Journal of
Plant Development Sciences
Meerut, India

RESEARCH ARTICLE

DUAL-METHOD QUANTIFICATION OF COPPER OXYCHLORIDE RESIDUES IN AGRICULTURAL SOILS: A COMPARATIVE STUDY OF TITRATION AND ATOMIC ABSORPTION SPECTROSCOPY ANALYSIS

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Received-02.11.2025, Revised-14.11.2025, Accepted-29.11.2025

Abstract: Copper oxychloride (COC) is extensively utilized in agriculture, but its accumulation in soil can threaten soil health and productivity. This research evaluated two analytical techniques—titration and atomic absorption spectroscopy (AAS)—to measure copper levels in soils from COC-treated and control areas in four districts of Kerala, India. A total of 64 samples were examined on the 15th and 30th days following COC application. Both methods consistently found low copper concentrations in control soils (<0.4 ppm), whereas treated soils exhibited significantly higher levels, ranging from 70.08±0.03 ppm to 132.00±1.10 ppm on day 15th and decreasing to 61.85±1.60 ppm to 104.90±0.85 ppm by day 30th in titration tests. AAS reported slightly higher values, demonstrating its superior sensitivity and precision. Both methods showed a 15–30% reduction in copper levels over time, indicating environmental dissipation. While titration is economical for routine assessments, AAS offers more dependable results for accurately monitoring copper contamination in agricultural soils.

Keywords: Copper oxychloride, Atomic absorption spectroscopy, Fungicide

INTRODUCTION

Copper based fungicides are in use in agriculture since a long time, Bordeaux mixture (a combination of lime and copper sulphate) being the first among them which was initially used to control downy mildew in vineyards. Presently, a variety of copper-based fungicides are used for crop protection, and these include copper oxychloride (COC), copper hydroxide, copper sulphate, bordeaux mixture and copper acetate (Chrisfield *et al*., 2021; Burandt*et al*., 2023; Gao *et al*., 2023). COC, a commonly used fungicide in agriculture was initially employed to control diseases particularly in orchards and vineyards. Its chemical composition, represented as $Cu_2(OH)_3Cl$, allows it to perform as a broad-spectrum plant protectant that helps to combat fungal pathogens like *Phytophthora infestans* responsible for late blight in potato, *Phytophthora palmivora* causing abnormal leaf fall and *Cornyespora cassicola* causing *Cornyespora* leaf fall in rubber and *Guignardia citricarpa* infesting citrus plants (Jayasuriya, 2006; Schutte *et al*., 2012; Ferreira *et al*., 2014; Keiblinger *et al*., 2018; Manju *et al*., 2019; Oghama *et al*., 2023).

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Application of COC in rubber plants retains the copper particles appropriately distributed on the leaf surface and stalks, even during high humidity and heavy rainfall ensuring proper protection from *Phytophthora palmivora*, thus improving the yield of rubber and rubber latex production. Research have demonstrated that COC accumulation in soil adversely affect soil microbial community including beneficial fungi and bacteria. Copper accumulation in soil disrupts soil fungal activity. Research indicates that COC residues in soil significantly reduce microbial biomass which alters the functional diversity of soil fungal community (Masaka and Muunganirwa, 2007; Keiblinger *et al*., 2018; Wang *et al*., 2018). Soil pH, chemical forms of copper and soil organic matter influence the bioavailability of COC in soil. Bioavailability of COC is higher compared to other copper-based fungicides like bordeaux mixture which leads to more environmental impacts (Schutte *et al*., 2012; Keiblinger*et al*., 2018). COC also has detrimental impacts on soil fauna, especially earthworms. Exposure to COC decreases earthworm growth and impacts their reproduction, which are vital for maintaining soil structure and fertility. A decrease in earthworm population leads to reduced soil aeration and nutrient availability (Eijsackers *et al*., 2005; Wang *et al*., 2018). In addition to these environmental implications, COC is also known to induce oxidative stress in plants. Even though copper is an essential plant micronutrient, higher concentration leads to the production of reactive oxygen species (ROS) that damages cellular structure thus impairing plant growth (Ferreira *et al*., 2014). Hence this study was performed to compare efficacy of two different methods for determining the copper concentration the active ingredient of COC in soil samples.

MATERIALS AND METHODS

Study Area and Sample Collection

Details of the study area are outlined in table 1. According to data from the COC application collected by the Regional Rubber Board (RRB) in Kerala, India, a total of 64 soil samples (192 in triplicate) were gathered from both COC-treated and untreated control sites (where no pesticides were applied) across four locations in the Kasaragod, Kannur, Kozhikode, and Wayanad districts of Kerala, India, as specified in figure 1. These samples were collected on the 15th and 30th days following COC application. Ongoing analysis indicated that pesticide contamination peaked on the 15th day after COC application, followed by a gradual decline, with the most notable reduction observed by the 30th day. Consequently, samples for further research were collected on the 15th and 30th day post-COC application. The samples were transported to the laboratory in ice boxes, air-dried, sieved through a 2mm mesh, and stored at room temperature for later analysis.

