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RESEARCH ARTICLE

Kenaf and soybean intercropping affects morpho-physiological attributes, antioxidant capacity and copper uptake in contaminated soil

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Abstract

Background and aims Intercropping can affect the growth and elemental absorption of crops. Copper (Cu) pollution has become a worldwide environmental problem due to its effect on human health and food security. Well-designed intercropping of crops could be an effective strategy for sustainable production and remediation of contaminated soil.

Methods A pot experiment was conducted to investigate the effects of monoculture and intercropping on morpho-physiological attributes, antioxidant capacity, root growth and Cu uptake of kenaf and soybean grown under different levels of Cu in soil.

Results Intercropping was conducive to the accumulation of biomass and chlorophyll in kenaf and soybean under Cu stress. Mutual intercropping of kenaf and soybean increased antioxidant enzymes activity and decreased malondialdehyde content in both the plants under Cu stress. In addition, intercropping increased soluble sugars, soluble proteins and proline content in kenaf and soybean under Cu stress except that proline content were not significantly different in kenaf. Moreover, intercropping alleviate the negative effects of Cu by reducing its soil availability and uptake in the plants. At the same time, bio-accumulation factor (BAF) and translocation factor (TF) were declined.

Conclusion Mutual intercropping of kenaf and soybean is favorable to promote plant growth and biomass production of both the species with reduced Cu uptake in plant tissues. Present findings strengthen our understanding about effectiveness of intercropping on Cu contaminated soils for sustainable crop production and soil remediation.

Keywords Intercropping · Kenaf · Soybean · Copper uptake · Physiology

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Introduction

Intercropping is the cultivation of two or more crops together in the same field for a period of time. It is an integrated strategy that enhances crop productivity through complementary benefits in nutrient absorption and disease resistance (Zhang et al. 2023; Li et al. 2021). Intercropping ensures yield enhancement, environmental security, production sustainability and greater ecosystem services (Maitra et al. 2021).

Soil pollution has become a major challenge due to heavy metals toxicity, persistence, and bioaccumulation (Mitra et al. 2022; Rehman et al. 2021). Excessive accumulation of heavy metals in the soil adversely affects the soil characteristics and plant growth (Pan et al. 2023; Zafar-ul-hye et al. 2021; Younis et al. 2020; Shah et al. 2020; Zafar-ul-hye et al. 2020; Rehman et al. 2019a; Dotaniya et al. 2020; Danish et al. 2019). Among various metals in soil, copper is a microelement and beneficial for plant growth however, its accumulation inhibit the growth and disrupt metabolism (Chen et al. 2022; Sağlam et al. 2016; Zhao et al. 2010; Chaffai et al. 2007). Natural and anthropogenic sources release Cu in soil. Natural sources mainly include weathering rocks, and anthropogenic sources include mining, smelting, sewage water, and Cu containing fertilizers and fungicides (Rehman et al. 2019a; Yang et al. 2018). Agricultural soils receive considerable quantity of Cu due to excessive application of Cu containing fertilizers and fungicides every year (Mir et al. 2021; Husak 2015). High level of Cu in plants causes reactive oxygen species(ROS) production which result metabolic pathways disturbance and cellular damage (Liu et al. 2018; Hegedus et al. 2001).

For the protection of agricultural lands and ecosystems, this is the time to search and implement economical and effective strategies to address heavy metal contamination. Among various possible agronomic strategies, the practice of intercropping is considered more economical, reliable, effective, ecologically friendly, and publically acceptable. This is a practice of growing two or more crops together on a piece of land for a time (Brooker et al. 2015), and can be applied for the phytoremediation of heavy metal contaminated soil (De Conti et al. 2019). Currently, intercropping has been applied to farmland potentially toxic metals (PTM) remediation, that can efficiently reduce PTM uptake in agricultural produce (Wang et al. 2020). Being an eco-friendly strategy, intercropping mitigate Cu stress in slightly or moderately contaminated soils (Brunetto et al. 2016) with advantages of increase in crop production rate and control of weeds (Liebman and Dyck 1993). This increase in crop yield and quality is associated with the effective use of resources such as soil fertility, sunlight, and water by adjacent plants under intercropping (Li et al. 2009). At present more than 100 intercropping combinations have been applied in China, and 70% of them are including leguminous crops (Li et al. 2014), for example, intercropping of tea and soybean (Sun et al. 2022). Several other studies reported the role of intercropping in regulation of heavy metals absorption by the plants such as *Solanum nigrum* L. significantly inhibited the Cd uptake in *Solanum melongena* L. under intercropping (Tang et al. 2017). Similarly, intercropping with *Thalia dealbata*, significantly decreased the Cd uptake of rice grains (Wang et al. 2020). Intercropping reduced Pb concentrations in the shoots and roots of ryegrass and alfalfa (Cui et al. 2018). There are few studies, however, addressing the physiological mechanisms behind this phenomenon (Sadeghzadeh et al. 2022).

Kenaf is a bast fiber crop, grown well in tropical climate and can be used to remediate heavy metals contaminated lands (Chen et al. 2021; Saleem et al. 2020). Arbaoui et al. (2013) investigated Zn and Cd accumulation in kenaf and *Z. mays* and found 82.5 mg/kg of Zn and 2.49 mg/kg of Cd in kenaf shoots and 10.19 mg/kg of Zn and 2.1 mg/kg of Cd in *Z. mays* shoots. Soybean is an oilseed crop and a source of high-quality protein and oil for human and livestock use (Adeyemi et al. 2020). Soybean being a leguminous crop can fix nitrogen symbiotically in the presence of effective strains of *Rhizobium* in the soil. Previous studies reported that legumes, with their ability to fix atmospheric N offers minimum competition for N nutrition (Vance 1998; Rerkasem et al. 1988) because N fixed by legumes becomes available to non-fixing neighboring plant (Carranca et al. 2015) that increased the possibility for sustainable production. Soybean–tea intercropping sustainably improve nutrient management, crop yield, and quality (Duan et al. 2019). Chu et al. (2004) verified that in the intercropping of sorghum and soybean about 32–58% of the N assimilated by sorghum was derived from soybean.

