



## Physiological responses of maize (*Zea mays* L.) to zinc (Zn) induced stress

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### Abstract

*Z. mays* L. is among the most essential staple crops globally. It has multifunctional uses both as food; feed as well as for other industrial purposes. Pollution of soil by heavy metals is however a huge factor limiting the optimal growth of this crop. The purpose of this study was to investigate the physiological responses of maize when induced with zinc (Zn) at five different concentrations (0%, 39%, 77%, 115% and 154%) respectively. The plant material was acquired from Agricultural Development Program, Rumuodumaya, Port Harcourt while soil was sterilized and analyzed using standard protocols. Planting was done under controlled environment. After 1 week of planting, growth parameters such as Plant height, number of leaves, Leaf length and leaf area were recorded at weekly interval for 9 weeks. The study revealed that growth responses of the maize studied decreased as the concentration increased for zinc (Zn). Higher treatments at 115% and 154% resulted in mortality of the maize from week three. There were conditions where growth performances fluctuated with variation in the treatments and there was also fluctuation in chlorophyll content as the concentration increases. It is therefore suggested that high concentration of heavy metal is detrimental to the growth of maize plant and the level of negative effect relies on the span of exposure.

**Keywords:** physiological, maize, zinc, stress

### Introduction

Maize is a crop belonging to the family of grasses, Poaceae, having the botanical name *Zea mays* (L.), and cultivated globally being one of the most important cereal crops worldwide. It is a tall, determinate annual C4 plant varying in height from 1 to 4 meters producing large, narrow, opposing leaves (about a tenth as wide as they are long), borne alternately along the length of a solid stem. Maize is wind pollinated and both self and cross pollination is usually possible. Shed pollen usually remains viable for 10 to 30 minutes, but can remain viable for longer durations under favorable conditions (Coe *et al.*, 1988). Maize crop is primarily a warm weather crop and it is grown in wide range of climatic conditions (ICAR, 2006). Maize can successfully be grown in areas receiving an annual rainfall of 60 cm, which should be well distributed throughout its growing stage. It needs more than 50% of its total water requirements in about 30 to 35 days after tasseling and inadequate soil moisture at grain filling stage results in a poor yield and shriveled grains.

In Nigeria it may be prepared alone or in combined with other food source or snacks such as Ogi, tuwo, donkunu, gwater, couscous, Egbo, maasa, apkle, nakia Abari, ajepasi. donkwa, Aadun, koror, elekute etc. (Abdurahaman and Kowakw, 2006). Maize serves as livestock feed, it has high fibre content and calories that are an outstanding product of energy, also it gives a yellow color to poultry, egg yolks and animal fat.

The plant residue after the ear has been removed called stover (contains 30-40% of total Nitrogen, 75% K, Mn S and almost all C) is utilized by majority of maize growers in less developed Countries as roughage feed for livestock (Dowswell *et al.*, 1996). Silage maize is essential feed in temperate region and consists of whole plant which is chopped and placed in a structure for anaerobic fermentation so as to allow for preservation. It can be processed into different products for diverse end users at the local and industrial level. Large quantity of products used in developing countries are obtained from local processing while industrial processing meets the bulk of the demand in developed countries. Due to its increasing importance, maize has developed into a major staple and cash crop for small stake farmers (Manyong *et al.*, 2000). Dowswell *et al.*, (1996) observed that maize is utilized for different uses more than other cereal: as food, as a feed, fodder, and for several industrial purposes due to its wide distribution globally, its low price, its different grain types, and its broad variety of industrial and Biological characteristics. When fermented it is utilized to make hydrolyzed dextrins, syrup and sugars (Sowunmi and Akintola, 2010). Most Africans are apt to eat maize mostly as a starchy base in a broad diversity of porridges, pastes, grits, and beer. The green freshly harvested maize cob is eaten either roasted or boiled. Another striking thing about this crop (maize grains) is that it could be stored indefinitely if the conditions for storage are right. The starch extracted from maize grain is applied in the manufacture of noodles and confectionary. The syrup produced from maize is very rich in fructose and other purposes as a sweetener and holds moisture once it is applied to some foods. It has high yield capacity, various uses, easy to transport, processes and has market values.

