Zinc Availability to Rice
From Seven Granular Fertilizers

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INTRODUCTION

Zinc deficiency has been documented throughout the rice growing areas of the world (Jones et al., 1982). Most of the reports concerning Zn deficiency in rice have been associated with soil pH values above 6.5 (Nene, 1966; Westfall et al., 1971; Sedberry et al., 1971; Wells et al., 1973; Katyal and Ponnamperuma, 1974; Mikkelsen and Brandon, 1975). Many of the aforementioned researchers observed yield increases resulting from application of Zn fertilizer. In Arkansas, application of Zn to the rice crop is recommended on silt loam soils that have a pH greater than 6.5 or on silt loam soils that have been precision-leveled within the past 2 years (Slaton et al., 1994). In the mid 1970s, most of the Zn applied to rice fields was granular $ZnSO_4$, but in the late 1980s Zn was also applied as dissolved powders of $ZnSO_4$ and Zn chelate. In addition, new sources have appeared on the market. Some are recommended by the manufacturer for blending with primary nutrients, and some claim to have better efficiency than $ZnSO_4$, which is considered the most immediately available source.

Zinc deficiency symptoms include a subtle chlorosis beginning at the base of the leaf blade, a purplish-brown discoloration known as bronz-

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ing, and loss of turgidity resulting in the plants floating on the surface of the water. The symptoms usually appear within 3 d after flooding, generally appear first on the lower (oldest) leaves first, and progress upward as the plant grows (Wells, 1980). If not corrected, a severe deficiency may result in the death of the rice seedlings.

Boawn et al. (1957) found that Zn applied as ground blast-furnace slag and Zn frits was not readily available to corn (*Zea mays* L.) or bean (*Phaseolus vulgaris* L.) plants. But ground $ZnSO_4$, $ZnCO_3$, ZnO, and $Zn_3(PO_4)_2$ were readily and equally available to corn and beans. Kang and Okoro (1976) observed that rice plants treated with ground $ZnSO_4$, ZnEDTA, fritted Zn, and metallic Zn showed an increase in dry matter yield (DMY) over that associated with no Zn treatment. They showed that uptake of fritted Zn was less than that of $ZnSO_4$ and ZnEDTA. These researchers demonstrated that even when the zinc is ground, some sources were not as effective as other Zn fertilizers.

Mordvedt (1992) concluded that powder forms of ZnO and $ZnSO_4$ were as effective as Zn fertilizers. He also found that at least 40% of the total Zn in granular fertilizers should be water-soluble to be fully effective for corn. Similarly, Amrani et al. (1997) stated that in corn, at least 50% of the total Zn in granular fertilizers should be water soluble in order to supply the needs of the current crop. If the water solubility of a Zn fertilizer is related to the efficiency of the fertilizer, water-soluble Zn could prove useful as a method to characterize Zn sources for rice. However, there is little information regarding the effect of granule size on Zn availability to plants. It would be expected that if placement and distribution of Zn sources play a key role in their agronomic efficiency (Mordvedt, 1991; Wells, 1980) the size of a granule should also influence the effectiveness of the source.

Since Zn deficiency was first observed in crop plants, many extracting solutions have been developed to establish a relationship between plant-available Zn, measured as either plant Zn concentration or total Zn uptake by the plant, and soil-extractable Zn (Martens and Lindsay, 1990). Because Zn deficiency in rice is more prevalent when the soil pH is greater than 6.5, most of the methods used are those that have been developed for crops other than rice grown under either calcareous or alkaline conditions. Selvarajah et al. (1982) and Singh and Takkar (1981) found a strong positive correlation between Zn extracted by the $EDTA(NH_4)_2CO_3$ (Ammonium carbonate ethylenediaminetetraacetic acid) method and Zn concentration in the tissue of the rice plant. However, Ponnamperuma et al. (1981) recommended the use of 0.05N HCl over that of $EDTA(NH_4)_2CO_3$ on the basis of the availability and superior performance of HCl during the chemical analysis when atomic absorption spectroscopy is used. They observed that

EDTA($NH_4)_2CO_3$ tended to clog the atomizer on the spectrophotometer. Ponnamperuma et al. (1981) found the 0.05N HCl method to be more effective in identifying Zn-deficient soils than DTPA (diethylene-triaminepentaacetic acid) and EDTA ($NH_4)_2CO_3$.

