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Germination and Survival of Maize and Beans Seeds: Effects of Irrigation with NaCl and Heavy Metals Contaminated Water

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Abstract

Metal toxicity and soil salinity at excessive levels in soils are toxic to plants. The main objective of this study was to demonstrate the effects of water salinity (NaCl) and heavy metal concentration on germination and seedling characteristics of maize and beans seeds. Different concentrations of NaCl (0 to 35000 mg/L) and Zn^{2+}, Cu^{2+} and Pb^{2+} with concentrations 0 to 600 µmol/L each were used separately. These solutions were used to irrigate maize and bean seeds for 30 days. Data was taken daily for the following parameters; "the number of germinated seeds, shoot length, shoot circumference, leaf area index, leaf colors, senescence, and disease occurrences." The crops were uprooted at the end of the 30 days and their wet masses were measured and recorded. Results indicated that seed germination reduced with an increase in the concentration of NaCl for both maize and beans with 0% germination recorded in concentrations > 5000 mg/L. The same trends were observed for other parameters, the shoot length, the leaf area index, and the shoot circumference. "For heavy metals, the trends were similar. Beans growth was more affected by Zn and Cu but maize growth was more affected by Pb." The dry masses and wet masses of the crops with higher concentrations of metals and salts were very low compared to the control experiments. For the effects of salinity and heavy metals on beans and maize seeds, there was a significant difference ($P \leq 0.001$) between all tested concentrations and the control for all growth parameters monitored. It was concluded from the results that salinity and heavy metals affect the germination and seedling characteristics of maize and beans; though some metals are essential, their presence in higher concentrations instead cause harm.

Keywords

Salinity, Heavy Metals, Pollution, Seedling Characteristics, Irrigation

1. Introduction

Environmental pollution continues to be a local and global threat to plants, humans as well as animals. It has negative effects on all components of the environment. The effluents from industries are the main sources of organic and inorganic pollutants [1]. Pollution of the land occurs from various degradable and non-degradable materials. These materials could be solid waste, trash, or chemicals including metals and salts. Soil can be described as a sink of most pollutants because it receives most of the solid waste, trash, or chemicals. One group of chemicals called salts (defined as any chemical compound resulting from acid and base reaction in which the metal or other cation replaces all or part of the hydrogen) presence in soils leads to soil salinity above certain levels. According to Irfan *et al.* [2], soils with electrical conductivity (EC) ≥4 dS/m have been widely accepted as the main definition of saline soils. This salinity originates from the long-term accumulation of salts through natural processes, a process called primary salinity. In this process, soluble salt such as chlorides of sodium, calcium, and magnesium found in the main rock material is released. Soil salinity also results from anthropogenic sources modifying the hydraulic balance of the soil from water applied (irrigation or rainfall) and that used by the crop (transpiration), a process called secondary salinity. The main causes are irrigation schemes using salt-rich irrigation water, insufficient drainage and land clearing [3]. It is estimated that about 800 million hectares of land representing 6% of the world's land are impacted by salinity [2]. Hence, to avoid land degradation in these environments, there is a need to keep surveillance on the quantity of salt accumulating on them [4]. Agricultural yield losses of 30% - 50% (depending on the level of soil salinity) occur on saline soils worldwide because salinity affects major plant functions [5]. One of the most negative salinity consequences comes from the accumulation of Na^+ as well as Cl^- ions in organs of plants in contact with soils at elevated NaCl concentrations [6]. Sodium-ion has an essential role in toxicity; this is because its absorption is very rapid by the root cells of plants [7]. Low plant productivity and even death can occur at higher concentrations of Na^+ because Na^+ ions inhibit K^+ uptake, which is a very important nutrient for crop growth and development [6]. Saline water also makes it difficult for plants to adsorbed soil moisture, influences the organization of membranes, affects vegetative growth and reproductive development, causes a decrease in germination rates, deteriorates the physical structure of the soil hence reducing water permeability, reduces cell division and expansion, causes oxidative stress, leads to ion toxicity, result in nutritional disorders, it alters metabolic processes, and leads to genotoxicity [2] [7] [8] [9] [10]. Equally, salinity leads to damage to the nutrient and hormone balances, during germination, leading to delay or inhibition in some cases of germination. These conditions generate reactive oxygen species (ROS) in chloroplasts, mitochondria, and the apoplastic causing membrane peroxidation; ion leakage; and damage to nucleic acids, cell membranes, and cellular structure. This leads to the poor quality and yield of the affected crop [10].

Heavy metal pollution is also a great threat to the biosphere due to the fact that the heavy metals cannot degrade, rather they persist and are accumulated, hence posing severe effects on humans, animals, and plants [11]. They are defined as metals with atomic density > 4 g/cm^3, or five times > that of water [12]. Agricultural soils in many regions of the world are contaminated with heavy metals in slightly to moderate amounts. This result from prolonged use of industrial waste, dust from smelters, phosphatic fertilizers, mineralogical backgrounds, sewage sludge application, and poor irrigation methods in agricultural farmlands [13] [14]. There is an equally need to monitor these heavy metals in soils which are used for growing crops for human consumption. Many body systems of living organisms can be damaged at excessive levels of heavy metals meanwhile at lower concentrations these organisms can tolerate them [15]. A number of heavy metals in appropriate concentrations are essential for plant development, like Fe, Cu, Zn, and Mn, while some are harmful to the plants. They include; cadmium, mercury, lead, and arsenic [13]. For example, high Pb and Cu concentration also induce oxidative stress in plants [16] leading to the destruction of macromolecules and perturbation of metabolic pathways. Vassilev *et al.* [17] in their study demonstrated how copper toxicity affects barley plant growth. This toxicity causes leaf chlorosis by degrading the photosynthetic elements. Heavy metals accumulate in soils are subsequently transported through the roots to the stem, leaves, and fruits [13].

Cereals are the main source of food for Sub-Saharan Africa contributing to about 50% of the total dietary energy source from 2007-2009 [18]. The most widely grown cereal is maize, grown on more than 33 million hectares each year in Sub-Saharan Africa. It had the best yield of 70,076,591 tons in Africa in 2012. In Cameroon, it is the main ingredient used in cattle and poultry feed manufacture as well as the brewery industries. It is consumed by more than 12 million Cameroonians and contributes about 5.6 billion CFA to Cameroon's GDP. The North West Region was ranked third nationally with an annual production of 176,473 tons in 2010. Equally, Mezam Division with Bamenda as its headquarters was also ranked third nationally in terms of maize production in 2012 [18]. Beans follow maize in terms of production and consumption in Cameroon and Africa in general [19]. It has a high content of protein, iron, and zinc. Like maize, it is cheap and consumed throughout Cameroon and throughout the year because it can easily be preserved at no cost. It also provides revenue to many homes in Africa especially the rural population, exceeding US$500 million annually with its sales value [19].

With the advent of modern Agricultural techniques, new brands of seedlings for maize and beans are developed every day in different countries around the world. These varieties of seeds will respond differently to varying conditions of the environment across the world. While several studies have been carried out on salinity effects particularly using NaCl and heavy metals in other countries [20] [21] [22] [23], no such study has been carried out in Bamenda North West Region of Cameroon using local maize and beans seeds. This paper is therefore aimed at studying the effects of water contaminated with NaCl, Pb, Zn, and Cu ions on the growth and survival of maize and beans, the two most cultivated and consumed crops in the North West Region of Cameroon. Water contaminated with NaCl at different concentrations as well as different concentrations of Pb, Zn, and Cu ions were used to irrigate the two crops, and growth parameters such as germination rate, shoot length, shoot circumference, leave area index, and wet and dry mass were used to characterize the growth of the two crops at tested conditions. The results of this study will help the Government educate the population on the effects of environmental pollution on food security, their livelihood, and health.

2. Introduction Materials and Methods

2.1. Soil Sampling and Characterization

The soil used for the experiment was collected from a school demonstration farm. Soil characteristics (Table 1) were adopted from the study carried out in 2018 at the school demonstration farm by the two departments of the COLTECH. Two samples from different positions on the farm were collected, homogenized, and used. The soil was collected using a hand shovel at 0 - 10 cm depth from two positions. These are sites where no cultivation activity has taken place, hence, very little possibility of heavy metal contamination from fertilizer application. Mixing can reduce salinity from site 1, obtain higher pH values, and improve the cationic exchange capacity (CEC) for site 1. Higher pH results in more neutral soil with a better root environment and a higher amount of nutrients available to plants [24]. Higher CEC values are favorable for the soil to hold cations. Thus, the ability of soil from site 1 to hold cations such as calcium, magnesium, and others will be greatly improved. This can improve its fertility for

Table 1. Soil characteristics of the school demonstration farm.

