

**INSTITUTO SUPERIOR DE AGRONOMIA  
UNIVERSIDADE DE LISBOA**

**MASTER THESIS**

**Effect of different organic amendments on soil quality, vines growth,  
grape production and wine quality of mechanically pruned vineyards.**

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Dissertation to obtain the degree of  
**European Master of Science in Viticulture and Enology**

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November 2014, Lisbon

## **Declaration**

I Kaushal Khairnar herewith declare that the Master Thesis submitted here with the title;

*Effect of different organic amendments on soil quality, vines growth, grape production and wine quality of mechanically pruned vineyards.*

This thesis has been composed by myself without any inadmissible help and without the use of sources other than listed in the list of references. All person and institutions that have directly or indirectly helped me with the preparation of the thesis, have been acknowledged and that this thesis has not been submitted wholly or substantially, as an examination document at any other institution.

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## **ABSTRACT**

The experiment with an aim to study the effect of pruning types and effect of different types of organic amendments on soil quality, vine growth, grape production and wine quality was conducted in two sites located in Vilar and Sardeal region of Portugal. The pruning treatments included manual spur (MAN) and mechanical hedge (MEC) type of pruning and types of organic amendments applied were biochar (BIOC), municipal solid waste compost (RSUC), cattle manure (ESTR), sewage sludge (ETAR) and control treatment (TEST).

The treatment effects were analyzed for chemical composition, mineral content and heavy metals content and sensorial analysis of wine samples. The analysis results show that there was no significant effect of type of pruning on wine composition except pH, total acidity, alcohol content, calcium and total nitrogen content of wine sample. Similar trend was also followed with the application of organic amendments; the composition of wine remained unchanged by the use of organic amendments except some effect on pH, total acidity, volatile acidity, total anthocyanins and potassium content of wine samples. There was no significant increase in the heavy metals content of wine samples after the treatment of organic amendment. The manual pruning generally showed higher content compared to mechanical pruning due to less number of bunches retained per shoots. Wine composition was more influenced by the site specific characters and climatic conditions than the effect of treatment applied. Sensory analysis along with chemical analysis shows that mechanical pruning can be used without having much significant effect on grape composition when sufficient yield compensation is achieved. Treatment which supplied higher amount of nitrogen (ETAR and ESTR) was generally marked by more negative sensory perception such as vegetal characters in wine.

On the face of climate change and increased competitive pressure use of mechanical pruning can be good strategy to reduce the cost of grape production and organic amendment can be applied to soil with an aim of increasing organic matter contents of soil. But the care should be taken to avoid excessive supply of nitrogen in order to avoid negative effect on wine composition over longer period of time. So the supply of amendment should be managed according to vine and soil nutritional status.

**Keywords:** Pruning, organic amendments, wine composition.

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# General Index

<b><i>1 Introduction</i></b>	<b>6</b>
<b><i>2 Bibliographic Review</i></b>	<b>8</b>
<b><i>2.1 Grapevine Pruning</i></b>	<b>8</b>
2.1.1 Manual Pruning	8
2.1.2 Mechanical Pruning	9
2.1.3 Minimal Pruning	9
<b><i>2.2 Use of Organic Amendments in Vineyard</i></b>	<b>11</b>
2.2.1 Cattle Manure	12
2.2.2 Sewage Sludge	13
2.2.3 Municipal Solid Waste Compost	14
2.2.4 Biochar	15
<b><i>2.3 Objectives of Project Work</i></b>	<b>16</b>
<b><i>3 Material and Methods</i></b>	<b>17</b>
3.1.1 Establishment of Experimental Vineyards	17
3.1.2 Wine Making	19
3.1.3 Physiochemical Analysis	21
3.1.4 Mineral Analysis	23
3.1.5 Heavy Metal Analysis	24
3.1.6 Sensory Analysis	24
3.1.7 Statistical Analysis	25
<b><i>4 Results and Discussion</i></b>	<b>26</b>
<b><i>4.1 Analysis during Grape Maturation and Vinification</i></b>	<b>26</b>
4.1.1 Analysis at Grape Stage	26
4.1.2 Analysis of must prior to Fermentation	31
<b><i>4.2 Analysis of Wine Samples after Bottling</i></b>	<b>33</b>
4.2.1 Analysis of Wine (pH, TA, Volatile Acidity, So <sub>2</sub> )	34
4.2.2 Analysis of Chromatic Characteristics	37
4.2.2 Mineral Analysis	42
4.2.3 Analysis of Heavy Metals and other elements	46
<b><i>4.3 Sensory Analysis of Wine Samples</i></b>	<b>55</b>
<b><i>5 Conclusion</i></b>	<b>64</b>
<b><i>6 Bibliography</i></b>	<b>67</b>
<b><i>7 Annex</i></b>	<b>74</b>

## 1. INTRODUCTION

Viticulture is sustainable when it considers soil building as foundation, minimizes off farm inputs, depends on economic profitability and concerns itself with environmental health and social equity. Due to increased competitive pressure all around globe it has become necessary to reduce cost associated with grape production. Reduced cost of mechanization and increased cost of labor has shifted viticultural practices towards mechanization.

Wine production is big business in Portugal. Viticulture, grape growing and production of wine represents an important economic activity of Portuguese agricultural sector. But most of the climate change research work, projects the potential risk due to climate changes in front of viticulture and adaptation measures that needs to be taken into consideration.

The image for the future seems less bright as in the large part of country the suitability for wine grape growing decreases as results of projected dryer and warmer climate in southern part of Europe. This is more prominent in southern and innermost part of Portugal. The thermal index is projected to increase, indicating excessive heat that may damage the vineyards. Humidity index is projected to decrease as a result of projected extreme periods of draughts for which especially South Eastern Portugal is vulnerable.

The study done by Fraga H. et al (2012) has shown the urgent need for the adaptation and mitigation measures that needs to be undertaken for Portuguese viticulture, including new grape varieties, genetic modification or adaption of agricultural practices like irrigation to cope up with climate changes.

There is intensifying competition in wine sector from new world wine making countries over the period of time. Because of this wineries try to search new channels for the sale and many vintners chose to develop other activities. Portugal is one of the European country which has several natural and technical constrains that may be resulting in modest performance regarding its position in the global wine market because of which competitiveness and dynamism are required to overcome these difficulties.

The price to quality ratio and reduction in the price of wine production can be part of strategies to face this intensifying competition. The cost of production can be controlled by adapting agricultural practices towards mechanization, reducing applications of fertilizer, limiting the use of herbicides to control weeds and increasing yield without affecting quality of grapes and wine.

Adapting towards mechanization has its benefits due to ease of operation, quick, consistent capacity and less time consumption with lower cost than manual labor over the period of time. Traditional pruning is one of the most important cultural operations carried out in the vineyard and after harvesting; it's most expensive and labor consuming operation (Tassie E. and Freeman B.M, 1992). Pruning is aimed to remove last year's growth and leaving behind number of buds that will determine number of shoots and ultimately production for next year. The cost of pruning can be reduced by adapting pruning practices towards mechanical pruning, hedge pruning or minimal pruning.

Another problem in front of viticulture is soil erosion. Most of important Portuguese vineyards are located on mountain terrains. This makes them potentially susceptible for the loss of soil due to erosion. The erosion is mainly due to improper soil management practices, concentrated rainfall and strong wind which carry the soil with it. Because of this reasons and many of the places represents soils originally poor in organic matter. It becomes necessary practice to optimize the organic matter content of soil in order to adapt towards mechanization and support the increase in production and quality. Organic matter is important biochemical indicator of soil quality. It can improve soil porosity and decrease the bulk density of soil (Pinamonti, 1998). Soil structural stability and water infiltration rate are improved by the use of organic matter (Goulet et al, 2004)

It has been observed that nutrient requirement for the vine will increase after minimal pruning to maintain the growth of more vigor, to supply nutrient to more number of retained buds. Also after mechanically pruning over period of time grape vine generally starts to decrease its vigor, this potential loss of vigor can ultimately effect on yield of upcoming year due to less accumulation of reserve. To counter the above problem of decrease in vigor and with an aim to achieve more yield generally grape growers carries addition of commercial nitrogen fertilizers.

The addition of fertilizers seems to put more additional cost on viticulture under adverse conditions due to climate, geographical constrains and trying to sustain under competitive pressure of low cost production. In such scenario it will be good idea to shift towards the use of organic amendments as an option to carry addition of organic material in soil. In many of scientific experiments its positive effect on grape vine has been proved. Soil organic compounds can act as source of energy and nutrient for microbial biomass. Total nitrogen content of soil is strongly related to organic carbon concentrations. Mineralization of organic matter is related to microbial activity and it produces large part of soil mineral nitrogen (Houot and Chaussod, 1995; Delphin, 2000). It can help to meet increased nutrient demand of vine and can be achieved with less expenditure.

With an aim of studying effect of organic amendment application on grapevine, the experiment work was carried. In this experiment organic amendments were applied at two different sites of Portugal, in the region of Lisboa and Tejo. These regions are characterized by the different types of soils, climatic conditions and terroir effect. Effect of manual and mechanical pruning system and different types of organic amendments such as sewage sludge (ETAR), municipal solid waste compost (MSW), cattle manure (ESTR) and biochar (BIOC) application on soil quality, vine growth, vines nutritional and sanitary status, grape production and wine quality was studied. The main aim of the experiment was to see the effect of applied organic amendment on wine quality, to compare the variation amongst the treatments in chromatic, sensorial as well as other parameters of the wine.

## 2. BIBLIOGRAPHIC REVIEW

### 2.1 Grapevine Pruning

#### 2.1.1 Manual Pruning

Canopy management practices have been established with the aim of optimizing the sunlight interception, photosynthetic capacity and fruit microclimate to improve yield and wine quality particularly in vigorous and shaded vineyards. The pruning of vine is an important canopy and crop management operation as it permits grape grower to establish particular training system and manipulate vine yield. Pruning allows selection of bearing woods (spurs and canes) and hence it influences location and development of canopy. Grapevine pruning is normally performed during winter dormancy period in order to remove most part of vegetative growth from previous year. Pruning of grapevine is performed to maintain the shape and size of vine, to control number of buds, for maintaining better distribution of potentially productive buds, controlling number of shoots, which ultimately controls number of bunches and their size (Winkler et al, 1974).

The grape vine stores its reserve in mature canes and cordons so many times along with winter pruning these reserves are lost. Excessive removal of cane and shoots can impede the vine capacity to ripe fruits. Reserves stored in canes, cordons are essential for initiating rapid growth in spring for grapevine. The excessive pruning and excessive over cropping can suppress the capacity of vine (Ronald Jackson, 2008). Hence the pruning should be balanced in order to avoid excessive vigor which can create problem for ripening of fruits and at the same time maintaining enough amounts of fruit to achieve better yields without influencing on final quality and composition of grapes.

Most of wine industries are globally facing strong competitive pressure. And rising cost of production in both national and international wine industries make it obligatory to implement cost reducing methods and practices. One of most cost effective operation during vineyard management is winter pruning besides its immense importance for achieving quality and controlling yield of grapes.

The winter pruning is costly operation as it requires 70 to 100 hr/ha of human labor depending on the training system (Baldini and Intrieri, 2004). During winter pruning almost 95% of year old wood is removed (Winkler et al, 1974) leaving 15-20 buds per linear meter of hedge wall (Intrieri and Filippetti, 2001). To reduce the cost of wine and dried grapes production mechanization of harvest and pruning operations has been already adopted in Australian Vineyards (Clingleffer, 2000). Viticulture in Portugal also needs to adapt towards mechanization and implement the practices which can benefit to produce the wine with lower cost without influencing on final quality of grapes. Use of mechanical pruning (hedge pruning) or minimal pruning can be part of such adaptations. While adapting towards this practices they should take into account conditions and constrains of local “terroir” and the suitability of vineyards for the implication of such practices.

### **2.1.2. Mechanical Pruning**

Use of mechanical pruning has been part of growing interest particularly vineyards belonging to new world wine growing regions. Mechanical pruning is being applied to approximately 65% of wine grapes in Australia. Pruning is done by hedging vines on sides and by topping, thus providing a box in cross section. The shapes may vary depending on the way pruning is done but by this way vines are treated in fully mechanized manner. Also there is other trend which is of increasing interest, pre-pruning followed by manual hand follow up. This pre-pruning generally works best for vineyards that are vertically positioned or lyre type of trellising system, where the canes can be arranged in vertical plane and can be easily cut down by the machines. Then later manual follow up is done in order to control number of buds to be maintained on the retained spurs and some other clean up can be done (Mark Greenspan, 2007).

The work by Gatti et al, (2011) to study long term effect of mechanical winter pruning on Barbera grapevine cultivar showed that winter pruning was performed in the case of mechanically hedged vines within less than 25 hr/ha which significantly reduced the labor demand from 54% to 70% in comparison of hand pruning. The experiment concluded, if all vineyard operations are also mechanized a single wire Barbera vineyard with mostly erect canopy can be managed in less than 70 worker hr/ha. Such a performance with overall unaffected yield and grape composition represents solid and reliable approach in a wine market that demands greater efficiency and competitiveness (Gatti et al, 2011)

The main distinguishing trait of mechanical pruning is the number of retained buds on vine is largely increased compared to the hand pruning. This can be compensated by the vines capacity to perform self yield regulation by the effect of reduced bud break rates (Poni et al, 2004) clusters per shoot (Clingeleffer, 1993), berry set (Jackson et al, 1984; Possingham 1994), cluster and berry weight (Freeman and Cullis, 1981) and even self pruning due to the abscission of immature wood (Clingeleffer 1984, 1993; Morris, 1993; Tassie and Freeman, 1992).

It has been proved by the field trials that if sufficient amount of yield compensation is achieved in case of mechanically pruned vines to higher canopy efficiency (i.e. earlier canopy formation and larger leaf area through the increased shoot numbers) then even mechanical pruning can produce similar or even higher amount of yield without alteration of grape composition (Clingeleffer, 1993; Keller et al, 2004; Zabadal et al, 2002).

### **2.1.3 Minimal Pruning**

Rising production cost in national and international wine industries makes it obligatory to carry implementation of cost reducing methods and procedures. Minimal pruning can be used as strategy to reduce production cost of wine. Minimal pruning is known for its low pruning severity, rapid speed and low cost (Clingeleffer 1983, 1984). It removes 10% or less of year old wood (Clingeleffer, 1988) which cuts down labor hour as low as 5-10 hr/ha, leaving behind bud number as high as 500-700 per meter of hedge wall.

The research work on minimal pruning when compared to normal hand pruning showed an increase of yield, number of bunches and shoots (Cirami et al, 1985; Clingeffer and Possingham, 1987; Clingeffer, 1988). Most of these reports showed that after minimal pruning sugar concentration as well as pH was reduced and total acid concentration increased by minimal pruning. In terms of wine color, flavor and quality variable results were reported depending on the climate and vigor conditions. Clingeffer work on the minimally pruned sultana vines showed that fruit distribution was improved in minimal pruned vines, bunches were smaller which altogether reduced losses from berry splitting and mould in wet season (Clingeffer, 1984).

With an aim of studying the effect of alternative pruning methods on the viticultural and oenological performance of 9 different wine grape varieties experiment was done in South Africa with different pruning treatments (hand, mechanical, minimal and no pruning) at three different sites (E.Archer and D.Van Shalkwyk, 2007). All alternative treatment achieved huge labor saving compared to hand pruning. A reduced vigor and increased yield was obvious for all the varieties. Experiment showed that adaptability towards alternative pruning varies as per varieties too. Varieties like Cabernet Sauvignon, Pinotage and Chardonnay performed well while Sauvignon Blanc and Merlot poorly. Chenin Blanc, Shiraz, Colombard and Ruby Cabernet showed acceptable performance. Idea of alternative pruning methods seemed to be possible especially for production of medium and low priced wines.

The alternative pruning in this experiment resulted in smaller bunch size, but due to increased number of bunches, it ensured that yield per hectare is increased. Alternative pruning method also induced better color in skins, wines and it's ascribed to better penetration of sunlight into bunch zone of these vines. It was found by the experiment that hand pruning together with all the necessary canopy management as well harvest requires 273.6 manual hr/ha compared to 43.8 and 14.4 manual hr/ha for minimal and mechanical pruning respectively. The saving was true for this particular experiment for all varieties and there is clear economical advantage suggested by the use of alternative pruning methods (E.Archer and D.Van Shalkwyk, 2007).

Results of minimal pruning trials are quite satisfactory as it provides smaller and loose bunches, even in case of vine health (Clingeffer and Possingham 1987; Possingham 1994, 1996; Clingeffer 2009a). So it will be good practice to shift towards minimal pruning to reduce the extra inputs associated viticulture management practices. But at the same time we need to take into consideration that minimal pruning is found to be sensitive for environmental conditions, management systems and minimal pruning is better adapted for warmer climatic conditions and early to mid season ripening varieties (Possingham, 1996). Trials in Italy showed that minimal pruning was positive for early ripening cultivars like Chardonnay (Poni et al, 2000) but were less than satisfactory for mid to late ripening cultivars like Sangiovese in Northern Italy (Intrieri et al, 2001).

Experiment with Semi Minimal Pruning hedge in Italy for Sangiovese cultivar showed that the yield for SMPH was 35-40 % higher than spur pruned cordon (Intrieri et al, 2011). Clusters appeared less compact and less susceptible to botrytis attack. Despite the fact that higher cropping was achieved, must titratable acidity, brix and pH did not differ in case of SMPH compared to control. The total anthocyanin concentration showed higher values in SMPH treatments compared to control. It was suggested by author that, higher concentration of anthocyanin may be partially due to smaller berry size but mainly was possible due to direct effect of biosynthesis in this case as its shown by the higher level of anthocyanin per gram of skin. It was also linked to greater light interception and higher leaf area in case of SMPH treatment. Effect was also possible due to higher loss of water from canopy of SMPH treatment which perceived higher light interception.

These results are in accordance to suggestions of Roby et al, 2004, that anthocyanin biosynthesis normally increases after water stress. Semi minimal pruning hedge practice can effectively address drawbacks associated with minimal pruning with medium to late ripening cultivars like Sangiovese. As there was no delay in ripening and biochemical properties do not differ significantly from spur pruned cordon grapes. Hence SMPH can be positive practice because of its reduced management cost and adaptability for full mechanization and improved yield and quality (Intrieri et al, 2011). Clingeffer (1993) work on Pruning of Cabernet Sauvignon showed that lighter pruning resulted in significant yield increase (2 fold) without influencing fruit composition and spectral properties, except slight delay in maturity. There was positive linear relationship between yield and nodes per bearer because of the increased shoot and bunch number.

## **2.2 Use of Organic Amendments in Vineyard**

Organic matter content of soil is widely used as biochemical indicator of soil quality. It improves moisture content of soil at field capacity (McConnell et al, 1993; Pinamonti et al, 1995). Use of organic matter helps to decrease bulk density of soil and increases soil porosity (Pinamonti, 1998). Soil structural stability and water infiltration rates are improved by organic matter (Goulet et al, 2004). Soil organic matter also acts as source of energy and nutrient for microbial biomass. Total nitrogen contents of soil are strongly related to organic carbon concentrations. Mineralization of organic matter is associated with soil microbial activity and produces large part of soil mineral nitrogen. This increased level of mineral nitrogen can be useful to support growth of increased grapevine vigor.

Application of organic nutrient should supply sufficient nutrient to prevent deficiency for plant but at the same time leaching of the excess of nutrient in ground water must be avoided. Soils with gravel and sandy textures, without cover crops, on steep slope without pruning wood restrictions and with tillage practices will show accentuated depletion of soil carbon. In such a case rational management of soil organic matter will be necessary without causing any adverse effect on environment or wine quality.

Decreased amount of organic matter in vineyard soils can be compensated by application of different types of organic amendments. Changes in climate has projected reduced level of rainfalls in many part of world, organic matter seems to be good indicator of crop productivity in semi arid regions as it affects positively on soil water holding capacity. Organic matter also acts as indigenous source of available nitrogen and it contains as much as 65% of total Phosphorus (Bauer and Black, 1994). Soil nitrogen content can affect and stimulate vine vigor, growth and yield (Conradie, 2001).

### **2.2.1. Cattle Manure (ESTR)**

Cattle Manure is waste product of bovine animal species. Application of cattle manure to farmland is an economical and environmentally sustainable mechanism for increasing crop production. Cattle manure is a combination of feces and manure, bedding material, wasted feed, and water. Composted cattle manure makes an excellent fine slow release fertilizer for use on a range of plants.

Experiment about use of organic amendment in case of Cabernet franc was carried with three types of treatments (Morlat and Chaussod, 2008), spent mushroom compost (8-16 t/ha), cattle manure (10-20 t/ha) and pruned vine wood (2 t/ha). Results showed that during the initial years of experiment there was no significant impact of the treatment but during later years there was decreased yield observed in case of cattle manure application 20 t/ha however average of fruit yield was always highest in case of pruned vine wood application (2 t/ha). Treatment with doubled rates during later stage of this experiment showed reduction in Pruning weight, yield and increased vine mortality, this effect was attributed to multiple reasons, like possible water supply reduction or an eventual depressive effect of a higher salinity of soil, but more probably was a toxic effect of higher nitrogen level.

