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Copper and zinc thresholds in EU topsoils: Insights from LUCAS and literature datasets

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ABSTRACT

Copper (Cu) and zinc (Zn) are essential micronutrients, critical for both crop growth and human health, yet imbalances can result in deficiency or toxicity. Assessments based on total metal concentrations miss key insights into metal bioavailability and mobility. This study addresses the existing gap in data and knowledge on readily available Cu and Zn in EU agricultural soils. We used a subset of the Land Use and Coverage Area frame Survey (LUCAS) topsoil database in 2015 to map distributions of available Cu and Zn in agricultural soils across the EU27 + UK using the electro-ultrafiltration (EUF) technique. The EUF results made it possible to identify regions where agricultural soils may require Cu and Zn fertilization or face toxicity risks. Using existing thresholds from the Austrian fertilization guideline, around one-third (32%) of the soils were deficient in Cu, mainly in Northern and Eastern Europe, while Zn deficiency affected 14% of samples, notably in Spain, Cyprus, and Finland. Only a small proportion (1.5%) of samples exceeded the estimated Cu toxicity threshold. Overall, our findings indicate that continental regions consistently exhibit higher available Cu and Zn levels than Northern Europe, where igneous parent materials and less weathered glacial soils prevail. In addition, in Southern and Eastern Europe and inland regions of Spain, where calcareous soils are common, low Zn availability was observed. While natural factors largely explain these regional differences, local anthropogenic activities further contribute to elevated metal concentrations. Our results contribute to the monitoring of soil Cu and Zn beyond total concentrations, helping guide fertilization practices that support optimal crop nutrition and ensure safe and nutritious food.

1. Introduction

Copper (Cu) and zinc (Zn) are essential micronutrients for both human health and plant development. In humans, Cu is important for skin and tissue health and red blood cell formation, and Zn is crucial for immune system and brain function (Osredkar and Sustar, 2011). Zn deficiency is associated with impaired growth, weakened immune

function, and heightened infection risk (EFSA, 2014). An estimated 17.3% of the world's population is at risk of inadequate Zn intake, often due to diets low in animal products and high in phytates, which inhibit zinc absorption (Wessells and Brown, 2012; Kumssa et al., 2015). In Europe, the prevalence of human Zn deficiency has varied over time and across regions, with earlier estimates ranging from 11 to 16% in 2004 (Sanghvi et al., 2007) and 3% in 2011 (Kumssa et al., 2015). More recent

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assessments indicate an average prevalence of around 9% (Ali et al., 2021), although specific demographic groups, such as young children in high-income Western European countries, can exhibit higher rates of deficiency (~31%; Vreugdenhil et al., 2021), highlighting regional and population-level heterogeneity. Copper deficiency in humans is less prevalent but can lead to serious health issues such as anaemia and neutropenia (Oliver, 1997). In the EU, due to the lack of precise biomarkers for Cu requirements, current recommendations are based on average intake levels in European populations, where no overt deficiency is evident (EFSA, 2015).

Conversely, Cu and Zn can be toxic to humans at a certain exposure level. Copper excess may increase the risk of liver diseases, neurological effects and the Alzheimer disease (Uriu-Adams and Keen, 2005). Chronic high Zn intake can lead to severe neurological disorders due to physiological Cu deficiency (Hedera et al., 2009; EFSA, 2014). Although the European Commission sets maximum levels for certain metals in food products, it has not defined limits for Cu and Zn. However, the European Food Safety Authority (EFSA) specified the required daily intake as 0.07 mg Cu kg⁻¹ body weight (EFSA, 2023) and 7.5 to 16.3 mg Zn day⁻¹ for adults (EFSA, 2014).

In agricultural systems, deficiencies of these micronutrients can limit crop productivity and food nutritional quality, whereas excessive soluble concentrations may result in toxicity for plants and humans. Despite the natural occurrence of these metals, anthropogenic sources significantly contribute to their elevated levels in soil. For Cu, key sources include copper-containing fungicides used in agriculture, particularly in vineyards and orchards, sewage sludge application, and industrial activities (Ballabio et al., 2018; Weil and Brady, 2017). For Zn, significant inputs derive from mining, smelters, industrial activities, and manure application in agricultural systems (Van Eynde et al., 2023a; Weil and Brady, 2017; Yunta et al., 2024).

Several studies include maps of Cu and Zn distributions in soils at the EU level with different resolutions (Albanese et al., 2015; Ballabio et al., 2018; Van Eynde et al., 2023a; Tóth et al., 2016a; Tóth et al., 2016b). These studies primarily report total concentrations of metals in soils, often determined using an aqua regia digestion, to assess their distribution and potential impact on agricultural systems. However, only a few studies at the EU level focus on metal mobility and bioavailability (Mann et al., 2015), which are critical for understanding deficiency risks, contamination, and broader ecological and health effects (Nolan et al., 2003). Additionally, threshold values used in regional and international contexts for assessing soil contamination and remediation requirement are based on total concentrations rather than bioavailable forms (Carlon et al., 2008; MEF, 2007). There is no widely established threshold value for soluble concentrations of metals in agricultural soils. The Geochemical Mapping of Agricultural Soils (GEMAS) project analysed 2,108 soil samples from the plough layer of agricultural land across Europe using the Mobile Metal Ion (MMI) extraction technique, which involves a mild organic-inorganic acid extractant, to assess spatial distribution of soluble Cu and Zn concentrations in the EU agricultural topsoils. This project suggested “normal” ranges for mobile forms of Cu (0.9–2 mg kg⁻¹) and Zn (0.45–1.3 mg kg⁻¹) as measured by MMI (Mann et al., 2015). However, to our knowledge, aside from GEMAS, no other comprehensive EU-wide dataset on soluble Cu and Zn concentrations in agricultural soils is currently available. Our study addresses this gap by providing EU-wide data on readily available Cu and Zn using the electro-ultrafiltration (EUF) technique and producing EU-wide maps of these metals through transfer functions.

The EUF method has been used in several countries, including Germany, Austria, Poland, Hungary, Croatia, and Moldova (Németh 1979, 1982; Akinrinde et al., 2006; Baumgarten, 2023) for measuring soil nutrient availability. Currently, the assessment of plant-available nutrient fractions using EUF is officially recognized in Germany (VDLUFA, 1997; VDLUFA, 2002) and Austria (Baumgarten, 2023) for all agriculturally relevant nutrients including Cu and Zn, with ca. 60,000 analyses being done annually in these two countries mostly for sugar

beet growers. The EUF method applies an electric field to a water-based soil suspension while temperature and voltage are varied, causing ions to migrate toward the cathode or anode, where they are separated through ultra-membrane filters (Németh, 1979, 1982). EUF measures the intensity, quantity, and rate of nutrient release, whereas traditional chemical extractions only give a static equilibrium snapshot (Nair, 2019; Sager, 2021). Unlike conventional single-step chemical extractions (e.g., CaCl₂, DTPA, or EDTA) it continuously removes dissolved ions, preventing back-reactions and interference, so the measured fraction more accurately reflects plant-available nutrients (Nair, 2019). The standard EUF method uses two steps (30 min at 20 °C and 5 min at 80 °C) to assess plant-available nutrients. However, the solubility of trace elements in water is low and some elements tend to precipitate when passing the electrodes. Adding diethylenetriaminepentaacetic acid (DTPA) in a third fraction enables extraction of these elements with similar or better results for Cu and Zn compared with CaCl₂/DTPA, while avoiding the overestimation observed with EDTA (Horn, 2006). Although the EUF method does not directly measure mechanistic bioavailability, it yields an operationally defined, labile extractable fraction indicative of short-term nutrient availability, which has been linked to plant nutrient uptake in pot and field studies (Akinrinde et al., 2006; Jelecevic et al., 2021; Sager, 2021).

