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Effects of phosphorus fertilizer on kenaf growth physiology and copper absorption in copper-contaminated soil

Muhammad Roman[1,2] · Rui Cui[1] · Jinzhan Yuan[1] · Michal Hejcman[2] · Lijun Liu[1]

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Abstract

Copper (Cu) contamination in agricultural soils is one of the health risks, due to its translocation to humans through the food chain. Therefore, optimized nutrient application is required to achieve higher yields with reduced Cu uptake, ensuring food security. One way to reduce soil contamination is phytoremediation. Phosphorus (P) application decreases oxidative stress, improves plant growth, and facilitates the phytoremediation potential of plants. This study investigated the phytoremediation potential of kenaf (*Hibiscus cannabinus*) with P fertilizer in Cu-polluted mining soil (2375 mg kg^{-1} Cu) of Hubei, China. A pot experiment was conducted to assess the effect of P on kenaf growth, gas exchange traits, antioxidant enzyme activities, Cu uptake, and soil health under different levels of P (0, 10, 15, and 20 g/15 kg of soil). P_{15} significantly improved plant growth by increasing plant height, stem diameter, number of leaves, and SPAD (relative chlorophyll index). Application of P improved net photosynthesis (Pn), transpiration rate (Tr), stomatal conductance (gs), and intercellular CO_2 concentration (Ci) while decreasing oxidative stress in kenaf leaves up to P_{15}. Contradictory, a high concentration of P_{20} was toxic to the morphological and physiological traits of the plants. Maximum Cu uptake was observed at P_{20} in roots, leaves, stems, and fibers. Additionally, P application significantly decreased soil pH and bulk density. Our findings revealed the effectiveness of P application in improving kenaf growth in heavily Cu-polluted mining soil.

Keywords Antioxidative enzymes · Copper-contaminated soil · Gaseous exchange attributes · *Hibiscus cannabinus* · Phosphate fertilizer · Phytoremediation

Introduction

Heavy metal pollution in Chinese soils represents a significant and pervasive issue. Mining regions cause a comparatively more substantial risk among various land use categories. In particular, the southeastern part of China experiences more severe soil pollution than the northwestern regions (Shi et al. 2023). Long-term mining activities cause heavy metal pollution in soil, posing a risk to human and plant health (Daryabeigi and Mühling, 2022; Yang et al. 2020). A report in 2000 revealed that mining activities in China had caused pollution on approximately 3 million hectares, with a yearly expansion rate of 46,700 hectares (Li 2006). Consequently, mining activities have resulted in the loss of large areas of fertile land in China, and this depletion has become a serious issue that substantially impacts the country's food security (Li et al. 2018a; Rehman et al. 2019a). Cu is a prevalent heavy metal in numerous areas across China (Rehman et al. 2021). As stated in China's National Soil Pollution Survey Bulletin, Cu contents in Chinese soil increased by 2.1% (Li et al. 2024). Copper in soil results from both natural processes and human activities. Contamination in large regions globally has been caused by mining, smelting, and agricultural practices. Countries such as Indonesia, China, and Japan have been particularly affected by the contamination, primarily due to Cu, Zn, and Cd (Herawati et al. 2000). Mining causes disruptions to various soil characteristics, encompassing soil pH level,

Muhammad Roman and Rui Cui contributed equally to this work.

✉ Lijun Liu
liulijun@mail.hzau.edu.cn

1 MOA Key Laboratory of Crop Ecophysiology and Farming Systems in the Middle Reaches of the Yangtze River, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

2 Department of Environment, Faculty of Environment, Jan Evangelista Purkyně University in Ústí nad Labem, Pasteurova 15, Labem CZ-400 96, Czech Republic

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electrical conductivity, and cation exchange capacity (Farjana et al. 2019). Hence, exploring strategies for ecological rehabilitation and removing these hazardous metals from soil is imperative. Phytoremediation is a promising strategy with the potential to mitigate risks to individual health and improve China's food security (Mwamba et al. 2016; Saleem et al. 2019a). Copper toxicity triggers imbalances in plant nutrition, thereby impeding plant growth (Feil et al. 2020). While plants necessitate only minute quantities of Cu, excessive Cu in the soil can harm crops. Throughout all growth stages, excessive Cu levels profoundly influence plant physical characteristics and physiological processes (Li et al. 2018b; Saleem et al. 2019a; Zhou et al. 2019). Additionally, numerous studies involving crops such as wheat, rice, and ramie have consistently demonstrated that excessive copper in the soil leads to a notable decrease in plant growth (Mostofa and Fujita 2013; Keller et al. 2015). In times of stress, like environmental challenges, plants activate internal signals that lead to oxidative stress. Reactive oxygen species can harm plants, but they can counteract or neutralize them using various antioxidants (Kamran et al. 2020; Khan et al., 2019). Further, toxic Cu contents in the soil resulted in heightened antioxidant activity (Chandrasekhar and Ray 2017; Saleem et al. 2020a). Over 16% of China's agricultural land is contaminated with heavy metals, and 2.1% is polluted with Cu (Chen et al. 2015).