Good correlations between nutrient concentrations in plants and DTPA soil extracts have been found not only for Zn, but also for copper, iron, and manganese (Lindsay and Norvell, 1978). This has resulted in the adoption of DTPA as a universal soil extractant for micronutrients (Martens and Lindsay, 1990). Tiller et al. (1979) found a correlation coefficient of 0.79 between DTPA-Zn and Zn concentration in the rice plants. Ziah et al. (1994) found similar results working with soils of Pakistan. He found DTPA-Zn to be positively correlated to tissue Zn concentration, plant Zn uptake, DMY, and number of tillers per plant.

In Arkansas, prior to 1987, micronutrients were extracted with DTPA. However, recommendations for Zn application to rice fields were based solely on soil pH rather than extractable Zn (Slaton et al., 1994). After 1987, the University of Arkansas Soil Testing Laboratory at Marianna and the Diagnostic Research Laboratory at Fayetteville adopted the Mehlich 3 extractant. Because Mehlich 3 is suitable for the extraction of macronutrients and micronutrients, many laboratories are adopting this method for routine soil analyses (Martens and Lindsay, 1990). The use of ICPAES (inductively coupled plasma atomic emission spectroscopy) for multi-element analysis lends itself as a complement to the Mehlich 3 extractant. The University of Arkansas Cooperative Extension Service established less than 1.1 mg of Zn kg^{-1} soil extracted by Mehlich 3 as a very low soil Zn level (Soil Test Recommendations Guide, University of Arkansas Cooperative Extension Service, 1998). Nevertheless, a critical level for rice has not been established.

Vocaseck and Friedericks (1994) found that Mehlich 3 correlates positively with DTPA. They developed the following equation to relate Mehlich 3-extractable Zn to DTPA-Zn:

$$\text{M3-Zn} = 0.104 + 1.709(\text{DTPA-Zn}) \qquad \text{[Eq. 1]}$$

In Arkansas it has been suggested that Mehlich 3 extracts approximately two times more Zn than DTPA (W.E. Sabbe, 1997 personal communication).

The objectives of this study were to (1) evaluate several granular Zn fertilizer sources for their effectiveness in increasing DMY, Zn concentration, and total Zn uptake by rice shoots and in preventing Zn deficiency symptoms in the greenhouse; (2) evaluate the solubility of Zn in water and

solubility of Zn in a 2% EDTA solution of these sources as potential indices of available Zn in various granular Zn sources; (3) determine the effect of granule size on the availability of Zn to rice from a low-solubility Zn oxysulfate; and (4) establish a relationship between Zn extracted with DTPA (Lindsay and Norvell, 1978), Mehlich 3 (Mehlich,1984), and 0.05 M HCl (Katyal and Ponnamperuma, 1974) and DMY, Zn concentration, and Zn uptake by rice grown on a Dewitt silt loam in the greenhouse.

MATERIALS AND METHODS

To achieve the given objectives, three greenhouse and laboratory experiments were conducted. Each experiment was conducted at the University of Arkansas Main Agricultural Experiment Station with Dewitt (formerly Crowley) silt loam (fine, montmorillonitic, thermic, Typic Albaqualfs) that was collected from the University of Arkansas Rice Research and Experiment Station.

The organic carbon of the soil was 1% , determined by dry combustion using a Leco CNS-2000 (Leco Corporation, St. Joseph, Mich.). The original DTPA-extractable Zn was determined to be 0.33 mg kg^{-1} by the method of Lindsay and Norvell (1978). The pH of the soil was increased from 6.8 to 7.5 on the basis of a buffer curve by addition of 1.2 g per pot (1.8 mg ha^{-1}) of reagent-grade $CaCO_3$ 15 d prior to planting to enhance the probability of Zn deficiency.

