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Evaluation and Responses of ZEA Mays Seedlings to Soil Micronutrients Toxicity

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Abstract: *Green-house experiment was conducted on zea mays planted on sandy soil to determine the toxic levels of Zn^{2+}, Mn^{2+}, Cu^{2+} and Fe^{2+} in the leaf of seedling of the plant. This was achieved by applying 250cm^3 of 10PPM, 50PPM, 100PPM, 150PPM, 200PPM and 500PPM of Zn^{2+}, Mn^{2+}, Cu^{2+} and Fe^{2+} at intervals until toxic level was indicated through change in colour of the leaves. Diethylene triaminepentaacetic acid (DTPA) was used to extract the micronutrients in the soil and the concentrations were evaluated using, Atomic Absorption Spectrophotometer (AAS). The concentrations of the micronutrients in leaves were also determined using AAS. The concentration of the micronutrients in leaves of the plant in the soil at toxic levels were 250PPM- 260PPM Zn^{2+}, 37.24PPM-55.90PPM of Cu^{2+}, 290-320 of Mn^{2+}420-700 of Fe^{2+}. The results were statistically treated using t-test to determine the significant difference in the uptake of the micronutrients byZea mays in sandy soil.*

Key Words: *Green-house, micronutrients, Zea mays, toxicities.*

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I. Introduction

Interest in trace elements Zn, Mn, Cu and Fe has been sparked by problems of toxicity resulting from an oversupply of those elements. Levels of trace elements toxic to plants may result from natural conditions, from pollution, or fromsoil-management practices [1].

If a level of nutrient availability has been reached, that is, sufficient to meet the plants needs, raising it further will have little effect on plant growth, although the concentration of the nutrient may continue to increase in the plant tissue [5]. At some level of availability, the plant will take up too much of the nutrient for its own good, causing adverse physiological reactions to take place [1]. As a result of the inference of pH on micronutrient solubility, anthropogenic processes which result in the lowering of soil pH can cause micronutrient toxicities; even if no extra micronutrient has been added to the system [4]. Plants cannot usually access the total pool of a micronutrient present in the growth soil. Instead, that fraction of the metal which plants can absorb is known as the available or bio-available fractions.

This study is aimed at determining the tolerance of Zea mays at seedling stage supplied on daily basis with 250cm^3 of 10, 50, 100, 150, 200 and 500 of Zn^{2+}, Mn^{2+}, Cu^{2+} and Fe^{2+} in excess grown on sandy soil and loamy soil with the view to ascertaining the toxic level of the micronutrients.

II. Materials And Methods

2.1 Materials

The seeds of the zea mays were purchased from Plateau Agricultural Development Pragramme (P.A.D.P.) A greenhouse experimentwas carried out using sandy soil collected around Federal College Education,Pankshinusing a hand trowel. The samples was transferred into polythene bags and transported to the laboratory. In the laboratory, the sample were exposed and allowed to dry at room temperature for a period of 2 weeks after which they were sieved using a 2mm screen. In order to maintain the integrity of the sample, the 2mm air-dried samples were kept in polythene bags and stored under dry conditions until the time of analysis.

2.2 Soil P_H Determination

A 10.0g of soil was crushed and about 25cm^3 of water was added. A pH-sensitive glass reference electrode was inserted into the soil-water suspension which stimulates the soil solution. The difference between the H^+ ion activation in the soil suspension and in the glass electrode gives rise to an electrometric potential that is related to the soil solution pH. A pH meter was then used to measure the electrometric potential [1].

2.3 Soil Organic Matter Determination

The soil organic matter was determined by ashing. A porcelain crucible was ignited in a hot Bunsen flame for one minute. The crucible was transferred to a desiccator to cool and weighted as w, 5g of the soil sample was weighed into the crucible and weighed again as W_2. The crucible and contents were heated gently on a Bunsen burner in a fume cupboard until smoking ceased, then it was transferred to a muffle furnace heated at $(550\text{-}570)^oC$ to burn off all the organic matter. The crucible was taken out immediately, covered and placed again as W_3[3].

