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Assessment of Efficiency of Nutrient Uptake of Different Sources of Zn, Mn, Cu and B in *Zea mays*

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Abstract: Advances in plant nutrition can be achieved by improving the delivery of micronutrients to the plants. The objective of this research was to compare the efficiency of uptake of different sources of zinc, copper and manganese (sulfates, Ethylenediaminetetraacetic acid (EDTA) and oxides) and boron (boric anhydride and colemanite). We conducted all experiments in maize, repeated the experiment twice, using five replicates per treatment, and used two different media. Results showed that for cations, the soluble sources of micronutrients (sulfate and EDTA) in both media were more efficiently taken up. One-way ANOVA with post hoc Tukey for multiple comparisons of means (95% confidence level) was used for all statistical analyses. Sulfate sources were significantly different when compared to the negative control and to the oxide sources. EDTA sources were significantly different when compared to the negative control and to the oxide sources. Oxide sources were not significantly different from the negative control. For boron, we found a similar trend, with boric anhydride being significantly different when compared to the negative control and to colemanite. Colemanite was significantly different when compared to the negative control. This study generated important information about uptake of soluble and insoluble sources of four micronutrients that can be used for the development of new formulations.

Keywords: zinc; copper; manganese; boron; sulfates; EDTA; oxides; boric anhydride; colemanite; nutrient uptake

1. Introduction

Micronutrient deficiency is one of the most important issues in global crop production. There are eight micronutrients that are essential in trace levels for normal and healthy plant growth, including boron, chloride, copper, iron, manganese, molybdenum, nickel and, zinc [1]. When the supply of one or more of these elements is low, yield and quality of the crop can decrease, with different crops having different nutrients needs and different physiological responses to these deficiencies. Severe micronutrient deficiencies in plants are accompanied by different symptoms, but deficiencies with no symptoms are also common [2]. Moreover, it is also important to avoid over-application of micronutrients because this can cause severe toxicity [2]. To have a better understanding of how deficient, adequate or excess supply of micronutrients can influence final crop yields, we can use the dose-response graph developed by Brady and Weil as reference [3]. The availability of the essential micronutrients to plants is often poorly correlated with the total quantity of the particular element in the soil. Soil properties such as pH, redox potential, organic matter content, microbial diversity, nutrient interactions, and environmental factors, such as soil water content, temperature and light, greatly influence the micronutrient availability in soil and its consequent uptake by plants [4,5].

Zinc is the most common and widely occurring micronutrient deficiency worldwide and correlates with human zinc-deficiency as one-third of the world population suffer from inadequate zinc in diets [6].

Zinc availability is affected by high pH, high calcite or organic matter contents, and high concentrations of sodium, calcium, magnesium, bicarbonate or phosphate in soil [7]. Zinc is an essential element in plant metabolism because of its strong tendency to form tetrahedral complexes with ligands of nitrogen, oxygen and sulfur; and hence, zinc plays a catalytic and structural role in metabolism [8].

Boron is the second most common micronutrient deficiency [9]. Boron leaches readily under high rainfall conditions in sandy, weathered or acidic soils and this can severely limit plant yield [9]. Conversely, with low rainfalls in alkaline or organic soils, boron accumulates and can become severely toxic to plants [10]. The optimum amount of boron needed is crop dependent; however, the range needed is narrow [10]. Under deficient boron conditions, plants show physiological and biochemical responses [11,12]. The most important function of boron in plants is the cross-linking of the cell wall component rhamnogalacturonan II [13].

Copper is an essential micronutrient; however, it can be phytotoxic [14], and induce detrimental physiological responses when supplied in excess [15,16]. Some of the induced changes due to toxicity can also manifest when copper is deficient which makes it hard to determine the cause of the symptoms [15].

Manganese is most commonly deficient in siliceous and calcareous sandy soils of neutral or alkaline pH [17]. Deficiency in plants is not correlated to quantity in soil, but to accessibility to bioavailable manganese [17]. This deficiency is difficult to overcome because of how quick manganese is oxidized in soil [17]. This results in plants having compromised growth and development as a result of reduced photosynthesis [18,19], reduction of auxin levels [20], which results in suppressed root development, and deficient synthesis of carbohydrates, which impacts regeneration of roots that results in plants lacking vigor [20].

