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Citrus wastewaters increase soil nitrate and improve nutrient translocation in a copper contaminated soil-lettuce (*Lactuca sativa* L.) system

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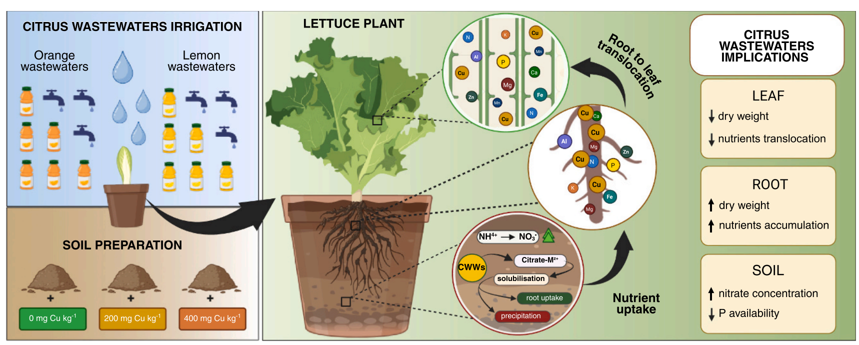
HIGHLIGHTS

- The effects of citrus wastewaters and copper were tested in a soil–plant system.
- Citrus wastewaters increased soil nitrate and promoted root growth.
- Nutrient translocations improved with citrus wastewaters under copper stress.
- Dilution enhanced the positive effects of citrus wastewaters on plant growth.

GRAPHICAL ABSTRACT



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1. Introduction

Water scarcity is concerned in many regions of the world, especially in arid and semi-arid areas where water availability has reached critical levels (Liu, J. et al., 2024; Ingrao et al., 2023). According to the expected increase in the global population growth rate (United Nations, Department of Economic and Social Affairs, Population Division, 2024), the estimated increase in food consumption will intensify the need for water for agricultural purposes.

Indeed, agricultural production is responsible for almost 70 % of total water withdrawals, while the industrial and domestic sectors account for approximately 22 % and 8 %, respectively (FAO, 2025).

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Consequently, agriculture emerges as the most water-intensive sector, significantly contributing to freshwater scarcity. This situation is being compounded by reduced water availability caused by climate change-induced droughts (Karimi et al., 2024; Dolan et al., 2021). To address these challenges and reduce the pressure on conventional freshwater sources, alternative and sustainable approaches must be implemented (Ssekyanzi et al., 2024).

In this context, the use of treated wastewater for agriculture practice is gaining attention as a strategy to alleviate freshwater scarcity (Muscarella et al., 2024; Paliaga et al., 2024; Yalin et al., 2023).

As a result, several recent studies have concentrated on identifying new alternative sources of water for irrigation practice (Christou et al., 2024). Among the possible unconventional water sources, citrus processing wastewaters (CWWs) from citrus industry represent a promising but still underexplored alternative for agricultural reuse.

The citrus fruit industry has significant global importance, producing approximately 150 million Mg in 2019 (FAO, 2021), and the European Mediterranean region, including Spain, Egypt, Turkey, and Italy, is a notable contributor to global production. Due to large-scale production, citrus fruit processing requires substantial amounts of water and, as a result, produces significant volumes of wastewater. Indeed, Zema et al. (2018) and Di Trapani et al. (2019) have estimated that the amount of CWWs accounts for approximately 1–17 m^3 per ton of processed fruits, depending on the plant's technology for fruit processing. Citrus wastewaters produced at the end of the processing are a mixture of fruit constituents and processing water. They are mainly derived from fruit washing, plants, and device cleaning, as well as cooling, essential oil extraction, and peel drying (Zema et al., 2019). Citrus wastewaters are characterized by low pH and high electrical conductivity. Moreover, they are rich in organic matter and contain nitrogen, phosphorus, some microelements (B, Al, Fe, Zn, Cu) and traces of essential oils (Ioppolo et al., 2020; Zema et al., 2018). However, due to the different processes contributing to their formation, the chemical properties of CWWs may be extremely variable, even daily (Lucia et al., 2022). Due to their chemical characteristics, CWWs could be used for crop irrigation without the need to be treated in a plant. Moreover, considering the significant role of citrus fruit production in the agri-food sector, the reuse of CWWs could help reduce the environmental impact of this industry and contribute to more sustainable agricultural practices. One of the former studies investigating the effect of CWWs on soil properties was that of Ioppolo et al. (2020). In such preliminary laboratory study, the authors moistened sandy-clay soil with CWWs and followed the dynamics of soil microbial biomass and activity, as well as the changes in chemical properties during 56 days of incubation. They found that total and extractable C pools, as well as microbial biomass C and N were stimulated by CWWs depending on their type and added dose. Moreover, Ioppolo et al. (2020) reported a greater stimulation effect of CWWs on fungi than on bacteria. They concluded that CWWs can play a key role in sustainable agriculture by improving the overall biological soil fertility.

However, due to their high concentration of organic acids that make them strongly acidic (pH ~ 3; Ioppolo et al., 2020), CWWs may increase the availability of soluble metals, in the form of both plant meso- and micro-nutrients or contaminants, especially in soil with a low buffer capacity. Recently, Pampinella et al. (2024) evaluated the effect of added CWWs on the dynamics of soil water-soluble metals (Ca, Mg, K, Na, Fe, Al, Co, Ni, Cu, Zn, and Cd) and acidity in a not calcareous soil for 28 days. They found that soil pH promptly decreased following the addition of CWWs but after 7 days it recovered the initial value. Because of the pH decrease, the concentration of almost all investigated metals sharply increased. The authors ascribed the increase in metals availability to the pH decrease for different causes: the exchange reaction between H^+ and cations adsorbed onto colloid surfaces, the addition of organic matter by CWWs that stimulated microbial activity, which in turn acidified soil, and the quantity and type of organic acids added via CWWs. Just after 7 days since the addition of CWWs, the concentration of divalent and trivalent cations was lower than the control soil, where tap water instead of CWWs was used to moist soil.

Among the eight micronutrients essential for plants, copper (Cu) is necessary for several enzymatic processes, acting as a co-factor, but also for the formation of chlorophyll and seeds (Tripathi et al., 2015). In fact, compounds holding Cu have been used as fertilizers over the years. However, Cu added at high doses to soil might become exceedingly toxic for plants, causing symptoms such as chlorosis, necrosis, stunted growth, leaf discoloration, and restricted root development (Pietrini et al., 2019). For example, routine use of Cu-based fungicides (like Cu sulphate and Cu oxychloride) to protect grapevine plants against pests has resulted in a long-term Cu accumulation in vineyard soils, reaching concentrations significantly higher than the trace amounts needed for healthy plant growth (Pietrzak and McPhail, 2004). Moreover, studies by Ambrosini et al. (2018) have shown that Cu toxicity not only induces changes in grapevine roots, such as increased root diameter and additional cortical layers, but also inhibits the uptake of essential nutrients like P and K. Indeed, the excess of Cu interferes with the plant's transport mechanisms, resulting in an imbalance in nutrient absorption and limiting the availability of crucial nutrients, like P and K, to the shoots. Besides its effects on plants, Cu plays a crucial role in soil biological processes, particularly nitrification. During nitrification, the ammonia monooxygenase (AMO) enzyme, which in the active site contains Cu, oxidizes NH_4^+ to hydroxylamine. This is further oxidized to NO_2^- and NO_3^- by hydroxylamine oxidoreductase and nitrite oxidoreductase enzymes, respectively (Preena et al., 2021; Li et al., 2018; Tao et al., 2017; Arp et al., 2002). Consequently, AMO activity, and thus soil nitrification, depends on Cu as a key co-factor (Gorman-Lewis et al., 2019; Wagner et al., 2019).

The availability of metals, among which Cu, in soil is determined by a variety of factors among which pH and organic matter (Azeez et al., 2006). Thus, any management practice affecting chemical and biochemical soil properties indirectly affect Cu availability.

To date the effect of CWWs on availability of nutrient and contaminant elements in calcareous soils, to our knowledge, has not been investigated. It is presumable that carbonates could buffer the acidity of CWWs, thus not decreasing soil pH and increasing nutrients availability. On the other hand, the addition of organic acids by CWWs, mainly citric acid, could lead to the formation of Cu-Al-hydroxide coprecipitates (Terzano et al., 2017) thus lowering Cu availability in soil. These implications highlight the need to investigate how CWWs influence Cu behavior in calcareous soils, particularly in regard to Cu availability, nutrient interactions, and microbial processes such as nitrification. Although the direct application of CWWs to agricultural soils is currently not permitted under Italian legislation (Legislative Decree 152/2006), their reuse may be allowed after treatment, particularly by small farms with on-site processing activities. This treatment may consist of simple accumulation or natural stabilization steps (Zimbone and Zema, 2009). Moreover, scientific evidence from controlled experiments such as this study is essential to support future regulatory assessments and guide the development of safe and sustainable practices for the reuse of CWWs in agriculture.

Therefore, within a circular economy perspective that includes the reuse of wastewater for crop irrigation, the aim of this study was twofold:

i) to investigate the effects of CWWs on soil Cu availability and, in turn, on nitrification; ii) to evaluate the impact of CWWs, applied in combination with different Cu concentrations, on the translocation and accumulation of selected elements in plants.For this purpose, a greenhouse experiment, which lasted 46 days, was set up using two different types of CWWs at three different concentrations. Calcareous soil was selected for this study as the impact of CWWs has not been studied under alkaline soil conditions. Moreover, the lettuce plant (*Lactuca sativa* L.) was chosen as a crop model, due to its sensitivity to nutrients availability and fast growth.

2. Materials and methods

2.1. Citrus wastewaters

Citrus processing wastewaters were collected at an early stage of the citrus transformation process, from a local citrus industry (Eurofood S.r.l., Messina, Italy), before being mixed with other effluents or treated as part of the facility's wastewater management. Although these materials contain fruit-derived organic compounds, they do not include industrial detergents or sewage components. They were obtained by cold pressing of peels from two different citrus species: *Citrus sinensis* (L.) Osbeck (orange) and *Citrus limon* (L.) Osbeck (lemon). CWWs were analyzed to determine their main chemical properties (Table 1).