% ash= $\frac{W3-W1}{W2-W1}$x100

Therefore, % organic matter = 100-%ash

2.4 Experimental Procedure

The soil samples were washed with $0.01MCaCl_2.2H_20$ before zea mays seed was planted on 1kg of the soil for 21days. At seedling stage, $250cm^3$ solution of the micronutrients (Zn, Mn, Cu and Fe) at various concentration (10, 50, 150, 200 and 500) were added to six polythene bags containing the Zea mays seedlings on daily basis until toxic effects were observed from the plant, i.e showing a change of colour on the leaves. The soil and the leaves were evaluated for the micronutrient concentrations and the data obtained were analysed statistically.

2.5 Leaf Sample Preparation

The leaves with the sign of toxicity were plugged, cleaned and placed in the oven at 110^0c for at least I hour. The dried leaves were removed from the oven, ground and sieved to pass through a 2mm screen. A 0.5g sample was dry-ashed in a porcelain crucible at 500^oc in a muffle furnace for 4-6 hours. The ash was dissolved in $25cm^3$ of 1.0MHCl.

2.6 Extraction of The Micronutrients From The Soil

a. Diethylenetriaminepentaacetic acid (DTPA) method was used to determine the micronutrient in the soil. 10g of the soil was weighed, air-dried and ground to pass through 2-mm screen which was then introduced into a $120cm^3$ extraction vessel. $20cm^3$ of 0.01M DTPA extraction reagent was added to the extraction vessel. Extraction vessel was placed on reciprocating mechanical shake for 2hours at 250^OC. The mixture was filtered and filtration was repeated if filtrate was cloudy [2](Lindsay and Norvel, 1978).

The atomic absorption spectrophotometer was thencalibrated using standard calibration solutions and applied to determine individually Zn, Mn, Cu and Fe.

b. The analysis for the micro nutrients in leaves was carried out on the solution of the leave ash using Atomic Absorption Spectrophotometer.

III. Results

TABLE 3.1: Concentration of Zn^{2+} in Zea Mays Leaf at seedling stage grown in sandy soil.

Standard addition on daily basis (ppm)	Concentration ofleaf(ppm)	Concentration ofsoil (Sandy)(ppm)
10	25.55	2.0
50	60.00	16.0
100	81.00	43.0
150	135.00	105.0
200	250.00	177.00
500	260.00	189.0

TABLE 3.2: Concentration of Zn^{2+} in Zea Mays Leaf at seedling stage grown in loamy soil.

Standard addition (ppm) on daily basis	Concentration of leaf (ppm)	Concentration of soil (loamy)(ppm)
10	12.75	33.11
50	33.25	20.0
100	67.00	19.0
150	89.00	14.0
200	175.00	12.0
500	200.0	79.0

Permissible value of Zn^{2+} is 150 (Tisdale, 1993)

Concentration of Zn^{2+} for control in;Sandy soil = 0.00120, leaf grown in sandy soil = 0.00015

Loamy soil = 0.00017, leaf grown in loamy soil = 0.00002

TABLE 3.3: Concentration of Cu^{2+} in Zea Mays Leaf at seedling stage grown in sandy soil.

Standard addition (ppm) on daily basis	Concentration in leaf (ppm)	Concentration of soil (Sandy)(ppm)
10	16.02	62.02
50	26.77	60.00
100	35.24	26.19

150	40.00	15.20
200	46.00	55.33
500	55.90	14.47

TABLE 3.4: Concentration of Cu^{2+} in Zea Mays Leaf at seedling stage grown in loamy soil.

