



Research Article

Effect of Copper Toxicity on the Growth Parameters of *Silybum marianum* (L.) Gaertn

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Abstract | This study investigated the effect of copper contamination (100 to 500 ppm) on the germination and growth of *Silybum marianum*. The germination of seed was maximum at 500 ppm and minimum in the control soil (0 ppm). Maximum biomass were investigate at 400 ppm of root, stem length and leaves, and number of flowers. Followed by control group consecutively investigate the least value. Dried leaf weight and total plant weight were highest at 400 ppm, whereas fresh leaf weight peaked at 200 ppm. Some characteristics, primarily biomass and blooming, were inhibited by copper concentrations of 200 and 500 ppm. Statistical analysis using One-Way ANOVA revealed significant differences in mean values across treatments, despite no significant variance in standard deviation. These results imply that copper has a concentration-dependent function as both an inhibitor and a growth enhancer, demonstrating *Silybum marianum*'s adaptive response to heavy metal pollution.

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Keywords | *Silybum marianum*, Copper toxicity, growth parameters, Heavy metal



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Introduction

At trace levels, heavy metals can have numerous harmful effects on plants, making them one of the most hazardous forms of metal pollution in the environment. According to Gonzalez *et al.* (2010), Rehman *et al.* (2019), and Zhang *et al.* (2014), the most common environmental contaminants in regions with high levels of intense human activity are heavy metals such as copper (Cu), nickel (Ni), cadmium (Cd), lead (Pb), and zinc (Zn). Pedological and climatic

conditions, such as mining, smelting, industrial wastes and effluents, and the application of various essential fertilisers, including fungicides and pesticides, have a significant impact on the distribution of heavy metals in soil (Li *et al.*, 2009; Rehman *et al.*, 2019; Yu *et al.*, 2016). Cu is one of the major heavy metals among other toxic metals, and for ecological and evolutionary reasons, its toxicity is a growing concern. However, copper is an essential micronutrient for plants and is required in trace amounts for a number of biological and physiological processes (Chen *et al.*,

2015; Husak, 2015; Li *et al.*, 2018; Rehman *et al.*, 2019). Photosynthesis, respiration, glucose, proteins, and cell wall metabolism all depend on copper, but too much of it in plants can change DNA, cell membrane integrity, and enzyme activity, all of which have an impact on crop output and plant productivity (Kohli *et al.*, 2018; Lu *et al.*, 2017; Rehman *et al.*, 2019; Rizwan *et al.*, 2017). Around 2.1% of China's agricultural land is contaminated by copper, while over 16% of the land is contaminated by various heavy metals (Chen *et al.*, 2015). The use of Cu-made agrochemicals, pesticides, fungicides, bactericides, and nematocides which increase crop production and yield and control pests is the primary cause of this, as they are the main sources of high Cu concentrations in agricultural soil (Husak, 2015; Rehman *et al.*, 2019). Several prior research have documented the phytotoxicity of copper in lowering plant growth and biomass (Celis-Plá *et al.*, 2018; Chen *et al.*, 2015a, b; Gao *et al.*, 2017; Liu *et al.*, 2018; Rehman *et al.*, 2019; Zaheer *et al.*, 2015). Through combining with organic matter, clay minerals, and hydrated oxides of iron (Fe), aluminium (Al), and manganese (Mn), toxic concentrations of copper in soil can lead to nutritional imbalance and reduce plant production. Additionally, too much copper (Cu) results in the production of reactive oxygen species (ROS) in plant tissues, including hydrogen peroxide (H_2O_2), superoxide radical (O^{2-}), and singlet oxygen (1O_2), which negatively impacts plant metabolism and cellular structure (Chen *et al.*, 2015; Gonzalez *et al.*, 2010).