The aim of our study was to address the existing data and knowledge gap regarding readily available Cu and Zn concentrations in EU topsoils using the EUF method, and to provide guidance on how to interpret such concentrations. More specifically, this study aimed to: (1) Improve the understanding of factors affecting soil EUF-extracted Cu and Zn concentrations by developing transfer functions, (2) Use the transfer functions to develop maps that model the distribution of dissolved Cu and Zn concentrations across the EU27 + UK using the EUF technique and (3) Determine possible threshold values for interpreting EUF-extracted Cu and Zn by using the Austrian fertilization guidelines, where EUF is an officially applied method, and by converting literature-based DTPA thresholds to EUF-extractable concentrations.

2. Materials and methods

2.1. LUCAS soil dataset in agricultural soils

The Land Use/Land Cover Area Frame Survey (LUCAS) is a project that monitors variations in land use and land cover in the EU. LUCAS also includes a soil module, which analysed around 20,000 soil samples for physical, chemical, and biological properties in 2009/2012, 2015 and 2018 (Orgiazzi et al., 2018). In 2009, LUCAS soil samples were also analysed for total element concentrations using aqua regia. For this study, the solubility of Cu and Zn in agricultural fields in the EU was determined by analysing a sub-set of soil samples from croplands and grasslands of the LUCAS 2015 topsoil archive. In 2015, the survey included 21,859 unique topsoil samples across EU27 + UK, of which 8973 were located in croplands and 4753 in grasslands (Jones et al., 2020). The selection of soil samples for the present study was based on proportional allocation, aiming to evaluate soil phosphorus (P) status across the EU. The selection criteria included soil pH, texture, soil organic carbon (SOC) and calcium carbonate (CaCO₃) contents, and soluble P based on the Olsen-P method (Olsen, 1954). Soil samples of the LUCAS 2015 subset originated from all EU27 + UK countries, comprising 1658 sampling points in croplands, including non-permanent crops (e.g., cereals, root crops, non-permanent industrial crops, dry pulses, vegetables and flowers, fodder crops) and permanent crops (e.g., fruit trees, other permanent crops), as well as agricultural bare lands with crop residue during the year of soil sampling (Table S1). Additionally, 690 soil samples originated from grasslands. The locations of all samples are shown in Fig. S1.

2.2. Soil analysis

Basic soil properties including soil texture, soil pH, electrical conductivity (EC), SOC, CaCO_3 , and total Cu and Zn concentrations (determined using aqua regia as an extracting agent), were previously determined as part of the standard data for LUCAS samples (Jones et al., 2020). For this study, Cu and Zn were extracted by the EUF method on a subset of 2348 LUCAS 2015 soil samples selected from croplands and grasslands across EU27 + UK. Sample selection aimed to ensure proportional representation across soil types and included quality control measures to maintain data reliability. For the EUF extraction, 5.0 g of samples were suspended in 50 mL of deionised water (1:10 soil:water), and the following fractionation procedure was applied: the suspension was extracted in the EUF chamber at 200 V and up to 15 mA at 20° C for 30 min as the 1st fraction. This was followed by a 2nd fraction, extracted for 5 min at 400 V and up to 150 mA at 80° C (Németh 1979, Németh 1982, VDLUFA, 1997; VDLUFA, 2002). EUF-soluble Cu and Zn (EUF-Cu, EUF-Zn) were determined in the 3rd fraction, for which the suspension was extracted another time for further 5 min at 400 V, max. 150 mA at 80° C by adding 0.002 M diethylenetriaminepentaacetic acid (DTPA) to the extraction cell (Horn, 2006). The concentrations of Cu and Zn in the extractant were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES; ICP Spectro Flame-Modula SOP). The limit of quantification (LOQ) of the EUF method was 8.58 $\mu\text{g L}^{-1}$ Cu and 13.99 $\mu\text{g L}^{-1}$ Zn. Converted to soil-based values these correspond to 86–240 $\mu\text{g Cu kg}^{-1}$ and 60–392 $\mu\text{g Zn kg}^{-1}$. These soil-based LOQs are presented as ranges, because the extract volume collected by EUF depends on soil properties, such as texture, and varied between ~50 and ~140 mL in the samples used in this study.

2.3. EUF thresholds

To support the interpretation of EU-scale data on concentrations of EUF-Cu and EUF-Zn, two complementary approaches were used: (i) sufficiency ranges defined by the Austrian fertilization guidelines (Baumgarten, 2023), and (ii) literature-based toxicity thresholds derived from DTPA-extractable concentrations and converted to EUF values using correlations (Table S2 and Fig. S2).

To estimate threshold values, we conducted a literature review to compile studies that reported crop Cu and Zn concentrations alongside DTPA-extractable soil concentrations. Data were extracted from both field trials and controlled pot experiments and included a variety of crop species, such as cereals, legumes, vegetables, and forage crops. In total, 17 studies ($n = 176$ for Cu and $n = 194$ for Zn) met the inclusion criteria (Table S2, Fig. S2). In addition, using data from Bodengesundheitsdienst GmbH in Ochsenfurt, Germany, a strong correlation between DTPA- and EUF-extracted Cu and Zn ($r > 0.95$, $n = 114$; Fig. S2) was observed, allowing conversion of DTPA-based thresholds to approximate EUF equivalents.

Moreover, samples were collected from a total of 11 field trials conducted either in 2021 or 2022 across a climate gradient in Europe (Table S3, Fig. S1) to determine total soil and grain Cu and Zn concentrations and EUF-Cu and EUF-Zn. Grain samples from maize, spring barley, spring wheat, and winter wheat were analysed from the treatment that received the reference fertilizers according to local recommendations. Total soil Cu and Zn concentrations were measured using ICP-MS (Agilent Technologies 7800) after microwave digestion with aqua regia. Soluble Cu and Zn concentrations in soils were analysed using the EUF method as described above. Grain samples were milled and analysed for Cu and Zn concentrations by ICP-OES (Perkin Elmer Optima 8300) after HNO_3 digestion in a microwave. Detailed descriptions of trial sites propertice, management and sampling, and analytical procedures were provided in Müller et al. (2024) and Frick et al. (2025).

2.4. Exploratory analysis and mapping of Cu and Zn in soils

Statistical analyses for the LUCAS subset and field trials were conducted using RStudio Server 2023.12.1 Build 402. No outliers were detected based on Cook's distance with a threshold of 1 for EUF-Cu and EUF-Zn values. Data were checked for normality using the Shapiro-Wilk test and Q-Q plots, and for homogeneity of variances using Levene's test and residuals vs. fitted plots. Linear correlations among variables in the field trials were analysed using Pearson's correlation.

Mapping EUF-Cu and EUF-Zn in EU agricultural soil involved the following steps: (1) collecting the relevant covariates for modelling the partitioning of soil total Cu and Zn content into EUF extractable content (2) deriving a transfer function based on these covariates and (3) applying the transfer function using existing maps of the covariates across EU agricultural land.

