

# Copper sulfate use in organic vineyards: Are wine yeast and bacteria affected by this fungicide?

In 2017, the organic vineyard areas had tripled in 10 years and approximately 5% of worldwide vineyards are now under organic certifications (Figure 1). The growth is also reflected in consumer consumption of organic wines, not only in Europe but all over the world where in Sweden for example, the growth is 51%, in France 36%, and in Australia, 120%.

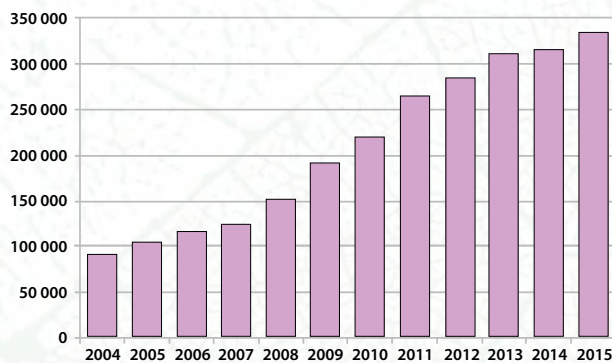


Figure 1. The evolution of the organic vineyard in the world  
Source: "La Bio dans le monde", Agence Bio 2017

Copper compounds, usually copper sulfate mixed with lime, have been used by grape growers since the late 1800's to fight fungi and bacteria on vines. For organic growers, who cannot use other fungicide sprays, copper sulfate is still an effective tool against downy mildew. With the increased production of organic vineyards in the world, the use of this fungicide has also grown with the expansion of this type of farming. Consequently it is possible to find this heavy metal to carry over from the vineyard to the cellar, during all stages of the vinification process. The amount found varies. For example, if we compare a vineyard without any copper-based sprays, then the level of copper in the juice is likely to be less than 0.5 mg/L. If copper-based sprays are used, then the level of copper can vary depending on the number of applications, total dose applied and the time between the last application and harvest. The copper content of the juice could therefore range from less than 1 mg/L to higher than 15 mg/L.

## How copper affects alcoholic fermentation and wine yeast

It is known that elevated concentrations of this metal can be toxic to yeasts and bacteria, interacting with cellular nucleic acids and enzyme active sites, although a principal initial site of Cu action is considered to be the plasma membrane. It can affect cell growth and activity especially at high levels above 10.2 mg/L, (Ohsumi et al., 1988) inducing sluggish fermentation (Azenha et al., 2000), and can impact aromatic compounds formation, especially thiols. The impact of Cu also affects not only selected yeast or bacteria, but also the indigenous flora although, it does not appear that one species is more affected than another (IFV, 2019).

A characterization study was conducted in order to understand how our selected wine yeast behave under different conditions and concentrations of copper, so that winemakers who wish to inoculate alcoholic fermentation for their organic wines can do so without any potential issues.

The first step was to evaluate the impact of copper on wine yeast in synthetic must; copper added at 7.5 mg/L exhibited different sensitivities, but mostly the impact was observed during the lag phase of the AF. As the concentration of copper increased, so did the impact. But overall, the time to complete fermentation was similar between the yeast strains.

In real must studies, wine yeast were tested with copper concentration in the range of 7.5 mg/L to 30 mg/L. The behaviour of the yeast in real must echoed that observed in synthetic must. There was a slight variation amongst the different yeast tested, especially in the lag phase, but at copper concentrations that were above those generally found in musts coming from vineyards sprayed with copper (Figure 2A and 2B).

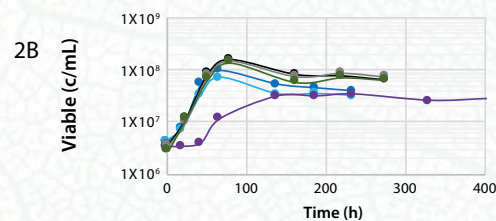
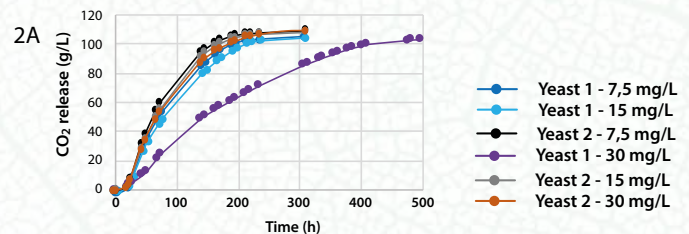


Figure 2A - B. Fermentation rate (A) and viability (B) in Chardonnay must (2017) with 2 different wine yeast inoculated at 25 g/hL and with different Cu concentration

In terms of cell viability, the pattern is similar with some wine yeast showing more sensitivity and slower to multiply and survive at higher concentration (30 mg/L) but eventually catching up towards the end of fermentation. The Cu concentration in which the wine yeast becomes affected is higher than those usually found in wine.