The *Silybum marianum* belong to Kingdom: Plantae, Clade: Tracheophytes, Clade: Angiosperm, Division: Pinophyta, Class: Pinopsida, Order: Asterales, Family: Asteraceae, Subfamily: Carduoideae, Genus: *Silybum* Milk thistle (*Silybum marianum* (L.) Gaertn. (Asteraceae)] is an annual herb, native to the Mediterranean and North African regions (Boulos, 2000). The plant can grow up to ten feet tall. It has an upright stem that is 20 to 150 cm high, with ridges and branches on top. Solitary composite flower heads made of purple disc florets, each about 2 inches in diameter, are found at the end of each stem. The leathery bracts that are also capped with stiff spines distinguish the flower heads of milk thistle from those of other thistles. The fruits are flat, smooth, shiny, dark brown, hard-skimmed achenes that are 6 to 8 mm in length. Fruits of *S. marianum* contain silymarin, an isomeric combination of flavonolignans (Morazzoni and Bombardelli, 1995). Silybin,

isosilybin, silychristin, and silydianin are the main constituents of silymarin. These compounds (silybin A, silybin B, isosilybin A, isosilybin B, silychristin A, and silychristin B) have diastereoisomers. The dihydroflavanol taxifolin is essentially connected to the coniferyl alcohol moiety by an oxeran ring to form the flavonolignan nucleus. One of the most studied plant extracts with a well-established mode of action is silymarin. It is used to treat toxic liver damage orally (Flora *et al.*, 1998). According to Deep *et al.* (2008), silymarin is also utilised to lower the risk of acquiring cancer. Individual flavonolignans have been shown to have distinct antiproliferative effects on human prostate cancer cells (Davis-Searles *et al.*, 2005). When compared to other flavonolignans, isosilybin B was the most effective at suppressing cell proliferation. According to Sy-Cordero *et al.* (2010), isosilybin B and, to a lesser extent, isosilybin A appear to be the most effective in a variety of prostate cancer chemopreventive and antiproliferative activity tests.

Materials and Methods

Selection of plant

The plant chosen for the study was the Asteraceae, member milk thistle (*Silybum marianum*). Originally from southern Europe and Asia. The plant was identified by Dr. Naveed Akhtar, a taxonomist from the Department of Botany at Islamia College Peshawar.

Collection of seed

The seed were obtained from the herbarium of university of agriculture Peshawar. The seeds were of same size and colour.

Experimental design

The experiment, which was carried out in triplicate in the botanical garden of Islamia College Peshawar, involved a total of eighteen pots. Six major groups, grouped in sequence lines, made up the experimental design. The control group, 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm groups were the primary groups.

Concentration of copper (Cu) used in experiment

Different concentration of copper sulphate ($CuSO_4$) was used in experimental groups. No copper was added to the control group while 0.1g, 0.2g, 0.3g, 0.4g, 0.5g $CuSO_4$ was added in solution form to each one pot of rest of the groups i.e., 100ppm group, 200ppm

group, 300ppm group, 400ppm group, 500ppm group, respectively. The five concentration of the Copper was prepared using the formula.

Procedure

For 100ppm solution, 0.1g of copper sulphate was dissolved in 1000 ml of tap water while for 200ppm solution 0.2g of copper sulphate was dissolved in 1000 ml of tap water. For 300ppm solution, 0.3g of copper sulphate was dissolved in 1000 ml of tap water, for 400ppm solution, 0.4g of copper sulphate was dissolved in 1000 ml of tap water and for 500ppm solution, 0.5g of copper sulphate was dissolved in 1000 ml of tap water.

Effect of copper on germination

The seed of *Silybum marianum* were ground on 15 March, 2023 and give solution of copper sulphate to the experiment groups. First, I visited after 7 days to the field and observed that the seed started germination. The effects of the used copper on the germination of the *Silybum marianum* seeds were recorded by counting the number of germinated seeds in each pot.