Kenaf is an ideal crop for this role due to its resilience to soil trace elements, rapid growth, high biomass production, minimal resource demands, compatibility with established agricultural methods, and versatility (Zhao et al., 2022). In prior research, fibrous plants like kenaf, industrial hemp, and ramie have exhibited resilience when exposed to Cu stress (Rehman et al. 2019c). In recent research, Zhao et al. (2022) found that kenaf is more tolerant to Cu-contaminated soil than flax and industrial hemp. Nonetheless, Cu-tolerant species may have lower Cu concentrations in their tissues or cells than Cu-sensitive species, possibly due to their substantial biomass and distinctive physiological adaptations (Saleem et al. 2019a). The direct impact of Cu phytotoxicity on soil is evident in its toxic effects on plant growth and biomass. Previous research has documented that elevated soil Cu concentrations decreased plant biomass (Rehman et al. 2019c; Saleem et al. 2020c). The application of soil treatments is helpful for plants, enhancing growth and development, even in challenging circumstances. Thus, plants must employ optimal fertilization practices to sustain their growth (Eissa and Roshdy 2018; Yang et al. 2020). To achieve optimum crop yield and biomass, it is essential to use balanced or ideal plant fertilizers. Even in low soil fertility conditions, where vital nutrients are lacking, fertilizers are imperative for promoting plant growth in metal-contaminated soils (Grames et al. 2019).

The proper fertilizers are essential for achieving optimal crop yields, especially in adverse conditions. Phosphorus fertilizer is important in different physiological steps, including photosynthesis and carbohydrate synthesis, and it increases the antioxidant defense mechanisms in challenging environments (Ahmad et al. 2017). Furthermore, Sofo et al. (2004) recommended that P fertilizer can enhance the resistance of plants to face adverse conditions and improve the mechanism of the plant antioxidant systems. Balanced or ideal P promotes plant growth and biomass production, but excessive P concentrations can harm plant growth (Yang et al. 2020; Saleem et al. 2020a). Alatawi et al. (2023) also recommended P fertilizer application for better jute yield and phytoremediation in Cu-contaminated soil. The P application is cost-effective and convenient. It can enhance plant resilience to adverse conditions and stimulate plant growth in toxic soil (Tang et al. 2022). In previous findings, Tang et al. (2022) discovered that P enhanced the heavy metal uptake in the upper plant parts and accelerated the absorption of these metals (Huang et al. 2020; Tang et al. 2022). Heavy metal contamination causes significant environmental concerns due to its non-biodegradable nature and adverse impacts on the environment, humans, and plants. An innovative and eco-friendly solution to this issue is phytoremediation, a way of using plants to extract toxic elements from soil. Considering the process underlying the phytoremediation potential of specific plants, such as phosphorus and kenaf, is crucial for addressing this problem, particularly in Cu-mining soil.

We hypothesized that the P fertilizer could enhance the effectiveness of phytoremediation in Cu-toxic soil. To investigate this, we designed a greenhouse experiment to examine the response of phosphorus and kenaf in Cu- contaminated soil for phytoremediation. Our findings offer a cost-effective approach for remediating polluted farmland, abandoned areas, and mining regions. This study focuses on the impact of varying P concentrations on kenaf plant growth, antioxidant defence mechanisms, and its potential for Cu accumulation in Cu-contaminated soil in Hubei Province, China. The research aims to test three key hypotheses: (1) Different P applications influence kenaf growth and biomass, (2) P increases copper content in kenaf, and (3) Diverse P levels affect antioxidant and gas exchange attributes in copper-rich soil. Our research provides unique insights into copper soil tolerance and accumulation in fibrous plant species.

Materials and methods

Experimental layout

In this research, kenaf seeds (Zhonghongma No. 12) were taken from the Bast, Fibrous Research Centre of Huazhong Agricultural University in Hubei Province, China. The study used Cu-mining soil from the mining site of Baisha Village, Daye County, Hubei, China (115.20 º E, 29.85 º N) at 0–20 cm depth. Soil properties were as follows: Soil pH (8.52), exchangeable K (12.24 g kg^{-1}), exchangeable N (0.17 g kg^{-1}), exchangeable P (0.18 g kg^{-1}), soil bulk density (1.86 g/cm³), EC (284 µS cm^{-1}), cation exchange capacity (18.3 cmol kg^{-1}), organic matter (3.95 g $kg^{-1)}$ and soil porosity (63.34%). Following soil collection, the soil was systematically mixed, moisture-adjusted, air-dried, and sieved through 5 mm screens before the pot experiment began. The study examined the effects of different P concentrations (0, 10, 15,20 g/pot) while keeping the Cu-impacted soil consistent at 15 kg/pot. The phosphate fertilizer is made of ordinary superphosphate, and the content of phosphorous pentoxide was 12%. The experiment occurred between April and September 2021 in a controlled glasshouse at Huazhong Agricultural University in Wuhan, Hubei, China. The plants were exposed to natural light, with temperatures ranging from 25 °C to 30 °C during day-night, and humidity levels of 70–80%. The research layout employed in this study followed a complete randomized design framework, incorporating 3 repetitions for each treatment condition. Each pot measured 30 cm in height and 40 cm in width, and firstly, 8 seeds were sown in each experiment pot and 3 plants after thinning.

Agronomic parameters

In September 2021, all plants were harvested to calculate plant height, fresh weight, and dry biomass. A weighing balance is used to measure plant fresh and dry weights. For fresh weight, all parts of the plant were measured, while oven-drying plant components ascertained dry weight at 65 °C for 72 h.

Before harvest, 9 plants (3 from each repetition) indicating each treatment were randomly chosen for data measurement. Stem diameter (mm) was calculated using a scale and digital vernier calliper. Plant height was calculated using the way mentioned in past studies (Saleem et al. 2020a).

SPAD and gaseous exchange traits

After 60 days of sowing in pots, the study measured the SPAD value from leaves and various gaseous exchange attributes. Chlorophyll (SPAD) values were determined using a Soil Plant Analysis Development Meter SPAD-502 plus (Konica Minolta, Inc., Japan) between 09:30 and 10:30 a.m. Leaf gas exchange parameters, transpiration rate (Tr), net photosynthesis (Pn), stomatal conductance (gs), and intercellular CO_2 (Ci) were determined after 60 DAS (date after sowing) by the method of Alharby and Fahad (2020).