Density of CWWs was determined by filling a 50 mL volumetric flask and weighing it to calculate the mass to volume ratio. Reaction (pH) and electrical conductivity (EC) of CWWs were determined on undiluted volumes after filtration through Whatman #42 paper using a pH meter (FiveEasy, Mettler Toledo Spa, Milan, Italy) and a conductometer (HI5321, Hanna Instruments Italia srl, Padua, Italy), respectively. Total organic carbon and nitrogen were determined by the acid digestion-oxidation method with potassium dichromate (Walkley and Black, 1934) and by micro-Kjeldahl acid digestion-distillation method (Bremner, 1996), respectively. Total carbohydrates were determined by the anthrone reaction method after acid hydrolysis of CWWs (Hedge et al., 1962), while soluble monosaccharides were determined by titration with Fehling's reagent as described by Yebra et al. (1993). Total fiber was determined according to the method described by Prosky et al. (1988). Ash content was determined after heating CWWs at 550 °C to dryness in a muffle. Total phosphorus was determined on an aliquot of the CWWs ashes by spectrophotometry (UVmini-1240, Shimadzu Italia srl, Milan, Italy), after forming a blue-colored complex with ammonium molybdate solution (Murphy and Riley, 1962). The total contents of Ca, Mg, K, Na, Al, Fe, Cu, Zn, Mn, Ni and Cd were determined by Microwave Plasma Atomic Emission Spectroscopy (MP-AES; Agilent 4210 MP-AES, Milan, Italy). This was performed after the acidification of the CWWs with ultrapure nitric acid. All reagents were provided by Merck KGaA (Darmstadt, Germany).

2.2. Sampling and experimental design

The experiment was carried out in a greenhouse at the Department of Agricultural, Food and Forest Sciences (University of Palermo, Italy, 38°06′24″N 13°21′02″E). Pots of 10x10x10 cm were filled with 500 g of orchard soil collected from an area within the Department. Before filling, the soil was air dried, sieved at <4 mm and mixed with 10 % of peat (*w/w*) to increase its fertility. The plant selected for pot experiment was *Lactuca sativa* L. due to its sensitivity to soil nitrogen availability and rapid growth. One plant at two leaves stage was transplanted in each pot. The main characteristics of the used soil were: clay 27 %, sand 51 %, silt 22 %, pH in water 7.9, electrical conductivity (1:2.5, *w/v*) 0.48 dS m^{-1}, total organic carbon 49.4 g kg^{-1}, total nitrogen 3.5 g kg^{-1}, total carbonates 17 %. The soil was moistened up to 50 % of its water holding capacity with a Cu sulphate ($CuSO_4$) solution to reach two different concentrations of total Cu added to soil: 200 and 400 mg kg^{-1}. A control group without Cu addition was also included. After planting the lettuce, pots were placed in the greenhouse in an open field. The lettuce growth lasted 46 days. During the first two weeks after planting, all pots were irrigated daily with tap water to support seedling establishment. From day 15 to day 35 (weeks 3–5), plants were irrigated three times per week with lemon wastewaters (LWWs) or orange wastewaters (OWWs), applied at three different dilutions (1/3, 2/3, or 3/3, corresponding to 138, 275, and 550 mL per pot, respectively, over the whole period) representing progressively increasing proportions of the wastewater. For the 1/3 and 2/3 dilutions, the residual volume was compensated with tap water. On the remaining days of each week, if necessary, only tap water was used for irrigation. The amount of water used for each irrigation was to maintain soil at 50 % of its water holding capacity and avoid nutrient leaching. This irrigation strategy was designed to simulate real field conditions and to minimize the use of tap water while avoiding salt accumulation, given the high electrical conductivity of citrus processing wastewaters (as reported by Ioppolo et al., 2020). During the last almost two weeks (days 36–46), all plants were again irrigated only with tap water. In total, the experiment included 63 pots: 2 types of wastewaters (LWWs, OWWs) × 3 dilution levels × 3 Cu concentrations (0, 200, 400 mg kg^{-1}) × 3 replicates, plus 9 control pots irrigated exclusively with tap water.

Table 1
Main chemical and physical properties of lemon and orange wastewaters (LWWs and OWWs, respectively). Different lowercase letters indicate significant differences ($p < 0.05$) between the wastewaters.

Parameters		LWWs	OWWs
pH		3.6 a	3.0 a
Electrical conductivity	dS m^{-1}	2.8 a	2.6 a
Density	g cm^{-3}	1.01 a	1.04 a
Total carbon	g L^{-1}	15.0 b	17.7 a
Total nitrogen	mg L^{-1}	51 b	366 a
Total soluble monosaccharides	%	1.4 b	4.6 a
Total carbohydrates	%	3.5 b	4.4 a
Total fibers	%	1.3 b	1.6 a
Citric acid	g L^{-1}	10.7 a	6.1 b
Total phosphorus	mg L^{-1}	45.9 b	56.5 a
Ca	mg L^{-1}	95.1 a	91.8 b
Mg	mg L^{-1}	32.2 b	42.4 a
K	mg L^{-1}	373 a	377 a
Na	mg L^{-1}	46.7 a	49.3 a
Al	mg L^{-1}	4.93 b	7.02 a
Fe	mg L^{-1}	0.91 a	0.93 a
Cu	mg L^{-1}	0.25 b	0.37 a
Zn	mg L^{-1}	0.12 b	0.55 a
Mn	mg L^{-1}	0.05 a	0.07 a
Ni	mg L^{-1}	0.01 a	0.02 a
Cd	mg L^{-1}	n.d.	n.d.

2.3. Soil and plant analysis

At the end of growth, lettuce plants were removed from the pots. Exchangeable ammonium and nitrate contents were determined on ten grams of fresh soil samples, placed in plastic bottles and added with 40 mL of K_2SO_4 0.5 M. Soil slurries were stirred at 75 rpm for 45 min and then filtered through Whatman #42 paper. The extract was used for the determination of ammonium, according to the Berthelot method (Mulvaney, 1996), and nitrate using the Spectroquant® Nitrate test (Merck KGaA, Darmstadt, Germany), where nitrate ions react in concentrated sulfuric acid with a benzoic acid derivative to form a red nitro compound that is determined through spectrophotometer (UVmini-1240, Shimadzu Italia srl, Milan Italy) reading at 525 nm. Air-dried soil samples from each pot were sieved at <2 mm and further analyzed for total Ca, Mg, K, Mn, Cu, Fe, Al and Zn, after acid digestion according to US EPA Method 3050B (EPA, U.S., 1996). Briefly, 10 mL of 1:1 (*v/v*) water-concentrated (65 %) HNO_3 solution was added to 0.5 g of soil sample in a porcelain crucible. The samples were then placed in a hot plate and heated to 95 ± 5 °C for about 15 min. After cooling, 5 mL of 65 % HNO_3 were added to crucibles and heated for a further 30 min. The addition of concentrated HNO_3 was repeated until no brown fumes evolved. The digests were again allowed to cool, then 2 mL of Milli-Q water and 3 mL of 30 % H_2O_2 were added and heated to 95 ± 5 °C. After cooling again, another 2 mL of 30 % H_2O_2 were added. Heating continued until the sample volumes reduced to approximately 5 mL. After cooling, the digested samples were quantitatively transferred to 20 mL volumetric flasks, brought up to total volume with 2 % HNO_3 and analyzed by MP-AES as above.

Soil available Cu was determined on diethylenetriaminepentaacetic acid (DTPA) soil extract (Lindsay and Norvell, 1978). The extraction solution had a pH of 7.3 and consisted of 5 mM DTPA, 0.01 M calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$) and 0.1 M triethanolamine. Ten grams of soil were weighed and 20 mL of the DTPA extraction solution was added (ratio 1:2, *w/v*). After 120 min of shaking at 80 rpm, the samples were filtered through Whatman #42 paper and analyzed by MP-AES. Available phosphorus was determined by the Olsen method (Olsen and Sommers, 1982) and total nitrogen (TN) by Kjeldahl method (Bremner, 1996).

The lettuce plants were removed from the pots after 46 days growth and the roots were rapidly washed, first with tap water and then with distilled water. Roots and leaves of the lettuce plants were separated and oven-dried at 60 °C for 48 h, and the dry weight was determined. Dried lettuce plants were ground and stored in plastic bottles for further analysis. Roots and leaves were mineralized by wet digestion with concentrated HNO_3 (65 %) and hydrogen peroxide (H_2O_2) at 30 % (Jones Jr. and Case, 1990). Briefly, 0.5 g of dried sample was weighed into a porcelain crucible and placed in a muffle furnace. The furnace temperature reached 500 °C in about 2 h and the samples were left overnight. The samples were then removed and, after cooling, 5 mL of 65 % HNO_3 and 5 mL of 30 % H_2O_2 were added. Then, they were placed in a hot plate at 250 °C for 2 h and the addition of HNO_3 and 30 % H_2O_2 was repeated if necessary. After cooling the digested samples were quantitatively transferred to 20 mL volumetric flasks, brought up to total volume with 2 % HNO_3 and analyzed by MP-AES.

The mineralized samples were analyzed for total P by the Spectroquant® Phosphate test (Merck KGaA, Darmstadt, Germany) where in the presence of vanadate, phosphates react with molybdates to form yellow phospho-vanadomolybdate. That is determined through spectrophotometer (UVmini-1240, Shimadzu Italia srl, Milan Italy) reading at 410 nm, while total Ca, Mg, K, Mn, Cu, Fe, Al and Zn by MP-AES. Total N was determined on dried biomass by the Kjeldahl method (Bremner, 1996).