Sensory characters of Cabernet franc were also strongly affected by use of organic amendments and due to vintage effect. Higher dosage rate of cattle manure showed decreased level of color intensity and anthocyanin concentration. Higher perception of grassy herbaceous characters can be possible due to lower degree of grape ripening due to higher dosage rate of cattle manure. Higher dosage rate of organic amendment that provided higher level of nitrogen through the mineralization of soil organic matter affected negatively on grape and wine characteristics. Total soluble solids, anthocyanin, tannin contents of grapes were decreased. Total N, total P, total K and pH were increased in juice. Effect of nitrogen as per dose and type of organic amendments depends on region, climatic conditions, soil type, composition and existing weather conditions Morlat R., (2008); Morlat and Symoneaux, 2008). Similar kind of trend was observed by the Bell and Robson, 1999, they observed that higher rates of nitrogen fertilizer application produced higher insoluble nitrogen in soil and had no favorable effect on growth and yield.

Long term application of cattle manure can result in increase of salinity of soil (Hao and Chang, 2003). Experiment work showed that repeated application of cattle manure and mushroom compost can result in salt accumulation leading to cause depressive effect on dry matter of grapevine. Soil electrical conductivity was increased with cumulative amount of cattle manure

over 25 years of continuous application. While other study showed stable electrical conductivity and concluded that applying livestock manure did not increase soil salinity over 8 year duration of the experiment (Clark et al, 1998).

### **2.2.2 Sewage Sludge (ETAR)**

Sewage sludge is insoluble residue from municipal waste water treatment after either aerobic or anaerobic digestion process. Increased cost of commercial fertilizer and growing issue of largely produced sewage sludge worldwide have made cropland application of this residue an attractive option. Chemical and biochemical composition of sludge depends on the composition of the wastewater (Melo et al, 2002). Sewage sludge is rich in organic matter and also contains plants nutrients such as nitrogen (N), phosphorus (P) and calcium (Ca) (Hue, 1988).

Sewage sludge can help to improve physical, chemical and biological properties of soil such as porosity, aggregate stability, bulk density, soil fertility, water movement and retention (Silveira et al, 2003). Sewage sludge can be possible means of recycling important plant nutrients like organic matter, N, P and other plant nutrients, hence agricultural application of sewage sludge is of increasing interest. Major problem which is associated with excessive application of sewage sludge is toxicity to plant due to heavy metals accumulation in soil (Mc Grath et al, 2000) and increase in salt content (Hao and Chang, 2003). Hence it's of immense important that application of sewage sludge should be balanced in order to gain its fertility benefits. Availability of heavy metals present in the sewage sludge and soil treated with sewage sludge depends upon many factors such as properties and amounts of heavy metals, their dispersal between solution and solid phase and soil characteristics (Jin et al, 1996). Also availability of heavy metals is affected by the quality and amount of organic matter that can react with heavy metals, forming complexes and chelates of variable stability (Leita et al, 1999).

Antoline et al (2005) reported that application of sewage sludge decreased soil pH and increased total soluble salts, organic carbon and cation exchange capacity of soil. Also they found that application of sewage sludge increased soil extractable heavy metals (Cd, Cu, Mn, Pd and Zn) and increased N-NH<sub>4</sub><sup>+</sup> content of soil. Increased level of organic matter and extractable metals (Cu, Zn, Ni, Pb, Mn and Fe) with addition of sludge and decrease of the pH was also reported by Mendoza et al, (2006). Increase in nutrient level by application of sludge is possible due to increased acid level by decomposition of sludge and its effect on some of soil minerals or release of the nutrients through sludge decay by microorganism's activity and also higher nutrient availability in soil can be attributed to high content of such elements in sludge used. Decomposition of organic manure produces acids such as fulvic acid, humic acid and carbonic acid this increase in the acid level can result in the decrease of the pH which ultimately affect on solubility of soil nutrient and improves their availability and supply to plant uptake.

The experiment was conducted to study the effect of Sewage Sludge compost on volatile organic compounds of Grenache wine (Korboulewsky et al, 2004). Treatment included application of urban sludge a byproduct of municipal waste water compost mixed with pine bark and green wastes the application rates were 10 t/ha, 30 t/ha, 90 t/ha and control. Application of sewage

sludge increased vine vigor this was possible due to increased input of mineral N via sewage sludge (Korboulewsky et al, 2002) which delayed maturity of grapes. Generally higher nitrogen content of soils favors vegetative growth and delays the maturity of grapes and results in lower volatile organic compounds in wines (Ribereau-Gayon and Peynaud, 1980; Hashizume and Samuta, 1999). Application of sewage sludge compost at higher rates in first year decreased volatile organic compounds concentration of wines, especially several compounds which contribute to a rich aromas and flavors to wine (Korboulewsky et al, 2004). Difference amongst treatment was not remained significant in second year after application of treatment compared to first year. Mineralization rate of applied organic amendments was much lower in second summer compared to first one and similar to controls (Korboulewsky et al, 2002).

### **2.2.3 Municipal Solid Waste Compost (RSUC)**

Compost obtained from municipal solid waste and other organic biomass can be used as organic fertilizer on agricultural soil. Good quality compost generally contains higher amount of stabilized organic matter and nutrients, which guarantees its agricultural advantages. At the same time compost also makes the reuse of the possible organic wastes. Application of organic compost can be beneficial to improve the organic matter contents of soil and they also participate in stimulation of soil microbial activity as well as biogeochemical cycles in which they participate (Perucci, 1990; Bustamante et al, 2010). Hence application of organic waste or compost can be beneficial for soil conservation especially in degraded soil and those susceptible to erosion. It's necessary to carry composting of solid waste and sewage sludge before using into soil due to the possibility of having negative impact. Composting is beneficial as it helps to recycle the organic waste by transforming them into stable, hygienic and humic rich materials that can be used as organic fertilizers in agricultural soil. Compost is beneficial for improvement of soil physical properties as it increases soil porosity, structural stability, available water content and reduction of the erosion (Guidi G. et al, 1988; He XT et al, 1992).

Presence of organic and inorganic contaminants in compost may constitute risk to environment especially heavy metals are factor which puts some restrictions on the use of compost in agriculture. Effect of compost use on environmental heavy metal contents shows generally it varies as per the soil type, compost quality and plant species (Woodbury, 1992).

If application of organic compost in vineyard is uncontrolled and repeated then it can result in increased content of metals such as Cu, Mn, Zn and Fe to potential toxic level (Ramos and Lopez - Acevedo 2004). Long term use of sewage sludge causes accumulation of Cu, Zn, Pb, Ni and Cd in soil and plants (Williams et al, 1980; Mulchi et al, 1991). Experiment carried by Bustamante et al, 2011 about effect of organic waste on characteristics of Calcareous sandy- loam soil, showed that application of organic amendments favored long term N availability especially the compost which were elaborated with grape marc, grape stalk, exhausted grape marc, sewage sludge and vinasse producing the release of inorganic nitrogen balance in order to satisfy vine demands, so that losses of excessively released N are avoided in soil. Addition of organic amendments also increased the level of available Zn, Cu and Mn at the end of experiment. Also

it increased availability of iron compared to control, effect was also possible due to chelating ability of humic and fulvic substances and stimulation exerted by organic components on microbial activity (Tagliavini and Rombola, 2001). Ramos (2006) also has reported increased level of Cu and Mn concentration in the vineyard soil amended with cattle manure. Compost can be also used as mulching compost by which growth of weeds can be restricted and requirement of herbicide application can be decreased with improvement in soil structure (Pinamonti et al, 1996).

#### **2.2.4 Biochar (BIOC)**

Positive attributes associated with use of biochar is its nutritive value, it supplies nutrient to plant either directly or indirectly by improving soil quality and at the same time by improving the efficiency of fertilizer use. Biochar has ability to retain nutrients hence it reduces the losses by leaching resulting in increased uptake by plant and higher production. Generally nutrient content of biochar is dependent upon feedstock and the way how pyrolysis operation was carried during processing of biochar. Despite having higher total N content (6.4%) biochar produced from sewage sludge was found to have negligible mineral N (Ammonium – N<sup>+</sup> nitrates- N) even after the incubation period of 56 days (Bridle and Pritchard, 2004) While contrastingly the available potassium content of biochar is generally higher and increased uptake of K has been frequently observed as result of biochar application (Lehman et al, 2003; Chan et al, 2007).

Many studies have shown lower uptake of nitrogen after biochar application, (Lehman et al, 2003). This may be possible as only small part of freshly produced biochar is easily mineralizable and also application may cause immobilization due to higher C/N ratio. Lehman et al, (2003) also observed ability of biochar to retain applied fertilizer against leaching and increased efficiency of applied fertilizer. This property can be possible due to charge and surface area properties of the biochar.

In an experiment in Delinat Institute in Valais treatment of biochar and compost together showed shoots with larger diameter stems than control, pure biochar and pure compost (Niggli and Schmidt, 2011). In same experiment, analysis of grape component reflected that anthocyanin content were higher in treatment with biochar and biochar-compost than control or pure compost treatment. Polyphenols content was higher in treatments with pure compost. Sugar content was higher in pure biochar treatment than control. Yeast Assimilable Nitrogen (YAN) was higher in treatment involving biochar compost and only compost treatment while non activated biochar showed low levels of YAN. There was slight decrease in acidity and increase of pH was observed with compost and non activated biochar treatment. Biochar has showed reduced emission of greenhouse gases from soil (Yanai et al, 2007; Van Zwieten et al, 2009). Biochar being pyrolyzed product, it's protected from rapid degradation by microbes so it's able to sequester carbon over longer period, providing good potential in mitigating greenhouse gas emission (Lehman et al, 2006).

The biochar has also shown improved effect on soil water holding capacity. This effect is related to porosity and higher specific surface area (Verheijjan et al, 2010) and magnitude of effect

depends upon feedstock type and pyrolysis conditions (Uzoma et al, 2011). Improved increase of soil capacity by 18% was observed in anthrosols rich in charcoal (Glaser et al, 2002). Similar increase was observed with sandy soil type (Lehman et al, 2011). Hence use of biochar can be good strategy to face problem of water scarcity in near future, especially among those regions which are projected to face extreme environmental conditions of water deficit and draught. Additional amount of water and soluble nutrients which are stored in micro pores of biochar are hypothesized to become available to plants as soils start to dry and matric potential decreases (Uzoma et al, 2011) but there is no much data available to confirm this kind of behavior.

Biochar addition could increase resilience of vineyard to drought so it can be possible measure for farmer as adaptation strategy for climate change. Many vine growers are trying to adapt towards reactive and anticipatory adaptation strategies which generally include modification of vineyard management practices such as irrigation, trellis system, row orientation. Biochar based strategy can also play a role as adaptation measure to tackle negative impact of drought. Use of biochar can be good way to use in AOC where to maintain the terroir effect irrigation is prohibited. In such a case use of biochar to ameliorate soil water status can be seen as a way, also it can be used as water saving measure in the AOC's where irrigation is not forbidden.

### **2.3 Objectives**

Aim of present work is focused on the adaptation measures that are necessary to be undertaken in near future due to projected climate changes specially focused on Portuguese viticulture. Work is associated with mainly two types of viticultural operations

- Use of different types of pruning – Mechanical vs Manual Pruning
- Use of different types of organic amendments – Control or Test, Biochar, Municipal Solid Waste Compost, Cattle Manure and Sewage Sludge.

Two types of pruning system were applied in the experimental site of vineyard; it includes use of mechanical hedge type of pruning and manual spur pruning. Aim of these two types of pruning is to compare adaptability of vines toward mechanization and comparing the effect amongst mechanical and manual types of pruning on quality of grapevine cultivar Shiraz. Along with two types of pruning vines were also subjected to different types of organic amendment treatment. Different types of organic amendments were applied with purpose of comparing effect on Wine and Grape composition and associated quality parameters. Effects of treatments were compared by chemical analysis as well as sensorial methods. Goal is to see best suitable organic amendments, linkage between applied amendment in vineyard and its effect on overall final quality of wine, variation within treatments and to see best suitability for the region and winery.

### 3. MATERIAL AND METHODS

#### 3.1.1 Establishment of experimental vineyard

Experimental vineyard was established at two different sites located in different region of Portugal. Quinta do Coro which is located at 6 km from the river Tejo near the village of Sardeal and Quinta do Gradil situated next to Sierra de Montejunto, near the town of Vilar under the Lisboa wine region. Vineyard was designed by using split plot method. First pruning treatment was randomized within full rows. Then for each block organic amendment was randomized along the row. For each type of treatment applied there were three replicates.

Details of treatment are as following –

**Table No. 1 Experimental vineyard design**

<b>Name of the winery</b>	<b>Pruning type</b>	<b>Treatment</b>	<b>Blocks</b>
Quinta do Coro	Manual	Test	1,2,3
Quinta do Coro	Manual	Biochar	1,2,3
Quinta do Coro	Manual	Municipal solid waste compost	1,2,3
Quinta do Coro	Manual	Cattle Manure	1,2,3
Quinta do Coro	Manual	Sewage Sludge	1,2,3
Quinta do Coro	Mechanical	Test	1,2,3
Quinta do Coro	Mechanical	Biochar	1,2,3
Quinta do Coro	Mechanical	Municipal solid waste compost	1,2,3
Quinta do Coro	Mechanical	Cattle Manure	1,2,3
Quinta do Coro	Mechanical	Sewage Sludge	1,2,3
Quinta do Gradil	Manual	Test	1,2,3
Quinta do Gradil	Manual	Biochar	1,2,3
Quinta do Gradil	Manual	Municipal solid waste compost	1,2,3
Quinta do Gradil	Manual	Cattle Manure	1,2,3
Quinta do Gradil	Manual	Sewage Sludge	1,2,3
Quinta do Gradil	Mechanical	Test	1,2,3
Quinta do Gradil	Mechanical	Biochar	1,2,3
Quinta do Gradil	Mechanical	Municipal solid waste compost	1,2,3
Quinta do Gradil	Mechanical	Cattle Manure	1,2,3
Quinta do Gradil	Mechanical	Sewage Sludge	1,2,3

Experimental work was carried by adapting already established vineyards. Vines were pruned by using mechanical machine pruner to make hedge type of pruning with each vine plant bearing approximately 34 buds. Other type of pruning treatment was traditional manual pruning in which grapes were spur pruned leaving behind generally six spurs with two buds per spur.



**Image No. 1 Manual pruning**



**Image No. 2 Mechanical pruning**

Five different types of organic amendments were used in vineyard (Table No.1). Application rates of each treatment were as following (Table No.2). Application rates for each amendment were based upon the calculation to supply 5000 kg/ha/year of organic matter to soil.

**Table No. 2 Application rates of organic amendment applied**

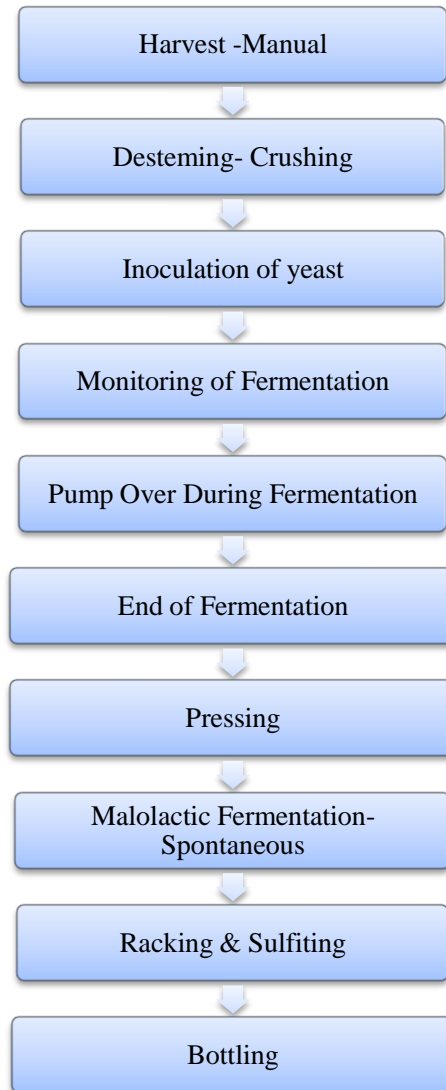
<b>Treatment type</b>	<b>Application rate (kg/ha)</b>
Test	None
Biochar	8600
Composted municipal solid waste	15500
Cattle manure	23000
Waste water treatment plant sludge	31300

### **3.1.2 Wine making**

Syrah (Shiraz) grape variety is used in this particular experiment. Style and flavor profile of wines made from this variety is influenced by climate where grapes are grown. Grapes were harvested on September 2013 manually at both wineries Quinta do Coro and Quinta do Gradil. Vinification was carried at experimental winery of Instituto Superior de Agronomia, Lisboa. Before harvest, grapes were regularly analyzed in order to control maturity indices. Parameter's that were monitored included pH, titratable acidity and total soluble solids content of grapes. After harvest at juice stage chromatic characteristics of grapes were analyzed by measuring total anthocyanins and total phenolic content of grapes. After receiving grapes at winery they were destemmed and crushed.

Fermentation was carried by inoculation of commercial yeast. Progress of alcoholic fermentation was daily monitored by checking density and temperature. Temperature during fermentation was monitored around 26°C. During fermentation daily pump over was carried in order to achieve better extraction of color compounds.

Spontaneous malolactic fermentation begun after alcoholic fermentation, temperature of fermented wine was allowed to rise till 25°C. Progress of malolactic fermentation was monitored by qualitative method of paper chromatography. Due to external cold temperature conditions during period of malolactic fermentation temperature of 25°C was maintained by using room heater. Malolactic fermentation for Quinta do Gradil was completed without any troubles but in case of Quinta do Coro wine samples took longer time to finish malolactic fermentation. Wines were kept long time with elevated temperature in order to facilitate the fermentation. Probable reason which was responsible for this was higher alcohol content especially in case of wines made from the manually pruned grapes of Quinta do Coro. After end of malolactic fermentation wines were racked in order to get rid of settled lees and other residues. After wines were adjusted to free sulfur level of 40 mg/L by addition of potassium meta-bisulfite (KMS) and then bottled.



**Image No. 3 Flowchart of vinification**

Above diagram of flow chart shows summarized view of methodology following for vinification of experimental wines.

### 3.1.3 Physiochemical analysis

Wines were analyzed for physical and chemical characterization in order to understand effects of each organic amendment addition and its effect on final composition of wines. Analysis of wines was performed at laboratory of Instituto Superior de Agronomia, Lisboa and analysis of heavy metals was performed at Instituto Nacional de Investigacao Agraria e Veterinaria located in Dois Portos near Lisboa. For analysis of basic parameters of wine, methods recommended by OIV were followed. Analysis of the specific methods, tannin power (Freitas Victor and Mateus N., 2001), and heavy metals (Catarino et al, 2006) was performed by referring protocols mentioned in specific research paper associated with them.

Analysis of pH plays important role in determining and maintaining quality of wine. Generally pH of wine is associated with its acid contents. Monitoring pH of wine is essential as acidic strength controls wine color, taste and storage abilities. The pH of wine was measured by using OIV method (Ref. No MA-E-AS313-15-pH). Total acidity is measured as sum of total acids found in wine; it was measured by using OIV method (Ref.No.MA-E-AS313-01-ACITOT). Acidity of wine helps to balance sensorial characters of wine on palate.

Volatile acidity consists of free form and volatile acids present in form of salts in wine. Generally it's associated with acetic acid content of wine. Its determination helps to understand health of wine. Higher level of acetic acid is an indication of bad storage conditions, microbial spoilage of wine and inappropriate fermentation. It was determined by OIV method (Ref.No MA-E-AS313-02-ACIVOL). Alcohol content of wine was determined by using the Ebulliometry method (Zoecklein et al, 1995). Alcohol content provides texture, structure and flavor to wine and contribute better mouth feel and body. It can also give an idea about initial sugar content of grapes and harvest conditions. Sulfur dioxide is one of the most promising antimicrobial and antioxidant agent in wine making. Determination of sulfur dioxide was done by using Ripper method described by OIV (Ref.No. MA-AS323-04B:R2009).

Progress of malolactic fermentation was monitored by using paper chromatography methods with use of Watman paper n°1 (Ribereau-Gayon 1954, Patrick Illand et al, 1993). Malolactic fermentation is done with an aim to reduce the aggressive acidity of malic acid, to reduce astringency and bitterness of tannins by release of polysaccharides from bacterial cell walls. Adverse conditions of wine alcohol, temperature and pH can act as inhibitory factor for progress or onset of malolactic fermentation (Constantin Croitoru, 2013).

