SCAP
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Stand factors related to sensory defects of cork planks

Fatores de campo relacionados com defeitos sensoriais da cortiça

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ABSTRACT

Wine bottle stoppers are the main bio-technological product obtained from cork. The 2,4,6-trichloroanisole (TCA) is considered the main responsible for "cork taint" in bottled wines. This study intent to prove the relation between TCA and *mancha amarela*-yellow spot (MA) and to identify field factors that can be related to the cork TCA presence. Cork planks with and without MA were collected from cork oak stands in ten different regions of the country and 15 variables were analyzed. Three samples were taken from each plank for TCA quantification. The results show that TCA and MA are significantly related and that the TCA level is higher in cork samples coming from stands with shrubs in the understorey.

Keywords: *mancha amarela*-yellow spot; 2,4,6-trichloroanisole; understorey

RESUMO

As rolhas de cortiça são o produto tecnológico mais importante obtido a partir da cortiça. O 2,4,6-tricloroanisol (TCA) é considerado o principal responsável pelo "gosto a rolha" no vinho engarrafado. Este estudo tem como objetivo provar a relação entre TCA e mancha amarela-*yellow spot* (MA) e identificar os fatores de campo que possam estar relacionados com a presença de TCA na cortiça. Pranchas de cortiça com a presença do defeito MA e sem a presença de MA foram colhidas em dez regiões do país e 15 variáveis foram analisadas. De cada prancha foram retiradas três amostras para quantificação do TCA. Os resultados mostram que a presença de TCA e da MA estão significativamente relacionadas e que os valores de TCA são mais altos nas parcelas com matos do que nas parcelas sem matos.

Palavras-chave: mancha amarela, 2,4,6-tricloroanisol, subcoberto

Introduction

The cork oak (*Quercus suber* L.) occupies part of the Portuguese landscape woodlands called *montado de sobro* in Portugal. It's a multiple use agro-silvo--pastoral system characteristic of Mediterranean - continental regions, sparsely covered by trees and understorey formed by grassland formations, supporting high levels of biodiversity (Costa and Pereira, 2007). This artificial ecosystem requires intensive and active management to ensure its survival (Bugalho *et al*., 2011). Apart from cork production, these stands are becoming valorized as places for recreation, tourism and residential uses (Almeida and Pinto-Correia, 2012).

Portugal has 730,000 hectares of cork oak stands, representing about 33% of its total area (APCOR, 2014). The cork oak is distributed only in the western Mediterranean region, especially in areas with Atlantic influence like Morocco, Algeria, Tunisia, Italy, France, Spain and Portugal (Pereira, 2007). The cork and its sub products represent 2% of Portuguese exportations and 30% of all Portuguese forest products exportations, where the cork bottle stopper production represents about 70% of the cork industry exports (INE, 2014).

Mancha Amarela-Yellow spot (MA), a cork sensory defect, and few studies were made on this subject. The MA causes modifications in cork's structure and optical properties being a potential cause of wine cork stoppers taint (Rocha *et al*., 2005). This sensory defect is described as a mouldy paper smell or non-airy wet basement and can make a bottled wine completely undrinkable (Vlachos *et al*., 2008). Cork planks with MA are rejected for cork stoppers productions, since its presence is empirically related to 2,4,6-trichloroanisole (TCA) values which compromises the quality of cork, thus causing a huge loss for cork industry (Neto, 2005; Rocha *et al*., 1996; Rocha *et al*., 2005). However the MA concept is still ambiguous and may vary between the industrial units that assign that defect's name.

Volatile and non-volatile compounds soluble in ethanol or water can migrate to the wine, thus contributing to the wine sensorial properties (Rocha *et al*., 2005).The TCA, a volatile chlorinated compound, was reported as the chief cause of cork off-flavours (Felter *et al*., 2010; Teixeira *et al*., 2006) and the filamentous fungi metabolism is the main responsible for the presence of TCA (Maggi *et al*., 2008; Oliveira, 2011).

The present study aims to clarify if the MA defect is related with TCA levels on raw cork planks and in parallel to identify field factors that can be related with TCA cork presence. Despite being an exploratory work, the results obtained can be regarded as a valuable expansion of the existing knowledge about the factors to which the MA defect is correlated.