In plant nutrition, the use of sulfates is widespread due to its high solubility and fast release for plant uptake [21,22]. Different sources of chelates have been shown to increase soil availability of multiple micronutrients in soil significantly better than other conventional sources [23]. Therefore, the use of metal-chelates could improve the uptake of micronutrients in plants under certain conditions. However, excessive application of Ethylenediaminetetraacetic acid (EDTA) or metal-EDTA can also be potentially harmful [24], eventually reducing yield, income and environmental quality. Moreover, oxides are insoluble in water and their availability for plant uptake is less than sulfates and chelates unless the rates in the oxide source are corrected to increase the amount of the cation applied to the soil [25–27]. In the case of boron, borax, boric acid and solubor are commonly used because of their high solubility. Colemanite, with lower solubility, can be used if the particle size is small enough [28]. This research was conducted to evaluate and compare plant uptake of different sources of four micronutrients: zinc, manganese, copper and boron.

2. Materials and Methods

Experiments were designed using a randomized complete design (RCD) to compare nutrient uptake differences in maize plants receiving different micronutrient sources. For zinc, copper and manganese, we tested sulfate, chelate (EDTA) and oxide sources, and for boron, we tested colemanite and boric anhydride. For all treatments, the negative control consisted of NPK application and no additional micronutrients. Plants were harvested 30 days after planting and plant nutrient uptake was subsequently analyzed. Plants were grown in a greenhouse at 24 °C for day temperature and 21 °C for night temperature; and no supplemental lighting was used.

The experiments were repeated two times in two different growing media: 0.7 kg Michigan Peat 5540 Garden Magic Top Soil (Michigan Peat Company BACCTO®, Houston, TX, USA, referred as top soil in this article) and 1 kg mixture of half top soil and half sand (measured by weight) for all treatments. The media, top soil and top soil and sand, used to grow the maize plants was analyzed by A&L Great Lakes Lab (Fort Wayne, IN, USA) to understand organic matter content (%), cation-exchange capacity (CEC, measured in meq/100 g) and micronutrient composition (Table 1). Based on the results, we applied 2 ppm of each corresponding micronutrient from the different sources.

For every experiment, 10 replicates were used for each treatment, and the five dry replicate samples with the greatest biomass were used for plant nutrient analysis.

Table 1. Media analysis was replicated 3 times in top soil, and in top soil and sand mixtures, and the amount of zinc, manganese, copper and boron (in ppm), the organic matter content (%) and CEC (meq/100 g) were determined.

Growing Media	Organic Matter (%)	CEC (meq/100 g)	Zn (ppm)	Mn (ppm)	Cu (ppm)	B (ppm)
Top Soil and Sand-1	3.5	9.5	1.3	10	0.5	0.2
Top Soil and Sand-2	3.8	9.1	1.2	9	0.5	0.2
Top Soil and Sand-3	3.8	9.6	1.3	8	0.5	0.2
Top Soil-1	8.7	16.8	2.1	7	0.9	0.4
Top Soil-2	10.4	19.7	2	5	0.9	0.3
Top Soil-3	9.5	17.9	1.8	6	0.7	0.2

All treatments received N-P-K at a rate of 112-56-56 kg/ha in granular form. The sources were urea for nitrogen, monoammonium phosphate for phosphorous, and potassium sulfate for potassium. The micronutrient sources were applied in powder form along with the NPK mixture before planting and mixed in the media. For all treatments, roots and shoots were collected and cleansed of all particles using water. After cleaning, the plants' fresh tissue was dried at 95 °C for 48 h. Then, all samples (roots and shoots) were weighed, packed and sent for analysis to A&L Great Lakes Lab.

