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Slow-release copper efficacy study on wheat: a sustainable solution for efficient crop micronutrient delivery

Farahnaz Nourmohammadian, Hessamoddin Solouki, Wilfried Dossou-Yovo, Cheng Jiang*

Research and Development Department, Lucent Biosciences Inc, Burnaby, British Columbia, Canada

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Abstract: This study evaluated a novel slow-release copper fertiliser (soileos Cu) as a sustainable alternative to conventional copper sources for improving wheat yield and nutrient use efficiency. Traditional Cu fertilisers are often limited by rapid leaching and low efficiency, especially on sandy soils with low organic matter, contributing to environmental pollution. They also exhibit low plant-use efficiency due to strong adsorption and immobilisation in soils rich in organic matter and clay minerals, thereby reducing copper availability in the soil solution and contributing to environmental pollution. A multi-scale approach was employed, including laboratory incubation, greenhouse experiments, and multi-site field trials. Copper release was quantified in water over 30 days. Greenhouse experiments compared soileos Cu with copper sulfate ($CuSO_4$) across multiple application rates, assessing grain yield, biomass, spike count, chlorophyll index (SPAD), and tissue and grain nutrient concentrations. Field trials were conducted at four sites in Canada and the United States with contrasting soil Cu availability. Soileos Cu exhibited controlled, non-linear Cu release with substantially reduced leaching compared to $CuSO_4$. In greenhouse conditions, soileos Cu achieved maximum grain yield, biomass, and spike number at 25–26% lower Cu application rates than $CuSO_4$, indicating higher nutrient use efficiency. Field trials confirmed that yield responses were strongly dependent on baseline soil Cu levels, with the greatest yield increase (up to 13.3%) observed at a Cu-deficient site. Overall, soileos Cu provides an effective and environmentally responsible strategy for improving Cu nutrition and wheat productivity, particularly under Cu-limiting conditions.

Keywords: bio-based fertiliser; field; greenhouse; yield improvement; circular economy

Improving nutrient use efficiency is essential for sustainable agricultural production, as rising fertiliser consumption places increasing pressure on agroecosystems (Yuvaraj et al. 2024). Conventional fertilisers often exhibit low efficiency, with 40–70% of applied nutrients lost through leaching or soil immobilisation, leading to soil degradation, groundwater contamination, eutrophication, and increased greenhouse gas emissions (Benlamlih et al. 2021, Malhi et al. 2021). Improving fertiliser efficiency is critical to sustaining crop productivity while reducing environmental impacts (Kumar et al. 2023). Wheat (*Triticum aestivum* L.) is a globally important crop, yet its productivity is frequently constrained by copper (Cu) deficiency, particularly in the Canadian Prairies (Rahman et al. 2021, Rahman and Schoenau 2022, Singh Dhaliwal et al. 2023). Copper is essential for enzyme activation, nitrogen metabolism, and chlorophyll synthesis (Kumar et al. 2021), underscoring the need for more effective Cu-delivery systems.

Although controlled- and slow-release fertilisers can improve nutrient efficiency, many existing formulations rely on petroleum-derived polymer coatings that contribute to the accumulation of microplastics and nanoplastics in soils and aquatic systems (Kumar et al. 2023). Environmentally responsible nutrient delivery technologies are urgently needed to enhance

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nutrient bioavailability while minimising ecological risks (Duan et al. 2023, Magaletti et al. 2023).

Soileos Cu, a novel biopolymer-based slow-release copper fertiliser, aims to slowly release the copper micronutrient to the crop, improve nutrient delivery, and positively impact crop growth and ultimately yield. This study evaluated a novel biopolymer-based slow-release copper fertiliser, soileos Cu, in greenhouse and field trials on wheat. Developed using patented technology (Branda et al. 2023), soileos Cu binds Cu ions to cellulose derived from agricultural by-products, reducing solubility and enabling controlled release in response to biological demand (Nourmohammadian et al. 2025). The formulation promotes circularity by utilising crop-processing residues and contains organic carbon that supports microbial activity and Cu mobilisation in the rhizosphere. The performance of soileos Cu was compared with conventional $CuSO_4$ by assessing wheat growth, yield components, SPAD values, and tissue and grain nutrient concentrations.

MATERIAL AND METHODS

Micronutrient release quantification. A leaching experiment was conducted to compare Cu release from soileos Cu and $CuSO_4 \cdot 5\ H_2O$ with three replicates per treatment in water. Soileos Cu was evaluated for long-term metal ion release in a 30-day laboratory incubation. For release assessment, 25 g of soileos Cu and 5.82 g of $CuSO_4$ were suspended in 500 mL of distilled water in 1 L glass Mason jars with tightly closed lids during the experiment, providing initial Cu concentrations of 2 953 mg/L. Due to the low Cu solubility in soileos, this loading was selected to ensure accurate measurement of Cu leaching within the lower limit of detection while minimising sampling error. At designated intervals, 0.25 mL aliquots were collected for Cu quantification by EDTA complexometric titration (Pižeta et al. 2015). Sampling occurred one hour after immersion, daily for the first four days, and 2–3 times per week thereafter for 30 days.

Greenhouse study. The greenhouse efficacy study was conducted in 2023 at Simon Fraser University, Burnaby, Canada. Spring wheat (*Triticum aestivum* L., cv. AAC Brandon) was grown in 5-L pots containing sterilised peat moss-perlite (70:30) under controlled conditions (16/8 h photoperiod, 450 ± 50 µmol/m^2/s photosynthetically active radiation (PAR), 22/18 ± 2 °C day/night, 45 ± 5% relative humidity (RH)) with specific irrigation and potting media detailed in Table 1. Six seeds were sown per pot and thinned to three plants at 7 days after sowing, fertigated twice weekly with Cu-omitted modified Hoagland solution (pH 5.8–6.5) to induce Cu deficiency (Hoagland and Arnon 1950).