Materials and Methods

Selection, sampling of cork and determining 2,4,6 - Trichloroanisol (TCA) values

In a cork factory several raw cork samples, were randomly collected from different stack which came from ten regions geographically different (Figure 1).

From each region three stacks were considered. Each stack has represented an individual cork stand.

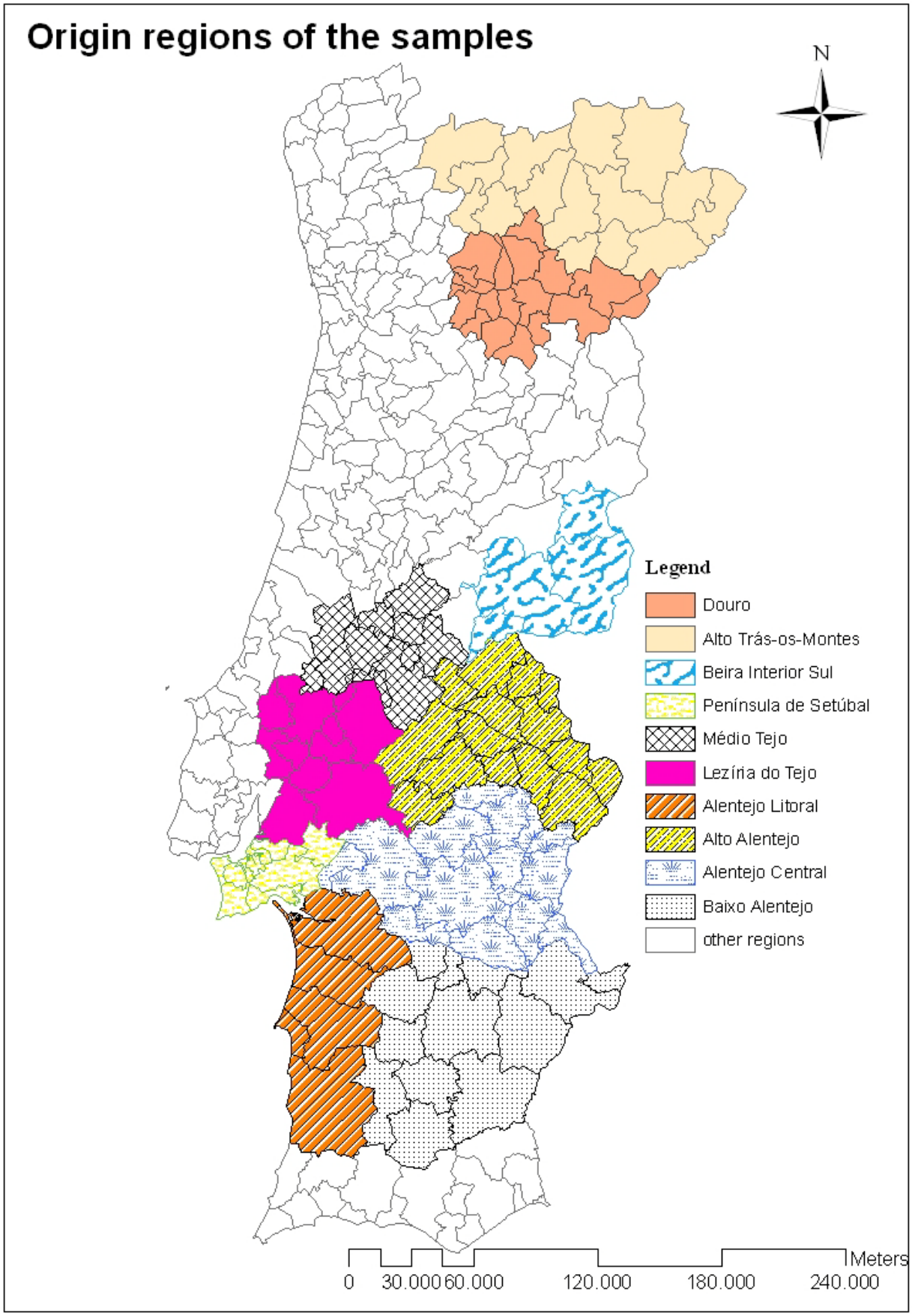

Figure 1 - Origin regions of the samples.