#### Analysis of chromatic characters

Color of wine is most easily recognizable character of wine. It reflects style of vinification, quality and evolution of wine over period of ageing. Application of fertilizers, organic amendment, nitrogen content, and pruning type can affect on the development of secondary metabolites during grape maturation period. In order to determine effect of application of organic amendments on wine quality, analysis of the chromatic characters was performed. Analysis was

performed by using spectrophotometer method proposed by Somers and Evans (1977). Chromatic characteristics of wine such as color intensity, ionization index, polymerization index, total anthocyanin and total phenol content of wines were analyzed. Color intensity was measured by checking absorbance of wine samples at 420, 520 and 620 nm (Somers and Evans, 1977).

Ionization index of anthocyanins was calculated by measuring absorbance of wine sample with presence of HCl and SO<sub>2</sub> solution. Ionization index was calculated by using following formula

$$\text{Ionization index} = \frac{(\text{abs } 520 \text{ nm}) - (\text{abs SO}_2 \text{ 520 nm})}{(\text{abs HCl } 520 \text{ nm}) - (5/3 \cdot \text{abs SO}_2 \text{ 520 nm})} \times 100$$

Total phenolic content were evaluated by direct spectrophotometric measurement of diluted wine sample at 280 nm wavelength. Dilution factor was considered while providing final result (Somers and Evans, 1977).

$$\text{Total phenol index (TPI)} = \text{abs } 280 \text{ nm} \cdot \text{dilution factor.}$$

To convert total phenolic index into actual concentration of phenolic compound (mg/L) standard curve of gallic acid was used.

Total anthocyanin which play vital role in color of red wines, were analyzed by measuring difference in absorbance of wine sample with introduction of hydrochloric acid (HCL) and sulfur dioxide (SO<sub>2</sub>) (Somers and Evans, 1977).

$$\text{Total anthocyanins (mg/L)} = 20\{\text{abs HCl (520 nm)} - 5/3 \text{ abs SO}_2 \text{ (520 nm)}\}$$

#### Tannin power

Astringency is known to be one of most important organoleptic properties of red wines. This sensation is normally associated to non-covalent interactions between salivary proteins and polyphenols present in wines. Reaction results into formation of insoluble aggregates which can precipitate, blocking lubrication of palate leading to sensation of dryness and constriction.

Procyanidin molecular structure contains several groups such as the aromatic rings and carbon – hydrogen skeleton of the pyranic ring which provides many sites of hydrophobic nature able to interact with proteins. To measure tannin power method was followed as described by Freitas and Mateus, (2001). Relative concentration of procyanidins was measured by ability of proteins present in BSA (bovine serum albumin) to bind with procyanidins and precipitate them in solution in which composition of resulting protein- tannin aggregate was directly measured by nephelometry. This method allows quantification of scattered light in solution resulting from

gradual formation of cloudy precipitates corresponding to tannin- protein aggregates (Freitas and Mateus, 2001)

Model solution for dilution of wine sample was prepared by hydro-alcoholic solution of 12% (v/v); pH 3.2, pH was reduced by using tartaric acid solution of 5 g/L. Solution was filtered (0.45  $\mu\text{m}$ ) prior to use. Wine sample after centrifugation was diluted by using model solution to 1:50. Then turbidity of this sample was measured by using the nephelometer (Hach 2100N). Instrument was calibrated before use by using formazin standards for calibration. Later in other test tube diluted wine sample (4 mL) was introduced by 150  $\mu\text{L}$  of BSA (Bovine Serum Albumin 0.8 g/L). It was mixed by using vortex and stored in dark place at ambient temperature for 45 minutes. After that turbidity of this wine sample along with BSA was also measured by using nephelometer.

Tannin power of wine sample was obtained by using following formula-

$$\text{Tannin power (NTU/mL)} = (d-d_0/0.08)$$

Where, d - turbidity of sample along with BSA

$d_0$  - turbidity of sample without BSA

### 3.1.4 Mineral analysis

Mineral analysis of wine sample was performed for quantification of potassium, calcium, copper and iron element. For analysis of iron method proposed by OIV was used (Ref.No.OIV-MA-AS322-05A). After achieving dilution of wine and removal of alcohol iron content of wine sample were determined directly by using atomic absorption spectrophotometer. Removal of alcohol was achieved by using rotary evaporator with thermostatically controlled water bath. Analysis of copper was performed as per method proposed by OIV (Ref.No.OIV-MA-AS322-06). After achieving suitable dilution copper content of wine samples was directly measured by using atomic absorption spectrophotometer. Calcium content of wine samples were measured directly by using atomic absorption spectrophotometer on diluted wine sample after the addition of ionization suppression agent (Ref.No.OIV-MA- AS322-04). In this case strontium chloride was used as ionization suppressing agent.

Potassium content of wine sample was analyzed on diluted wine sample, after addition of cesium chloride to suppress ionization of potassium. Contents were directly measured by using atomic absorption spectrophotometry( Ref.No.OIV-MA-AS322-02A). For all minerals standard solutions were prepared to make calibration curve as per the methods described in each of reference method. Total nitrogen content of wine sample were analyzed by sample was wet ashed using sulfuric acid in presence of catalyst and then ammonia liberated by sodium hydroxide was determined titrimetrically.

### 3.1.5 Heavy metal analysis

#### Apparatus

Analysis of heavy elements present in wine sample was done by using semi quantitative method with use of inductively coupled plasma-mass spectrometry (Catarino et al, 2006). The analytical measurements were carried out with a Perkin Elmer SCIEX Elan 9000, ICP-MS (Perkin- Elmer SCIEX, Nowalk, CT, USA), apparatus equipped with a cross flow nebulizer, and a Scott- type chamber made of Ryton and Nickel cones. A peristaltic sample delivery pump model Gilson, was used. Autosampler Perkin- Elmer AS-93 Plus was used. Application software Elan-6100 Windows NT (version 2.4) was used.

#### Materials and reagent

For the optimization of the ICP-MS monoelement standard solutions of Be, Co and In (1000 mg/L) and solution with Mg, Cu, Rh, Cd, In, Ba, Ce, Pb and U (10 µg/L) were used. Concentrated HNO<sub>3</sub>, C<sub>2</sub>H<sub>5</sub>OH were used for the wash and preparation of standard solutions. For the internal standardization of ICP-MS solutions of Rh and Re (1000mg/L) were used. ICP- MS calibration was established with a multielement standard solution with 30 elements: Li, Be, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ag, Cd, In, Cs, Ba, Hg, Ti, Pb, Bi, U (10mg/L). The preparation of the solutions was done using only plastic materials. All the materials was soaked in 20% HNO<sub>3</sub> (v/v) for at least 24h and rinsed several times with purified water before use. The purified water was produced by using a Seralpur Pro 90CN apparatus (Seral, Ransbach-Baumbach, Germany).

### 3.1.6 Sensory analysis

Sensory analysis of wines play huge role in understanding perception of consumers towards wine. It covers various aspects of wine evaluation such as preference determination, assessment of specific attributes and development of flavor profiles. It can act as supplementary for confirmation of results that are obtained during laboratory chemical analysis. It can be helpful to understand the trends and to associate linkage between physiochemical and sensorial analysis of wine.

Advantage of analytical sensory analysis is that, statistical methods can be applied to determine if trends seen are due to random chance or reflect a significant difference. Analysis was performed in tasting room of Instituto Superior de Agronomia. Ideal conditions of lighting, adequate air and physically isolated space was provided, also taster's interactions were limited.

Each of wine samples was given random code, totally 13 numbers of tasters were participated in tasting. All tasters were professionally skilled in sensory analysis of wines; they had good knowledge and background to evaluate the wines. Tasting scorecard was recommended by Instituto superior de Agronomia. It consisted of four different parameters color, aroma, taste and global evaluation of the wine. For each of the parameters certain set of descriptive notes were

given. Scale of 1 to 5 was provided for all descriptive notes. Example of tasting score card is provided in annex.

### **3.1.7 Statistical analysis**

It's important to process statistically absolute values obtained during laboratory analysis and information gathered during observations, as it gives better understanding of effect, interference and variation amongst each types of treatment used under experimental work.

Results presented in this work are generally the average value of two analytical repetitions. For calculating final result from observations recorded after physiochemical analysis Microsoft Excel 2007 was used.

In order to check statistical significance of treatments software Statistix 9 was used. All treatments were checked by using analysis of variance test (ANOVA). Effect of each organic amendment treatment was checked on each analytical parameter one by one, similar was performed in order to check effect of pruning type. By using the p value it was possible to verify differences between various values are statistically significant or not. In order to show significance of p value star (\*) symbol was used (equal and below 0.05 - \*, below 0.01 - \*\*, below 0.001 - \*\*\*, below 0.0001 - \*\*\*\*) and for non significant p value, symbol 'ns' was used.

## 4. RESULTS AND DISCUSSION

Observations were made from maturation stage of grapes prior to harvest until sensorial analysis after final bottling of wine. This part present result obtained for all different types of analysis carried during entire period of experiment. Analysis carried were physiochemical analysis which included analysis of basic parameters of wine, mineral analysis, heavy metal analysis and finally sensorial analysis of wine samples.

Entire result obtained can be summarized into three broad categories as following:

- Results obtained during maturation stage and vinification
- Results obtained after bottling of wine samples
- Results obtained after sensorial analysis.

### 4.1 Analysis during grape maturation and vinification

#### 4.1.1. Analysis at grape stage

Grape samples were analyzed for both wineries after harvest at juice stage. Analysis results presented here reflects effect of organic amendments addition and pruning type on grape juice composition for each block under experiment. Analysis of each block at must stage shows that parameters that are analyzed have close values in all blocks with some exceptions. Grapes for Quinta do Coro were harvested on 12<sup>th</sup> September 2013 results below show data for analytical results obtained for each block under treatment.

**Table No. 3 Quinta do Coro - analysis of grapes for each block**

<b>Quinta do Coro - block 1</b>						
<b>Treatment</b>	<b>Weight of 100 berries (g)</b>	<b>Brix (%)</b>	<b>pH</b>	<b>Total acidity (g/L)</b>	<b>Total anthocyanins (mg/L)</b>	<b>Total phenol index</b>
MAN TEST	125.3	26.8	3.58	5.0	1473.8	59
MAN BIOC	132.5	26.1	3.52	4.9	1206.2	47
MAN RSUC	124.9	26.7	3.52	5.0	1492.3	57
MAN ESTR	121.8	26.1	3.48	5.0	1923.1	72
MAN ETAR	135.2	25	3.49	4.9	932.3	37
MEC TEST	92.5	27.6	3.62	4.8	2329.2	94
MEC BIOC	100	27.7	3.59	4.5	2418.5	87
MEC RSUC	107.5	27	3.55	4.5	2132.3	80
MEC ESTR	106.5	25.9	3.52	4.8	1787.7	70
MEC ETAR	111.4	25.3	3.48	4.7	1726.2	65

**Table No. 4 Quinta do Coro - analysis of grapes for each block**

<b>Quinta do Coro - block 2</b>						
<b>Treatment</b>	<b>Weight of 100 berries (g)</b>	<b>Brix (%)</b>	<b>pH</b>	<b>Total acidity (g/L)</b>	<b>Total anthocyanins (mg/L)</b>	<b>Total phenol index</b>
MAN TEST	126.3	26.5	3.54	5.1	1904.6	69
MAN BIOC	103.9	28.4	3.61	5.3	2338.5	91
MAN RSUC	148.5	25.5	3.56	5.0	1569.2	62
MAN ESTR	132.8	26.2	3.53	5.1	1756.9	70
MAN ETAR	122.3	25.2	3.60	4.8	2089.2	79
MEC TEST	113.6	27.9	3.60	4.8	1978.5	81
MEC BIOC	97	26.2	3.55	5.1	2230.8	91
MEC RSUC	116.1	25.8	3.47	4.8	1824.6	75
MEC ESTR	110.6	25.7	3.53	5.0	1960.0	62
MEC ETAR	112.7	24.7	3.54	4.8	1858.5	72

**Table No. 5 Quinta do Coro - analysis of grapes for each block**

<b>Quinta do Coro - block 3</b>						
<b>Treatment</b>	<b>Weight of 100 berries (g)</b>	<b>Brix (%)</b>	<b>pH</b>	<b>Total acidity (g/L)</b>	<b>Total anthocyanins (mg/L)</b>	<b>Total phenol index</b>
MAN TEST	133.2	24.6	3.56	5.3	1935.4	74
MAN BIOC	144.5	25.7	3.54	4.8	1630.8	61
MAN RSUC	131.4	26	3.62	4.5	1864.6	73
MAN ESTR	127.7	26.3	3.58	4.8	1840.0	67
MAN ETAR	136	25.7	3.56	4.7	1963.1	73
MEC TEST	110.7	26.2	3.53	5.1	1944.6	77
MEC BIOC	119.5	26.6	3.59	5.1	1633.8	64
MEC RSUC	122.5	26.4	3.61	5.2	1763.1	64
MEC ESTR	129.3	24.9	3.54	5.1	1433.8	54
MEC ETAR	126.6	24.5	3.49	5.1	1572.3	58

Grape samples for Quinta do Gradil were harvested on 26<sup>th</sup> September 2013. After harvest analysis of must was performed for each block. Following are values recorded for parameters under analysis.

**Table No. 6 Quinta do Gradil - analysis of grapes for each block**

<b>Quinta do Gradil - block 1</b>						
<b>Treatment</b>	<b>Weight of 100 berries (g)</b>	<b>Brix (%)</b>	<b>pH</b>	<b>Total acidity (g/L)</b>	<b>Total anthocyanins (mg/L)</b>	<b>Total phenol index</b>
MAN TEST	176.3	22	3.46	5.6	953.8	27.4
MAN BIOC	157.5	20.3	3.4	5.0	1032.3	31.8
MAN RSUC	151.9	20.7	3.43	5.4	1129.2	35.3
MAN ESTR	151.9	19.8	3.48	4.8	940.0	27.3
MAN ETAR	166.7	19.9	3.46	5.3	1047.7	32.0
MEC TEST	125.1	20.6	3.32	5.4	980.0	32.4
MEC BIOC	122.4	20.3	3.44	4.8	1338.5	46.8
MEC RSUC	112.7	19.2	3.35	5.9	1143.1	36.8
MEC ESTR	148.2	19.5	3.31	5.4	1161.5	39.3
MEC ETAR	146.7	18.8	3.35	5.1	843.1	28.5

**Table No. 7 Quinta do Gradil - analysis of grapes for each block**

<b>Quinta do Gradil-block 2</b>						
<b>Treatment</b>	<b>Weight of 100 berries (g)</b>	<b>Brix (%)</b>	<b>pH</b>	<b>Total acidity (g/L)</b>	<b>Total anthocyanins (mg/L)</b>	<b>Total phenol index</b>
MAN TEST	141.2	21.9	3.49	5.0	1626.2	56.8
MAN BIOC	181.9	19.4	3.35	5.3	924.6	29.0
MAN RSUC	160.4	20.1	3.43	5.0	1276.9	40.2
MAN ESTR	162.7	20.1	3.41	5.3	1156.9	37.3
MAN ETAR	179.5	18.9	3.29	5.9	950.8	28.6
MEC TEST	133.0	20.6	3.37	5.7	1400.0	45.5
MEC BIOC	164.0	18.9	3.34	5.6	1049.2	34.1
MEC RSUC	116.1	19.5	3.33	5.9	1249.2	40.6
MEC ESTR	132.8	18.5	3.29	5.9	926.2	30.1
MEC ETAR	167.7	18.4	3.26	5.6	818.5	26.6

**Table No. 8 Quinta do Gradil - analysis of grapes for each block**

<b>Quinta do Gradil - block 3</b>						
<b>Treatment</b>	<b>Weight of 100 berries (g)</b>	<b>Brix (%)</b>	<b>pH</b>	<b>Total acidity (gm/L)</b>	<b>Total anthocyanins (mg/L)</b>	<b>Total phenol index</b>
MAN TEST	166.3	21.1	3.45	5.1	1378.5	48.3
MAN BIOC	154.7	20.8	3.37	6.0	1206.2	45.2
MAN RSUC	172.6	19.4	3.33	5.7	732.3	28.5
MAN ESTR	152.1	20.9	3.49	5.0	1320.0	47.6
MAN ETAR	170.7	20.9	3.38	5.3	1323.1	48.8
MEC TEST	123.4	18.5	3.30	5.7	1123.1	42.1
MEC BIOC	131.8	18.9	3.35	5.3	1043.1	38.7
MEC RSUC	148.8	18.9	3.30	5.0	652.3	28.4
MEC ESTR	132.8	19.9	3.33	5.4	1175.4	46.9
MEC ETAR	144.0	18.5	3.3	5.3	735.4	30.1

**Statistical results**

**Table No. 9 statistical results for Quinta do Coro (grapes analysis for each block)**

<b>Treatment</b>	<b>Weight of berries (g)</b>	<b>Brix (%)</b>	<b>Potential alcohol (% v/v)</b>	<b>pH</b>	<b>Total acidity (g/L)</b>	<b>Total anthocyanins (mg/L)</b>	<b>Total phenol index</b>
<b>Man</b>	129.8 a	26.0	15.3	3.56	4.9	1805.97	70
<b>Mec</b>	112.6 b	26.0	15.3	3.54	5.0	1819.97	71
<b>Sig</b>	<b>***</b>	<b>Ns</b>	<b>ns</b>	<b>ns</b>	<b>ns</b>	<b>ns</b>	<b>ns</b>
<b>TEST</b>	119.6	26.4 a	15.5 a	3.56	5.0	1862.6	76
<b>BIOC</b>	116.2	26.7 a	15.7 a	3.57	5.0	1942.2	76
<b>RSUC</b>	126.8	26.0 ab	15.3 ab	3.56	4.8	1761.7	68
<b>ESTR</b>	119.1	25.8 ab	15.1 ab	3.54	5.0	1687.7	64
<b>ETAR</b>	124.3	25.0 b	14.7 b	3.54	4.8	1810.6	68
<b>Sig</b>	<b>ns</b>	<b>**</b>	<b>**</b>	<b>ns</b>	<b>ns</b>	<b>ns</b>	<b>ns</b>

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, ns - not significant )

**Table No. 10 statistical results for Quinta do Gradil (grapes analysis for each block)**

Treatment	Weight of berries (gm)	Brix (%)	Potential alcohol (% v/v)	pH	Total acidity (g/L)	Total anthocyanins (mg/L)	Total phenol index
<b>Man</b>	163.1 a	20.4 a	12.0 a	3.41 a	5.3	1133.2	38
<b>Mec</b>	136.6 b	19.3 b	11.3 b	3.33 b	5.4	1042.6	36
<b>Sig</b>	****	***	***	****	<i>ns</i>	<i>ns</i>	<i>ns</i>
<b>TEST</b>	144.2	20.8	12.2	3.40	5.4	1243.6	42
<b>BIOC</b>	152.1	19.8	11.6	3.38	5.3	1099.0	38
<b>RSUC</b>	143.8	19.6	11.5	3.36	5.5	1030.5	35
<b>ESTR</b>	146.8	19.8	11.6	3.39	5.3	1113.3	38
<b>ETAR</b>	162.6	19.2	11.3	3.34	5.4	953.1	32
<b>Sig</b>	<i>ns</i>	<i>Ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, *ns* - not significant )

For both wineries there is marked effect of type of pruning on berry weight (Table No. 9 and 10). Mechanically pruned grapevines shows reduced amount of berry weight compared to manual pruning. Similar results with mechanical pruning were obtained by Reynolds (1988) and Morries et al, (1981). This may be possible due to higher number of buds retained by mechanical pruning (34 buds/vine) compared to manual pruning 14 buds/vine (Gatti et al, 2011). There was no effect on overall yield due to decreased bunch weight by mechanical pruning as the number of bunches per vine was higher enough to compensate the decreased bunch weight (Archer and Shalkwyk, 2007). Weight of berries for Quinta do Coro was lower than Quinta do Gradil because of the difference in their berry stage when harvested. Harvest of Quinta do Coro was delayed and because of which there might be excessive loss of water from vegetative tissue of berries giving concentration effect with loss of berry weight. While Quinta do Gradil was harvested later than Quinta do Coro grapes were intact and well shaped also event of rainfall before harvest could have been accounted for higher weight of berries.