At A&L Great Lakes, samples were ground, using a Wily Mill Grinder (Thomas Scientific, Swedesboro, NJ, USA), and sieved through a 20-mesh screen. Plant tissue samples were digested in an open vessel microwave oven procedure. Samples were weighed to approximately 0.2 g, and the final weight was recorded to use for the final dilution factor. Then, 2 mL of nitric acid were added to each sample, and these were microwaved in 2 steps. In the first step, the microwave was programmed to ramp up to 90 °C and hold at that temperature for 90 s. After the samples cooled below 50 °C, 1 mL of peroxide was added. The samples were returned to the microwave a second time and the temperature was ramped up to 105 °C and held for 10 min. After the samples had cooled, they were brought to a final volume of 25 mL (~1:125 dilution) capped, mixed and analyzed. For mineral analysis, an Inductively Coupled Argon Plasma (ICAP) AOAC 985.01 ran on Thermo iCAP 6500 (Thermo Fisher Scientific, Waltham, MA, USA) was used. After analysis, nutrient uptake was calculated for all samples using A&L data and dry weight of samples. The analysis prepared by A&L Great Lakes Lab shows the concentration of the different micronutrients in parts per million (ppm). By multiplying the concentration (in ppm) to the total dry weight of the plant (in grams) and dividing by 1000, we obtain the plant nutrient uptake in milligrams.

For the statistical analysis of plant nutrient uptake, we did one-way ANOVA statistical analyses in the R-Studio software (v. 1.1.456, RStudio, Inc., Boston, MA, USA), using post hoc Tukey multiple comparisons of means with a 95% confidence level ($p = 0.05$) to identify treatment differences.

3. Results and Discussion

Increases in yield and quality of crops from application of zinc, boron, copper and manganese occur in many parts of the world [1]. Growers and scientists are aware of how micronutrient deficiencies can limit yields even when plants only need small amounts [28]. In this research we investigated the nutrient efficiency uptake of different sources of four micronutrients. Nutrient efficiency uptake can be defined as the amount of a nutrient taken up by a plant and how that compares with the other sources of that same nutrient (i.e., for zinc, manganese and copper, it is the comparison of the sulfate, chelate and oxide sources; and for boron, it is the comparison of the boric anhydride and colemanite sources).

The two different media used in this investigation (top soil and top soil and sand) have differences in the organic matter, CEC and micronutrient composition. The higher levels of organic matter and CEC in the top soil increase this media's ability to attract, retain and exchange cations (Zn, Cu and Mn) compared to the top soil and sand media; which increases the level of nutrition available to plants in the soils with the higher CEC. This is correlated with the observed results for the three cations when present in soluble forms (sulfate and EDTA) as the plant nutrient uptake was higher in the top soil media (Table 2). Additionally, in the case of boron, the top soil and sand media will have more leaching due to the higher amount of sand, and we can observe that in the plant, nutrient uptake results for all boron sources (Table 2).

In the zinc trials, we found that zinc sulfate had more efficient uptake by maize in both media. In the top soil experiments, plants treated with zinc sulfate had on average 9.19% more zinc uptake than plants treated with zinc EDTA; plants treated with zinc EDTA had an average of 73.65% more zinc uptake than plants treated with zinc oxide; and plants treated with zinc oxide had an average of 59.28% more zinc uptake than plants treated only with NPK (Table 3). In the top soil and sand experiments, plants treated with zinc sulfate had an average of 12.31% more zinc uptake than plants treated with zinc EDTA; plants treated with zinc EDTA had an average of 96.32% more zinc uptake than plants treated with zinc oxide; and plants treated with zinc oxide had an average of 119.79% more zinc uptake than plants treated only with NPK (Table 3). Additionally, we found that plants grown in top soil had greater uptake of zinc than plants grown in top soil and sand, by 16.25% when treated with zinc sulfate, 19.58% when treated with zinc EDTA, 35.19% when treated with zinc oxide, and 86.55% when not treated with supplemental zinc (NPK only) (Table 4). No significant differences were observed when comparing zinc sulfate to zinc EDTA and zinc oxide to NPK for the amount of zinc uptake in both media; moreover, when comparing zinc sulfate to zinc oxide, zinc sulfate to NPK, zinc EDTA to zinc oxide and zinc EDTA to NPK, there were significant differences (Table 5). The observed differences in the uptake of the different sources of zinc can be explained by the solubility of those sources. Zinc sulfate and zinc EDTA are more easily used by plants due to their high solubility, while zinc oxide is insoluble and hence has a slower release. Nevertheless, zinc oxide was shown to be a usable source when compared to the negative control (NPK only). The results show that zinc uptake by plants in top soil was more efficient than in top soil and sand, which can be explained by the higher CEC and organic matter content.