Several studies were conducted on kenaf or soybean, but most of the studies were only on the sole crop of kenaf or soybean and we still lack an adequate understanding of how intercropping kenaf and soybean affects the growth and Cu absorption in adjacent plant for sustainable crop production. Therefore, we hypothesized that intercropping of kenaf and soybean could be effective to promote plant growth and to reduce the Cu uptake in contaminated soil. To this end, in present study, kenaf and soybean were mutually intercropped at different levels of Cu contaminated soil, and the sound effects of this intercrop combination on the morphology, physiology and Cu uptake in both of the species were investigated.

Materials and methods

Materials

The soil was collected from an experiment field in Guangxi, China at 0–20 cm depth. Kenaf cultivar 'Zhongsi 1' seeds were obtained from the laboratory of Plant Genetics and Breeding, College of Agriculture, Guangxi University, China. The seeds of soybean local cultivar Guixia 1 were obtained from market.

Experimental design

The experiment was conducted in a glasshouse at the Guangxi University (22° 50' 47" N, 108° 17' 17" E) during 2022. The soil was air dried, crushed and passed through 5 mm sieve. Later the sieved soil was thoroughly mixed. The basic physical and chemical properties of the soil were: pH 6.2 (1:2.5 w/v soil: water suspension), available phosphorus 28.7 mg/kg, available potassium 143 mg/kg, organic matter 17.3 g/kg, and total Cu 34.9 mg/kg. The soil was artificially spiked with copper using $CuSO_4·5H_2O$ and equilibrated for 60 days by four saturation cycles using distilled H_2O and air drying. The experiment comprised of three levels of Cu in soil (0 Cu: 0 mg/kg, 300 Cu: 300 mg/kg, and 600 Cu: 600 mg/kg Cu on dry soil basis) and three cropping treatments (monoculture of kenaf, monoculture of soybean, and intercropping of kenaf and soybean), with three replicates. The seeds were soaked in 1.5% NaClO (AR) for 10 min to get their surface sterilized and then washed with deionized water. Subsequently, the seeds were soaked in water for 2 h at 28 °C for imbibition. Finally, these seeds were sown in germination trays. Seven days after germination, seedlings of uniform growth from both the species were selected and removed to transplant into the prepared pots containing 10 kg of dry soil. Four plants of kenaf or soybean were planted in each pot under monoculture condition. For the mutual intercropping of two species, two plants of kenaf and two plants of soybean were planted in each pot. A space of 10 cm was maintained between the pots. The margin effect was reduced by every so often changing the pots positions during whole study. A controlled temperature of 28 °C, light intensity of 350 μmol/m^2/s, and light/dark period of 16/8 h were maintained throughout the growth period. Deionized water was added in the soil to maintain 70% (w/w) of water-holding capacity (WHC) till the crop harvest. Weeds were removed at different intervals and the pots were kept weed free.

Sampling, harvesting and data collection

After 40 days of cultivation the upper mature leaves of each species were selected to measure chlorophyll using a hand-held, portable and non-destructive SPAD-502 (Minolta) Chlorophyll Meter. Then the upper fully expanded leaves were collected during 09:00–10:30 a.m., cleaned and immediately stored at -80 °C. Shoots of both the species were harvested by cutting stems at 5 cm above the soil surface. The roots were uprooted and adhered soil on the root surface was removed. The roots were washed thrice with distilled water, and once with deionized water. Shoots and roots were dried in an oven at 70 °C for 72 h and finally weighed using a digital weighing balance machine to determine their dry weights.

Determination of root morphological attributes

The roots were carefully separated from the soil, rinsed with distilled water, and then scanned using an imagery Scan Screen (Epson Expression 11000XL, Regent Instruments, Canada) to investigate root morphology, and the WinRHIZO 2003a programme (Regent Instruments, Canada) was used to measure root attributes.

Determination of antioxidant enzyme activity, content of MDA, proline, soluble sugars and soluble protein

Superoxide dismutase (SOD) activity was determined by measuring its ability to inhibit photochemical reduction of nitro blue tetrazolium chloride (NBT), and the activity of peroxidase (POD) was determined by guaiacol method (Chen et al. 2020). Malondialdehyde (MDA) level was measured following the Hodges et al. (1999) method to assess membrane lipid peroxidation and the absorbance of the supernatant was measured at 532 nm. The method outlined by Bates et al. (1973) was used to measure proline content and the proline concentration was estimated using a calibration curve. Anthrone colorimetric method was used to measure the soluble sugar content as reported by (Feng et al. 2021). The concentration of soluble protein was calculated by using bovine albumin serum (BSA) standard curve (Bradford 1976).

Determination of copper in plant tissues

To obtained uniform samples, crushed dry roots, leaves and stem were passed through 100-mesh sieve separately. Dry roots, leaves and stem samples were digested in HNO_3-$HClO_4$ mixture (volume ratio: 3:1), and analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent, USA) for Cu content. Bioaccumulation factor (BAF) was calculated as the ratio of Cu content in plant tissue to the soil Cu content and translocation factor (TF) was calculated as the ratio of Cu content in leaf or stem to the Cu content in root (Rehman et al. 2019b).

Determination of soil pH and soil available copper

The soil of each pot was dried naturally and ground into fine powder to determine pH at 1:2.5 (w/v) soil water suspension, using an automated pH meter (Lu 1999). The DTPA (diethylenetriaminepentaacetic acid) extraction method was used for estimating the soil availability of Cu.

Data analysis

Data were analyzed by two-way analysis of variance (ANOVA), followed by Tukey HSD (honestly significantly difference) test with Statistix 8.01 (Analytical Software, Tallahassee, F.L., United States). Statistical variations of the data were showed as standard deviation and significance of the data was calculated at $p \leq 0.05$. Graphical presentation was done using MS-Excel and GraphPad Prism.