Parameter	Soil Samples	
	Sample 1	Sample 2
pH	6.3 (slightly acidic)	5.7 (moderately acidic)
Electrical conductivity (dS/m)	16 (extremely saline)	8 (moderately saline)
Organic carbon content (mg/kg)	1.44 (medium)	2.48 (high organic carbon content)
Organic matter (%)	2.1 (low organic matter)	2.1 (low organic matter)
Total nitrogen (mg/kg)	1.14 (high nitrogen content)	1.21 (high nitrogen content)
Available phosphorus (mg/kg)	6.83 (very low phosphorus)	6.26 (very low phosphorus)
Calcium content (mg/kg)	1.14 (very low calcium content)	1.54 (very low calcium content)
Magnesium content (mg/kg)	0.22 (Low magnesium)	0.7 (Low magnesium)
Potassium content (mg/kg)	0.023 (very low potasium)	0.01 (very low potasium)
Sodium content (mg/kg)	0.041 (very low sodium)	0.02 (very low sodium)
Cation exchange capacity (meq/100g)	6.72 (low CEC)	11.36 (low CEC)

crop germination. Total nitrogen Magnesium, Phosphorus, calcium, sodium, and potassium were determined using Atomic Absorption Spectrometry (ASS) by FASSA Laboratory University of Dschang while pH (H_2O) was determined by APERA multi-pH meter (PC60-Ph/Cond/TDS/sal. Tester). Soil organic matter and carbon content were determined using the loss on ignition method at about 440˚C in a furnace (ASTM D 2974 Method C). Cation exchange capacity was determined after leaching extraction of cations in 1M NH_4OAc solution (pH 7.0).

2.2. Crop Selection

The crops used here were maize and beans. This is because these are conventional crops. Yellow and sweet improved maize seeds ATP (Acid Tolerance Population) very much used by local farmers were bought from IRAD (National Institute of Agricultural research and development). Improved red beans seeds were bought from MIDENO (North West Development Authority*)*. A quick seed germination test was done on the seeds to be used. Here, 10 maize seeds and 10 bean seeds were soaked in water for 8 hours. A double thick paper was moistened with water and folded in half, the paper was opened and these seeds were placed in it. The paper was folded back and placed in a plastic bag. This was placed in a warm environment with a temperature of about 25˚C - 30˚C (propagator temperature) and checked every day for germination. The germination percentages were determined from the number of germinated seeds compared to the number used as indicated in Equation (1).

$$\text{germination rate} = \frac{\text{number of germinated seeds}}{\text{total number of seeds}} \times 100 \quad (1)$$

There was 100% germination for maize and 90% for beans. With these germination rates, the seeds were used for the study.

2.3. Preparation of Salt and Heavy Metals Solutions

Salt contaminated water solutions of 500 - 35,000 mg/L were prepared by dissolving 5 g, 10 g, 50 g, 100 g, 200g, and 350 g of NaCl in 10 L of distilled water respectively. Similarly, 40, 200, and 600 μmol/L concentrations of each metal were used. Three metals were studied; Zn, Cu, and Pb. They were prepared by dissolving appropriate masses of each of the metal salts ($ZnSO_4$, $CuSO_4$, and $Pb(NO_3)_2$) in 10 L of water. The analyte-grade chemicals were used.

2.4. Experimental Procedure

Soil samples were collected from 2 spots and uniformly mixed. The soil samples were sun-dried for 3 days. Polythene bags were labeled and 1 kg of soil was filled in all of them. 12 g of urea fertilizer was measured and placed in each of the polythene bags containing the soil and properly mixed prior to planting. Six seeds, 3 maize, and 3 bean seeds were planted in each polythene bag (18 for salinity studies, 27 for the three heavy metals, and three for the control). The polythene bags were irrigated with the appropriate solutions (0.70 L) twice a week for 4 weeks with data being taken daily for the different parameters (number of germinated seeds, shoot length, shoot circumference, and leaf area index). The control was irrigated with tap water. After the 4th week, the plants were harvested from the polythene bags, weighed, and their fresh weights measured and recorded. The crops were sundried for 2 weeks and their dry masses measured and recorded. All the experiments were done in triplicates and the reported results are averages.

2.5. Statistical Data Analysis

The Microsoft Excel 2010 version was used to record and compute the data from the study and OriginLab 8.0 was used for graphical analysis. SigmaPlot 12.0 was used for descriptive analysis.

3. Results and Discussion

3.1. Effects of NaCl

3.1.1. Number of Germinated Seeds

Results of NaCl effects on the quantity of germinated seeds are presented in Figure 1(a) for maize seeds and Figure 1(b) for bean seeds. The seed germination rates for both maize and beans varied greatly with the concentration of the NaCl. With beans being highly affected as the highest number of germinated seeds occurred in the control which is just 2 out of 3. Maize had the highest, 3 which occurs in the control. For both crops, at concentrations ≥ 10,000 mg/L, no germination was observed. Salinity has adverse effects on the germination of seeds, as suggested by Hafeez *et al.* [22]. According to him, the germination of seeds is highly affected by salinity due to the creation of an osmotic potential that inhibits water uptake or damages to the embryo due to the toxic effects of ions. This also agrees with the fact that salinity delays and reduces the rate of germination [24] [25]. For maize, germination rate standard deviation values of 0.583, 0.434, 0.551, 0.000, 0.000, 0.000 and 0.900 were obtained for 500, 1000, 5000, 10,000,

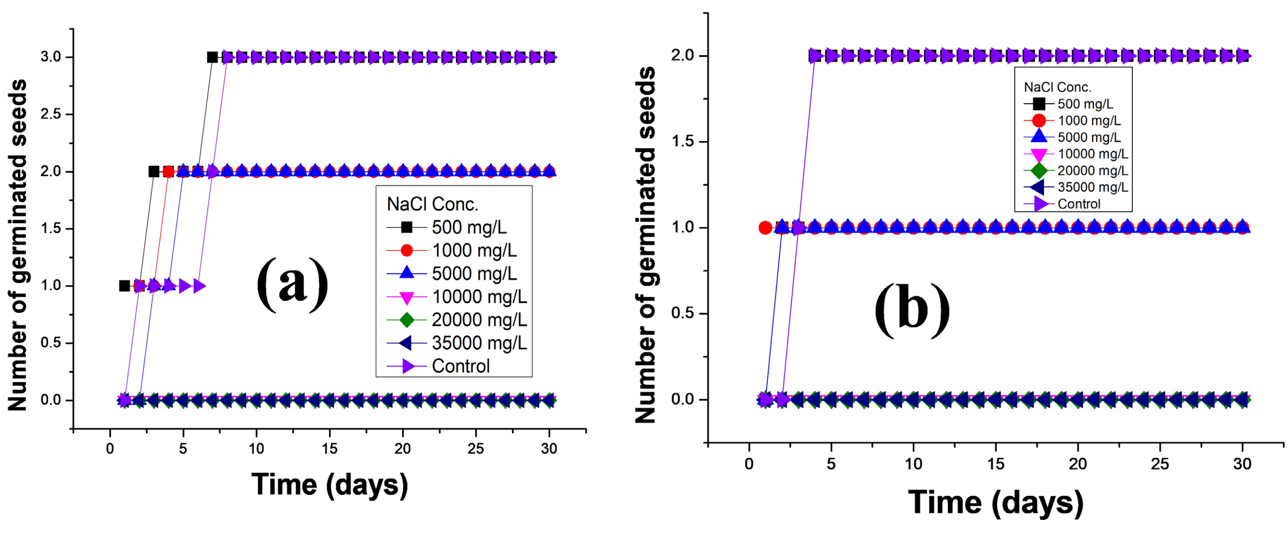

Figure 1. Number of germinated seeds. (a) Maize; (b) Beans at different concentrations of NaCl.

20,000, 35,000 mg/L salt concentrations and the control respectively. There is a significant difference (P ≤ 0.001) between all tested salt concentrations and the control except the 500 mg/L (P = 0.445), indicating the effect of salinity on the germination rate for maize. There is also a significant difference (P ≤ 0.001) between lower concentrations such as 500 mg/L, 1000 mg/L and 5000 mg/L and higher concentrations of 1000, 20,000 and 35,000 mg/L each. The high value of standard deviation value (0.900) in the control is due to the fact that germination in the control occurred in four phases (day 1, zero germinated seed, days 2 to 6, 1 germinated seed, day 7, 2 germinated seeds, and days 8 to 30, 3 germinated seeds) compared to three for 500 (days 1 to 2, 1 germinated seed, days 3 to 6, 2 germinated seeds, days 7 to 30, 3 germinated seeds), 1000 (day 1, zero germinated seed, days 2 to 3, 1 germinated seed, days 4 - 30, 2 germinated seeds) and 5000 (days 1 to 2, zero germinated seed, days 3 to 4, 1 germinated seed, days 5 to 30, 2 germinated seeds) mg/L concentrations and only one phase (no germination) for 10000, 20,000 and 35,000 mg/L concentrations. For beans, standard deviation values of 0.434, 0.00, 1.83, 0.000, 0.000, 0.000 and 0.531 were obtained for 500, 1000, 5000, 10,000, 20,000, 35,000 mg/L salt concentrations and the control respectively. Similar to maize, the patterns of statistical significance were the same.

