

# Study of Copper Phytotoxicity on Maize Plants (*Zea mays* L.) Irrigated by Water Treated with Copper Sulfate as Algaecide

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**Abstract:** *The phytotoxicity of Copper Sulfate used as algaecide in irrigation water on maize plants tissues (*Zea mays* L.) and its accumulation in soil has been studied, using two irrigation modes: Basin Irrigation and Sprinkler Irrigation. Disinfected seeds were inoculated in plastic columns containing clay loam soil. Experimental plants in columns were irrigated with water treated by copper sulfate ( $\text{CuSO}_4$ , 1ppm), and control crops by tap water. The morphological effect of copper (Cu) on the growth of maize plants was followed weekly during two months in comparison with control plants. Experimental plants showed a decrease of leaves surfaces by 8%, without any foliar toxicity symptoms or significant effect on shoot height. However, the use of copper sulfate in irrigation water have decreased the total dry mass by a value greater than 11%, which is closer to 10%, This latest occurs when the element is at the critical toxic concentration in plant tissues. The bioaccumulation of copper in maize tissues (roots, shoots, and leaves) was measured in both irrigation modes. Average total masses of Cu in plants irrigated by basin and sprinklers modes showed an increase of Cu masses by 35% and 46%, respectively, compared to their corresponding control plants. In basin, copper bioaccumulation has occurred in root tissues through roots uptake, whereas by sprinkler method, the bioaccumulation has occurred in roots and leaves through roots uptake and leaves diffusion. Furthermore, the deposition of copper on leaves and shoots by sprinkler irrigation reduces greater than 8% the growth of plants in comparison with basin irrigation mode, and thus, increased further the phytotoxicity rate. The vertical distribution of total copper in soils irrigated by copper treated water was also examined. At the end of irrigation period (two months), retention profiles showed that the concentrations of retained copper in the upper 0.2 cm of the soil are 28.23 ppm and 22.35 ppm when basin and sprinkler irrigation are applied respectively, and then decrease for the following depths. Therefore, this study has confirmed that the use of copper sulfate in irrigation water leads to copper bioaccumulation in plant tissues and with time, to its accumulation in soil. Consequently, this entails an increase of phytotoxicity rate. It proved as well the impact of irrigation modes on copper bioaccumulation rate and uptake pathways. Finally, this document showed the need to look for another alternative of copper sulfate as algaecide.*

**Keywords:** Phytotoxicity, algaecide, copper sulfate, biomass, dry matter, bioaccumulation

## 1. Introduction

Copper sulfate is worldwide applied as biological inhibitor to control algae growth in surface water [1], [2], [3]. Algal bloom in lakes and rivers is related to the increase of nitrate and phosphate concentrations in receiving water from the discharge of untreated domestic wastewater and the runoff on agriculture land [4], [5], [6], [7]. A high proliferation rate of photosynthetically algae occurred at the surface of water, restricts the sunlight penetration into water column leading to the decrease of dioxygen production, mass mortality of wild species, microbial proliferation, and hydro-dynamical disturbance [6], [7], [8].

Canal 900 is an artificial open irrigation canal, and one of the most eutrophicated surface water in Lebanon [9], [10]. The water is pumped from the Qaroun Lake, and irrigates approximately 2000 hectares located in South Bekaa Valley, during dry hot summer season [11]. Algal proliferation causes a decrease of water flow, an obstruction of irrigation drippers, bad odors resulting in complaints from farmers who hesitate to subscribe to the canal water deliveries [9], [10]. Litani River Authority uses copper sulfate as algaecide, and pretends this action has not toxic impact on aquatic life. Yearly, in June and July, the concentration of copper sulfate

added in irrigation water is 0.1 mg/L and 1 mg/L in August [10].

Nevertheless, previous studies demonstrate that concentrations of copper sulfate required to inhibit algae are 10 to 100 times than those known to be lethal for beneficial zooplankton [12], [13]. It was found that copper concentration between 0.021 and 0.04 mg/L is the maximum acceptable toxicant concentration for bluegills [13]. Copper in soil is an essential element for plant growth [14], [15], however, its bioaccumulation at high amount in plant tissues may cause crops phytotoxicity. Also, copper from treated irrigation water will accumulate in the soil and infiltrate to groundwater [16]. At long term, the use of copper in irrigation presents a toxic effect on human health [17], [18], [19]. Sheldon and Menzies [19] observed that Rhodes grass treated with Cu (1.17  $\mu\text{M}$ ) showed a 10% of growth reduction. Mateos-Narania et al. [20] found that Cu bioaccumulation in *A. halimus* decreases the net photosynthetic rate and reduces the growth [21]. Root microscopy analyses of *Chloris gayana* Knuth plants treated by copper solution (0.66  $\mu\text{M}$ ) have thick and cracked root meristem and cuticle [19].