To screen for *Mancha Amarela*-Yellow spot (MA) cork planks were observed and three cork planks with MA were randomly taken from each lot. Cork samples of 20x20x20cm size were taken from each plank (Figure 2). Twelve samples from planks without MA were also collected as controls.

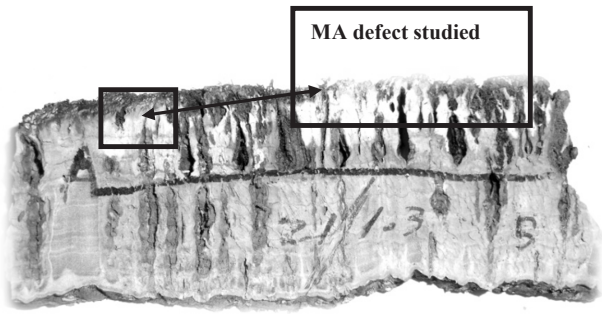

Figura 2 - Cork plank with Mancha Amarela-Yellow spot defect studied in this work.

In Table 1 are listed the field data from the oak stands where the surveyed cork planks were obtained. Also are listed laboratorial variables based on the direct observation of the outer surface of the cork planks.

Table 1 - List of field and laboratorial variables registered.

Field Variables	
Country region	Grazing and cattle type
Stripping date	Moisture of the stand
Cork oaks average age	Slope
Understorey	Mortality
	Presence of flathead oak borer (*Coraebus undatus* F.)

Laboratorial Variables	
White/ Grey mould	Green mould
Lichens	Musty smell

For TCA analysis chips with 200x30x3 mm size were removed from each sample and the remaining material was suitably packed into a sterile bag, labelled and sent to laboratory. The TCA values were determined by Gas Chromatography. Each chip was cut into small pieces of cork (5x5x5 mm) which were introduced into flasks. After, 20 ml of a 12% ethanol solution (value close to the alcohol content of wine) was added to measure the amounts of TCA. The same flasks were individually labelled with the reference region number, the lot number and the sample number. The equipment used was a GC / ECD- Varian CP3800 chromatograph; 20 mL vials with 20 mm magnetic capsules and silicone septum; polidimetilsiloxane fiber (PDMS) of 100 m and nonpolar column.

Statistical analysis

The TCA values were statistically correlated to the MA cork samples variable, the field and laboratory variables. A multiple correspondence analysis (MCA) was used to assess the most relevant variables that would later be individually analyzed (Oliveira, 2002), with non parametric statistical methods (Kruskall-Wallis, Mann-Whitney tests), after evaluating data normality (Levene test). Statistical treatments were made using Statistic Software (version 6.1 - StatsoftInc, 2003).

Results

The results revealed that TCA values of all control samples had TCA levels below 1 ng/L, as expected.

The statistical analysis of the results showed that the parametric methods could not be applied (Levene test: $F(1,43)= 8,0071$; $p= 0,0071$) and the frequencies distribution histogram of TCA values did not follow the normality.

The Mann-Whitney test revealed statistically significant differences between the TCA values of control samples and those of MA samples (Z adjusted =5.0913; $p<0,05$) and is represented in Figure 3.