The accessibility of dependable research products according to 2011 *et al.*, (2012) edible oil is derived from the seeds of maize, which is an all-purpose cooking oil. Levulinic acid, a chemical obtain from maize, is applied as ingredient in antifreeze and has possibility of substituting the toxic petroleum –based ingredients use. Stock from maize is used in production of fabrics and Plastic. The Ethanol derived from maize can serve as a biomass fuel. Female corn-flowers stigmas called corn silk can function as herbal supplements. Maize straw is an economical source of energy and is capable of being used in home-heating furnaces.

Heavy metals accumulation due to pollution from industries causes serious soil degradation and rigorously inhibits plant growth and yield leading to Agricultural loss and is also harmful to human when it goes into the food chain (Micheal *et al.*, 2012, Metwali *et al.*, 2013, Youssef and Azooz, 2013) <sup>[19, 37]</sup>. Bhatia and Singh, (2012) noted that abuse of pesticides and chemical fertilizer contributes largely to high level of heavy metal found in the soil. The harmful property of heavy metals depends largely on the extent of exposure of the plant to the heavy metal and the physical as well as ion's chemical characteristics to bypass the physiological obstruction (Seregin and Kozhenikova, 2008). Studies have shown that the injurious effect of heavy metal is noticeable as oxidative stress, as a consequence of the generation of free oxygen radical (Gao *et al.*, 2011) <sup>[12]</sup>. Zinc is amongst the major nutrients needed by maize plant for adequate growth however excess of these nutrients may be detrimental to this plant species. A higher concentration leads to undesirable effects which include growth reduction in the plants resulting to death of the plant (Chen *et al.*, 2000) <sup>[9, 10]</sup>. Zinc also is a heavy metal micronutrient required by maize for adequate growth. It plays a role in various metabolic activities but it is toxic in excess amount and leads to growth inhibition (Vaillant *et al.*, 2005) <sup>[33]</sup> and induction of oxidative harm in diverse plant species (Panda *et al.*, 2003). Excess concentration of Zinc (Zn) can severely disrupt mitosis. The rationale of this project is to evaluate the Physiological responses of *Z. mays* to Copper and Zinc induced stress.

Zinc (Zn) is a micronutrient in spite of not possessing any redox action is mainly involved in many crucial physiological activities in plants (Sagardoy *et al.*, 2009) <sup>[28]</sup>. Zn is an imperative constituent of particular proteins called as zinc fingers that attach to DNA and RNA and plays major role in their stabilization and regulation (Gupta *et al.*, 2011) <sup>[13]</sup>. It is in addition a component of different enzymes, which includes transferases, oxidoreductases, hydrolases, and also ribosome (Mousavi, 2013) <sup>[25]</sup> and functions in carbohydrate production, chlorophyll and growth of root. In divalent form ( $Zn^{2+}$ ), Zn is the highly pervasive state found in the earth crust and obtained by plants (Broadley *et al.*, 2007) <sup>[7]</sup>. Zn bioavailability/phytoavailability depends on different variables like the overall Zn content in the crust, amount of lime, organic matter, type of clay, and other HMs availability, soil's pH, and the amount of salt in the substrate (Aref, 2011) <sup>[2]</sup>. Of all aforementioned, pH is the most vital issue affecting Zn accessibility, Broadley *et al.*, (2007) <sup>[7]</sup> and high pH is commonly connected to the reduced Zinc assimilation by plants (Aref, 2011) <sup>[2]</sup>. Accumulation of Zn in the earth crust up to 150 - 300  $\mu g\text{-}g^{-1}$  is highly toxic (Yadav, 2009) <sup>[36]</sup> and its toxic effect, together with the biological availability factors, relies on the kind of plant and stage of growth (Baran, 2013) <sup>[5]</sup>. Optical indication of danger in plants due to Zn harmful effects are revealed to be discoloration in immature leaves as an outcome of Fe or Mg shortage (Sivasankar *et al.*, 2012) <sup>[30]</sup> and symptoms of purplike-red color found in leaves due to Phosphorus shortage, which shows that surplus  $Zn^{2+}$  can effortlessly surpass other metals, particularly those that has related ionic radii of the energetic enzymes sites or transporters (Fukao, 2011) <sup>[11]</sup>. Moreover, necrotic spot around the veins found in the blade of matured leaves and innermost gently sloping at the margins leaf are credited to Zn toxic activities.