Experiment 1

For the first greenhouse study, three rice plants (cv. Cypress) were grown in black polyethylene pots 15 cm (6 in.) in diameter containing 1.5 kg (3.3 lb) of the soil described above. Seven Zn sources described in Table 1 were utilized in this study. The Zn sources were applied prior to planting and mixed with the top 2 cm (0.78 in.) of soil at the rates of 0, 5.6, 11.2, 16.8, and 22.4 kg of Zn ha^{-1} (i.e., 0, 5, 10, 15, and 20 lb acre^{-1}). All sources were granules between 1 and 2 mm in diameter (Table 2). To promote Zn deficiency, phosphorus (P) was applied as reagent-grade $Ca(H_2PO_4)_2$ at a rate of 0.56 g per pot (81 kg of P ha^{-1}; 72 lb of P acre^{-1}; 165 lb of P_2O_5 acre^{-1}) prior to planting.

Ten pregerminated rice seeds were lightly embedded in the soil, which had a water content by weight of 33% (Q_w = 0.33). This water content was approximately 80% of saturation. The rice plants were thinned to three per pot 10 d after seeding. Nitrogen (N) was applied pre-flooding as a solution of urea to provide 0.2 g of N per pot (100 kg N ha^{-1}; 89 lb acre^{-1}) 15 d after seeding. The flood was established 16 d after seeding and maintained

at a depth of 5 cm during the remainder of the experiment.

Plants were evaluated for the occurrence of bronzing (a purplish-brown discoloration, typical of Zn-deficient rice) 7 d after flooding. The shoots of the rice plants were harvested 20 d after flooding, passed through a 0.2% phosphate-free detergent solution, rinsed twice with deionized water, dried

Table 1. Characteristics of Zn sources used for Experiment 1.

Source	Abbreviation	Manufacturer[†]	Total Zn Concentration			
			Labeled	Measured[‡]	WS[§]	ES
			——————— (%) ———————			
Zn Sulfate	CoZinco	CoZinco, Inc.	31	31	100	100
Zn Lignosulfonate	Ruffin	Ruffin Micronutrients, Inc.	10	11	76	87
Zn Chelate[¶]	F12	Frit Industries, Inc.	12	16	71	65
Zn Oxy-Sulfate	F20	Frit Industries, Inc.	20	27	54	39
Zn Oxy-Sulfate	F36	Frit Industries, Inc.	36	28	4	11
Zn Sulfate	FZnS	Frit Industries, Inc.	36	32	100	100
Zn Carboxylate	Granusol	American Minerals, Inc.	36	31	3	49

[†] Note: The mention of a trade name does not constitute endorsement of that product.

[‡] Analysis of Zn was performed in accordance to the Official Methods of the AOAC and inductively coupled plasma atomic emission spectroscopy.

[§] WS = water-soluble zinc; ES = EDTA-soluble Zn.

[¶] According to MSDS this product contains fulvic and citric acids.

Table 2. Average number of granules per pot added to supply four Zn application rates[†] for Experiment 1.

Source	Granules (kg ha⁻¹)			
	5.6	11.2	16.8	22.4
	——————— (no. per pot) ———————			
CoZinCo	5	9	14	18
Ruffin	14	26	40	51
F12	13	29	43	68
F20	5	9	13	17
F36	3	7	9	14
FZnS	3	5	8	10
Granusol	4	8	11	15

[†] Rates based on total Zn from product label.

to constant weight at 60°C, ground with a Wiley Mill to pass a 0.850-mm (0.033-in.) screen, digested in nitric acid (HNO_3), and analyzed by ICPAES (Jones and Case, 1990) on a Spectro Model D (Spectro Analytical Instruments, Finchburg, Mass.). Total Zn, water-soluble Zn, and EDTA-soluble Zn were determined following the methods described by the Association of Official Analytical Chemists (now known as AOAC International) (1995) (Table 1).