3.1.2. Shoot Length (cm)

The shoot length of both maize and beans reduced as the salt concentration increased as seen in Figure 2(a) for maize and Figure 2(b) for beans. Shoot length for maize reduced as concentration increased up to 1000 mg/L concentration though at different rates with the control making a remarkable difference (Figure 2(a)). For beans, the crop could survive at just 500 mg/L concentration. That for 1000 mg/L germinated and died before the 6th day, the control still making a remarkable difference (Figure 2(b)). This is probably because salinity hinders cell division and retards enlargement in the growth regions as stated by Hasegawa

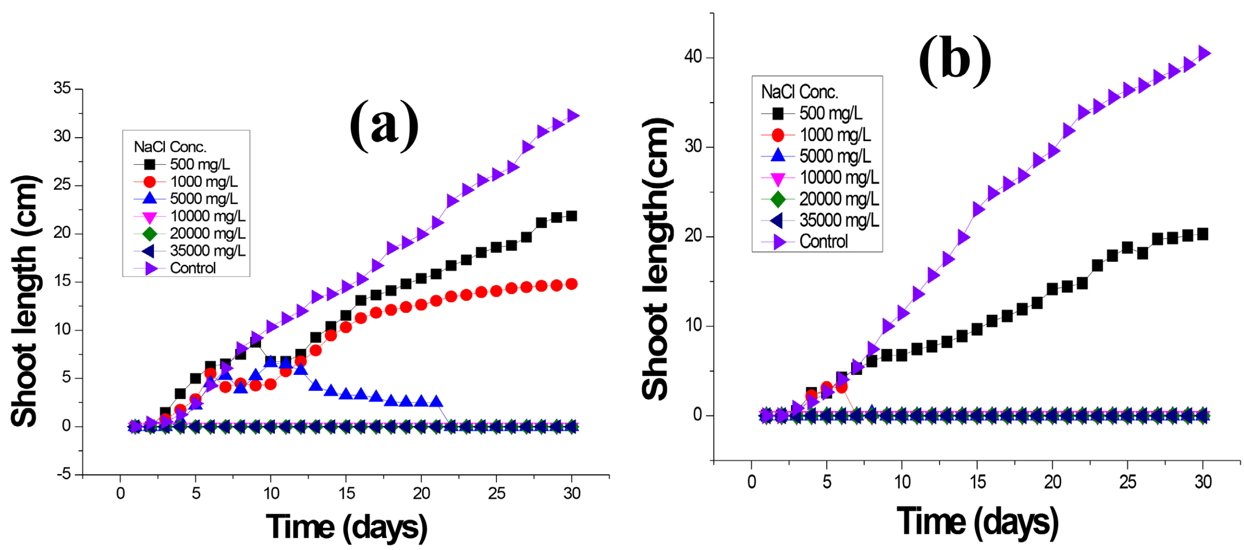

Figure 2. Shoot lengths. (a) Maize; (b) Beans at different concentrations of NaCl.

et al. [26]. Shoot length standard deviation values of 6.497, 0.878, 0.0456, 0.000, 0.000, 0.000 and 14.025 were obtained for 500, 1000, 5000, 10,000, 20,000, 35,000 mg/L salt concentrations and the control respectively for beans with a statistical significance ($P \leq 0.001$) between shoot lengths at tested concentrations and the control. For maize, standard deviation values were calculated as 6.570, 5.088, 2.251, 0.000, 0.000, 0.000 and 10.085 for 500, 1000, 5000, 10,000, 20,000, 35,000 mg/L salt concentrations and the control respectively with a statistical significance ($P \leq 0.001$) between shoot lengths at tested concentrations and the control.

3.1.3. Shoot Circumference (cm)

The shoot circumference results for maize and beans are presented in **Figure 3(a)** and **Figure 3(b)** respectively. Shoot circumference reduced as the concentration of sodium chloride increased for both maize and beans. Maize irrigated with 500 mg/L NaCl has a closer value to the control experiment than at higher concentrations (**Figure 3(a)**). At the initial stage of growth, salts affect many plant functions like photosynthesis, cell elongation, and many others. This accounts for the reduced shoot circumference of the crops as concentrations of salts increases. As for germination rate and shoot length, there is a significant difference ($P \leq 0.001$) in shoot circumference between tested salt concentrations and the control for both beans and maize. The maize shoot circumference standard deviation values of 0.385, 0.232, 0.475, 0.000, 0.000, 0.000, and 0.406 for 500, 1000, 5000, 10,000, 20,000, 35,000 mg/L salt concentrations and the control respectively were obtained. The corresponding standard deviation values for beans were: 0.171, 0.178, 0.0913, 0.000, 0.000, 0.000, and 0.318.

3.1.4. Leaf Area Index

The leaf area index for the two crops reduced with an increase in the concentration of sodium chloride (**Figure 4**). Beans leaf area index showed a more significant effect with increasing NaCl. From **Figure 4(a)**, for maize, the control starts forming leaves on the fifth day followed by seedlings irrigated with 500 mg/L sodium chloride, and then that for 1000 mg/L sodium chloride. Maize seeds irrigated

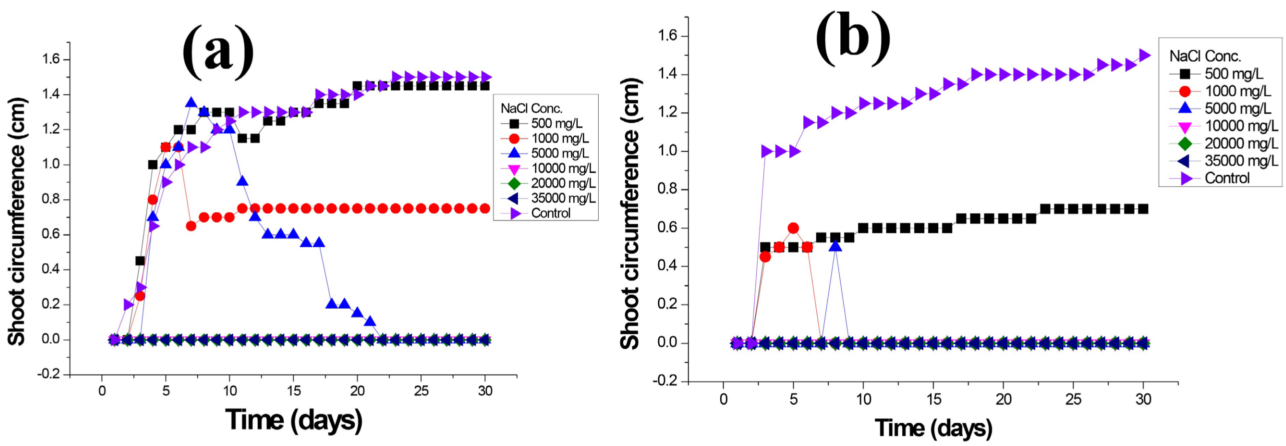

Figure 3. Shoot circumference. (a) Maize; (b) Beans at different concentrations of NaCl.

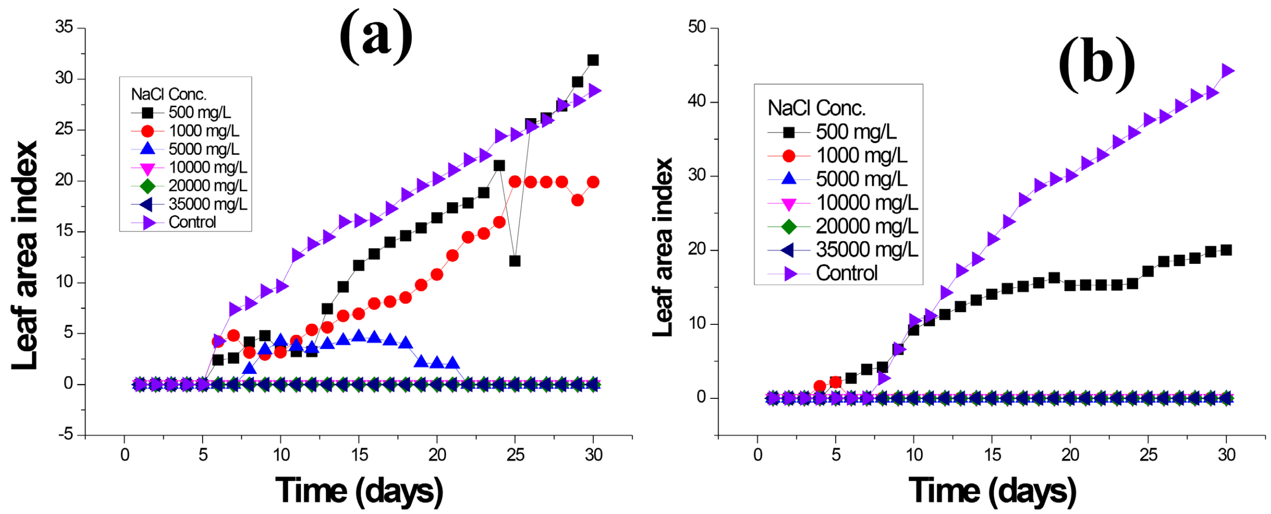

Figure 4. Leaf area index. (a) Maize; (b) Beans at different concentrations of NaCl.

with 5000 mg/L sodium chloride produced leaves on the 7th day but eventually died down before the 25th day. For beans (**Figure 4(b)**), the control produced leaves from the 8th day, almost the same day with seedlings irrigated with 500 mg/L sodium chloride. The remaining seedlings for higher concentrations of sodium chloride did not produce leaves at all. This is because due to salinity, leaf initiation and expansion are suppressed accompanied by internode growth, and acceleration of leaf abscission hence reducing shoot growth [27]. Also, the number of elongating cells and leaf growth rate reduces with salinity [28]. As for germination rate, shoot length and shoot circumference there is a significant difference ($P \leq 0.001$) in leaf area index between tested salt concentrations and the control for both beans and maize. Leaf area standard deviation values of maize were obtained as: 9.896, 6.984, 1.883, 0.000, 0.000, 0.000 and 9.376 for 500, 1000, 5000, 10,000, 20,000, 35,000 mg/L salt concentrations and the control respectively. The corresponding standard deviation values for beans were; 8.803, 6.721, 0.483, 0.000, 0.000, 0.000 and 15.616.