Surplus  $Zn^{2+}$  in the cells is able to make ROS and negatively manipulate combination and porosity of membrane (Hosseini *et al.*, 2013). Zn toxicity, similar to other HMs, obstructs the activities and effectiveness of photosynthetic structure in diverse plant species (Vassilev *et al.*, 2011) <sup>[34]</sup> in bean, Mirshekali *et al.*, (2012) in Sorghum (*S. bicolor.*), and Lalelou *et al.*, (2013) <sup>[17]</sup> in uncovered pumpkin showed that glut amount of  $Zn^{2+}$  decreased the component of photosynthetic pigments contents including Chl *a* and Chl *b* via distressing the assimilation and movement of Mg and Fe into chloroplast. The high content of  $Zn^{2+}$  is revealed lead to a reduction in early and highest Chl fluorescence, resulting in suppression of activities of PSII. Zinc in extreme is established to possess genotoxic properties on plants, resulting in genetic aberrations and injury to plants Species. Oladele *et al.*, (2013) <sup>[26]</sup> established that elevated amount (100  $mg\text{-}L^{-1}$ ) of Zn in cells results to mutated chromosomes, accompanied by a sultry metaphase and untimely segregation of genetic material in *Vigna subterranean* (Bambara groundnut). Furthermore, Truta *et al.*, (2013) <sup>[32]</sup> noted that the level of ana-telophase abnormality was 2-3 times more than control when seedlings of barley (*Hordeum vulgare* L.) when treated with 250- 500  $\mu M$  of  $Zn^{2+}$ . This study therefore aims at evaluating the physiological responses of *Zea mays* to zinc (Zn) induced stress.

## Materials and Methods

### Description of study area

The research was carried out in the Department of Plant Science and Biotechnology Screen House, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria.

### Sources and collection of materials

Maize seeds (Oba 98) were obtained from Agricultural Development Program Rumuodumaya, Rivers State, and Port Harcourt, Nigeria. Copper (Cu) was obtained from Copper (II) Sulfate Pentahydrate which was of analytical grade.

### Soil sample collection and sterilization

13 kg of soil sample (loamy soil) used for the research was collected and sterilized at the Department of Crop and Soil Experimental farm, Faculty of Agriculture, University of Port Harcourt, Rivers State before it was transferred into the planting buckets and were taken to the Screen House.

### Determination of the physicochemical properties of the experimental soil

The physicochemical properties of the experimental soil were determined using atomic absorption spectrophotometer.

### Determination of heavy metals

10ml of well mixed Perchloric, nitric and Sulphuric was added to 1g of dried soil sample which was heated using heating mantle for 10-20mins. It was allowed to cool and 20ml of distilled water was added and solution was subjected to boiling to bring metal into solution. After cooling the solution was filtered using whatmann filter paper into 100ml standard flask. And it was relocated to 100ml plastic container. The samples were then measured using AAS (Atomic Absorbance Spectrophotometer).

### Soil pollution, planting of seeds and measurement of growth parameters

Soil was polluted with Zinc (Zn) at 0g, 5g, 10g, 15g and 20g which gave the concentration 0%, 39%, 77% 115% and 154% w/w. The soil was homogenized with the pollutants and then was allowed for three weeks (21 days) before planting was done. Five seeds were planted per bucket with five replicates using Randomized Complete Block Design (RCBD) at the depth of 4cm and were watered every 2 to 4 days throughout the experimental periods. Data collection took effect from second week and various growth parameters measured included plant height, leaf length; number of leaves, and leaf width.