The efficacy of two Cu fertiliser sources on wheat yield was evaluated using a randomised complete block design with five replications and two subsamples per treatment. Each pot was filled with 1.6 kg of steriliesd peat moss-perlite mixture (70:30). Copper treatments were applied before sowing by thoroughly mixing the appropriate amount of Cu fertilisers into the media to achieve target concentrations of 0, 3, 6, and 12 ppm of actual Cu (equivalent to 0, 1, 2, and 4.3 lbs/acre actual Cu) using soileos Cu (containing 5.9% Cu) and $CuSO_4$ (containing 25% Cu) as Cu sources. Soileos Cu and conventional $CuSO_4$ were applied at four rates (0, 3, 6, and 12 ppm) by mixing fertilisers into 1.6 kg of potting media per treatment. To promote nutrient mobilisation, enhance microbial activity, and prevent microbial infections, 1 g of root shield (*Trichoderma harzianum* strain T-22) and 100 mL of a 1% Serenade (*Bacillus subtilis* Group 44 strain QST713) solution were also incorporated into the media. The treated media were then placed in the pots, and wheat seeds were sown in each pot.

Sampling and measurements. Leaf physiological status, elemental composition, and yield components were assessed during the growing cycle and at harvest. Leaf chlorophyll content was measured at BBCH growth stage 32 using a handheld SPAD chlorophyll analyser (Konica Minolta 502, Tokyo, Japan). Measurements were taken from the youngest fully expanded leaf at three positions (apex, middle, and base) on each plant, and mean values were calculated per pot (Song et al. 2021). Before harvest, flag leaf samples were collected for elemental analysis, and concentrations of P, K, Mg, Zn, Cu, Fe, Mn, and B were determined using inductively coupled plasma-mass spectrometry (Thermo Scientific iCAP™Q ICP-MS). Yield components were evaluated by recording the number of tillers per plant at 90 days after sowing. Plants were harvested at BBCH growth stage 92 (105 days after sowing), and above-ground dry biomass, spike number, and grain weight were measured separately for each plant.

Field study. The field study was conducted at four different locations in the United States and Canada, as shown in Figure 1. (A) 3MG North, Olivia, Minnesota, US; (B) ABG AG Services, Toronto, South Dakota, US; (C) New Era Ag Technologies Inc., Swan River, Manitoba, Canada and (D) Mackenzie Applied Research Association, Fort Vermilion, Alberta, Canada in 2024.

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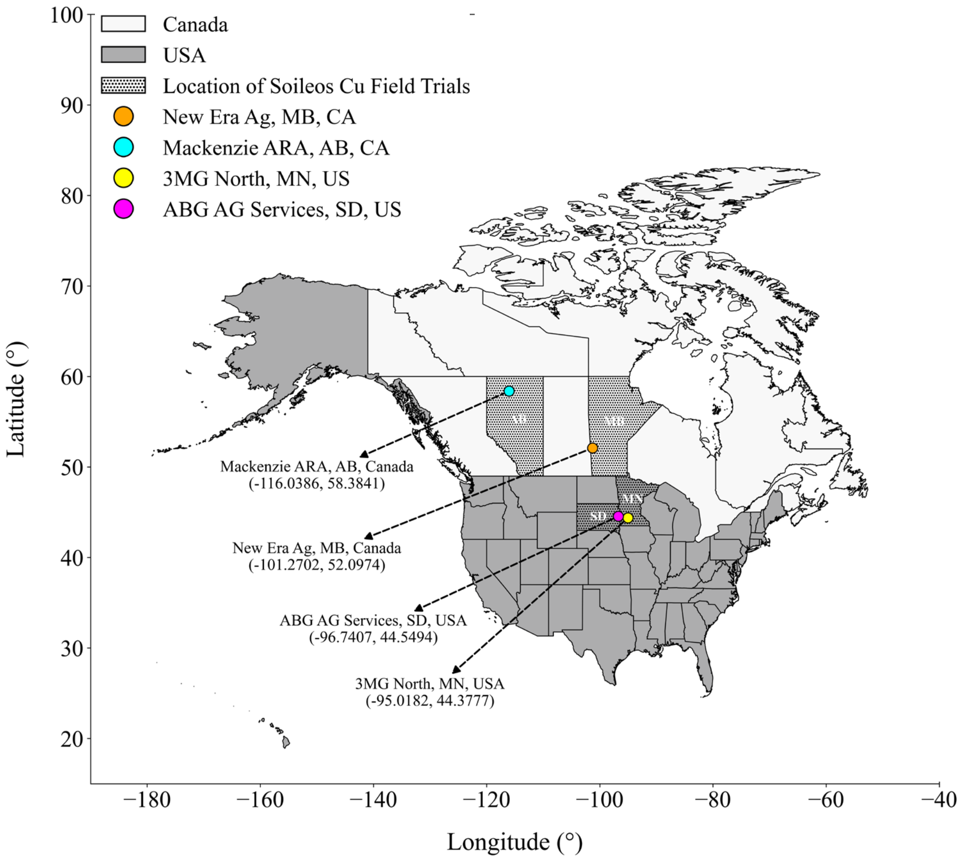

Figure 1. Field trial locations in the United States and Canada

Uniform seeds of spring wheat (*Triticum aestivum* L.) were used in all experiments. Given the likely differences in climatic conditions and variety adaptability, AAC Viewfield, Wheatland, Starflex, and SY Valda were used by MacKenzie, New Era, 3MG, and ABG, respectively. Soil sampling and analysis were conducted before seeding at each site (a composite sample from the depth of 0–15 cm). Soil samples from 3MG and ABG were analysed for Cu using DTPA extraction in the US, while those from New Era and MacKenzie were analysed using Mehlich-3 extraction in Canada. The soil physicochemical properties are presented in Table 2.