3.1.5. Discussion on Effects of Salinity

The results for salinity, as represented in **Figures 1-4** are similar to results gotten by Hafeez *et al.* [22] in which soil of higher electrical conductivity affected both germination and morphology of germinated seeds, when different cultivars of sunflower were subjected to soils with varying electrical conductivities. The poor morphological characteristics of the crop seedlings agree with the conclusion made by [26] [29]. They concluded that the main processes required for successful initial plant growth (photosynthesis, protein synthesis, enzyme activity and energy, and lipid metabolism) are affected by salinity. This results in speedy aging of older leaves and the appearance of toxicity symptoms (chlorosis, necrosis) on mature leaves. Plants are noted to experience water stress at the initial stage of growth causing a reduction in leaf expansion. When the plant is exposed to salinity, the osmotic effects are immediately observed. And there is further inhibition of cell expansion and cell division along with the closure of stomata due to continuous exposure to salt. Results presented also show that beans suffer more from the effects of salinity compared to maize. This could be explained by the fact that maize can tolerate salinity and toxicity to a higher extent than beans. Because some plants have the characteristics that can help them tolerate salts to a particular concentration.

3.2. Effects of Heavy Metals

3.2.1. Effects of Zn^{2+}

1) Number of germinated seeds

From Figure 5(a), the number of germinated seeds for maize seeds irrigated with 40 μmol/L Zn^{2+} is higher, similar to the control, which is 100%. Two out of 3 seeds germinated for the higher concentrations, a percentage of 66. For beans (Figure 5(b)), a similar trend was observed, but only 1 out of 3 seeds germinated (a percentage of 33) for each 40, 200, and 600 μmol/L Zn^{2+} concentrations. This could be attributed to the fact that there is a rapid breakdown of food materials stored in seeds when heavy metals are used [30]. Statistical analysis of maize seeds germinated showed that there is a statistically significant difference ($P \leq 0.001$) in germination rate between the control and each tested Zinc concentration. Meanwhile, there was no significant difference between the following pairs; control and 40 ($P = 0.446$) and 200 and 600 ($P = 0.495$). For beans seeds, there was a significant difference ($P \leq 0.001$) in germination rate between the control and each tested Zinc concentration. However, there was no significant difference ($P = 1.000$) in germination rate between the three concentrations.

2) Shoot length (cm)

The shoot lengths as indicated in Figure 6(a) increased for maize as Zn^{2+} concentration increased. The seedlings irrigated with 600 μmol/L Zn^{2+} have the highest shoot lengths, closer to that of the control experiment. However, the shoot length for beans shows a contrary pattern, with a reduction as the concentration

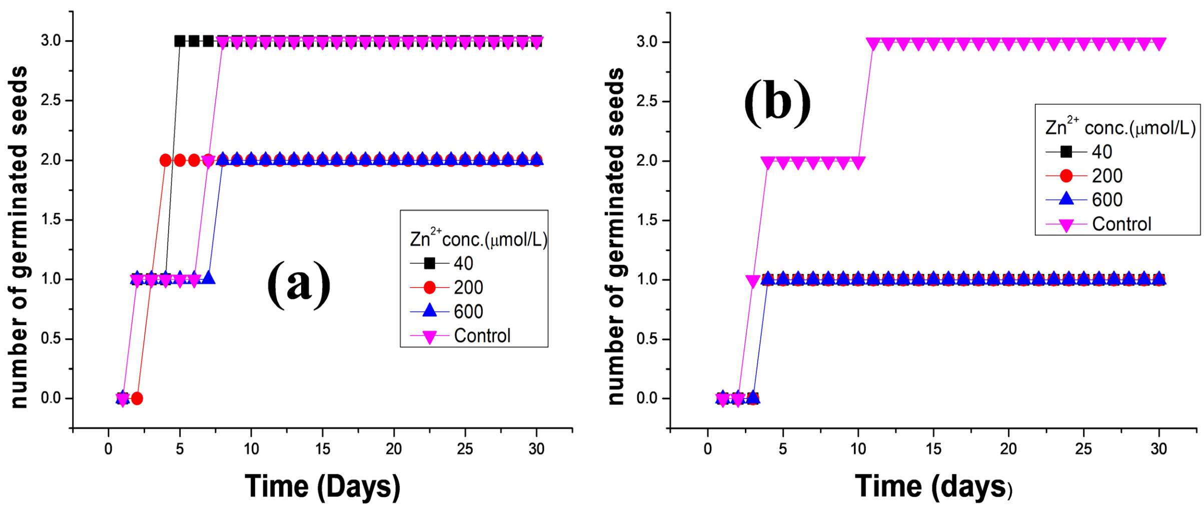

Figure 5. Number of germinated seeds. (a) Maize; (b) Beans at different concentrations of Zn^{2+}.

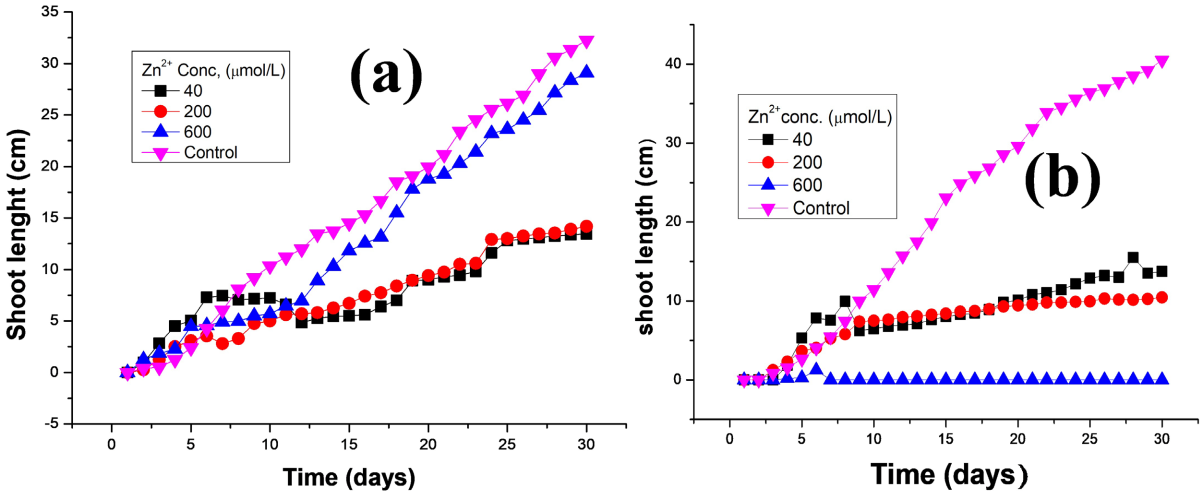

Figure 6. Shoot length. (a) Maize; (b) Beans at different concentrations of Zn^{2+}.

increased (**Figure 6(b)**). Zinc causes the decrease in growth in plant organs through alteration in metabolism processes which stems from induction of oxidative damage [31]. Shoot length standard deviation values of 3.65, 4.37, 9.21, and 10.09 cm were obtained for 40, 200, and 600 µmol/L $Zn^{2+}$and control respectively for maize seed. Statistical analysis indicated a statistically significant difference ($P \leq 0.001$) for shoot length at different zinc concentrations in relation to the control. In the case of beans, there was a significant difference ($P \leq 0.001$) in shoot length between the control and each tested Zinc concentration as well as between the following initial Zinc concentration pairs; 40/600 and 200/600. There was no significant difference ($P = 0.586$) in shoot length for beans between zinc concentrations of 40 and 200.

3) Shoot circumference (cm)

Shoot circumference for seedlings irrigated with varying Zn^{2+} concentrations are given in **Figures 7(a)** and **Figure 7(b)**. From **Figure 7(a)** for maize, shoot circumference increased for all tested concentrations to the 9th day, then a reduction is observed for 40 µmol/L of Zn^{2+} up to about the 20th day. While a maximum shoot circumference of about 1.1 cm is obtained for 200 and 600 µmol/L on the 30th day, the value of about 1.3 cm is finally obtained for 40 µmol/L concentration closer to that of the control with about 1.5 cm (**Figure 7(a)**). The reduced shoot circumference for a short period with 40 µmol/L of Zn^{2+} might be due to insufficient assimilation of Zn caused by abiotic stress while improved maize shoot circumference with increasing Zn supply indicated that adequate Zn led to the assimilation of the available supply [32]. The highest shoot length for beans of 1.0 cm is obtained when irrigated with 40 µmol/L (**Figure 7(b)**). Standard deviation values of 0.367, 0.330, 0.296 and 0.406 cm were obtained for 40, 200, 600 µmol/L Zn^{2+} and control respectively for maize seed. Statistically, analysis shows there is a significant difference ($P = 0.030$) for maize shoot circumference at different Zinc initial concentrations relative to the control. For the case of beans, there was a significant difference ($P \leq 0.001$) in shoot circumference between