### Chlorophyll Content determination

0.1g of sample was weighed and added into a vial containing 10ml acetone. The young plant were harvested from the screen house about three weeks old and some amount of acetone was introduced into the curvet to clean off every other chemical that would have altered the result and also to prepare the cuvette and the spectrophotometer for better result. 0.1g sample was weighed and added into a vial containing 10ml of acetone and kept under room temperature for 48hours (2days) to facilitate visibility of the green pigment thereafter the extract or the chlorophyll content was introduced or poured into the cuvette and was determined or read using the Spectrophotometer with different wavelength of 660nm and 643nm respectively. The acetone helps to increase the appearance of the chlorophyll determination. Spectrophotometer is the instrument utilized to determine the chlorophyll content.

Chlorophyll-mg/c (Ch) was calculated following the formula beneath;

$$12 \times \text{Abs at } 660 + 16.8 \times \text{Abs at } 643 \times 100$$

### Statistical analysis

The data were analyzed using One-way analysis of variance (ANOVA) to determine the significant difference within factors. Also, multiple comparisons were employed to determine significant disparity between paired factors. Descriptive statistics like mean and standard deviation were also used.

### Results

The result of the physicochemical properties of the experimental soil and heavy metals shows that pH was 4.97, electrical conductivity 51.2, Total Nitrogen 0.086%, Zinc 0.234mg/kg and Copper 0.105mg/kg (Table 1).

**Table 1:** Result of Soil sample analysis and heavy metals

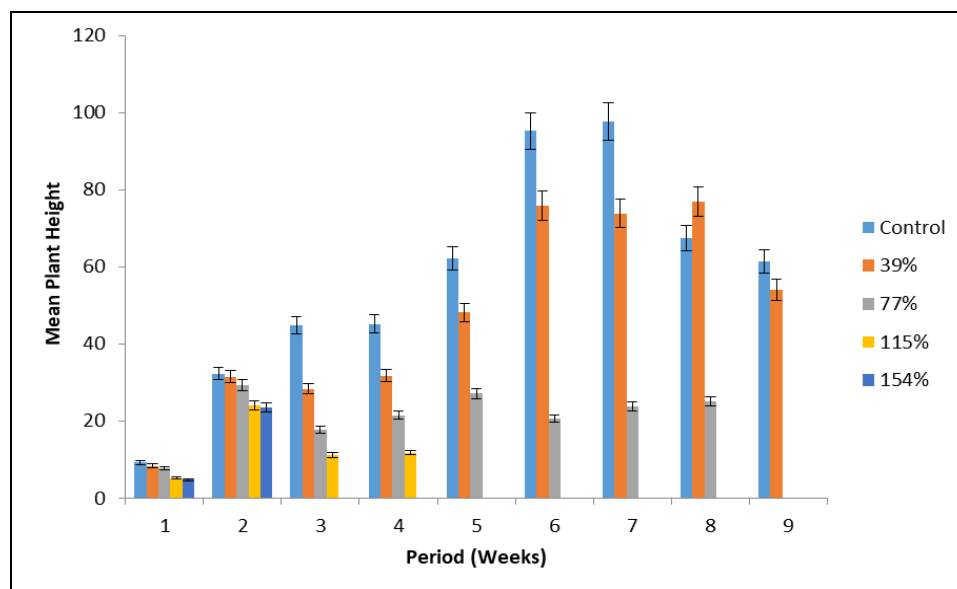
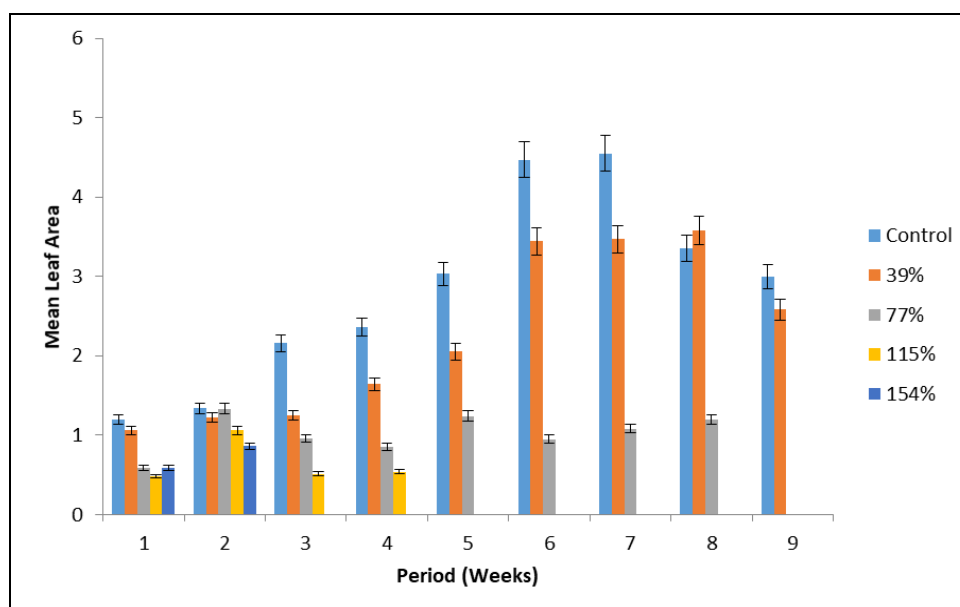
Parameter/Unit	Method	Sample 1-UTS
Physiochemical		
pH	ASTMD1293B	4.97
Electrical conductivity	ASTMD1125	51.2
Total Nitrogen %	ASTME258	0.086
Phosphorus, mg/kg	ASTMD5198	16.43
Cation Exchange Capacity, meq/100g		1.806
Cations, mg/kg		
Potassium	API-RP45	2.374
Heavy metals, mg/kg		
Zinc	API-RP45	0.234
Copper	API-RP45	0.105

