

## COPPER ACCUMULATION IN VINEYARD SOILS FROM NEMEA, GREECE

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### Abstract

We present for the first time the extent and magnitude of Cu accumulation in calcareous vineyard soils from Nemea, Greece, as result of intensive application of Cu-based fungicidal sprays. Surface (0-20 cm) soil samples were collected from 40 vineyard plots covering the whole agricultural region of Nemea devoted to viticulture. In 20 randomly selected vineyards, we also collected soil at 50 cm depth. Major soil properties were determined (pH, electrical conductivity, organic carbon and equivalent calcium carbonate contents, particle size distribution). Copper concentrations in Nemea vineyard soils (33.1 - 291 mg kg<sup>-1</sup>) were similar to those reported in the soils of vineyards in other parts of Europe. Copper has migrated down the soil profile since the levels at 50 cm depth are higher than the known background concentrations, probably as result of soil disturbance by tillage practices. We did not find differences in Cu availability between the surface and deep soil samples after applying the DTPA chemical extraction. The DTPA-extracted Cu concentrations were largely dependent on the total soil Cu content. The excessive application of Cu-based fungicides should be avoided with the aim to ensure that Cu accumulation does not reach levels that may inhibit plant growth.

**Keywords:** chemical extractions, heavy metals, soil contamination, agricultural activities.

### Περίληψη

Στην εργασία αυτή παρουσιάζουμε το μέγεθος και τη σημασία της συσσώρευσης χαλκού σε ασβεστούχα εδάφη αμπελώνων στη Νεμέα της Ελλάδας, ως αποτέλεσμα της εντατικής εφαρμογής χαλκούχων μυκητοκτόνων. Συλλέχθηκαν επιφανειακά (0-20 cm) δείγματα εδαφών από 40 αμπελώνες αντιπροσωπεύοντας το σύνολο της γεωργικής γης της Νεμέας το οποίο είναι αφιερωμένο στην αμπελοκαλλιέργεια. Επίσης, σε 20 τυχαία επιλεγμένους αμπελώνες συλλέξαμε έδαφος μέχρι το βάθος των 50 cm. Στα εδαφικά δείγματα προσδιορίστηκαν βασικές ιδιότητες των εδαφών. Οι συγκεντρώσεις του χαλκού (33.1 - 291 mg kg<sup>-1</sup>) στους αμπελώνες της Νεμέας είναι παρόμοιες με αυτές που αναφέρονται σε εδάφη αμπελώνων και σε άλλες περιοχές της Ευρώπης. Ο χαλκός έχει μετακινηθεί σε βαθύτερες στρώσεις του εδάφους με τα επίπεδά στο βάθος 50 cm να παραμένουν υψηλότερα από τις γνωστές συγκεντρώσεις αναφοράς, πιθανόν ως αποτέλεσμα της ανατάραξης του εδάφους λόγω καλλιεργητικών τεχνικών (άρωση). Η εκχύλιση των διαθέσιμων μορφών του χαλκού επιτεύχθηκε με τη βοήθεια του εκχυλιστικού DTPA. Δεν εντοπίσαμε διαφορές στη διαθεσιμότητα χαλκού ανάμεσα στα

επιφανειακά δείγματα εδάφους και στα δείγματα βάθους 50 cm. Προτείνονται ο έλεγχος της εφαρμογής και η αποφυγή υπερβολικής χρήσης χαλκούχων μυκητοκτόνων διασφαλίζοντας ότι η συσσώρευση του χαλκού στα εδάφη να μην φτάσει σε επίπεδα που να εμποδίζουν την ανάπτυξη φυτών.