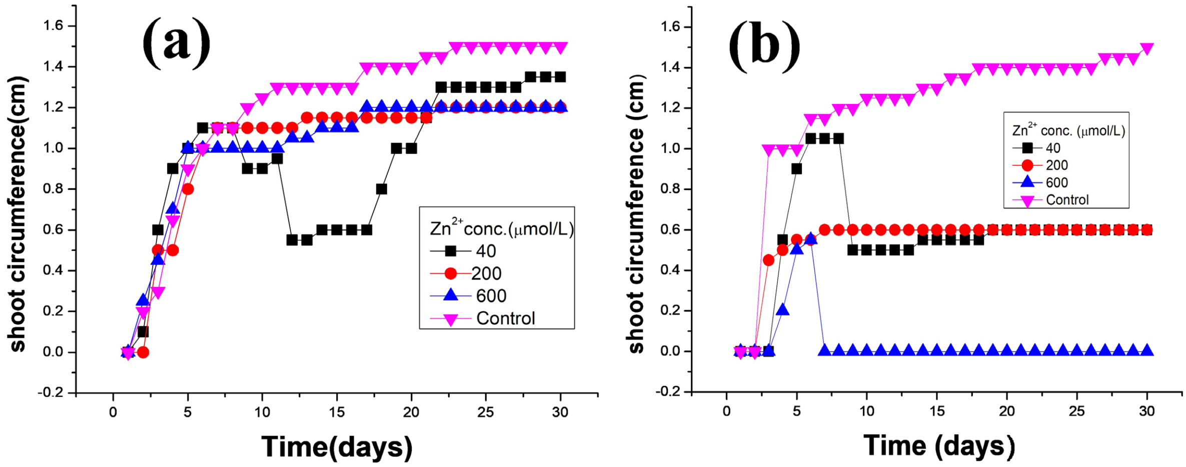

Figure 7. Shoot circumference. (a) Maize; (b) Beans at different concentrations of Zn^{2+}.

the control and each tested Zinc concentration as well as between the following initial zinc concentration pairs; 40/600 and 200/600. There was no significant difference ($P = 0.749$) in shoot circumference for beans between zinc concentrations of 40 and 200.

4) Leaf area index

Leave area index values of less than 15 are observed for beans (**Figure 8(b)**) against values of about 30 for maize (**Figure 8(a)**). Other observed physical characteristics in crops irrigated with Zn were the appearance of a yellow and purplish-red color in leaves of maize plants and beans treated with 600 mg/L Zn^{2+}. This observation agrees with the findings of Ebbs and Kochain [33] which showed that higher concentrations of Zn cause chlorosis in younger leaves of plants with the possibility of attacking the older leaves as well. The purple leaves were also observed on the leaves of seedlings with 600 μmol/L Zn^{2+}. This purple color intensified as the growth continued and as the crop was being irrigated. This is a result of a deficiency in phosphorus caused by high Zn concentrations as demonstrated by Lee *et al.* [34]. Average values of 8.94, 12.28, 11.79, and 15.30 were obtained for 40, 200, and 600 μmol/L Zn^{2+} and control respectively for maize seed. There is no statistically significant difference ($P = 0.081$) for maize seed leaf area index at different zinc concentrations compared to the control. Statistical analysis on bean seed leaf area index followed the same pattern as for shoot length and shoot circumference.

3.2.2. Effects of Cu^{2+}

1) Germinated seeds number

Figure 9(a) presents germinated seeds number for maize seeds irrigated with Cu^{2+} of different concentrations, which is 40 μmol/L, 200 μmol/L, and 600 μmol/L. 2 out of 3 seeds germinated for those irrigated with 40 μmol/L of Cu^{2+}, a percentage of 66. The control had three germinated seeds, a percentage of 100 while 1 seed each germinated for those treated with 200 μmol/L and 600 μmol/L. **Figure 9(b)** shows the trend for beans where the germination rate was very low as

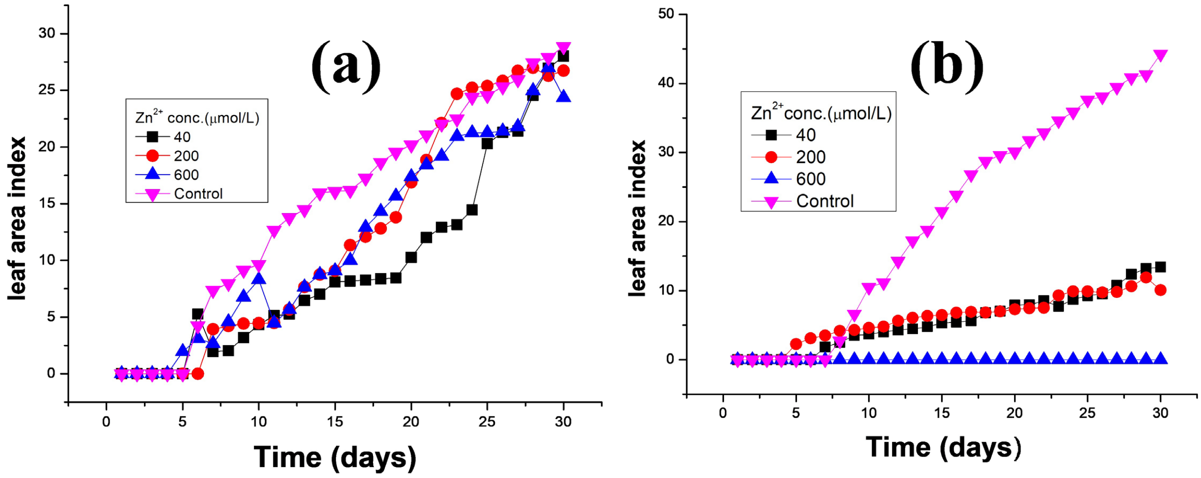

Figure 8. Leaf area index. (a) Maize; (b) Beans at different concentrations of Zn^{2+}.

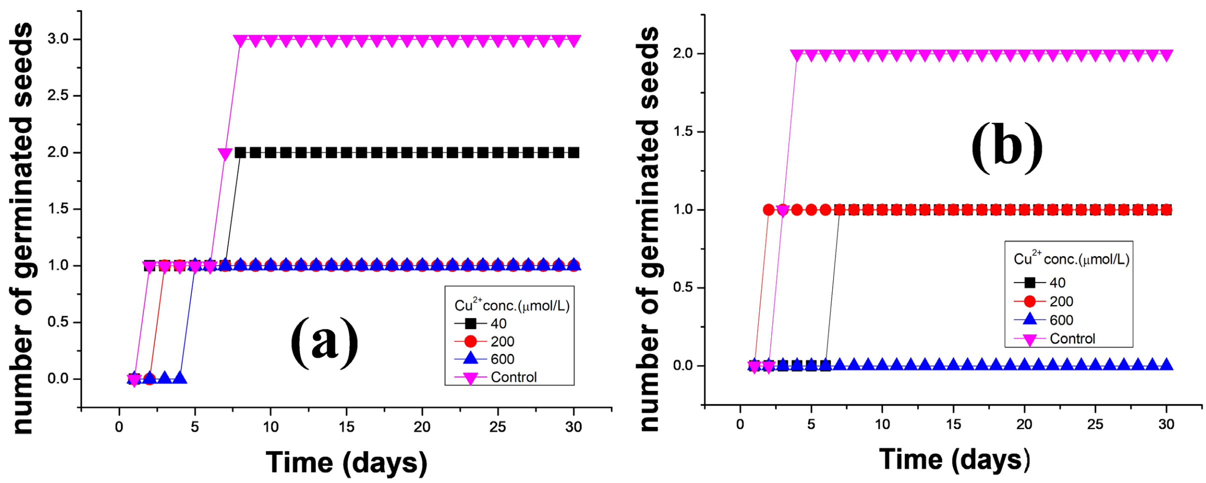

Figure 9. Number of germinated seeds. (a) Maize; (b) Beans at different concentrations of Cu^{2+}.

the control had 2 germinated seeds out of 3. The seeds irrigated with Cu^{2+} had only 1 germinated seed for 40 μmol/L and 200 μmol/L and zero for 600 μmol/L. Statistical analysis of maize seeds germinated showed that there is a statistically significant difference ($P \leq 0.001$) in germination rate between the control and each tested copper concentrations. Meanwhile, there was no significant difference between the 200 and 600 ($P = 0.647$). For beans seeds, there was a significant difference ($P \leq 0.001$) in germination rate between the control and each tested copper concentration. However, there was no significant difference ($P = 0.065$) in germination rate between 40 and 200 μmol/L concentrations. For maize standard deviation values were respectively 0.521, 0.254, 0.346 and 0.900 for 40, 200, 600 μmol/L and control in the case of maize and correspondingly 0.407, 0.183, 0.000 and 0.531 for beans.