A randomised complete block design (RCBD) was used for these field experiments with six replications and three treatments with consideration of farmers' traditional practices: T1 – Grower Standard (GS) as described in the following; T2 – 11.2 kg/ha soileos Cu (0.62 kg/ha of Cu); and T3 – 22.4 kg/ha soileos Cu (1.23 kg/ha of Cu). Additionally, depending on soil conditions at each site, they received a basal macronutrient application (N, P, and K). At 3MG, grower standard nutrients were applied at 134.5 kg N/ha, 19.5 kg P/ha, and 55.9 kg K/ha. At ABG, 67.3 kg N/ha was applied, with no P or K. At New Era, fertilisation consisted of 154.7 kg N/ha, with no P or

Table 1. The physicochemical characteristics of (A) irrigation water and (B) potting media

	Conductivity (mS/cm)	pH	Na	Cl	Ca	K	N	P	Cu
(A)			(ppm)						
	0.05	6.5	2.1	2.2	5.8	~0	~0	~0	~0
(B)	Cation exchange capacity (mmol$_+$/100g)	pH	media texture		organic carbon (%)	K	N	P	Cu
						(ppm)			
	16	6.8	70% PeatMoss + 30% Perlite		28.1	17	1	3	0.2

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Table 2. The physicochemical characteristics of soil

Trial	Soil texture	Cation exchange capacity ($mmol_+$/100 g)	pH	Organic carbon (%)	P	K	Cu
					(ppm)		
(A) 3MG	clay-loam	21.4	7.1	2.8	68	306	1.7
(B) ABG	silty-clay-loam	14.1	5.3	2.5	38	92	1
(C) New Era	sandy-clay-loam	30	6	3.5	17	238	0.73
(D) MacKenzie	sandy-loam	7.7	7.1	1.3	32	131	0.36

K applied. At MacKenzie, nutrients were applied at 144.6 kg N/ha, 0 kg P/ha, and 11.2 kg K/ha. Agronomic practices are represented in Table 3.

Yield. After the growing cycle, the plants were harvested, and the grain weight was carefully recorded per plot, expressed as kilograms per hectare (kg/ha). Detailed information regarding the harvest date and plot size for each trial is summarised in Table 3, providing a comprehensive overview of the conditions under which the data were collected.

Statistical analysis. Statistical analyses were conducted using the R statistical language (version 4.5.1; R Core team 2025). The tidyverse meta-package (version 2.0) was used for data preprocessing and visualisation (Wickham et al. 2019), the mgcv package (version 1.9.4) for the General Additive Modelling (GAM) (Wood 2023), and the least square difference (*LSD*) test was performed using the Agricolae package (version 1.3.7) (de Mendiburu 2023).

The fertiliser release rate was determined by fitting the logarithm base 10:

$$Y = \log (X) \quad (1)$$

where: Y – outcome (soileos Cu leaching rate); X – time (days).

The GAM framework, an extension of generalised linear models, allows flexible modelling of non-linear relationships (Chambers and Hastie 1992) and has been widely applied in agricultural and ecological studies (Guillermo et al. 2021, Wellington et al. 2023). The general model form was:

$$\eta = \beta_0 + \sum_{j=1}^{p} f_j(x_j) \quad (2)$$

where: β_0 – intercept and $f_j(x_j)$ are smooth functions of predictor variables. In this study, GAMs were used to quantify wheat yield responses to Cu fertilisation under field conditions.

RESULTS

Micronutrient release quantification in water. Regression analysis showed that Cu leaching from soileos Cu followed a non-linear pattern over time. A $\log_{10}$ model provided a good fit for the leaching data (R^2 = 0.56, P < 0.05), as reflected in the cumulative release pattern (Figure 1). Leaching increased linearly from 1% to 3% during the first five days, then transitioned to a non-linear trend, reaching a maximum of 3.3% by day 13. The average Cu leaching rate over 30 days was approximately 0.1% per day (Figure 2). In contrast, $CuSO_4$ dissolved completely within 2 days.

Greenhouse study. Copper application significantly improved wheat growth and physiological performance compared with the untreated and grower standard controls (Figure 3).

Plants without Cu exhibited severe reductions in grain weight, biomass, spike number, and SPAD values, confirming the effects of Cu deficiency. Both $CuSO_4$ and soileos-Cu markedly increased grain weight (from 0.6 to ~9–13 g/plant), above-ground biomass (from 3.6 to ~21–27 g/plant), spike count (from 3.7 to ~11.9 spikes/plant), and SPAD values (from 25.8 to ~40.2). Growth responses increased sharply from 0 to 3 ppm Cu and then plateaued, indi-

Table 3. Agronomic practices of field trials

Trial	Cultivar	Plot dimension (m^2)	Sowing date (2024)	Harvest date (2024)	Previous crop	Tillage
(A) 3MG	Starflex	20	Jun 10	Oct 04	soybean	15 cm of conventional till
(B) ABG	SY Valda	13.9	Apr 10	Aug 23	soybean	
(C) New Era	Wheatland	20.8	May 13	Sep 02	soybean	
(D) MacKenzie	AAC Viewfield	7.8	May 23	Sep 06	fallow	

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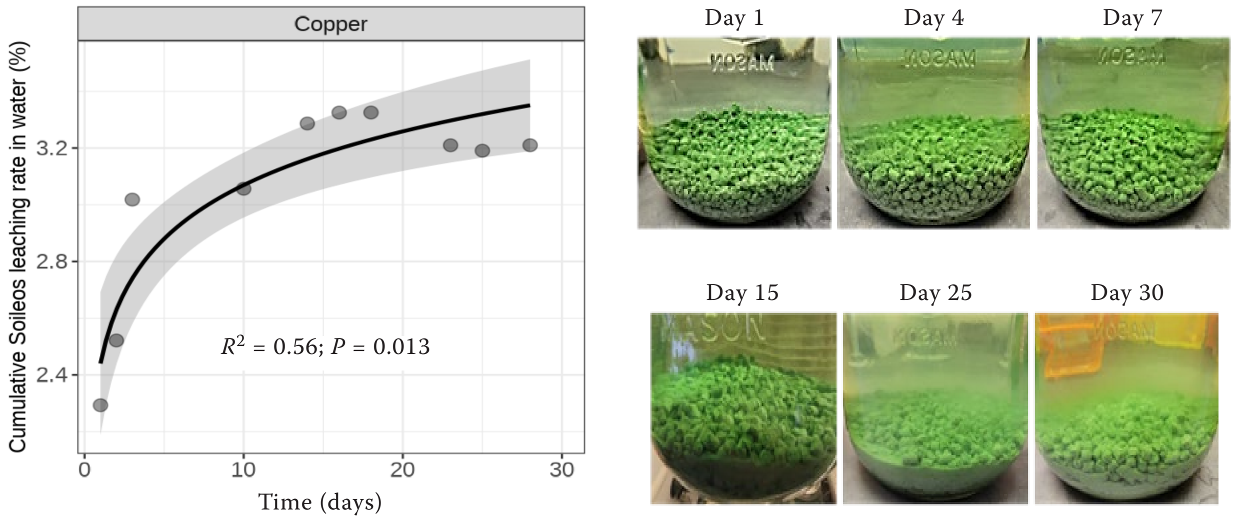