Transfer functions (Groenberg et al., 2010; Römkens et al., 2004) were developed to map EUF-Cu and EUF-Zn, predicting EUF concentrations based on total soil metal content and other relevant covariates (detailed below). To do so, the dataset containing EUF measurements from the 2015 LUCAS soil samples was merged with the 2009 LUCAS database containing total Cu and Zn concentrations (Ballabio et al., 2018; Van Eynde et al., 2023a). Samples with total and EUF Cu and Zn concentrations below the LOQ, missing covariates data, or higher EUF extractable concentrations than total content were excluded. This led to a dataset of 1947 and 1958 observations for Cu and Zn, respectively. Covariates used in the transfer functions included key soil properties and climatic variables known to influence Cu and Zn partitioning. Soil data were sourced from the LUCAS 2015 topsoil database and included: clay and sand content (affecting metal adsorption and fixation via clay minerals and metal (hydr)oxides (Groenberg et al., 2010; Van Eynde et al., 2022a), SOC (the primary adsorption surface for Cu and Zn in most pH ranges; Dijkstra et al., 2009; Van Eynde et al., 2022b), pH measured in 0.01 M CaCl_2 (influencing charge-dependent adsorption and mineral fixation; Buekers et al., 2007; Dijkstra et al., 2009), and CaCO_3 content (which buffers acidity and indirectly affects metal mobility). Soil type was obtained from the European Soil Data Centre based on the World Reference Base (WRB) classification (Panagos et al., 2022). Additionally, climatic covariates, mean annual temperature (BIO1) and precipitation (BIO12), were extracted from WORLDCLIM rasters (Noce et al., 2020), as these were found previously to affect the solubility and distribution of Cu and Zn across Europe (Moreno-Jiménez et al., 2022).

Transfer functions were derived using Generalized Additive Model (GAM). GAMs can handle different distributions for the dependent variable, the EUF extractable metal content, as well as non-linear effects of the independent variables through smooth functions (Wood, 2017). Initial GAM models were fitted using the *mgcv* package in R (Wood, 2017), using 80% of the data. We tested log and Box-Cox transformations of the dependent variable, as well as scaled t and Gaussian distributions, and tested effects of both the individual covariates and their interactions. Model performance was assessed by qualitative inspection of residuals using worm plots (detrended QQ plots). Due to unsatisfactory fits, we proceeded with the Generalized Additive Model for Location, Shape and Scale (GAMLSS) using the *gamlss* package (Stasinopoulos et al., 2006). The optimal distribution was selected based on the lowest Akaike information criterion (AIC) when fitting the dependent variable without covariates, using the *fitDist* function. Initially, we did not include additional parameters for sigma, nu and tau parameters.

Model performance was validated on the remaining 20% of the data. To assess the effect of each covariate on the EUF-Cu and EUF-Zn concentrations, accumulated local effects (ALE) plots were constructed. These plots show the magnitude and direction of the effect of one variable on the predicted outcome while accounting for the influence of the other variables (Molnar, 2022).

The final model was used to upscale predicted EUF-Cu and EUF-Zn

across EU27 + UK agricultural land, using existing maps of the covariates (Ballabio et al., 2018, 2019; Noce et al., 2020; Van Eynde et al., 2023a). The area of agricultural land was defined based on the Corine Land Cover (EEA, 2018). Although applying the model with map-derived covariates may introduce additional uncertainty due to their estimated nature, the model was calibrated using actual measurements to ensure the development of robust transfer functions for future applications. The map was validated using the independent set of data points that was also used to validate the GAMLSS model, and the Root Mean Square Error (RMSE) was calculated.

3. Results and discussion

3.1. Sufficiency and toxicity thresholds for Cu and Zn

According to the Austrian fertilization guidelines, the sufficient ranges for Cu and Zn in agricultural soils, i.e., concentrations at which fertilization is not required, are 1–2.5 mg EUF-Cu kg⁻¹ and 0.5–2.5 mg EUF-Zn kg⁻¹, respectively. Values below these ranges indicate a potential need for fertilization, while values above the upper limit may suggest excess availability, though not necessarily toxicity. Comparable sufficiency interpretations using the DTPA extraction method showed similar trends. For wheat grown on prairie soils, DTPA-Cu > 1.2 mg kg⁻¹ is considered sufficient, with negligible yield response to Cu fertilization (Karamanos et al., 2003). Other studies report adequate DTPA-Cu levels of 0.5 mg kg⁻¹ for soybean, 1.0 mg kg⁻¹ for upland rice, 1.5 mg kg⁻¹ for corn, and up to 8.5 mg kg⁻¹ for wheat in highly weathered Oxisols (Fageria, 2000), highlighting the influence of soil type and crop species. Using correlations between DTPA and EUF, these thresholds corresponded approximately to 0.4–6.3 mg kg⁻¹ EUF-Cu. Similarly for Zn, the DTPA values for wheat ranged from a critical threshold of 0.5 mg kg⁻¹ in calcareous soils (Cakmak et al., 1996) to a sufficiency threshold of 1 mg kg⁻¹ in non-calcareous Mollisols (Martínez Cuesta et al., 2021), while for maize the critical level was 0.8 mg kg⁻¹ (Lindsay and Norvell, 1978), increasing to 1.3 mg kg⁻¹ (Barbieri et al., 2017). Also, Australian guidelines suggested DTPA-Zn sufficiency of 0.5–0.8 mg kg⁻¹ for cereals (Reuter and Robinson, 1997; Zou et al., 2008). Using correlations between DTPA and EUF, these thresholds corresponded approximately to 0.3–0.8 mg kg⁻¹ EUF-Zn, matching the Austrian sufficiency range for Zn.

Copper toxicity thresholds vary among crop species, with tolerance levels reported as 10 mg Cu kg⁻¹ dry weight (DW) in common bean (Fageria, 2000), 20 mg Cu kg⁻¹ DW in maize and rice (Borkert et al., 1998; Mocquot et al., 1996), and up to 30 mg Cu kg⁻¹ DW as upper critical concentration in aerial parts of food crops (Adrees et al., 2015). The maximum permissible Cu content in complete feed for dairy cow is 30 mg Cu kg⁻¹ (EFSA, 2016), whereas the EU Commission has not set a maximum Cu level for food (European Commission, 2023). Our analysis of previous studies estimated that when soil DTPA-Cu exceeds 20 mg kg⁻¹, plant Cu concentrations can occasionally surpass the critical toxicity threshold of 30 mg Cu kg⁻¹ DW (Fig. S2). This finding aligns with Fageria (2000), who reported toxic DTPA-Cu thresholds in soil as 18 mg kg⁻¹ for common bean, 28 mg kg⁻¹ for upland rice and wheat, and 32 mg kg⁻¹ for maize. Additionally, correlation analysis of DTPA-Cu and EUF-Cu estimated that a DTPA-Cu toxicity threshold of 20 mg kg⁻¹ corresponds to an EUF-Cu concentration of approximately 15 mg kg⁻¹ (Fig. S2).