Statistical analysis

The statistical analysis and graph were done through graph prism pad version 10.0

Results

The results indicate that the fresh and dry weights of plant stems, leaves, roots, and flowers using different treatment groups, including a control and treatments with varying concentrations (100 ppm to 500 ppm). The analyzing the treatments influence plant biomass distribution and overall growth. In the control group, sample A shows no recorded biomass across all parts, indicating either a lack of growth. Samples B and C exhibit total weights of 3.3g and 2.0g, respectively, with leaves contributing the most significant portion of both fresh and dry weights. At 100 ppm, sample A records a total weight of 6.0g, nearly doubling the biomass compared to the control samples. This increase is primarily due to enhanced leaf and stem growth, indicating a positive response to the treatment at this concentration. Samples B and C, however, show total weights of 4.0g and 3.2g, respectively, suggesting variability in response among replicates. ASamples A (1.7g) and B (2.9g) have a lower total biomass after the 200 ppm treatment, while sample C has a higher total weight of 6.2g. Although the conflicting results between samples indicate diversity in plant responses, the increased leaf weight in sample C suggests a potential stimulatory effect at this concentration. Samples A and C have lower total weights at 300 ppm, with values of 2.9g and 2.0g, respectively, while

Table 1: Effect of copper on fresh weight of stem, fresh weight of leaves, dry weight of stem, dry weight of leaves, roots, flowers and total weight of all parts of *Silybum marianum*.

Groups		Fresh weight of stem	Fresh weight of leaves	Dried weight of stem	Dried weight of leaves	Dry weight of root	Dry weight of flower	Total weight
Control	A	0	0	0	0	0	0	0
	B	0.22	1.4	0.11	1	0.14	0.64	3.3
	C	0.12	1.3	0.07	0.6	0.09	0	2
100 ppm	A	0.48	2.8	0.17	1.27	0.2	0.47	6
	B	0.14	2.93	0.04	0.82	0.12	0	4
	C	0.39	1.1	0.16	0.74	0.11	0.46	3.2
200 ppm	A	0.16	1.1	0.05	0.49	0.13	0	1.7
	B	0.02	2.33	0.01	0.97	0.1	0	2.9
	C	0.07	4.9	0.01	1.48	0.33	0	6.2
300 ppm	A	0.03	2.05	0.01	0.61	0.18	0	2.9
	B	0.03	4.63	0.01	1.38	0.26	0	5.8
	C	0.12	1.14	0.05	0.5	0.07	0.25	2
400 ppm	A	0.83	2.9	0.3	1.09	0.22	0.69	8.3
	B	0.1	2.91	0.02	0.93	0.11	0	3.8
	C	0.37	2.1	0.15	1	0.16	0.1	4.2
500 ppm	A	0.08	1.64	0.03	0.71	0.09	0	2.2
	B	0.1	2.3	0.01	0.88	0.1	0	3
	C	0.04	0.77	0.02	0.38	0.06	0	1.5

sample B has a higher total weight of 5.8g, primarily due to higher leaf biomass. The 400 ppm treatment results in the highest recorded total weight in sample A at 8.3g, with significant contributions from both stems and leaves. For total weights, B = 3.8 g, C = 4.2 g which suggests that this concentration may give a better promotion for some, while others grow the same without much difference among individuals. But in the higher concentration of 500 ppm compared to 400 ppm, sample A = 2.2g, sample B = 3.0g, Sample C = 1.5 g have shown more or less declined amount of the total biomass growth compared to this one. The using of One-Way ANOVA there was statistically difference in mean values while there was no statistical difference in standard deviation.