Results

Intercropping improved biomass, and chlorophyll of kenaf and soybean under Cu stress

Excess Cu impaired kenaf and soybean growth that was confirmed by reduced dry weight of shoot and root (Fig. 1). However, intercropping of kenaf and soybean alleviated this reduction in growth under Cu stress compared with monoculture. Intercropping increased the shoot dry weight by 26.8% (Fig. 1a), and root dry weight by 12.5% (Fig. 1b) in kenaf under 300 mg/kg Cu in soil. In case of soybean, at 300 mg/kg Cu in soil, intercropping increased the shoot dry weight by 12.8% and root

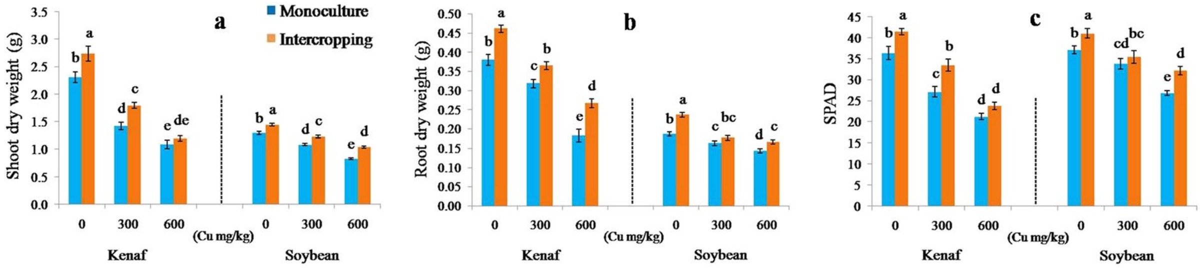

Fig. 1 Effects of intercropping on **a** shoot dry weight/plant, **b** root dry weight/plant, and **c** chlorophyll (SPAD index) of kenaf and soybean grown in copper contaminated soil. Bars are mean ± SD ($n = 3$). Different letters on bars indicate significant difference ($p \leq 0.05$)

dry weight by 12.5%, compared to monoculture. Furthermore, compared with monoculture, at 600 Cu, intercropping increased shoot dry weight by 10.1%, and root dry weight by 50.0%, in kenaf. In case of soybean, at 600 Cu, intercropping increased shoot dry weight by 25.3%, and root dry weight by 21.4%, compared to monoculture. Excess Cu also affected the chlorophyll (SPAD index) of kenaf and soybean. SPAD values were decreased with the increasing Cu in soil (Fig. 1c). However, intercropping of kenaf and soybean increased the SPAD values at all levels of Cu in soil. Leaf SPAD values were increased by 13.9 and 22.2% in kenaf grown under intercropping at 0 and 300 Cu levels respectively while, it did not differ at 600 Cu, compared to monoculture. Moreover, intercropping increased SPAD values in soybean by 10.8, 2.90, and18.5% at 0, 300 and 600 Cu respectively, compared to monoculture.

Intercropping improved root growth of kenaf and soybean under Cu stress

Similar to the shoot growth in kenaf and soybean, root growth was also reduced under incremented Cu in soil. However, intercropping alleviate the effects of Cu on root morphology of kenaf and soybean (Fig. 2). Under the same Cu concentration, monocultured kenaf and soybean roots were shorter than those from intercropping. Our results showed that intercropping increased root total length in kenaf by 21.5, and 16.5% at 0 and 300 Cu respectively, and in soybean by 15.9, and 28.7% at 300 and 600 Cu respectively, compared to monoculture (Fig. 2a). However, no significant differences in the root total length in kenaf at 600 Cu and in soybean at 0 Cu treatment were found between monoculture and intercropping, compared to control. Root projected area and root surface area of kenaf were lower in monoculture than

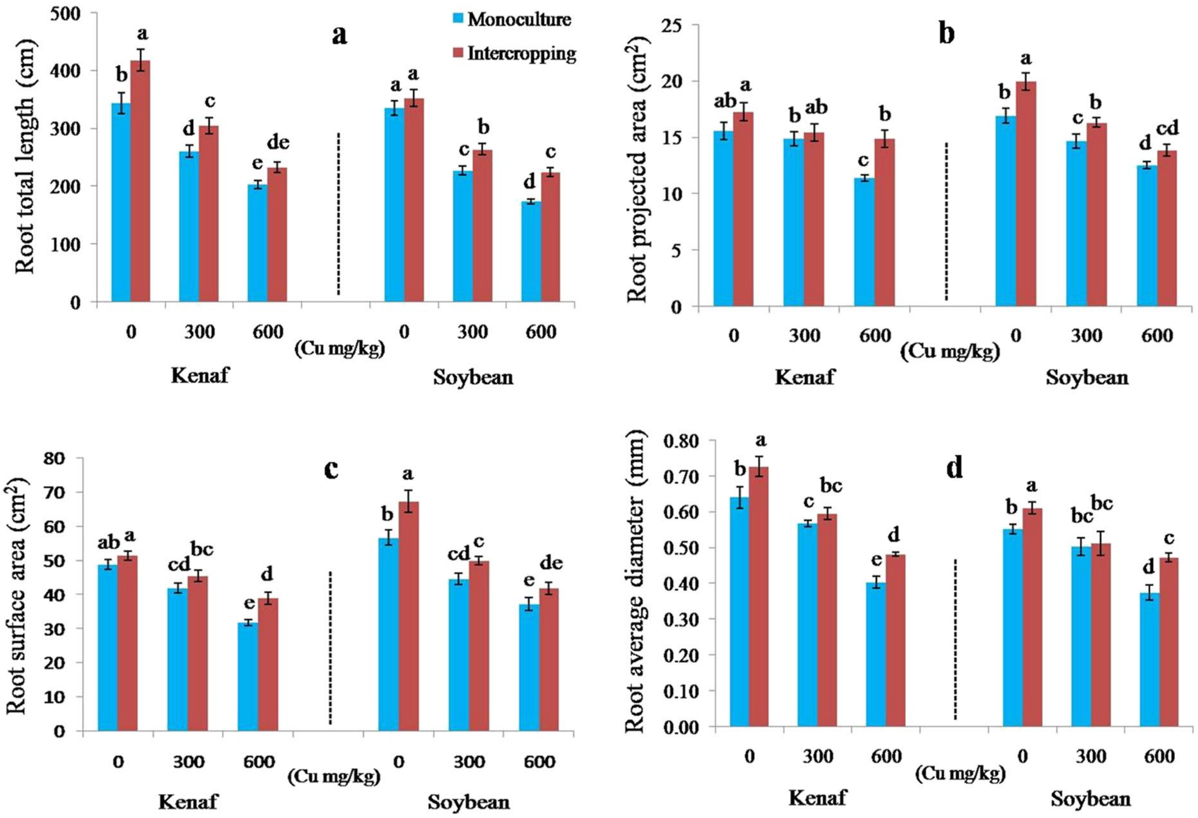

Fig. 2 Effects of intercropping on **a** root length, **b** root projected area, **c** root surface area, and **d** root average diameter of kenaf and soybean grown in copper contaminated soil. Bars are mean ± SD ($n = 3$). Different letters on bars indicate significant difference ($p \leq 0.05$)

that of intercropping treatment (Fig. 2b, c). Compared with monoculture, intercropping led to a significant increase in kenaf root projected area and root surface area by 30.5, and 22.4% respectively, under 600 Cu. No significant differences in the root projected area and root surface area of kenaf were found between monoculture and intercropping at 0 and 300 Cu treatments. In case of soybean, intercropping caused an increase in root projected area by 18.1, and 11.1% at 0 and 300 Cu respectively, and in root surface area by 18.8% at 0 Cu, compared to monoculture. No significant differences in the root projected area at 600 Cu and root surface area at 300 and 600 Cu were found in soybean between monoculture and intercropping treatments. Furthermore, compared with monoculture, intercropping resulted in a significant increase in kenaf root average diameter by 14.1, and 20.0% and in soybean by 10.9, 23.9% at 0 and 600 Cu respectively. However, the root average diameter was not different statistically under intercropping at 300 Cu in both the crops (Fig. 2d).