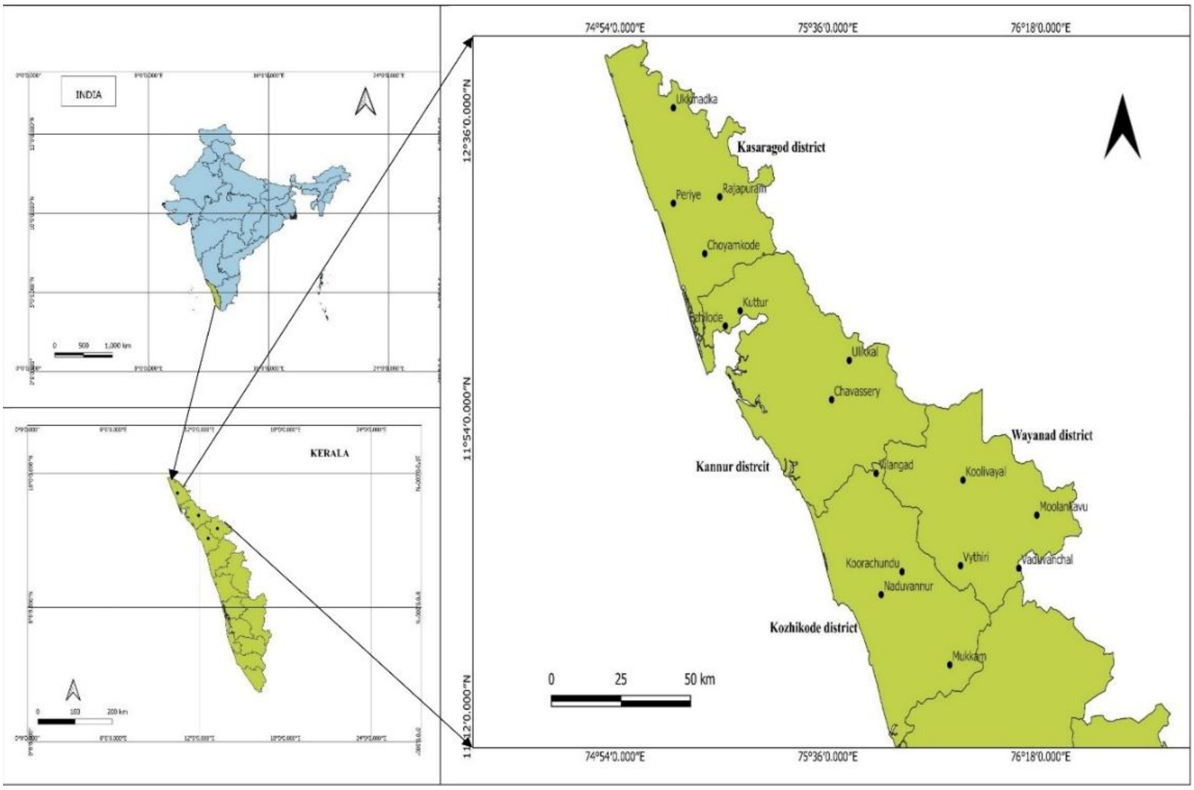

Figure 1: Soil Sampling Sites

COC Analysis

COC concentration in soil samples was determined using titration method and atomic absorption spectroscopy (AAS).

Determination of COC concentration in soil samples using titration method

Approximately 0.20 g of soil sample was placed in a 250 ml conical flask, to which 3 ml of concentrated nitric acid and 20 ml of distilled water were added, and the mixture was shaken for 5 minutes. After cooling, 1 g of urea was added and the solution was boiled for 5 minutes. Once cooled, 1 g of sodium carbonate was added. Subsequently, 5 ml of a 10% acetic acid solution was introduced to dissolve the precipitate. Then, 5 ml of a 30% potassium iodide solution was added, and the solution was titrated with 0.1N standard sodium thiosulphate until a pale straw yellow colour appeared. Following this, 2 g of potassium thiocyanate and 1 ml of a 1% starch solution were added, and the titration continued until the blue colour disappeared (Sridevi *et al*., 2011). The copper concentration was calculated and expressed in ppm.