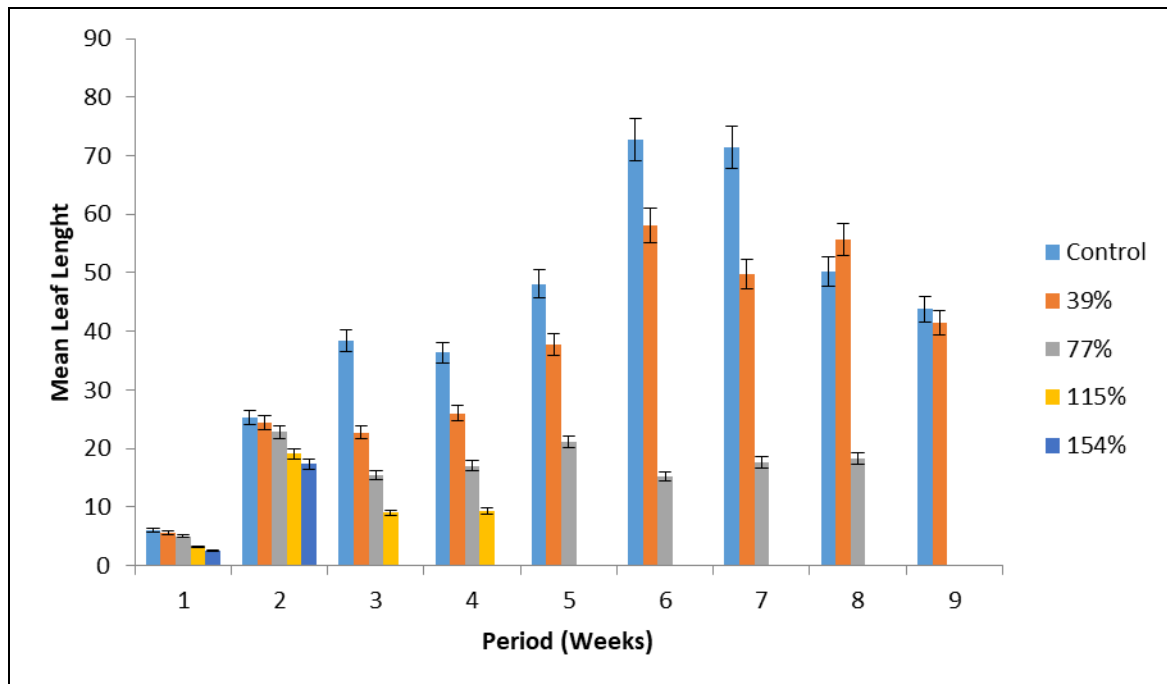
The results of the chlorophyll content determination shows that as the concentration of the Zinc (Zn) increases there was fluctuation in the chlorophyll content and 77% Zn had the lowest chlorophyll content of 194.0Mg/Kg (Table 2).