The translocation factor of each element was calculated as the ratio between the concentration of a given element in leaves (C_{leaves}) and the concentration of the same element in roots (C_{roots}) (Hussain et al., 2022; Zhang et al., 2002), as follows:

$$Translocation\ Factor = \frac{C_{leaves}}{C_{roots}} \tag{1}$$

The accumulation factor of each element, except for nitrogen and phosphorous, was calculated as the ratio between the concentration of a given element in the root (C_{roots}) and the concentration of the same element in soil (C_{soil}) (Hussain et al., 2022; Deng et al., 2004) as follows:

$$Accumulation\ Factor = \frac{C_{roots}}{C_{soil}} \tag{2}$$

To facilitate comparison among treatments, translocation and accumulation factors of each element were normalized using min–max scaling. This method transformed each value to a range between 0 and 1, according to the following formula:

$$X_{norm} = \frac{x - x_{min}}{x_{max} - x_{min}} \tag{3}$$

where x is the value of the variable, and x_{min} and x_{max} are the minimum and maximum values of the variable within the same Cu concentration. The corresponding standard deviation was normalized using the same scaling factor:

$$SD_{norm} = \frac{SD}{x_{max} - x_{min}} \tag{4}$$

2.4. Statistical analysis

Reported data are arithmetic means ± standard deviations of three replicates. Data related to soil properties, plant parameters, plant nutrient contents, translocation and accumulation factors were subjected to three-way ANOVA, including irrigation water type (LWWs or OWWs), CWWs dilution (1/3, 2/3, 3/3) and Cu concentration (0, 200, 400 mg kg^{-1}) as fixed factors. The control treatment irrigated only with tap water was not included in the factorial ANOVA model. For each dependent variable, the proportion of variance explained by each factor and their interactions was estimated by calculating the percentage of Eta Squared (η^2), defined as the ratio between the sum of squares of each effect (SS_{effect}) and the corrected total sum of squares ($SS_{corrected\ total}$):

$$Explained\ variance\ (\%) = \frac{SS_{effect}}{SS_{corrected\ total}} \times 100 \tag{5}$$

This index represents the percentage of total variance attributable to each factor or interaction (Richardson, 2011). Given the number of factors and response variables considered in this study, reporting the percentage of explained variance allowed us to clearly assess the relative importance of each factor and their interaction. Tukey's HSD post-hoc test was used to assess significant differences among factor levels ($p < 0.05$). To enhance figure readability, significance letters were omitted but are provided in Supplementary Tables S1–S9.

3. Results

3.1. Total and available Cu

Total Cu in soil without the addition of Cu was on average 34 mg kg^{-1} of which 13 % was plant available (Table 2). Following the addition of Cu sulphate, total and available Cu, as expected, were proportionally increased. Therefore, the most significant factor was the addition of Cu, which explained 94 % of the variance in total Cu and 97 % in available Cu. They were also significantly affected ($p < 0.01$) by the interaction between water type and Cu concentration, although this interaction explained <1 % of the variance. Specifically, soil added with 400 mg Cu kg^{-1} and irrigated with LWWs showed lower values of total and available Cu compared to soil irrigated with OWWs ($p < 0.05$).

3.2. Soil mineral nitrogen and available phosphorus

Soil ammonium (NH_4^+-N) was detected only in soils irrigated with not diluted OWWs regardless of Cu concentration (Table 3).

Soil nitrates (NO_3^--N) concentration were mainly affected by the interaction among the three factors: water type, CWWs dilution, and Cu concentration (39 % of explained variance) (Table 3). In the absence of Cu addition, NO_3^--N increased in soils irrigated with CWWs, with an average increase of 112 % with OWWs and 95 % with LWWs compared to the control. Increasing LWWs concentration led to a decrease in NO_3^--N concentrations, whereas no consistent trend was observed for OWWs. In soils with Cu addition, NO_3^--N concentration was not significantly affected by either water type or CWWs dilution. On the other hand, in soil irrigated with tap water, Cu addition increased soil NO_3^--N by approximately 122 %, with no significant differences between 200 or 400 mg Cu kg^{-1} treatments.

Soil available P (Fig. 1) was affected by Cu concentration ($p < 0.01$) and its interaction with water type ($p < 0.01$) which together explained 59 % of variance. In soils irrigated with tap water, available P decreased with the addition of Cu. Moreover, without the addition of Cu, soils irrigated with LWWs showed lower available P compared to OWWs ($p < 0.01$), regardless of dilution.

3.3. Total N and P in leaves and roots

Total nitrogen (N) in leaves ranged from 27 to 68 mg (Fig. 2a) and was affected mainly by Cu concentration ($p < 0.001$), the interaction between water type and dilution ($p < 0.01$), and by the three-way interaction among water type, dilution and Cu concentration ($p <$

Table 2
Total and available copper in soil cropped with lettuce in soil without the addition of Cu, soil added with 200 mg Cu kg^{-1}, soil added with 400 mg Cu kg^{-1} and irrigated with tap water (TAP), and with orange (OWWs) and lemon (LWWs) wastewaters at three different dilutions (1/3. 2/3 and 3/3). Different lower-case letters indicate significant differences ($p < 0.05$) among dilution within the same wastewater (OWWs and LWWs). Different upper-case letters indicate significant differences ($p < 0.05$) between wastewaters at the same dilution (1/3, 2/3 and 3/3). Numbers in bold indicate significant differences ($p < 0.05$) compared with the control (TAP) at the same Cu addition (0, 200 and 400 mg Cu kg^{-1}). D.L., detection limit. For each variable, the percentage of variance explained by water type, CWWs dilution, Cu concentration, water type x CWWs dilutions interaction, water type x Cu concentration interaction, water dilution x Cu concentration interaction and water type x CWWs dilution x Cu concentration interaction is also reported.

Treatments	Cu added	Dilution	Total Cu	Available Cu
	mg kg^{-1}		mg kg^{-1}	
TAP			35 ± 4	4.4 ± 0.2
		1/3	32 Aa	4.7 Aa
OWWs		2/3	35 Aa	**3.9** Bb
	–	3/3	33 Aa	5.2 Aa
		1/3	32 Aa	4.5 Aa
LWWs		2/3	38 Aa	4.3 Aa
		3/3	33 Aa	4.0 Ba
TAP			241 ± 17	79.1 ± 3.1
		1/3	260 Aa	**72.3** Ba
OWWs		2/3	247 Ab	75.8 Aa
	200	3/3	234 Ab	76.8 Aa
		1/3	**282** Aa	**84.9** Aa
LWWs		2/3	**213** Bb	77.2 Ab
		3/3	**206** Bb	72.7 Ab
TAP			464 ± 60	159.7 ± 6.0
		1/3	512 Aa	147.5 Aa
OWWs		2/3	490 Aa	148.5 Aa
	400	3/3	500 Aa	**135.6** Aa
		1/3	390 Bab	**134.6** Aa
LWWs		2/3	**258** Bb	**122.5** Bb
		3/3	417 Bab	**123.5** Bb
Water			*1.4****	*0.2**
Dilution			*0.3**	*0.3***
Cu			*94.2****	*97.1****
Water x Dilution			*0.1 ns*	*0.04 ns*
Water x Cu			*2.2****	*0.5***
Dilution x Cu			*0.6***	*0.4**
Water x Dilution x Cu			*0.3 ns*	*0.3 ns*

*, **, and *** significant at p ≤ 0.05, p ≤ 0.01, and p ≤ 0.001, respectively; ns, not significant.

Table 3
Total and mineral (ammonia and nitrate) nitrogen pools in soil cropped with lettuce in soil without the addition of Cu, soil added with 200 mg Cu kg^{-1}, soil added with 400 mg Cu kg^{-1} and irrigated with tap water (TAP), and with orange (OWWs) and lemon (LWWs) wastewaters at three different dilutions (1/3. 2/3 and 3/3). Different lower-case letters indicate significant differences ($p < 0.05$) among dilution within the same wastewater (OWWs and LWWs). Different upper-case letters indicate significant differences ($p < 0.05$) between wastewaters at the same dilution (1/3, 2/3 and 3/3). Numbers in bold indicate significant differences ($p < 0.05$) compared with the control (TAP) at the same Cu addition (0, 200 and 400 mg Cu kg^{-1}). D.L., detection limit. For each variable, the percentage of variance explained by water type, CWWs dilution, Cu concentration, water type x CWWs dilutions interaction, water type x Cu concentration interaction, water dilution x Cu concentration interaction and water type x CWWs dilution x Cu concentration interaction is also reported.

Treatments	Cu added	Dilution	NH_4^+-N	NO_3^--N
	mg kg^{-1}		mg kg^{-1}	
TAP			<D.L.	5.0 ± 0.2
		1/3	<D.L.	6.4 Bb
OWWs		2/3	<D.L.	**16.9** Aa
	–	3/3	**4.0** Aa	**8.5** Ab
		1/3	<D.L.	**14.8** Aa
LWWs		2/3	<D.L.	6.5 Bb
		3/3	<D.L.	7.8 Ab
TAP			<D.L.	11.80 ± 1.3
		1/3	<D.L.	**14.6** Aa
OWWs		2/3	<D.L.	9.5 Ab
	200	3/3	**2.5** Aa	8.3 Ab
		1/3	<D.L.	11.1 Aa
LWWs		2/3	<D.L.	10.6 Aa
		3/3	<D.L.	10.8 Aa
TAP			<D.L.	10.4 ± 1.0
		1/3	<D.L.	14.7 Aab
OWWs		2/3	<D.L.	**15.3** Aa
	400	3/3	**6.7** Aa	9.7 Bb
		1/3	<D.L.	8.7 Ab
LWWs		2/3	<D.L.	11.0 Aa
		3/3	**0.3** Ba	**14.2** Aa
Water			*12.0****	*1.5 ns*
Dilution			*26.1****	*5.4**
Cu			*2.3 ns*	*5.8****
Water x Dilution			*24.0****	*13.7****
Water x Cu			*1.8 ns*	*1.3 ns*
Dilution x Cu			*4.6 ns*	*6.3 ns*
Water x Dilution x Cu			*3.5 ns*	*39.3****

*, **, and *** significant at p ≤ 0.05, p ≤ 0.01, and p ≤ 0.001, respectively; ns, not significant.