The table presents data on plant growth parameters of stem length, root length, number of leaves, and number of flowers across different treatment groups such as control, 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm. Each group comprises three groups (A, B, and C), indicating replicates or distinct samples within each treatment. Control group: All measured parameters are zero, suggesting either no growth occurred or data was not recorded. B and C exhibited growth with stem lengths of 4.2 cm and 2.2 cm, root lengths of 6 cm and 6.9 cm, and leaf counts of 10 and 6, respectively. 100 ppm treatment A and C Showed increased stem lengths (6 cm and 7 cm) and root lengths (7.5 cm and 6.7 cm) compared to the Control. Both had 8 leaves, and each produced one flower. B displayed a stem length of 3.5 cm, root length of 9.9 cm, 6 leaves, and no flowers. 200 ppm treatment A, B, and C demonstrated reduced stem lengths (3.6 cm, 1.1 cm, and 1 cm) compared to the 100 ppm group. Root lengths varied, with B having the longest roots at 11.7 cm. Leaf counts ranged from 5 to 7, with no flowers observed in this treatment. 300 ppm treatment A, B, and C stem lengths were relatively low (2.5 cm, 1.7 cm, and 2.5 cm). Root lengths ranged from 8 cm to 9.8 cm. Leaf counts varied between 5 and 8. C produced a flower. 400 ppm treatment A notably higher stem length (10.5 cm) compared to other subgroups and treatments. Root length was 7 cm, with 9 leaves and one flower. B and C stem lengths of 2.5 cm and 8.6 cm, root lengths of 7.2 cm and 14.5 cm, and leaf counts of 8 and 10, respectively. Only C produced a flower. 500 ppm treatment A, B, and C displayed reduced stem lengths (1.9 cm, 2 cm, and 1.9 cm) and root lengths (4.5 cm, 6 cm, and 6.8 cm). Leaf counts ranged from

6 to 7, with no flowers observed in this treatment. The data suggests that the treatment concentration significantly influences plant growth parameters: Low to Moderate Concentrations (100 ppm to 400 ppm): Generally associated with increased growth metrics compared to the Control, with some variability among subgroups. Notably, the 400 ppm treatment, Subgroup A, exhibited the highest stem length (10.5 cm). High Concentration (500 ppm) associated with reduced growth across all parameters, indicating potential inhibitory effects at this concentration.

Table 2: *Effect of Copper on length of stem, length of root, no of leaves and no of flowers.*

Groups		Length of stem	Length of root	No of leaves	No of flowers
Control	A	0	0	0	0
	B	4.2	6	10	1
	C	2.2	6.9	6	0
100 ppm	A	6	7.5	8	1
	B	3.5	9.9	6	0
	C	7	6.7	8	1
200 ppm	A	3.6	4.8	5	0
	B	1.1	11.7	6	0
	C	1	8.2	7	0
300 ppm	A	2.5	8	5	0
	B	1.7	9.8	8	0
	C	2.5	8.9	6	1
400 ppm	A	10.5	7	9	1
	B	2.5	7.2	8	0
	C	8.6	14.5	10	1
500 ppm	A	1.9	4.5	7	0
	B	2	6	7	0
	C	1.9	6.8	6	0

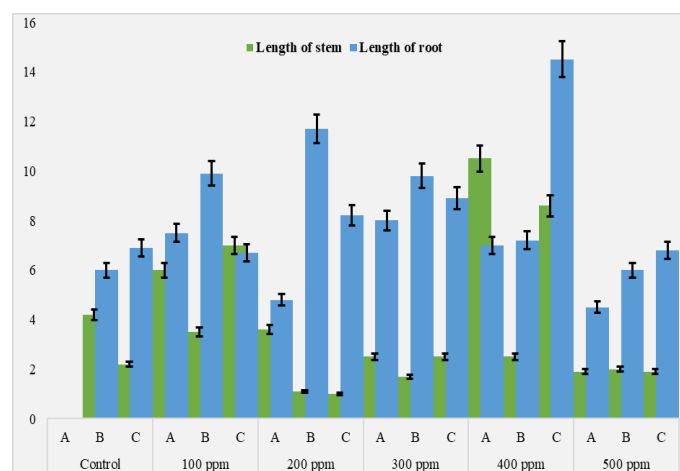


Figure 1: *Effect of copper on stem length, root length across different treatment. The using of One-Way ANOVA there was statistically difference in mean values while there was no statistical difference in standard deviation.*