The aim of this study was therefore to increase the knowledge about the bioaccumulation of copper in plant

tissues and its phytotoxicity. Moreover, the effects of irrigation mode on the rate of phytotoxicity, and the profile distribution of copper in soil depth are examined. Actually, maize seeds (*zea mays* L.) are cultivated in plastic columns containing clay loam soil and irrigated by copper treated water ( $\text{CuSO}_4$  1ppm) during 2 months, using two irrigation modes: basins and sprinklers. The growth and the morphological modification of maize plants were followed weekly. The concentrations of cu in plants tissues and at different soil depths were measured.

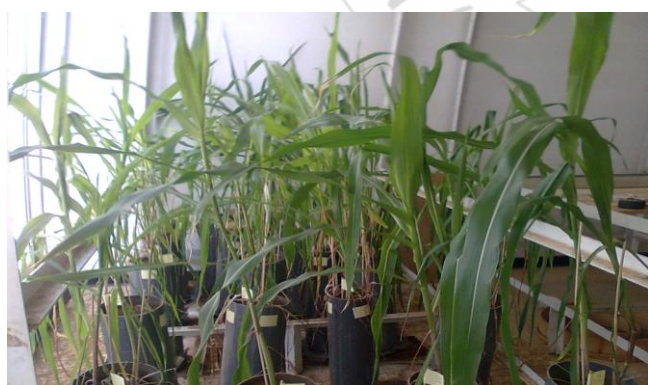
## 2. Methodology

### 2.1. Seeds Preparation

Before inoculation, the disinfection of seeds surfaces is necessary in order to remove bacteria and fungi. A spoon of maize seeds was placed in a beaker, washed with a commercial detergent, shaken vigorously for 2 minutes (min), drained, washed 5 times with sterile distilled water, drained, treated with 70% ethanol for 30 seconds (sec), and drained. Then, maize seeds were aseptically disinfected with 5% sodium hypochlorite for 10 min, followed by the addition of 2 drops of Tween 20 for 10 min, drained, and washed 2 times with sterile distilled water for 2 and 5 min and 3 times for 10 min to completely remove the disinfectants.

### 2.2. System Description

Sweet corns seeds (*Zea mays* L.) are cultivated in columns containing clay loam soil and irrigated with treated water containing copper sulfate 1ppm. Twelve plastic columns with 16 cm of diameter and 45 cm of height were used (Figure 1). Each column contains a layer of sieve, 5 cm of gravel, 35 cm (11 Kg) of a clay loam soil with a texture comparable to the soil in South Bekaa Valley [22]. A part of the chemical composition of the soil is shown in table 1.



**Figure 1:** Growing maize plants in plastic columns

**Table 1:** Elemental analysis of the clay loam soil used to cultivate maize crops.

Elements	Concentration (ppm)
Sodium	9066
Potassium	816
Copper	9.1
Iron	2822
Zinc	96
Lead	20.1

Columns were divided into two groups of six columns referring to irrigation methods: Basin Irrigation (BI) and Sprinkler Irrigation (SI). The first group is composed of six control columns [3(BI-C) + 3(SI-C)] irrigated by tap water for reference, while the second is composed of six experimental columns [3(BI-Cu) + 3(SI-Cu)] irrigated by a tap water treated by copper sulfate solution 1 ppm. The chemical composition of the tap water used to irrigate the control columns and the copper treated water used to irrigate the experimental columns are illustrated in table 2. Each column was inoculated by four seeds, receiving equal volume of two liters a week and irrigated three times. A manual sprinkler was used to apply the (SI) method. After the first week of growth, three seedlings were removed from each column leaving one to grow during two months at 25-30°C. Results are the average of observed, measured or analyzed parameters on the three cultivated plants of each category.