Zinc deficiency is one of the most critical health problems, affecting one-third of world population and this is related to the fact that zinc is the most deficient micronutrient worldwide [1]. For this reason, research focus is shifting to increasing zinc levels in different crops [29–32]. The total zinc content in soils varies from 3 to 770 mg/kg with the world average being 64 mg/kg; contents below 10 mg/kg are considered deficient; and contents above 200 mg/kg are usually due to contamination [7]. On average, crops remove 5 kg of zinc per hectare every year [33], and application rates are crop-dependent, with 11 kg Zn/ha for wheat and rice; 5.5 kg Zn/ha for maize, soybean and sugarcane [6]. Therefore, finding a zinc fertilizer that can be a source of zinc throughout the season is important. The most commonly used zinc fertilizer is zinc sulfate because of its high solubility and availability; however, this compound can precipitate rapidly in calcareous soils. In this kind of soil, zinc chelates, like zinc-EDTA are preferably used [34]. It has been established that at least 40% to 50% of fertilizers must contain a water-soluble source of zinc [6], with individual applications of either zinc sulfate or zinc EDTA working efficiently at providing zinc to plants [35]. Insoluble sources such as zinc oxide may also be used as a zinc source. Zinc oxide was shown to be a satisfactory source of zinc (comparable to zinc sulfate) when mixed well in soil [36]. Furthermore, zinc oxide worked as efficiently as zinc sulfate to correct deficiencies as demonstrated by Schulte and Walsh [37]. Based on the results from this research and past research, these three zinc sources were shown to be reliable sources of zinc with different availability, which is dependent on their solubility.

Table 2. Mean and standard error (SE) of, zinc, boron, manganese and copper, uptake for the two experiments in top soil and top soil and sand. All units for Mean and SE are in milligrams (mg). The average mean values were used to calculate percentage differences. Percentage Difference (%) = (Mean 2 − Mean 1) × 100/Mean 1.

Zinc Trials	**Top Soil Experiment 1**		**Top Soil Experiment 2**		**Top Soil—Average**	**Top Soil and Sand Experiment 1**		**Top Soil and Sand Experiment 2**		**Top Soil and Sand—Average**
Treatments	**Mean**	**SE**	**Mean**	**SE**		**Mean**	**SE**	**Mean**	**SE**	
NPK	0.0451	0.0023	0.0528	0.0059	0.0489	0.034	0.004	0.0184	0.0022	0.0262
Zinc EDTA	0.1363	0.0063	0.1343	0.0166	0.1353	0.1147	0.0086	0.1116	0.0326	0.1131
Zinc Oxide	0.0839	0.004	0.0719	0.0169	0.0779	0.0836	0.0039	0.0317	0.0026	0.0576
Zinc Sulfate	0.148	0.0045	0.1474	0.023	0.1477	0.1245	0.0133	0.1297	0.013	0.1271
Manganese Trials	**Top Soil Experiment 1**		**Top Soil Experiment 2**		**Top Soil—Average**	**Top Soil and Sand Experiment 1**		**Top Soil and Sand Experiment 2**		**Top Soil and Sand—Average**
Treatments	**Mean**	**SE**	**Mean**	**SE**		**Mean**	**SE**	**Mean**	**SE**	
NPK	0.012	0.0013	0.0086	0.0018	0.0103	0.0085	0.0005	0.012	0.0013	0.0103
Manganese EDTA	0.0265	0.0034	0.0237	0.0033	0.0251	0.0292	0.0041	0.0265	0.0034	0.0278
Manganese Oxide	0.0159	0.0028	0.0143	0.0017	0.0151	0.0115	0.0029	0.0159	0.0028	0.0137
Manganese Sulfate	0.0339	0.005	0.0304	0.0034	0.0322	0.0259	0.0041	0.0339	0.005	0.0299
Copper Trials	**Top Soil Experiment 1**		**Top Soil Experiment 2**		**Top Soil—Average**	**Top Soil and Sand Experiment 1**		**Top Soil and Sand Experiment 2**		**Top Soil and Sand—Average**
Treatments	**Mean**	**SE**	**Mean**	**SE**		**Mean**	**SE**	**Mean**	**SE**	
NPK	0.0022	0.0001	0.0026	0.0003	0.0024	0.0022	0.0004	0.002	0.0002	0.0021
Copper EDTA	0.0086	0.0016	0.0078	0.0017	0.0082	0.0065	0.0005	0.0052	0.0007	0.0058
Copper Oxide	0.004	0.0003	0.0041	0.0005	0.0041	0.0034	0.0003	0.0031	0.0003	0.0032
Copper Sulfate	0.0091	0.0013	0.0097	0.0008	0.0094	0.0069	0.0008	0.0059	0.0004	0.0064
Boron Trials	**Top Soil Experiment 1**		**Top Soil Experiment 2**		**Top Soil—Average**	**Top Soil and Sand Experiment 1**		**Top Soil and Sand Experiment 2**		**Top Soil and Sand—Average**
Treatments	**Mean**	**SE**	**Mean**	**SE**		**Mean**	**SE**	**Mean**	**SE**	
NPK	0.0065	0.0014	0.0041	0.0004	0.0053	0.0037	0.0007	0.0029	0.0003	0.0033
Colemanite	0.0407	0.0029	0.0242	0.0032	0.0324	0.018	0.0041	0.0191	0.006	0.0185
Boric Anhydride	0.0718	0.0048	0.0631	0.0039	0.0675	0.0403	0.0034	0.0408	0.0038	0.0405