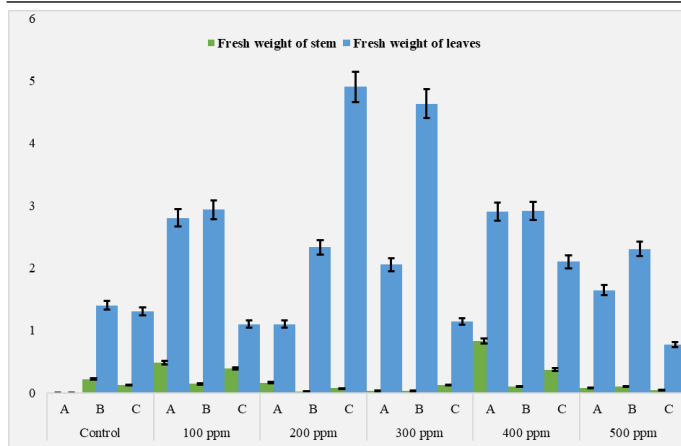


Figure 2: Effect of Copper on fresh weight of stem and leaves, across different treatment. The using of One-Way ANOVA there was statistically difference in mean values while there was no statistical difference in standard deviation.

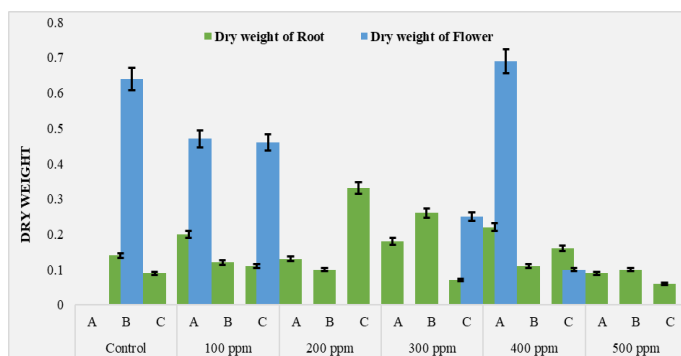


Figure 3: Effect of Copper on dry weight of root and flowers across different treatment. The using of One-Way ANOVA there was statistically difference in mean values while there was no statistical difference in standard deviation.

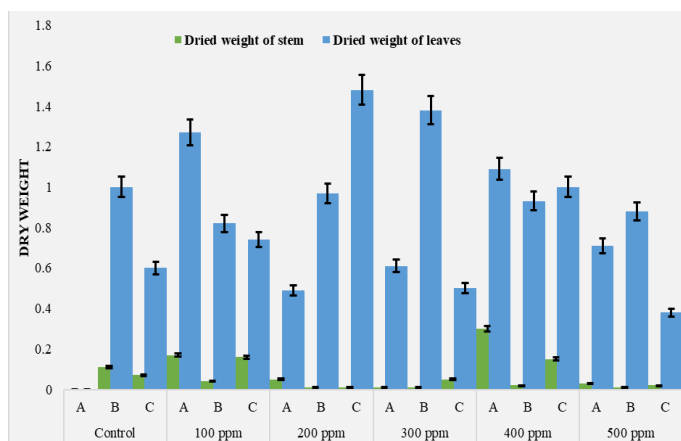


Figure 4: Effect of copper on dry weight of stem and leaves across different treatment. The using of One-Way ANOVA there was statistically difference in mean values while there was no statistical difference in standard deviation.

Discussion

The effect of copper on the growth parameters of *Silybum marianum*, commonly known as milk thistle, is a critical consideration for farmers and researchers involved in its cultivation (Htwe *et al.*, 2020). Copper,

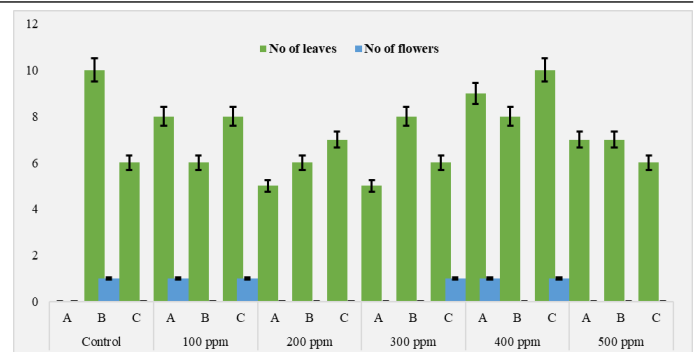


Figure 5: Effect of copper on number of leaves, and number of flowers across different treatment. The using of one-way ANOVA there was statistically difference in mean values while there was no statistical difference in standard deviation.