Intercropping increased antioxidant enzyme activity, and decreased MDA content of kenaf and soybean under Cu stress

In present study high concentration of Cu in soil increase oxidative stress in kenaf and soybean. Moreover, intercropping increase the antioxidant enzymes activities while, reduce lipid per oxidation in kenaf and soybean (Fig. 3). In kenaf, intercropping increased the SOD activity by 66.0, 20.0, and 11.0% at 0, 300, and 600 Cu respectively, and POD activity by 23.5, 27.0, and 37.6% at 0, 300, and 600 Cu respectively, compared to monoculture. In case of soybean, intercropping increased the SOD activity by 18.8, 15.7, and 18.1%, and POD activity by 10.7, 26.9, and 9.91% at 0, 300, and 600 Cu respectively, compared to monoculture (Fig. 3a, b). Furthermore, our results confirmed that Cu stress significantly improved the kenaf and soybean leaf MDA content representing Cu-induced oxidative damage. However, intercropping alleviate Cu-induced damage by decreasing MDA content in both the kenaf and soybean (Fig. 3c). Compared with monoculture, intercropping exhibited significant reduction in MDA content in kenaf by 34.8, 31.1, and 11.3% and in soybean by 48.5, 16.0, and 23.2% at 0, 300, and 600 Cu, respectively.

Intercropping increased content of proline, soluble sugars and soluble protein of kenaf and soybean under Cu stress

According to present results increment of Cu in soil reduces soluble sugars and soluble proteins in kenaf and soybean. However, intercropping exhibited significant increase in soluble sugars and soluble proteins in both the crops, compared to monoculture (Fig. 3d, e). In kenaf, intercropping increased the soluble sugars by 8.78, 11.8, and 22.5%, and soluble proteins by 6.70, 16.3, and 13.2% at 0, 300, and 600 Cu respectively, compared to monoculture. While, in case of soybean, intercropping exhibited significant increase in soluble sugars by 14.0, 11.4, and 9.96% at 0, 300, and 600 Cu respectively, and soluble proteins by 20.3, and 15.9% at 300, and 600 Cu respectively, compared to monoculture. Intercropping has no significant difference for soluble proteins in soybean at 0 Cu, however values were higher than monoculture. Kenaf and soybean leaf proline was also significantly increased with the increasing levels of Cu in soil. Intercropping explained a significant amount of variation on proline content in soybean. However, intercropping exhibit no significant difference in proline content for kenaf (Fig. 3f). Compared with control, intercropping exhibited significant increase in soybean proline content by 12.1, and 12.2% at 0, 300 Cu respectively while, there was no significant difference at 600 Cu.

Intercropping effected copper uptake, bioaccumulation factor (BAF) and translocation factor (TF) of kenaf and soybean under Cu stress

Cu content in the roots, leaves and stem of kenaf and soybean were significantly different under different Cu concretions and cropping treatments (Table 1). The content of Cu in kenaf and soybean tissues were gradually raised with increment of Cu in soil. In this study, kenaf and soybean roots accumulated more Cu as compared to their aerial parts. However, intercropping alleviate Cu stress due to significant reduction of Cu content in kenaf roots, leaves and stems by 13.4, 15.7, and 29.6% respectively at 300 Cu, and by 12.3, 21.5, and 12.7% respectively at 600 Cu, compared to monoculture. Similarly, intercropping significantly decreased the Cu content in soybean roots, leaves and stems by 19.7, 23.8, and 11.8% respectively at