Standard addition (ppm) on daily basis	Concentration in leaf (ppm)	Concentration of soil (loamy)(ppm)
10	7.37	4.12
50	13.75	26.28
100	20.65	6.00
150	37.32	48.12
200	38.75	90.00
500	45.25	62.02

Permissible value of Cu^{2+} is 20 (Tisdale, 1993)
Concentration () of Cu^{2+} for control in Sandy soil = 0.00060,leaf grown in sandy soil = 0.00007, Loamy soil = 0.00036, leaf grown in loamy soil = 0.00001

TABLE 3.5: Concentration of Mn^{2+} in Zea Mays Leaf at seedling stage grown in sandy soil.

Standard addition (ppm) on daily basis	Concentration in leaf (ppm)	Concentration of soil (Sandy)(ppm)
10	25.30	5.00
50	28.50	7.01
100	66.75	6.00
150	200.20	27.23
200	290.72	16.02
500	320.00	8.44

TABLE 3.6: Concentration of Mn^{2+} in Zea Mays Leaf at seedling stage grown in loamy soil.

Standard addition () on daily basis	Concentration in leaf ()	Concentration of soil (loamy)()
10	20.03	4.11
50	21.47	16.08
100	34.33	20.25
150	86.05	27.33
200	105.00	60.09
500	255.23	84.22

Permissible value of Mn^{2+} is 200 (Tisdale, 1993)
Concentration () of Mn^{2+} for control in; Sandy soil = 0.000076,leaf grown in sandy soil = 0.00001, Loamy soil = 0.00052, leaf grown in loamy soil = 0.00004

TABLE 3.7: Concentration of Fe^{2+} in Zea Mays Leaf at seedling stage grown in sandy soil.

Standard addition (ppm) on daily basis	Concentration in leaf (ppm)	Concentration of soil (Sandy)(ppm)
10	17.00	9.13
50	180.00	4.07
100	190.00	28.05
150	200.00	30.17
200	420.00	19.22
500	700.00	56.08

TABLE 3.8: Concentration of Fe^{2+} in Zea Mays Leaf at seedling stage grown in loamy soil.

Standard addition (ppm) on daily basis	Concentration in leaf (ppm)	Concentration of soil (loamy)(ppm)
10	24.35	7.33
50	62.00	8.24
100	85.65	10.77
150	89.77	28.00
200	117.00	20.10
500	450.00	38.25

Permissible value of Fe^{2+} is 400 (Tisdale, 1993)
Concentration () of Fe^{2+} for control in Sandy soil = 0.00021,leaf grown in sandy soil = 0.00003, Loamy soil = 0.00017, leaf grown in loamy soil = 0.00009

TABLE 3.9: Difference in the Concentration of Zn^{2+} in Zea Mays Leaf at seedling stage Grown in Loamy and Sandy Soil.

Concentration in leaf (ppm) (Sandy soil)	Concentration in leaf (ppm) (loamy)	Difference in Concentration (di) (ppm)
25.55	12.75	12.80
60.00	33.25	26.75
81.00	67.00	14.00
135.00	89.00	46.00
250.00	175.00	75.00
260.00	200.00	60.00

TABLE 3.10: Mean, Standard Deviation and Error Mean Analysis of Concentration of Zn^{2+} in Zea Mays Leaf at seedling stage Grown in Loamy and Sandy Soils.

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Concentration in leaf Sandy soil (ppm)	135.2583	6	99.36790	40.56678
	Concentration in leaf Loamy soil (ppm)	96.1667	6	75.91437	30.99191

TABLE 3.10: Correlation between Concentration of Zn^{2+} in Zea Mays Leaf Grown in Sandy and Loamy Soils.

		N	Correlation	Sig.
Pair 1	Concentration in leaf Sandy soil (ppm)	6	.993	.000
	Concentration in leaf Loamy soil (ppm)			

TABLE 3.12: T-test Analysis of the Concentration of Zn^{2+} in Zea Mays Leaf at seedling stage Grown in Loamy and Sandy Soils.