2) Shoot length (cm)

Figure 10(a) shows the shoot length of seedlings for maize seeds irrigated with different concentrations of Cu^{2+} where the control recorded the highest

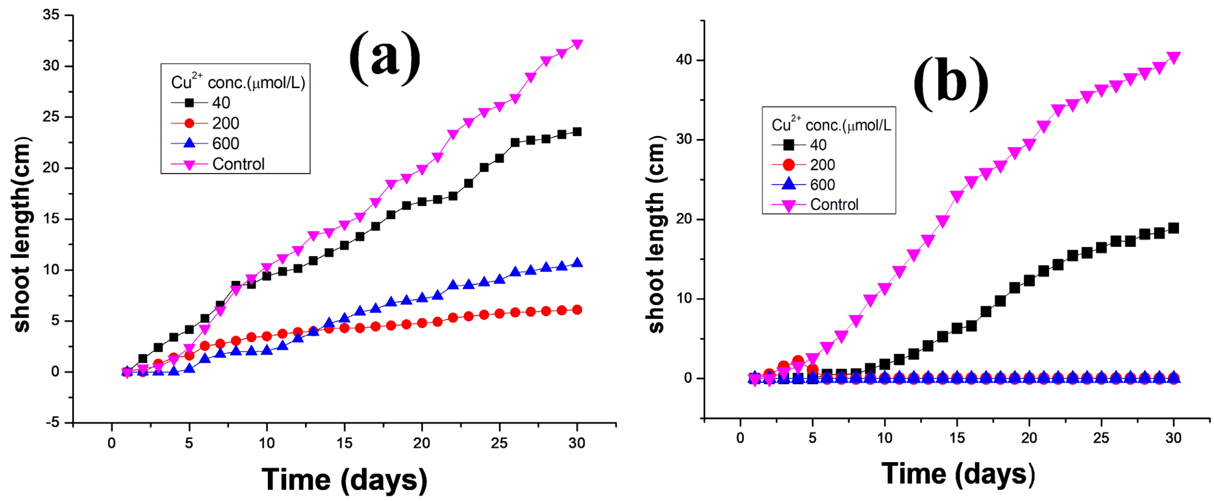

Figure 10. Shoot length. (a) Maize; (b) Beans at different concentrations of Cu^{2+}.

shoot length, followed by those seedlings irrigated with 40 μmol/L of Cu^{2+}. Those irrigated with 200 μmol/L and 600 μmol/L grew but at a very slow rate. Similar trends were observed for beans (**Figure 10(b)**) where the control had the highest shoot length, followed by the seedlings irrigated with 40 μmol/L of Cu^{2+}. Shoot length standard deviation values of 7.133, 1.774, 3.675, and 10.085 cm were obtained for 40, 200, and 600 μmol/L $Cu^{2+}$and control respectively for maize seed and correspondingly 7.057, 0.506, 0.000, and 14.025 for beans seeds. Statistical analysis indicates a statistically significant difference ($P \le 0.001$) for shoot length at different copper concentrations in relation to the control for both maize and beans. However, there was no significant difference in shoot length for maize between copper concentrations of 40 and control ($P = 0.227$); and between 200 and 600 ($P = 0.476$). In the case of beans, there was no significant difference ($P = 0.931$) in shoot length for beans between copper concentrations of 200 and 600 μmol/L.

3) Shoot circumference (cm)

The shoot circumferences for the seedlings irrigated with Cu^{2+} are shown in **Figure 11**. In **Figure 11(a)**, the shoot circumference for maize showed the highest circumference of 1.6 cm in the control followed by that irrigated with 40 μmol/L of Cu^{2+} at 1.4 cm. Those irrigated with 200 μmol/L and 600 μmol/L had lower values, about 0.6 cm. **Figure 11(b)** represents similar trends for beans. However, seedlings irrigated with 200 μmol/L reached a circumference of 0.9 cm but died down while the circumference continued for 40 μmol/L attaining a maximum of 0.6 cm. Shoot circumference standard deviation values of 0.250, 0.157, 0.205 and 0.406 were obtained for 40, 200, 600 μmol/L $Cu^{2+}$and control respectively for maize seed and correspondingly 0.224, 0.237, 0.000 and 0.358 for beans seeds. Statistical analysis indicates a statistically significant difference ($P \le 0.001$) for shoot circumference at different copper concentrations in relation to the control for both maize and beans. However, between copper concentrations of 40 and control, there was no significant difference in shoot circumference for

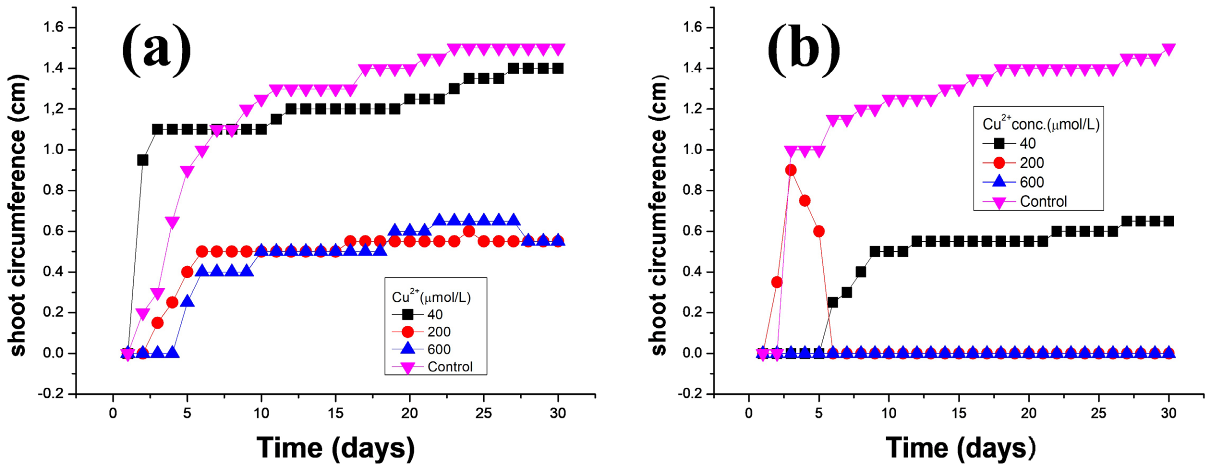

Figure 11. Shoot circumference. (a) Maize; (b) Beans at different concentrations of Cu^{2+}.

maize (P = 0.912) and for beans (P = 0.168). There was also no significant difference in shoot circumference for maize (P = 0.849) between Cu concentrations of 200 and 600 μmol/L.

4) Leaf area index

The graphs for the leaf area index are represented in Figure 12. The leaf area index for maize is in Figure 12(a) in which the control has the same high value of 38 with the seedlings irrigated with 40 μmol/L of Cu^{2+}, though growing at different rates. That irrigated with 200 μmol/L started producing leaves earlier, same as the control, but grows slowly compared to the control. That irrigated with 600 μmol/L started producing leaves right on the 11th day and the leaf expansion rate was very slow. Figure 12(b) represents the trend for beans with the control having a high leaf area index of about 48 and seedlings irrigated with 40 μmol/L Cu^{2+} with a low value of about 19. Other observed characteristics on the leaves of the crops irrigated with Cu^{2+} like Zn^{2+} are yellowing of leaves of maize plants and beans treated with 600 μmol/L. Leaf area standard deviation of 11.976, 3.227, 3.357, and 11.803 was obtained for 40, 200, and 600 μmol/L and control for maize with corresponding values of 9.381, 0.000, 0.000, and 15.616 for beans. There is a statistically significant difference (P ≤ 0.001) for maize seed and beans seed leaf area index at different copper concentrations compared to the control. Maize seeds leaf area index statistical analysis follows the same pattern as for shoot circumference while that of beans seed follows the same pattern as that for shoot length.

3.2.3. Effects of Pb^{2+}

1) Number of germinated seed

Figure 13(a) below shows the number of germinated seeds for maize at different concentrations of Pb^{2+}. The highest number of seeds germinated with a germination percentage of 100 was recorded in the control experiment followed by those seeds irrigated with 40 μmol/L of Pb^{2+}, having percentage germination of 66%, this against 33% for 200 and 600 μmol/L of Pb^{2+}. Figure 13(b) represents

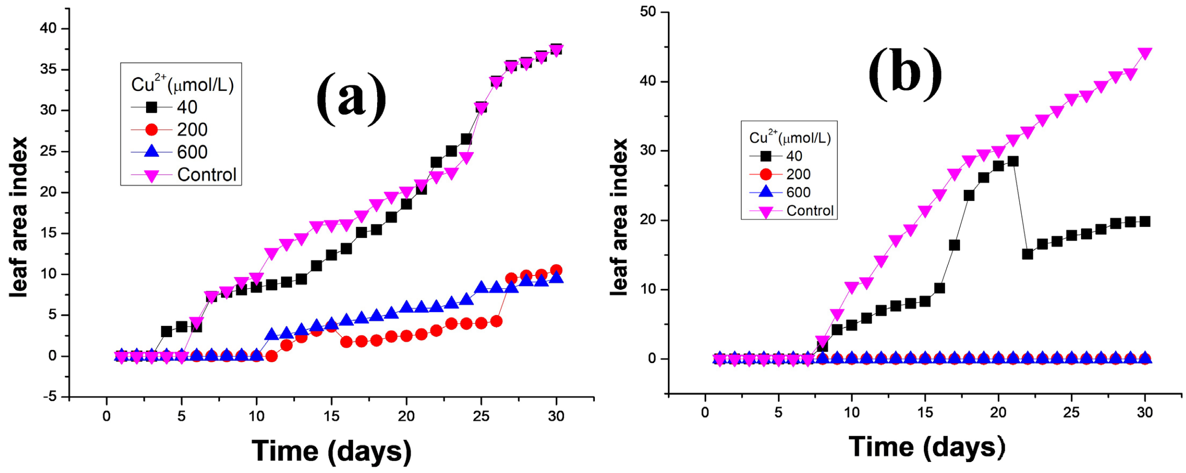

Figure 12. Leaf area index. (a) Maize; (b) Beans at different concentrations of Cu^{2+}.