**Λέξεις κλειδιά:** εκχυλίσεις, βαρέα μέταλλα, ρύπανση εδάφους, αγροτικές δραστηριότητες.

## 1. Introduction

Since the end of the 19<sup>th</sup> century, the intensive application of copper (Cu)-based fungicides in vineyards to fight vine fungal diseases, such as downy mildew caused by *Plasmopara viticola*, has resulted to increased Cu concentrations in the receiving soils (Komárek *et al.*, 2010). The wash-off of Cu from the vine leaves, the deposition of the senescent leaves and the accidental spills of the fungicides are the major source processes of Cu to the vineyard soils. However, the Cu accumulation in the soil over the years is the result of its strong fixation to soil organic matter, Fe- and Mn-(hydr)oxides and clay minerals (Komárek *et al.*, 2008 and Pietrzak and McPhail, 2004). This geochemical behavior combined with the neutral-alkaline soil pH of most of vineyard soil (Mackie *et al.*, 2012) do not favor the migration of Cu throughout the soil profile. As a result, a decrease in Cu concentrations with increasing depth has been reported, and Cu commonly reaches background levels within 40-50 cm depth (Deluisa *et al.*, 1996 and Pietrzak and McPhail, 2004). Referring to the chemical methods used in the laboratory to determine the Cu content in vineyard soil, the *aqua regia* extraction is frequently applied, especially in the European studies, but with low correlation with the phytoavailability of this element. For that reason, a variety of single stage chemical extractions has been proposed for mimicking the processes that operate at the soil-plant interface providing more useful information for the fraction of Cu that is available for plant uptake (Kelepertzis *et al.*, 2015).

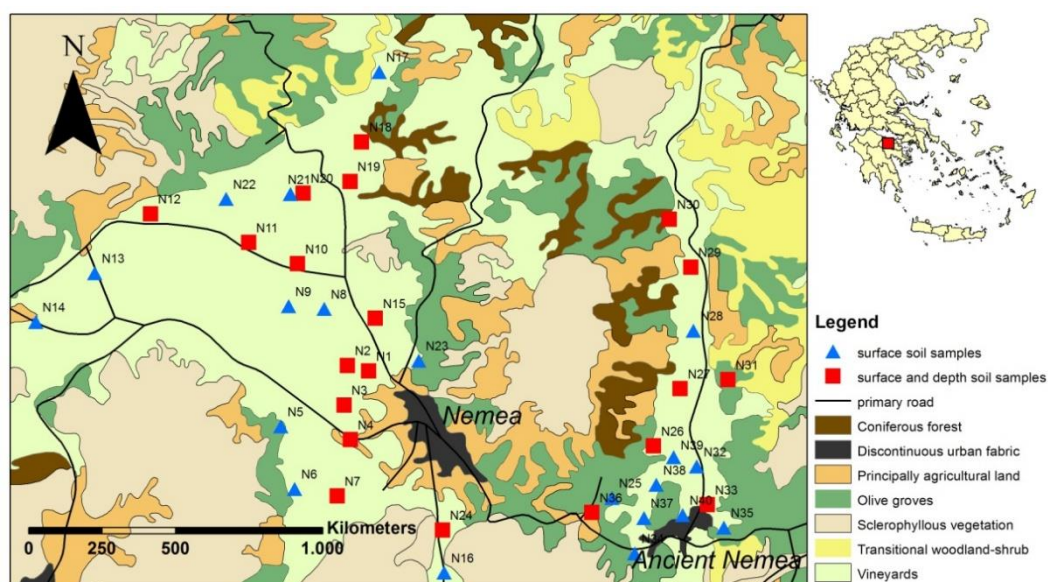
In Greece, the favorable calcareous substrate and the Mediterranean climatic conditions have allowed the wide spread of viticulture since the early historic years. In North Peloponnese, the region of Nemea has enjoyed a long tradition of intensive grapevine growing for the production of its indigenous *Agiorgitiko* red wine variety. In this area, Cu-based fungicides, such as copper sulfate (CuSO<sub>4</sub>), have been extensively applied to the vineyards, and as a result there is a high potential risk for soil contamination with Cu. An investigation regarding the Cu concentration levels in agricultural soils from different regions of Greece, including some vineyard soils located in Nemea, has previously been undertaken (Vavoulidou *et al.*, 2005). Nevertheless, this study was based on a reduced set of samples without delineating the magnitude of Cu accumulation throughout this region and without investigating the potential Cu migration in deeper soil layers. The aim of the present survey was to assess the extent and magnitude of Cu accumulation in Nemea vineyard soils by using the *aqua regia* and diethylene triamine pentaacetic acid (DTPA) chemical extractions and to investigate the potential for the soil to retain Cu at greater depths.