Zinc toxicity in crops is far less common than Zn deficiency (Broadley et al., 2007), and the EU Commission has not set a Zn concentration threshold for food. Crops vary in their tolerance to Zn, with leaf Zn concentrations considered potentially toxic ranging from about 100 mg kg⁻¹ DW to over 300 mg kg⁻¹ DW (Broadley et al., 2007; Marschner, 1995). In animal feed, the maximum permissible Zn content for dairy cows is 100 mg kg⁻¹ (EFSA, 2014). Our analysis of previous studies estimated that soil DTPA-Zn above 30 mg kg⁻¹, can occasionally lead to plant Zn concentrations exceeding 100 mg kg⁻¹ DW (Table S2, Fig. S2). This finding fits within the range of previous research, which found a

critical toxicity level of 14.4 mg kg⁻¹ DTPA-Zn in forage grass (Nardis et al., 2018) and toxic levels causing 10% yield loss at 25 mg kg⁻¹ in beans and 60 mg kg⁻¹ in maize (Fageria, 2000). A strong correlation ($r = 0.96$, $n = 114$) between soil DTPA-Zn and EUF-Zn (Fig. S2) resulted in an EUF-Zn toxicity threshold estimate of 18 mg kg⁻¹. EUF is not standardized across all EU countries, and the toxicity thresholds presented here are based on literature and on conversions from DTPA values rather than direct plant-toxicity experiments. Thus, while useful as a starting point, these thresholds should not be regarded as definitive and must be interpreted with caution. Future studies should refine these estimates through additional plant uptake experiments.

According to field trials, EUF-Cu and EUF-Zn values did not correlate with total soil concentrations (Fig. S3). For example, in the Spanish trial, total Zn exceeded 60 mg kg⁻¹ while EUF-Zn was only 0.347 mg kg⁻¹ (Table 1), well below sufficiency thresholds, implying that total soil metal concentrations might not be an accurate proxy for plant-available micronutrients. Similar discrepancies were observed for Cu across sites. Grain Cu and Zn concentrations were generally adequate (Table 1), yet correlations with EUF or total soil metals were weak. Only Zn showed a positive correlation between its total concentration in soil and its concentration in grains ($r = 0.62$). This aligns with previous studies showing that correlations between total (Nan et al., 2002; Chojnacka et al., 2005) or labile (Asensio et al., 2018; Karami et al., 2009; Singh and Myhr, 1998) metal contents in soil and their concentrations in grains are often low. Genotype effects (Breure et al., 2023; Zhao et al., 2009), crop-specific partitioning, and plant strategies (Chandrakar et al., 2020; Xu et al., 2022) regulate metal uptake further weaken these correlations. As a consequence, our experimental set-up, comprising 11 locations with varying soil properties (Table S3), four different crops (Table 1), and different varieties within the same crop type, may hamper finding strong correlations between grain and soil concentrations. In addition, soil micronutrient levels are generally a better indicator of total crop nutrient uptake than grain concentrations, which are influenced by factors such as crop species and dilution effects from high biomass (Van Eynde et al., 2023b). However, in northern regions like Finland, lower yields can make total nutrient uptake appear small even when soil micronutrient levels are adequate. These observations suggest that more controlled and homogeneous experimental conditions could help clarify the relationships between soil and plant micronutrient status, while detailed studies on crop-specific metal partitioning and plant uptake mechanisms may improve interpretation of EUF-based assessments.

3.2. Distribution of EUF-Cu in agricultural soils in the EU27 + UK

Soluble Cu, as determined by EUF extraction, ranged from 0.09 to 98 mg kg⁻¹, with a median concentration of 1.4 mg kg⁻¹ across all soil samples (Table S4 and Fig. 1). The median EUF-Cu in the LUCAS 2015 subset is only 10% of the median total Cu concentration (aqua regia extraction; Table S4). About 32% of samples have EUF-Cu values below 1 mg kg⁻¹, suggesting a need for further assessment of Cu fertilization according to the Austrian fertilization guidelines using the EUF extraction technique (Baumgarten, 2023). On the other side, 24% of samples had EUF-Cu values above 2.5 mg kg⁻¹ (Fig. 1), exceeding the crop sufficiency range of 1–2.5 mg Cu kg⁻¹ (Baumgarten, 2023). Only 1.5% of samples had EUF-Cu values above 15 mg kg⁻¹ (Fig. 1), potentially indicating a risk of Cu toxicity in plants. A maximum EUF-extracted concentration of 98 mg kg⁻¹ was observed in areas with permanent crops, including olive groves, vineyards, nurseries, and permanent industrial crops (Fig. 1).

The GAMLSS model to predict EUF-Cu was found to follow a t family type 2 distribution (Rigby et al., 2019), with a Box-Cox transformation applied to achieve normality (Eq. (1)). The covariates were included only for the location parameter (μ) as this already resulted in a satisfactory worm plot (Fig. S4a):

$$Cu_{EUF}(\text{boxcoxtransformed}) \sim TF2(\mu, \sigma, \nu) \quad (1)$$

Table 1

Grain yield, Cu and Zn concentrations in harvested grain, along with total and EUF-extracted Cu and Zn in soil, measured in field trials in the LEX4BIO project.

Country	Austria	Denmark	Finland		France		Germany	Hungary		Spain	
Trial*	P-trial	N-trial	N-trial	P-trial	N-trial	P-trial	N-trial	N-trial	P-trial	N-trial	P-trial
Crop/Year	Maize 2021	S. barley 2021	S. wheat 2022	S. barley 2021	Maize 2021	Maize 2021	Maize 2021	W. wheat 2022	S. barley 2021	W. wheat 2021	W. wheat 2021
Grain yield (kg ha ⁻¹)	3934	6449	4247	3715	16,571	17,314	19,098 ^g	8036	4816	2877	2387
<i>Cu</i> (mg kg ⁻¹)											
Grain conc. (DW)	1.81	2.98	4.99	12.9	1.43	1.44	4.65	3.52	6.01	6.68	6.50
Critical grain conc.	1–3.8 ^a	2–3 ^b	1.2–1.5 ^c	2–3 ^b	1–3.8 ^a		1–3.8 ^a	1.2–1.5 ^c	2–3 ^b	1.2–1.5 ^c	
Soil EUF conc.	6.25	3.03	3.68	3.43	4.28	2.70	2.94	2.96	2.18	1.50	1.38
LUCAS EUF conc.	1.92	1.53		1.44		1.51	1.73		2.23		0.89
Soil total conc.	31.6	7.58	32.6	41.8	24.5	19.9	16.0	13.5	15.2	22.4	18.7
Limit value**	60	40		100		100	40		75		210
<i>Zn</i> (mg kg ⁻¹)											
Grain conc. (DW)	23.3	27.8	30.0	55.1	13.3	14.2	25.5	23.3	23.9	32.9	34.7
Critical grain conc.	18–30 ^d	10–15 ^e	15–20 ^f	10–15 ^e	18–30 ^d		18–30 ^d	15–20 ^f	10–15 ^e	15–20 ^f	
Soil EUF conc.	1.71	1.28	1.68	0.719	0.928	0.889	1.77	0.929	0.648	0.700	0.347
LUCAS EUF conc.	1.54	2.13		0.61		1.47	2.73		0.91		0.57
Soil total conc.	91.8	30.1	129	137	57.7	55.4	75.5	52.7	53.4	68.6	60.4
Limit value**	200	100		150		300	130		200		450

Critical grain conc.: Critical grain concentrations correspond to the nutrient level at which 95% of the maximum crop yield is achieved; LUCAS EUF conc.: Median values of EUF-extracted Cu and Zn measured in cropland soils from the LUCAS 2015 subset; Limit value: Maximum soil threshold limits set by each country's regulations; S. barley: Spring barley; S. wheat: Spring wheat; W. wheat: Winter wheat; DW: dry weight.

* P and N refers to the P and N response trials in LEX4BIO project. The N-trials in Spain faced drought, and in France, they were irrigated.

Yield (kg ha⁻¹) represents the aboveground biomass for silage maize in Germany.