Oxidative responses and antioxidant systems

Leaf samples from each treatment were collected at 60 DAS between 09:00 and 10:00 a.m. MDA (µg g^{-1} FW) in kenaf leaves was measured by the thiobarbituric (TBA) method (Chen et al. 2020). Leaf proline (µg g^{-1} FW), SOD (U g^{-1} FW), and POD (U g^{-1} FW) were determined by the procedure of Alharby and Fahad (2020).

Cu determination, bulk density, and soil pH

Copper contents in leaves, stems, shoots, and fibers were determined by the recently used method of Saleem et al. (2020b). Soil bulk density was measured in a core way using the mass of soil (g) in relation to the volume of soil (cm^3) (Throop et al. 2012). The soil pH was measured by a pH meter (Model: HANNA HI 8520) (Chi and Wang 2010).

Statistical analysis

In assessing significance, standard deviation (SD) was determined to be statistically significant when the Significant differences were less than 0.05, based on comparisons made using the Tukey post hoc (HSD) test. Differences in various morphological and physiological traits were analyzed using a one-way analysis of variance (ANOVA). The data were graphically represented using Origin software.

Results

Kenaf growth

This research details kenaf plant growth and biomass under various P levels in Table 1. The findings revealed that, under consistent Cu concentrations, plant growth and biomass increased as the P dosage increased. Specifically, growth and biomass indicators peaked at P_{15}, but at P_{20}, they began to decline. The analysis of variance (ANOVA) confirmed P fertilization's significant influence on growth and biomass production. It was noted that Cu (2375 mg kg^{-1}) had a notable negative effect on kenaf plant growth, and P fertilization notably improved kenaf growth. Maximum plant height was noticed (203 ± 2.64 cm) at P_{15}, whereas a further addition of P_{20} caused a significant decline in plant

Table 1 Impact of P on plant fresh weight(g), dry weight (g), plant height (cm), SPAD, stem diameter, no. Of leaves in Cu-mining soil

Treatment	Fresh weight	Dry weight	Plant height	Stem diameter	SPAD	No. of leaves
P_0	88 ± 3.78 ^{c}	32 ± 1.99 ^{c}	180 ± 2.08 ^{c}	7.1 ± 0.0.20 ^{c}	36 ± 1.09 ^{c}	20 ± 1.63 ^{c}
P_{10}	104 ± 2.51 ^{b}	42 ± 1.52 ^{b}	192 ± 3.60 ^{b}	7.7 ± 0.17 ^{b}	39 ± 0.99 ^{bc}	26.6 ± 2.08 ^{bc}
P_{15}	115 ± 2.64 ^{a}	49 ± 1.70 ^{a}	203 ± 2.64 ^{a}	8.2 ± 0.11 ^{a}	44 ± 1.40 ^{a}	31.2 ± 1.52 ^{a}
P_{20}	111 ± 3.04 ^{ab}	38 ± 2.46 ^{bc}	187 ± 1.48 ^{bc}	7.7 ± 0.18 ^{b}	42 ± 0.76 ^{ab}	29.1 ± 1.45 ^{ab}

Letter-different bars represent mean ± standard deviation ($n=3$). Significant differences ($P<0.05$) between the treatments are shown by different letters on the bars

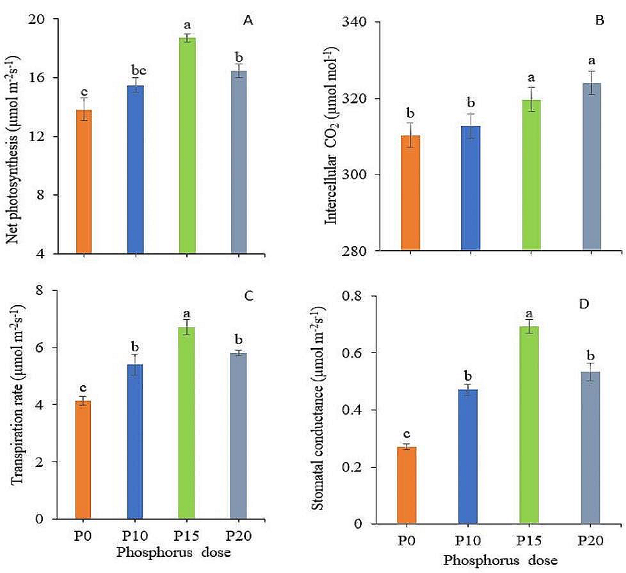

Fig. 1 Phosphorus effect on net Pn (**a**), Ci (**b**), Tr (**c**), and gs (**d**) in kenaf leaves. Letter-different bars represent mean ± standard deviation ($n=3$). Different letters on the bars show significant differences ($P<0.05$) between the treatments

height by 7.8%. Additionally, plant fresh (115 ± 2.64 g) and dry biomass (49 ± 1.70 g) were maximum at P_{15}, whereas a further increase in P (P_{20}) caused a decrease in plant fresh (3.4%) and dry (22%) biomass in P_{20}. Higher levels of P fertilization (P_{20}) negatively affected kenaf growth compared to other treatments. Furthermore, among various treatments, the maximum increase in plant height was found at P_{15} (203 ± 2.64 cm), followed by P_{10} (192 ± 3.60 cm), and P_{20} (187 ± 1.48 cm) compared to the control treatment (180 ± 2.08 cm).