Figure 2. Cumulative copper (Cu) leaching of soileos-Cu in distilled water over 30 days, fitted using the $y = \log(X)$ function

cating Cu sufficiency, with no significant differences among the higher rates (Figure 4). The improvement in grain weight (yield), biomass, and SPAD values achieved at 3 ppm Cu were statistically significant. The improvement in Cu rate higher than 3 ppm was not significant, indicating sufficient Cu for the plant.

General additive model (GAM) analysis showed that both soileos-Cu and $CuSO_4$ achieved peak grain yield, biomass, spike count, and SPAD values at moderate Cu application rates, followed by declines at higher rates (Figure 5). Importantly, soileos-Cu consistently reached equivalent maximum responses at lower Cu inputs than $CuSO_4$. Peak grain yield (~14.2–14.4 g/plant) and spike count were achieved at approximately 6–7 ppm for soileos-Cu compared with 8 ppm for $CuSO_4$, representing a ~25% reduction in Cu require-

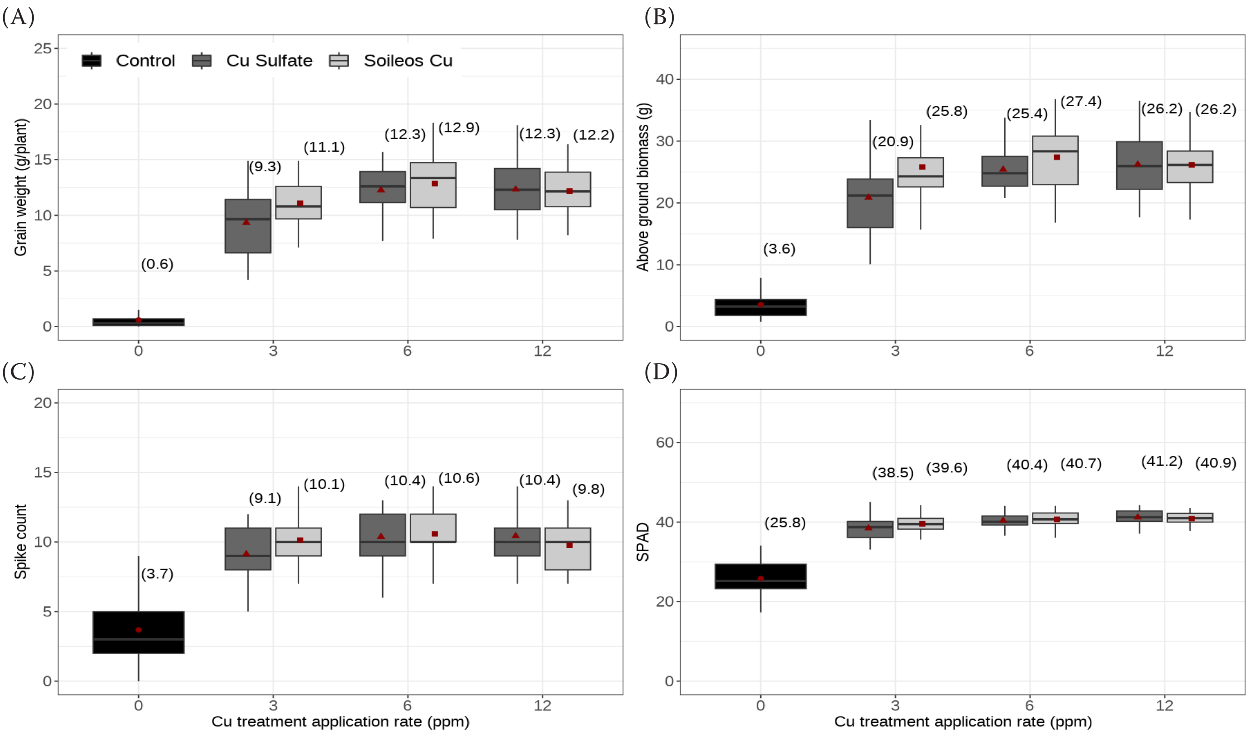

Figure 3. Boxplots displaying the effect of copper (Cu) treatment rate on (A) grain weight; (B) above-ground biomass; (C) spike count, and (D) chlorophyll index (SPAD). Points and bracketed values indicate treatment medians

