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# TOXICITY OF MICRONUTRIENTS TOWARD CORN LEAF CATALASE

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Presently science is rather sure about the essentiality of 15 elements for plant growth. These 15 are: H, B, C, N, O, Mg, P, S, K, Ca, Mn, Fe, Cu, Zn, and Mo. Of these fifteen B, Mn, Fe, Cu, Zn, and Mo are required only in small amounts and are called micronutrient elements or trace elements. The others are used in comparatively large amounts and may be referred to as macronutrient elements. Iron was the first micronutrient element found to be essential to plant life, and the essentiality of the other five has been established only in recent years.

The literature contains some references which pertain to the effects of micronutrients on the relative activity of catalase. Rather than measure the toxic effects of any micronutrient element, these investigations in the literature more nearly determine the relative amount of catalase formed without the micronutrient element or with varying amounts of the micronutrient in the nutrient solutions. Bailey and McHargue (1944) working with tomato leaves found that maximum catalase activity occurred when 0.01 p.p.m. copper was added to the nutrient solutions, and that with higher concentrations of copper there was a decrease in catalase activity. They also reported that catalase activity in tomato fruits diminished progessively with additions of 0.01, 0.05 and 0.1 p.p.m. copper to the nutrient solutions, and that catalase activity in alfalfa leaves was at a maximum when 1 p.p.m. zinc was added to the nutrient solutions as compared to 0, 0.5 and 2 p.p.m.

The catalase activity of alfalfa was reported by Bailey and McHargue (1944) to be depressed by the addition of 1 and 2 p.p.m. manganese to the nutrient solutions. However, Pattanaik (1950) found that additions of manganese as high as 10 p.p.m. to rice plants growing in culture solutions increased the catalase activity, and it was postulated by him that the catalase activity was affected by the direct influence of manganese on the iron containing prosthetic group of the catalase molecule. There are also conflicting reports for the catalase activity in squash plants. Alexander (1942) found that the catalase activity in squash plants was increased by boron deficiency, whereas Bailey and McHargue (1944) reported that catalase activity in alfalfa was at a maximum with 1 p.p.m. boron as compared with 0.1, 0.25 and 0.5 p.p.m.

The general effect of inorganic ions on plant catalases was previously reviewed (Eyster, 1953).

## METHODS AND MATERIALS

The method described previously (Eyster, 1953) was used for the quantitative determinations of catalase activity. A "dilution" method was used for copper and

zinc salts which were effective in such small concentrations that the amount could not be weighed and added directly to the macerated leaf suspension. In this "dilution" method, the same macerated sample was handled in the following sequence: (a) two catalase measurements reducing volume from 25 ml. to 21ml.,

TABLE 1

*Toxicity of Micronutrients toward Activity of Catalase in Macerated Corn Leaves. Catalase Value is Expressed as Milliliters of $O_2$ in a 5-Minute Interval.*

| Micronutrient Compound | Concentration of Micronutrient Element | Catalase Activity* A Without Compound | Catalase Activity* B With Compound | B/A |
|---|---|---|---|---|
| | p.p.m. | ml. | ml. | % |
| $Na_2B_4O_7.10H_2O$ | 100 B | 5.52 | 5.40 | 98 |
| | 500 B | 11.88 | 11.54 | 97 |
| | 2000 B | 12.10 | 11.06 | 91 |
| | 2500 B | 11.84 | <8.42 | <71 |
| $Na_2MoO_4.2H_2O$ | 100 Mo | 6.54 | 6.41 | 98 |
| | 500 Mo | 7.40 | 7.20 | 97 |
| | 2000 Mo | 7.76 | 7.26 | 94 |
| | 3000 Mo | 6.72 | 6.43 | 96 |
| | 5000 Mo | 6.72 | 7.20 | 107 |
| | 15000 Mo | 7.13 | 6.32 | 89 |
| $MnCl_2.4H_2O$ | 75 Mn | 8.24 | 8.04 | 98 |
| | 100 Mn | 8.23 | 7.46 | 91 |
| | 100 Mn | | 0.24† | |
| | 125 Mn | 8.46 | 8.40 | 99 |
| | 200 Mn | 8.21 | 7.38 | 90 |
| | 300 Mn | 8.13 | 7.60 | 94 |
| | 2000 Mn | 7.40 | 6.45 | 87 |
| | 2500 Mn | 8.26 | 7.44 | 90 |
| | 3000 Mn | 7.59 | 5.86 | 77 |
| | 3000 Mn | | 0.66† | |
| | 3500 Mn | 8.33 | 5.60 | 67 |
| $KMnO_4$ | 100 Mn | | 12.26† | |
| | 100 Mn | 8.62 | 9.46 | 110 |
| | 200 Mn | 9.50 | 11.80 | 124 |
| $FeSO_4.7H_2O$ | 0.1 N‡ | 11.54 | 9.84 | 85 |
| | | | 3.25† | |
| $FeCl_3.6H_2O$ | 0.1 N‡ | 10.91 | 5.16 | 47 |
| | | | 6.04 | |
| $ZnCl_2$ | 50 Zn | 7.17 | 3.38 | 47 |