For Quinta do Coro application of organic amendment has effect on total soluble solids (i.e. potential alcohol) present in grape juice. The control and BIOC treatment application shows higher values compared to ETAR (Table No. 10). This is possible due to higher supply of nitrogen in case of ETAR treatment (Delas and Pouget, 1984)

In wine samples from Coro pH and total acidity values were very close and there was no significant impact of application of organic amendment and pruning type except in case of Quinta do Gradil there was significant effect of type of pruning on pH values, mechanically pruned vine had lower pH compared to hand pruning (Table 11). Archer and Schalkwyk (2007) observed similar results, grapes from alternative pruning methods, like mechanical pruning, minimal pruning and no pruning had lower pH compared to hand pruned vines and this effect

was also carried in final wine, similar results was observed in our experiment. Effect of higher pH in case of hand pruned vines can be due to higher shade levels in bunch zone for vertically shoot position (VSP) trained plots. Anthocyanin content of grape must were unaffected by treatment as well type of pruning (Table 9 and 10). Higher level of anthocyanins and total phenolic index was observed in grape juice from the biochar treated plot while lowest value was observed in plot treated with cattle manure (Table 9). Morlat and Symoneaux (2008) also found that higher dosage of cattle manure showed decrease in color intensity and lower anthocyanin concentration in wine. Value of total anthocyanin and total phenolic index for Quinta do Coro is higher than Quinta do Gradil, these results can be due to concentration effect as harvest for Quinta do Coro was delayed and it can be noted from elevated level of potential alcohol. Another possible reason can be ascribed to climatic conditions, there was frequent event of rainfall prior to harvest occurred in case of Quinta do Gradil. This may have lead to dilution effect in overall berry composition.

#### 4.1.2 Analysis of must prior to fermentation

Grapes which were obtained from all three different blocks were treated altogether. Analysis of juice was carried and results obtained were as following.

**Table No. 11 Quinta do Coro analysis of must (after mixing blocks together)**

<b>Treatment</b>	<b>Degree brix (%)</b>	<b>pH</b>	<b>Total acidity (g/L)</b>
MAN TEST	26.0	3.54	4.95
MAN BIOC	25.5	3.55	4.95
MAN RSUC	26.2	3.57	4.8
MAN ESTR	25.9	3.58	4.95
MAN ETAR	26.2	3.57	5.1
MEC TEST	26.3	3.46	5.25
MEC BIOC	25.9	3.48	5.25
MEC RSUC	24.5	3.48	5.1
MEC ESTR	24.7	3.48	5.1
MEC ETAR	23.3	3.42	5.4

**Table No. 12 Quinta do Gradil analysis of must (after mixing blocks together)**

Treatment	Degree brix (%)	pH	Total acidity (g/L)
MAN TEST	20.7	3.56	5.6
MAN BIOC	20.2	3.57	6.0
MAN RSUC	20.1	3.50	6.0
MAN ESTR	20.1	3.54	5.6
MAN ETAR	19.2	3.48	6.3
MEC TEST	19.1	3.44	6.5
MEC BIOC	19.1	3.52	6.0
MEC RSUC	18.6	3.40	6.8
MEC ESTR	18.6	3.42	6.6
MEC ETAR	17.9	3.40	6.2

Pruning treatment showed significant effect on total soluble solids content, pH and total acidity of grape juice. Grape juice coming from manually pruned vines had higher level of pH, total soluble solids and lower acidity. Application of organic amendments did not show any significant effect on grape juice composition of above mentioned parameters.

**Table No. 13 Statistical results for Quinta do Coro analysis of must (after mixing blocks together)**

Treatment	Degree brix (%)	pH	Total acidity (g/L)
MAN	26.0	3.56 a	5.0 b
MEC	24.9	3.46 b	5.2 a
Sig.	<i>ns</i>	****	**
TEST	26.2	3.5	5.1
BIOC	25.7	3.52	5.1
RSUC	25.4	3.53	5.0
ESTR	25.3	3.53	5.0
ETAR	24.8	3.50	5.3
Sig.	<i>ns</i>	<i>ns</i>	<i>ns</i>

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, *ns* - not significant)

**Table No. 14 Statistical results for Quinta do Gradil analysis of must (after mixing blocks together)**

Treatment	Degree brix (%)	pH	Total acidity (g/L)
MAN	20.1 a	3.53 a	5.9 b
MEC	18.7 b	3.44 b	6.4 a
Sig.	**	*	*
TEST	19.9	3.50	6.1
BIOC	19.7	3.55	6.0
RSUC	19.4	3.45	6.4
ESTR	19.4	3.48	6.1
ETAR	18.6	3.44	6.3
Sig.	<i>ns</i>	<i>ns</i>	<i>ns</i>

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, *ns* - not significant )

Statistical analysis of data shows that there is significant effect of type of the pruning on pH and total acidity. Value of pH is higher for manual pruning compared to mechanical pruning. Regarding total soluble solids (degree brix) there is significant effect of type of pruning in case of Quinta do Gradil (Table No. 14), These results are consistent Di Collalto et al (1988) within their mechanical trials for Sangiovese grape variety for four years; mechanical pruned vines showed increase of yield, almost 30 % more compared with hand pruned vines yield and thus must total soluble solids concentration decreased.

When we compare data amongst two wineries, there is big difference between results of parameters analyzed. Values for degree brix for Coro are in between 24°Brix to 26°Brix while for Gradil they are comparatively lower in between 18°Brix to 20°Brix. For value of pH there is not much difference within wineries but value of total acidity are different, Coro has lower total acidity values compared to Gradil.

Variation between degree brix and total acidity was due to terroir effect specific for each winery. For Coro values were higher also due to delayed harvest beyond maturity and hence there was concentration effect on total soluble solids caused by loss of water from berries. At same time there was effect of the dilution in case of Quinta do Gradil where frequent rainfall events occurred prior to harvest period. Similar results were found in Tuscany (Italy) by Storchi et al, (2005). Large amount of variation of yield and berry composition variables was determined by soil type and soil water holding capacity. High water availability from veraison to harvest induced more vegetative growth and reduced sugar, color and phenol concentrations in berries.

#### **4.2 Analysis of wine Samples after bottling**

After analysis of grape juice, it went through alcoholic fermentation. Later wines were allowed to undergo natural malolactic fermentation. To facilitate malolactic fermentation after alcoholic fermentation sulfur dioxide was not added and also temperature of wine was maintained around 25°C.

Malolactic fermentation for Quinta do Gradil was finished earlier than Quinta do Coro. All wines of Gradil finished malolactic fermentation in between end of October month to first week of November. For Quinta do Coro malolactic fermentation took much longer than expected but this delay of malolactic fermentation for Quinta do Coro can be ascribed to higher level of alcohol in wine. This may have reduced and delayed activity of malolactic bacteria. After malolactic fermentation, wines were analyzed for further parameters in order to understand exact effect of addition of organic amendments and type of pruning on final wine quality. For each parameter there were two replicates, results presented here is average of two analytical replicates.

#### 4.2.1 Analysis of wine (pH, total acidity, volatile acidity, sulfur dioxide)

##### Analysis of wine samples - Quinta do Coro

Analysis results show that pH of wine sample increased after alcoholic fermentation. It's normal to have increase in pH of wine after alcoholic as well as malolactic fermentation. Alcohol content of wine sample is quiet high in control treatment for both manual as well as mechanical type of pruning. This elevated alcohol level can significantly affect on sensory perception of this wines. Almost all wines have alcohol level above 15 % v/v for this particular winery.

**Table No. 15 Analysis of wine - Quinta do Coro**

Pruning	Treatment	pH	Total acidity (g/L)	Alcohol (% v/v)	Volatile acidity (g/L)	Free sulfur dioxide (mg/L)	Total sulfur dioxide (mg/L)
MAN	TEST	3.48	6.3	17.1	0.41	14	38
MAN	BIOC	3.53	6.2	16.7	0.46	16	54
MAN	RSUC	3.60	6.2	16.7	0.43	14	59
MAN	ESTR	3.84	5.1	17.1	0.45	30	102
MAN	ETAR	3.54	6.0	17.1	0.34	13	38
MEC	TEST	3.45	6.3	17.9	0.40	10	50
MEC	BIOC	3.49	6.1	16.6	0.43	11	53
MEC	RSUC	3.63	5.6	15.1	0.41	45	90
MEC	ESTR	3.67	5.4	15.8	0.42	40	94
MEC	ETAR	3.45	6.1	14.8	0.36	34	90

**Table No. 16 Statistical results for analysis of wine - Quinta do Coro**

Treatment	pH	Total acidity (g/L)	Alcohol (% v/v)	Volatile acidity (g/L)	Free sulfur dioxide (mg/L)	Total sulfur dioxide (mg/L)
MAN	3.60	5.96	16.94	0.42	17.44	58.40
MEC	3.54	5.88	16.04	0.40	27.84	75.20
<i>Sig.</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
TEST	3.47 b	6.3 a	17.50	0.41 ab	12.00	44.00
BIOC	3.51 ab	6.2 a	16.65	0.45 a	13.60	53.60
RSUC	3.62 ab	5.9 ab	15.90	0.42 a	29.60	74.80
ESTR	3.76 a	5.3 b	16.45	0.43 a	34.80	97.60
ETAR	3.50 b	5.9 ab	15.95	0.34 b	23.20	64.00
<i>Sig.</i>	*	*	<i>ns</i>	*	<i>ns</i>	<i>ns</i>

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, *ns* - not significant )

When results were processed statistically, there was significant effect of type of organic amendments applied on pH and total acidity of wine sample (Table No. 16). Wine sample treated with cattle manure (ESTR) has higher pH and lower total acidity values compared to other treatments. This may be possible due to higher supply of available potassium from this particular treatment (Table No.24). Mineral analysis of wine sample confirmed that the wine sample treated with this organic amendment (cattle manure) has higher potassium levels compared to other treatments. There is no significant effect of type of pruning on alcohol level for this winery. But alcohol level is over all higher in all wine samples from this winery compared to Quinta do Gradil. Probably because harvest was done beyond optimum maturity level, it may have decreased difference of initial degree brix between two types of pruning due to concentration effect caused by loss of water.

There is statistically significant effect of type of organic amendment on volatile acidity of wine sample; Sewage sludge (ETAR) has lowers volatile acidity levels in both types of pruning. This may be possible due to higher supply of the nitrogen provided by ETAR. Fermentation by *Saccharomyces cerevisiae* can produce some amount of acetic acids depending on must composition and strain type. Bely et al, (2003) observed in there experiment about the influence of assimilable nitrogen on volatile acidity production by *Saccharomyces cerevisiae* during high sugar fermentation, they concluded that, production of volatile acidity was inversely correlated with maximum cell population and assimilable nitrogen concentration. Higher is nitrogen concentration, less volatile acidity is produced. This might be reason for having lower volatile acidity in case of ETAR treatment in our experiment, but we can't conclude its only reason for it, as we didn't analyze the assimilable nitrogen level and yeast cell population. Malolactic fermentation can also contribute to the part of volatile acidity level increase of the final wine.

The sulfur dioxide levels of wine sample were adjusted to 40 mg/L prior to bottling. But there are some of the wines in the results mentioned here have low free sulfur dioxide levels ranging in between 10-14 mg/L, because analysis was performed before addition of the sulfur. At final

bottling of wines care was taken to adjust free sulfur dioxide level for all wines to 40 mg/L, wines were well protected from any damage caused by oxidation and microbial spoilage.

### Analysis of wine samples - Quinta do Gradil

Analysis of wine sample for Gradil shows that pH of wine sample has increased after fermentation. Wine sample pH for Gradil is significantly higher than Coro especially in the case of manually pruned vines. These results can be due to higher level of the potassium recorded for this winery as compared to level of the potassium recorded in the wine sample from Coro (Table No. 26).

**Table No. 17 Analysis of wine - Quinta do Gradil**

Pruning	Treatment	pH	Total acidity (g/L)	Alcohol (% v/v)	Volatile acidity (g/L)	Free sulfur dioxide (mg/L)	Total sulfur dioxide (mg/L)
MAN	TEST	3.72	4.5	12.4	0.31	32	85
MAN	BIOC	3.78	4.4	11.8	0.30	38	77
MAN	RSUC	3.76	4.4	11.6	0.33	32	77
MAN	ESTR	3.72	4.5	11.8	0.33	22	78
MAN	ETAR	3.68	4.5	11.3	0.45	35	72
MEC	TEST	3.55	4.8	11.0	0.37	35	77
MEC	BIOC	3.63	4.7	10.9	0.35	29	69
MEC	RSUC	3.48	5.3	10.5	0.27	42	93
MEC	ESTR	3.50	5.4	10.5	0.39	26	93
MEC	ETAR	3.48	5.4	10.3	0.23	24	75

**Table No. 18 Statistical results for analysis of wine - Quinta do Gradil**

Treatment	pH	Total acidity (g/L)	Alcohol (% v/v)	Volatile acidity (g/L)	Free sulfur dioxide (mg/L)	Total sulfur dioxide (mg/L)
MAN	3.73 a	4.42 b	11.78 a	0.34	32.00	77.76
MEC	3.53 b	5.14 a	10.64 b	0.32	31.04	81.28
<b>Sig.</b>	<b>***</b>	<b>**</b>	<b>**</b>	<b>ns</b>	<b>ns</b>	<b>ns</b>
TEST	3.64	4.7	11.70	0.34	33.6	80.8
BIOC	3.71	4.6	11.35	0.33	33.6	72.8
RSUC	3.62	4.9	11.05	0.30	36.8	84.8
ESTR	3.61	4.9	11.15	0.36	24	85.6
ETAR	3.58	5.0	10.80	0.34	29.6	73.6
<b>Sig.</b>	<b>ns</b>	<b>ns</b>	<b>ns</b>	<b>ns</b>	<b>ns</b>	<b>ns</b>

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, ns - not significant)

Statistical results show significant effect of type of pruning on the pH of wine sample (Table No. 18) Manual pruning has higher values of pH compared to mechanical pruning, this can be possible due to lower number of bunches and so higher accumulation of the potassium in berries compared to mechanical pruned vines where bunches number was higher. Davies et al (2006) also observed during potassium content analysis at grape stage, that excessive Potassium concentration in the fruits at harvest can results in increased pH. Also pH increase can result due to shading effect. There is no significant effect of organic amendment on pH, total acidity. But ETAR which is sewage sludge compost it has higher vegetation in vineyard, had lower pH and higher acidity. The alcohol content for this winery was below 12 % v/v which is much lower than Quinta do Coro, this is possible because either grapes were yet to achieve optimum maturity level or the rainfall events which occurred prior to harvest can be responsible for dilution effect. Type of pruning has significant effect on alcohol content of wine manual pruning has higher alcohol than mechanical pruning. This is ascribed to higher number of bunches in case of mechanical pruning which may have resulted in the lower accumulation of the overall berry sugar levels.

#### 4.2.2 Analysis of Chromatic Characteristics

##### Analysis of chromatic characteristics of wine samples - Quinta do Coro

Along with the other visual features such as clarity, fluidity and effervescence, a wine color can provide information on integrity of wine. Analysis results don't show any significant particular trend associated with specific organic amendment addition in the vineyard. Most probable reason for this is delayed harvest beyond the optimum maturity level. This delayed harvest may have caused concentration effect in grapes, overcoming effect of each individual organic amendment added. For total Phenolic index which represents amount of phenolic compounds present in wine, higher values are generally observed in control treatment.

**Table No. 19 - Analysis of chromatic characteristics of wine samples - Quinta do Coro**

Treatment	Total phenol (mg/L)	Total Phenol Index	Total antho. (mg/L)	Color intensity	Color hue	Ionization index (%)	Total pigment	Poly. pigment	Poly. index (%)	Tannin power (NTU/ml)
MAN TEST	2305.5	70	470.2	22.26	0.56	29.1	32.88	5.62	17.1	277.2
MAN BIOC	1964.1	59	452.4	18.43	0.57	24.2	30.55	4.76	15.6	282.0
MAN RSUC	2371.8	72	515.5	22.09	0.58	26.2	34.69	5.35	15.4	279.0
MAN ESTR	2106.5	63	419.5	11.94	0.72	4.3	29.44	5.08	17.3	269.0
MAN ETAR	2396.1	72	475.0	22.37	0.58	27.1	33.43	5.81	17.4	288.0
MEC TEST	2640.5	80	510.6	24.32	0.54	29.9	35.70	6.11	17.1	240.1
MEC BIOC	2501.3	76	473.1	23.15	0.55	30.9	33.13	5.69	17.2	298.4
MEC RSUC	1941.4	58	420.2	10.85	0.67	9.7	26.97	3.58	13.3	222.3
MEC ESTR	1936.6	58	400.7	11.29	0.65	11.1	26.16	3.68	14.1	226.1
MEC ETAR	1740.8	52	354.4	9.81	0.65	9.0	23.68	3.58	15.1	258.2

**Table No. 20 - Statistical results for chromatic characteristics of wine samples - Quinta do Coro**

Treatment	Total phenol (mg/L)	Total Phenol Index	Total antho. (mg/L)	Color intensity	Color hue	Ionization index (%)	Total pigment	Poly. pigment	Poly. index (%)	Tannin power (NTU/ml)
MAN	2228.8	67	466.5	19.42	0.60	22.2	32.20	5.32	16.6	279.04
MEC	2152.1	65	431.8	15.88	0.61	18.1	29.13	4.53	15.4	249.01
<i>Sig.</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
TEST	2473.0	75	490.4	23.29	0.55	29.5	34.29	5.87	17.1	258.68
BIOC	2232.7	68	462.8	20.79	0.56	27.6	31.84	5.23	16.4	290.19
RSUC	2156.6	65	467.9	16.47	0.63	18.0	30.83	4.47	14.4	250.64
ESTR	2021.6	61	410.1	11.62	0.69	7.7	27.80	4.38	15.7	247.53
ETAR	2068.5	62	414.7	16.09	0.62	18.1	28.56	4.70	16.3	273.08
<i>Sig.</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, *ns* - not significant)

Statistical analysis of results obtained shows no significant effect of the type of pruning or type of organic amendments added on the chromatic characteristics of wine. Overall the values of the chromatic characters were higher for manual pruning compared to mechanical pruning. This is because of the higher number of bunches retained in case of the mechanical pruned vines and other possible reason is may be due to shading effect on fruit zone, in case of the mechanical pruning due to increased shoot density.

In an experiment about long term effect of mechanical winter pruning with Barbera Grapevines by Gatti et al, (2011) they also observed lower amount of anthocyanins content in semi-mechanical pruning treatment compared to manual pruning. They attributed this result due to the effect of increased shoot density and shading effect on fruiting zone in case of mechanical pruned vines. Similar kind of difference between anthocyanin, flavonols and proanthocyanidin content was observed in model wine solution between shaded and exposed bunches of Pinot Noir grapes in an experiment carried in Oregon, with lower concentration observed in shaded bunches, (Cortell and Kennedy, 2006).

Wine made from control treatment has higher levels of total phenolic compounds and anthocyanin content. Percentage of polymerized anthocyanin content is also higher in control treatment. Lowest value of polymerized anthocyanin is observed in Cattle Manure treated wine. In our experimental case also wines made from Cattle Manure (ESTR) have lower values of anthocyanin as well as total phenolic content as compared with remaining other treatments. This effect is possible due to the excessive supply of the nitrogen content to the grapevine by application of cattle manure resulting in increased vegetative growth. For same treatment value for ionization index i.e. fraction of anthocyanin in color form is very less. This is possible due to higher pH values in this particular treatment resulting in most part of anthocyanin in colorless hemiacetal form. As pH of red wine decreases there is an increase in the proportion of red or

flavylium form of anthocyanin and hence at an equivalent anthocyanin concentration wines will appear redder at a lower pH (Reynolds A.G., 2010).

There is no statistical significant difference in values for color intensity and color hue for different types of organic amendments added and type of pruning method used. Wines produced from cattle manure treatment has lowest color intensity compared to control and other organic amendment added. These results are in accordance with experiment carried by Morlat and Symoneaux (2008) with use of cattle manure, spent mushroom compost and dry crushed pruned vine wood to study effect of this organic amendment addition on wine characteristics. They also noted that treatment with higher dosage of cattle manure had both lowest color intensity and aromatic persistency and also had more herbaceous, animal odors and aromas which are less desirable descriptors.

Wine produced from manually pruned grapevines showed higher values of tannin power as compared with mechanically pruning. Tannin Power which is observed highest in biochar treated wine samples and lowest was observed in wine sample produced from cattle manure treated grapevines (Table No. 20). But similar trend was not observed in wine samples arrived from Quinta do Gradil (Table No. 22).

By observing absolute values for polymerized pigment it's clear that mechanically pruned control and biochar and manually pruned sewage sludge compost has higher values than rest of all other treatments (Table No. 19). All this treatments has higher levels of tannin power as well as anthocyanin content providing more sites for the polymerization of the pigment. Nitrogen analysis of all of this three treatment shows they have moderately higher level of nitrogen content in final wine samples. The other possible explanation of higher polymerized pigment in these particular treatments can be due to moderate to highly expose bunches. This can provide higher ratio of skin tannins to anthocyanins than extensively shaded bunches (Herderich et al, 2006).

Value of polymerization index for Quinta do Gradil is very less as compared to Quinta do Coro this is possible due to presence of the less amount of tannin in wine samples compared to total anthocyanins. As tannin quantity is less there is less reactivity between tannin and anthocyanin polymerization reaction leading to lower values of polymerization index.