0.01), which together explained 58 % of the variance. Overall, irrigation with CWWs decreased total N in leaves compared to soil irrigated with tap water. Generally, the addition of Cu increased N in leaves, especially with the addition of 400 mg of Cu per kilogram of soil.

Total N in roots was mainly affected by CWWs dilution with 25 % of explained variance ($p < 0.001$), with values ranging from 49 to 10 mg. Regardless Cu concentration, soils irrigated with undiluted CWWs, resulted in lower roots N content ($p < 0.01$) compared to the other two dilution levels (1/3 and 2/3). Total N in roots increased by increasing Cu concentration with a significant difference between soils without the addition of Cu and soils added with 400 mg of Cu per kilogram of soil ($p < 0.05$). Total phosphorus (P) in leaves and roots was generally higher in soils irrigated with tap water, followed by OWWs and LWWs, regardless of Cu concentration (Fig. 2b). Total P in leaves was significantly influenced by water type, CWWs dilution, and Cu concentration, as well as by several interactions. The highest contribution to variance was explained by the interaction between dilution and Cu concentration (25 % of explained variance; $p < 0.001$). Notably, P in leaves decreased with increasing Cu concentrations and in soils irrigated with undiluted CWWs. Total P in roots was primarily affected by Cu concentration and CWWs dilution ($p < 0.001$), which respectively explained 18 % and 17 % of the variance. Therefore, root P was the lowest in soils irrigated with undiluted LWWs. Cu addition increased P in roots in most treatments, especially at 400 mg Cu kg^{-1}.

3.4. Translocation and accumulation factor

Generally, translocation (TF) and accumulation (AF) factors were mainly affected by Cu concentration and by its interaction with water type or CWWs dilution.

Nitrogen translocation factor (N-TF, Fig. 3a) increased by increasing Cu concentration, especially in soils irrigated with tap water. Across the Cu concentration levels, N-TF was affected mainly by CWWs dilution (37 %, $p < 0.001$) rather than CWWs type ($p = 0.065$). Indeed, by increasing CWWs concentration N-TF increased, regardless water type.

Instead, phosphorus translocation factor (P-TF, Fig. 3b) was mainly affected by water type, which explained 20 % of the variance ($p < 0.001$). LWWs treatments resulted in a significant decrease in P-TF compared to tap water and OWWs treatments ($p < 0.05$), regardless of CWWs dilution and Cu concentration. The calculation of accumulation factor of N and P was not possible due to the significant difference in the order of magnitude of the concentrations of N in roots and soil nitrate, and P in roots and soil available P.

Both copper translocation (Cu-TF) and accumulation factors (Cu-AF)

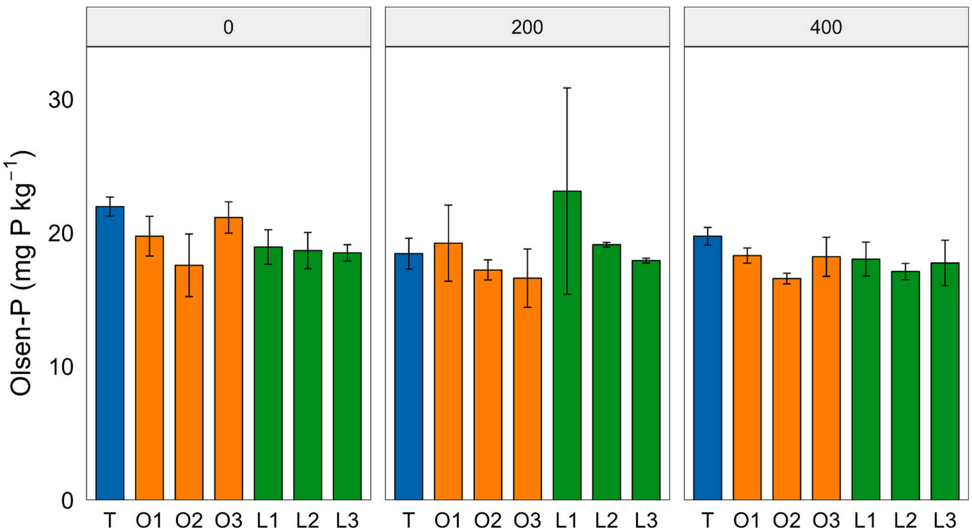

Fig. 1. Available phosphorus in soil cropped with lettuce in soil without the addition of Cu (0), soil added with 200 mg Cu kg^{-1} (200), soil added with 400 mg Cu kg^{-1} (400). Each error bars represents standard deviation (n = 3). T = tap water; O = OWWs; L = LWWs; 1 = 1/3; 2 = 2/3; 3 = 3/3.

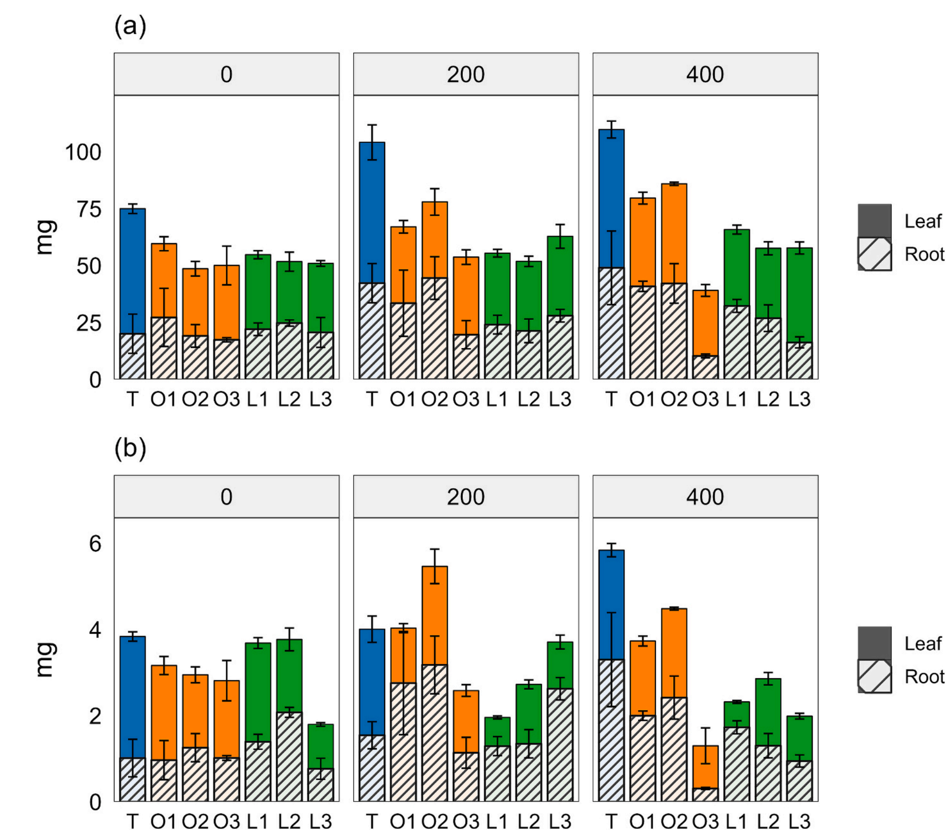

Fig. 2. Total nitrogen (a) and total phosphorus (b) in lettuce in soil without the addition of Cu (0), soil added with 200 mg Cu kg^{-1} (200), soil added with 400 mg Cu kg^{-1} (400). Each error bars represents standard deviation (n = 3). T = tap water; O = OWWs; L = LWWs; 1 = 1/3; 2 = 2/3; 3 = 3/3.

(Fig. 4) were significantly affected by the three factors (water type, CWWs dilution and Cu concentration) and by their interaction. However, the relevance of each factor was different. Indeed, Cu-TF was mainly affected by the interaction between water type and Cu concentration (28 %). Instead, Cu-AF by Cu concentration (43 %). However, soils irrigated with OWWs, generally, resulted in significantly higher Cu-TF ($p < 0.05$) and lower Cu-AF ($p < 0.05$), compared to LWWs.

The TF and AF of calcium (Ca), potassium (K), and manganese (Mn) showed similar trends in response to Cu concentration (Figs. 5–6). For all three elements, TF decreased while AF increased with increasing Cu concentration, which was the main factor explaining the variance in Ca-TF (43 %), Ca-AF (41 %), and K-AF (59 %).

Additionally, K-TF was significantly influenced by water type (30 %), with LWWs showing lower values than OWWs and tap water, regardless of CWWs dilution. Ca-TF was also affected by water type, although no consistent trend was observed across all treatments.