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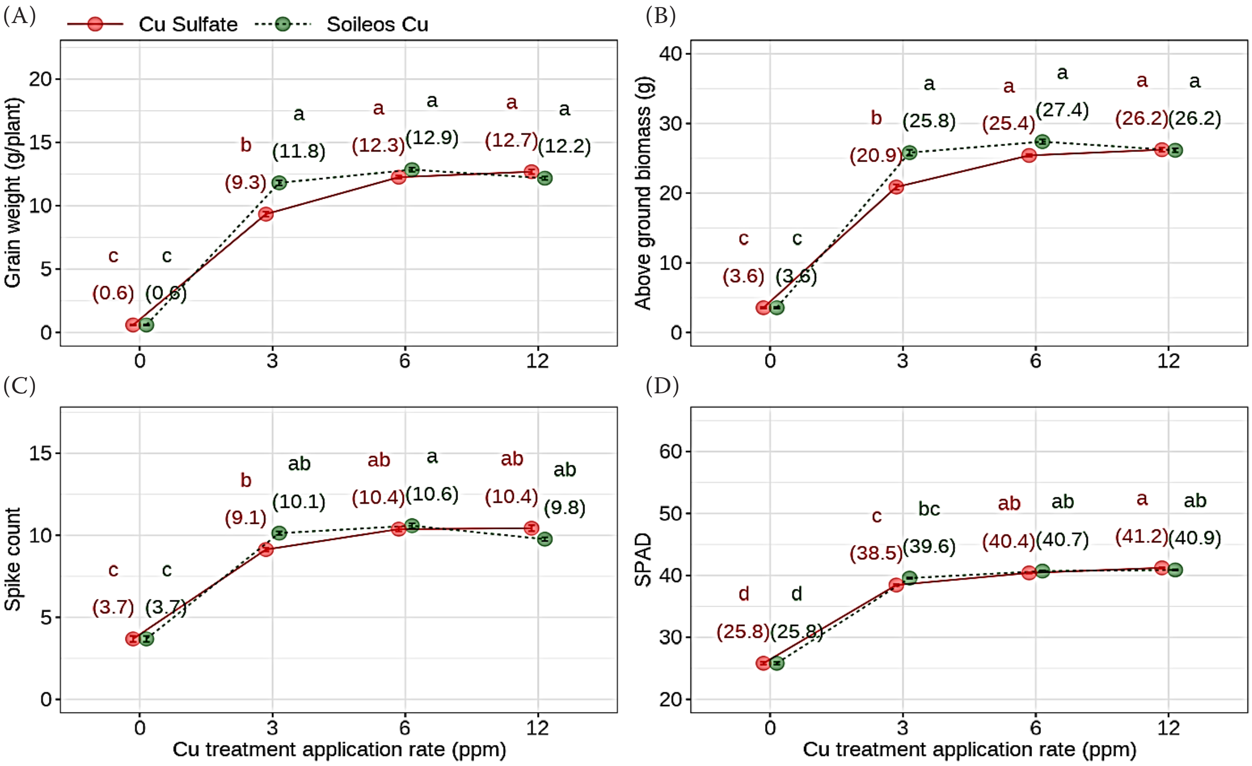

Figure 4. Effect of different copper (Cu) application rates on (A) grain weight; (B) above-ground biomass; (C) spike count, and (D) chlorophyll index (SPAD)

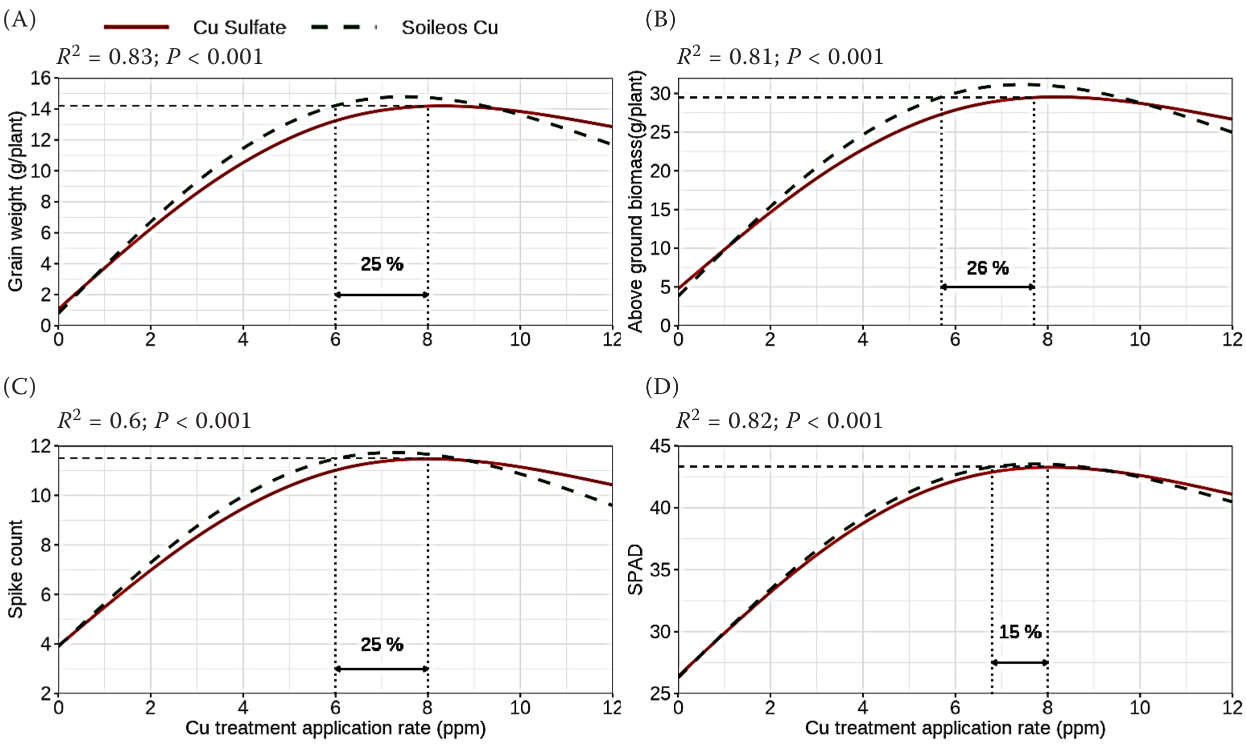

Figure 5. Modelled relationship (general additive model (GAM)) between copper (Cu) application rate and (A) grain weight; (B) above-ground biomass; (C) spike count, and (D) chlorophyll index (SPAD)