Table 3. Percentage differences (%) of plant uptake of different sources of micronutrients tested in two different media. These differences show that soluble sources of micronutrients (sulfate and EDTA for the cations and anhydride for the anion) are more efficiently taken up by plants in the short term.

Zinc	**Top Soil Differences**			**Zinc**	**Top Soil and Sand Differences**		
	NPK	**Oxide**	**Sulfate**		**NPK**	**Oxide**	**Sulfate**
EDTA	176.59	73.65	9.19	EDTA	331.50	96.32	12.31
NPK		59.28	202.01	NPK		119.79	384.63
Oxide			89.61	Oxide			120.50
Sulfate				Sulfate			
Manganese	**Top Soil Differences**			**Manganese**	**Top Soil and Sand Differences**		
	NPK	**Oxide**	**Sulfate**		**NPK**	**Oxide**	**Sulfate**
EDTA	143.74	66.10	28.15	EDTA	170.61	102.75	7.45
NPK		46.74	212.35	NPK		33.47	190.77
Oxide			112.85	Oxide			117.86
Sulfate				Sulfate			
Copper	**Top Soil Differences**			**Copper**	**Top Soil and Sand Differences**		
	NPK	**Oxide**	**Sulfate**		**NPK**	**Oxide**	**Sulfate**
EDTA	241.18	101.32	14.34	EDTA	177.92	81.20	10.08
NPK		69.47	290.11	NPK		53.38	205.94
Oxide			130.20	Oxide			99.47
Sulfate				Sulfate			
Boron	**Top Soil Differences**			**Boron**	**Top Soil and Sand Differences**		
	Colemanite	**NPK**			**Colemanite**	**NPK**	
Anhydride	107.97	1170.43		Anhydride	118.77	1121.28	
Colemanite		510.87		Colemanite		458.25	
NPK				NPK			