**Table 2:** Results of Chlorophyll content determination

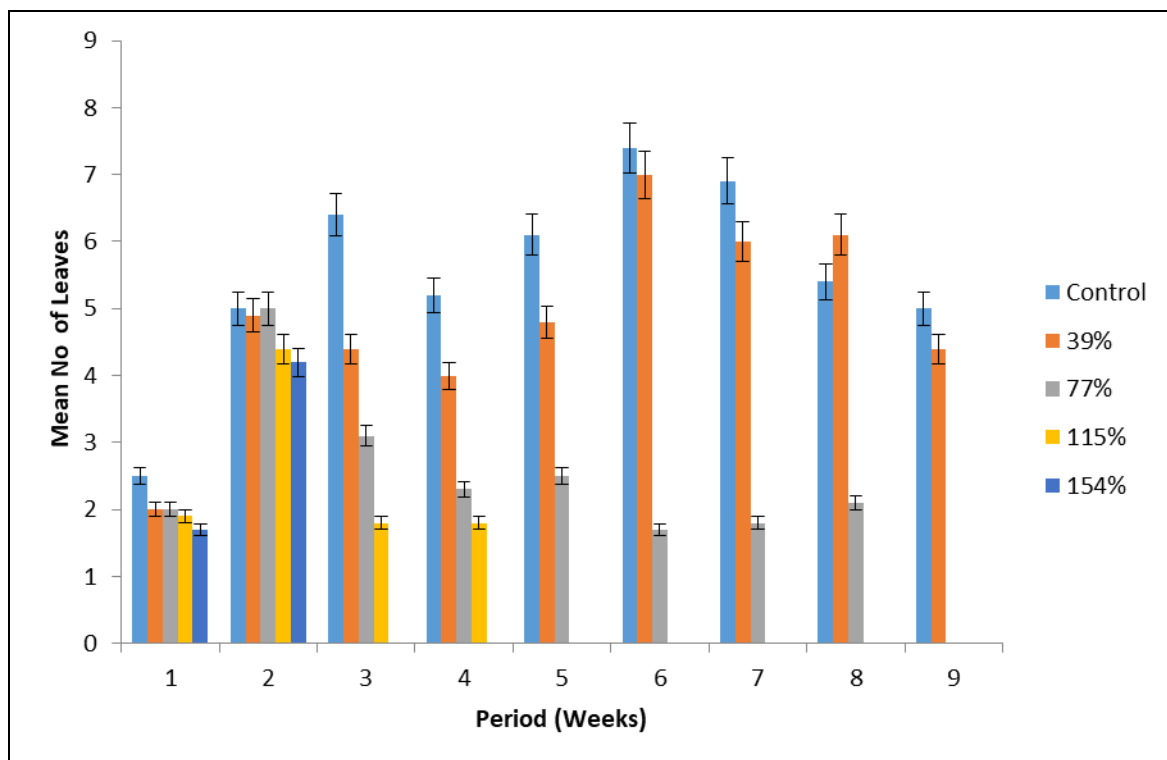
S/N	Concentration	Total chlorophyll (Mg/kg)
1.	0% of Zn	2022.0
2.	39% of Zn	1309.0
3.	77% of Zn	194.0
4.	115% of Zn	264.0
5.	154% of Zn	332.0

The results of the growth studies shows that as the concentration of the heavy metal (Zn) increases there was reduction in the growth performances of *Zea mays* and at 115% and 154% resulted in complete mortality of the crop from week 3 (Figures 1-4).