Volatile acidity production is also follows the same pattern, however an effect is already observed at 15 mg/L, and is also wine yeast strain dependent, as shown in Figure 3.

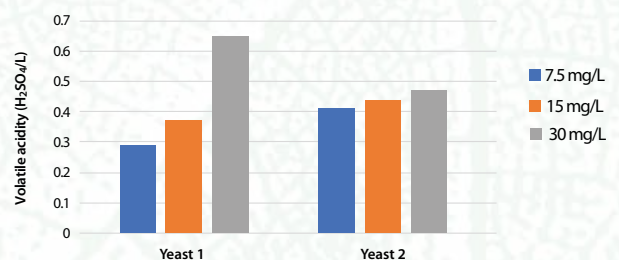


Figure 3. Volatile acidity production during AF by 2 different wine yeasts with different Cu concentrations.



Consequently, levels of copper below 15 mg/L are unlikely to affect the rate of fermentation and viability of our wine yeasts but it does impact the lag phase. However, if other inhibitory factors are also present (e.g. agrochemical residues, acetic acid, high ethanol, high or low pH etc), then it is possible that such levels of copper might influence fermentation rate. For example, in a study to evaluate the impact of pH coupled with copper levels on cell viability and volatile acid production, it seems that there is a link between higher pH and higher copper concentration on cell viability decrease (Figure 4) as well as volatile acidity increase (Figure 5). With climate change having a strong influence on wine pH and acidity (higher pH, lower acidity), it can be assumed if copper concentrations are higher (> 10 mg/L), then extra care must be taken to assure that the fermentation is well managed through proper yeast rehydration and nutrition.

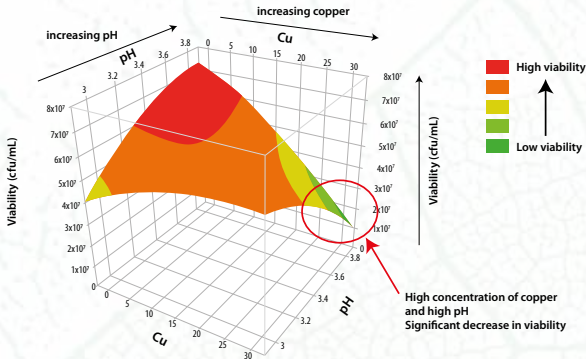


Figure 4. Impact of increased Cu concentration and increased pH on wine yeast viability

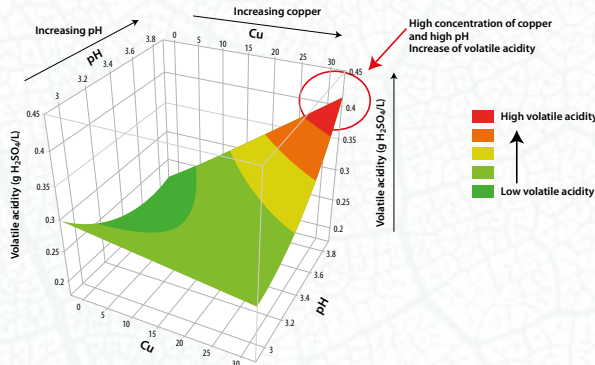


Figure 5. Impact of increased Cu concentration and increased pH on volatile acidity production

### How copper affect malolactic fermentation and wine bacteria

A similar approach was done to study the impact of copper on malolactic fermentation and wine bacteria. Similar patterns were seen with wine bacteria during co-inoculation, in that, unless the concentrations of Cu are very high, above typical juice levels (>15mg/L), wine bacteria and malolactic fermentation are not strongly affected. At high concentration, it appears to be strain specific (Figure 6). However, below 15mg/L, there is no impact. This is similar for both co-inoculation and sequential inoculation in red wines. In sequential inoculation, as the wine bacteria are inoculated into a wine matrix where other factors can come into play, and also because the residual Cu is usually within OIV regulations (< 1 mg/L), it did not impact our wine bacteria in terms of both MLF and viability. In white wines, when Cu concentrations in must are higher than 7.5 mg/L, some wine bacteria can be affected, thus it is recommended to use a sequential inoculation rather than do a co-inoculation.