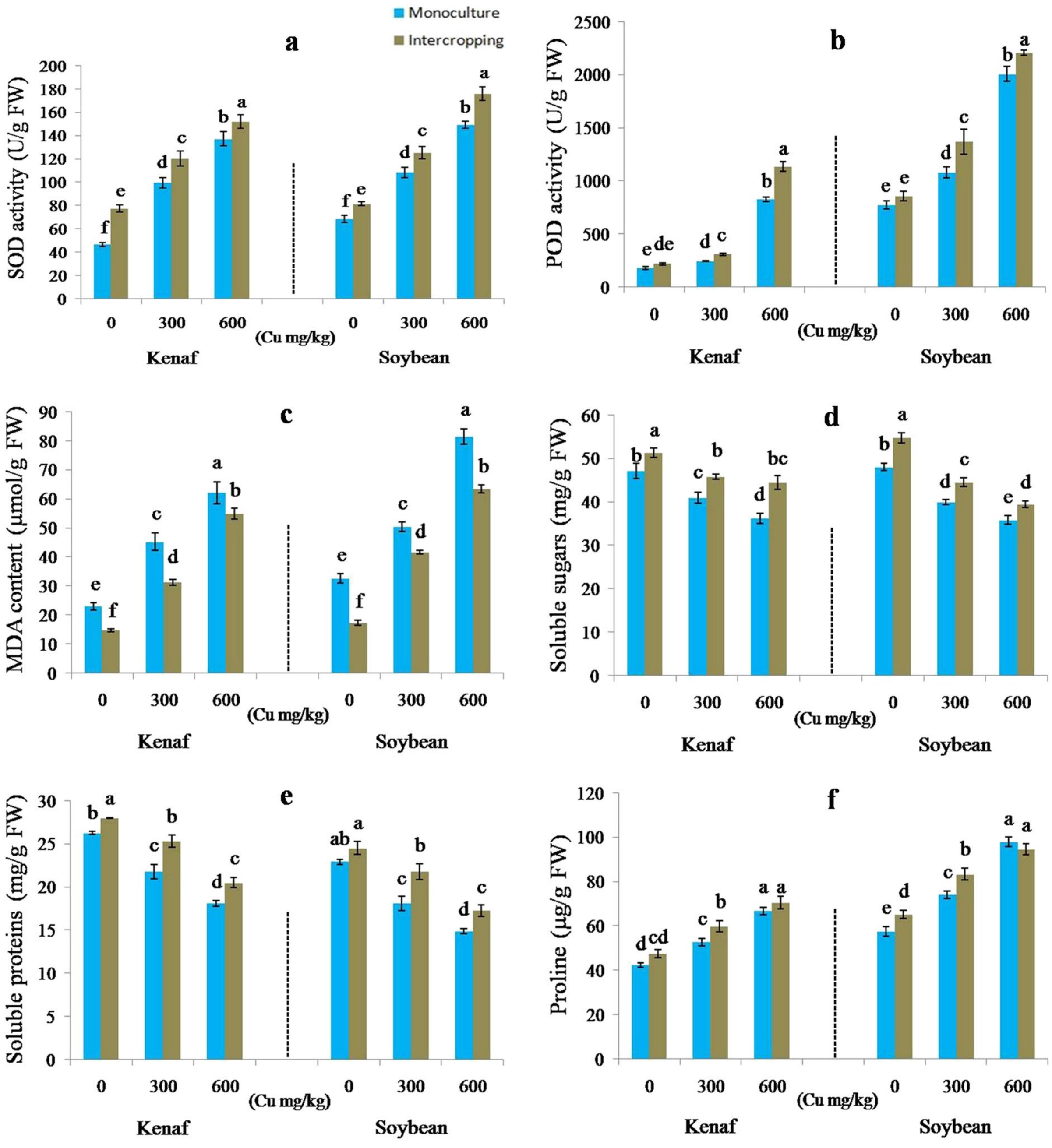

Fig. 3 Effects of intercropping on **a** superoxide dismutase (SOD) activity, **b** peroxidase (POD) activity, **c** malondialdehyde (MDA) content, **d** soluble sugars, **e** soluble protein, and **f** proline content of kenaf and soybean grown in copper contaminated soil. Bars are mean ± SD ($n = 3$). Different letters on the bars indicate significant difference ($p \leq 0.05$)

300 Cu, and by 14.9, 33.0, and 22.8% respectively at 600 Cu, compared to monoculture. BAF and TF could be applied to evaluate phytoextraction level by a plant. Therefore, phytoextraction of Cu in kenaf and soybean were measured by estimating the values of BAF and TF (Table 2). Intercropping reduced the BAF for root, leaf and stem in kenaf and soybean at 300 and 600 Cu in soil. According to our results,

Table 1 Uptake and distribution of copper in root, leaf, and stem of kenaf and soybean grown under monoculture and intercropping at different levels of copper in soil

Copper (mg/kg)	Cropping	Kenaf			Soybean		
		Root	Leaf	Stem	Root	Leaf	Stem
0	Mono.	13.54 ± 0.49^{d}	9.67 ± 0.32^{e}	5.92 ± 0.32^{d}	13.65 ± 0.95^{e}	13.27 ± 0.56^{d}	9.35 ± 0.60^{e}
	Inter.	8.24 ± 0.21^{d}	7.02 ± 0.23^{f}	5.80 ± 0.15^{d}	10.40 ± 0.46^{e}	7.23 ± 0.43^{e}	8.04 ± 0.15^{e}
300	Mono.	82.46 ± 3.15^{b}	13.67 ± 0.63^{c}	8.58 ± 0.32^{c}	101.71 ± 2.99^{c}	23.78 ± 1.19^{b}	21.88 ± 1.05^{c}
	Inter.	71.40 ± 2.18^{c}	11.52 ± 0.53^{d}	6.04 ± 0.22^{d}	81.67 ± 1.37^{d}	18.11 ± 0.97^{c}	19.29 ± 0.33^{d}
600	Mono.	95.10 ± 1.73^{a}	21.32 ± 0.67^{a}	11.63 ± 0.64^{a}	195.95 ± 4.72^{a}	32.20 ± 1.01^{a}	44.12 ± 0.95^{a}
	Inter.	83.36 ± 3.12^{b}	16.74 ± 0.62^{b}	10.15 ± 0.49^{b}	166.74 ± 8.70^{b}	21.59 ± 0.70^{b}	34.06 ± 1.66^{b}

Values in the table are means ± SD ($n=3$). Different letters within a column indicate significant difference ($p \leq 0.05$). Mono: monoculture, Inter: Intercropping

Table 2 Bioaccumulation factor (BAF) and translocation factor (TF) for copper in kenaf and soybean grown under monoculture and intercropping at different levels of copper in soil

Crop	Copper	Cropping	Bioaccumulation factor			Translocation factor	
			Root	Leaf	Stem	Leaf	Stem
Kenaf	300	Mono.	0.246	0.041	0.026	0.166	0.104
		Inter.	0.213	0.034	0.018	0.161	0.085
	600	Mono.	0.150	0.034	0.018	0.224	0.122
		Inter.	0.131	0.026	0.016	0.201	0.122
Soybean	300	Mono.	0.304	0.071	0.065	0.234	0.215
		Inter.	0.244	0.054	0.058	0.222	0.236
	600	Mono.	0.309	0.051	0.069	0.164	0.225
		Inter.	0.263	0.034	0.054	0.129	0.204

Mono: monoculture, Inter: Intercropping

bio-accumulation factor (BAF) and translocation factor (TF) also declined under intercropping system. Overall intercropping significantly affected the studied parameters of kenaf and soybean under Cu stress. This indicated that intercropping of kenaf and soybean could also be a viable option for their sustainable production with reduced Cu content in plant tissues.