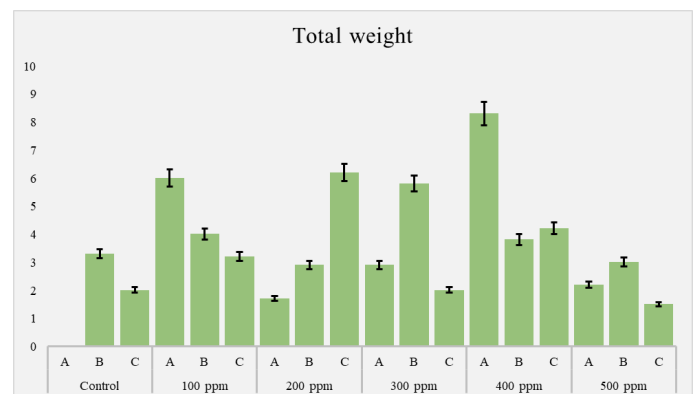


Figure 6: Effect of Copper on total weight of plant parts, stem length, root length, number of leaves, and number of flowers across different treatment. The using of one-way ANOVA there was statistically difference in mean values while there was no statistical difference in standard deviation.

like other essential micronutrients, plays a crucial role in the growth and development of plants (Zhao *et al.*, 2012). However, its impact on *Silybum marianum* can vary depending on several factors, and it's important to strike a balance between providing sufficient copper for growth and avoiding toxicity. The present study was investigated that the effect of copper on growth parameters of *Silybum marianum*. The germination of seed was maximum at 500 ppm and minimum in the control soil (0 ppm). Maximum biomass was observed at 400 ppm in roots, stem length, leaves and number of flowers. The lowest biomass was recorded in the control group. While dried leaf and overall plant weights peaked at 400 ppm, fresh leaf weight peaked at 200 ppm. Some characteristics, primarily biomass and blooming, were inhibited by copper concentrations of 200 and 500 ppm. Similar research was conducted by Kumar *et al.* (2009), who examined the effects of copper (Cu) concentrations of 0, 0.5, 1.0, 1.5, 2.0, and 2.5 mg kg⁻¹ on wheat plants cultivated in an entisol-based alluvial soil under glass house conditions. At 1.5 mg kg⁻¹ Cu, the number of tillers was at its lowest,