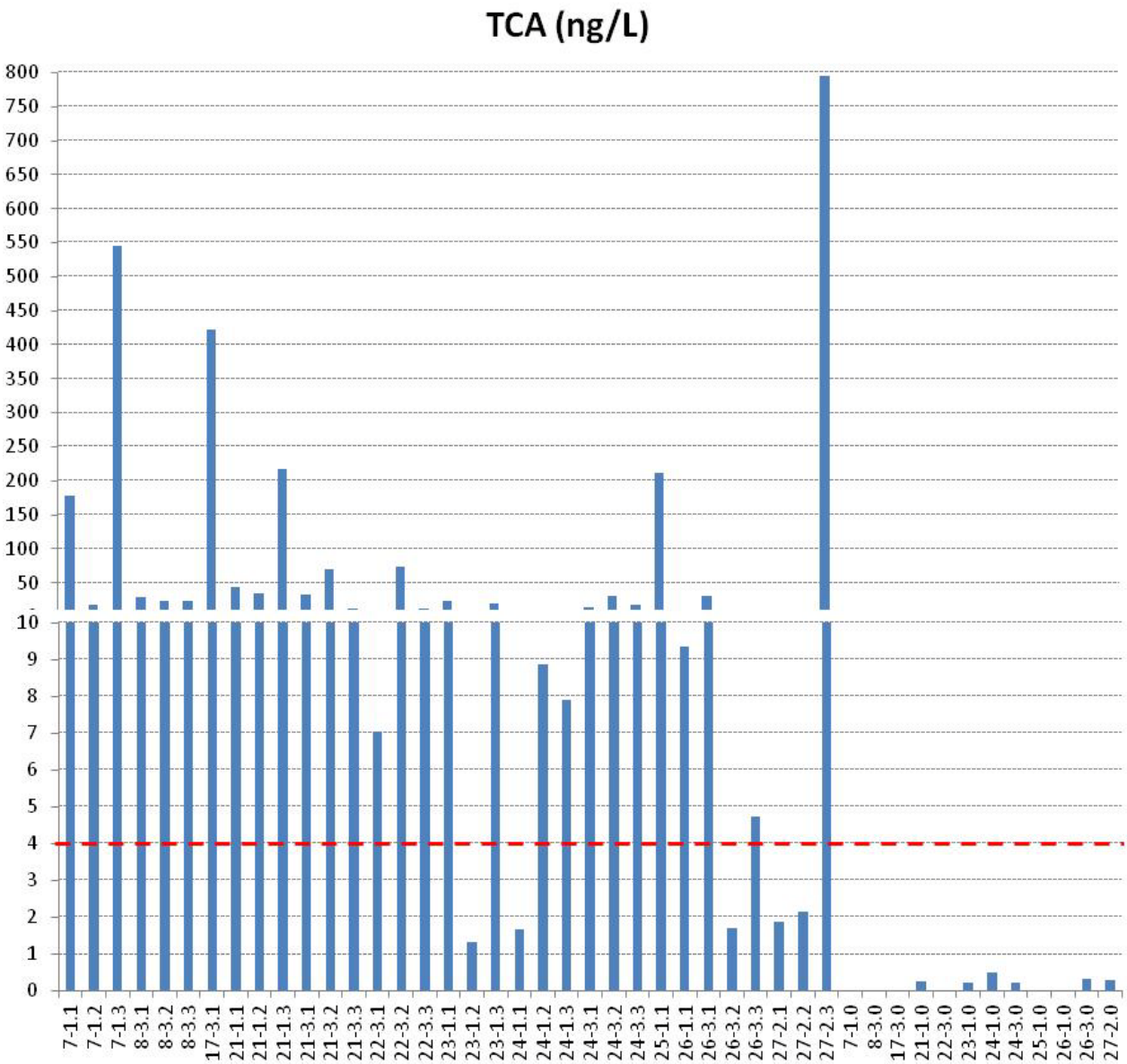

Figure 3 - Values of TCA (ng/L) obtained in cork samples. Dotted red line represents TCA threshold considered by industry as MA free. Control samples are the 12 samples placed on the far right (codes end with zero).

The Multiple Correspondent Analyses [MCA] adjusted to the field variables highlighted as more relevant for the MA and high TCA presence, the understorey and slope (positive), the region of origin (negative) (Fig. 4a) and the moisture of the stand (Fig. 4b). Laboratory variables white/ green mould, musty smell and lichens are also strongly related to MA and high TCA levels.