## 2. Materials and Methods

### 2.1. Soil sampling and preparation

Soil samples were collected during May and June 2015 from a total of 40 vineyard plots evenly distributed throughout the Nemea agricultural region devoted to grapevine production (Fig. 1). We specifically followed the recommendations given by Wightwick *et al.* (2006) who suggested the collection of one composite sample from as many vineyards in a region as possible instead of collecting many samples from fewer vineyards. In each vineyard, soil samples were collected from the surface soil (20 cm depth) from the middle row in the vineyard. Within this row, soil was collected from locations one-fourth, halfway and three-fourths of the way down the row, and the sub-samples were mixed in order to constitute a representative composite sample from each vineyard. Following the conclusions reached by Pietrzak and McPhail (2004) and Mirlean *et al.* (2007) who

claimed that Cu concentrations are higher at the vines than between the vine lines, we collected the surface soil samples within 30 cm of the vine line instead of collecting soil from the middle of the row. This soil sampling strategy was accomplished with the aim to detect the highest concentrations of accumulated Cu in the studied vineyard soil. Additionally, at 20 randomly selected vineyards, we collected soil at a depth of 50 cm using a soil auger. The samples were air dried at 45°C for three days, sieved through a 2 mm plastic sieve and well homogenised. Representative portions obtained by quartation were further sieved through a 100- $\mu$ m sieve and stored in room temperature.



**Figure 1 - Land use map of the area according to the CORINE database showing the location of the investigated vineyard plots.**

## 2.2. Soil analysis

Soil pH and electrical conductivity were determined using a 1:1 ratio (w/v) of soil and deionised water based on the < 2 mm sample fraction. Total organic carbon (TOC) was determined on the < 100  $\mu$ m fraction according to the method described by Walkley and Black (1934). Particle size distribution in the sand, silt and clay fractions was determined using the hydrometer sedimentation method. The equivalent calcium carbonate content was determined by HCl attack. The applied chemical extractions were performed on the < 100  $\mu$ m fraction. Copper was extracted from soil samples using the *aqua regia* attack ( $\text{HNO}_3/\text{HCl}$ , 1:3) at the Acme Analytical Laboratories of Canada and Cu concentrations were measured using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Hereafter and for simplicity reasons, the Cu concentrations determined by this method will be referred to as total. The availability of Cu was evaluated by the DTPA extraction because of its effectiveness in neutral to alkaline soils (Lindsay and Norvell, 1978). Concentrations of Cu in the DTPA extracts were determined by flame atomic absorption spectrometry.

## 3. Results

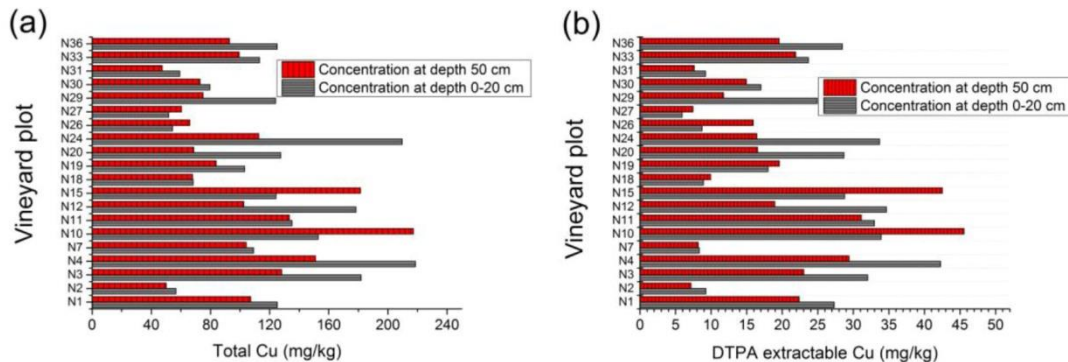
Basic physico-chemical properties and extracted Cu concentrations of the studied vineyard surface soils are given in Table 1. The strong influence of the carbonate bedrock on soil chemistry is reflected by the alkaline soil pH (median 7.8) and the high eq.  $\text{CaCO}_3$  content (up to 68.9%). The soils exhibit a low TOC content (median 1.34%, max 1.99%) and a wide variation in the electrical conductivity values ranging from 256 to 1150  $\mu\text{S}/\text{cm}$ . The texture in most of the vineyard-devoted soils is characterised by the predominance of the clay fraction (median 44.3%), which is expected

given that the weathering of carbonate rocks result to soil parent materials that give rise to soils with a fine texture.