whereas growth characteristics such as plant height, fresh and dry matter yield, and percentage dry matter increased as Cu levels rose. Compared to the control, the grain yield at 1.5 mg kg⁻¹ Cu increased by 62.9%. When compared to the control (32.58 g), the weight increase of 1000 grains varied from 33.93 to 41.35 g. Additionally, the harvest index (%) rose, ranging from 39.42 to 47.73. Bean plants (*Phaseolus vulgaris* L. var. Zargana Kavala) were cultivated with increasing Cu concentrations in the growth medium (0.5–160.5 µM) in order to examine the findings of [Cook et al. \(1998\)](#). In general, plant development was hazardous at Cu doses of 10.5–160.5 µM, optimal at 1.5–10.5 µM, and inadequate at 0.5–1.5 µM. Significant increases in Cu concentrations in plant tissue were linked to Cu toxicity. In both the ideal and Cu-deficient growth circumstances, Cu was mostly found in the leaves. It was mostly trapped in the roots under Cu toxicity. The growth stage of cucumber leaves determines how they react to the addition of copper. While mature leaves demonstrated a notable decrease in photosynthesis, young expanding leaves displayed a loss in leaf area. In comparison to the control, maize (*Zea mays* L. cv. Cargill 350 Hybrid) plants' roots, shoots, and leaves shrank when greater concentrations of copper (10–3 mM and above) were added to the nutritional solution ([Benimali et al., 2010](#)). Similarly, high Cu dosages caused a linear decrease in maize plant height ([Barbosa et al., 2013](#)). Furthermore, in maize, a 15-days treatment with 15.7 µM Cu decreased root length by 90.6% compared to the control, but treatments with 78.7 and 157 µM Cu had nearly no root growth ([Ali et al., 2002](#)). With 10 µM Cu, maize has demonstrated a shoot length reduction of roughly 23% ([Mocquot et al., 1996](#)). Similarly, following 15 days of exposure to a 100 µM Cu level, two maize cultivars showed toxic effects of Cu excess on their growth, which led to a considerable decrease in the number of roots per plant and the length of the shoots ([Aly and Mohamed, 2012](#)). During six days of Cu stress, maize roots were noticeably black and degraded, and shoot and root lengths decreased by 10–3 M ([Lin et al., 2003](#)). In contrast, after two days at 10–2 M Cu, the roots of maize were rotting and became yellow-green ([Jiang et al., 2001](#)). Similarly, with 50 ppm Cu concentration in nutritional medium for 6 days, wheat root and shoot length dramatically decreased to 72% and 31%, respectively, compared to control ([Gang et al., 2013](#)). When copper levels in nutritional solution reach 25 parts per million, mung beans' root length is inhibited ([Azmat and Riaz, 2012](#)).

Furthermore, overexposure to copper was found to be poisonous to the roots of cucumbers (*Cucumis sativus* L.) and tomatoes (*Solanum lycopersicum* L.) after seven days of treatment. Cu was more harmful to cucumber roots than tomato roots at the same Cu treatment, and both species' root lengths shrank as the medium's Cu levels rose ([Işeri et al., 2011](#)).

Conclusions

The overall experimental results depicted that presence of copper affects the growth of *S. marianum* different part like root, stem, flowers, and leaves at different concentration such as 100ppm, 200ppm, 300ppm, 400ppm, and 500ppm. The maximum growth was found at 400ppm for *S. marianum* and the minimum growth was shown by 500ppm concentration on different parts of the plant. Based on current research work it is recommended that the *Silybum marianum* plant cannot tolerate high concentration of copper. However, to some low level of copper in soil cannot affect the plants growth therefore it is recommended that the plant must not be grown in soil where the copper concentration is high.

Novelty Statements

- This study is the first to comprehensively evaluate the dose-dependent effects of copper toxicity on the growth and seed germination of *Silybum marianum*, an important medicinal plant native to the Mediterranean and South Asia.
- Unlike conventional studies that report a linear toxic response to heavy metals, our findings demonstrate a biphasic response, where moderate copper concentrations (especially 400 ppm) enhance growth parameters including stem length, leaf number, and flower production.
- The study reveals a paradoxical effect of high copper concentration (500 ppm) in stimulating seed germination, despite its inhibitory impact on biomass and flowering highlighting the species' adaptive resilience and stress signaling mechanisms.
- By analyzing growth parameters using One-Way ANOVA, the study provides statistically robust evidence of copper's dual role as both a micronutrient and phytotoxin in *Silybum marianum*.
- This research provides foundational data for using *Silybum marianum* in phytoremediation

and cultivation in heavy metal-contaminated soils, suggesting its potential for eco-toxicological monitoring and agricultural practices in marginal lands.

Author's Contribution

Uzma Sahib and Atta Ur Rahman: Designed and carried out the research.

Sajjad Ahmad, Kaleem Ullah and Muskaan Zaman: Collected the data and did fieldwork.

Fahmeeda Kausar and Sana Khan: Provided logistical support.

Sumaira Noor: Performed the data analysis.

Sajjad Ahmad and Fawad Khan: Wrote the manuscript.

Sobia Gul, Riffat Sabar Yousafzai and Tariq Zaman: Conducted the literature review necessary for writing the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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