		Mean	Std. Deviation	Std. Error Mean	95% Confidence interval of the Difference		t	df	Sig. (2tailed)
					Lower	Upper			
Pair1	concentration in leaf Sandy soil (ppm) minus concentration in leaf Loamy soil (ppm)	39.0917	25.4850	10.4045	12.34669	65.8364	3.757	5	.013

The critical value of t is 2.57 for 5 degrees of freedom. Since t-observed is greater than t-critical, then it is concluded that there was a significant difference in the concentration of Zn^{2+} in leaf of Zea Mays grown in the two soils (sandy and loamy).

TABLE 3.13: Difference in the Concentration of Cu^{2+} in Zea Mays Leaf at seedling stage Grown in Loamy and Sandy Soil.

Concentration(ppm) (Sandy soil)	Concentration(ppm) (loamy soil)	Difference in Concentration (di) (ppm)
16.02	7.37	8.65
26.77	13.75	13.02
37.24	20.65	16.59
40.00	35.32	4.68
46.00	38.75	7.25
55.90	45.25	10.65

TABLE 3.14: Mean, Standard Deviation and Error Mean Analysis of Concentration of Cu^{2+} in Zea Mays Leaf at seedling stage Grown in Loamy and Sandy Soils.

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Concentration in leaf Sandy soil (ppm)	36.9883	6	14.08602	5.75059
	Concentration in leaf Loamy soil (ppm)	26.8483	6	15.10914	6.16828

TABLE 3.10: Correlation between Concentration of Cu^{2+} in Zea Mays Leaf Grown in Sandy and Loamy Soils.

		N	Correlation	Sig.
Pair 1	Concentration in leaf Sandy soil (ppm)& Concentration in leaf Loamy soil (ppm)	6	.960	.002

TABLE 3.16: T-test Analysis of the Concentration of Cu^{2+} in Zea Mays Leaf at seedling stage Grown in Loamy and Sandy Soils.

		Mean	Std. Deviation	Std.Error Mean	95% Confidence interval of the Difference		t	df	Sig.(2-tailed)
					Lower	Upper			
Pair1	concentration in leaf Sandy soil (ppm) minus Concentration in leaf Loamy soil (ppm)	10.1400	4.25537	1.73725	5.67426	14.60574	5.837	5	

The critical value of t is 2.57 for 5 degrees of freedom at 95% confidence level. Since t-observed is greater than t-critical, then it is concluded that there was a significant difference in the concentration of Cu^{2+} in leaf of Zea Mays grown in the two soils (sandy and loamy).

TABLE 3.17: Difference in the Concentration of Mn^{2+} in Zea Mays Leaf at seedling stage Grown in Loamy and Sandy Soil.

Concentration(ppm) (Sandy soil)	Concentration in leaf (ppm) (loamy Soil)	Difference in Concentration (di) (ppm)
25.30	20.03	5.25
28.50	21.47	7.03
66.75	34.33	32.42
200.20	86.05	114.15
290.72	105.00	185.72
320.00	255.23	64.77

TABLE 3.18: Mean, Standard Deviation and Error Mean Analysis of Concentration of Mn^{2+} in Zea Mays Leaf at seedling stage Grown in Loamy and Sandy Soils.

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Concentration in leaf Sandy soil (ppm)	155.2450	6	132.89093	54.25250
	Concentration in leaf Loamy soil (ppm)	87.0183	6	89.64321	36.59669

TABLE 3.19: Correlation between Concentration of Mn^{2+} in Zea Mays Leaf Grown in Sandy and Loamy Soils.

		N	Correlation	Sig.
Pair 1	Concentration in leaf Sandy soil (ppm) & Concentration in leaf Loamy soil (ppm)	6	.869	.024

TABLE 3.20: T-test Analysis of the Concentration of Mn^{2+} in Zea Mays Leaf at seedling stage Grown in Loamy and Sandy Soils.