Various P levels had a noticeable influence on shoot fresh biomass, as depicted in Table 1. It was noticed that a notable rise in shoot fresh biomass was achieved where P was applied at P_{15} (115 ± 2.64 g), followed by P_{20} (111 ± 3.04 g), P_{10} (104 ± 2.51 g), and as compared to control P_0 (88 ± 3.78 g). Further, different P applications also influenced maximum plant fresh weight, and the maximum was found at P_{15} (49 ± 1.70 g), followed by P_{10} (42 ± 1.52 g), P_{20} (38 ± 2.46 g) in comparison with P_0 (32 ± 1.99 g). The results indicated that higher Cu concentrations substantially decreased the leaves quantity and stem diameter. In contrast, P fertilizer increased both the No. of leaves and stem diameter of kenaf significantly. Maximum No. of leaves/plants were noted where P was applied at P_{15} (31.2 ± 1.52), followed by P_{20} (29.1 ± 1.45), P_{10} (26.6 ± 2.08) as compared to control (20 ± 1.63). The highest increase in stem diameter of kenaf was found at P_{15} (8.2 ± 0.11), followed by P_{10} (7.7 ± 0.17) and P_{20} (7.7 ± 0.18) as compared to P_0 (7.1 ± 0.20).

Chlorophyll and gas exchange traits

This experiment showed that SPAD values and gas exchange attributes were at their lowest levels under low P conditions. However, as P concentration increased, there was a significant rise in SPAD up to a certain threshold (Table 1; Fig. 1). Additionally, results indicated that Cu significantly reduced the SPAD values in kenaf. However, the application of P significantly increased SPAD up to P_{15}. However, a further increase in P at P_{20} significantly decreased chlorophyll in kenaf plants). The maximum SPAD contents were found at P_{15} (44 ± 1.40), followed by P_{20} (42 ± 0.76) and P_{10} (39 ± 0.99) as compared to control treatment P_0 (36 ± 1.09). Similarly, the maximum Pn (18.7 μmol $m^{-2}s^{-1}$), gs (0.69 μmol $m^{-2}s^{-1}$), Tr (6.71 nmol m^2s^{-1}), and Ci (235 μmol m^{-1}) levels were observed at P_{15}, whereas increase in P dose caused a significant decline in Pn, gs, Tr and Ci by 11%, 23%, 13.56%, and 1.56%, and a no significant difference were noted between P_0 and P_{10}. Different levels of P significantly influenced gas exchange traits, i.e., net Pn, Tr, gs, and Ci (Fig. 1). The Cu excess resulted in a significant decrease in Pn, Tr, gs, and Ci. In addition, P doses up to P_{15} showed an increase in Pn, Tr, gs, and Ci. Moreover, gas exchange parameters significantly decreased (except Ci) in response to additional P (P_{20}).

MDA and proline

The findings indicated that excessive Cu presence significantly increased MDA in kenaf leaves, a copper oxidative damage marker. The notable increase in MDA (71.66 ± 2.08 μmol g^{-1} FW) was measured at $P_{0,}$ followed by P_{10} (57.01 ± 2 μmol g^{-1} FW), P_{15} (47.33 ± 2.10 μmol g^{-1} FW), and P_{20} (57.66 ± 1.52 μmol g^{-1} FW). Kenaf leaf proline was also significantly increased in Cu-polluted soil,

Table 2 Cu uptake (mg kg^{-1}) by roots, leaf, stem, and fiber under different levels of P applications

Treatment	Cu in Root	Cu in Leaf	Cu in Stem	Cu in Fiber
P_0	73 ± 4.04^{d}	17 ± 2.06^{b}	4.44 ± 0.31^{c}	1.2 ± 0.16^{c}
P_{10}	99 ± 4.16^{c}	22 ± 1.52^{b}	5.26 ± 0.21^{b}	1.6 ± 0.06^{bc}
P_{15}	112 ± 3.05^{a}	27 ± 2.50^{a}	6.03 ± 0.25^{a}	1.77 ± 0.07^{ab}
P_{20}	129 ± 3.69^{b}	32 ± 2.51^{a}	6.66 ± 0.24^{a}	2.06 ± 0.17^{a}

Letter-different bars represent mean ± standard deviation ($n = 3$). Different letters on the bars show significant differences ($P < 0.05$) between the treatments

Table 3 Removal of Cu contents (mg kg^{-1}) from polluted soil by kenaf plants

Treatment	Initial Cu in soil	Total plant uptake	Remaining Cu in soil	Removal %
$\mathbf{P_0}$	2375	97 ± 3^{d}	2280 ± 4^{d}	4.04
$\mathbf{P_{10}}$	2375	123 ± 4^{c}	2248 ± 5^{c}	5.38
$\mathbf{P_{15}}$	2375	142 ± 4^{b}	2229 ± 4^{b}	6.18
$\mathbf{P_{20}}$	2375	179 ± 6^{a}	2202 ± 6^{a}	7.15

Letter-different bars represent mean ± standard deviation ($n = 3$). Different letters on the bars show significant differences ($P < 0.05$) between the treatments

while P fertilization significantly decreased the proline in leaves. The highest proline was found at P_0 (27.85 ± 0.79 µg g^{-1} FW), followed by P_{10} (23.77 ± 0.93 µg g^{-1} FW), P_{15} (17.20 ± 0.73 µg g^{-1} FW), and P_{20} (20.37 ± 0.59 µg g^{-1} FW).