In the manganese trials, we found that manganese sulfate was most efficiently taken up by the maize plants in top soil. In the plants grown in top soil and sand, manganese EDTA had uptake at similar levels compared to manganese sulfate. In the top soil experiments, plants treated with manganese sulfate had an average of 28.15% more manganese uptake than plants treated with manganese EDTA; plants treated with manganese EDTA had an average of 66.1% more manganese uptake than plants treated with manganese oxide; and plants treated with manganese oxide had an average of 46.74% more manganese uptake than plants treated only with NPK (Table 3). In the top soil and sand experiments, plants treated with manganese sulfate had an average of 7.45% more manganese uptake than plants treated with manganese EDTA; plants treated with manganese EDTA had an average of 102.75% more manganese uptake than plants treated with manganese oxide; and plants treated with manganese oxide had an average of 33.47% more manganese uptake than plants treated only with NPK (Table 3). Additionally, we found that plants grown in top soil had greater uptake of manganese than plants grown in top soil and sand, by 7.6% when treated with manganese sulfate, 10.13% when treated with manganese oxide, and 0.17% when not treated with supplemental manganese (NPK only). Conversely, it was found that plants grown in top soil and sand had greater uptake of manganese than plants grown in top soil by 10.84% when treated with manganese EDTA (Table 4). No significant differences were observed when comparing manganese sulfate to manganese EDTA and manganese oxide to NPK uptake; moreover, when comparing manganese sulfate to manganese oxide, manganese sulfate to NPK, manganese EDTA to manganese oxide and manganese EDTA to NPK, there were significant differences (Table 5). The observed differences in the uptake of the different sources of manganese can be explained by the solubility of those sources. Manganese sulfate and manganese EDTA are more easily used by plants due to their high solubility, while manganese oxide is insoluble and hence has a slower release. Nevertheless, manganese oxide was shown to be a usable source when compared to the negative control (NPK only). The results show that manganese uptake by plants in top soil was more efficient than in top soil and sand for all sources except manganese EDTA.

Table 4. Percentage differences (%) in plant uptake of different sources of micronutrients between the two different media. These differences show that plant nutrient uptake in the top soil media was more efficient than in the top soil and sand media.

Zinc		**Average—Top Soil and Sand Experiment**			
		EDTA	**NPK**	**Oxide**	**Sulfate**
Average—Top Soil Experiment	**EDTA**	19.58	415.97	134.76	6.47
	NPK	131.31	86.55	17.82	159.79
	Oxide	45.22	197.13	35.19	63.1
	Sulfate	30.57	463.39	156.33	16.25
Boron		**Average—Top Soil and Sand Experiment**			
		Anhydride	**Colemanite**	**NPK**	
Average—Top Soil Experiment	**Anhydride**	66.39	264.01	1932.08	
	Colemanite	24.99	75.03	877.09	
	NPK	663.53	249.01	59.95	
Manganese		**Average—Top Soil and Sand Experiment**			
		EDTA	**NPK**	**Oxide**	**Sulfate**
Average—Top Soil Experiment	**EDTA**	10.84	144.15	82.92	19.1
	NPK	170.15	0.17	33.24	190.28
	Oxide	84.1	46.99	10.13	97.82
	Sulfate	15.62	212.88	134.42	7.60
Copper		**Average—Top Soil and Sand Experiment**			
		EDTA	**NPK**	**Oxide**	**Sulfate**
Average—Top Soil Experiment	**EDTA**	41	291.85	155.48	28.08
	NPK	141.98	14.85	33.54	166.38
	Oxide	42.79	94.64	26.9	57.18
	Sulfate	61.22	348.06	192.13	46.45

Manganese deficiency varies geographically, with most of the manganese in soil being unavailable for plant uptake [38]. Individual applications of either manganese sulfate or manganese EDTA are the most common fertilizer sources of manganese [38]. However, chelated sources of manganese have been found to potentially increase manganese deficiency due to an iron-manganese imbalance that converts the manganese chelate into a more stable iron chelate [38]. Manganese deficiency is most likely to occur in neutral to high pH soils that are rich in organic matter [39]. Considering these facts and based on the results from this research, a fertilizer containing different sources of manganese would be ideal for crop requirements throughout the season and for providing this micronutrient in different soil types.