**Table 1 - Descriptive statistics of the analysed soil properties and Cu concentrations extracted by the aqua regia and DTPA chemical extractions for the surface soil samples ( $n=40$ ).**

Variable	Mean	Median	Minimum	Maximum
pH	7.8	7.8	7.4	8.1
Electrical Conductivity ( $\mu\text{S}/\text{cm}$ )	484	465	256	1150
Sand (%)	26.7	25.5	12.8	51.8
Silt (%)	31.4	31.6	20.6	39.6
Clay (%)	41.9	44.3	24.6	58.6
$\text{CaCO}_3$ (%)	42.7	41.0	9.8	68.9
TOC (%)	1.35	1.34	0.68	1.99
Cu (aqua regia), $\text{mg kg}^{-1}$	116	111	33.1	291
Cu DTPA, $\text{mg kg}^{-1}$	22.6	21.3	3.2	59.4

The total Cu concentrations in the surface vineyard soils varied from 33.1 to 291  $\text{mg kg}^{-1}$  with a median value of 111  $\text{mg kg}^{-1}$ . The average Cu content observed in the 0-20 cm soil layer by the DTPA extraction was 22.6  $\text{mg kg}^{-1}$ , with a maximum concentration of 59.4  $\text{mg kg}^{-1}$ . No significant differences could be found in Cu variation with depth ( $p > 0.05$ , one way ANOVA) based on the results from the 20 vineyard plots where soil samples were collected from both the surface and deeper soil horizons. An inspection of the variation in Cu amounts as a function of soil depth (Figure 2a and 2b) indicates that the classical expected decrease with depth is not evident for all the vineyards; for some vineyards, the Cu concentrations (for both metal forms) can remain either the same (e.g. for the investigated vineyards coded as N7, N11 and N18) or considerably increased (e.g. for the vineyards coded as N15 and N10).



**Figure 2 - Total (a) and DTPA (b) extractable Cu concentrations at two depths (0-20 cm and 50 cm) in 20 vineyard soils from Nemea.**

#### 4. Discussion

The vineyard soils from Nemea presented elevated Cu concentrations when compared to the world average Cu content of 20  $\text{mg kg}^{-1}$  for non-contaminated soils (Kabata-Pendias and Pendias, 2001). Copper has a tendency to be excluded from carbonate rocks, sandstones and calcareous shales (Kabata-Pendias and Pendias, 2001). Since the local geology is characterised by the presence of such rocks we consider that weathering and erosion has minimum contribution to the observed soil

Cu enrichment. Moreover, Cu concentrations in background uncultivated soil samples from the adjacent Argolida area, which is characterized by occurrences of similar rock types and therefore similar soil parent material, ranged from 17.24 to 42.81 mg kg<sup>-1</sup> (Kelepertzis, 2014). Such data clearly demonstrate that the source of Cu in the studied vineyard soils could be attributed to the application of Cu-based fungicides. Furthermore, the low contents of Zn and Cd in the studied soils (data not presented in this study) provide further evidence that the fungicide treatments should be regarded as the principal source for the established soil Cu enrichment. These elements are typical tracers of fertilizer and manure application in agricultural soils (Kelepertzis, 2014).

Our results are comparable to those reported from studies in vineyards within other European countries. In particular, the total Cu concentrations in the present study are consistent with those found in the soils of vineyards in Italy (mean 130 mg kg<sup>-1</sup>, Deluisa *et al.*, 1996), France (mean 99 mg kg<sup>-1</sup>, Brun *et al.*, 1998) and NW Iberian Peninsula (mean 163 mg kg<sup>-1</sup>, Fernández-Calviño *et al.*, 2009). The levels of concentrations tend to be lower than those found in our study for the Australian vineyards (Wightwick *et al.*, 2006) and for vineyards from the Czech Republic (Komárek *et al.*, 2008), probably due to the lower average annual usage of Cu-based fungicides. On the contrary, Cu concentrations in Brazilian vineyards and in vineyards from Champagne area, France, can reach up to 3200 mg/kg<sup>-1</sup> and 1500 mg/kg<sup>-1</sup> respectively, because of the exceptionally high volumes of fungicides that are needed in areas with greater humidity and precipitation for diminishing downy mildew attacks (Mirlean *et al.*, 2007).