		Mean	Std. Deviation	Std.Error Mean	95% Confidence interval of the Difference		t	df	Sig. (2-tailed)
					Lower	Upper			
Pair1	concentration in leaf Sandy soil (ppm) minus concentration in leaf Loamy soil (ppm)	68.22667	70.59217	28.81913	-5.85528	142.30861	2.367	5	.064

The critical value of t is 2.57 for 5 degrees of freedom. Since t-observed is greater than t-critical, thenit is concluded that there was no significant difference in the concentration of Mn^{2+} in leaf of Zea Mays grown in the two soils (sandy and loamy).

TABLE 3.21: Difference in the Concentration of Fe^{2+} in Zea Mays Leaf at seedling stage Grown in Loamy and Sandy Soil.

Concentration(ppm) (Sandy soil)	Concentration(ppm) (loamy soil)	Difference in Concentration (di) (ppm)
17.00	9.13	7.87
180.00	4.07	175.93
190.00	28.05	161.95
200.00	30.17	169.83
420.00	19.22	400.78
700.00	56.08	643.92

TABLE 3.22: Mean, Standard Deviation and Error Mean Analysis of Concentration of Fe^{2+} in Zea Mays Leaf at seedling stage Grown in Loamy and Sandy Soils.

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Concentration in leaf Sandy soil (ppm)	284.5000	6	240.64393	98.24249
	Concentration in leaf Loamy soil (ppm)	135.5917	6	158.24053	664.60143

TABLE 3.23: Correlation Between Concentration of Fe^{2+} in Zea Mays Leaf Grown in Sandy and Loamy Soils.

		N	Correlation	Sig.
Pair 1	Concentration in leaf Sandy soil (ppm) & Concentration in leaf Loamy soil (ppm)	6	.936	.006

TABLE 3.24: T-test Analysis of the Concentration of Cu^{2+} in Zea Mays Leaf at seedling stage Grown in Loamy and Sandy Soils.

		Mean	Std. Deviation	Std.Error Mean	95%Confidence interval of the Difference Lower	Upper	t	df	Sig (2-tailed)
Pair1	concentration in leaf Sandy soil (ppm) minus Concentration in leaf Loamy soil (ppm)	148.90833	107.97441	44.08037	35.59614	262.22052	3.378	5	.020

The critical value of t is 2.57 for 5 degrees of freedom at 95% confidence level. Since t-observed is greater than t-critical, then it is concluded that there is a significant difference in the concentration of Fe^{2+} in leaf of Zea Mays grown in the two soils (sandy and loamy).

IV. Discussion

The toxic levels of Zn^{2+}, Cu^{2+}, Fe^{2+} and Mn^{2+} of Zea mays at seedling stage have been investigated in loamy and sandy soils. From Tables 1 and 2, the toxic level of Zn^{2+} was found to be 250-260 in sandy soil and 175-200 in loamy sand. Tisdale (1993) observed that the toxicity of Zn^{2+} in seedlings occur when concentration of Zn^{2+} exceeds 150.

From Tables 3 and 4, the toxic level of cu^{2+} was found to be 37.24-55.90 in sandy soil and 35.32-45-25 in loamy soil. Tisdale (1993) recorded that toxicity of Cu^{2+} occur when concentration of Cu^{2+} exceeds 20.

Tables 5 and 6, showed that the toxic level of Mn^{2+} was found to be 290-320 in sandy soil and 255 in loamy soil. Tisdale (1993) observed that the toxicity of manganese occur in seedlings when concentration in the leaf exceeds 200.

From Tables 7 and 8, the toxic level of Fe^{2+} was found to be 420-700 in the leaf of Zea Mays in sandy soil and 450 in the leaf of Zea mays grown in loamy soil. Tisdale (1993) observed that the toxicity of Manganese occur in seedlings when concentration in leaf exceeds 400.