**Table No. 21 - Analysis of chromatic characteristics of wine samples - Quinta do Gradil**

Treatment	Total phenol (mg/L)	Total antho. (mg/L)	Color intensity	Color hue	Ionization index (%)	Total pigment	Poly. pigment	Poly. index (%)	Tannin power (NTU/ml)
MAN TEST	1438.7	482.3	6.53	0.66	7.9	26.71	1.56	5.9	144.9
MAN BIOC	1314.1	453.6	5.74	0.66	7.5	24.92	1.35	5.4	112.0
MAN RSUC	1428.0	442.4	6.38	0.66	8.5	24.59	1.48	6.0	146.2
MAN ESTR	1358.9	347.0	6.05	0.70	5.8	20.81	2.08	10.0	156.4
MAN ETAR	1009.4	260.8	3.73	0.70	5.3	15.10	1.24	8.2	128.8
MEC TEST	1554.7	461.3	8.67	0.56	13.7	26.06	1.80	6.9	180.2
MEC BIOC	1295.8	396.9	6.57	0.60	10.8	22.27	1.46	6.5	112.6
MEC RSUC	1305.5	393.5	5.40	0.63	8.0	21.97	1.38	6.3	167.4
MEC ESTR	1329.8	398.8	5.44	0.62	7.7	22.32	1.43	6.4	102.1
MEC ETAR	1239.2	264.5	5.02	0.61	4.4	15.96	1.64	10.3	183.1

Analysis of chromatic characteristics of wine samples from Quinta do Gradil shows that values for all parameters analyzed for this particular winery are much lower than Quinta do Coro. This strong difference between values of two wineries can be attributed to frequent rainfall event that occurred at Quinta do Gradil near to harvest period. Other reason is harvest timing and maturity levels of grapes at harvest time. By looking at analysis of maturity indices, it's clear that, at Quinta do Coro grapes were over matured beyond optimum ripening level, while at Gradil grapes didn't achieved sufficient maturity level which is indicated by low level of alcohol. Also rainfall event must have lead to overall dilution effect on berry composition of Gradil.

Another possible explanation is difference in climatic conditions observed between two locations. Quinta do Coro which is located at Sardoal more in center of the Portugal away from influence of ocean. Average monthly higher temperature at this site is more (25°C) during duration of grape maturation period than Quinta do Gradil (20°C). While average lower temperatures at Quinta do Gradil (16°- 17°C) is higher than Coro (15°C). Although difference between two wineries for monthly average low temperature is not much significant this can be one factor responsible for accumulation of the higher phenolic compounds at the wine samples from Quinta do Coro compared to Quinta do Gradil.

Yamane et al, (2006) observed in their experiment with an aim to study effect of temperature on anthocyanin biosynthesis in grape berry skins from one to three week after coloring began, high and low temperature during ripening likely to affect production and/ or degradation of abscisic acid (ABA) in berry skins and ultimately endogenous ABA levels affects the expression VvmybA1; product of VvmybA1 then controls expression of anthocyanin biosynthetic enzyme genes. It appears that below higher excessive temperature (30°- 35°C) and reduction in diurnal temperature variation lead to an increase in anthocyanin production (Cohen et al, 2008).

Analysis results of wine samples from Quinta do Gradil displays that there is no statistical significant effect of type of pruning on chromatic characteristics of wines. Phenolic compounds were present highest in control treatment as compared with wines coming from plots applied with different types of organic amendments. Highest amount of anthocyanin was also found in control treatment while plot treated with waste water treatment plant sludge compost has lowest values of both total phenolic compounds and total anthocyanin. These results can be possible due to higher vegetative growth conferred by application of sewage sludge treatment. Vines grown under this particular plot has strong vegetative growth leading to shading effect on grape bunches.

Lower value in all other treatment compared to control for phenolic compounds can be possible may be by the application of organic amendment maturity of grapes was delayed. Also it's possible that nitrogen supplied by the means of organic amendment may have lead in alteration of source sink relationship where most of nitrogen supplied may have used for vegetative growth than reproductive growth of vine.

Statistical analysis of results shows that there is significant effect of type of organic amendment added on total anthocyanin and total pigments of wine produced. TEST, BIOC and RSUC treatment showed higher level of anthocyanin while ETAR and ESTR displayed lower amount of anthocyanin level. These results can be related to amount of nitrogen supplied by this particular treatment to grape vine. Polymerized pigment and tannin content for this winery is much lower than Quinta do coro. This is correlated with the overall lower content of total phenolic compounds.

**Table No. 22 - Statistical results for chromatic characteristics of wine samples - Quinta do Gradil**

Treatment	Total Phenol (mg/L)	Total antho. (mg/L)	Color intensity	Color hue	Ionization index (%)	Total pigment	Poly. pigment	Poly. index (%)	Tannin power (NTU/ml)
MAN	1309.8	397.2	5.69	0.68 a	7.0	22.43	1.54	7.1	137.7
MEC	1345.0	383.0	6.22	0.60 b	8.9	21.72	1.54	7.3	149.1
<i>Sig.</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	**	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
TEST	1496.7	471.8 a	7.60	0.61	10.8	26.39 a	1.68	6.4	162.6
BIOC	1304.9	425.3 a	6.16	0.63	9.2	23.6 a	1.41	6.0	112.3
RSUC	1366.7	418.0 a	5.89	0.65	8.3	23.28 a	1.43	6.2	156.8
ESTR	1344.4	373.0 ab	5.75	0.66	6.8	21.57 a	1.76	8.2	129.3
ETAR	1124.3	262.7 b	4.38	0.66	4.9	15.53 b	1.44	9.3	156.0
<i>Sig.</i>	<i>ns</i>	**	<i>ns</i>	<i>ns</i>	<i>ns</i>	**	<i>ns</i>	<i>ns</i>	<i>ns</i>

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, *ns* - not significant)

Morlat and Symoneaux, 2008 studied in their experiment, effect of long organic amendment addition on fruit composition and chemical and sensory characteristics of Cabernet franc wine. They observed that addition of organic amendments in higher dosage rate particularly cattle manure (at the rate of 20 t/ha) delayed berry maturation. Total soluble solids, anthocyanins and

tannin content in juice were lowered, whereas pH, nitrogen, phosphorus and potassium were significantly increased compared with control treatment. They concluded that higher dosage rate of organic amendments that supplied heavy supply of the nitrogen through the mineralization of soil organic matter affected negatively on grape and wine characteristics. Similar reasons can be possible in case of ETAR showing less total phenolic compounds, where supplied nitrogen was may be too higher leading to excessive vegetative growth of vine. (Table No. 22). Korbulesky et al, (2002) also noted that application of sewage sludge compost increased vine vigor may be because of higher mineral nitrogen input via sewage sludge which ultimately delayed grape maturity.

### **4.2.3 Mineral Analysis**

#### **Mineral analysis wine samples - Quinta do Coro**

Addition of organic amendments has marked influence on overall quantity of plant available elements in soil. It has been observed in several studies that these organic wastes are important sources of nutrients such as nitrogen, phosphorus and other elements so they can be beneficial to reduce cost of fertilization. Mineral analysis of final wine sample results shows that higher amount of the total nitrogen was generally obtained in wine sample coming from TEST and ETAR treatment. Possible explanation for this is the availability of the nitrogen present originally in soil and capacity of mineralization of nitrogen added in form of organic amendment. Sewage sludge chemical composition is dependent upon waste water composition. Sewage sludge is rich in organic matter and also contains plants nutrients such as nitrogen (N), phosphorus (P) and calcium (Ca) (Hue, 1988). Plant available form of nitrogen is released for several years after application of sewage sludge by decomposition of organic matter (Korboulesky et al, 2001, Hernandez et al, 2002).

Nitrogen content in wine sample coming from biochar treatment is lower than control probably this is associated with ability of biochar to retain nutrient in soil and nitrogen present in biochar product has very low availability. While potassium content for same wine sample is higher than control. It has been observed in field trails associated with biochar produced from sewage sludge despite having higher total N content mineral N fraction of biochar was negligible so less availability of nitrogen to plant (Bridle and Pritchard, 2004). Lehman et al, (2003) and Chan et al, (2007) noted that available potassium content of biochar is generally higher and increased uptake of K has been observed often as a results of application of biochar.

Potassium values for all wine samples are within normal potassium content range of wine (600-1000 mg/L). Even though there is no statistical significant effect of type of the organic amendment added on the potassium content, but by looking at absolute values it's clear that addition of cattle manure (ESTR) has strong effect on the final Potassium content of the wine (Table No. 23). This increased potassium level has also resulted in elevated pH level for this

particular treatment (Table No. 16). It has been observed in several trials that addition of cattle manure has resulted in increased potassium level in wine sample (Morlat and Symoneaux, 2008).

**Table No. 23 Mineral analysis of wine samples - Quinta do Coro**

Pruning	Treatment	Total nitrogen (mg/L)	Potassium (mg/L)	Calcium (mg/L)	Copper (mg/L)	Iron (mg/L)
MAN	TEST	225.21	809.41	8.91	0.17	2.01
MAN	BIOC	169.60	895.22	11.07	0.13	1.62
MAN	RSUC	208.53	940.97	8.20	0.18	2.16
MAN	ESTR	241.89	1054.32	9.31	0.08	1.83
MAN	ETAR	305.84	804.41	7.98	0.19	2.30
MEC	TEST	266.92	726.5	7.02	0.18	1.86
MEC	BIOC	241.89	791.01	6.90	0.17	2.17
MEC	RSUC	219.65	827.4	7.39	0.12	1.71
MEC	ESTR	191.85	881.96	8.07	0.08	1.80
MEC	ETAR	269.70	769.27	7.43	0.12	1.96

**Table No. 24 Statistical results for mineral analysis of wine samples - Quinta do Coro**

Treatment	Total nitrogen (mg/L)	Potassium (mg/L)	Calcium (mg/L)	Copper (mg/L)	Iron (mg/L)
MAN	230.21	900.87	9.09 a	0.15	1.98
MEC	238.00	799.23	7.36 b	0.13	1.90
<b>Sig.</b>	<i>ns</i>	<i>ns</i>	*	<i>ns</i>	<i>ns</i>
TEST	246.07	767.96	7.97	0.18	1.94
BIOC	205.75	843.12	8.99	0.15	1.90
RSUC	214.09	884.19	7.80	0.15	1.94
ESTR	216.87	968.14	8.69	0.08	1.82
ETAR	287.77	786.84	7.71	0.16	2.13
<b>Sig.</b>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, *ns* - not significant)

Plot treated with municipal solid waste compost (RSUC) also showed increased level of potassium content in final wine sample (Table No. 24). This is because addition of municipal solid waste compost increases organic matter content of soil, which increases available phosphorus and exchangeable potassium content of the soil (Pinamonti, 1998). Calcium, copper and iron content of all wine samples is within general range. Application of cattle manure seems to have increased effect on calcium content of final wine sample, but copper content was reduced with similar organic amendment compared to control wine sample (Table No. 24). Calcium content of wine sample is lower than levels where it may lead to problems associated with

tartrate instability in wine. Iron content of wine sample is lower; this means there is no contamination via winery equipment and also no potential risk of formation of iron casse in wine. Because generally iron instability problem begin when iron concentration gets above 4-5 mg/L. There is only with treatment ETAR value of iron is higher compared to control treatment. There is no effect of the addition of organic amendment on copper level in wine samples. Copper can catalyze oxidation of wine phenols at higher levels but in these wines coming from all treatment copper content is within general norms of 0.1-0.3 mg/L.

### Mineral analysis of wine samples - Quinta do Gradil

Mineral analysis of wine samples displays strong difference between two wineries. Total nitrogen level for wine sample coming from Coro is much higher than wine samples from Gradil. While potassium and calcium content of wine samples coming from Gradil is higher than Coro. Regarding copper and iron both wineries has almost equal values.

Soil analysis of plots for both wineries was carried. And from soil analysis it's clear that there is already difference in the nitrogen and potassium content of soil. Quinta do Coro which has higher level of plant available form of nitrogen in soil compared to Quinta do Gradil. Soil analysis shows that nitrate content and mineralizable nitrogen are also higher in Coro compared to Gradil. Contrary to nitrogen content the potassium content of Coro is lower than Gradil this is also related to original nutrient content of the soil, as soil analysis shows that potassium oxide (K<sub>2</sub>O) content of Coro is in between 39-62 mg/kg while for Gradil it ranges between 142-186 mg/kg. Exchangeable cation analysis shows lower level of exchangeable potassium in Coro compared to Gradil. There is no much difference amongst iron and copper content in two wineries except calcium which has higher level in Gradil compared to Coro.

**Table No. 25 Mineral analysis of wine samples - Quinta do Gradil**

Pruning	Treatment	Total nitrogen (mg/L)	Potassium (mg/L)	Calcium (mg/L)	Copper (mg/L)	Iron (mg/L)
MAN	TEST	197.41	1095.95	14.43	0.11	2.57
MAN	BIOC	189.07	1157.69	13.72	0.13	2.46
MAN	RSUC	186.29	1083.91	13.82	0.10	2.57
MAN	ESTR	164.04	1116.07	12.01	0.10	2.48
MAN	ETAR	125.12	986.77	13.4	0.05	1.85
MEC	TEST	102.88	1009.10	17.27	0.13	2.01
MEC	BIOC	119.56	1151.20	17.25	0.13	2.37
MEC	RSUC	127.90	1109.23	16.93	0.12	1.95
MEC	ESTR	125.12	1027.62	17.53	0.10	1.99
MEC	ETAR	97.31	943.84	15.46	0.18	2.27

Statistical analysis for mineral content of wine samples shows significant effect of type of pruning on nitrogen content. Wine made from manually pruned grapes has higher level of

nitrogen compared to mechanically pruning. This is because number of nodes retained per vine with mechanically pruning is higher than manual pruning. This lead to the more use of nitrogen for growth of vegetative part of vine and there is less accumulation in berry. There is significant effect of type of organic amendment added on the potassium content of wine sample. Wine produced from ETAR treatment has lowest potassium content compared to other treatment. Study done by Miah et al, (1999) also concluded that sewage sludge is poor source of potassium and therefore can result in deficiency, prolonged application can cause restriction of growth and yield of plants.

**Table No. 26 Statistical results for mineral analysis of wine samples - Quinta do Gradil**

<b>Treatment</b>	<b>Total nitrogen (mg/L)</b>	<b>Potassium (mg/L)</b>	<b>Calcium (mg/L)</b>	<b>Copper (mg/L)</b>	<b>Iron (mg/L)</b>
MAN	172.39 a	1088.08	13.48 b	0.10	2.38
MEC	114.55 b	1048.20	16.89 a	0.13	2.12
<i>Sig.</i>	**	<i>ns</i>	***	<i>ns</i>	<i>ns</i>
TEST	150.14	1052,53 ab	15.85	0.12	2.29
BIOC	154.31	1154,45 a	15.49	0.13	2.42
RSUC	157.09	1096,57 ab	15.38	0.11	2.26
ESTR	144.58	1071,85 ab	14.77	0.10	2.23
ETAR	111.22	965,31 b	14.43	0.11	2.06
<i>Sig.</i>	<i>ns</i>	*	<i>ns</i>	<i>ns</i>	<i>ns</i>

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, *ns* - not significant )

Wine coming from plot treated with biochar has higher value of potassium, this is may be because available potassium content of biochar is generally higher and increased uptake of K has been observed as results of biochar application (Lehman et al, 2003; Chan et al, 2007). Nitrogen content of wines from ETAR is very low compared to other treatments; this result is completely contradictory to trend that was observed in the wine sample from Coro with same treatment. Possible reason can be most part of nitrogen supplied from treatment was used for growth of vegetative part of vine plant and hence there was very less accumulation in berries. Type of pruning has significant effect on calcium content of final wine sample with mechanical pruning showing higher content compared to wine sample of manually pruned grapes.

#### **4.2.4 Analysis of Heavy Metals and other Elements**

##### **Analysis of heavy metals and other elements - Quinta do Coro**

Repeated and higher application of organic amendment can result in accumulation of heavy metals in soil. Hence determination of some of this element is of interest due to their toxicological or physiological properties and due to their harmful effect on environment. Besides some of them can result in spoilage related with wine. It's also necessary to control some of this element within legal limits proposed by OIV.

Almost 28 elements were analyzed in wine samples in order to understand effect of organic amendment addition on final wine content. For most of the elements analyzed there is no much significant effect of type of organic amendment added on final wine composition. There is significant effect of type of pruning on concentration of Nickel (Ni), Gallium (Ga), Yttrium (Y), Terbium (Tb), Thulium (Tm), Lutetium (Lu), Titanium (Ti), and Rubidium (Rb) element.

For all of these elements where effect of pruning type is significant, it shows that concentration of element is more in wine samples coming from manually pruned grape vines compared to mechanically pruned. This may be because number of bunches per shoot are higher in mechanically pruned vines compared to manually pruned. This gives chances of having higher concentration effect of these elements in bunches coming from manual pruning.

**Table No. 27 Metal analysis of wine samples - Quinta do Coro**

<b>Quinta do Coro - Manual pruning</b>					
<b>Element</b>	<b>MAN TEST</b>	<b>MAN BIOC</b>	<b>MAN RSUC</b>	<b>MAN ESTR</b>	<b>MAN ETAR</b>
	<b>Ave. (µg/L)</b>	<b>Ave. (µg/L)</b>	<b>Ave. (µg/L)</b>	<b>Ave. (µg/L)</b>	<b>Ave. (µg/L)</b>
<b>Li</b>	2.25 (0.02)	1.99 (0.03)	2.34 (0.01)	2.2 (0.1)	2.0 (0.1)
<b>Co</b>	1.8 (0.1)	1.3 (0.1)	1.360 (0.004)	1.42 (0.01)	1.52 (0.03)
<b>Ni</b>	41 (1)	24 (1)	20 (1)	20.650 (0.003)	15 (1)
<b>Ga</b>	3.42 (0.23)	3.3 (0.1)	3.75 (0.04)	3.5 (0.1)	3.4 (0.1)
<b>As</b>	0.22 (0.03)	0.20 (0.02)	0.26 (0.01)	0.2 (0.1)	0.21 (0.01)
<b>Y</b>	0.49 (0.03)	0.37 (0.01)	0.53 (0.01)	0.50 (0.02)	0.49 (0.03)
<b>Zr</b>	0.003 (0.001)	0.0045 (0.0001)	0.0017 (0.002)	0.0040 (0.0001)	0.005 (0.002)
<b>Cs</b>	82 (1)	48 (1)	59.3 (0.5)	60 (2)	117 (9)
<b>Ce</b>	0.2761 (0.0001)	0.25 (0.03)	0.2 (0.1)	0.20 (0.04)	0.19 (0.02)
<b>Pr</b>	0.03 (0.01)	0.03 (0.01)	0.03 (0.02)	0.0283 (0.0004)	0.04 (0.01)
<b>Nd</b>	0.132 (0.005)	0.08 (0.03)	0.1 (0.1)	0.125 (0.004)	0.09 (0.02)
<b>Sm</b>	0.05 (0.01)	0.05 (0.01)	0.0433 (0.0004)	0.04 (0.01)	0.05 (0.02)
<b>Eu</b>	0.17 (0.01)	0.16 (0.02)	0.18 (0.01)	0.16 (0.01)	0.15 (0.01)
<b>Tb</b>	0.009 (0.002)	0.009 (0.001)	0.010 (0.002)	0.009 (0.002)	0.015 (0.005)
<b>Dy</b>	0.04 (0.01)	0.02 (0.01)	0.05 (0.01)	0.048 (0.002)	0.05 (0.02)
<b>Ho</b>	0.010 (0.002)	0.009 (0.002)	0.019 (0.002)	0.03 (0.01)	0.02 (0.01)
<b>Er</b>	0.030 (0.003)	0.031 (0.002)	0.043 (0.002)	0.033 (0.004)	0.038 (0.001)
<b>Tm</b>	0.016 (0.002)	0.016 (0.003)	0.015 (0.002)	0.017 (0.003)	0.012 (0.001)
<b>Yb</b>	0.030 (0.003)	0.03 (0.01)	0.03 (0.01)	0.0366 (0.0003)	0.043 (0.001)
<b>Lu</b>	0.009 (0.001)	0.010 (0.002)	0.01481 (0.00003)	0.020 (0.001)	0.013 (0.005)
<b>Tl</b>	3.3 (0.1)	3.8 (0.1)	3.90 (0.03)	4.0 (0.1)	3.4 (0.2)
<b>Pb</b>	10.7 (0.1)	8 (1)	5.3 (0.2)	4.35 (0.03)	5.8 (0.3)
<b>Element</b>	<b>MAN TEST</b>	<b>MAN BIOC</b>	<b>MAN RSUC</b>	<b>MAN ESTR</b>	<b>MAN ETAR</b>
	<b>Ave. mg/L</b>	<b>Ave. mg/L</b>	<b>Ave. mg/L</b>	<b>Ave. mg/L</b>	<b>Ave. mg/L</b>
<b>Al</b>	0.0876 (0.0002)	0.060 (0.005)	0.07(0.01)	0.050 (0.003)	0.06 (0.01)
<b>Mn</b>	0.58 (0.01)	0.52 (0.02)	0.523 (0.005)	0.56 (0.02)	0.58 (0.03)
<b>Zn</b>	0.58 (0.01)	0.41 (0.03)	0.47 (0.01)	0.48 (0.01)	0.51 (0.02)
<b>Rb</b>	5.7 (0.1)	5.7 (0.2)	6.5 (0.1)	6.5 (0.3)	5.7 (0.4)
<b>Sr</b>	0.46 (0.01)	0.40 (0.01)	0.374 (0.004)	0.39 (0.01)	0.33 (0.03)
<b>Element</b>	<b>MAN TEST</b>	<b>MAN BIOC</b>	<b>MAN RSUC</b>	<b>MAN ESTR</b>	<b>MAN ETAR</b>
	<b>Ave. (ng/L)</b>	<b>Ave. (ng/L)</b>	<b>Ave. (ng/L)</b>	<b>Ave. (ng/L)</b>	<b>Ave. (ng/L)</b>
<b>Nb</b>	0.14 (0.02)	0.06 (0.01)	0.07 (0.01)	0.077 (0.002)	0.11 (0.02)