The experiment was arranged as a completely randomized factorial design with four Zn rates, seven Zn sources, and five replications. Five control pots were also grown to provide a means for comparison. Analysis of variance and regression analyses were conducted with the PROC GLM procedure in SAS (SAS Institute, Inc., 1988).

Experiment 2

A second greenhouse experiment was conducted by placing 8 kg (17.6 lb) of soil in black polyethylene pots 25.4 cm (10 in.) in diameter. In order to promote Zn deficiency, P was mixed with the top 2 cm of soil as reagent-grade $Ca(H_2PO_4)_2$ at a rate of 1.6 g per pot (81 kg of P ha^{-1}; 72 lb of P acre^{-1}; 165 lb of P_2O_5 acre^{-1}) prior to planting. Zinc was incorporated as granules to the top 2 cm of soil 1 d prior to planting at a rate of 57 mg of Zn per pot (11.2 kg ha^{-1}; 10 lb acre^{-1}).

The source utilized to provide the Zn treatments was 36% Zn oxysulfate, a product containing 36% total Zn, of which 4% was water-soluble (Table 1). One hundred grams of the Zn oxysulfate was placed in a nest of sieves consisting of the following sizes: 2.83, 2.36, 2.00, 1.70, and 1.40 mm (0.11, 0.09, 0.08, 0.07, and 0.06 in.). The contents of the 2.36-, 2.00-, and 1.40-mm sieves were used as treatments and were designated as 2.5, 2.0, and 1.5 mm, respectively. The powder treatment consisted of Zn oxysulfate ground with a mortar and pestle. A more detailed description of the particle size distribution of the powder is shown in Table 3.

Twenty pregerminated rice seeds (cv. Cypress) were lightly embedded in the soil, which was saturated with water (3 mm of water was standing on the surface of the soil). The rice plants were thinned to 10 per pot 10 d after planting. Urea was applied at a rate of 1.85 g per pot dissolved in 50 mL of double deionized water in order to provide 853 mg of N per pot (equivalent to 150 lb of N acre^{-1}) 1 d prior to flooding. The flood was established 15 d after planting and maintained at a depth of 10 cm (4 in.) for the remainder of the experiment.

The plants were evaluated for symptoms of Zn deficiency 7 d after flooding and harvested 20 d after flooding. Measurements were made for plant height and number of tillers per plant. The plants were then dried,

Table 3. Approximate particle size (X) of 36 % Zn oxysulfate used as "Powder" treatment in Experiment 2.

Particle Size[†]	Distribution (%)
X > 1 mm	5.8
1 mm > X > 0.420 mm	24.3
0.420 mm > X > 0.250 mm	19.1
0.250 mm > X > 0.149 mm	34.5
0.149 mm > X	16.3

[†] Based on a 25-g sample.

weighed, ground, and analyzed for Zn as previously described.

The experiment had a single factor (granule size) and was arranged in a completely randomized design with five treatments and four replications. The five particle-size treatments were powder (< 1.5 mm), 1.5, 2.0, 2.5 mm, and an untreated check. Analysis of variance and regression analyses were performed with the PROC GLM procedure in SAS (SAS institute, 1988).

Experiment 3

One of the limitations to making accurate Zn fertilizer recommendations is the absence of a suitable soil test procedure. As a preliminary approach to developing a Zn soil test method, a greenhouse study was conducted to determine whether extractable Zn determined by three methods could be related to rice dry matter and Zn uptake. The greenhouse experiment was implemented by filling black polyethylene pots (15 cm in diameter) with 1.5 kg of the Dewitt silt loam described above. Zinc was incorporated as granules of CoZinco $ZnSO_4$ at rates of 0, 5.6, 11.2, 16.8, and 22.4 kg of Zn ha^{-1} calculated on an area basis. Nitrogen was applied as urea at a rate of 0.2 g of N per pot (100 kg ha^{-1}; 89 lb acre^{-1}) dissolved in 100 mL of double deionized water 6 h prior to flooding.