Antioxidant enzyme activity

This study found that soil contaminated with Cu increased oxidative stress in kenaf plants. The elevation in antioxidant levels in the absence of P under conditions of high Cu concentration indicates oxidative stress attributed to elevated levels of MDA within leaf tissues. The SOD and POD contents decreased by 23.18% and 38.24% at P_{15} than control treatment (Fig. 1). Antioxidant enzyme activity was significantly reduced with increased levels of P, and resulted in minimum values of SOD (49.92 ± 1.88 U g^{-1} FW), and POD (71.72 ± 1.05 U g^{-1} FW) at P_{15} comparatively with P_0 (73.34 ± 1.51,93.35 ± 2.40 U g^{-1} FW), P_{10} (66.46 ± 1.17, 86.09 ± 1.56 U g^{-1} FW), and P_{20} (61.05 ± 1.53, 82.17 ± 1.49 U g^{-1} FW). Furthermore, the application of P up to P_{15} led to a notable decline in SOD and POD contents. However, with an additional increase in the P value, SOD and POD activities also increased.

Cu accumulation

In this study, kenaf plants exhibited a capacity to accumulate a substantial quantity of Cu in their roots comparatively with upper plant parts (Table 2). The results demonstrated that more P in the Cu soil significantly increased Cu concentration across all plant parts. Further, the maximum accumulation of Cu was noted under P_{20} in roots (129 ± 3.69 mg kg^{-1}), leaf (32 ± 2.51 mg kg^{-1}), stem (6.66 ± 0.24 mg kg^{-1}), and fibers (2.06 ± 0.17 mg kg^{-1}), which is 76%, 146%, 50% and 71% more than the control treatment (P_0). Moreover, with increasing P levels, the Cu uptake in plant parts gradually increased (Table 2).

The copper in the soil before kenaf sowing and after harvesting was also measured to analyze how kenaf and P fertilizers affected Cu-contents in the soil (Table 3). After the kenaf harvest, a decrease in Cu content in the soil was observed compared to its initial levels in Cu soil. The removal of Cu contents from soil gradually increased with increasing P application.

Table 4 Effects of phosphate fertilizer application on soil pH and soil bulk density mg.m^{-3} in copper soil

Treatment	pH	Decreased %	Bulk Density	Decreased %
P_0	8.51 ± 0.01 a	0%	1.85 ± 0.02^{a}	0.56
P_{10}	8.30 ± 0.11 ab	2.58	1.75 ± 0.02^{b}	2.68
P_{15}	8.11 ± 0.08 bc	4.81	1.69 ± 0.03^{b}	5.91
P_{20}	7.88 ± 0.12 c	7.51	1.56 ± 0.01^{c}	16.12

Letter-different bars represent mean ± standard deviation ($n = 3$). Different letters on the bars show significant differences ($P < 0.05$) between the treatments

Soil pH and bulk density

As shown in Table 4, the pH of the copper-contaminated soil was significantly higher than phosphorous-treated soil in our experiment. The soil pH gradually decreased with increasing P levels; the maximum was found at P_{20}. The minimum soil pH decreased by 2.58%, 4.81%, and 7.51% compared to P_0. In contrast, soil bulk density increased with increasing P levels, and maximum soil bulk density was found at P_{20}. The soil bulk density decreased by 2.68%, 5.91%, and 16.12% compared to P_0.

Pearson's correlation

Pearson's correlation graph was generated to illustrate the relationship between Cu uptake and different growth parameters of kenaf (Fig. 2). This correlation demonstrated a strong connection between growth traits and Cu accumulation in kenaf. The correlation between various plant growth and antioxidant enzyme analysis parameters under varying P levels is shown in Fig. 2. The pH, FW, DR, NL, SPAD, and SD are positively correlated with net Pn, gs, Tr, Ci, Cu contents in roots, leaf, shoot, and fibers. In addition, these