Mn-TF responded similarly, decreasing with increasing Cu, but was influenced by all three factors (water type, dilution, and Cu) without a

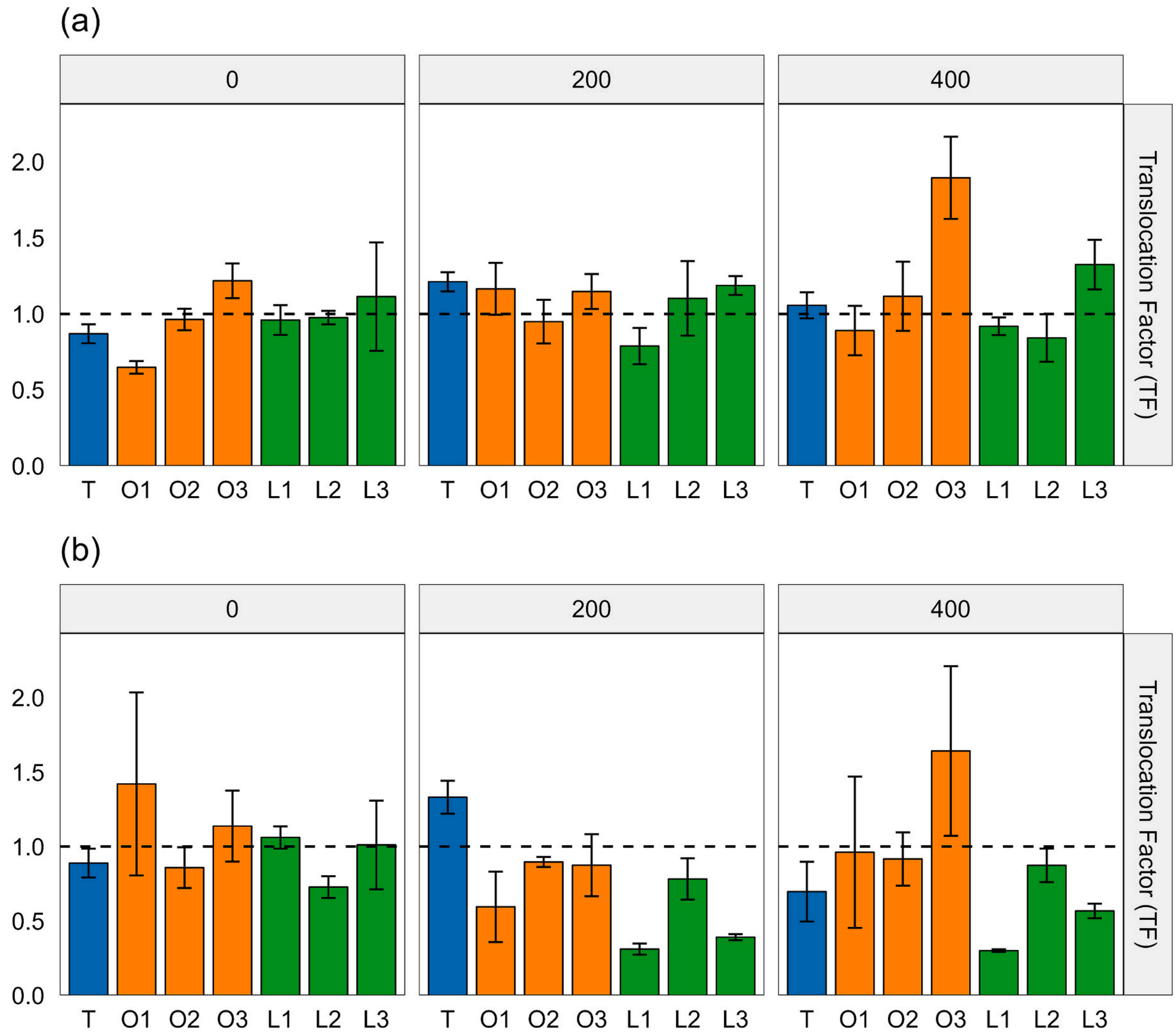

Fig. 3. Translocation factor of nitrogen (a) and phosphorus (b) in lettuce in soil without the addition of Cu (0), soil added with 200 mg Cu kg^{-1} (200), soil added with 400 mg Cu kg^{-1} (400). Each error bars represents standard deviation (n = 3). T = tap water; O = OWWs; L = LWWs; 1 = 1/3; 2 = 2/3; 3 = 3/3.

single dominant effect. In the absence of Cu, LWWs significantly reduced Mn-TF compared to OWWs and tap water ($p < 0.05$), while the opposite occurred when Cu was added.

In contrast, magnesium (Mg) showed a different pattern (Fig. 5). Mg-TF was mainly influenced by water type (34 %), with higher values under OWWs irrigation compared to LWWs. Meanwhile, Mg-AF was strongly and significantly affected by Cu concentration (69 % of explained variance; $p < 0.001$). Soils treated with 400 mg Cu kg^{-1} showed higher Mg-AF under tap water, but lower under CWWs treatments.

Iron (Fe), aluminum (Al) and zinc (Zn) generally behaved in the same manner with regard both TF and AF (Figs. 7–8). The translocation of both elements was mostly influenced by the interaction between CWWs dilution and Cu concentration, which explained 20 %, 23 % and 23 % of variance for Fe, Al and Zn respectively ($p < 0.001$). This result is mostly explained by the treatment with undiluted OWWs combined with 400 mg Cu kg^{-1}, which resulted in the highest TF values for all three elements. Moreover, OWWs and LWWs affected differently Fe-TF and Al-TF. Indeed, without the addition of Cu, translocation of Fe and Al was generally higher in OWWs treatments, compared to LWWs treatments. The addition of 200 mg Cu kg^{-1} led to a decrease in TF and a corresponding increase in AF of both Fe and Al, especially under OWWs treatments.

Normalized translocation factors were higher for several elements (e.g. Zn, Mn, Mg, K, Fe, Ca, and Al) with CWWs irrigation, particularly at 200 mg Cu kg^{-1}, compared to those irrigated with tap water (Figs. S1-S9). In contrast, accumulation factors were generally lower under CWWs treatments.

3.5. *Plant dry weights*

Total plant dry weights were affected mainly by CWWs dilution, decreasing as the concentration of CWWs increased (Fig. 9). The lowest value (3.9 g) resulted in soil treated with 400 mg Cu kg^{-1} and irrigated with undiluted OWWs. Leaves dry weight was primarily affected by CWWs dilution (40 %), followed by water type (22 %). Leaves weight generally decreased in treatments with CWWs, being such decrease more consistent with not diluted CWWs, and in soils irrigated with OWWs rather than LWWs.

Root dry weight was mainly affected by CWWs dilution (22 %) and the interaction between CWWs dilution and Cu concentration (12 %; $p < 0.05$). Generally, the addition of Cu increased roots wight, except in soils irrigated with not diluted CWWs, where root weight was reduced, particularly in the OWWs treatments. In soil without the addition of Cu, weight of roots was not significantly affected by water type or dilution, except for LWWs, which resulted in higher values compared to OWWs.

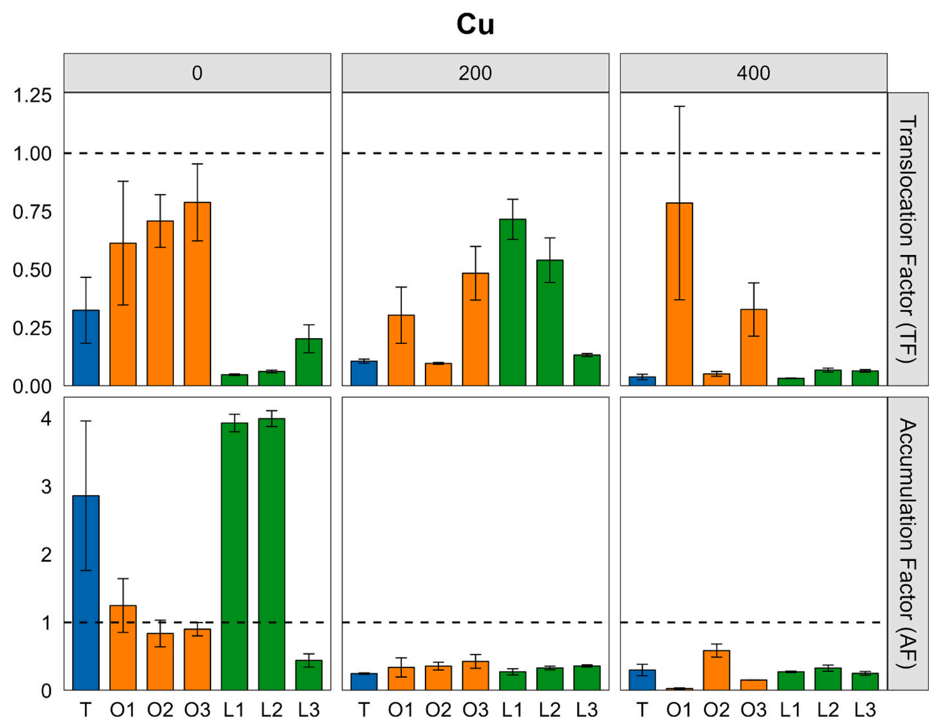

Fig. 4. Translocation factor (TF) and accumulation factor (AF) of copper (Cu) in lettuce in soil without the addition of Cu (0), soil added with 200 mg Cu kg^{-1} (200), soil added with 400 mg Cu kg^{-1} (400). Each error bars represents standard deviation (n = 3). T = tap water; O = OWWs; L = LWWs; 1 = 1/3; 2 = 2/3; 3 = 3/3.

4. Discussion

4.1. Effect of CWWs on available Cu

Understanding how CWWs affect Cu availability in soil is an important prerequisite when considering their use in irrigation practice, particularly in the context of a circular economy. In this study, the effect of CWWs on Cu availability was assessed using a soil added with different Cu concentrations, i.e. below, equal to and above the threshold value for agricultural soils (200 mg Cu kg^{-1}) provided by Italian law (Italian Legislative Decree 46/2019). Results suggested that in soils without the addition of Cu or added with 200 mg kg^{-1} of Cu, the irrigation with CWWs, regardless of their dilution, did not significantly alter Cu availability. This could be ascribed to the timing of soil sampling, which occurred one week after the last CWWs application. During this week, plants were irrigated with tap water thus nullifying the acidifying effect of CWWs and allowing Cu to reprecipitate in less available forms. In contrast, in soil amended with 400 mg Cu kg^{-1} and irrigated with LWWs, the concentration of available Cu decreased. Such results may be due to the coprecipitation of Cu hydroxide as aluminum and iron hydroxides (Terzano et al., 2017), promoted by the organic acids (mainly citric acid) present in LWWs. However, this effect was observed only at the highest Cu dose (400 mg kg^{-1}), suggesting that LWWs may reduce Cu availability under severe contamination conditions. Although the interaction was statistically significant, it explained <1 % of the total variance. Nevertheless, this finding may be relevant for potential agronomic applications, especially in Cu-contaminated soils. Further studies are needed to clarify the underlying mechanisms to evaluate whether this effect can be reproduced under field conditions. A similar pattern was not observed in soil irrigated with OWWs, probably due to the lower content of organic acids compared to LWWs (Table 1).