**Table 2:** Chemical analysis of the water used to irrigate maize crops.

Elements	Concentration (ppm)	
	Tap water	Copper treated water
Sodium	0.733	0.733
Potassium	0.062	0.062
Copper	0.007	0.400
Iron	0.027	0.027
Zinc	0.024	0.024
Lead	0.028	0.028

### 2.3. Evaluation of morphological modification

The symptoms of the irrigation by treated water on crops growth and morphological modifications were monitored during two months and the following parameters were recorded on weekly basis: height, shoot diameter, leaves number, and leaves surfaces. Plants heights and shoots diameters were measured using a Meter Ruler and Caliper, respectively. Leaves number was counted. At the end of the experiment, the total leaves surfaces were determined for each plant.

### 2.4. Sampling methods and Chemical analysis

After two months and at the end of their development, plants were uprooted manually and samples were prepared in order to determine their concentration in copper using Atomic Absorption Spectrometry (AAS).

#### 2.4.1. Plant samples

All plant parts were taken aside (roots, shoots, and leaves), washed, and dried at 70°C for 24 hours in an oven in order to determine their dry weight. Then, plant samples were blended and homogenized using a mortar. A wet mineralization is made by stirring 0.5 g of each sample in 7 mL of nitric acid 14 M (65%) and 1 mL of hydrogen peroxide (30%) at 95 °C for 30 min in a Microwave (Speed wave-Berghof). After acid digestion, solutions are transferred into 50 mL Erlenmeyer flasks and completed with deionized water. Samples were stored at 4°C and elemental analysis was performed by using an AAS (RayleiGh- WFX-210).

### 2.4.2. Soil samples

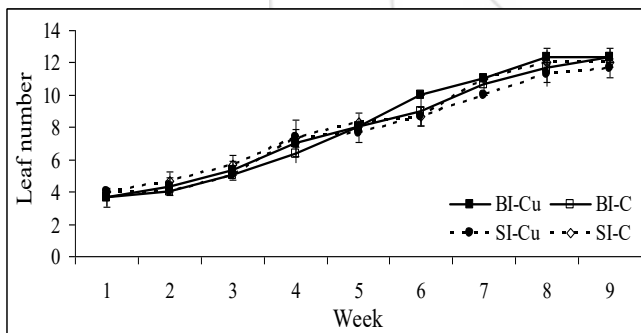
From each column, soil samples were taken at different depths (0.2, 1, 3, 5, 10, 15, and 20 cm) in order to study the downward movement of copper in soils. 10 g of samples were blended, homogenized using a mortar, dried at 105°C for 24 hours in an oven. Then 2 g were ashed at 500°C for 6 hours and 0.5 g of ash is mineralized by adding 9 mL of hydrochloric acid (37%) and 3 mL of nitric acid 14 M (65%) under 140 °C for 3 hours. After acid digestion, soil samples are submitted as for plant samples to an elemental analysis using an AAS (RayleiGh- WFX-210).

## 3. Results and Discussions

### 3.1. Morphological Modification

#### 3.1.1. Leaves

The evolution of leaves number of maize crops irrigated with and without Cu was followed on weekly basis and illustrated in figure 2. After one week of growth, all seedlings in the 12 cylinders have 4 leaves each one. Over weeks, the number of leaves increases at the same rate for all irrigated systems, to reach 12 leaves by the ninth week. This suggests that the irrigation with water at 1 ppm concentration of copper sulfate and the irrigation method don't affect the evolution of leaves number over time. No foliar symptoms were observed as result of copper phytotoxicity. However, at the end of growth, a decrease of 8% of the total leaves surfaces for (BI-Cu) and (SI-Cu) crops were noted comparing to those of control crops. Such observation indicates that the addition of copper to irrigation water reduces the development of leaves.



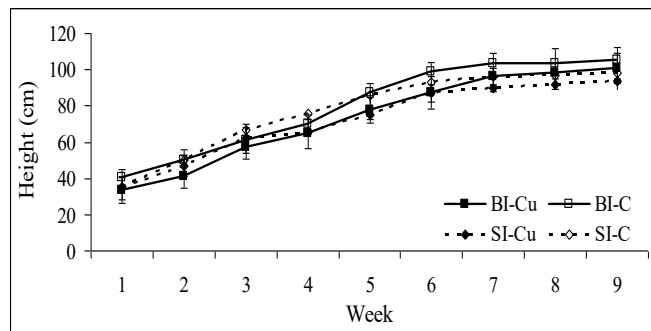
**Figure 2:** Temporal evolution of leaves number for Zea mays L. crops irrigated by basin irrigation (BI) and sprinkler irrigation (SI). Crops are irrigated with (BI-Cu/SI-Cu) and without (BI-C/SI-C) copper treated water

#### 3.1.2. Shoots

Shoot heights show a similar pattern as leaves number. All systems have similar curve trend (Figure 3). The height rises slowly from the first week until the seventh week, with 1.18 as weekly growth rate. The height remains then stable, in a stationary phase to the ninth week. However, the experimental crops have a growth rate slightly lower than control crops along weeks, by 0.85-0.90 times. Thereby the addition of copper sulfate at 1ppm to irrigation water doesn't have a significant effect on maize height.