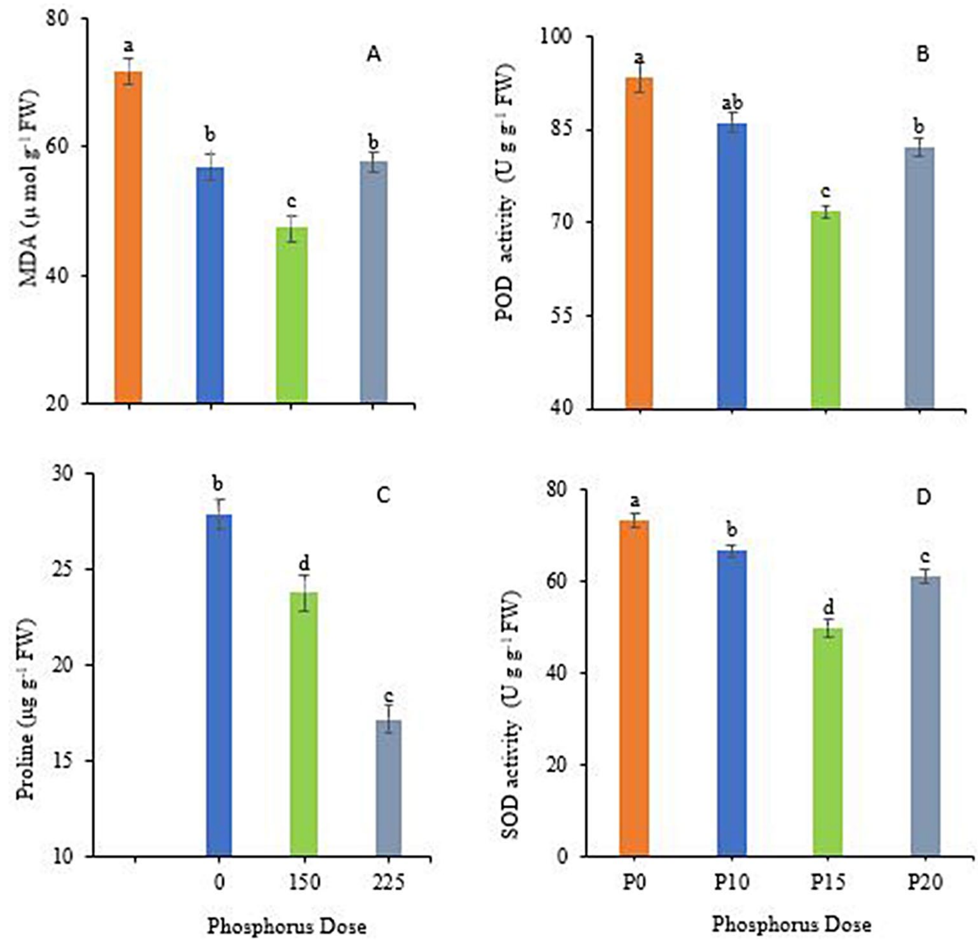

Fig. 2 Correlation between different growth parameters. PH (plant height), FW (plant fresh weight), DR (plant dry weight), NL (No. of leaves), SPAD, SD (stem diameter), NP (net photosynthesis), SC (stomatal conductance), TR (transpiration rate), Ci (intercellular CO_2), Cu L (Cu in leaves), Cu S (Cu in shoots), Cu R (Cu in the roots), Cu F (Cu in fibers), MDA (malondialdehyde contents), POD (peroxidase activity), Pro(proline), pH(soil pH), BD (bulk density), SOD (superoxide activity)

parameters are negatively correlated with MDA, POD, Proline, soil pH, BD, and SOD.

Discussion

Agronomic parameters

This study illustrated the effectiveness of using P fertilizer in conjunction with Cu-contaminated soil, which increased Cu resistance in kenaf by promoting growth, photosynthetic activity, antioxidant defence, and the uptake of soil Cu. According to our findings, the maximum plant height, fresh and dry biomass, SPAD value, stem diameter, and No. of leaves were noted at P_{15} (Table 1). The increased biomass production may be due to improved photosynthetic processes. Similar findings were obtained by Saleem et al. (2020a), mentioning that adding P fertilizer promoted the growth and biomass of jute in Cu-contaminated soil. Recently, Huang et al. (2024) found that P fertilizer at 600 mg/kg increased the height of the plant, dry weight, and SPAD contents (1.28, 1.27, and 1.19 times more) in ryegrass. Previous research studies have consistently indicated that P significantly promotes plant growth and biomass (Chotchutima et al. 2016; Khanam et al. 2016), which is aligned with our research findings. In our research, fresh and dry biomass were significantly decreased when no P was used in highly Cu-polluted soil. Saleem et al. (2019b) found that Cu-contaminated soil mainly changed the ultrastructure of chloroplasts in jute plants. The decline in fresh biomass may be attributed to heavy metal's ability to suppress photosynthesis, subsequently decreasing overall plant productivity (Kastori et al. 1992). The plant growth parameters also start decreasing when P application increases to P_{20}. Earlier studies have shown that increasing P concentration minimized seed proteins and plant growth (Yang et al. 2020), which is aligned with our results. Our results confirmed that kenaf plants can survive in Cu-toxic soil up to $P_{15,}$ and a further increase in P can decrease plant growth. The decline in plant growth and biomass under low P concentrations (P_0) is likely attributed to the limited P uptake by kenaf in contaminated soil. Furthermore, higher P concentrations caused a decrease in kenaf growth, and possibly heavy metal stress affected the photosynthetic process, consequently impacting plant biomass and growth (Ahmad et al. 2017; Dai et al. 2017). Our research findings showed that the maximum dose of P was harmful to kenaf growth, as shown in different jute varieties (Saleem et al. 2020a). The poor growth and biomass might be due to the low absorption of water and nutrients, poor stomatal conductance, and different plant metabolic systems (Akram et al. 2018; Khan et al. 2019b). In our experiment, the leaves per plant and stem diameter increased with the rising P dose up to a certain point. These results are consistent with Kim and Li (2016), who stated that P fertilizer enhanced vegetative growth by maximizing the No. of leaves and stem width in Lantana.

Chlorophyll and gaseous exchange attributes

According to our results, the SPAD value and gaseous exchange attributes were at their lowest levels at P_0. When P application increased, there was a significant rise in SPAD value and photosynthesis up to a specific level (Table 1; Fig. 1). The highest SPAD (44 ± 1.40) was observed at P_{15}. In contrast, a further addition in P dose resulted in a significant decrease in SPAD by 18% compared with P_0 (Table 1). Similarly, the maximum Pn (18.7 µmol $m^{-2}s^{-1}$), gs (0.69 µmol $m^{-2}s^{-1}$), Tr (6.71 nmol m^2s^{-1}), and Ci (235 µmol m^{-1}) levels were noted at the P_{15} level. Recently, Saleem et al. (2020a) stated that different levels of P applications significantly affected Pn, gs, Tr, and Ci in jute, and maximum Pn, gs, and Tr were found at 60 kg P ha^{-1}, which is aligned with our research findings. In another research, Rehman et al. (2021) also revealed that nitrogen applications significantly altered the net-photosynthesis transpiration rate, stomatal conductance, and SPAD value in ramie. Excessive copper in the soil negatively impacted the leaf SPAD value. These results might be due to the displacement of magnesium (Mg) ions, a crucial element in chlorophyll biosynthesis (Marques et al. 2018; Zaheer et al. 2015).Furthermore, leaf chlorophyll content is a key parameter for assessing plant stress. In our findings, the minimum SPAD value was found at P_0, and the maximum level was found at P_{15}. Previous studies indicated improper Cu concentration in soil negatively impacts leaf chlorophyll content in plants (Marques et al. 2018; Saleem et al. 2020b), which is similar to our study findings. In conditions with low P and a high Cu in the soil, reduction in SPAD can be attributed to the interaction of specific enzymes with P. This interaction may potentially contribute to the degradation of SPAD values (Table 1). The decline in chlorophyll and photosynthesis in plants may be associated with a reduction in the maximum quantum efficiency of PSII and the electron transport chain (Garcia-Molina et al. 2011; Habiba et al. 2015). Nonetheless, an excessive P concentration harms leaf chlorophyll and diminishes photosynthesis, as documented (Saleem et al. 2020a).