** Yunta et al., (2024).

^a Cu normal range 1–5 mg kg⁻¹ (Anderson et al., 1991) and critical 3.8 mg kg⁻¹ in high yielding corn grains (Roberts and Rhee, 1993).

^b Alloway (2005); Kirchmann and Eskilsson (2010).

^c Brennan (1994); Reuter and Robinson (1997).

^d Rashid and Fox (1992); Roberts and Rhee (1993).

^e Kenbaev and Sade (2002); Reuter and Robinson (1997).

^f Zn critical 15 mg kg⁻¹ (Rashid and Fox, 1992; Reuter and Robinson, 1997); 20–24 mg kg⁻¹ in rainfed wheat (Rafique et al., 2006).

$$\mu = pb(\log Cu - AR) + pb(\log SOC) + pb(pH) + pb(CaCO_3) + pb(Clay) + pb(Bio1) + pb(Bio12) + WRB + LandUse$$

where the scale parameter (σ) was estimated at -0.6383 , and the shape parameter (ν) at 1.199 , to account for skewness and heteroscedasticity.

Among the tested covariates, total Cu showed the strongest association with EUF-Cu concentrations followed by SOC, pH, and CaCO₃ as the main drivers of EUF-Cu (Fig. 2). In the common pH range (4.2–7.5) in agricultural soils, SOC is the dominant adsorption surface for Cu (Wiersma et al., 2025). The model indicated that SOC levels higher than 20 g kg⁻¹ reduced EUF-Cu concentrations (Fig. 2). One explanation might be that in soils with high SOC, the Cu:SOC ratio becomes small, likely reflecting a shift in Cu speciation toward stronger organic complexes, and high-affinity binding sites, limiting its mobility and extractability. As soil pH increased from 3.4 to about 6.5, the EUF-Cu also increased. In very acidic soils, Cu is highly soluble but much of the dissolved Cu can be lost by leaching, which may result in lower concentrations measured with extractants. Additionally, in the EU dataset, the most acidic soils are often derived from igneous parent materials, which are naturally low in Cu. At pH values above neutral, however, EUF-Cu decreased, likely reflects adsorption onto negatively charged surfaces and precipitation as hydroxides or carbonates, reducing Cu mobility under conditions of high Cu concentration or abundant carbonate (Oorts, 2013). Similarly, increasing CaCO₃ content reduced EUF-Cu. Temperature, precipitation and clay content had only a

minor effect on the EUF-Cu content (Fig. 2).

The model described in Eq. (1) was used to extrapolate predictions of EUF-Cu across EU agricultural land (Fig. 4). The model's accuracy was assessed using the independent subset of LUCAS points, with an RMSE of 2.5 mg kg⁻¹ based on the median predicted value (Fig. 4). This RMSE is relatively high compared to the data (median value of 1.4 mg kg⁻¹). However, the validation of the model in terms of the worm plot (Fig. S4) gives confidence for interpretation of probabilities, as done in the following section.

Using our GAMLSS model we calculated the probability of EUF-Cu exceeding thresholds for toxicity or being below levels considered deficient. In line with the measurements, the probability of EUF-Cu exceeding 15 mg kg⁻¹ was nearly zero (results not shown), except in some regions of Northern Italy, Greece, and Southern France, where their median predicted values were higher (Fig. 4). On the contrary, the probability of EUF-Cu being lower than 1 mg kg⁻¹ can be high in some regions. Notably, in Lithuania, Estonia, Spain, Latvia, and Poland, the model predicts a high probability of EUF-Cu to be below 1 mg kg⁻¹ (Figs. 3 and 4). For these countries, more than 50% of the sampling points had EUF-Cu values below 1 mg kg⁻¹ (Table S5), and our results suggest a more detailed assessment of their Cu fertilization requirements.

In general, the model (results not shown) and the measurements (Table S4; Fig. 2) indicated that croplands in the EU have higher concentrations of EUF-Cu than grasslands, probably originating from

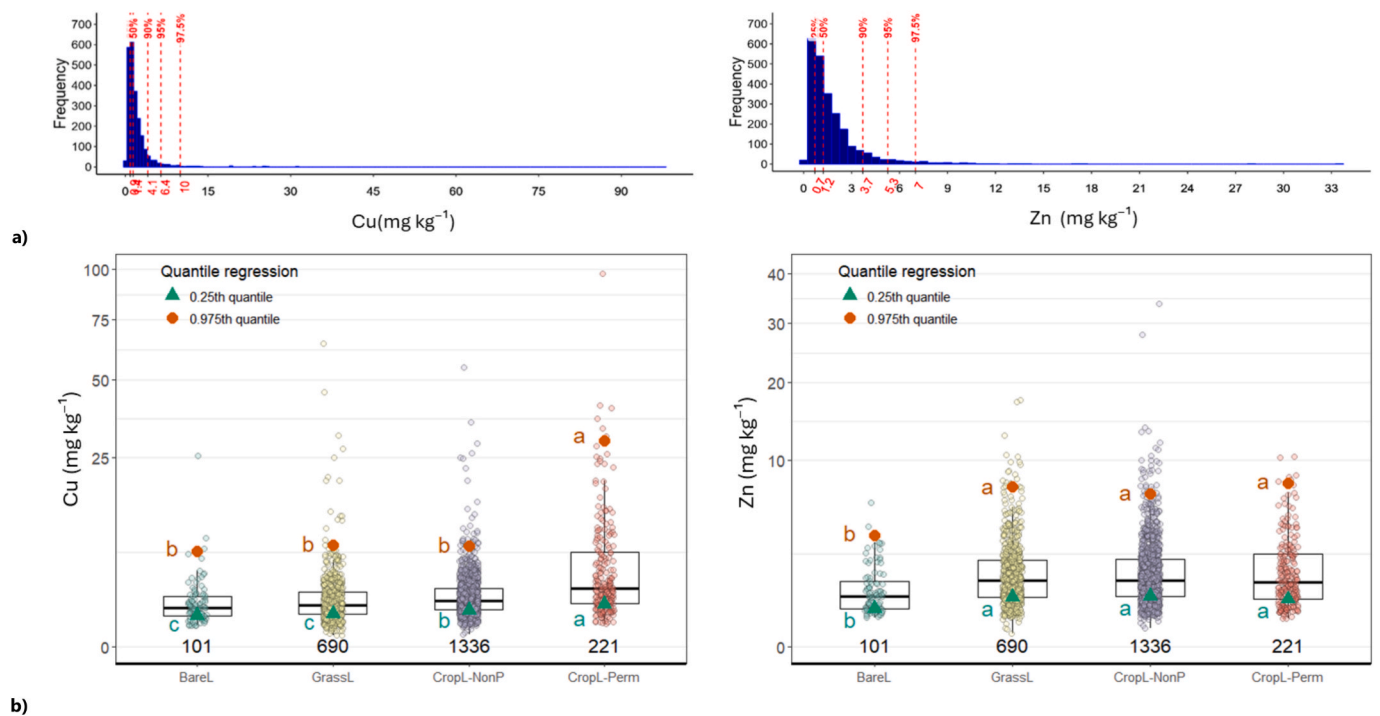


Fig. 1. Distribution of EUF-extractable copper (Cu) and zinc (Zn) concentrations (mg kg^{-1}) in soil samples from the LUCAS 2015 subset ($n = 2348$) across EU27 + UK. (a) Histograms showing overall concentration distributions with percentile values marked in red. (b) Boxplots grouped by land cover types: BareL (temporarily bare agricultural fields), GrassL (grassland), CropL-NonPerm (cropland with non-permanent crops), and CropL-Perm (cropland with permanent crops) illustrating variability across quantiles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