**Table No. 28 Metal analysis of wine samples - Quinta do Coro**

<b>Quinta do Coro - Mechanical pruning</b>					
<b>Element</b>	<b>MEC TEST</b>	<b>MEC BIOC</b>	<b>MEC RSUC</b>	<b>MEC ESTR</b>	<b>MEC ETAR</b>
	<b>Ave. (µg/L)</b>	<b>Ave. (µg/L)</b>	<b>Ave. (µg/L)</b>	<b>Ave. (µg/L)</b>	<b>Ave. (µg/L)</b>
<b>Li</b>	2.78 (0.02)	2.6 (0.1)	2.1 (0.3)	3 (1)	1.9 (0.1)
<b>Co</b>	1.4 (0.1)	1.60 (0.03)	1.1 (0.1)	1.2 (0.2)	1.33 (0.03)
<b>Ni</b>	20.2 (0.1)	20.3 (0.3)	12 (1)	12 (2)	13.2 (0.1)
<b>Ga</b>	3.4 (0.1)	3.0 (0.1)	2.6 (0.3)	2.5 (0.3)	2.16 (0.04)
<b>As</b>	0.245 (0.005)	0.21 (0.02)	0.15 (0.04)	0.17 (0.04)	0.225 (0.003)
<b>Y</b>	0.49 (0.02)	0.44 (0.01)	0.23 (0.02)	0.26 (0.04)	0.26 (0.05)
<b>Zr</b>	0.0060 (0.0005)	0.020 (0.002)	0.004 (0.001)	0.0024 (0.0001)	0.007 (0.001)
<b>Cs</b>	62.1 (0.4)	59 (1)	50 (4)	32 (6)	101.6 (0.4)
<b>Ce</b>	0.25 (0.04)	0.25 (0.03)	0.2 (0.1)	0.18 (0.05)	0.22 (0.02)
<b>Pr</b>	0.03 (0.02)	0.030 (0.001)	0.021 (0.001)	0.022 (0.001)	0.031 (0.003)
<b>Nd</b>	0.08 (0.01)	0.098 (0.003)	0.07 (0.01)	0.1 (0.1)	0.08 (0.01)
<b>Sm</b>	0.04 (0.01)	0.05 (0.01)	0.03 (0.01)	0.038 (0.002)	0.07 (0.01)
<b>Eu</b>	0.14 (0.01)	0.17 (0.02)	0.13 (0.02)	0.2 (0.1)	0.129 (0.003)
<b>Tb</b>	0.006 (0.001)	0.011 (0.002)	0.00273 (0.00004)	0.003 (0.001)	0.005 (0.002)
<b>Dy</b>	0.04 (0.01)	0.038 (0.002)	0.013 (0.003)	0.019 (0.002)	0.04 (0.01)
<b>Ho</b>	0.013 (0.003)	0.02 (0.01)	0.007 (0.001)	0.011 (0.003)	0.009 (0.003)
<b>Er</b>	0.035 (0.002)	0.04 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.02)
<b>Tm</b>	0.010 (0.004)	0.012 (0.002)	0.006 (0.002)	0.020 (0.001)	0.009 (0.002)
<b>Yb</b>	0.028 (0.001)	0.0286 (0.0005)	0.0061 (0.0001)	0.031 (0.002)	0.03 (0.01)
<b>Lu</b>	0.01 (0.01)	0.006 (0.004)	0.005 (0.001)	0.007 (0.002)	0.003 (0.001)
<b>Tl</b>	3.1 (0.1)	2.9 (0.1)	2.4 (0.2)	2.0 (0.4)	3.6 (0.1)
<b>Pb</b>	12.1 (0.4)	21.8 (0.4)	5 (1)	4 (1)	5.3 (0.2)
<b>Element</b>	<b>MEC TEST</b>	<b>MEC BIOC</b>	<b>MEC RSUC</b>	<b>MEC ESTR</b>	<b>MEC ETAR</b>
	<b>Ave. mg/L</b>	<b>Ave. mg/L</b>	<b>Ave. mg/L</b>	<b>Ave. mg/L</b>	<b>Ave. mg/L</b>
<b>Al</b>	0.067 (0.002)	0.074 (0.002)	0.043 (0.001)	0.06 (0.01)	0.06 (0.01)
<b>Mn</b>	0.68 (0.01)	0.65 (0.02)	0.49 (0.04)	0.5 (0.1)	0.488 (0.004)
<b>Zn</b>	0.63 (0.01)	0.49 (0.01)	0.69 (0.04)	0.4 (0.1)	0.488 (0.002)
<b>Rb</b>	5.0 (0.1)	5.1 (0.2)	3.9 (0.3)	4 (1)	3.806 (0.005)
<b>Sr</b>	0.420 (0.004)	0.41 (0.01)	0.33 (0.02)	0.4 (0.1)	0.310 (0.002)
<b>Element</b>	<b>MEC TEST</b>	<b>MEC BIOC</b>	<b>MEC RSUC</b>	<b>MEC ESTR</b>	<b>MEC ETAR</b>
	<b>Ave. (ng/L)</b>	<b>Ave. (ng/L)</b>	<b>Ave. (ng/L)</b>	<b>Ave. (ng/L)</b>	<b>Ave. (ng/L)</b>
<b>Nb</b>	0.074 (0.003)	0.06 (0.02)	0.08 (0.02)	0.067 (0.003)	0.061 (0.001)

When compared wines samples for effect of treatment of organic amendments, it's clear that applied amendment has significant effect on final concentration of some of elements analyzed. Concentration of Aluminum (Al), Manganese (Mn), Zinc (Zn), Strontium (Sr), Lead (Pb),

Ytterbium (Yb), Caesium (Cs), Zirconium (Zr), Nickel (Ni) and Cobalt (Co) were significantly affected by type of treatment applied [Table No.29, 30, 31]. For Al, Mn, Zn, Co and Ni higher concentrations were found in wine samples from control treatment [Table No. 29 and 31]. Concentration of these elements lower in samples coming from other treatment can be possible, because bioavailability of heavy metals in soil is affected by the amount and quality of organic matter that can react with heavy metals and forming complexes and chelates of varying stability (Leita et al, 1999). Probably this may lead to have decreased availability of these metals to plant uptake. From human health and concern associated with heavy metals accumulation by application of organic amendments this results are good. But results are contrasting to observations noted by Antoline et al (2005) they found that application of sewage sludge increased soil extractable heavy metals (Cd, Cu, Mn, Pb and Zn) and increased N-NH<sub>4</sub><sup>+</sup> content of soil. Increase in extractable metals (Cu, Zn, Ni, Pb, Mn and Fe) was also noted by Mendoza et al, 2006 with application of sewage sludge to soil. Municipal Solid Compost (RSUC) application does not show any signs of contamination effect. Effect of compost use on environmental heavy metal levels shows it varies as per soil type, compost quality and plant species (Woodbury, 1992).

Content of Aluminum are within normal range which indicates that there is no contamination during vinification. Concentration of very few numbers of elements in wine is currently under regulation; list of this potentially toxic element may increase in future due to increasing concern for health effect and risk to environment. International Organization of Vine and Wine (OIV) defines maximum concentration for elements Cu (1 mg/L), Zn (5 mg/L), As (0.2 mg/L), Cd (0.01 mg/L) and Pb (0.2 mg/L) in wine (OIV 2005). Content of all of these metals in our wine samples is much below levels regulated by OIV. Cadmium (Cd) concentration was not determined as it was not detected by inductively coupled plasma - mass spectrometry (ICP-MS).

**Table No. 29 Statistical result of metal analysis of wine samples - Quinta do Coro**

<i>Stat</i>	<b>Li</b> (µg/L)	<b>Co</b> (µg/L)	<b>Ni</b> (µg/L)	<b>Ga</b> (µg/L)	<b>As</b> (µg/L)	<b>Y</b> (µg/L)	<b>Zr</b> (µg/L)	<b>Cs</b> (µg/L)	<b>Ce</b> (µg/L)	<b>Pr</b> (µg/L)	<b>Nd</b> (µg/L)
MAN	2.17	1.49	24.05 a	3.50 a	0.22	0.47 a	0.004	73.44	0.224	0.030	0.106
MEC	2.56	1.34	15.60 b	2.72 b	0.20	0.34 b	0.008	60.88	0.221	0.027	0.090
<b>Sig.</b>	<i>ns</i>	<i>ns</i>	*	***	<i>ns</i>	**	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
TEST	2.51	1.63a	30.41a	3.43	0.23	0.49	0.004	72.05b	0.263	0.030	0.115
BIOC	2.29	1.46ab	21.61ab	3.16	0.21	0.41	0.012	53.72bc	0.249	0.028	0.088
RSUC	2.22	1.23b	15.96b	3.17	0.21	0.38	0.003	54.47bc	0.198	0.025	0.089
ESTR	2.82	1.30ab	16.49ab	2.99	0.19	0.38	0.003	46.04c	0.191	0.025	0.128
ETAR	1.97	1.43ab	14.07b	2.8	0.22	0.38	0.006	109.48a	0.204	0.035	0.087
<b>Sig.</b>	<i>ns</i>	*	*	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	****	<i>ns</i>	<i>ns</i>	<i>ns</i>

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, *ns* - not significant )

**Table No. 30 Statistical result of metal analysis of wine samples - Quinta do Coro**

<i>Stat</i>	<b>Sm</b> (µg/L)	<b>Eu</b> (µg/L)	<b>Tb</b> (µg/L)	<b>Dy</b> (µg/L)	<b>Ho</b> (µg/L)	<b>Er</b> (µg/L)	<b>Tm</b> (µg/L)	<b>Yb</b> (µg/L)	<b>Lu</b> (µg/L)	<b>Ti</b> (µg/L)	<b>Pb</b> (µg/L)
MAN	0.047	0.167	0.010 a	0.043	0.016	0.035	0.015 a	0.033	0.013 a	3.68 a	6.81
MEC	0.044	0.152	0.006 b	0.030	0.012	0.028	0.010 b	0.025	0.007 b	2.80 b	9.59
<b>Sig.</b>	<i>ns</i>	<i>ns</i>	**	<i>ns</i>	<i>ns</i>	<i>ns</i>	**	<i>ns</i>	*	***	<i>ns</i>
TEST	0.043	0.154	0.007	0.038	0.011	0.032	0.013	0.029ab	0.012	3.19	11.39ab
BIOC	0.052	0.167	0.010	0.030	0.014	0.037	0.014	0.028ab	0.008	3.35	14.83a
RSUC	0.038	0.156	0.006	0.032	0.013	0.030	0.011	0.017b	0.010	3.14	4.95b
ESTR	0.040	0.177	0.006	0.034	0.018	0.027	0.017	0.034a	0.003	3.03	4.29b
ETAR	0.055	0.142	0.009	0.048	0.013	0.031	0.01	0.036a	0.003	3.48	5.56b
<b>Sig.</b>	<i>Ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	*	<i>ns</i>	<i>ns</i>	**

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, *ns* - not significant )

**Table No. 31 Statistical result of metal analysis of wine samples - Quinta do Coro**

<i>Stat</i>	<b>Al (mg/L)</b>	<b>Mn</b> (mg/L)	<b>Zn</b> (mg/L)	<b>Rb</b> (mg/L)	<b>Sr</b> (mg/L)	<b>Nb</b> (ng/L)
MAN	0.066	0.552	0.490	6.021 a	0.390	0.090
MEC	0.060	0.559	0.543	4.317 b	0.367	0.068
<b>Sig.</b>	<i>ns</i>	<i>ns</i>	<i>ns</i>	****	<i>ns</i>	<i>ns</i>
TEST	0.077a	0.631a	0.608a	5.36	0.438a	0.109
BIOC	0.067ab	0.587a	0.448b	5.44	0.402ab	0.056
RSUC	0.057ab	0.507a	0.576ab	5.16	0.350bc	0.074
ESTR	0.052b	0.521a	0.452b	5.16	0.380abc	0.072
ETAR	0.061ab	0.533a	0.499ab	4.73	0.321c	0.084
<b>Sig.</b>	*	*	*	<i>ns</i>	***	<i>ns</i>

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, *ns* - not significant )

### **Analysis of Heavy Metals and other Elements - Quinta do Gradil**

Metal analysis of wine sample shows that in between two wineries there is no big difference in concentration for most of elements analyzed by ICP-MS. Except Yttrium (Y), Cesium (Ce), Dysprosium (Dy), Holmium (Ho), Titanium (Ti), Rubidium (Rb) all of this element shows much higher values in Quinta do Coro compared to those obtained in the Gradil. Lead (Pb) content was much higher in Quinta do Gradil compared to Coro. These results are supposed to be site specific for each winery depending on soil characteristics and nutrient composition of soil.

Pb content of wine samples from all organic amendment treatments of Gradil is more than above of 10 µg/L with control sample showing the highest value of 68 µg/L. While for Coro all the wine samples has Pb content almost close 10 µg/L. But overall for both wineries wine samples Pb content are much lower than legal limit of 0.2 mg/L. Difference in wine samples for lead content between two wineries can be accounted to may be original composition of soil, atmosphere surrounding vineyard site and probably technological sources due to equipment used for handling of product during entire procedure of vinification. Most probable chances of having such high level of Pb in wine sample of Quinta do Gradil manually pruned test treatment compared to other treatment is possible due to contamination during the vinification.

There is statistically significant effect of type of vine pruning on final composition of some of metals in wine samples (Table No. 34, 35, 36). The concentration of Lithium (Li), Beryllium (Be), Cobalt (Co) and Gallium (Ga) was higher in wine samples coming from manually pruned vines (Table No.34). While Zirconium (Zr), Indium (In), Zinc (Zn), Strontium (Sr) and Niobium (Nb) has higher concentration in mechanically pruned vine wine samples (Table No.34 and 36).

There was significant effect of type of organic amendment applied on final metal concentration of wine samples but most of cases higher concentration of metal was found in control treatment. The level of Beryllium and Lithium content was higher in wines samples from control treatment this can be possible due to their presence in soil composition. Wine sample with sewage sludge (ETAR) treatment shows higher content of the Cobalt (Co) and Nickel (Ni) composition (Table No. 34). There is statistically significant effect of the ETAR treatment on the Caesium (Cs) content (Table No. 35). Sample from ETAR shows much higher values of Caesium compared to other treatment.

**Table No. 32 Metal analysis of wine Samples - Quinta do Gradil**

<b>Quinta do Gradil - Manual Pruning</b>					
<b>Element</b>	<b>TEST</b>	<b>BIOC</b>	<b>RSUC</b>	<b>ESTR</b>	<b>ETAR</b>
	<b>Ave.(µg/L)</b>	<b>Ave.(µg/L)</b>	<b>Ave.(µg/L)</b>	<b>Ave.(µg/L)</b>	<b>Ave.(µg/L)</b>
Li	4.00 (0.04)	3.1 (0.1)	3.6 (0.2)	2.8 (0.1)	3.64 (0.02)
Be	0.27 (0.01)	0.28 (0.04)	0.22 (0.01)	0.18 (0.03)	0.225 (0.002)
Co	1.345 (0.02)	1.52 (0.02)	1.9 (0.1)	1.11 (0.02)	2.26 (0.02)
Ni	24.1 (0.5)	26 (2)	27 (1)	19 (1)	24 (1)
Ga	1.892 (0.005)	1.65 (0.04)	1.81 (0.01)	1.64 (0.01)	1.53 (0.01)
As	0.294 (0.005)	0.28 (0.03)	0.31 (0.01)	0.25 (0.01)	0.29 (0.02)
Y	0.19 (0.01)	0.19 (0.03)	0.17 (0.01)	0.11 (0.02)	0.14 (0.03)
Zr	0.006 (0.001)	0.005 (0.001)	0.005 (0.001)	0.0063 (0.0002)	0.003 (0.001)
In	0.0019 (0.0002)	ND	0.005 (0.003)	0.002 (0.001)	0.006 (0.002)
Sb	0.024 (0.002)	0.04 (0.01)	0.05 (0.05)	0.015 (0.003)	0.028 (0.004)
Cs	1.68 (0.03)	1.50 (0.02)	2.0 (0.1)	1.63 (0.01)	3.73 (0.04)
Ce	0.19 (0.02)	0.10 (0.08)	0.2 (0.1)	0.13 (0.02)	0.12 (0.01)
Pr	0.04 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)
Eu	0.169 (0.004)	0.17 (0.01)	0.153 (0.001)	0.1888 (0.0004)	0.12 (0.01)
Gd	0.029 (0.004)	0.010 (0.003)	0.0123 (0.002)	0.010 (0.004)	0.02 (0.01)
Tb	0.01 (0.01)	0.002 (0.001)	0.001 (0.001)	0.00166 (0.00003)	0.003 (0.001)
Dy	0.03 (0.01)	0.019 (0.002)	0.014 (0.003)	0.004 (0.001)	0.02 (0.01)
Ho	0.016 (0.002)	0.006 (0.001)	0.005 (0.002)	0.008 (0.002)	0.010 (0.001)
Tm	0.016 (0.001)	0.011 (0.001)	0.006 (0.002)	0.008 (0.001)	0.008 (0.001)
Lu	0.009 (0.002)	0.008 (0.002)	0.007 (0.004)	0.003 (0.002)	0.009 (0.001)
Tl	0.084 (0.005)	0.082 (0.004)	0.07 (0.02)	0.0732 (0.0002)	0.192 (0.004)
Pb	46 (1)	9 (1)	10.5 (0.4)	7.5 (0.1)	8.4 (0.1)
<b>Element</b>	<b>TEST</b>	<b>BIOC</b>	<b>RSUC</b>	<b>ESTR</b>	<b>ETAR</b>
	<b>Ave(mg/L)</b>	<b>Ave(mg/L)</b>	<b>Ave(mg/L)</b>	<b>Ave(mg/L)</b>	<b>Ave(mg/L)</b>
Al	0.051 (0.003)	0.2 (0.2)	0.045 (0.001)	0.063 (0.001)	0.038 (0.001)
Zn	0.546 (0.001)	0.5 (0.1)	0.46 (0.02)	0.44 (0.01)	0.49 (0.02)
Rb	1.20 (0.02)	1.20 (0.01)	1.91 (0.04)	1.07 (0.01)	2.3 (0.1)
Sr	0.325 (0.004)	0.29 (0.01)	0.36 (0.01)	0.2931 (0.0003)	0.29 (0.01)
<b>Element</b>	<b>TEST</b>	<b>BIOC</b>	<b>RSUC</b>	<b>ESTR</b>	<b>ETAR</b>
	<b>Ave.(ng/L)</b>	<b>Ave.(ng/L)</b>	<b>Ave.(ng/L)</b>	<b>Ave.(ng/L)</b>	<b>Ave.(ng/L)</b>
Nb	0.053 (0.003)	0.079 (0.020)	0.075 (0.018)	0.053 (0.012)	0.047 (0.020)