Determination of COC concentration in soil samples using AAS method

The protocol by Shah *et al*. (2013) was employed to determine the active copper ingredient in COC. A standard solution of COC fungicide was obtained from Sigma-Aldrich Chemicals Private Limited, Bangalore, India. About 1 g of powdered soil sample was placed in a conical flask, and 10 ml of HNO_3 was added. The solution was left to stand for 24 hours. Then, 5 ml of $HClO_4$ was added and the solution was heated until its volume reduced to 3 ml. After complete cooling, the solution was filtered using Whatman No 42 filter paper. The filtrate was then diluted to 25 ml in a volumetric flask using double-distilled water. The copper concentration in the filtrate was measured at a wavelength of 324.80 nm using atomic absorption.

RESULTS AND DISCUSSION

Copper oxychloride (COC) application led to markedly elevated copper concentrations in test soil samples across all four districts (Kasaragod, Kannur, Kozhikode, and Wayanad), as determined by both titration and atomic absorption spectroscopy (AAS). Control samples consistently showed low baseline levels (<0.4 ppm), while test samples exhibited concentrations far exceeding this. Titration results revealed sharp increases post-COC application. Copper values on day 15th found in the range between in 0.11±0.01 ppm and 0.39±0.02 ppm control and in the test samples from 70.08±0.03ppm to 132.00±1.10 ppm. On 30th day in the control samples values ranged between 0.08±0.01ppm and 0.32±0.03 ppm and, in test samples it was found in the range between 59.10±0.40 ppm and 99.01±0.23 ppm. AAS results confirmed these findings, often showing slightly higher peaks. On day15th of COC application copper values were observed in the range between 0.07±0.03 ppm and 0.40±0.03 ppm in control and, from 75.10±0.24 ppm to 135.08±1.08 ppm in the test samples. By day 30th, test concentrations declined but remained elevated. On 30th day in the control samples, values ranged between 0.04±0.01 ppm to 0.38±0.06 ppm and in test samples it was found to be in the range between 61.85±1.60 ppm to 104.90±0.85 ppm. Both methods confirmed a consistent 15–30% decline from day 15th to 30th across districts. Both techniques exhibited strong spatial consistency across 16 sites, validating COC persistence, but AAS demonstrated superior sensitivity, particularly for subtle district variations (e.g., Kozhikode's lower peaks). Titrimetry proved simpler and cost-effective for field screening, though its indirect iodide-based endpoint may underestimate tightly bound copper fractions released more completely by AAS digestion. Standard errors overlapped minimally, indicating high reproducibility; AAS is recommended for precise regulatory monitoring in copper-contaminated soils. These trends align with prior reports of copper accumulation from COC and similar copper based-fungicides (Aarya and Mathew, 2020; Kakutey *et al*., 2023; Matse *et al*., 2024). The reductions over time likely stem from leaching, soil/plant uptake, or environmental factors like moisture and temperature influencing bioavailability (Droz *et al*., 2021; Cheng *et al*., 2024). Excess copper, while an essential micronutrient, proved toxic at these concentrations. It disrupts plant membrane integrity, enzyme/photosynthetic activity, root growth, biomass, and causes chlorosis/necrosis (Kumar *et al*., 2021; Mir *et al*., 2021; Al-Jayashi *et al*., 2023; Feil *et al*., 2023). Soil nutrient availability (P, K, Mn, Fe) diminishes via reactions with oxides and organic matter, curbing crop yields (Schutte *et al*., 2012; Ambrosini *et al*., 2018). Copper also harms soil biota, particularly fungi. COC inhibits saprobic and rhizosphere fungal diversity (e.g., lowest alpha diversity in fungicide-treated tea plantation rhizospheres) (Mallano *et al*., 2023) and impairing nutrient cycling, fertility, and ecosystem resilience (Eijsackers *et al*., 2005; Ferreira *et al*., 2014; Keiblinger *et al*., 2018; Wang *et al*., 2018; Lasota *et al*., 2019; Golubeva *et al*., 2020; Marini *et al*., 2024). Regular monitoring is essential to sustain optimal soil copper levels and mitigate these risks in COC-treated farmlands.