Oxidative responses and antioxidant enzymes

In the current experiment, P fertilizer improved plant growth in Cu soil by scavenging free ROS, as evidenced by the reduction in MDA levels. The results indicated that excessive levels of Cu significantly increased MDA levels in kenaf leaves, signifying oxidative damage caused by Cu exposure. Our findings are aligned with Saleem et al. (2020d), who used different P applications in Cu-contaminated soil. In the jute plants, Alatawi et al. (2023) found that P application decreased MDA contents in Cu-toxic soil, which is aligned with our findings. The maximum increase in MDA (71.66 µmol g^{-1} FW) was measured at P_0, followed by P_{10} (57.01 µmol g^{-1} FW), P_{15} (47.33 µmol g^{-1} FW), and P_{20} (57.66 µmol g^{-1} FW). Increased Cu concentration in soil can increase lipid peroxidation (Li et al. 2018b). As MDA indicates the presence of oxidative stress, it can cause oxidative damage in leaves and cells (Zaheer et al. 2015; Rehman et al. 2019c; Saleem et al. 2020d). Leaf proline significantly increased in Cu-polluted soil, while P significantly decreased the proline in kenaf leaves (Fig. 3c). The maximum level of proline was found at P_0 (27.85 µg g^{-1} FW), followed by P_{10} (23.77 µg g^{-1} FW), P_{15} (17.20 µg g^{-1} FW) and P_{20} (20.37 µg g^{-1} FW). In addition, plants subjected to excessive Cu developed high proline concentrations in their tissues to combat the Cu stress (Monteoliva et al., 2014; Rehman et al. 2019a). In our research, we applied P fertilizer in Cu soil to enhance plant growth by neutralizing free reactive oxygen species. Recently, Huang et al. (2024) found that phosphorus fertilizer significantly increased the activity of antioxidant defence mechanisms. Under heavy metal stress conditions, the antioxidative defence mechanism protects plants from harm and ensures their survival (Karimi et al. 2013). Our research found that phosphorus fertilizers increased antioxidant defence mechanisms in Cu-toxic soil. The SOD and POD contents decreased by 23.18%, 38.24%, at P_{15} compared to P_0. Furthermore, antioxidant activities were closely associated with kenaf growth and biomass. In recent research, Alatawi et al. (2023) found that increasing levels of Cu concentration in soil caused a significant ($P < 0.05$) increase in SOD, POD, and CAT contents, which is also aligned with our research findings. Reactive oxygen species (ROS) produced in plant cells and tissues are harmful, and plants have a robust antioxidant defence system to neutralize and remove ROS. Plants produce various antioxidants, including SOD and POD, which activates when plants face environmental stress leading to oxidative damage (Adrees et al. 2015; Kamran et al. 2019; Zafar et al. 2019). Furthermore, Ahmad et al. (2017) found that maize treated with different P doses showed decreased antioxidant activities, improving plant growth.