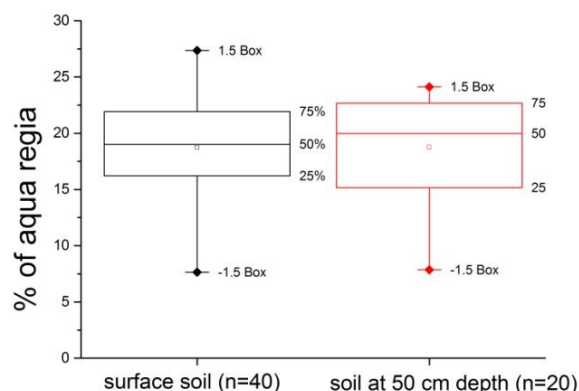
Interestingly, a wide variation in the Cu concentrations is observed for the soils of the studied vineyards since the values are fluctuating from 33.1 to 291 mg kg<sup>-1</sup> (Table 1). In general, differences in the Cu concentrations among vineyards from the same region could be ascribed to variation in soil properties, topography and Cu application history. In the present study and in line with findings by Vavoulidou *et al.* (2005) and Deluisa *et al.* (1996), no significant correlation was found between the analysed soil parameters and the Cu concentrations, probably due to the relatively narrow range of values of the soil properties. Only a weak positive correlation was found between the organic content and the Cu total concentrations ( $r=0.37$ ,  $p=0.02$ ) indicative though of Cu-humic complexes as a result of the long history of Cu application and the humification processes. The dispersion of Cu-enriched soil particles via runoff and soil erosion could be an influencing factor for the observed variation in Cu concentrations; nonetheless, the majority of the studied vineyards is exclusively located in the plain area (approximately 300 m a.s.l.), and as a result the topography can be reasonably regarded to have a relatively minor influence on the extent of Cu accumulation. According to Mackie *et al.* (2012) the effect of topography on Cu accumulation in vineyard soil is rather unclear and probably is masked when other determinants are taken into consideration. We speculate that the vineyard age is the main parameter that determines Cu concentrations since some vineyards in the Nemea region have been cultivated for more than 100 years. Previous studies have demonstrated that as vineyards age they are subjected to prolonged periods of fungicide treatments resulting to a more pronounced Cu accumulation (Romić *et al.*, 2014; Rusjan *et al.*, 2007). The Bordeaux mixture [CuSO<sub>4</sub>+Ca(OH)<sub>2</sub>] has been widely used since the end of 19<sup>th</sup> century in various countries, for example in Croatia (Romić *et al.*, 2014) and in France (McBride *et al.*, 1981), but we have not been able to verify the exact time period that this material was first used in the area of Nemea.

Copper accumulates mainly in the topsoil following fungicidal sprays application because of its complexation with organic matter and affinity for Fe oxyhydroxides and decreases with depth (Komárek *et al.*, 2010); this pattern was also seen for almost half of the vineyard plots where a soil sample from a deeper soil horizon was obtained (Fig. 2a). However and contrary to the general concession that Cu eventually returns to natural levels at a maximum depth of -50 cm, the studied vineyards at this particular depth exhibited a median soil Cu concentration of 96.1 mg kg<sup>-1</sup> ( $n=20$ ) which is considerably higher than the background concentrations (Kelepertzis, 2014). Furthermore, in some cases Cu concentrations remain either the same or even increase down to 50 cm depth (Fig. 2a) pointing out to a specific process that results to Cu migration deeper into the soil profile.

Considering the alkaline pH of the investigated vineyard soils that does not favor the mobility of Cu, leaching should be excluded. Moreover, the predominance of clay fraction provides plethora of cation exchange sites for Cu adsorption and neither promotes the opening of transport routes of water for the removal of minerals and soil particles nor shows any cracks during the dry summer period due to minor water application in vines; as a result, the process of Cu eluviation in these soils should be restricted. Soil disturbance by tillage should be the key reason for the redistribution of Cu-enriched particles within the profile (Romić *et al.*, 2014) resulting to the significant accumulated Cu levels at 50 cm depth.