**Table No. 33 Metal analysis of wine samples - Quinta do Gradil**

<b>Quinta do Gradil - Mechanical pruning</b>					
<b>Element</b>	<b>TEST</b>	<b>BIOC</b>	<b>RSUC</b>	<b>ESTR</b>	<b>ETAR</b>
	<b>Ave.(µg/L)</b>	<b>Ave.(µg/L)</b>	<b>Ave.(µg/L)</b>	<b>Ave.(µg/L)</b>	<b>Ave.(µg/L)</b>
Li	3.30 (0.03)	3.3 (0.1)	3.3 (0.1)	2.62 (0.02)	2.92 (0.04)
Be	0.17 (0.03)	0.226 (0.001)	0.19 (0.01)	0.19 (0.01)	0.16 (0.01)
Co	1.25 (0.02)	1.5 (0.1)	1.29 (0.04)	1.16 (0.05)	1.24 (0.01)
Ni	23.8 (0.40)	26 (1)	25 (2)	24 (1)	34 (2)
Ga	1.74 (0.05)	1.58 (0.03)	1.6 (0.1)	1.5 (0.1)	1.39 (0.02)
As	0.42 (0.01)	0.28 (0.01)	0.34 (0.02)	0.31 (0.02)	0.26 (0.03)
Y	0.22 (0.02)	0.17 (0.01)	0.2 (0.1)	0.18 (0.02)	0.16 (0.02)
Zr	0.0070 (0.0002)	0.007 (0.001)	0.0117 (0.0001)	0.009 (0.001)	0.006 (0.003)
In	0.0085 (0.0005)	0.007 (0.001)	0.006 (0.002)	0.004 (0.001)	0.006 (0.002)
Sb	0.050 (0.002)	0.03 (0.01)	0.04 (0.01)	0.03 (0.01)	0.04 (0.004)
Cs	1.65 (0.01)	1.19 (0.03)	1.72 (0.01)	1.6 (0.1)	5.91 (0.08)
Ce	0.32 (0.02)	0.09 (0.03)	0.3 (0.1)	0.2 (0.1)	0.219 (0.004)
Pr	0.029 (0.004)	0.017 (0.003)	0.034831 (0.000002)	0.020 (0.001)	0.021 (0.002)
Eu	0.19 (0.02)	0.200 (0.03)	0.16 (0.02)	0.21 (0.02)	0.14 (0.02)
Gd	0.0352 (0.0001)	0.02 (0.01)	0.02 (0.02)	0.02 (0.02)	0.03 (0.01)
Tb	0.0063 (0.0005)	0.00303 (0.00005)	0.004 (0.004)	0.004 (0.001)	0.0029 (0.0004)
Dy	0.02 (0.01)	0.017 (0.004)	0.02 (0.01)	0.003 (0.002)	0.02 (0.01)
Ho	0.006 (0.001)	ND	0.007 (0.002)	0.008 (0.002)	0.006 (0.001)
Tm	0.011 (0.003)	0.011 (0.003)	0.006 (0.001)	0.01 (0.01)	0.005 (0.002)
Lu	0.011 (0.002)	0.006 (0.002)	0.008 (0.002)	0.008 (0.002)	0.007 (0.001)
Tl	0.106 (0.002)	0.08 (0.01)	0.07 (0.01)	0.08 (0.01)	0.24 (0.02)
Pb	90 (2)	12.0 (0.1)	22 (1)	20 (4)	15.9 (0.5)
<b>Element</b>	<b>TEST</b>	<b>BIOC</b>	<b>RSUC</b>	<b>ESTR</b>	<b>ETAR</b>
	<b>Ave(mg/L)</b>	<b>Ave(mg/L)</b>	<b>Ave(mg/L)</b>	<b>Ave(mg/L)</b>	<b>Ave(mg/L)</b>
Al	0.077 (0.001)	0.1 (0.1)	0.08 (0.02)	0.11 (0.01)	0.061 (0.003)
Zn	1.62 (0.01)	0.98 (0.02)	0.65 (0.04)	0.54 (0.02)	0.68 (0.01)
Rb	9 (11)	1.60 (0.04)	1.589 (0.003)	1.37 (0.05)	1.83 (0.01)
Sr	0.4352 (0.0004)	0.41 (0.01)	0.388 (0.005)	0.39 (0.01)	0.35 (0.003)
<b>Element</b>	<b>TEST</b>	<b>BIOC</b>	<b>RSUC</b>	<b>ESTR</b>	<b>ETAR</b>
	<b>Ave.(ng/L)</b>	<b>Ave.(ng/L)</b>	<b>Ave.(ng/L)</b>	<b>Ave.(ng/L)</b>	<b>Ave.(ng/L)</b>
Nb	0.109 (0.016)	0.085 (0.021)	0.07 (0.01)	0.08 (0.01)	0.128 (0.028)

There is no significant effect of organic amendment application on composition of Aluminium (Al), Zinc (Zn), Rubidium (Rb) element. Higher concentration of Zn and Rb is found in control treatment (Table No. 36), while higher concentration of Aluminium was observed with plot treated by Biochar (BIOC) treatment (Table No. 36). There is no problem in wine associated

with aluminium stability, as this problem generally rise at the concentration above 10 mg/L, the recommended upper limit for Al is generally 3 mg/L (P.Szefer and J.Nriagu, 2006). Zinc content in wine sample is generally coming from soil and fungicides, insecticides used and possibly from vinification equipments. Concentration of zinc when it goes above limit of OIV 5 mg/L it can create cloudiness in wine. In our all the wine samples the concentration is much lower than recommended limit hence there is no potential risk of contamination by Zinc in wine samples.

**Table No. 34 Statistical result of metal analysis of wine samples – Quinta do Gradil**

<i>Stat</i>	<b>Li</b> (µg/L)	<b>Be</b> (µg/L)	<b>Co</b> (µg/L)	<b>Ni</b> (µg/L)	<b>Ga</b> (µg/L)	<b>As</b> (µg/L)	<b>Y</b> (µg/L)	<b>Zr</b> (µg/L)	<b>In</b> (µg/L)	<b>Sb</b> (µg/L)	<b>Cs</b> (µg/L)
MAN	3.44 a	0.23 a	1.62 a	24.05	1.7 a	0.28	0.16	0.005 b	0.003 b	0.029	2.11
MEC	3.07 b	0.19 b	1.28 b	26.64	1.58 b	0.32	0.19	0.008 a	0.006 a	0.037	2.40
<i>Sig.</i>	*	**	*	<i>ns</i>	*	<i>ns</i>	<i>ns</i>	**	*	<i>ns</i>	<i>ns</i>
TEST	3.65 a	0.22	1.30	23.97	1.82a	0.36a	0.20a	0.006	0.005	0.037	1.67b
BIOC	3.19 ab	0.26	1.50	26.11	1.62bc	0.28a	0.18a	0.005	0.005	0.033	1.35b
RSUC	3.44 a	0.20	1.57	26.38	1.72ab	0.33a	0.19a	0.008	0.005	0.042	1.87b
ESTR	2.73 b	0.18	1.14	21.64	1.59bc	0.28a	0.14a	0.007	0.002	0.021	1.59b
ETAR	3.28 ab	0.18	1.75	28.62	1.46c	0.28a	0.15a	0.004	0.005	0.033	4.81a
<i>Sig.</i>	**	<i>ns</i>	<i>ns</i>	<i>ns</i>	***	*	*	<i>ns</i>	<i>ns</i>	<i>ns</i>	***

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, *ns* - not significant)

**Table No. 35 Statistical result of metal analysis of wine samples - Quinta do Gradil**

<i>Stat</i>	<b>Ce</b> (µg/L)	<b>Pr</b> (µg/L)	<b>Eu</b> (µg/L)	<b>Gd</b> (µg/L)	<b>Tb</b> (µg/L)	<b>Dy</b> (µg/L)	<b>Ho</b> (µg/L)	<b>Tm</b> (µg/L)	<b>Lu</b> (µg/L)	<b>Ti</b> (µg/L)	<b>Pb</b> (µg/L)
MAN	0.14	0.02	0.16	0.02	0.004	0.017	0.009	0.010	0.007	0.10	16.44
MEC	0.21	0.02	0.18	0.02	0.004	0.017	0.006	0.008	0.008	0.12	32.04
<i>Sig.</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
TEST	0.25a	0.03	0.18ab	0.03	0.01a	0.027a	0.011	0.013a	0.01	0.10b	68.34a
BIOC	0.09b	0.02	0.18ab	0.01	0.002b	0.018ab	0.005	0.011ab	0.007	0.08b	10.70b
RSUC	0.23a	0.03	0.16bc	0.02	0.003b	0.017ab	0.006	0.006b	0.008	0.07b	16.11b
ESTR	0.14ab	0.02	0.20a	0.01	0.003b	0.004b	0.008	0.009ab	0.006	0.08b	13.96b
ETAR	0.17ab	0.02	0.13c	0.02	0.003ab	0.017ab	0.008	0.007b	0.008	0.22a	12.11b
<i>Sig.</i>	*	<i>ns</i>	***	<i>ns</i>	*	**	<i>ns</i>	**	<i>ns</i>	***	***

( $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, *ns* - not significant)

**Table No. 36 Statistical result of metal analysis of wine samples - Quinta do Gradil**

<i>Stat</i>	<b>Al (mg/L)</b>	<b>Zn (mg/L)</b>	<b>Rb (mg/L)</b>	<b>Sr (mg/L)</b>	<b>Nb (ng/L)</b>
MAN	0.072	0.489 b	1.54	0.31 b	0.06 b
MEC	0.079	0.896 a	3.11	0.39 a	0.10 a
<i>Sig.</i>	<i>ns</i>	**	<i>ns</i>	***	**
TEST	0.064	1.085	5.19	0.38	0.08
BIOC	0.128	0.741	1.40	0.35	0.08
RSUC	0.062	0.553	1.75	0.37	0.07
ESTR	0.088	0.493	1.22	0.34	0.06
ETAR	0.049	0.588	2.07	0.32	0.09
<i>Sig.</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

(  $p \leq 0.05$  - \*,  $p < 0.01$  - \*\*,  $p < 0.001$  - \*\*\*,  $p < 0.0001$  - \*\*\*\*, *ns* - not significant )

It has been shown in many studies with sewage sludge that application of excessive sewage sludge can increase toxicity to plant due to heavy metal accumulation in soil. Application of organic amendment shows significant effect on Arsenic (As) and Gallium (Ga) content (Table No. 34), with control showing higher level than rest of treatment applied. The level of Arsenic is much lower in the wine sample (0.28-0.36 µg/L Table No. 34) than legal limit of 0.2 mg/L so there is no risk effect associated with amendment application. Most possible contamination source of Arsenic in wine has been found in Soil composition, also the residue left by the arsenic pesticides which were used in vineyard treatment.

### 4.3 Sensory Analysis of Wine Samples

#### Sensory analysis of wine samples - Quinta do Coro

Application of organic amendment affects on soil quality and grape composition. This can modify overall sensorial profile of wine. To evaluate consumer preference for wines coming from different treatment and to see variation amongst wines under experiment, sensory analysis of wine samples was performed.

13 tasters were participated for tasting of wines, tasting for both wineries wine samples was performed on same day within interval of 15 minutes. Specially designed tasting sheet provided opportunity to compare effect of each treatment individually. Data represented here shows average values for all 13 tastes for each parameter considered during tasting of wine samples. Data was also processed by ANOVA in order to compare effect of treatment. End results are presented in format of spider chart.

Sensorial analysis of wine samples of Quinta do Coro shows that there is no significant effect of type of pruning on taster's preference (Table No.37). By looking at absolute values between two types of pruning it's clear that for all tasting attributes higher score is given to wine sample coming from manually pruned vines compared to mechanical pruned except bitterness. Color

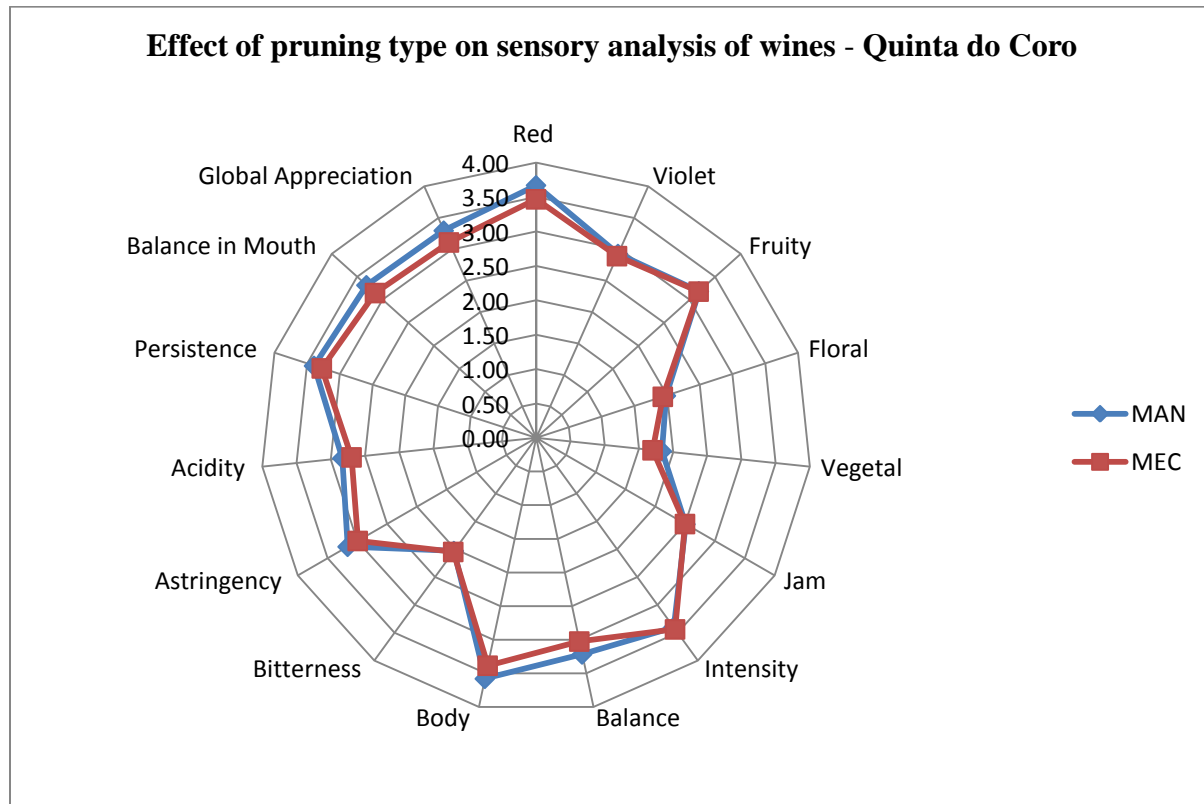
concentration seem to be perceived more in manually pruned grapes which is coherent to result of analysis of chromatic characters. Concentration of phenolic compounds and anthocyanin was overall higher in manually pruned treatment. Result seems similar to experimental work done by Reynold and Wardle (1993) they also observed reduction in overall wine quality due to loss of color with mechanically pruning.

Perception of aromatic characters such as floral, fruity and jam is almost same in both types of pruning. Normally perception of vegetative characters can be perceived more in the mechanically pruned grapes as grapes require more time to achieve optimum maturity. But in our wine sample the vegetative characters was perceived more in case of manual pruned wine sample, this can be possible because bunches were shaded. Gustatory characters such as acidity, bitterness, astringency and body were perceived more in wine sample coming from manual pruning treatment. Overall wine samples coming from manual type of pruning were more appreciated compared to mechanical pruning although difference between two is not significant. This means even though by mechanical pruning higher yield was achieved but quality was not affected. Finally wine produced from mechanically pruned grapes was perceived as equal as wine sample of manually pruned grapes by tasting panel.

**Table No. 37 Effect of pruning type on sensory analysis of wine Quinta do Coro**

	Tasting attributes (Average values)	Pruning type		Statistical significance
		MAN	MEC	
<b>Color</b>	Red	3.67	3.47	ns
	Violet	2.93	2.89	ns
<b>Aroma</b>	Fruity	3.18	3.18	ns
	Floral	1.98	1.94	ns
	Vegetal	1.84	1.70	ns
	Jam	2.51	2.50	ns
	Intensity	3.40	3.44	ns
<b>Taste</b>	Balance	3.22	3.02	ns
	Body	3.58	3.38	ns
	Bitterness	2.03	2.05	ns
	Astringency	3.16	2.99	ns
	Acidity	2.83	2.70	ns
	Persistence	3.39	3.27	ns
	Balance in mouth	3.32	3.15	ns
	Global appreciation	3.30	3.11	ns

**Table No. 38 Spider chart for effect of pruning type on sensory analysis of wines Quinta do Coro**



Effect of organic amendments addition on wine quality was also evaluated by sensorial analysis. Statistical results of data obtained shows that there is no significant effect of application of organic amendment on final quality of wine samples tasted. Absolute average values obtained from the tasters score shows that color characteristics were perceived more in control treatment. Probably nitrogen content supplied by the amendments applied was more which may have resulted in more vegetative growth of vine leading to less accumulation of phenolic compounds. Higher level of nitrogen can result in reduced soluble solid and skin color (Delas and Pouget, 1984).

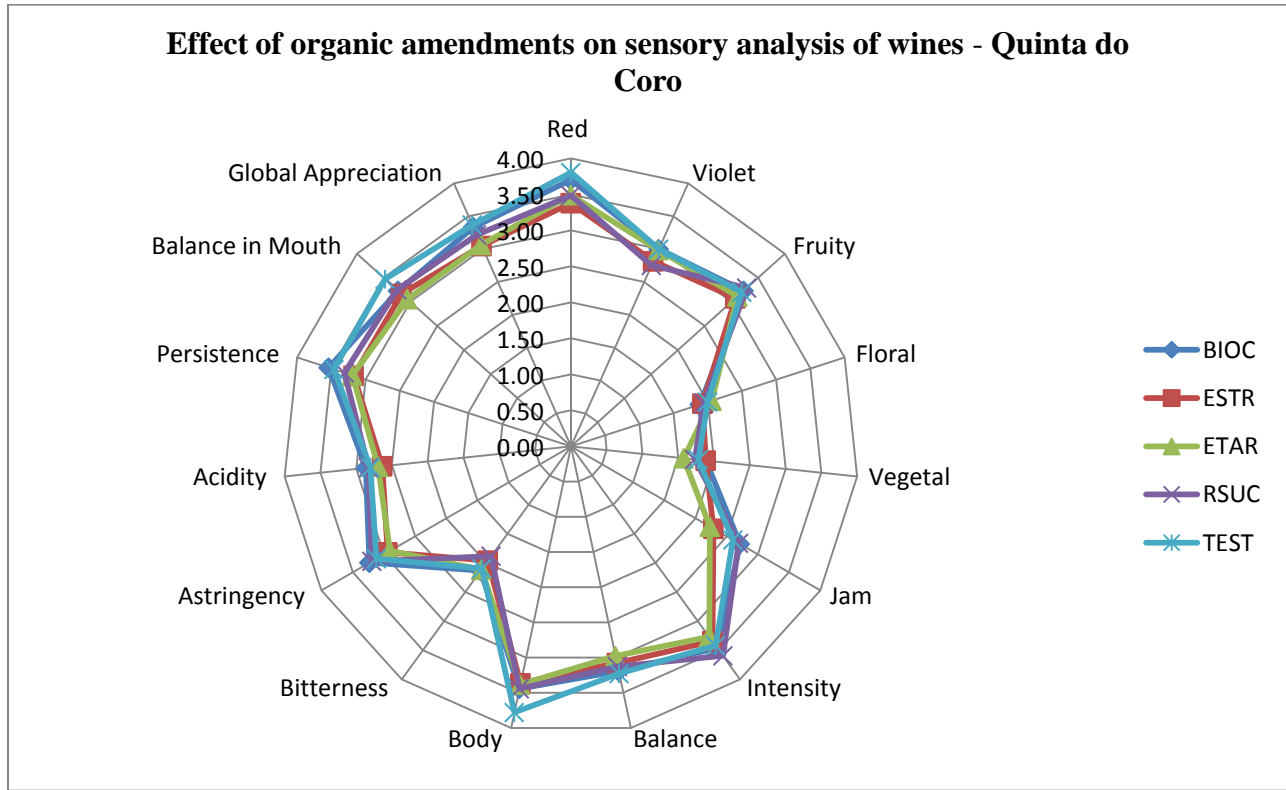
Lowest score for color was given to wines samples coming from Cattle Manure (ESTR) treatment. Morlat and Symoneaux (2008) also observed with higher dosage of cattle manure decrease in color intensity and lower anthocyanin concentration in wine. Overall perception of aromatic characters was perceived equal to control in all treatment. Perception of vegetative characters was more marked in treatment by cattle manure (ESTR) and biochar (BIOC). Application of cattle manure has resulted in grassy/herbaceous characters in wine made from Cabernet franc variety in an experiment with an aim to study effect of organic amendment on sensorial characters of wine made from Cabernet franc on calcareous sandy soil (Morlat and Symoneaux, 2008). Wine body and overall balance in mouth perceived more in wine from

control treatment. While bitterness characters which is generally considered as negative descriptor is perceived lowest in the control treatment.