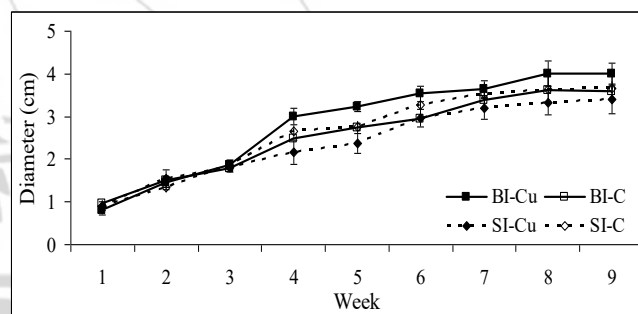
This result is analogous to those found in literature, mentioning that shoot growth is marginally affected at low

copper concentration, but it is significantly inhibited at a higher concentration. [18], [23].



**Figure 3:** Temporal evolution of the height of Zea mays L. crops irrigated by basin irrigation (BI) and sprinkler irrigation (SI). Crops are irrigated with (BI-Cu/SI-Cu) and without (BI-C/SI-C) copper treated water.

Furthermore, all corn crops have temporally similar diameter rise during the first three weeks (Figure 4). Later and along weeks, BI-Cu crops have a diameter slightly larger than those of control crops by 0.5 cm. However, SI-Cu crops have thinner diameter during the same period.



**Figure 4:** Temporal evolution of the diameter of Zea mays L. crops irrigated by basin irrigation (BI) and sprinkler irrigation (SI). Crops are irrigated with (BI-Cu/SI-Cu) and without (BI-C/SI-C) copper treated water.

### 3.2. Copper concentration in plants

The comparison of copper concentration measured in the tissues of plants irrigated by tap water as well by basin irrigation method as by sprinklers one shows similar pattern (Table 3). Analysis of copper results indicates that the concentration of copper in roots was doubled during the irrigation by basin, whereas its concentration in shoots and leaves was increased slightly. Thus, the irrigation by copper treated water leads to the bioaccumulation of copper in root tissues through roots uptake, and to non-significant translocation to harvestable parts. Those results are similar to that published observations [19], [24], [25], [26].

**Table 3:** Copper concentration in different parts of Zea mays L. plants, after 9 weeks of growth

Irrigation methods	Copper (ppm)		
	Roots	Shoots	Leaves
BI-C	6.1± 2.4	5.5± 1.7	11.8 ± 2.1
BI-Cu	13.7±1.8	7.1± 1.3	14.2 ± 1.3
SI-C	5.2± 1.9	6.2± 2.2	12.2 ± 1.8
SI-Cu	10.1±2.2	8.8± 3.1	20.6 ± 4.3

Furthermore, the irrigation by sprinkler method shows that the concentration of copper in roots as well as in leaves was doubled, whereas a slight increase of copper concentration in shoots is observed. The higher concentration of copper observed in leaves is explained by a diffusion phenomenon of copper to leaves cells when treated water is sprayed on them. Thus, the irrigation of plants by sprinkler method using copper treated water leads to the accumulation of copper in plant tissues through roots uptake and leaves diffusion. Globally, the measurement of the average accumulation of copper mass in experimental plants are 0.097 mg for BI-Cu and 0.123 mg for SI-Cu, while those in control plants are approximately 0.072 mg for BI-C and 0.084 mg for SI-C. Therefore, increasing rates by 35% and 46% are noticed.

### 3.3. Dry Matter

The measurements of dry matter (Table. 4) of *Zea mays* L. showed a global reduction in growth of experimental crops greater than 11 % compared to values obtained for control crops. With 1 ppm concentration of copper sulfate, this result is closer to the growth reduction of 10% admitted to consider the concentration of an element in plant tissues at the critical toxic concentration [27].

Therefore, the application of copper sulfate at (0.4 ppm Cu) concentration as algacide in irrigation water leads to the bioaccumulation of copper in plant tissues under a concentration that may be greater than the copper critical toxic concentration for *Zea mays* L. and may have a toxic effect on plant development.