Intercropping increased the soil pH and decreased soil available copper

Effects of intercropping kenaf and soybean on soil pH and soil available Cu are shown in Table 3. The soil pH under intercropping of kenaf and soybean was increased at 0, 300 and 600 mg/kg Cu in soil than that of the monoculture of kenaf and monoculture of soybean. However, the soil pH of intercropping kenaf and soybean were similar with monoculture of kenaf under 0 and 600 Cu, respectively. Compared

Table 3 Effects of intercropping kenaf and soybean on soil pH and soil available opper in contaminated soil

Copper (mg/kg)	Cropping	Soil pH	Available Cu (mg/kg)
0	Mono. kenaf	6.20 ± 0.02^{ab}	10.72 ± 1.14^{cd}
	Mono. soybean	6.17 ± 0.03^{bc}	10.92 ± 0.89^{cd}
	Inter. kenaf and soybean	6.26 ± 0.05^{a}	07.03 ± 0.59^{d}
300	Mono. kenaf	5.95 ± 0.03^{d}	27.90 ± 2.61^{b}
	Mono. soybean	5.90 ± 0.05^{d}	28.43 ± 1.50^{b}
	Inter. kenaf and soybean	6.11 ± 0.02^{c}	14.96 ± 0.55^{c}
600	Mono. kenaf	5.47 ± 0.03^{ef}	34.35 ± 3.01^{a}
	Mono. soybean	5.45 ± 0.03^{f}	36.03 ± 1.46^{a}
	Inter. kenaf and soybean	5.55 ± 0.03^{e}	26.13 ± 1.67^{b}

Values in the table are means ± SD ($n=3$). Different letters within a column indicate significant difference ($p \leq 0.05$). *Mono: monoculture, Inter: Intercropping with monoculture, maximum increase in soil pH was observed under intercropping of kenaf and soybean at 0 Cu in soil. The soil available Cu under intercropping of kenaf and soybean was significantly decreased than that of the monoculture of kenaf and monoculture of soybean at 300 and 600 Cu in soil. However, the soil available Cu of intercropping kenaf and soybean was similar with monoculture of kenaf and monoculture of soybean under 0 Cu in soil. Furthermore, minimum soil available Cu was observed under intercropping of kenaf and soybean at 0 Cu in soil.

Discussion

Intercropping improved biomass of kenaf and soybean under Cu stress

The reduction in biomass production is a common response of plants under heavy metal stress. However, intercropping can lead to the safe production of crops with benefits of promoting biodiversity, reducing pest related issues as well as improving growth and crops yields (Xu et al. 2023; Qian et al. 2022; Mir et al. 2022a; Huss et al. 2022; Tilman 2020; Mala et al. 2020; Jurik and Van 2004; Zhang and Li 2003; Li et al. 2001). Our results showed that mutual intercropping of kenaf and soybean significantly improved shoot and root dry weight of both the species, compared with monoculture (Fig. 1). Increase in plant biomass of both the species might be associated with enhanced utilization efficiency of resources such as light, water, and nutrients (Yin et al. 2020). High productivity under intercropping could be attributed to complementarity between crop species in patterns of resource acquisition (Hinsinger et al. 2011) as well as reduction in the diseases, pests and weeds (Cong et al. 2014; Zhu et al. 2000). Additionally, intercropped legume has also been shown to increase nutrient supplies by fixing or transferring N to non legumes (Shao et al. 2020). Intercropping pea and barley improved the plants efficiency to exploit the environmental sources for their higher growth (Hauggaard-Nielsen et al. 2009). Findings of several other studies also reported similar benefits from intercropping for example, intercropping increased the crops yields compared to monoculture (Qian et al. 2018). Intercropping greatly influenced the sugarcane root fresh weight by 14.7% and root dry weight by 26.0% over monoculture (Malviya et al. 2021). Beedy et al. (2010) and Awal et al. (2006) have confirmed that intercropping has yield benefits over monoculture. A meta-analysis by Feng et al. (2021) shows that maize/peanut intercropping is more efficient in the land use than monoculture, and attains yield benefit. Intercropping kenaf and maize raised the farm income ratio compared with sole crop system (Budi and Cholid 2018). According to Tilman (2020) growing crops in an intermingled way can produce up to 25% more food than sole crop. Moreover, intercropping can raise soil carbon, soil nitrogen (Cong et al. 2015), and soil phosphorus (Dissanayaka et al. 2015), thereby promoting the crop growth and increasing yield. In present study increase in plant growth and biomass in Cu contaminated soil might be associated to reduced Cu availability in soil under intercropping treatment. Previous studies also reported improved plant growth by reducing heavy metal stress in eggplant (Pan et al. 2021), water spinach (Tang et al. 2020), and tomato (Xie et al. 2021).

Intercropping increased chlorophyll of kenaf and soybean under Cu stress

Chlorophyll is important for the plant growth and metabolism (Wei et al. 2011) and indispensable for photosynthesis (Wang and Grimm 2021). However, heavy metals e.g., Cu and Cd can reduce chlorophyll and carotenoid levels and leave harmful effects on photosynthesis (Pan et al. 2023; Rehman et al. 2021; Chandra and Kang 2016). Present study revealed that intercropping enhanced the relative content of chlorophyll (SPAD index) in the leaves of kenaf and soybean, compared to monoculture (Fig. 1c), which is consistent with the findings of Pan et al. (2021) and Xie et al. (2021) that photosynthetic pigments in tomato and eggplant seedlings were improved under intercropping treatment. Excess of Cu disturbed thylakoid membranes integrity in chloroplasts and altered the composition of their fatty acid (De Vos et al. 1991) as well as inhibited enzymes related to chlorophyll biosynthesis (John et al. 2009).However, in this study, intercropping increased the SPAD index of kenaf and soybean grown in Cu contaminated soil which might be attributed to the productive use of the light, available water and nutrients (Nyawade et al. 2019; Gitari et al. 2018; Adeniyan et al. 2014)

Present results are consistent with Hussein et al. (2019) that intercropping significantly improve the growth of fenugreek and wheat plants, and Zeng et al. (2018) that intercropping of *A. donax* L. and *M. alba* L. (a woody plant) significantly improved *M. alba* L. photosynthesis and biomass along with contaminated soil remediation. Furthermore, intercropping of lettuce and pakchoi improved the chlorophyll content of lettuce (Liang et al. 2023). A previous study on intercropping of *Tagetes minuta* and soybean under Pb-contaminated soil revealed that *T. minuta* can give a photoprotection role for soybean, to increase the plant growth (Vergara Cid et al. 2020). This specify that intercropping could promote the growth and production of kenaf and soybean by increasing chlorophyll content and reducing oxidative stress and Cu uptake under Cu toxicity. Moreover, root exudates could also have favorable effects on the plant growth by altering rhizospheric environmental conditions and reducing the heavy metals toxicity (Choppala et al. 2014; Inal et al. 2007). Leguminous plants can develop available soil N through atmospheric leguminous nitrogen fixation (Wang et al. 2019; Zang et al. 2018; Li et al. 2016a, b). This favors adjacent plants and coupled with P acquisition for adjacent plants in a response to protons released from the legumes (Liao et al. 2020; Zhang et al. 2017).