In the copper trials, we found that copper sulfate was most efficiently taken up by the maize plants. In the top soil experiments, plants treated with copper sulfate had an average of 14.34% more copper uptake than plants treated with copper EDTA; plants treated with copper EDTA had an average of 101.32% more copper uptake than plants treated with copper oxide; and plants treated with copper oxide had an average of 69.47% more copper uptake than plants treated only with NPK (Table 3). In the top soil and sand experiments, plants treated with copper sulfate had an average of 10.08% more copper uptake than plants treated with copper EDTA; plants treated with copper EDTA had an average of 81.2% more copper uptake than plants treated with copper oxide; and plants treated with copper oxide had an average of 53.38% more copper uptake than plants treated only with NPK (Table 3). Additionally, we found that plants grown in top soil had greater uptake of copper than plants grown in top soil and sand, by 46.45% when treated with copper sulfate, 41% when treated with copper EDTA, 26.9% when treated with copper oxide, and 14.85% when not treated with supplemental copper (NPK only) (Table 4). No significant differences were observed when comparing uptake of copper sulfate with copper EDTA, and copper oxide to NPK. Significant differences were observed when comparing copper sulfate with copper oxide, copper sulfate to NPK, and copper EDTA to NPK ($p < 0.05$). No significant differences were observed when comparing copper sulfate to copper EDTA, and copper oxide to NPK; moreover, when comparing copper sulfate to copper oxide, copper sulfate to NPK, copper EDTA to copper oxide and copper EDTA to NPK, there were significant differences (Table 5). The observed differences in the uptake of the different sources of copper can be explained by the solubility of those sources. Copper sulfate and copper EDTA are more easily used by plants due to their high solubility, while copper oxide is insoluble and hence has a slower release. Nevertheless, copper oxide was shown to be a usable source when compared to the negative control (NPK only). The results show that copper uptake by plants in top soil was more efficient than in top soil and sand, which can be explained by the higher CEC and organic matter content.

Table 5. Comparison of raw materials for the different micronutrients tested in two different media, using a one-way ANOVA statistical analyses and post hoc Tukey multiple comparisons of means.

Zinc Trials	**Top Soil**	**Top Soil and Sand**
NPK—Zinc EDTA	<0.0001	<0.0001
Zinc Oxide—Zinc EDTA	0.0001	0.0027
Zinc Sulfate—Zinc EDTA	0.7139	0.7728
Zinc Oxide—NPK	0.0806	0.1529
Zinc Sulfate—NPK	<0.0001	<0.0001
Zinc Sulfate—Zinc Oxide	<0.0001	0.0002
Boron Trials	**Top Soil**	**Top Soil and Sand**
Colemanite—Boric Anhydride	<0.0001	<0.0001
NPK—Boric Anhdride	<0.0001	<0.0001
NPK—Colemanite	<0.0001	0.0007
Manganese Trials	**Top Soil**	**Top Soil and Sand**
NPK—Manganese EDTA	0.0001	<0.0001
Manganese Oxide—Manganese EDTA	0.017446	0.0016
Manganese Sulfate—Manganese EDTA	0.1061	0.9008
Manganese Oxide—NPK	0.4587	0.6826
Manganese Sulfate—NPK	<0.0001	0.0004
Manganese Sulfate—Manganese Oxide	<0.0001	0.01
Copper Trials	**Top Soil**	**Top Soil and Sand**
NPK—Copper EDTA	<0.0001	<0.0001
Copper Oxide—Copper EDTA	0.0009	<0.0001
Copper Sulfate—Copper EDTA	0.6056	0.7237
Copper Oxide—NPK	0.3343	0.1935
Copper Sulfate—NPK	<0.0001	<0.0001
Copper Sulfate—Copper Oxide	<0.0001	<0.0001

Copper complexes with organic matter, oxides, or metal adsorbed on clays have low availability for plant uptake compared to copper complexes with soluble sources, making copper sulfate and copper EDTA the most commonly used fertilizers for quickly correcting deficiencies compared to copper oxide [39]. Crops remove less than 0.11 kg/ha per year, and higher copper concentrations can be toxic to plants. Fertilizers based on the three sources tested in this research would work best to prevent deficiency and toxicity caused by copper. By including soluble sources with a faster release such as copper sulfate and copper EDTA, deficiencies can be reduced during the first stages of growth and development, and by including insoluble sources with a slower-release such as copper oxide, deficiency is preventable during the reproductive stages of the crop.