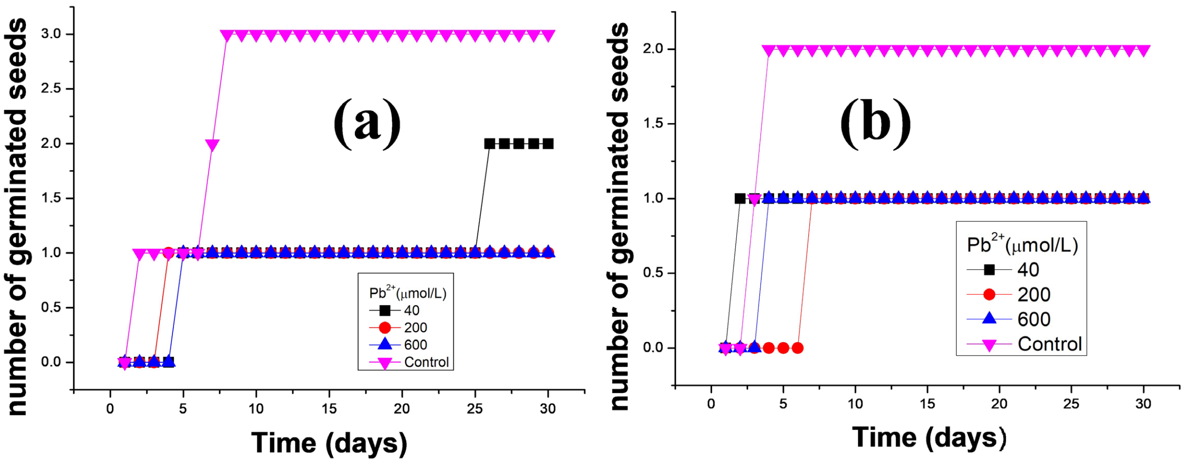

Figure 13. Number of germinated seeds. (a) Maize; (b) Beans at different concentrations of Pb^{2+}.

the amount of germinated seeds for beans. The control registered the highest number of seeds germinated with 66%. The beans seeds irrigated with 200 and 600 μmol/L of Pb^{2+} water had only one germinated seed (33%). This is because lead is toxic to plant seeds and thus inhibits germination [35]. Statistical analysis of the number of germinated seeds in Pb contaminated soil showed statistical significance ($P \le 0.001$) between tested Pb concentrations and the control for both maize and beans seed. However, there was no statistical significance between the following concentrations for maize 40 and 600 ($P = 0.604$), 40 and 200 ($P = 0.606$), 200 and 600 ($P = 0.824$) and 40 and 600 ($P = 0.497$), 40 and 200 ($P = 209$), 200 and 600 ($P = 0.522$) for beans. For beans seeds germination rate, the standard deviation values obtained were: 0.183, 0.407, 0.305 and 0.531 for 40, 200, 600 μmol/L of Pb^{2+} and control. The corresponding values for maize seeds were: 0.556, 0.305, 0.346 and 0.900.

2) Shoot length (cm)

Figure 14(a) shows the shoot length of seedlings for maize seeds irrigated with different concentrations of Pb^{2+} where the biggest value of shoot length was

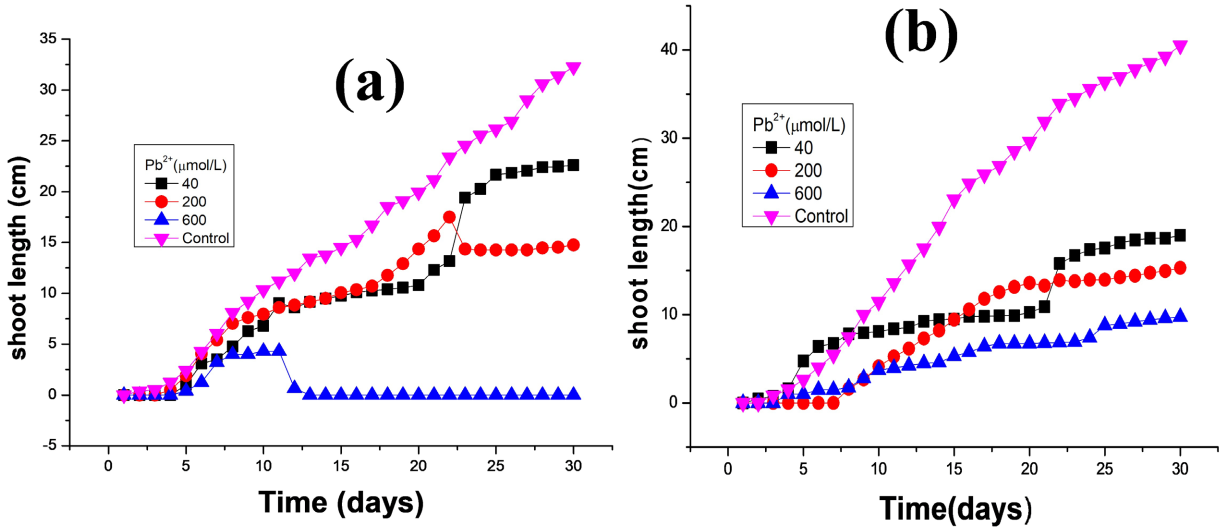

Figure 14. Shoot length. (a) Maize; (b) Beans at different concentrations of Pb^{2+}.

obtained in the control, and seedlings irrigated with 40 μmol/L of Pb^{2+}. Maize seeds irrigated with 200 μmol/L grow but at a very slow rate while the maize seeds irrigated with 600 μmol/L lead reached a length of about 5 cm but died down. Figure 14(b) shows the shoot length for beans where it indicates a decrease with increment in lead initial concentration. The control had the highest shoot length, followed by the seedlings irrigated with 40 μmol/L of Pb^{2+}. Those for 200 μmol/L and 600 μmol/L were much lower (Figure 14(b)). Statistical analysis of shoot length data for Pb shows a significant difference ($P \leq 0.001$) between tested concentrations and control for beans and maize. However, there was no significant difference ($P = 0.541$) between 40 and 200 μmol/L for maize, and the pairs 40 and 200 μmol ($P = 0.341$); 200 and 600 μmol ($P = 0.250$); for beans. Standard deviation values for beans were; 5.790, 5.981, 3.157 and14.025 for 40, 200, 600 μmol/L Pb and control while the corresponding values for maize were; 7.679, 5.267, 1.498, and 10.085.

3) Shoot circumference (cm)

The shoot circumference for the seedlings irrigated with Pb^{2+} is shown in Figure 15. Figure 15(a) shows the shoot circumference for maize in which the highest was recorded in the control, 1.6 cm followed by that irrigated with 40 μmol/L of Pb^{2+} at 1.2 cm. Those irrigated with 600 μmol/L reached a circumference of 0.9 cm then died down. The reduced shoot circumference only for a short period with 40 μmol/L of Pb^{2+} might be due to insufficient assimilation of Pb caused by abiotic stress which when overcomes improved maize shoot circumference. However, a sharp reduction in shoot circumference at 600 μmol/L indicated sufficient assimilation toxic to the plant. Figure 15(b) represents the results for beans. The control had a higher value of about 1.6 cm while lower values were obtained in succession for 40, 200, and 600 μmol/L Pb^{2+} water. Statistical significance ($P \leq 0.001$) on shoot circumference was obtained between tested Pb concentrations and the control except between control and 200 μmol/L

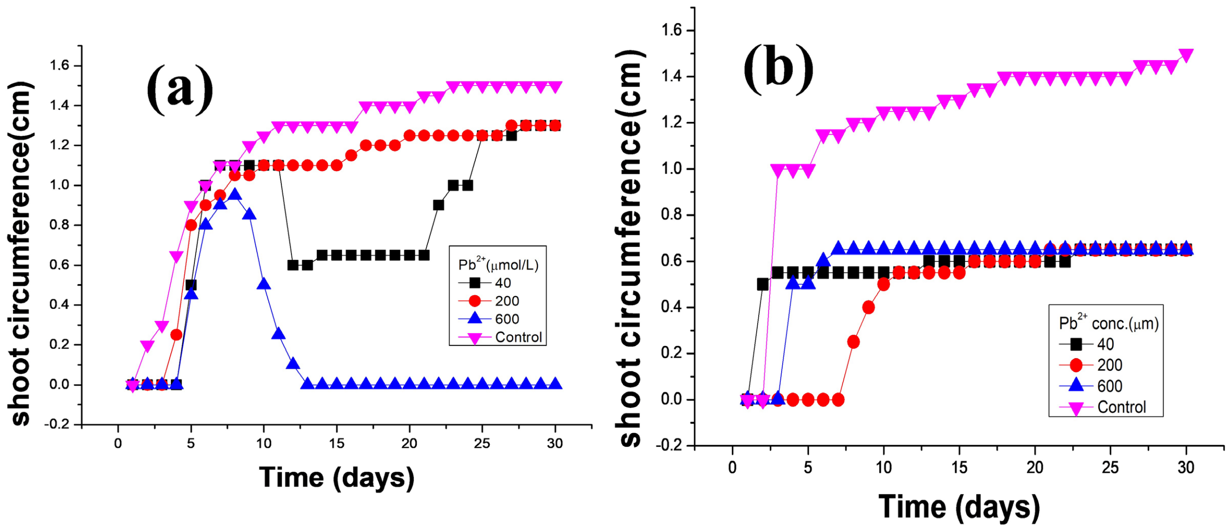

Figure 15. Shoot circumference. (a) Maize; (b) Beans at different concentrations of Pb^{2+}.