Intercropping improved root growth of kenaf and soybean under Cu stress

Plant roots are organs that absorb water and nutrients from the soil, provide mechanical support to crop plants and consequently playing a significant role to affect economic yields (Fageria and Moreira 2011). In the soil, the activation of physical, chemical, and biological phenomenon is due to the root growth and function (Layek et al. 2018; Duchene et al. 2017). Productivity of a plant is not only affected by aboveground parts but also affected by the belowground root interactions. This could be involved in underground interspecific interactions (Xia et al. 2013). In present study, root growth of both kenaf and soybean were significantly affected under their mutual intercropping treatment (Fig. 2). Previous researches have also confirmed that intercropping can promote or restrict the root growth and modify root distribution (Ren et al. 2017; Liu et al. 2020). Our results demonstrated that the root dry weight as well as morphological attributes such as root total length, root projected area, root surface area and root average diameter of kenaf and soybean were reduced under increasing levels of Cu in soil while, intercropping exhibit significant increase in all above root morphological attributes at each level of Cu, compared with monoculture. Ben-chuan et al. (2022) reported that in intercropped maize and soybean system the root length density and root surface area density were significantly higher as compared to their corresponding monocultures. In contrary, the specific root length of maize and peanut was decreased under maize–peanut intercropping system (Gao et al. 2016). Broad bean and maize intercropping altered the root morphology of crops and enhanced the effective space for the absorption of water and nutrients (Li et al. 2010). Previous studies revealed that variation in morphological attributes of roots can support the uptake of nutrients (Hermans et al. 2006; Cahill et al. 2010).

Intercropping activated antioxidant enzymes, and reduced MDA content of kenaf and soybean under Cu stress

Stress conditions caused synthesis of harmful by-products in plants. Reactive oxygen species (ROS) are substances generated through O_2 reduction in the plant tissue (Sahu et al. 2022). ROS production in plants subjected to heavy metal stress is an important biochemical process (Berni et al. 2019; Kohli et al. 2017). Plants activate their own antioxidant systems when exposed to external stress. The generation of ROS and their scavenging phenomenon is rather antagonistic (Sahu et al. 2022; Sewelam et al. 2016). Plants activate their own antioxidant defense system for ROS detoxification and protection from oxidative damage due to stress. For maintaining homeostasis in the plant cells, enzymatic components and non-enzymatic antioxidants firmly control the concentration of ROS and protect from oxidative injuries (Sahu et al. 2022). In heavy metal polluted soils, intercropping could be effective to reduce the oxidative damage in plants by improving antioxidant enzymes activity and their resistance to metal stress (Cui et al. 2018). In our results, intercropping kenaf and soybean enhanced the antioxidant enzymes activity while, reduced MDA content in both the species compared with monoculture. Enzymatic antioxidants reduced ROS through antioxidant enzymes activation such as peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) (Rehman et al. 2019b, 2021; Dias et al. 2019). In this study, ROS reduction is predominantly controlled through SOD and POD catalytic reaction and the increase in SOD and POD activity in kenaf and soybean associated with the exposure of increasing Cu in soil (Fig. 3). In a previous study, fenugreek and wheat intercropping reduced oxidative stress and increase antioxidant activity to improve heavy metals resistance in plants (Hussein et al. 2019). Similar results from the studies on intercropping were reported by Xie et al. (2021) and Cui et al. (2018). However, effects of intercropping could be varied due to the enzyme category, type of plant used as the main crop or an intercrop, as well as other environmental and experimental factors (Curtright and Tiemann 2021). Several other research have reported that intercropping reduced heavy metal toxicities in plants by improving their antioxidant enzyme system (Pan et al. 2021; Xie et al. 2021; Vergara Cid et al. 2020; Tang et al. 2017).

Intercropping increased content of proline, soluble sugars and soluble protein of kenaf and soybean under Cu stress

Soluble carbohydrates preserve protein's structure, possibly by interacting with their hydroxyl groups. Hydrophilic groups found in soluble carbohydrates can act as a substitute for water molecules to stop protein denaturation and subsequent breakdown (Trouvelot et al. 2014). Another study gives an explanation that soluble sugars are potent antioxidants, with sucrose having a far higher antioxidant capacity than glucose (Van den Ende and Valluru 2009). In our results, intercropping increase the soluble sugars, soluble proteins and proline content in kenaf and soybean under increasing levels of Cu in soil. Proline generation of plants in response to Cu toxicity, might be linked with the plant cells protection against oxidative damage and signal transduction (Tie et al. 2014). Excess of Cu might has broken osmoregulatory solutes and stimulated the synthesis of proline in plants (Chen et al. 2015). Similarly, a previous study also reported significant increase in free proline content under heavy metal stress (Mostofa and Fujita 2013). By preventing the root water absorption and lowering the leaf water content, the metal concentration in liquid media affects the osmotic balance in the plant cells (Cenkci et al. 2010). It was already reported that proline, soluble sugar, and starch accumulation resulted in reduced cell osmotic potential, and that plants required to take in water from the environment to keep their cells turgor and maintain proper osmotic balance. Enhanced cellular acidosis and imbalance of the NADH^{+}/NADPH ratio could be a possible explanation of proline accumulation under stress conditions (Bali et al. 2019).