From the Tables 9, 10, 11 and 12, the statistic t-test for the pairs of the data that is, the concentration of the micronutrients at toxic levels in leaf of Zea mays grown in loamy and sandy soils were computed. From the t-test result, the t-observed for Zn^{2+} was 3.78 which is greater than the t-critical 2.57 at 95% levels under degree of freedom of 5. Also the t-observed of Cu^{2+} was 5.85 which again is greater than t-critical of 2.57 at 95% confidence level under degree of freedom of 5. In Fe^{2+} t-observed is 3.38 which again is greater than t-critical of 2.57 at 95% confidence level under degree of freedom 5. For the micronutrients Mn^{2+}, t-observed was 2.37 which is less than the t-critical of 2.57 at 95% confidence level under the freedom of 5. From the results obtained, it shows that there was a significant difference in the rate of absorption of Zn^{2+}, Cu^{2+} and Fe^{2+} by the Zea mays in the two soils. On the other hand, there was no significant difference in the rate of absorption of Mn^{2+} in the leaf of the Zea mays grown in sandy and loamy soils.

From the correlation results in tables 11, 15, 19 and 23, all the values fall between -1 and +1 indicating that there was a relation between the concentration of the micronutrients (Zn, Cu, Mn, Fe) in Zea mays leaves grown in sandy and loamy soils.

Generally, there was significant plant absorption of the micronutrients. However, those zea mays grown on loamy soils have the lowest plant absorption of the nutrients. This may be due to high organic matter content.

Summary

The statistic t-test for pairs of data, that is concentration of the micronutrients at toxic level of zea mays grown in loamy and sandy soils were computed.

The toxic level Zn^{2+} was 250-260 in sandy and 175-200 in loamy soil and t-observed was 3.78. Comparing with the critical value at the level of significance under degree of freedom of 5 at 95% confidence level is 2.57. Since t-observed was greater than t-critical, it shows that there was a significant difference in the rate of absorption of Zn^{2+} by the zea mays in the two soils.

The toxic level of Cu^{2+} was 37.24-55.90 in sandy and 35.32-45.25 in loamy soil and the t-observed for Cu^{2+} was 5.83. Comparing again with the critical value at the level of significance under degree of freedom of 5 at 95% confidence is 2.57. Since t-observed was greater than t-critical, it also shows that there was a significant difference in the rate of absorption of Cu^{2+} by the zea mays in the two soils.

For the micronutrient Fe^{2+}, the toxic level was 420-700 in sandy soil and 450 in leaf grown in loamy soil and the t-observed was 3.38, and the t-critical is 2.57. Since t-observed was greater than t-critical, it shows that there was a significant difference in the rate of absorption of Fe^{2+} by the zea mays in sandy and loamy soils.

The toxic level of Mn^{2+} was 290-320 in sandy soil and 255 in loamy soil and the t-observed for Mn^{2+} was 2.37. Comparing with the critical value at the level of significance under degree of freedom of 5 at 95% confidence level is 2.57. Since t-observed is less than t-critical, it shows that there was no significant difference in the concentration of Mn^{2+} in leaf of zea mays grown in the sand and loamy soils.

Generally, there was a significant micronutrient transfer factor in the leaves of all the leaves analyzed. Those grown in loamy soils have the lowest plant uptake factor of the micronutrients due to high organic matter content.

Conclusion

This research work concentrated on the toxic level of the four micronutrients Cu, Zn, Fe, and Mn in the leaf of zea mays at seedling stage grown on sandy and loamy soils.

From the t-test analysis carried out, it is concluded that there was significant difference in rate of absorption of Zn^{2+}, Cu^{2+} and Fe^{2+}zea mays in sandy and loamy soils. This is due to the nature of the soil.

Recommendations

- Similar research should be carried out with boron, molybdenum, nickel and cobalt to ascertain their toxic levels at seedling stage.
- More research should be carried out onzea mays at grain filling stage to help assess the level of these micronutrients. Since toxic level of the micronutrients constitute health hazard to human.

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