In the boron trials, we found that boric anhydride was most efficiently taken up by the maize plants. In the top soil experiments, plants treated with boric anhydride had an average of 107.97% more boron uptake than plants treated with colemanite; and plants treated with colemanite had an average of 510.87% more boron uptake than plants treated only with NPK (Table 3). In the top soil and sand experiments, plants treated with boric anhydride had an average of 118.77% more boron uptake than plants treated with colemanite; and plants treated with colemanite had an average of 458.25% more boron uptake than plants treated only with NPK (Table 3). Additionally, we found that plants grown in top soil had greater uptake of boron than plants grown in top soil and sand, by 66.39% when treated with boric anhydride, 75.03% when treated with colemanite, and 59.95% when not treated with supplemental boron (NPK only) (Table 4). Significant differences were observed when comparing boron uptake of boric anhydride to colemanite, boric anhydride to NPK, and colemanite to NPK (Table 5). The observed differences in the uptake of the different sources of boron can be explained by the solubility of those sources. Boric anhydride is more easily used by plants due to its high solubility, while colemanite is insoluble and hence has a slower release. Nevertheless, colemanite was shown to be a usable source when compared to the negative control (NPK only). The results show that boron uptake by plants in top soil was more efficient than in top soil and sand, which can be explained by the higher possible leaching in top soil and sand due to the higher sand content.

Boron fertilizer is one of the most common micronutrient fertilizers applied, and borax, solubor and boric acid are the most commonly used [40]. Boron needs to be applied to soil before sowing and seedling emergence for best results [41]. Boron deficiencies occur on low-organic matter, acid, sandy and silt loam soils [42]. Borax and boric acid are popular boron fertilizers; however, they readily leach in sandy soils [28], as it was shown in this investigation. Solubor is used in soil and as a foliar fertilizer due to its very high solubility; however, it has been found to also readily leach from sandy soils [43]. Insoluble sources of boron are not commonly used [40,43] and this can be due to the fact that boron deficiencies are usually corrected until symptoms are observed, at which point quick-acting soluble sources are needed [42].

These experiments demonstrated that for the cations tested, sulfate and chelate sources are similar in plant nutrient uptake; although in formulation they behave differently, and hence, there is an advantage in having multiple sources [44]. Considering the different chemistries of these two soluble and readily-available products, a fertilizer containing a ratio of these products for all cations should work well. Furthermore, a ratio of sulfate to chelate would also allow the plant to get a constant supply of desired cation by the shuttle effect mechanism [44]. Oxide sources are insoluble and hence have a slow release, which translates into a slow uptake by plants. Since these cations are not immediately available, they ensure that plants can receive the desired metal throughout the season, especially close to the end of the season when most of the soluble sources of the micronutrients are lacking [25–27]. Therefore, for the three cations tested, zinc, copper and manganese, a fertilizer that contains a sulfate and chelate for immediate release and shuttle-effect technology, and an oxide, which has a slow-release mechanism, would ensure a constant supply of desired micronutrient to the plant. For boron, a fertilizer containing the soluble boric anhydride (which readily transforms into boric acid when mixed with water) and the less soluble colemanite would ensure constant supply of this micronutrient to the plant.

This combination of soluble and insoluble sources of micronutrients can help prevent, correct and minimize deficiencies due to the constant supply of fast-release and slow-release sources.

As the global production of crops has increased in the last 50 years, there is a need to match the nutrient needs of those crops [45], and micronutrients play an important role. World demand for fertilizer is calculated to increase at the same annual rate of ~1.9% as between 2012–2016 [46]. Additionally, there are differences in the fertilizer needs for the same crops in different parts of the world. For example, China is known for overusing fertilizers [47], while Kenya is known for underusing them [47]. This is important because there is need for a balance that meets the 4Rs of nutrient stewardship that provide the right source of nutrient, at the right time, source and time. As the use of micronutrients for enhancing yield and quality of different crops has been historically ignored compared to the most commonly used N-P-K [1], there also needs to be more work towards educating growers about the need for micronutrients in production agriculture. In this way, the formulation of a micronutrient product that provides fast-release and slow-release sources of specific elements for the plant to use during all growth stages, would help growers in the transition of starting to use more micronutrients.

4. Patents

Babu, T.; Martin, E.; Geiger, R.A.; Gaige, A.R. Multi-source micronutrient composition and methods of treating soil with the same. 2019 *U.S. Patent Application No. 16/252,120*.

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