Intercropping reduced copper uptake, bioaccumulation factor (BAF) and translocation factor (TF) of kenaf and soybean under Cu stress

In present study, increasing level of Cu in soil increased the content of Cu in the roots, leaves and stem of kenaf and soybean. However, higher Cu content in the roots and lower Cu content in the leaves and stems under 300 and 600 Cu indicates that kenaf and soybean both have an efficient mechanism in their root system for the prevention or reduction in translocation of Cu to aerial parts. For that reason, these findings revealed that the root is a primary Cu storage site and that a large proportion of Cu does not leak out of the root for translocation to the shoot. On the whole, kenaf and soybean grown in monoculture shown Cu toxicity in terms of lower biomass and reduction in growth attributes at both the levels of Cu contamination (300 and 600 Cu) in soil. Meanwhile, these results showed significance of intercropping under toxicity of Cu. Compared with monoculture, intercropping significantly reduced Cu in roots, leaves and stem of kenaf and soybean at each level of Cu contamination in soil (Table 1). Intercropping exhibited beneficial influence on kenaf and soybean specially to reduce Cu in leaves and stem. The permissible values of Cu set by FAO/WHO is 73.3 mg/kg for vegetable crops (Mensah et al. 2009). Our results are consistent with Cui et al. (2018) that intercropping significantly reduced the Pb concentration in roots and shoots of ryegrass and alfalfa, compared with monoculture. Moreover, intercropping with pakchoi significantly decreased the root and shoot Cd content (Liang et al. 2023). However, in opposing above findings, intercropping increased Cd accumulation in rape and *Brassica oleracea* seedlings which might be due to the variation in the rhizosphere flora and planting systems (Cao et al. 2020). Present findings provide support for the cultivation of kenaf and soybean in

Cu contaminated soils under intercropping because, the harmful effects caused by Cu (particularly on dry biomass) were mitigated by intercropping system. However, the effectiveness of this recovery was fewer when kenaf and soybean were grown at 600 Cu treatment in the soil. The root, leaf and stem dry biomasses accumulated by kenaf and soybean under 600 Cu were less as compared to control plants (monoculture in 0 Cu soil). However, under highly Cu polluted mining soil, it would be necessary to integrate other remediation approaches with intercropping to guarantee higher crop production. Plant species could be a main factor affecting plant and soil metal contents under intercropping system. Heavy metal content can be reduced by using legumes as intercropped plants (Liu et al. 2023). Furthermore, reduced effects of Cu on kenaf and soybean under intercropping could be attributed to the reason that the nutritional requirements of both the species rather than monoculture, has to be fulfilled, therefore limiting available heavy metals in the rhizosphere. Intercropping improves soil ecosystem multifunctionality by increased available nutrients but it depends on the regional factors (Ma et al. 2022). Bioaccumulation factor (BAF) and translocation factor (TF) are two major indicators for the accumulation and translocation of metals in plants (Rehman et al. 2019b). In this experiment, intercropping of kenaf and soybean significantly decrease the Cu content of the leaves, compared to monoculture. Guo et al. (2023) reported that kenaf uptake a significant level of Cd from soil through phytoremediation. Lin et al. (2014) reported higher Cd contents in the shoots of plants. The high content of Cu in roots suggests that intercropping of kenaf and soybean did not support Cu transfer from roots to the shoots of kenaf or soybean. According to a previous study intercropping ryegrass with alfalfa (*Medicago sativa* L.) decreased Pb uptake in test plants (Cui et al. 2018). Intercropping affects biomass distribution in plants and accumulation of metal, or soil environment has also an influence on metal absorption. The change of forms of metals due to root exudates linked with varied uptake of Cu by plants grown under Cu stress. Some of the studies have shown contradicted results that intercropping exhibit non-significant influence on metals content in main plants, even with enhanced heavy metal content. Another study by Cui et al. (2021) reported that ryegrass and Indian mustard intercropping enhanced antibiotics removal rate but there was slight effect on metal removal. Cadmium and Pb accumulation were increased under intercropping with faba bean and *Arabis alpina*, as compared to monocropping. The reason is that *Arabis alpina* intercropping activated metals in the soil through an increase in organic acids exudation from roots (Li et al. 2019). Varied results might associate with the different plant species and other environmental factors (Liu et al. 2023). According to our findings intercropping system seems to be a valuable tool to counteract Cu toxicity in kenaf and soybean. Similarly, in a previous study, Marastoni et al. (2019) also proven that intercropping is a valuable approach to reduce Cu toxicity in grapevine but the extent of this alleviation effect is species dependent because, selection of species can decrease competition between plants and increase the yield and quality of the crops (Thayamini and Brintha 2010).

Intercropping increased the soil pH and decreased soil available copper

In this study, mutual intercropping of kenaf and soybean has increased the soil pH and reduced the soil available Cu in contaminated soil (Table 3). our results showed that intercropping of kenaf and soybean has a significant effect on soil pH with an increase as compared to monoculture under all the Cu treatments in soil. Soil pH was highest under intercropping of kenaf and soybean at 0 Cu in soil, that presents a progressive effect of intercropping on soil pH. Our results are consistent with a previous study by Bian et al. (2018) who reported an increase in soil pH after intercropping of *S. plumbizincicola* with *Phyllostachys praecox* as compared to monoculture. In contrast, Zou et al. (2021) reported that intercropping of wheat and *S. plumbizincicola* decreased soil pH. The contrasting result might associated to differences in physical and chemical properties of the soil used in study for example difference of buffering capacity for acids. Soil pH is important for the morphological transformation of metals, affecting both their dissolution and availability (Tao et al. 2003). In present study, the content of available Cu in the soil under intercropping treatment decreased with increasing pH, compared with monoculture. Similarly, De Conti et al. (2019) reported that intercropping young grapevines with *Paspalum plicatulum* and *Axonopus affinis* reduced the bioavailability of Cu in soil. In another previous field study the content of soil available Cd and Zn were reduced following interplanting of bamboo with *S. plumbizincicola*. In this study, increase in soil pH under intercropping might increased the amount of Cu held by clay and organic matter, thereby reduced the availability of Cu. Intercropping reduced the contents of soil organic matter, available nutrients, and Cd and Cu in rhizosphere soils (Bian et al. 2021). Moreover, intercropping affects the bioavailability and mobility of heavy metals in the soil by affecting the chemical properties of the soil, such as organic acids excreted by roots, pH and redox the potential of the rhizosphere soils (Jones et al. 2003).

Conclusions

Under copper stress, intercropping of kenaf and soybean contribute to the plants growth in term of higher shoot and root biomass, and relative chlorophyll content of both the species by reducing oxidative damage and increasing antioxidant enzymes activity, soluble sugars, soluble proteins and proline content. Intercropping alleviated the negative effects of copper by reducing the copper contents in roots, leaves and stems of both the species. At the same time bioaccumulation factor and translocation factor also performed reducing trend which could be the result of existing effects of copper stress and biomass distribution under intercropping. In addition, intercropping increased the soil pH and reduced soil available copper. Therefore, the findings of this study could be served as an environment friendly practice to get more return from the kenaf and soybean under intercropping in copper polluted soils. Furthermore, this study provide a base to understand interactive effects of copper in soil and cropping system using bast fiber crops and leguminous crops.

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Data availability Data will be made available on a reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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