Fifteen pregerminated rice seeds (cv. Cypress) were lightly embedded in the soil, which had a water content by weight of 33% ($Q_W = 0.33$). This water content was approximately 80% of saturation. The rice plants were thinned to five per pot 10 d after planting. The flood was imposed 15 d after planting and maintained at a depth of 5 cm throughout the remainder of the experiment.

The plants were evaluated for symptoms of Zn deficiency 7 d after

flooding. Plants were harvested 20 d after flooding. The shoots were separated from the roots and washed in a 0.2% phosphate-free detergent solution, and then rinsed two times with double deionized water. The plant tissue was dried to constant weight at 80°C in a forced-air oven, weighed, and ground with a Wiley Mill to pass through a 0.850-mm screen. A 0.5-g sample was digested with HNO_3 by the method described by Jones and Case (1990) and analyzed by ICPAES on a Spectro Model D.

A soil sample was obtained from each observation prior to planting, and extracted by DTPA (Lindsay and Norvell, 1978), Mehlich 3 (Mehlich, 1984), and 0.05M HCl (Katyal and Ponnamperuma, 1974) following the procedure described by the original authors of each method. The extraction was performed in 60-ml polyethylene bottles at 200 reciprocations per minute on a horizontal shaker. The extracted soil was filtered through 125-mm Whatman 42 paper and collected in 20-ml polyethylene vials. The extracts were analyzed by ICPAES.

The experiment had a single factor, Zn rate, and was arranged in a completely randomized design with five treatments and five replications. The treatments were 0, 5.6, 11.2, 16.8, and 22.4 kg of added Zn ha^{-1} (0, 5, 10, 15, and 20 lb acre^{-1}). Analysis of variance was performed to determine the effect of rate on plant parameters, and regression analysis was used to establish a relationship among soil test methods and plant parameters with SAS (SAS Institute, 1988).

RESULTS AND DISCUSSION
Experiment 1

Occurrence of Symptoms. The frequency of the occurrence of bronzing is shown in Figure 1. When analyzed in a contingency table using a chi-squared test of homogeneity, the Zn sources fell into two significantly (a = 0.05) different groups. Group 1: CoZinCo, F12, F20, Ruffin, and FZnS had bronzing showing in 24% of the pots. Group 2: F36, Granusol, and the control showed bronzing in 67% of the pots. Thus the sources in group 1 appeared to be effective in reducing the occurrence of bronzing. In most of the pots, bronzing symptoms had attenuated by harvest time. The self-recovery of Zn-deficient rice has been previously reported (Nene, 1966).

Dry Matter Yield. Rice DMY did not show a statistically significant interaction between the Zn source and the rate applied. Application of CoZinco, F12, F20, and Ruffin significantly increased DMY compared with the control (Fig. 2). There was also an increase in DMY due to application of FZnS, but this increase was significantly lower than the increase obtained from the sources previously mentioned. Granusol and F36 failed to increase DMY.

A significant linear increase in DMY was observed with increasing rates of Zn (Fig. 3). The high coefficient of determination ($R^2 = 0.92$) indicates a strong relationship between DMY and Zn fertilizer rate. On this soil, the DMY increased an average of 69 mg per pot (34 lb acre^{-1}; 38 kg ha^{-1}) for each additional kilogram of Zn ha^{-1} applied.