**Fig 1:** Effect of Zinc (Zn) induced stress on mean plant height of *Zea mays* at 1-9 weeks after planting (WAP)**Fig 2:** Effect of Zinc (Zn) induced stress on mean leaf area of *Zea mays* at 1-9 weeks after planting (WAP)



**Fig 3:** Effect of Zinc (Zn) induced stress on mean leaf length of *Zea mays* at 1-9 weeks after planting (WAP)



**Fig 4:** Effect of Zinc (Zn) induced stress on number of leaves of *Zea mays* at 1-9 weeks after planting (WAP)

### Discussion

From the result it has shown that Zinc (Zn) is essential micronutrient for normal plant growth and development, but it could be poisonous either reversibly or irreversibly to changes in metabolism, known to respond to a good number of stressors such as natural and xenobiotic Singh and Tewari, (2003). The outcome of this research proves that when maize was induced with Zn at various concentration (0%, 39%, 77%, 115% and 154%), there were momentous differences in the growth performance. It was noticed that growth rate reduced as the concentration increases which is in accordance with an investigation by Aly *et al.*, (2012) and Chen *et al.*, (2000) [9, 10]. Zinc (Zn) significantly (5%) induced stress on the plant height, leaf length, leaf area and number of leaves with increased concentration compared to the control.

The mortality at 154% concentration could be as a consequence of elevated concentration of the metals Chen *et al.*, (2000) [9, 10] which probably implies that excess of zinc metal is detrimental to maize plant (Leo *et al.*, 2018). Adequate amount of Zinc (Zn) is needed by maize for better growth. Crops cultivated on soil with an elevated

content of Zinc (Zn) are characterized by inhibited growth, reduce transpiration, chlorosis of leaves, limited seed germination (Trans and Popova, 2013) and the consequences are stronger during developmental stages. One key role of Zinc (Zn) is that they are essential for proper functioning of plants (MacCall, *et al.*, 2000).

Growth parameters and structure of plant parts are shown to be negatively affected by Zinc toxicity. Todeschini *et al.*, (2011) <sup>[31]</sup> demonstrated that Zinc in poplar (*Populus alba*) drastically changed leaf morphology and ultrastructure and caused the formation of calcium-oxalate crystals. Vijayarengan and Mahalakshmi (2013) showed that Zinc toxicity decreased the length of root and shoot as well as area of leaves in tomato (*Solanum lycopersicum* L). Zinc is an essential trace metal that despite having no redox activity is particularly involved in many vital physiological events in plants (Sagardoy *et al.*, 2009) <sup>[28]</sup>. Zinc is an indispensable component of special proteins known as zinc fingers that bind to DNA and RNA and contribute to their regulation and stabilization (Gupta *et al.*, 2012) <sup>[14]</sup>. Moreover, it is a constituent of various enzymes, for example, oxidoreductases, transferases, and hydrolases (Mishra and Dubey 2005) <sup>[21]</sup> as well as ribosome Mousavi *et al.*, (2013) <sup>[25]</sup>, and plays a role in the formation of carbohydrates and chlorophyll and root growth (Kleckerova *et al.*, 2011) <sup>[16]</sup>. Zinc bioavailability/phytoavailability is dependent on various variables including the total Zinc concentration in soil, lime content and organic matter of soil, clay type, and presence of other heavy metals, soil's pH, Zinc at high soil concentrations (150 to 300  $\mu\text{g}\cdot\text{g}^{-1}$ ) is strongly toxic (Yadav, 2010) <sup>[36]</sup> and its phototoxicity, in addition to the bioavailability factors, depends on plant type and plant development stage (Baran, 2013) <sup>[5]</sup>. Visual signs of trouble in plants as a result of Zinc toxicity are reported to be chlorosis in young leaves due to iron or manganese deficiency (Sivasankar *et al.*, 2012) <sup>[30]</sup> and appearance of purplish-red colour in leaves due to phosphorus deficiency. Yadav (2010) <sup>[36]</sup> indicated that  $\text{Zn}^{2+}$  in excess can easily supersede other metals, especially those with similar ionic radii in the active sites of enzymes or transporters (Fukao *et al.*, 2011). Moreover, necrotic spotting between the veins in the blade of mature leaves (Miyasaka *et al.*, 2002) and inward rolling at leaf margins (Sagardoy *et al.*, 2009) <sup>[28]</sup> are attributed to Zinc toxicity.