*Each value is an average of two successive measurements.
†Value for blank, using micronutrient compound alone.
‡The concentration here is expressed in normality instead of parts per million. 0.1 N $FeSO_4.7H_2O$ represents 2800 p.p.m. Fe, and 0.1 N $FeCl_3.6H_2O$ represents 1867 p.p.m. Fe.

(b) addition of 4 ml. of dissolved micronutrient to bring volume back to 25 ml., (c) two more catalase determinations, final volume 21 ml., (d) addition of 4 ml. of dissolved micronutrient, final volume 25 ml., (e) two more catalase determinations, final volume 21., (f) addition of 4 ml. of dissoved micronutrient, final volume 25 ml., (g) two more catalase determinations. The concentration of the micronutrient

varied in (b), (d), and (f), and the concentration was computed so that it made a known final concentration in the 25 ml., allowing in (d) and (f) for the amount previously added. As for example in the case of $ZnCl_2$, 4 ml. of 6.25 p.p.m. Zn as $ZnCl_2$ was added in (b) giving a final concentration of 1 p.p.m. Zn, 4 ml. of

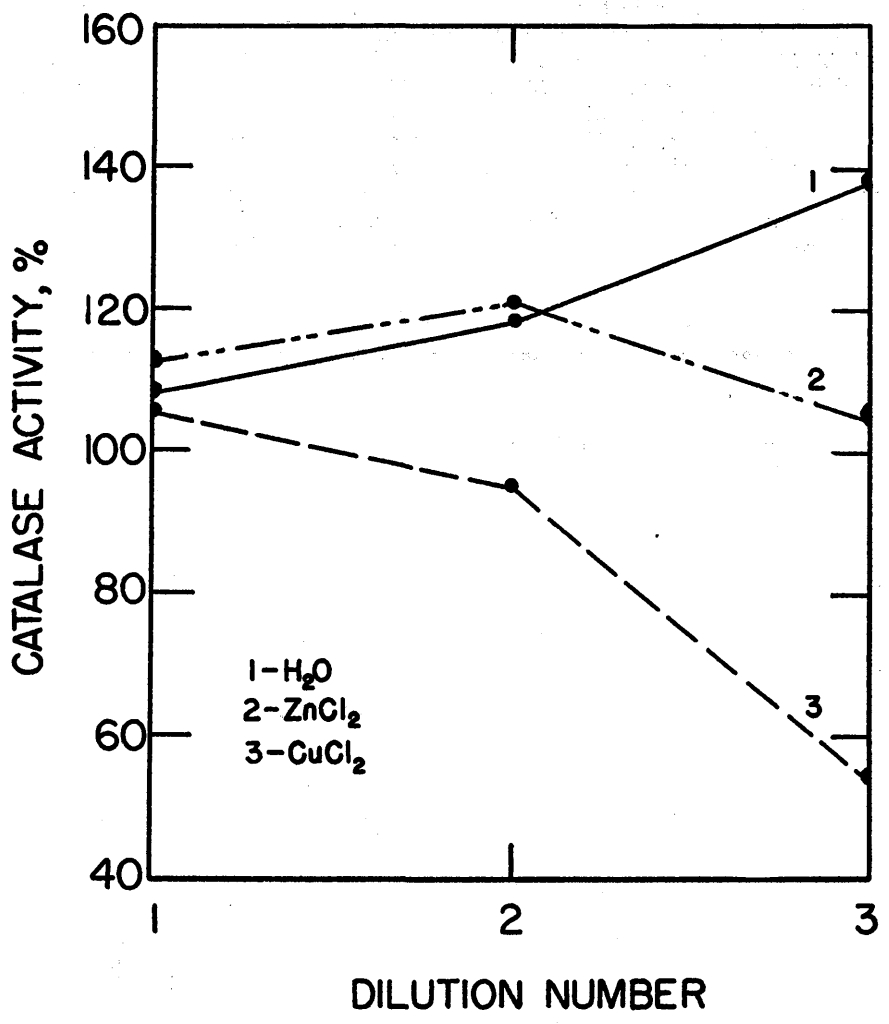


FIGURE 1. Toxicity effects of copper and zinc micronutrients toward catalase activity of macerated corn leaves determined by the "dilution" method. Concentrations: $ZnCl_2$—1 p.p.m. Zn, 10 p.p.m. Zn, and 50 p.p.m. Zn at dilutions 1, 2 and 3 respectively; $CuCl_2$—1 p.p.m. Cu, 10 p.p.m. Cu, and 50 p.p.m. Cu at dilutions 1, 2, and 3, respectively.

57.25 p.p.m. was added in (d) making 10 p.p.m. Zn, and 4 ml. of 260 p.p.m. was added in (f) making 50 p.p.m. Zn. It should be emphasized that concentrations refer to the pertinent element only and not the whole compound. The results were compared with a run in which 4 ml. of water was used for the additions in (b), (d), and (f).