**Table 4:** Total Dry Matter masses and global growth reduction of *Zea mays* L.

Irrigation method	Dry matter(g)	Growth Reduction
BI-C	9.09	11%
BI-Cu	8.05	
SI-C	8.65	14%
SI-Cu	7.43	

On the other hand, the comparison of dry matter between experimental crops showed lower mass by 8% with sprinkler irrigation. The deposition of copper on leaves and shoots by sprinkler irrigation reduces further the growth of plants and thus, increasing the rate of phytotoxicity. These results are similar to those obtained by Sheldon and Menzies [19], observing that Rhodes grass treated with even lower concentration of copper showed a growth reduction greater than 10%.

### 3.4 Accumulation profile of copper in soil

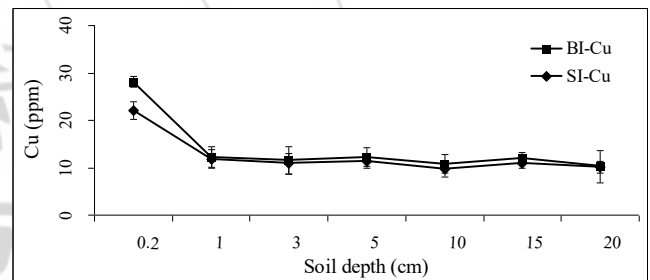
The vertical distribution of total copper in soils irrigated by copper treated water was also examined (Figure 5). Retention profiles shows that retained copper in the upper 0.2 cm of the soil are 28.23 ppm and 22.35 ppm when basin and sprinkler irrigation are applied respectively. The total copper concentration in soil decreases to be near the uncontaminated soil (9.1 ppm - see table 1) below approximately 1 cm, and remains constant for the following depths. Similarly, in their study of copper retention profile in

column leaching experiment using 18.7 kg CuSO<sub>4</sub>/ha/year, Salam and El-Fadel [16], indicated that copper is retained mainly in the upper 2 to 3 cm of the soil. Also, Rusjan et al. [28] found that the highest content of copper was identified in the surface soil layer on terraces (110 mg/kg), where it decreases with increasing depth. Pietrzak and McPhail [29] indicated that in vineyard soils, total Cu concentration decreases with increasing depth.

Indeed, the total mass of copper added during two months on each experimental column was 7.13 mg. 25 % are found accumulating near the surface (upper 0.2 cm), when columns were irrigated by basin method while 20 % are found with sprinkler irrigation. The difference is explained by the deposition of a part of copper on leaves and shoots surfaces during sprinklers irrigation.

Thus, copper from treated irrigation water accumulates in the surface of agricultural soil with higher rate when basin irrigation is applied, leading to potential negative impacts on the growth of cultivating maize plants.

We conclude that the use of copper in irrigation water leads with time to its accumulation in soil and so, to an increase of its phytotoxicity. This result may limit the use of Copper sulfate as algacide in irrigation [16].



**Figure 5:** The distribution of total copper concentration in soil depth of columns irrigated by basin irrigation (BI) (■) and sprinkler irrigation (SI) (◆).

## 4. Conclusion

Phytotoxicity of copper sulfate used as an algacide in irrigation water was studied on maize plants (*Zea mays* L.), cultivated in plastic columns and irrigated by two modes: Basin and Sprinkler. After two months of growth, a global reduction of dry matter of 11% was obtained. This rate of growth reduction is closer to the critical limit of phytotoxicity which is 10%. Morphological modification was shown in leaf surfaces and slightly on shoot height. The irrigation mode affects the copper bioaccumulation rate and its uptake pathway. Compared to control plants, the total copper bioaccumulation in experimental plants is increased by 35% and 45% (mass) during the irrigation by basin and sprinkler modes, respectively. In basin irrigation, the copper accumulation process is occurred through roots uptake, whereas by sprinkler, it is carried through roots uptake and leaves diffusion. The copper bioaccumulation in plants tissues is favored further by the sprinkler irrigation mode with more than 8%.

Distribution of copper retention profile in the soil is studied as well. The copper is mostly retained in the upper surface of the soil (0.2 cm) and decreases with depth, leading with time to its accumulation in the surface of agricultural soil, with higher rate when basin irrigation is applied. Thus, copper accumulation will cause an increase of phytotoxicity and have potential negative impacts on the growth of cultivated maize plants. This result may limit the use of copper sulfate as algaecide in irrigation water and requires the necessity to find out an alternative product.

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