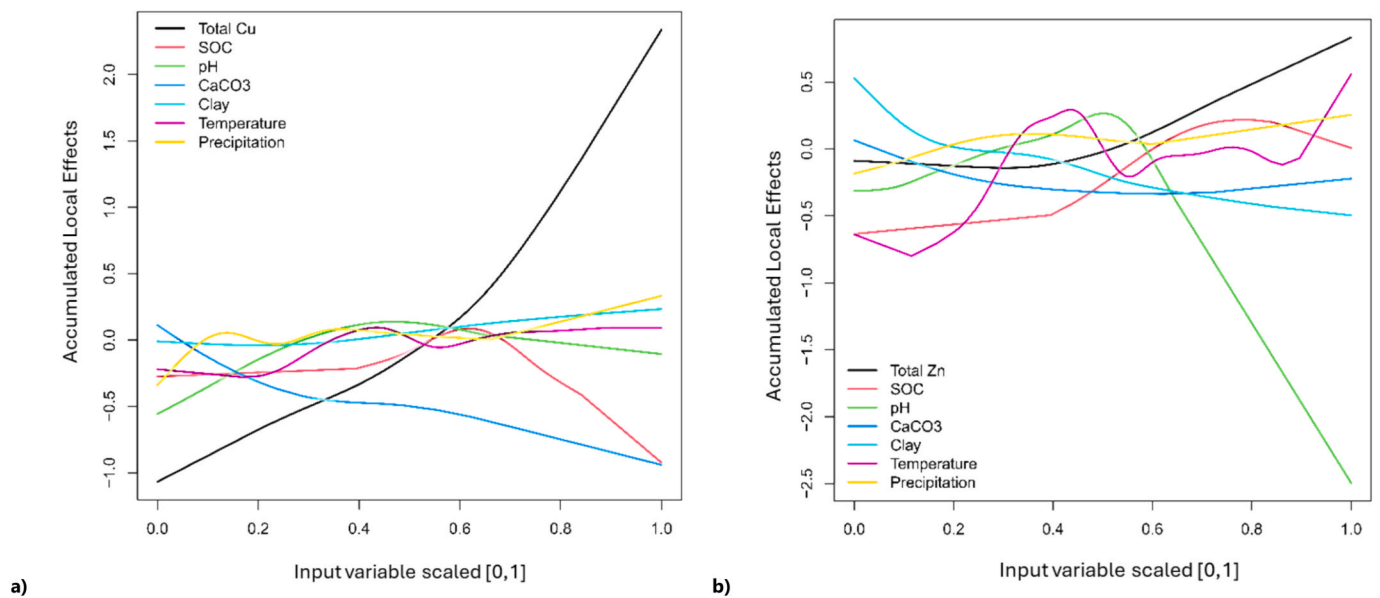


Fig. 2. The accumulated local effects (ALE) of the continuous covariates on the median predicted EUF-extractable Cu (a) and Zn (b) concentrations in soil. Covariates include total Cu and Zn concentrations, soil organic carbon (SOC), soil pH, calcium carbonate (CaCO_3) content, clay content, and climatic variables (mean annual temperature and precipitation). A larger vertical range of the ALE indicates a stronger influence of the covariate on the predicted concentration. Upward curves show that higher covariate values lead to increased predicted metal concentrations, while downward curves indicate the opposite.

anthropogenic inputs to croplands and a negative effect of high SOC from grasslands on EUF-Cu concentrations (Fig. 2). Previous analyses have also identified high Cu hotspots in croplands (average 18.91 mg kg^{-1}), with greater Cu levels observed in permanent crops like olive groves and vineyards (Ballabio et al., 2018). The highest total concentrations of Cu were reported in wet areas of the Mediterranean region due to frequent fungicide treatments in soils of permanent crops such as

vineyards and tree crops (Ballabio et al., 2018; Micó et al., 2006). Tamm et al. (2022) reported that olive, grapevine, and almond production account for nearly 80% of annual Cu use in organic farming across 12 surveyed EU countries. In our study, the map of EUF-Cu distribution indicates higher levels in soils of the Mediterranean and continental regions (Fig. 4), particularly in Italy. Italy had the highest proportion of sampling points with EUF-Cu exceeding the crop sufficiency (2.5 mg Cu

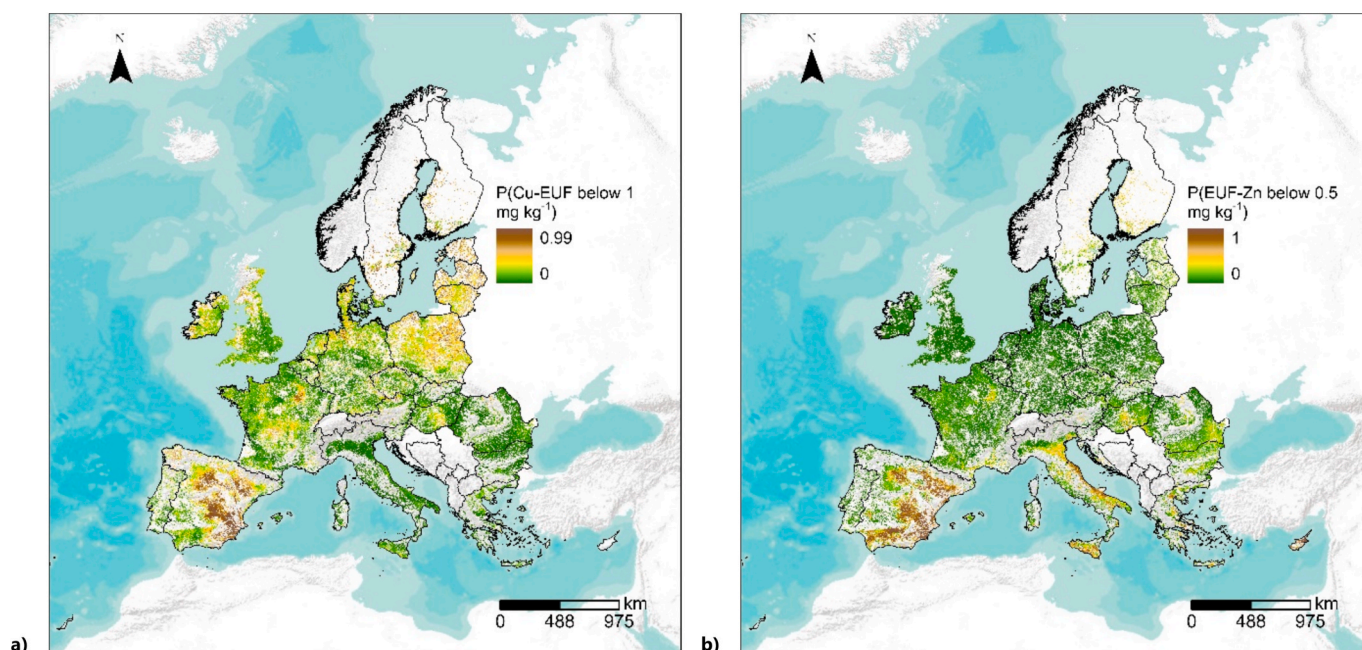


Fig. 3. The probability of EUF-extractable Cu being below 1 mg kg^{-1} (a) and EUF-extractable Zn below 0.5 mg kg^{-1} (b) across agricultural land in the EU27 + UK, based on GAMLSS models (Eqs. (1) and (2), respectively). Higher probabilities indicate a greater likelihood that soils in a given location have extractable metal concentrations below these thresholds.