Pb^{2+} (P = 0.058) and between lead concentrations of 40 and 200 μmol/L (P= 0.068) for maize. Similar statistical significance was obtained for beans, however, no statistical significance was observed between the following lead concentration; 40 and 200 (P = 0.149), 200 and 600 (P = 0.102), and 40 and 600 (P = 1.000). The shoot circumference standard deviation values were; 0.409, 0.399, 0.312 and 0.406 for 40, 200, 600 μmol/L Pb^{2+} and control for maize while the respective values for beans were; 0.117, 0.264, 0.198 and 0.358.

4) Leaf area index

The leaf area index for crops treated with Pb^{2+} kept reducing as the concentration increased. This agrees with the conclusion of Hussain *et al.* [36] that at higher concentrations, root and stem elongation and leaf expansion are inhibited by lead. That for maize is represented in Figure 16(a) while that for beans is represented in Figure 16(b). The highest values were recorded in the control experiment. The values for beans were higher than for maize. This is because beans have broader leaves. The appearance of yellow leaves on the 24th day for treatment with 600 μmol/L Pb^{2+} was also observed. For Leaf area index, similar trends compared to statistical significance ($P \leq 0.001$) on shoot circumference were obtained between tested Pb concentrations and the control except between control and 40 μmol/L Pb^{2+} (P = 0.081) and between lead concentrations of 40 and 200 μmol/L (P= 0.107) for maize. The pattern was equally similar for beans, however, no statistical significance was observed between the following lead concentrations; 40 and 200 (P = 0.726), 200 and 600 (P = 0.558), and 40 and 600 (P = 0.480). The Leaf area index standard deviation values for maize were; 8.233, 7.175, 0.000, and 9.379 for 40, 200, 600 μmol/L Pb^{2+} and control while the respective values for beans were; 7.571, 8.770, 5.513, and 15.616.

5) Discussion on the effects of heavy metals

From the results presented on the effect of heavy metals, it is observed that beans to a great extent are more affected by Zn and Cu than maize (Figures 6-12). The

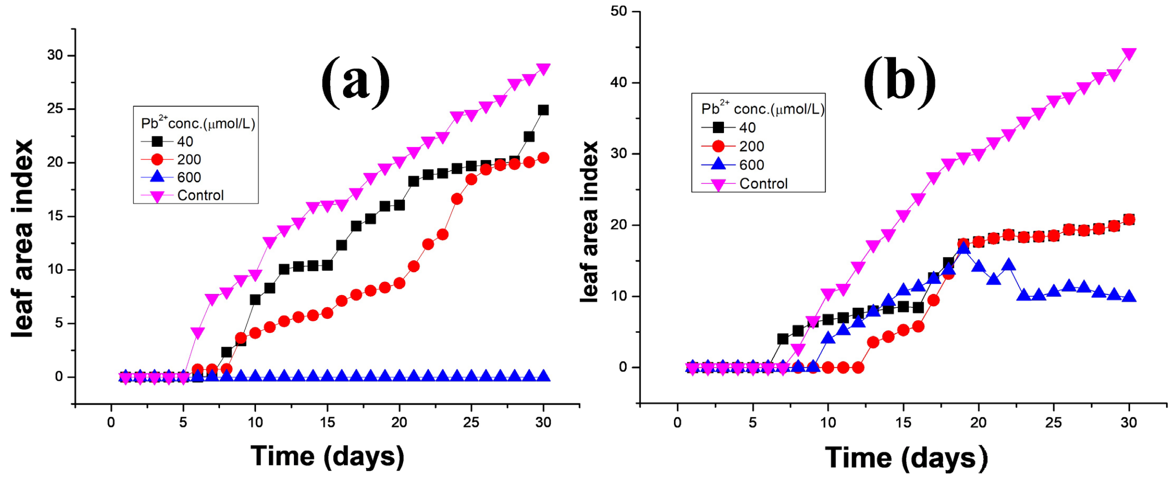

Figure 16. Leaf area index. (a) Maize; (b) Beans at different concentrations of Pb^{2+}.

Table 2. Wet and dry weights of maize seedlings (g) at different NaCl and heavy metals concentrations.

Chemical	Dose	Wet Weight (g)	Dry weight (g)
NaCl	500 (mg/L)	7.245 ± 0.068	1.313 ± 0.057
	1000 (mg/L)	3.15 ± 0.100	0.9 ± 0.144
	5000 (mg/L)	0	0
	10000 (mg/L)	0	0
	20000 (mg/L)	0	0
	35000 (mg/L)	0	0
Zn^{2+}	40 (μmol/L)	11.23 ± 0.125	1.16 ± 0.102
	200 (μmol/L)	8.23 ± 0.135	1.655 ± 0.088
	600 (μmol/L)	7.615 ± 0.723	1.93 ± 0.117
Cu^{2+}	40 (mg/L)	16.45 ± 0.308	2.70 ± 0.085
	200 (mg/L)	7.53 ± 0.156	1.36 ± 0.023
	600(mg/L)	1.28 ± 0.060	0.22 ± 0.021
Pb^{2+}	40 (μmol/L)	8.76 ± 0.207	1.37 ± 0.047
	200 (μmol/L)	8.69 ± 0.284	1.00 ± 0.171
	600 (μmol/L)	0	0
Control		16.51 ± 0.010	2.38 ± 0.017

shoot length, shoot circumference, and leaf area index for copper and Zn reduced as the concentration increased attaining zero at 200 and 600 μmol/L Cu and Zn for beans. Meanwhile, these values are significant for maize even with increasing Cu or Zn concentration. Contrary, maize growth performance was more negatively affected by Pb (Figures 14(a)-16(a)) compared to the beans (Figures 14(b)-16(b)). The negative effect of Pb on maize is also observed with wet and dry biomasses (Table 2 and Table 3) where these values are higher for beans

Table 3. Wet and dry weights of beans seedlings (g) at different NaCl and heavy metals concentrations.

Chemical	Dose	Wet Weight (g)	Dry weight (g)
NaCl	500 (mg/L)	9.26 ± 0.203	1.57 ± 0.020
	1000 (mg/L)	0	0
	5000 (mg/L)	0	0
	10000 (mg/L)	0	0
	20000 (mg/L)	0	0
	35000 (mg/L)	0	0
Zn^{2+}	40 (μmol/L)	8.04 ± 0.089	1.33 ± 0.044
	200 (μmol/L)	7.83 ± 0.047	1.19 ± 0.038
	600 (μmol/L)	0	0
Cu^{2+}	40 (mg/L)	9.88 ± 0.083	1.26 ± 0.034
	200 (mg/L)	0	0
	600(mg/L)	0	0
Pb^{2+}	40 (μmol/L)	10.03 ± 0.025	1.16 ± 0.030
	200 (μmol/L)	9.39 ± 0.055	1.655 ± 0.035
	600 (μmol/L)	4.7 ± 0.301	0.72 ± 0.079
Control		16.05 ± 0.050	2.83 ± 0.020

than maize with lead presence. Heavy metals effects are mostly felt at higher concentrations because they are either essential metals for plant growth or they become toxic above threshold values. The reduction in the various seedling characteristics as concentrations of heavy metals increases is also similar to results gotten by Ogundele *et al.* [1] in which it was found that metals and other chemicals affect germination, root, stem, and shoot elongation of the various crops used.

3.3. Measured Dry Masses

The various dry masses for the different crops, maize and beans are represented in **Table 2** and **Table 3** respectively at different NaCl and heavy metals concentrations. The dry and wet masses for maize in the control experiments are bigger than those irrigated with the different concentrations of both metals and salts (**Table 2**). As the concentration of the metals or salts increases, the wet masses are reduced. The same trend goes with the dry and wet masses of beans (**Table 3**). Fresh and dry plant weight reduction with increasing salinity and metal amounts is due to protein degradation at higher concentrations through amino acid metabolism. This is equally due to a reduction in carotenoid and chlorophyll contents due to an increase in heavy metals or salinity amounts [37].

4. Conclusion

After the text edit has been completed, the paper is ready for the template. Duplicate The effects of NaCl salinity and lead, copper, and Zinc on the growth performance of maize and beans, the two most cultivated and consumed crops in the North West Region of Cameroon were investigated in this study. The germination of beans and maize is highly reduced with salinity. This reduction was as the concentration of salt in the growth medium increased, which is from 500 mg/L to 35,000 mg/L NaCl. NaCl concentration up to 500 mg/L was found safe for maize seed germination. Maximum NaCl of 500 mg/L provides better plant growth, highlighting the use of lower concentrations for efficient plant growth and development. At higher concentrations, germination, seedling growth, and every other parameter were very low attaining 0% germination. Plants grown on soils contaminated with heavy metals observe retardation in growth. The principal cause is the modification of their physiological as well as biochemical activities. This is particularly relevant in cases where the growth and development of plants have no relationship with heavy metals of interest. The results for heavy metals were similar in trend to those of salinity, with increasing effects with an increase in concentration. At 600 μmol/L of each metal, the effects on plants were severe compared to 200 μmol/L of the same metal. Thus, the findings of this research elucidate the adverse effects of salt and heavy metals on plants when certain limits are exceeded. Results of this study show the risk in food availability in the zone of this project with beans and maize as the most grown and consumed crops if steps are not taken to properly preserve the farmlands from different pollution especially anthropogenic activities. It is recommended that further study on the growth performance of different improved maize and beans at different heavy metals concentrations (from 0, 0.01, 0.02 mg/L, etc.) be carried out to set levels that favour their growth.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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