Tissue Zn Concentration. A significant interaction between Zn source and Zn rate was observed for Zn concentration in the rice plant. The tissue Zn concentration of the control plants was 19 mg kg^{-1} (19 ppm) (Table 4). This concentration is in the 'likely deficient range' of 15 to 20 mg of Zn kg^{-1}, which has been reported by Tanaka and Yoshida (1970) and Sedberry et al. (1971). The mean tissue Zn concentration of plants receiving CoZinco, FZnS, F12, F20, and Ruffin were significantly higher than the tissue Zn concentration of plants in the control pots at all except at the 5.6-kg ha^{-1} rate. However, the mean Zn concentrations of plants treated with Granusol and F36 at 5.6, 11.2, and 16.8 kg ha^{-1} were either lower or the same as plants in the control pots (Table 4).
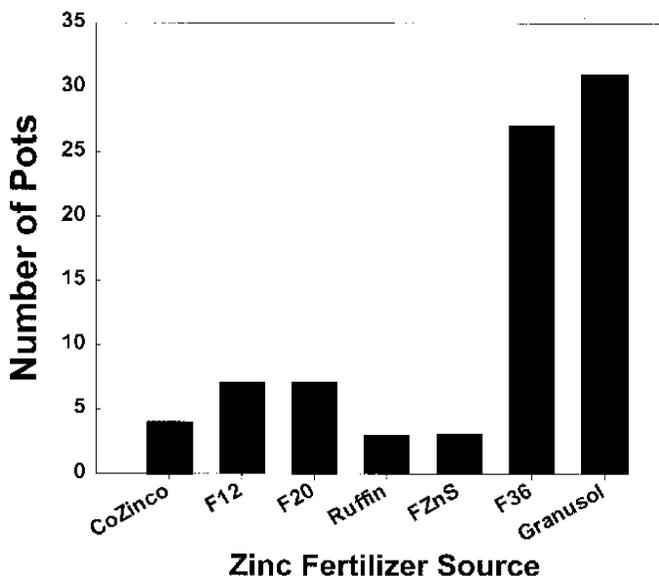

Figure 1. Comparison of the frequency of Zn deficiency symptoms associated with seven Zn fertilizer sources in Experiment 1.
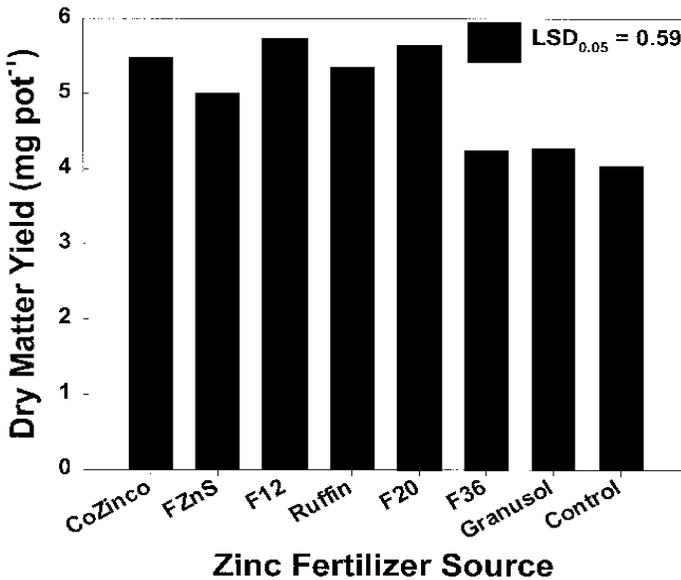
Figure 2. Rice dry matter yield 20 d after flooding as influenced by Zn fertilizer source in Experiment 1.

Total Zn Uptake. An interaction between Zn fertilizer rate and source significantly affected total Zn uptake (Table 4). At the rates of 11.2, 16.8 and 22.4 kg of Zn ha^{-1}, all sources significantly (α = 0.05) increased Zn uptake compared with the untreated control, except for the low-water-solubility sources, F36 and Granusol. Increasing the Zn rate resulted in increased Zn uptake for all sources except for F36. None of the sources increased uptake significantly (α = 0.05) over that of the control at the 5.6 kg of Zn ha^{-1} rate. The solubility of the source producing the greatest Zn uptake (F12) was 71% in water and 65% in EDTA (Table 1). The number of granules per pot (Table 3), coupled with the complexing ability of the fulvic acid and citric acid contained in this product, may have contributed to the greater increase in Zn uptake for F12. Fulvic acid, a common component of organic matter, has been found to form stable chelates in soils (Stevenson, 1991) while citric acid has been shown to increase absorption of Zn by plants grown in calcareous soil (Al-Bahrany and Al-Shabaan, 1995). Because of the low mobility of Zn from a granule (Mordvedt, 1992), it would