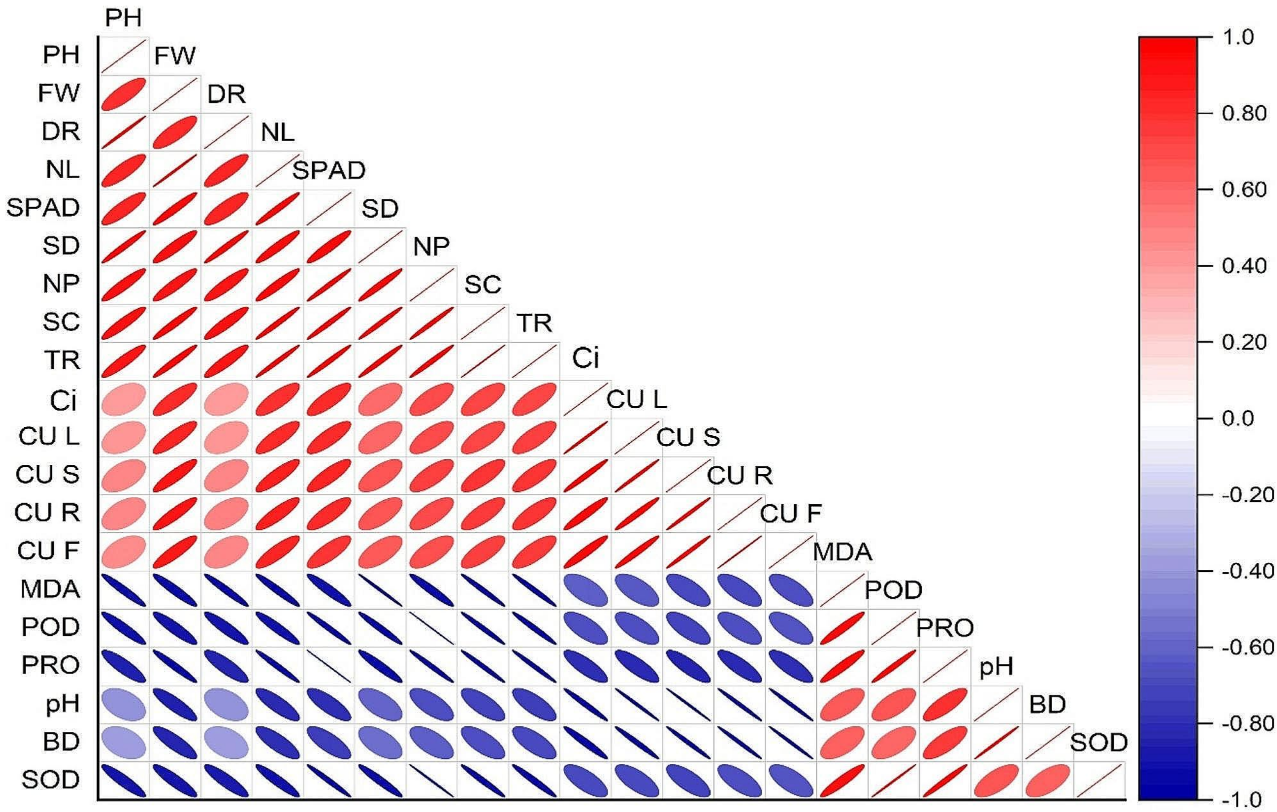

Fig. 3 Variations in (**a**) MDA, (**b**) proline, (**c**) SOD, and (d) POD activity in leaves under different P levels. Letter-different bars represent mean ± standard deviation ($n=3$). Different letters on the bars show significant differences ($P<0.05$) between the treatments

Cu uptake, soil pH, and bulk density

The range of 5 to 30 mg kg^{-1} of Cu in the soil does not harm plants when they usually grow (Li et al. 2018b; Shabbir et al. 2020; Alatawi et al. 2023). The absorption and movement of harmful elements in various plant parts are based mainly on growth conditions, metal availability, and the type of plant (Husak 2015; Marques et al. 2018). Our results showed that, compared to other plant components, kenaf plants could absorb significant levels of Cu in their roots (Table 2). The Cu accumulation in roots (129 mg kg^{-1}), leaf (32 mg kg^{-1}), stem (6.66 mg kg^{-1}), and fibers (2.06 mg kg $^{-1}$) was 76%, 146%, 50%, and 71% (P_{10}, P_{15}, P_{20}) more than the control treatment. According to Huang et al. (2024), phosphorus fertilizer can modify the pectin in plant cell walls, promoting the uptake of Cd in plant tissues and improving phytoremediation capacity (Cd contents were 1.12 times higher than control treatment. Further, Cu concentrations in wheat grains were higher when P fertilized respectively with other fertilizers (Sabiha-Javied et al. 2023). Recently, Li et al. (2024) found that P fertilizer increased the Cadmium content in rice plants and found more in roots than leaf sheaths, stems, and leaves, consistent with our research findings. Our results are supported by the fact that the P application increased Cu uptake in roots and shoots (Saleem et al. 2020a). According to Wang et al. (2024), the underground portion of herbaceous plants had higher Cu concentrations than the above-ground portion, which is aligned with our results. The increased Cu uptake by roots was associated with a decreased translocation of Cu into shoots in Cu-resistant plants (Liu et al. 2004). This mechanism can be attributed to the increased transpiration rate of kenaf under P application, leading to higher Cu accumulation and transportation to plant parts, as shown in Fig. 1. When taken as a whole, these investigations offer more proof of the beneficial influence of P fertilizer on the uptake and removal of harmful substances by plants.

Soil pH is an essential element for the bioavailability of orthophosphates. The soil pH level can influence the concentration of metal cations, which react with orthophosphate, and the ability of Fe and Al oxides to hold P ions (Hinsinger, 2001; Plante, 2006). The normal pH range (6.5 to 7.0) is optimal for P availability in soils (Penn and Camberato, 2019). In our results, as shown in Table 4, the pH of the copper-contaminated soil was significantly higher than that of the phosphorous-treated soil in our experiment. The soil pH gradually decreased with increasing P levels; the maximum was found at P_{20}. Our findings are aligned with Ali et al. (2014), who also discovered that P fertilizer decreased soil pH significantly, positively affecting micronutrients' solubility. In previous research, Nadian et al. (1998) found that decreased P application increased soil compaction up to a bulk density of 1.60 mg m^{-3}, aligned with our research in low P application, where soil bulk density was minimal. Further, an increase in soil bulk density caused a significant reduction of root length, reduced O_2 content in soil, and increased ethylene production (Nadian et al. 1998).

Conclusion

According to our findings, the impact of varying P concentrations on kenaf plant growth, biomass, SPAD values, antioxidant responses, and Cu uptake in different plant parts was assessed. It can be summarized that kenaf plants exhibited greater tolerance to Cu-contaminated soil when P_{15} was applied, leading to enhanced plant growth and biomass. Our findings showed Cu contents in the roots, leaves, shoots, and fibers increased under different P levels. Our findings indicated that the P augmented the hyperaccumulation potential of the plant species. Consequently, it can be inferred that externally applying P enhances kenaf copper uptake potential, growth, and development, especially in Cu-contaminated soil. Additionally, it suggests the need for field testing to determine the potential of P application in remediating soils polluted with heavy metals.

Author contributions The research was organized by L.L. M.R. and C.R. completed the research layout, data collection, analysis, and manuscript writing. L.L., J.Y., and M.H. reviewed the manuscript. All authors are agree to publish.

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Data availability Data will be made available on request.

Declarations

Ethics approval and consent to participate Not applicable.

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