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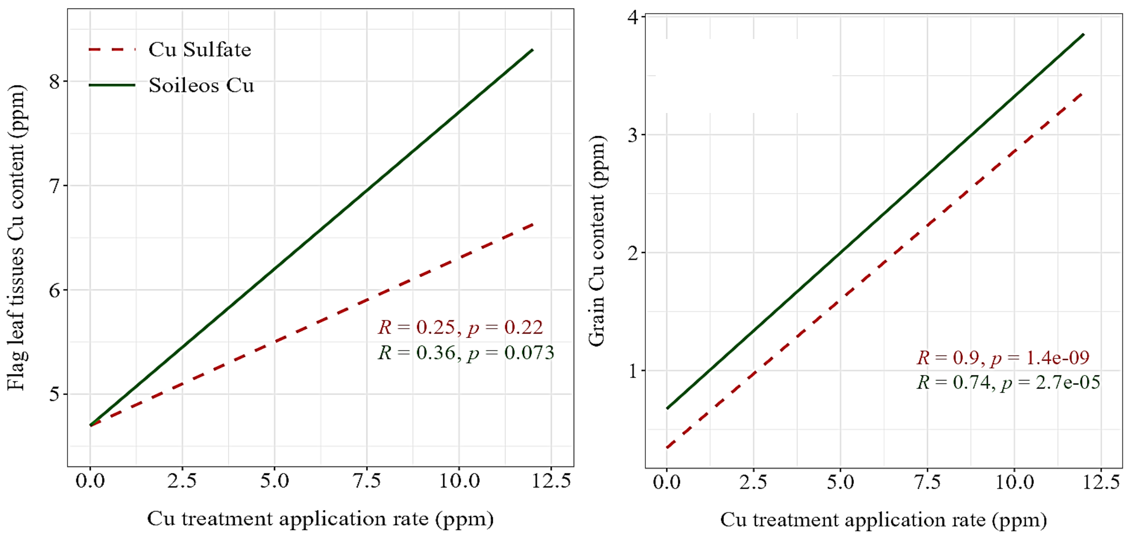

Figure 6. Flag leaf and grain elemental analysis at BBCH 97 growth stage

ment. Similarly, maximum biomass and SPAD values were attained with 15–26% less Cu using soileos-Cu. These results demonstrate the greater nutrient use efficiency of the soileos-Cu formulation.

Correlation analysis showed that soileos-Cu enhanced Cu uptake compared with $CuSO_4$ (Figure 6), reflecting improved Cu bioavailability and sustained release. This increased availability promoted Cu absorption and translocation from vegetative tissues to grain (Figure 6).

Soileos-Cu not only improved Cu uptake but also enhanced the absorption of several other nutrients, including phosphorus, potassium, zinc, and iron, compared with $CuSO_4$, while leaf boron levels remained unaffected (Figure 7).

Field study. Field trials confirmed the effectiveness of soileos-Cu in increasing wheat grain yield compared with the grower standard (GS) (Figure 8). The strongest response occurred at the Cu-deficient MacKenzie site (0.36 ppm soil Cu), where soileos-Cu

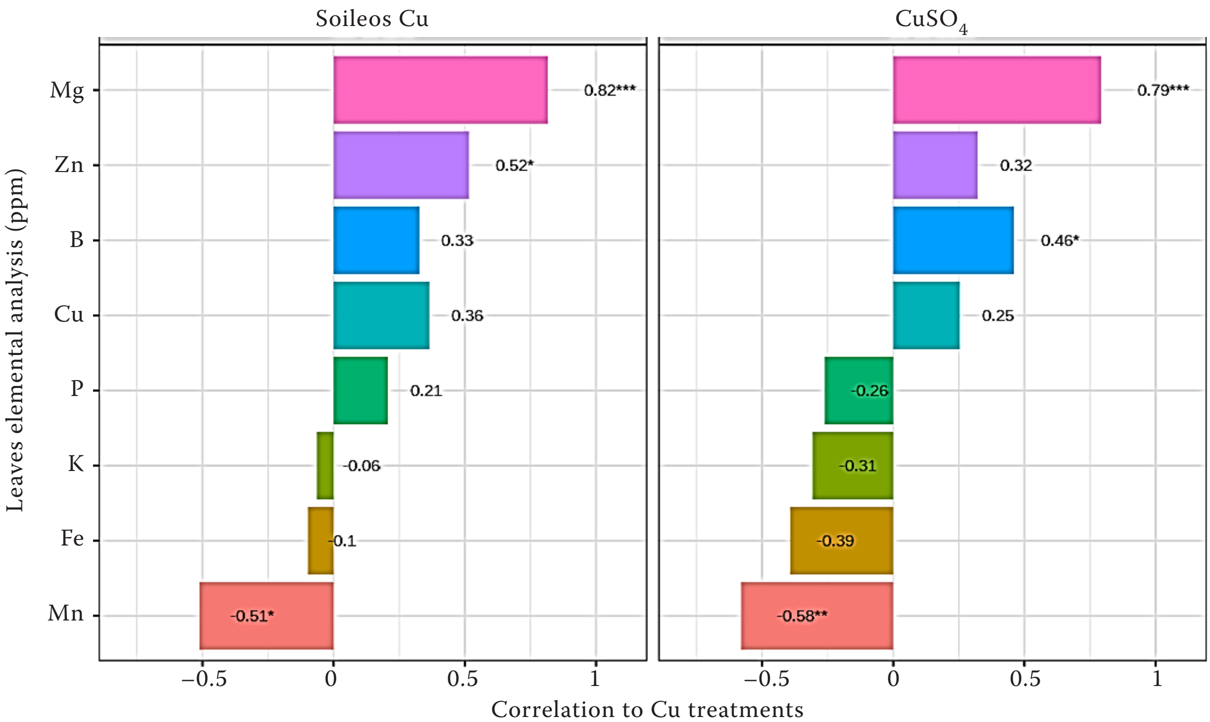

Figure 7. Tissue elemental analysis 65 days after sowing. BBCH stage 39 or Zadoks stage 47–49, once the flag leaf is fully developed and expanded

