearly equal.

The results are summarized below:

- The awareness-raising studies are required in all three districts due to the high rate of pouring waste vegetable oils into sinks or trash.
- Most people in all three districts do not follow the news about this subject.
- The surveyed people are unaware of the Waste Vegetable Oil Collection Centers near their area.

The following recommendations can be given as a result of the study;

- It has been thought that giving an incentive gift for collecting waste vegetable oils in homes will increase the return of the public on waste oil collection.
- Also, more broadcasting about waste vegetable oil collection should be done on television and radio, which is the only way to increase awareness of the Bursa people.

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