**Table No. 39 - Effect of type of organic amendment applied on sensory analysis of wine Quinta do Coro**

	Tasting attributes	Type of organic amendment					Statistical significance
		(Average values)	TEST	BIOC	RSUC	ESTR	
<b>Color</b>	Red	3.81	3.70	3.49	3.38	3.49	ns
	Violet	3.00	3.00	2.75	2.81	2.99	ns
<b>Aroma</b>	Fruity	3.20	3.23	3.29	3.08	3.12	ns
	Floral	2.00	1.88	1.94	1.92	2.06	ns
	Vegetal	1.78	1.88	1.73	1.88	1.58	ns
	Jam	2.60	2.71	2.69	2.29	2.23	ns
	Intensity	3.42	3.46	3.60	3.35	3.27	ns
	Balance	3.23	3.19	3.13	3.08	2.98	ns
<b>Taste</b>	Body	3.78	3.44	3.44	3.37	3.38	ns
	Bitterness	2.10	2.13	1.88	1.96	2.12	ns
	Astringency	3.12	3.23	3.19	2.94	2.90	ns
	Acidity	2.80	2.87	2.83	2.63	2.69	ns
	Persistence	3.46	3.54	3.31	3.17	3.19	ns
	Balance in mouth	3.47	3.23	3.25	3.17	3.06	ns
	Global appreciation	3.37	3.33	3.22	3.05	3.06	ns

**Table No. 40 Spider chart for effect of organic amendment applied on sensory analysis of wine Quinta do Coro**



Wine sample coming from control treatment was preferred by all tasters compared to wine samples from other organic amendments. Sample coming from ETAR and ESTR had lowest preference. It has been previously also reported that wine with higher repeated application of sewage sludge compost had higher animal odor and leading to decrease in overall quality of wine (Korboulewsky et al, 2004).

Although wine samples from organic amendments application were not highest preferred by the tasters, still it can be noted that the difference between control and applied treatment is not significant. The overall tasting attributes perceived by tasters doesn't show much difference between control and applied organic amendment.

### Sensory analysis of wine samples - Quinta do Gradil

Before looking at sensory analysis of wine samples from Quinta do Gradil it's important to take into consideration, grape and wine sample analysis of Quinta do Gradil showed that grapes were not completely matured in this particular winery compared to Coro. Also because of frequent rainfall event near grape ripening stage, overall grape composition was diluted.

There is statistically significant effect of type of pruning on taster's perception for some of tasting attribute in this winery. Color and aromatic characters perception was always higher in

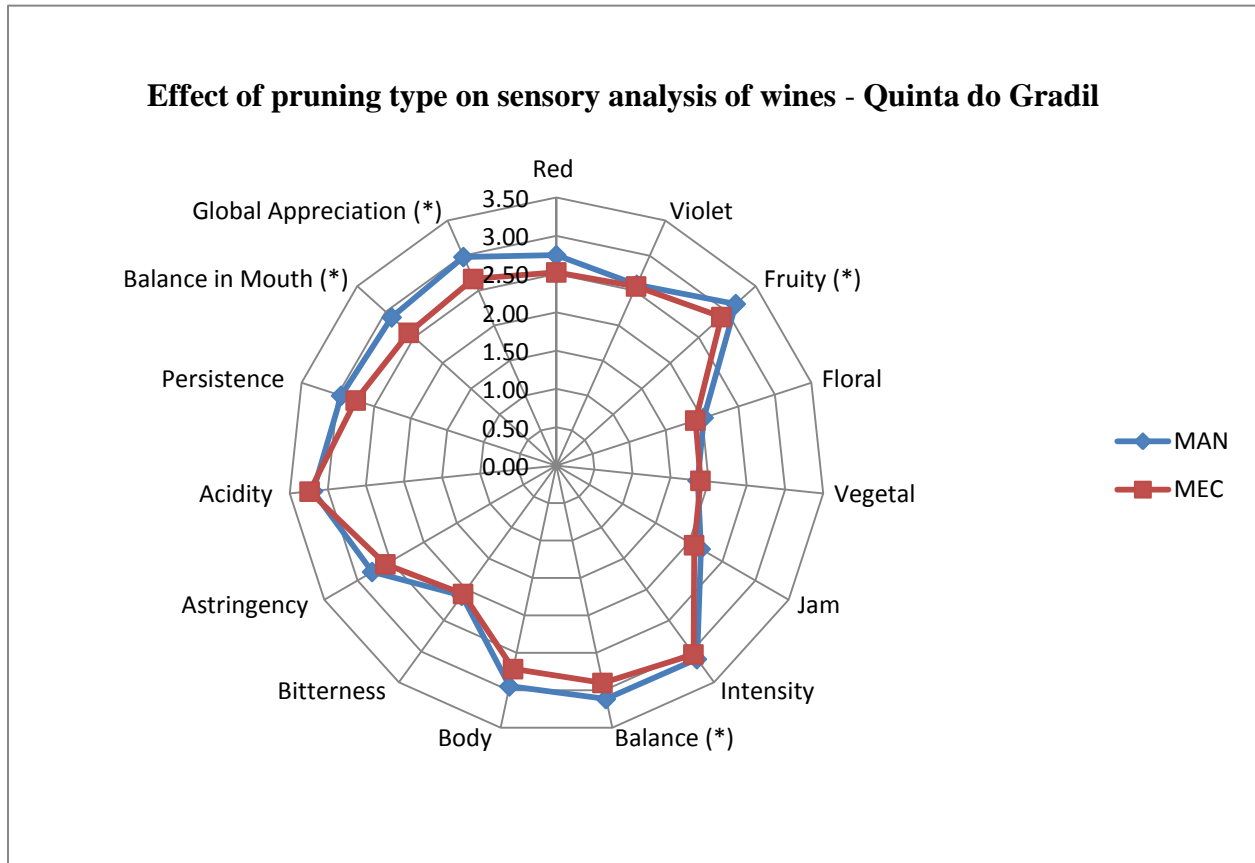
manually pruning treated wine samples. There is significant effect of pruning type on fruity characters perception. Manual pruning has higher fruit aroma perception while mechanical pruning treatment showed more perception of the vegetative aroma in wine samples [Table No.41]. This means that grape samples of manually pruning were may be more matured at the time of harvest compared to mechanical pruning treated grapes.

Overall all positive attributes associated with aroma characters of wine such as jam, intensity and balance of aroma was higher in wine samples coming from manual pruning treatment. Regarding perception of gustatory attributes also trend of having higher perception in manually pruning treated wine sample continues. Wine volume, bitterness and astringency perception was more in wine samples of manual pruning treatment. Acidity perception of wine samples from mechanical pruning treatment was more than manual pruning treatment. This can be correlated to chemical analysis of wine samples from mechanical pruning treatment which showed higher level of total acidity [Table No.18]

**Table No. 41 - Effect of pruning type on sensory analysis of wine Quinta do Gradil**

	Tasting attributes (Average values)	Type of pruning		Statistical significance
		Man	Mec	
<b>Color</b>	Red	2.75	2.52	ns
	Violet	2.58	2.56	ns
<b>Aroma</b>	Fruity	3.15 a	2.90 b	*
	Floral	2.02	1.91	ns
	Vegetal	1.85	1.89	ns
	Jam	2.18	2.08	ns
	Intensity	3.12	3.05	ns
	Balance	3.12 a	2.90 b	*
<b>Taste</b>	Body	2.95	2.72	ns
	Bitterness	2.10	2.07	ns
	Astringency	2.78	2.58	ns
	Acidity	3.19	3.24	ns
	Persistence	2.95	2.75	ns
	Balance in mouth	2.89 a	2.59 b	*
	Global appreciation	2.98 a	2.67 b	*

**Table No. 42 Spider chart for effect of pruning type on sensory analysis of wines Quinta do Gradil**



Balance in mouth and overall global appreciation for wine samples was significantly affected by pruning type. Wine samples coming from manual pruning treatment was perceived better and rated higher by taster's panel than mechanically pruning treated wine samples. This preference is surely affected by overall sensorially balanced wine samples of manual pruning treatment.

Sensory analysis of wine samples shows that there is statistically significant effect of type of organic amendment applied. Perception of color was more in control treatment. This is possible due to presence of higher total phenol and anthocyanin content in wine sample from control treatment [Table No. 22].

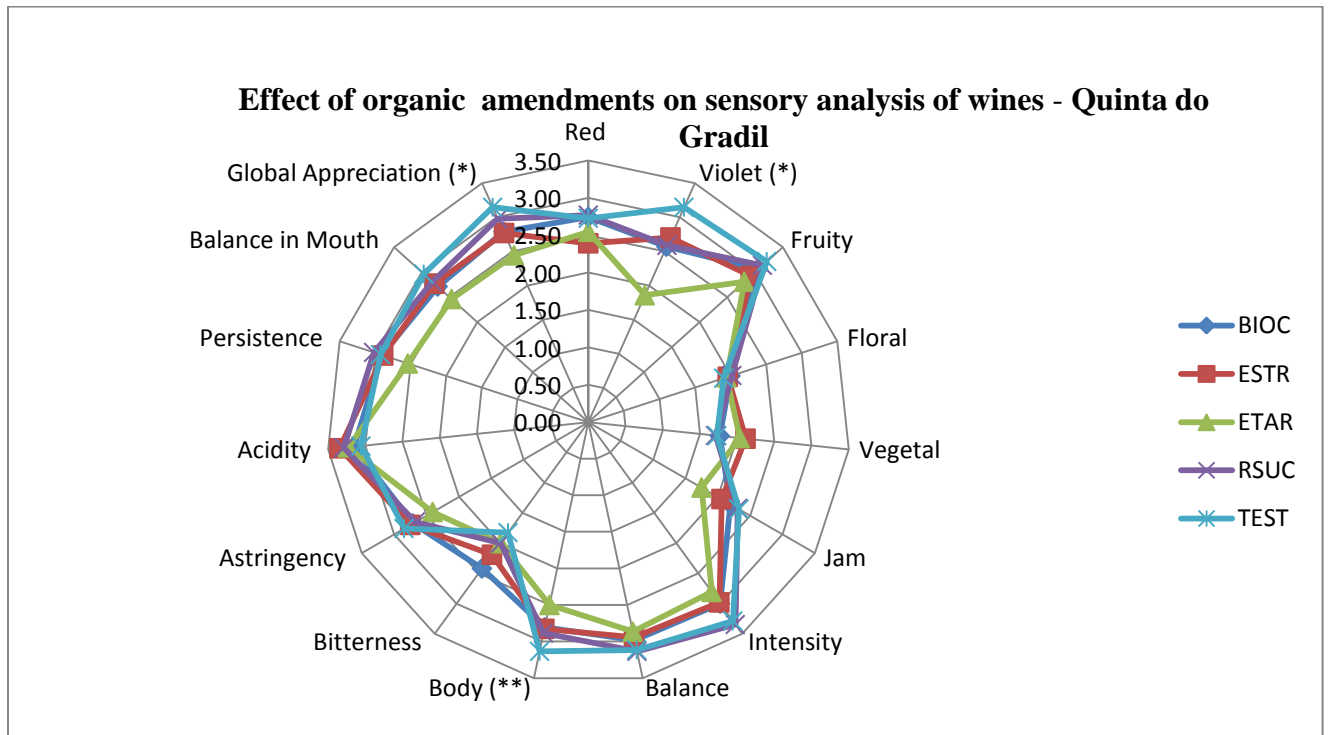
**Table No. 43 - Effect of type of organic amendment applied on sensory analysis of wine Quinta do Gradil**

	Tasting attributes	Type of organic amendment					Statistical significance
		(Average values)	TEST	BIOC	RSUC	ESTR	
<b>Color</b>	Red	2.73	2.74	2.77	2.39	2.55	ns
	Violet	3.15 a	2.57 ab	2.59 ab	2.70 a	1.86 b	*
<b>Aroma</b>	Fruity	3.21	3.06	3.13	2.92	2.81	ns
	Floral	1.90	2.00	2.02	1.96	1.94	ns
	Vegetal	1.73	1.77	1.71	2.12	2.04	ns
	Jam	2.33	2.19	2.31	2.06	1.75	ns
	Intensity	3.29	3.00	3.35	2.98	2.81	ns
	Balance	3.12	2.98	3.13	2.94	2.87	ns
<b>Taste</b>	Body	3.13 a	2.81 ab	2.88 ab	2.83 ab	2.50 b	**
	Bitterness	1.83	2.42	2.00	2.19	2.00	ns
	Astringency	2.85	2.71	2.67	2.75	2.40	ns
	Acidity	3.06	3.15	3.29	3.35	3.23	ns
	Persistence	2.92	2.90	3.02	2.88	2.54	ns
	Balance in mouth	2.96	2.71	2.81	2.77	2.46	ns
	Global appreciation	3.15 a	2.79 ab	2.98 ab	2.77 ab	2.44 b	*

Application of organic amendments doesn't show any statistically significant effect on aromatic characters in wine. Fruity characters were perceived more in wine coming from control treatment while ETAR and ESTR treated wine sample had more vegetal characters than rest of other treatment [Table No.43]. Jam aromas, intensity of flavors and overall balance was also higher in control treatment compared to applied organic amendments. This results with control treatment are almost similar to experimental work done by Korboulewsky et al, (2004) in order to study effect of sewage sludge compost on volatile organic compounds of wine from Grenache variety, They also observed that control wines had higher concentration of 3-methylbutyl acetate, ethyl 3-methylbutanoate, and 1,1- diethoxyethane, which gives more desirable organoleptic quality with a high fruity aromas. Sensory analysis shows that attributes related with perception on palate was also higher in control treatment. Wine volume was perceived higher in control treatment, while astringency and acidity perception was lower in control [Table No.43]. Wine characters were more persistent and balanced in control treatment. Taster's showed statistically significant preference to wine sample belonging to control treatment. These results can be attributed to application of organic amendment which might have delayed maturity of grapes leading to have perception of more vegetal and undesirable characters in wine. Also wine sample coming from control treatment was marked by perception of more volume in mouth along with good fruity characters. These positive characters may have lead to gain highest preference amongst tasters

for wine samples coming from control treatment compared to other organic amendments treated wine samples. There was no big difference for scores given by tasters for wine sample coming from TEST versus RSUC and BIOC. It Suggest that this two types of treatment can be good possible candidate to use as organic amendment without having much adverse effect on final wine quality.

**Table No. 44 Spider chart for effect of organic amendment applied on sensory analysis of wine Quinta do Gradil**



In between sensorial analysis result of two wineries there is much stronger difference, which can be accounted to specific characters of each site and climatic conditions near harvest. Perception of color as well as aromatic characters was scored higher for wine sample coming from Quinta do Coro compared to Quinta do Gradil. Wine samples of Coro were marked with more Jam, fruity characters with rich intensity of flavors on the nose. Contrastingly the Gradil samples had more perception of vegetal characters suggesting poor maturity levels of grapes at time of harvest. From perspective of gustatory attributes it's noted that body of wine, astringency, acidity and overall well balance was perceived more in wine samples of Coro than Gradil. As wine sample of Coro had higher score for almost all the positive attributes associated with wine sensory analysis, globally these samples were appreciated more by the tasting panel compared to sample of Gradil.

## 5. CONCLUSION

The present work was aimed to study the effect pruning type and application of organic amendments on final composition of Syrah Wine made from experimental plot. As data obtained from experiment is from two different sites it's necessary to conclude each site separately and then draw general conclusion about the experimental work performed. Along with the influence of pruning type and applied organic amendment, final results are also affected by characteristics specific to each site. Climatic condition near harvest also affected significantly on final composition of wine sample.

At Quinta do Coro red wine quality parameters such as total phenolic content, total anthocyanin, color intensity, polymerization index and tannin power were unaffected by type of pruning or type of organic amendment used. All other parameters associated with wine chromatic characteristics were not affected by the application of organic amendment. The pH of wine sample was higher with application of cattle manure which can be related to higher supply of potassium content received by application of cattle manure. Mineral analysis of the wine sample showed significant effect of pruning type only for content of calcium. This increase can be associated with less number of nodes retained in manual pruning per shoots compared to mechanical pruning type.

Concentration of element such as Ni, Ga, Y, Tb, Tm, Ti and Rb was affected by type of pruning applied in the vineyard. Higher concentration for all of these elements was generally observed with manual pruning treatment, probably due to less number of bunches retained per shoots. This less number bunches may have resulted in more accumulation of elements. Effect of organic amendment on heavy metal content was significant for Al, Mn, Zn, Sr, Pb, Yb, Cs, Zr, Ni and Co. Higher concentration of Al, Mn, Zn, Co and Ni was observed in control treatment compared to applied organic amendment. This may be possible because probably the content of this element was already more in control soil. Application of organic amendment resulted in increased content of organic matter in this soil. Bioavailability of heavy metals in soils is affected by amount and quality of organic matter that can react with heavy metals and also it can form complexes and chelates with heavy metals of varying stability. This might be one reason to have low concentration of heavy metals in wine sample coming from amendment treated plot compared to control plot.

For Quinta do Gradil chemical analysis of wine samples shows that pH, total acidity, alcohol, color hue, total nitrogen and calcium content of wine samples were significantly affected by type of pruning done. Total nitrogen content, pH, alcohol and color hue were observed higher in manual type of pruning. Higher nitrogen content was may be possible due to concentration effect in less of number berries in manual pruning compared to mechanical pruning type. Type of organic amendment application shows significant effect only on total anthocyanin, total pigment and final potassium content of wine sample. Higher potassium content was observed in biochar treatment. Increased uptake of potassium after application of biochar has been observed in several other studies. The total anthocyanin content observed between TEST, BIOC and RSUC

was similar; hence it's possible to use these amendments without having much negative effect on final wine composition.

At Quinta do Gradil pruning type has significant effect on composition of elements such as Li, Be, Co, Ga, Zr, In, Zn, Sr and Nb. Higher concentration of Li, Be, Co and Ga was observed in wine samples of manual pruning treatment. While Zr, In, Zn, Sr and Nb concentration was higher in wine samples coming from mechanical pruning treatment. Type of organic amendment applied affected significantly on composition of some of the metals analyzed. Concentration of Li, Ga, As, Y, Ce, Eu, Tb, Dy, Tm and Pb was higher in the control treatment compared with other organic amendment applied while concentration of Cs and Ti was higher in wine samples treated with ETAR. Even though there was some increase in concentration of some elements analyzed but level of elements was always below legal limits prescribed by OIV. This suggests that organic amendments can be used without having much effect on final concentration heavy metals and other elements in wine.

Sensory analysis of wine samples for both wineries shows that effect of pruning treatment and application of organic amendment was significant only for Quinta do Gradil while in case of Quinta do Coro there was no such significant effect recorded by tasters. Sensory analysis of Quinta do Coro shows that mechanical pruning and organic amendment can be used without affecting on final quality of wine.

Goal of this work was to suggest an idea to Portuguese viticulture in order to tackle the problem raised by climate changes and increase the competitiveness of Portuguese viticulture by reducing cost of overall wine production. Effect of projected climatic changes of more dryer and less rainfall event can be sorted by using organic amendment which can improve soil water infiltration and water holding capacity of soil along with providing important plant nutrient.

From our experimental work we can conclude that mechanical pruning can be used successfully with low money input. Also if sufficient yield compensation is achieved in mechanical pruned vines to gain higher efficiency of canopy then this can benefit in yield similar or even higher compared to hand pruning without changing grape composition. Application of organic amendment can be also good strategy in this region where soils are marked with low organic matter. Application of organic amendments can be good strategy to supply nutrients to vine without investing heavy cost for chemical fertilizers besides this amendments are beneficial for improving soil structure, porosity and water holding capacity of soil. Municipal solid waste compost and biochar amendments can be used in soil which does not require excess of nitrogen supply. Organic amendments can be also used as mulching compost so that cost of herbicides to control natural weeds can be avoided, simultaneously providing supply of organic matter to soil. Although in our experimental case there was no increase in heavy metal content of soil by application of organic amendment but in future it will be good idea to analyze effect of similar treatment over longer period of time to see if there is any adverse effect of organic amendment application on soil metal content.

Also while applying organic amendments into soil in order to increase organic matter content of soil viticulturist must take into consideration the cumulative effect of its application on wine quality over long period and should make adjustment accordingly. While carrying further study related with similar kind of experiment it will be good idea to start analyzing the mineral content and heavy metals content from grape and soil stage of the experimental plot.

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## 7. ANNEX

### 1. Sensory Analysis Score Card

INSTITUTO SUPERIOR DE AGRONOMIA											
The Tasting Sheet for Red Wine											
Name:								Date : / /		Sessoir :	
Tate the Wine in the order presented and sort different attributes using the following scales:											
For Color, Aroma and Taste :			1. Non existent	2.little Intence	3.Moderately Intenc	4. Intence	5. Very Intence				
For Equilibrium (Aroma and Taste) and Global Appreciation											
			1.Mediocre	2.Satisfactory	3. Good	4.Very Good	5.Excellent				
WINES/SAMPLE CODES											
COLOR:	RED										
	Violet										
	FRUITY										
AROMA:	FLORAL										
	VEGETAL										
	INTENSITY										
	BALANCE										
TASTE:	BODY										
	BITTERNESS										
	ASTRINGENCY										
	ACIDITY										
	PERSISTENCY										
	BALANCE										
GLOBAL APPRECIATION:											
Observations:											