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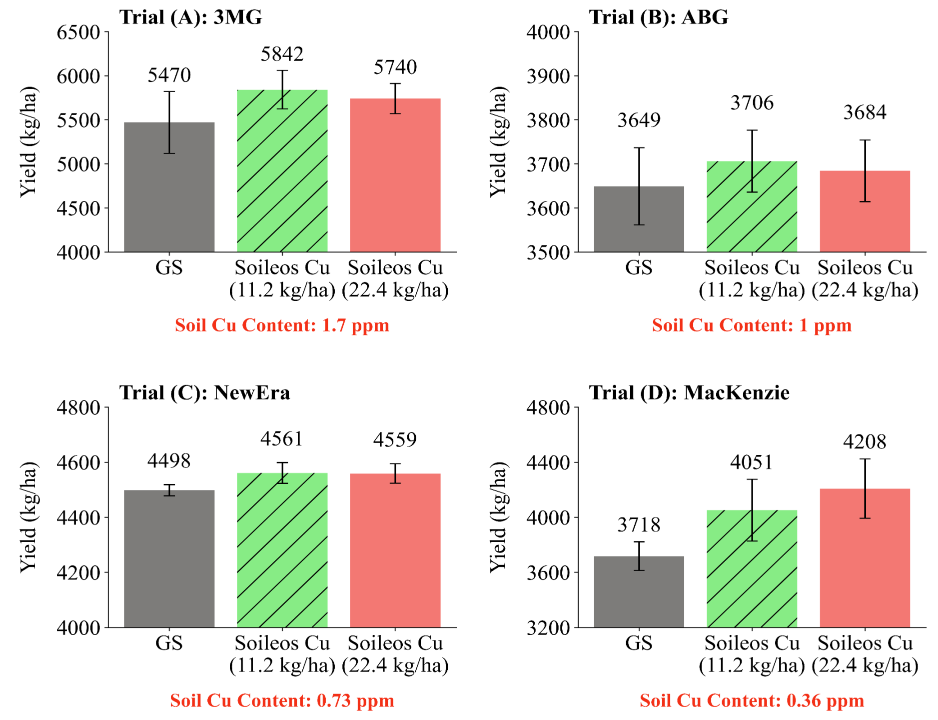

Figure 8. Effect of two application rates of soileos copper (Cu) on wheat (*Triticum aestivum* L.) yield compared to control under various soil copper content scenarios

increased yield by 13.3% and 9.1% at application rates of 22.4 and 11.2 kg/ha, respectively (Figures 8 and 9). Yield improvements were also observed at other sites, including increases of 6.8% at 3MG, and smaller gains at ABG (1.8%) and New Era (1.4%) with 11.2 kg/ha. Overall, these results demonstrate that soileos-Cu is most effective at enhancing wheat productivity, and a higher application rate is more efficient under Cu-deficient soil conditions (Figure 9).

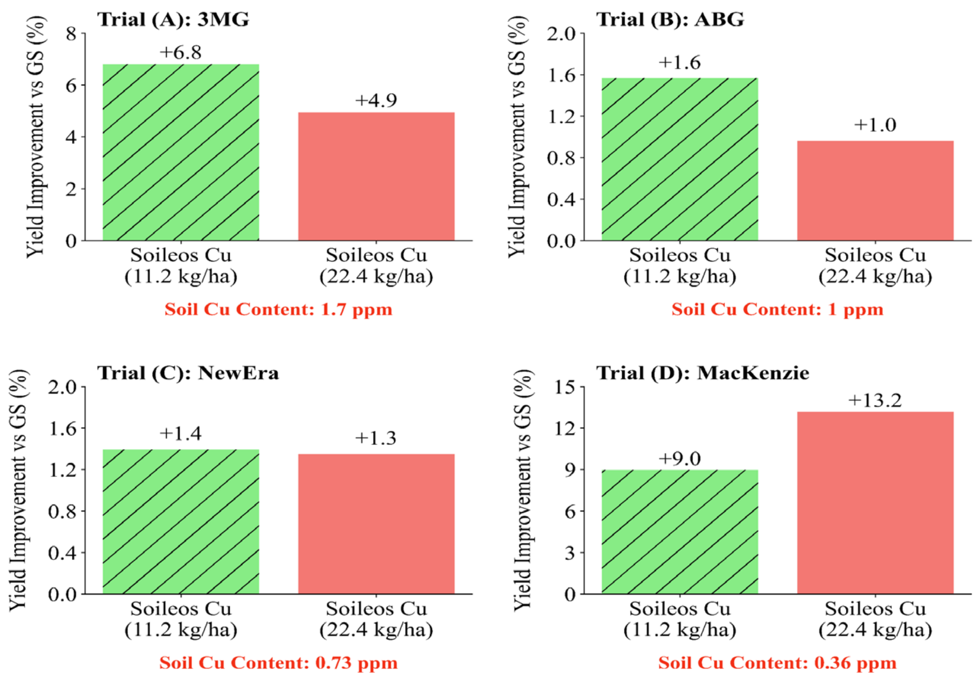

Figure 9. Comparison of yield improvement at two application rates of soileos copper (Cu) compared to control under various soil copper content scenarios

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DISCUSSION

As expected, 98% of $CuSO_4$ dissolved within 2 h (Chiaregato and Faez 2021). However, since Cu in soileos is chemically bonded to cellulose fibres (Branda et al. 2023), less than 4% of the Cu was released from soileos after 30 days of water incubation. The initial ~3% Cu release likely originated from readily available, unbound Cu, followed by a slow, sustained release consistent with first-order kinetics (Wei et al. 2019) (Figure 2).

Due to the antimicrobial properties of Cu (Lamichhane et al. 2018), no visible microbial growth was observed in soileos Cu suspensions until day 25. The formulation binds Cu to biopolymers derived from food-processing by-products, substantially reducing water solubility and extending nutrient longevity. Cu release is governed by gradual microbial decomposition of the cellulosic carrier, enabling synchronised nutrient availability with plant demand (Nourmohammadian et al. 2025).

Greenhouse study. Boxplots showed that Cu fertilisation significantly improved wheat growth and physiological traits, including grain weight, above-ground biomass, spike count, and SPAD values, compared with the untreated control (Figure 3). Grain weight increased from 0.6 g/plant in the control to 9.3–12.9 g/plant with Cu application, with the greatest response observed at 3 ppm ($P < 0.05$). Higher Cu rates did not yield additional gains, indicating that Cu requirements for maximum grain production were met at lower application levels, consistent with previous findings (Alloway 2008). Copper uptake by plants occurs primarily through roots as Cu^{2+} ions in the soil solution and is mediated by membrane transport proteins such as members of the COPT/CTR transporter family, which regulate copper absorption and homeostasis in plant cells (Wairich et al. 2022, Xu et al. 2024).