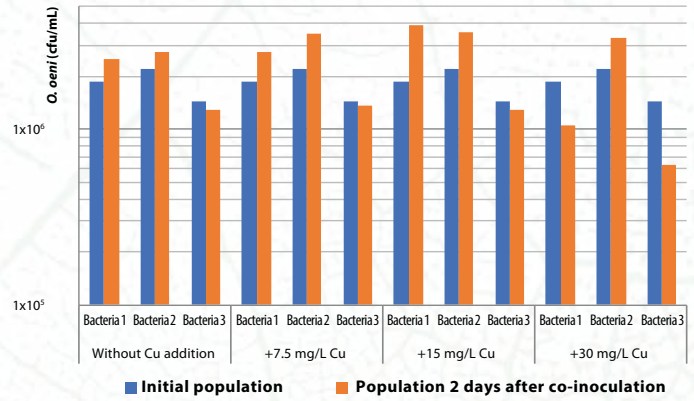


Figure 6. Bacterial population (cfu/mL) - Initial and 2 days after co-inoculation

### Sensory protection under high Cu concentration

It has been shown that copper reacts with thiols, so it can affect the varietal aromas of wines, especially those varieties where thiols play a major role, such as Sauvignon Blanc. Since the production of thiols is wine yeast strain dependant, a study done in collaboration with ICV has shown that even at very low concentration of Cu (0.8 to 1.8 mg/L), it can have a significant impact on the thiols (Figure 7). We have also shown that it can impact on the biosynthesis Cu some esters and higher alcohols as shown in Figure 8) especially at quite high Cu concentration (> 15 mg/L).

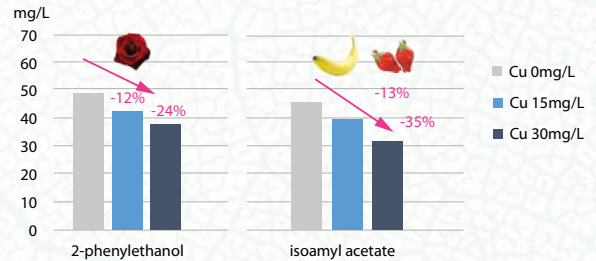


Figure 7. Ester productions during AF with different Cu concentrations

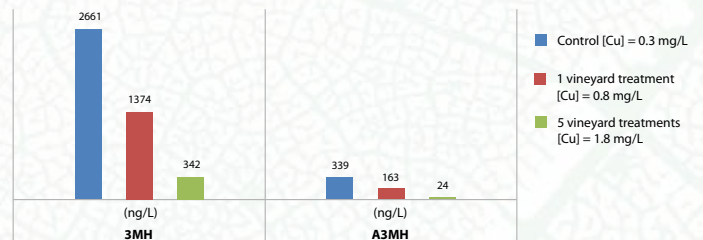


Figure 8. Concentration of volatile thiols in wine at the end of AF (Grenache rosé)

### To summarize

Wine yeast and bacteria do not appear to be significantly affected by Cu concentrations higher than 15 mg/L, except in the case of malolactic fermentation in white wines, where concentration > 7.5 mg/L can impact the MLF. High pH and high Cu concentrations can impact wine yeast viability and lag phase. As Cu affects the plasma membrane of the yeast cell, it is good practice to protect it during rehydration with high sterols containing product (GoFerm™ range) and appropriate organic nutrition. Copper does impact some aromatic compounds such as thiols at low levels and some esters at higher concentration. Volatile acidity, which can alter the quality of wine is also affected by increased Cu concentrations, especially under high pH conditions. It is important to remember that other factors (pH, SO<sub>2</sub>, temperature, alcohol, etc) could interact with and increase the impact of copper on microorganisms. Our selected wine yeast and bacteria can be used in organic winemaking and can help secure a complete fermentation while maximizing its sensory potential.