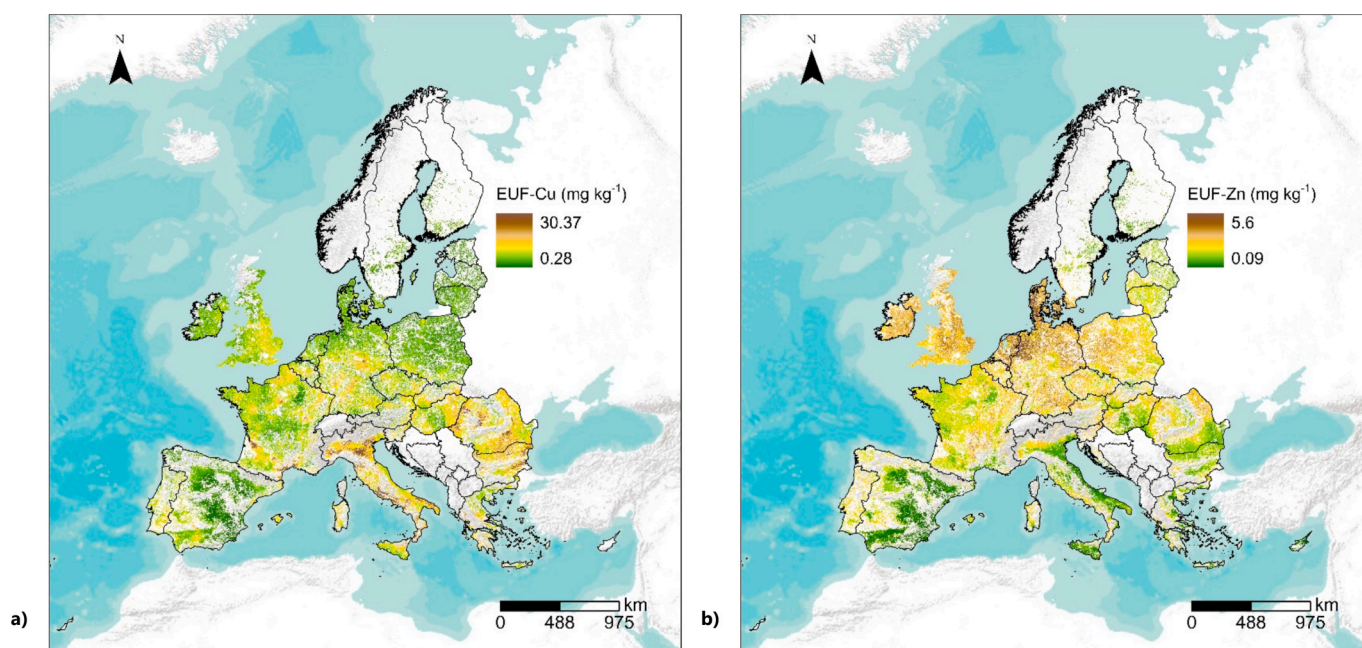


Fig. 4. The distribution of median predicted values of EUF extractable Cu (a) and Zn (b) in EU27 + UK agricultural land, based on GAMLSS models (Eqs. (1) and (2), respectively).

kg^{-1}), and 13 out of 36 points exceeding 15 mg Cu kg^{-1} . Consistent with these findings, Mann et al. (2015) also reported the highest soluble Cu concentrations in vineyard soils in France and Italy, where copper-based fungicides are used, with the highest soluble Cu level (62.1 mg kg^{-1} , measured by MMI) recorded in a vineyard near Bordeaux, France. Tamm et al. (2022) reported that Italy, among the 12 EU countries surveyed, had the highest annual Cu usage in organic farming (approximately 1550 t), with olives and grapevine alone accounting for 72% of the total Cu use in organic farming in the country. Based on our EUF-Cu distribution, Mediterranean and continental regions, particularly Italy,

should closely oversight Cu inputs to safeguard long-term soil health.

3.3. Distribution of EUF-Zn in agricultural soils in the EU27 + UK

Soil EUF-Zn ranged from 0.06 to 34 mg kg^{-1} , with a median of 1.23 mg kg^{-1} (Table S4 and Fig. 1). The median EUF-Zn was less than 3% of the median total Zn concentration (aqua regia extraction) in the LUCAS 2015 subsets (Table S4). Our results showed that the median EUF-Zn was similar in grasslands and croplands but significantly lower in temporarily bare agricultural fields (Fig. 1). About 13.7% of samples

have EUF-Zn values below 0.5 mg kg⁻¹, indicating a need for further assessment of Zn fertilization per Austrian guidelines (Baumgarten, 2023). Previous studies have also highlighted the deficiency of Zn in agricultural soils across European drylands, testing 149 LUCAS sampling points (Moreno-Jiménez et al., 2022). Approximately 19% of samples have EUF-Zn values above 2.5 mg kg⁻¹ (Fig. 1), exceeding the crop sufficiency range of 0.5–2.5 mg Zn kg⁻¹ (Baumgarten, 2023).

The GAMLSS model to predict EUF-Zn was found to follow a generalized *t* distribution (Rigby et al., 2019), with a Box-Cox transformation applied (Eq. (2)). The covariates were included only for the μ , as this resulted in a satisfactory worm plot (Fig. S4b):

$Zn - EUF(\text{boxcoxtransformed}) \sim GT(\mu, \sigma, \nu, \tau)$

$$\begin{aligned} \mu = & pb(\log Zn - AR) + pb(\log SOC) + pb(pH) + pb(CaCO_3) + pb(Clay) \\ & + pb(Bio1) + pb(Bio12) + WRB + LandUse \end{aligned} \quad (2)$$

where the scale (σ), shape (ν), and tail (τ) parameters were estimated at -0.239, 0.9017, and 0.9412, respectively.

Unlike Cu, not the total content but soil pH is the most important variable explaining EUF-Zn (Fig. 2b). The relationship between pH and EUF-Zn concentration followed a pattern similar to that observed for Cu (Fig. 2b). EUF-Zn concentrations increased with rising pH up to around pH 7, above which it decreased sharply. In alkaline soils, the formation of Zn-containing minerals may become important (Duffner et al., 2014), and Zn in these minerals may not be dissolved by the EUF extraction. It has previously been reported that pH is more important for explaining the speciation and extractability of Zn than of Cu (Van Eynde et al., 2022b), which is also confirmed in our findings. Total Zn appeared to have only a little effect on EUF-Zn at concentrations below 20 mg kg⁻¹ but showed a positive effect at higher total Zn concentrations (Fig. 2). Unlike Cu, where high SOC strongly reduces EUF-Cu due to strong organic complexation, SOC has a lower affinity for Zn adsorption (Wiersma et al., 2025), and increasing SOC level in our study resulted in higher EUF-Zn. The negative effect of high SOC levels on EUF-Zn was found to be rather small (Fig. 2b), similar to the effect of land use (results not shown). Clay had a negative effect on EUF-Zn. While clay surfaces are not major adsorption sites for Zn at typical soil pH (roughly pH 5–7; Dijkstra et al., 2004), Zn can be incorporated into clay-sized minerals that are not extracted by EUF (Degryse et al., 2011), which could explain the observed trend. Temperature seems to have a more prominent effect on EUF-Zn than on EUF-Cu (Fig. 2). EUF-Zn concentrations increased with mean annual temperatures up to about 9–10 °C, after which the relationship became negative. A similar pattern was found for total soil Zn concentrations in EU soils (Van Eynde et al., 2023a). This decline likely reflects more alkaline soils in warm southern regions, compared to acidic soils in cooler northern Europe (Fabian et al., 2014). In a global study, Moreno-Jiménez et al. (2022) attributed the negative temperature effects on soil Zn levels to increased weathering rates and precipitation-driven leaching. In addition, regional differences in manure application may contribute to the observed pattern of EUF-Zn, as manure inputs are generally highest in central Europe and lower in northern and southern regions (Batoool et al., 2025).