4.2. Nitrogen dynamics in the soil-plant system

The application of CWWs and Cu significantly affected soil NH_4^+-N and NO_3^--N concentrations, that in turn affected plant nitrogen content. The presence of NH_4^+-N only in soil irrigated with undiluted OWWs, independently of Cu concentration, is probably due to the high input of organic N by not diluted OWWs (Table 1), which, being mineralized is transformed in NH_4^+-N (Breza and Grandy, 2025; Brady and Weil, 2008). This suggests that the organic nitrogen supplied is easily mineralized and the ammonium provided exceeded the immediate microbial nitrification capacity, allowing its temporarily accumulation. Despite this, soil NO_3^--N increased in almost all CWWs treatments compared to the control. This effect may be explained by the organic N supply, as well as by the stimulation of microbial biomass and activity promoted by the organic compounds in CWWs, as reported by Ioppolo et al. (2020). Interestingly, the combination of Cu and CWWs did not result in an additive effect on soil nitrate. As reported in Table 3, the addition of Cu to water treatments significantly increased NO_3-N concentrations. This increase could be ascribed to the enhanced activity of ammonia-oxidizing microorganisms (Gwak et al., 2020; Matse et al., 2022; Wagner et al., 2019). However, nitrate levels in CWW-treated soils remained relatively unchanged regardless of the amount of Cu added. This suggests that, although CWWs stimulated nitrification, they may have simultaneously reduced Cu bioavailability for microorganism through the formation of stable organic complexes. Indeed, organic compounds in CWWs can bind Cu ions, limiting their interaction with soil microbes and thus mitigating Cu-induced effects (Liu, M. et al., 2024; Nogueira et al., 2009). Finally, comparing plant N content and soil NO_3^--N, it can be observed that, unlike the control, plants irrigated with CWWs showed lower total N content compared to the control, thus indicating reduced N uptake. This could be linked to the temporally changes in soil pH caused by repeated CWWs irrigation, which is known to affect nutrient availability and assimilation (Magray et al., 2022; Neina, 2019).

4.3. Phosphorus dynamics in the soil-plant system

Copper addition reduced soil available P, probably due to multiple mechanisms. The formation and precipitation of poorly soluble copper phosphate salts may immobilize phosphate in stable mineral forms (Madsen, 2005). Additionally, in calcareous soils, Cu can interact with

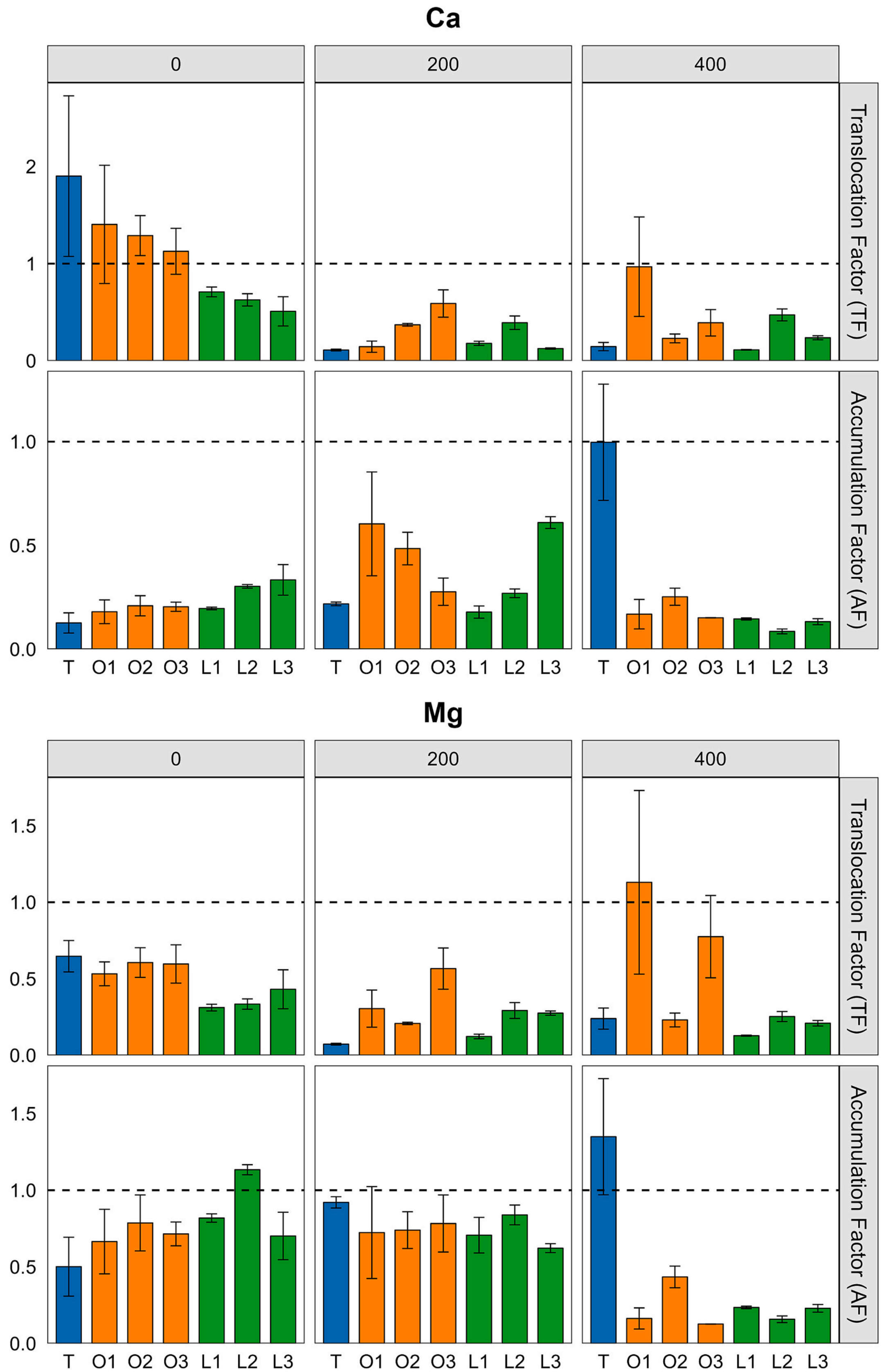

Fig. 5. Translocation factor (TF) and accumulation factor (AF) of calcium (Ca) and magnesium (Mg) in lettuce in soil without the addition of Cu (0), soil added with 200 mg Cu kg^{-1} (200), soil added with 400 mg Cu kg^{-1} (400). Each error bars represents standard deviation (n = 3). T = tap water; O = OWWs; L = LWWs; 1 = 1/3; 2 = 2/3; 3 = 3/3.

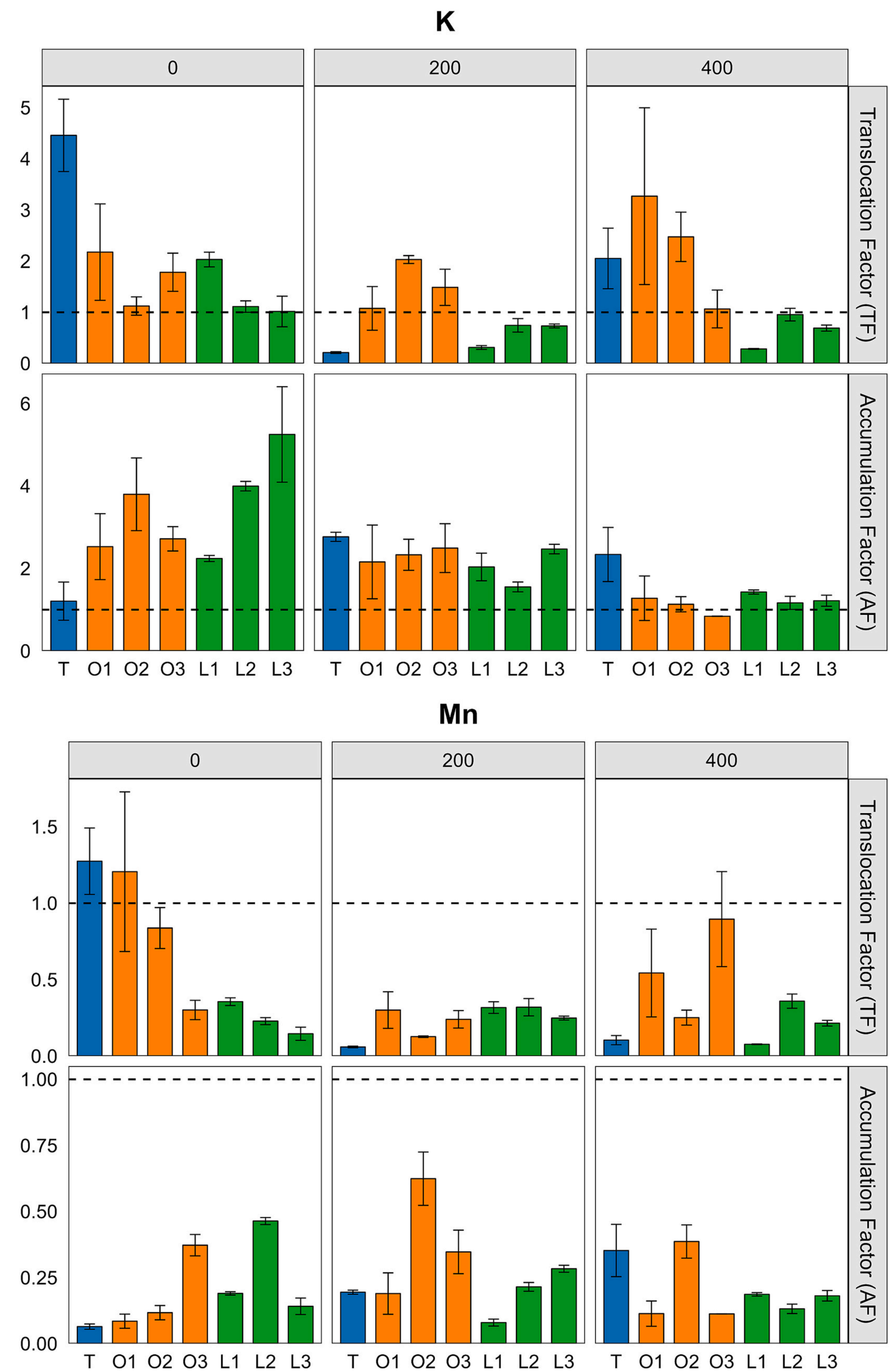

Fig. 6. Translocation factor (TF) and accumulation factor (AF) of potassium (K) and manganese (Mn) in lettuce in soil without the addition of Cu (0), soil added with 200 mg Cu kg^{-1} (200), soil added with 400 mg Cu kg^{-1} (400). Each error bars represents standard deviation (n = 3). T = tap water; O = OWWs; L = LWWs; 1 = 1/3; 2 = 2/3; 3 = 3/3.