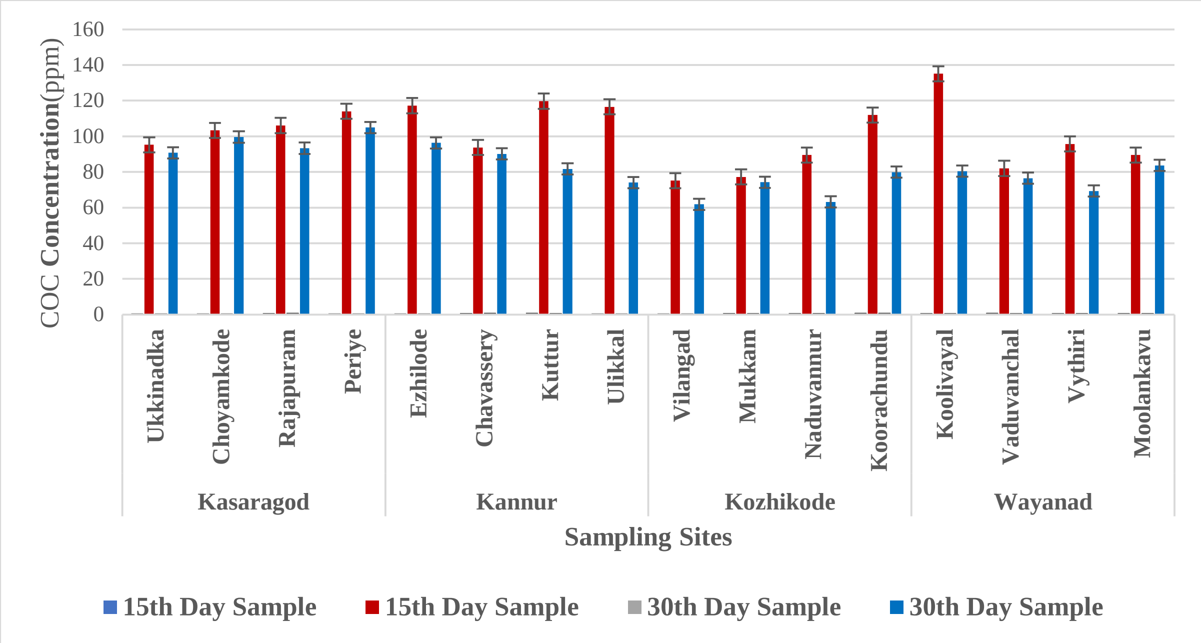

Figure 2: Concentration of COC in Northern Kerala analysed using AAS

Table 1. Concentration of COC in soil samples using titrimetry

District	Sampling site	Sampling Day			
		Day 15		Day 30	
		Control*	COC applied*	Control*	COC applied*
Kasaragod	Ukkinadka	0.11±0.01	92.00±1.02	0.14±0.02	81.00±0.86
	Choyamkode	0.23±0.09	91.00±0.03	0.18±0.05	89.01±0.73
	Rajapuram	0.32±0.02	103.00±0.91	0.29±0.06	83.00±0.66
	Periye	0.08±0.01	101.00±1.05	0.09±0.05	99.01±0.23
Kannur	Ezhilode	0.16±0.01	86.00±1.20	0.13±0.01	79.00±1.02
	Chavassery	0.18±0.01	90.00±1.03	0.15±0.02	88.00±0.98
	Kuttur	0.32±0.03	99.00±0.92	0.28±0.02	78.00±0.83
	Ulikkal	0.20±0.01	105.00±0.83	0.12±0.02	94.00±0.69
Kozhikode	Vilangad	0.17±0.03	71.00±0.01	0.12±0.01	60.18±0.55
	Mukkam	0.09±0.01	70.08±0.03	0.06±0.03	59.10±0.40
	Naduvannur	0.39±0.02	72.00±0.05	0.32±0.01	61.00±0.36
	Koorachundu	0.10±0.01	98.00±1.05	0.08±0.01	86.00±1.03
Wayanad	Koolivayal	0.28±0.09	132.00±1.10	0.27±0.01	71.00±0.92
	Vaduvanchal	0.12±0.03	109.00±0.03	0.11±0.02	73.00±0.80
	Vythiri	0.37±0.12	80.00±0.23	0.32±0.03	67.00±1.03
	Moolankavu	0.26±0.10	79.50±0.18	0.24±0.02	75.00±0.03

***mean±SE of concentration of COC in ppm**

CONCLUSION

This research reveals that applying copper oxychloride (COC) significantly raises copper levels in agricultural soils, with concentrations greatly surpassing those in untreated areas across all four districts studied. Both titration and atomic absorption spectroscopy (AAS) successfully identified these increases, but AAS consistently offered higher sensitivity, better precision, and more dependable quantification of copper residues. Although copper levels decreased by 15–30% between the 15^{th} and 30^{th} days post-application, the concentrations in treated soils remained notably high, indicating the persistence of COC in the environment. These results highlight the importance of regularly monitoring copper contamination in plantation soils, especially in regions with frequent fungicide use. Titration is a practical, cost-effective method for initial assessments, while AAS is recommended for regulatory, research, and long-term environmental studies. Overall, responsible management of COC application is crucial to protect soil quality, microbial diversity, crop productivity, and the broader ecosystem's health.

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