Above-ground biomass increased from 3.6 g to 25–27 g/plant ($P < 0.01$), accompanied by higher SPAD values, suggesting improved photosynthetic capacity and nutrient assimilation. Spike count also increased significantly with Cu treatment, from 3.7 to approximately 10 spikes/plant ($P < 0.05$), reflecting enhanced reproductive development (Marschner 2012). SPAD values were significantly higher in Cu-treated plants (mean ≈ 40) than in the control (25.8), confirming the role of Cu in chlorophyll synthesis and photosynthetic efficiency (Giunta et al. 2002). Uniform responses beyond 3 ppm indicate that relatively low Cu inputs are sufficient to restore optimal physiological function in Cu-deficient wheat.

Effect of copper treatments on wheat growth and physiology. Copper application significantly influenced wheat growth and physiological traits, including grain weight, above-ground biomass, spike count, and SPAD values (Figure 4). Both $CuSO_4$ and soileos-Cu exhibited similar response patterns, with severe reductions observed at 0 ppm Cu, confirming the essential role of Cu in plant metabolism as a structural component of several enzymes involved in photosynthetic electron transport, respiration, and oxidative stress regulation, thereby supporting plant growth and productivity under adequate Cu supply, photosynthesis, and reproductive development (Chia et al. 2025). Growth responses increased sharply between 0 and 3 ppm Cu, reflecting correction of Cu deficiency, and plateaued beyond 6 ppm, indicating Cu sufficiency. At the optimal rate of 3 ppm, soileos-Cu produced higher grain weight (11.8 *vs.* 9.3 g/plant), biomass (25.8 *vs.* 20.9 g/plant), and SPAD values (39.6 *vs.* 38.5) than $CuSO_4$, suggesting improved Cu availability due to slow-release kinetics. Maximum responses for $CuSO_4$ occurred at 6 ppm, with no further gains at higher rates. These results support the hypothesis that soileos-Cu provides more controlled Cu release and improved bioavailability without inducing toxicity, while both sources meet crop demand once Cu sufficiency is reached.

Modelling the dose-response relationship. The GAM analysis quantified the Cu dose-response relationship for both fertilisers, with strong model fits ($R^2 = 0.6–0.83$, $P < 0.001$), indicating that Cu concentration explained most variation in growth and SPAD parameters (Figure 5). Modelled response curves identified optimal Cu application rates of 6–8 ppm, consistent with experimental results (Figure 4), followed by slight declines at higher rates, suggesting potential Cu excess effects. Although maximum responses occurred within this range, they were not statistically different from those at 3 ppm Cu. Notably, soileos-Cu achieved peak grain yield, spike count, biomass, and SPAD values at 15–26% lower Cu input than $CuSO_4$, demonstrating greater nutrient use efficiency.

Effects of copper fertiliser sources on micronutrient accumulation. At 65 days after sowing (BBCH 39; Zadoks 47–49), distinct nutrient interaction patterns were observed between Cu sources (Figure 7). Both soileos-Cu and $CuSO_4$ showed strong positive correlations between Cu application and leaf Mg concentration ($r = 0.82$ and 0.79, respectively), indicating a synergistic relationship during late vegetative growth, likely associated with enhanced metabolic activity (Setiawati et al. 2025).

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Soileos-Cu also exhibited a significant positive correlation with Zn ($r = 0.52$, $P < 0.05$), whereas the response under $CuSO_4$ was not significant, suggesting improved micronutrient bioavailability or shared divalent cation transport pathways under the slow-release formulation. In contrast, Mn showed significant negative correlations with Cu for both sources ($r = -0.51$ to -0.58), consistent with competitive Cu-Mn interactions during uptake or translocation (Wairich et al. 2022).

Other nutrients displayed weak or source-dependent relationships with Cu. Under $CuSO_4$, P and Fe showed negative trends, whereas these associations were weak or slightly positive under soileos-Cu. Boron exhibited moderate positive correlations with Cu under both treatments, significant only for $CuSO_4$. Overall, soileos-Cu promoted more favourable synergistic nutrient interactions than $CuSO_4$, indicating that Cu release characteristics influence micronutrient balance in wheat tissues.

Effect of soileos Cu on wheat yield in different field soils. Field trials across four sites (3MG, ABG, New Era, and MacKenzie) showed that wheat yield responses to soileos-Cu were strongly dependent on baseline soil Cu availability (Figure 8). Yield increases were modest at sites with near-sufficient soil Cu, including 3MG (1.7 ppm) and ABG (1.0 ppm), ranging from approximately 1% to 6.8%, consistent with soils near the critical Cu threshold for wheat (Bolland and Brennan 2006).

In contrast, substantially greater yield responses were observed at Cu-deficient sites, particularly at MacKenzie (0.36 ppm) and New Era (0.73 ppm), where soileos-Cu increased grain yield by up to 13.3% at 22.4 kg/ha. These results highlight wheat's sensitivity to Cu deficiency, especially during reproductive development, when Cu-dependent enzymes and pollen viability are critical for grain set (Yruela 2009). Due to high variability in field conditions, the differences are not statistically significant, unlike those observed in the greenhouse under controlled conditions. Overall, soileos-Cu showed the greatest agronomic efficiency under Cu-limiting soil conditions, supporting targeted micronutrient management (Figure 9).

Dose response and application rate effects. Both 11.2 and 22.4 kg/ha application rates of soileos-Cu increased wheat yield across all sites, although responses were not consistently dose-dependent. At the 3MG and ABG sites, the lower rate achieved comparable or slightly greater yield gains, indicating that 11.2 kg/ha was sufficient to meet crop Cu demand. This response reflects a typical diminishing returns pattern for micronutrient fertilisation, where yield gains plateau once sufficiency is achieved. The absence of significant differences between rates highlights the efficiency of the soileos-Cu formulation, which provided consistent yield benefits across environments, including increases of up to 13.3% in Cu-deficient soils. It supports its high nutrient-use efficiency and its suitability for sustainable nutrient management. Consequently, in Cu-deficient soils, applying 22.4 kg/ha of soileos Cu can increase yield by up to 13%. In soils with low to marginal or even adequate Cu levels, applying 11.2 kg/ha of soileos Cu can improve yield by 1.4–6.8% compared to the grower standard.

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