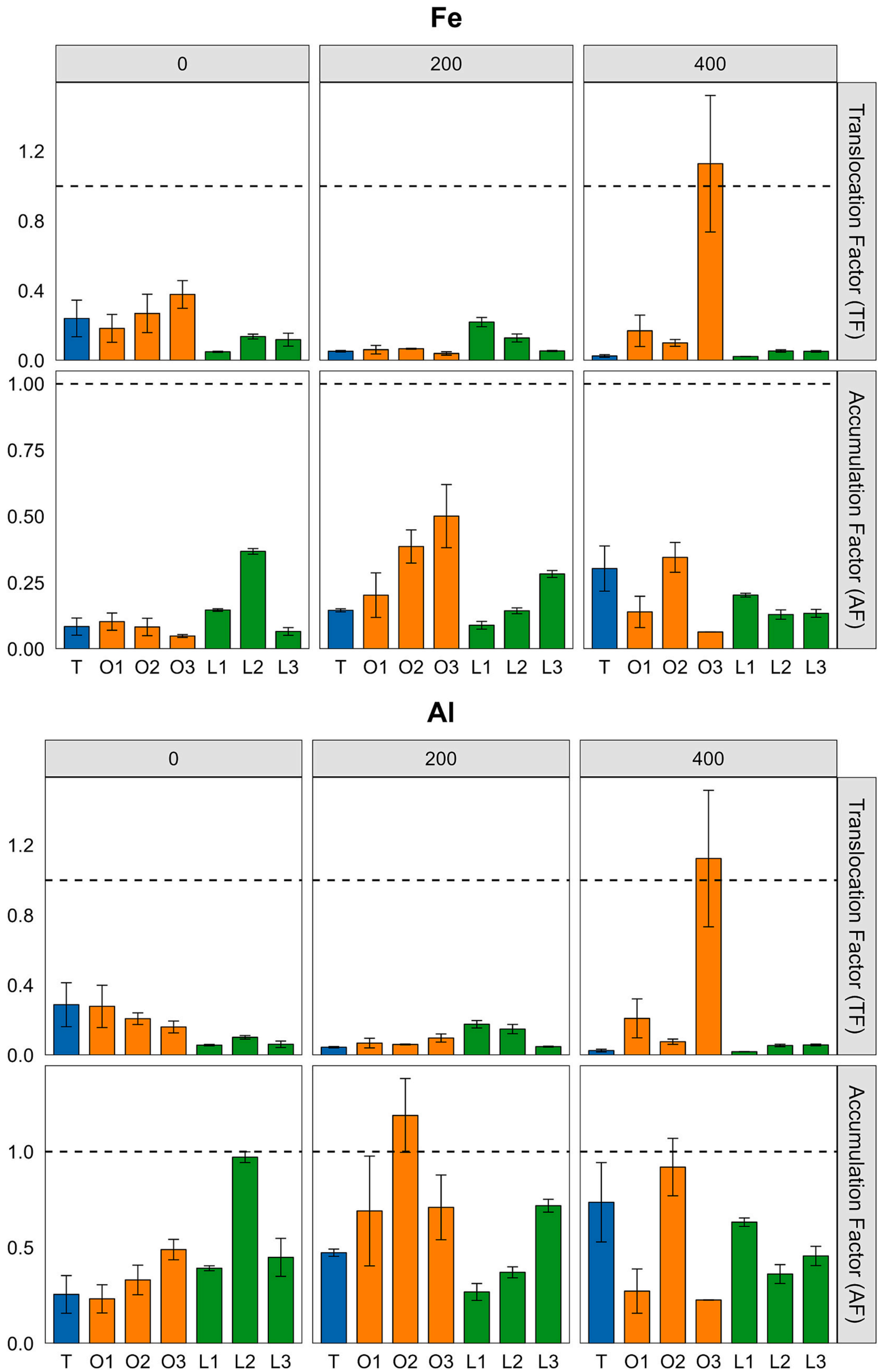

Fig. 7. Translocation factor (TF) and accumulation factor (AF) of iron (Fe) and aluminum (Al) in lettuce in soil without the addition of Cu (0), soil added with 200 mg Cu kg^{-1} (200), soil added with 400 mg Cu kg^{-1} (400). Each error bars represents standard deviation (n = 3). T = tap water; O = OWWs; L = LWWs; 1 = 1/3; 2 = 2/3; 3 = 3/3.

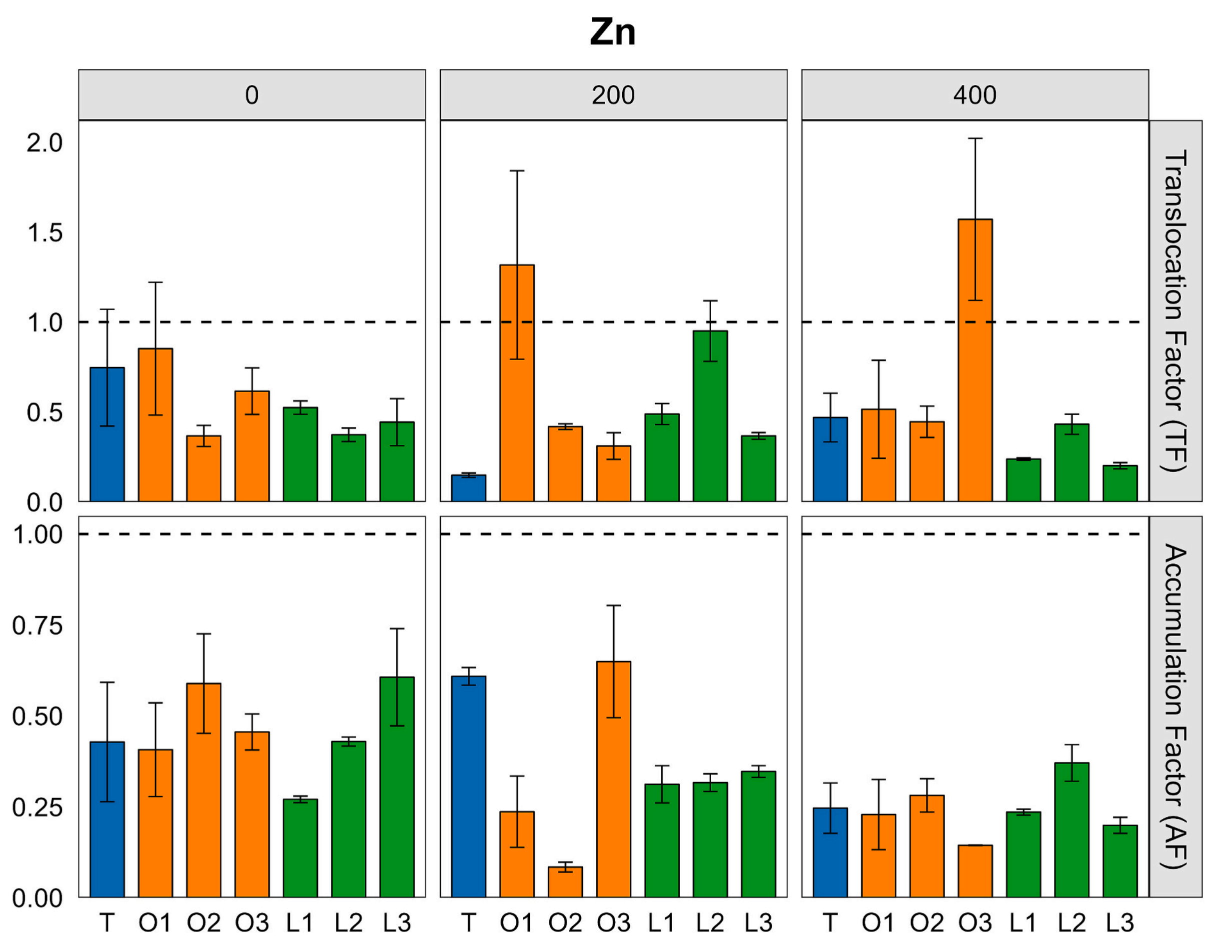

Fig. 8. Translocation factor (TF) and accumulation factor (AF) of zinc (Zn) in lettuce in soil without the addition of Cu (0), soil added with 200 mg Cu kg^{-1} (200), soil added with 400 mg Cu kg^{-1} (400). Each error bars represents standard deviation (n = 3). T = tap water; O = OWWs; L = LWWs; 1 = 1/3; 2 = 2/3; 3 = 3/3.