## RESULTS AND DISCUSSION

Toxicity effects of micronutrient compounds are recorded in tables 1 and 2. Only two micronutrient elements were found to have any marked degree of toxicity toward catalase activity. These were copper and zinc. The toxicity of copper chloride toward catalase activity became noticeable between 1–10 p.p.m. Cu, and that of zinc chloride between 10–50 p.p.m. Zn (fig. 1). The toxicity limits of the other micronutrient elements were found to be: about 2500 p.p.m. of Mn (0.041 M $MnCl_2.4H_2O$), about 2000 p.p.m. of B (0.043 M $Na_2B_4O_7.10H_2O$), and no toxicity at 5000 p.p.m. of Mo (0.052 M $Na_2MoO_4.2H_2O$). The toxicity of iron compounds was somewhat difficult to determine because of their "catalase" properties. However, 0.1 N $FeSO_4$ (2800 p.p.m. of Fe) still permitted 85 percent of full catalase activity. Measurements with ferric and ferrous compounds, as well as with permanganate and manganous compounds may indicate that catalase is extremely stable. The reducing environment of ferrous sulfate appeared to be more favorable than the oxidizing environment of ferric chloride.

TABLE 2

*Toxicity of Copper and Zinc Compounds Toward Catalase Activity by the "Dilution" Method.*

| Compound | Dilution | Concentration of of Element | Catalase Activity† |
|---|---|---|---|
| | | p.p.m. | % |
| Water* | 1st | | 108 |
| | 2nd | | 118 |
| | 3rd | | 139 |
| $CuCl_2$ | 1st | 1 Cu | 106 |
| | 2nd | 10 Cu | 96 |
| | 3rd | 50 Cu | 55 |
| $ZnCl_2$ | 1st | 1 Zn | 113 |
| | 2nd | 10 Zn | 121 |
| | 3rd | 50 Zn | 106 |

*Control.

†Based on the average of two measurements, the milliliters of $O_2$ actually produced in a 5-minute interval divided by the expected and then expressed in per cent, where the expected $O_2$ production was calculated by assuming direct proportional decrease by dilution.

## SUMMARY

Of the six micronutrients (Fe, Mn, B, Zn, Cu and Mo) only Cu and Zn compounds showed any marked toxicity toward catalase activity. The toxicity of copper chloride became noticeable between 1–10 p.p.m. Cu, and that of zinc chloride between 10–50 p.p.m. Zn.

## LITERATURE CITED

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