Excess  $\text{Zn}^{2+}$  in cells can produce ROS and adversely influence integration and permeability of membrane (Mishra *et al.*, 2010; Hosseini and Poorakbar, 2013) <sup>[22, 15]</sup>. Zinc toxicity, hampers the functionality and efficiency of photosynthetic system in different plant species. Vassilev *et al.*, (2011) <sup>[34]</sup> in bean plants, Mirshekali *et al.*, (2012) <sup>[23]</sup> in sorghum (*Sorghum bicolor* L.), and Lalelou *et al.*, (2013) <sup>[17]</sup> in naked pumpkin (*Cucurbita pepo*) showed that excessive concentration of  $\text{Zn}^{2+}$  reduced the content of accessory photosynthetic pigments including Chl *a* and Chl *b* by disturbing the absorption and translocation of Fe and Mg into chloroplast. This research shows that as the concentration of Zinc increases the chlorophyll content reduces. Chlorophyll content has the capability to alter even before the physical changes (Ayeni *et al.*, 2012) <sup>[3]</sup>. Scientific records exists concerning reduction in chlorophyll content as a result to heavy metal toxic activities in plants (Baldantoni *et al.*, 2009; Calheiros *et al.*, 2007; Bonanno and Lo Giudice, 2010; Bragato *et al.*, 2009).

Zinc in excess is found to have genotoxic effects on plants, resulting in genetic-related disorders and damages to plants. Oladele *et al.*, (2013) <sup>[26]</sup> demonstrated that high levels of Zinc (100  $\text{mg}\cdot\text{L}^{-1}$ ) in cells resulted in abnormal chromosomes, which was followed by a sticky metaphase and premature separation of chromosomes in bambara groundnut (*Vigna subterranean*). Also, Truta *et al.*, (2013) <sup>[32]</sup> observed that the rate of ana-telophase aberrations was 2-3 times higher than control treatment when barely seedlings (*Hordeum vulgare* L.) were treated with 250 to 500  $\mu\text{M}$   $\text{Zn}^{2+}$ . Moreover, it is a constituent of various enzymes, for example, oxidoreductases, transferases, and hydrolases (Mishra *et al.*, 2005) <sup>[21]</sup> as well as ribosome (Mousavi *et al.*, 2013) <sup>[25]</sup> and plays a role in the formation of carbohydrates and chlorophyll and root growth (Kleckerova *et al.*, 2011) <sup>[16]</sup>.

## Conclusion

Maize is a staple crop and one of the most imperative both for consumption and for industrial uses globally. Adequate nutrition of this crop is a requirement for its optimal growth whereas excess of these nutrients is disadvantageous to the plant. Finding from this research demonstrates that appropriate amount of nutrient such as and Zinc (Zn) potentially to enhance maize growth however, excess amount of this nutrient hinder the growth. The morphological examination of the plant indicated that growth rate reduced as the treatment increased. Chlorophyll content also reduced with increased concentration of Zinc (Zn). The discovery of this research therefore paves way for further studies.

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## Conflict of Interest

There is no conflict of interest in research. The research is an original work carried out by the authors.

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