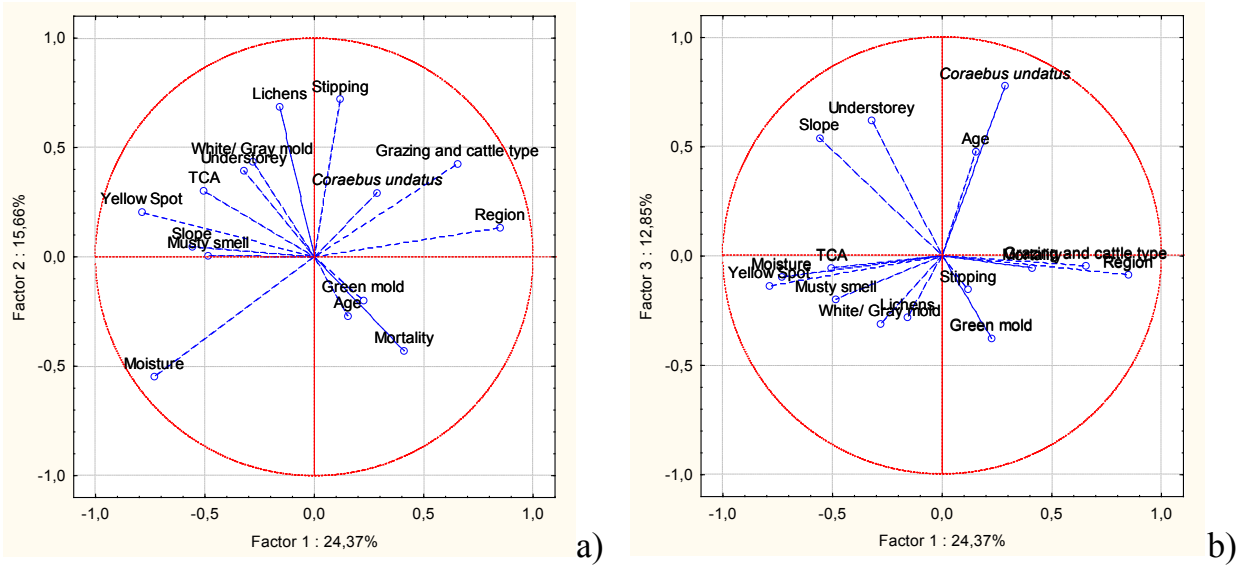

Figure 4 - Projection of the variables on the factor planes: a) Factors 1x2. b) Factors 1x3. In each Factor axis are calculated.

The results of nonparametric tests to assess the variation of TCA levels according to the field variables considered most relevant (Kruskal-Wallis test and Mann-Whitney test) are on Table 2.

Table 2 - Statistical analysis results of non-parametric tests (Kruskal-Wallis test and Mann-Whitney) adjusted to TCA values and relevant field variables.

Field variables	Statistical results		
Slope	$H_{(2,45)}$= 0.5538	p= 0.7581	NS
Moisture of the stand	$H_{(2, 45)}$= 2.2740	p= 0.3208	NS
Region of samples origin	$H_{(9, 45)}$= 6.4585	p= 0.6933	NS
Understorey	U= 155.00	p= 0.0411	*

Statistically not significant (NS) and statistically significant (*)

Only the understorey variable seems to affect significantly the TCA values (U = 155.00, p = 0.0411). The average levels of TCA in planks coming from stands with bushes are higher than the average levels of TCA in planks coming from oak stands without understorey (Table 3).

Table 3 - TCA average levels related with understorey.

stands with understorey	stands without understorey
101.86 ± 192.81	10.72 ± 9.69

Finally, according to the results of the nonparametric tests, the TCA levels are not statistically correlated to none of the laboratory variables (Table 4)

Table 4 - Statistical analysis results of non-parametric tests (Mann-Whitney) adjusted to TCA values and relevant laboratorial variables.

Laboratorial Variables	Results		
Musty smell	U=42.00	p= 0.1100	NS
White/grey mould	U=90.00	p= 0.0852	NS
Lichens	U=103.00	p= 0,.935	NS

Statistically not significant (NS)

Discussion and Conclusions

Cork stoppers are a natural product, and cork can be contaminated in different ways that can affect its proprieties (Rocha *et al.,* 2005).

Yellow-Spot (MA) is a cork sensory defect that, supposedly, is the result of filamentous fungi development, because some of them are usually associated with the presence of 2,4,6-trichloroanisole (TCA) (Maggi *et al.,* 2008; Oliveira, 2011).

The purpose of this study was to relate MA defect with TCA levels present in the cork, which was confirmed. Therefore cork planks with MA should be taken away from cork stopper industry. Are the filamentous fungi responsible for that relationship? To answer this question further studies are already being carried out.

Other purpose of this study was to relate the stand factors, obtained from a query to the owners, with TCA levels in the cork. From all variables considered, only the presence/absence of understorey on the cork oak stands was highly significantly correlated with TCA levels. In fact, the planks with high TCA levels were obtained from stands with shrubs in the understorey. Have the filamentous fungi found good conditions for their development on stands with shrubs in the understorey? Are those conditions humidity, food, and host or is there anything else more?

It should be emphasized that this was an exploratory study that allowed to reveal directions to more detailed studies that should be fulfilled in the near future about the conditions that induce the presence of TCA in cork planks.

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