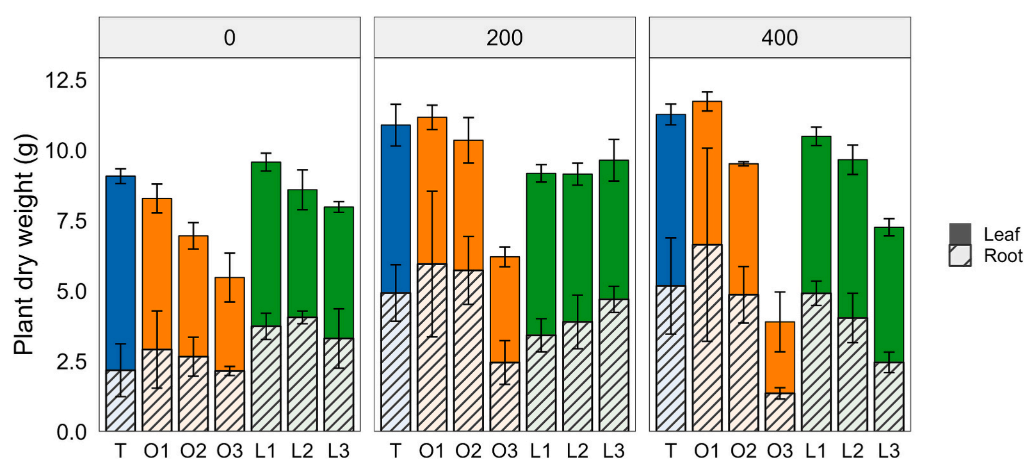

Fig. 9. Dry weight of lettuce leaves and roots cultivated in soil without the addition of Cu (0), soil added with 200 mg Cu kg^{-1} (200), soil added with 400 mg Cu kg^{-1} (400). Each error bars represents standard deviation (n = 3). T = tap water; O = OWWs; L = LWWs; 1 = 1/3; 2 = 2/3; 3 = 3/3.

mineral surfaces, occupying sorption sites or modifying surface charge properties (Bouray et al., 2024; Azeez et al., 2015). Both mechanisms may contribute to a reduction in phosphorus availability.

In contrast, the effect of CWWs irrigation on available P varied depending on the water type. Indeed, in the absence of Cu addition, OWWs had not significant effect, showing values of available P comparable to those of the control (Fig. 1). Conversely, LWWs reduced available P. Generally, citric acid is employed to mobilize soil P, as well as organic-P, in the form of insoluble salts with calcium, aluminum and iron. It is used to achieve an optimum soil pH for P assimilation by plants (Rahman et al., 2023; Zhu et al., 2018). However, the addition of LWWs, which have a high concentration of citric acid, more than OWWs (Table 1), resulted in a reduction in available P, particularly under 3/3 condition. It is therefore hypothesized that, although citric acid in LWWs may initially facilitate the solubilization of some forms of P, it may also contribute to the release of calcium, aluminum and iron from soil minerals. These, in turn, may precipitate as a complex with P reducing its availability (Wang et al., 2023; Geng et al., 2022; Johan et al., 2021; Penn and Camberato, 2019).

The interaction of CWWs with Cu affects the distribution of P between roots and leaves. Notably, at the highest Cu addition (400 mg kg^{-1}) and CWWs concentration (3/3), total P in roots drastically decreased, mainly due to a reduction in root biomass. In OWW-treated plants, the decrease in biomass was particularly marked, while in LWW-treated plants it was less pronounced but still sufficient to reduce total P in roots.

4.4. Element accumulation and translocation in the soil-plant system

Results of this study provided evidence that, without the addition of Cu, the application of CWWs, in particular LWWs, reduced the translocation of nutrients from roots to leaves compared to tap water, with the exception of N and P.

This suggested that although CWWs can provide essential nutrients, they could negatively affect the nutrient translocation, thus increasing nutrient accumulation in roots.

This behavior was likely linked to the high concentration of organic acids in CWWs that, in turn, decreased soil pH. The temporary acidification caused by CWWs may have increased the solubilization of some nutrients (e.g., Ca, Mg, K) in soil, enhancing their availability for root uptake (Pampinella et al., 2024). Such high availability in the root zone could be resulted in an accumulation of nutrients in the roots, being their translocation within the plant slower (Souri et al., 2019; Wendling et al., 2016; Wang et al., 2006).

By increasing Cu concentration, the role of CWWs becomes more significant. The excess of Cu is known to negatively affect essential nutrients dynamics due to various mechanisms (Kumar et al., 2021; Feil et al., 2020; Shabbir et al., 2020). For example, Shams et al. (2019) observed that *Lactuca sativa* L. treated with high levels of $CuSO_4$ (200 and 400 μM) showed a reduction in the uptake and translocation of N, K, P, Si, Zn and Mg and an increase in the concentration of B in the roots. This indicates that Cu exhibits antagonistic effects on the absorption of N, K, P, Si, Zn, and Mg while stimulating B accumulation in lettuce roots. Additionally, studies reviewed by Shabbir et al. (2020) have indicated that Cu tends to accumulate in roots, where it might outcompete other nutrients for common transporters, potentially limiting their translocation to aerial parts. However, CWWs alleviated some of these adverse effects by promoting nutrient translocation from roots to leaves in soils added with Cu. However, despite this mitigating effect, the translocation of nutrients to the leaves remains limited. Normalized translocation and accumulation factors, calculated for each element and Cu concentration, were used to highlight relative differences across treatments. It confirms that, especially at 200 mg Cu kg^{-1}, elements such as Zn, Mn, Mg, K, Fe, Ca, and Al exhibited higher translocation factors under CWWs irrigation compared to tap water. Conversely, accumulation factors were generally lower under CWWs treatments. However, it is important to interpret these values with a critical approach, as they may not always indicate improved nutrient transport efficiency. Indeed, in some cases, and in particular for undiluted OWWs treatment, higher translocation factor values could result from a concentration effect (Han et al., 2021; Guiz et al., 2018; Jarrell and Beverly, 1981). This phenomenon is due to the reduced growth of both roots and leaves in plants as occurred with OWWs 3/3. With reduced biomass, nutrients are concentrated in the limited tissue available, leading to an apparent increase in the translocation factor.

The lack of a univocal pattern of accumulation and translocation factors highlighted the complexity of the soil-plant system interacting with CWWs and Cu addition. This complexity is due to a strong dependence on nutrient dynamics and translocation processes on soil characteristics (e.g. pH, texture, organic matter) and plant-specific properties (e.g. root architecture, nutrient demand). Citrus wastewaters are chemically complex, containing a variety of organic and inorganic compounds that likely interact with soil and plant roots. Therefore, it is a challenge to predict the behavior of the soil-plant system under these conditions thus suggesting a deeper investigation about the effect of CWWs in the presence of Cu for each plant species.

4.5. Plant response to CWWs and Cu addition

The increase of soil nitrates stimulated plant root growth that could have positively affected water and nutrient uptake (Sun et al., 2017; Mantelin and Touraine, 2004; Chen et al., 2004). The stimulation of root growth could be ascribed also to a compensation effect of the plant to counteract the lack of nutrient adsorption and translocation by Cu (Lopez et al., 2023). Studies suggest that in the early development stages, plants may temporarily increase root length in response to nutrient scarcity, potentially as a strategy to explore deeper soil layers for essential nutrients (Xue et al., 2014; Peng et al., 2012). However, if the assimilation and transport of these nutrients from the roots to the leaves is compromised by Cu, the nutrients will remain concentrated in the roots and the leaves will receive less. This can lead to underdeveloped leaves with nutrient deficiencies (Marzec and Melzer, 2018). On the other hand, CWWs type and dilution plays a critical role in plant response. Undiluted OWWs significantly reduced plant biomass, likely due to their higher content of soluble sugars compared to LWWs (Table 1). Higher levels of soluble sugars may have increased the osmotic potential in the rhizosphere thus limiting water uptake by roots. Therefore, this may negatively affect plant development (Cui et al., 2010; Shohael et al., 2006; Al-Khayri and Al-Bahrany, 2002). In contrast, the lower sugar and higher citric acid content of LWWs compared to OWWs likely resulted in milder osmotic stress and a less pronounced negative effect on plant biomass. Furthermore, the composition of undiluted OWWs may have contributed to physical alterations in the soil, such as reduced porosity and water infiltration capacity. These probably affecting plant physiological responses and overall crop performance.

5. Conclusion

This study revealed both the potential and the challenges of using citrus processing wastewaters (CWWs) in agriculture, with a particular focus on their interaction with Cu in calcareous soil. Among the two tested CWWs, lemon wastewaters (LWWs) appeared to be more effective in mitigating Cu availability. This is probably due to the higher citric acid content, which may promote the formation of Cu-organic complexes or co-precipitates with Al and Fe. This effect was most evident at the highest Cu concentration. Both LWWs and orange wastewaters (OWWs) increased soil nitrate levels, indicating stimulated nitrification, although this was not reflected in greater nitrogen uptake by plants, likely due to temporary pH alterations affecting nutrient assimilation. Notably, under moderate Cu contamination, both LWWs and OWWs contributed to enhanced translocation of several nutrients, including Zn, Mn, Mg, K, Fe, Ca, and Al. These effects suggest that, despite some limitations, CWWs may help sustain nutrient movement from roots to leaves, partially counteracting Cu competition with other nutrients. In addition, CWWs provide a range of nutrients, including nitrogen, potassium, and calcium, which can contribute to soil fertility and nutrient cycling. However, when applied undiluted, particularly OWWs, they negatively affect plant growth. Based on results of this study, future research should assess CWWs effects on more resilient crop species, more adapted to the chemical complexity of these wastewater. Furthermore, it would be valuable to investigate whether CWWs can be used undiluted on such crops or whether sustainable dilution strategies can be developed. Indeed, as a potential environmental solution, diluted CWWs can help to balance their benefits while minimizing their adverse effects, making them more versatile for agricultural use. Overall, these results suggest that CWWs have the potential to play a key role in sustainable agriculture if properly managed.

CRediT authorship contribution statement

Daniela Pampinella: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Caterina Lucia:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Luigi Badalucco:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Vito Armando Laudicina:** Writing – review & editing, Writing – original draft, Supervision, Methodology, 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.179633.

Data availability

Data will be made available on request.

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