In the present study, we applied the DTPA chemical extraction for assessing the Cu availability. This chemical reagent uses a strong ligand that allows Cu desorption from the solid matrix through the mechanism of complexation. It was originally developed for the determination of Cu, Zn, Fe and Mn deficiency in neutral and alkaline soils (Lindsay and Norvell, 1978), and is has been frequently adopted for assessing Cu availability in vineyard soils (examples given by Brun *et al.*, 1998; Deluisa *et al.*, 1996 and Romić *et al.*, 2014). The average available Cu concentrations were almost identical for both the surface and deep soil samples (18.7 and 22.6 mg kg<sup>-1</sup>, respectively) and were quite similar with the mean value of 15.4 mg kg<sup>-1</sup> reported by Vavoulidou *et al.* (2005) for vineyard soils from the same area. The availability ratios that were calculated by dividing the Cu amounts obtained by the DTPA extraction over the total soil content, were almost the same for both the surface soils and the soils collected at 50 cm depth (Fig. 3). Such data further support the evidence for soil disturbance in Nemea area, as result of vineyard soil tilling by the local landowners. In the case of vineyard soils from Czech Republic (Komárek *et al.*, 2008) and Italy (Deluisa *et al.*, 2006), the Cu availability, both in terms of the actual values and relative to the total content, was significantly higher in the superficial soil layer, perhaps as a result of specific local cultivation tillage implements that are restricted to the surface soil. In addition, an excellent positive correlation coefficient ( $r=0.94$ ,  $p=0.000$ ) was found between the DTPA-extractable Cu and the respective total Cu content for the investigated soil samples from Nemea, in agreement with findings by Deluisa *et al.* (1996) and Romić *et al.* (2014). Linear regression analysis demonstrated that the available Cu concentrations can be accurately predicted by the corresponding total content ( $Cu_{DTPA} = -2.82 + 0.220Cu_{total}$ ,  $R^2_{adj} = 87.4\%$ ). Considering that the available Cu is part of the total Cu concentration, this strong correlation indicates a common source of both metal forms, which is the intensive fungicide application in the vineyards of Nemea area.

Copper is an essential micronutrient for plants and is involved in several metabolic processes. Despite its essentiality, excess Cu in soil has phytotoxic effects, including plant growth retardation and leaf chlorosis (Kabata Pendias and Pendias, 2001). The range of Cu concentrations determined in this study is not expected to cause phytotoxicity considering both monocotyledon and dicotyledon plants (toxicity thresholds are 18-537 mg kg<sup>-1</sup> and 36-698 mg kg<sup>-1</sup>, respectively) (Oorts, 2013). In addition and based on the threshold of human dietary toxicity for Cu (10 mg kg<sup>-1</sup>), the critical concentrations of total and available (DTPA) Cu in the soil were found to be 430 and 269 mg kg<sup>-1</sup> for pakchoi, 608 and 313 mg kg<sup>-1</sup> for celery, and 835 and 339 mg kg<sup>-1</sup> for Chinese cabbage, respectively (Yang *et al.*, 2002). The total and DTPA-extracted Cu concentrations as well as the alkaline pH values that determined in the investigated soils do not support potential phytotoxicity problems in the agricultural region of Nemea.



**Figure 3 - Ratio of the Cu concentrations extracted by the DTPA method relative to the total soil content in the vineyard soils from Nemea.**

## 5. Conclusions

Similar to agricultural areas around the world where Cu-based fungicides have been extensively applied, the calcareous vineyard soils from Nemea display elevated Cu concentrations that are much higher than the background levels. A wide range of Cu concentrations has been determined and the extent of Cu accumulation could be linked to the age of individual vineyard and the associated Cu application history, but such information cannot be easily acquired. Copper has been migrated through the soil profile, at least to a 50 cm depth, and this pattern is attributed to soil disturbance by tillage agricultural practices. As a consequence, the availability of Cu, as was evaluated by the DTPA extraction, does not demonstrate differences between the surface and the deeper soil samples. The DTPA-extracted Cu concentrations correlate significantly with the respective Cu total content in the soil indicating a common source of both metal forms in the soils of the area. Although the alkaline nature of the studied vineyard soils is certainly a limiting factor of plant uptake, the application rates of Cu-based fungicidal sprays should be reduced in order Cu accumulation does not reach levels that can inhibit plant growth.

## 6. Acknowledgments

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