The model described in Eq. (2) was used to extrapolate EUF-Zn predictions across EU agricultural land (Fig. 4b). The RMSE using the independent subset of LUCAS points was 1.7 mg kg⁻¹ based on the median predicted value. As with Cu, this RMSE is relatively high when considering the data (*i.e.* median value of 1.23 mg kg⁻¹). However, the validation in terms of the worm plot (Fig. S4) gives confidence for interpreting the probabilities calculated with the model. The model was used to calculate the probability of EUF-Zn exceeding or being less than thresholds for toxicity and deficiency respectively. The model predicts high probability of EUF-Zn being lower than 0.5 mg kg⁻¹ in Spain and Cyprus, where soils are calcareous, in Finland with with mean temperature of ~4 °C, and some regions of Italy. For these countries, more than

34% of the sampling points had EUF-Zn values below 0.5 mg kg⁻¹ (Table S5). Our results are consistent with previous studies reporting low total Zn concentrations (<30 mg kg⁻¹, aqua regia extraction) in Northern European countries, such as Finland, where there is an increased risk of Zn deficiencies in crop production, as well as in the calcareous soils of Spain (Van Eynde et al., 2023a). Dietary Zn deficiency in humans is a global health concern ranking as the 11th leading health risk globally in terms of disease burden with prevalence ranging from 25% in Africa to about 3% in Europe (WHO, 2002; Stein, 2010). Humans Zn deficiency is believed to be closely linked to regions where soils have low plant-available Zn (Cakmak, 2009), although human Zn status also depends on factors such as food processing and dietary patterns. In Zn-deficient areas, fertilization strategies have been proposed to boost Zn levels in staple food and forage crops, potentially supporting dietary Zn intake (Ylivainio et al., 2024).

Our findings confirm that elevated levels of EUF-Zn are rare across the sampled areas, with only two samples exceeding 18 mg kg⁻¹, with values of 34 mg kg⁻¹ in the Netherlands, located near a Zn smelter as reported by Van Eynde et al. (2023a), and 28 mg kg⁻¹ in Italy. In line with the measurements and the mean predicted values (Fig. 4b), the probability of EUF-Zn exceeding 18 mg kg⁻¹ was nearly zero (results not shown). However, Belgium (84%) and the Netherlands (64%), which have the highest livestock densities and manure production in the EU (Eurostat, 2023), stood out with the highest proportion of samples exceeding 2.5 mg kg⁻¹. Additionally, 8% of samples in Belgium exhibited values above the 97.5th percentile of 7 mg Zn kg⁻¹ (Fig. 1). This pattern is further reflected in the Zn distribution map, which highlights higher available Zn contents in Belgium and the Netherlands, and in Atlantic regions (Fig. 4b). The high livestock intensity in these regions (Eurostat, 2023), combined with the use of Cu and Zn as feed supplements that end up in manure, likely contributes to the elevated metal levels. These suggest that EU guidelines on manure management and micronutrient fertilization should pay attention to regions with intensive livestock production, such as Belgium and the Netherlands, to prevent long-term Zn accumulation.

In general, unlike total Zn concentrations, which show a north–south gradient with higher levels in Southern Europe, EUF-Zn exhibited a more scattered pattern, with notably low values in Southern and Eastern Europe, including parts of Spain, Italy, southern Hungary, eastern Greece, eastern Romania, and eastern Bulgaria. While not all of these areas fall within the core Mediterranean zone, many share agroecological characteristics such as calcareous soils and semi-arid to sub-humid climates, which may contribute to low soil available Zn. In contrast, northern Europe showed uniformly low concentrations of both total and EUF-Cu and Zn, particularly in areas of quaternary origin (Albanese et al., 2015; Ballabio et al., 2018; Tóth et al., 2016a; Van Eynde et al., 2023a). These lower concentrations are primarily attributed to the dominance of parent materials such as igneous rocks, which have lower concentrations of trace elements compared to sedimentary rocks (Systra, 2010). Additionally, the younger soils of Northern Europe, formed on glacial overburden, have undergone less extensive weathering processes contributing to lower their trace element concentrations (Négrel et al., 2018). However, human activities can locally increase soil-available Cu and Zn, for example, high Cu levels in vineyards (Mann et al., 2015), elevated Zn levels in regions with intensive manure application, or through mining and proximity to mineral deposits (Van Eynde et al., 2023a). In future studies, incorporating data layers such as livestock density and Cu use across different land-cover types could help better distinguish the effects of natural versus anthropogenic influences.

4. Conclusions

Our study provides new insights into the availability of Cu and Zn in agricultural soils across the EU27 + UK, using EUF method. Our spatial assessment showed that toxicity risks for Cu and Zn are generally low in EU + UK agricultural land, while probability of Cu and Zn being below

sufficiency thresholds was more widespread. Our transfer functions, linking total concentrations to EUF-extractable fractions, revealed that for similar total Cu and Zn concentrations, EUF extractable concentrations can vary widely depending on other soil and climatic properties. This highlights the limitations of relying solely on total metal concentrations in soil monitoring, especially when assessing risks towards human and ecosystem health. Croplands, especially in Mediterranean regions, showed higher available Cu than grasslands. This is likely due to anthropogenic inputs, such as Cu-based fungicides in croplands. In contrast, higher SOC levels in grasslands reduced EUF-Cu extractability. For Zn, availability was strongly controlled by soil pH, with elevated deficiency risk in calcareous soils of Southern and Eastern European regions, and in Northern European soils of quaternary origin. Our results emphasize the importance of understanding soluble metal fractions and their spatial distribution to develop precise, regionally tailored fertilization strategies that both address Cu and Zn deficiencies and avoid potential nutrient excesses. No consistent relationship was found between soil Cu and Zn and grain metal contents, likely due to the heterogeneity of fields and crops in our dataset. Future studies should focus on more homogeneous conditions to measure total and grain Cu and Zn uptake, examine crop-specific metal partitioning, and refine EUF-based thresholds through plant experiments. Furthermore, as the thresholds used here are based on literature and method conversions rather than direct plant experiments, they should be interpreted with caution and validated across diverse soils and climates.

CRedit authorship contribution statement

Mina Kiani: Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Elise Van Eynde:** Writing – review & editing, Visualization, Validation, Software, Methodology, Formal analysis. **Jakob Santner:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Dietmar Horn:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Panos Panagos:** Writing – review & editing, Supervision, Resources, Methodology. **Alicia Hernandez-Mora:** Writing – review & editing, Resources, Methodology. **Olivier Duboc:** Writing – review & editing, Resources, Methodology. **Herbert Eigner:** Resources, Methodology, Formal analysis. **Gereon Heller:** Methodology, Formal analysis, Data curation. **Stefan Geyer:** Resources, Methodology. **Arwyn Jones:** Resources, Methodology. **Kari Ylivainio:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2026.110133>.

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Data availability

Data will be made available on request.

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