Table 4. Influence of Zn fertilizer source and rate on rice dry matter yield and tissue concentration 20 d after flooding in Experiment 1.

| Zn Rate	Zn Tissue Concentration							Total Zn Uptake						
	CoZinco	FZnS	F12	Ruffin	F20	F36	Granusol	CoZinco	FZnS	F12	Ruffin	F20	F36	Granusol
	———————————— (mg kg^{-1}) ————————————							———————————— (mg per pot) ————————————						
0				19.7							81.4			
5.6	21.4	20.5	23.1	22.1	23.7	17.1	18.0	109.1	103.0	123.0	117.2	125.5	77.5	68.2
11.2	26.9	24.6	32.0	29.1	27.3	17.0	20.1	151.7	138.4	176.1	135.9	153.7	58.4	80.4
16.8	35.0	35.0	43.7	34.9	33.0	19.1	21.9	200.8	156.3	264.7	168.6	189.7	93.1	94.7
22.4	42.6	42.2	44.4	40.1	32.8	21.4	21.0	224.5	212.4	267.4	255.1	183.7	93.1	107.2
LSD	6.8		44.6											
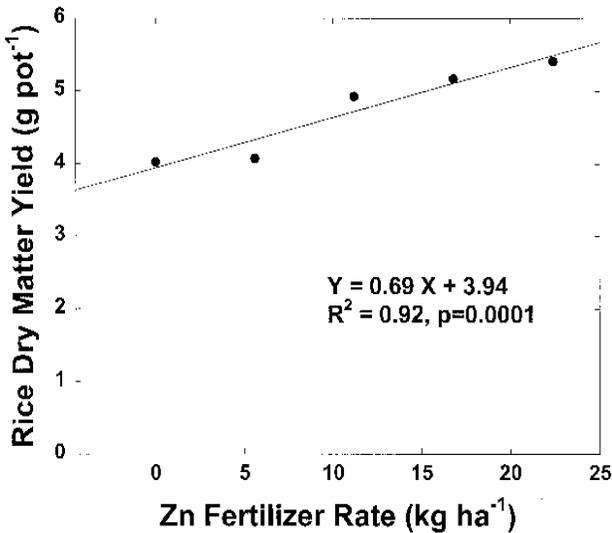
Figure 3. Relationship between rice dry matter yield 20 d after flooding and of Zn fertilizer rate in Experiment 1.

be expected that a greater number of granules per pot would enhance the effectiveness of a granular Zn fertilizer as a result of a more even distribution throughout the soil.

   Solubility in Water and in EDTA. Sources with 100% water-soluble Zn did not improve uptake over the 71% water-soluble Zn source (Fig. 4). The source with 54% water-soluble Zn improved uptake over that of plants in the control pots. Sources with 4% or less water-soluble Zn did not provide a significant increase in DMY, Zn concentration, and total Zn uptake over that of plants in the control pots (Fig. 2, Table 4). Mordvedt (1992) and Amrani et al. (1997) found that at least 40% or 50%, respectively, of the total Zn in granular fertilizers should be water-soluble to be effective.

   Zinc uptake had both a linear and a quadratic response to water-soluble Zn and EDTA-soluble Zn (Fig. 4). The larger values of R^2 for water-soluble Zn and the smaller levels of significance associated with them (Table 5) indicate that water-soluble Zn is more strongly correlated to Zn uptake and is a better predictor of uptake than EDTA-soluble Zn. With EDTA-soluble Zn, the quadratic response was significant ($\alpha = 0.05$) at only the rates of 11.2 and 16.8 kg ha^{-1} (Table 6). A value of $R^2 = 0.64$ (the highest obtained in

this experiment) indicated that only 64% of the variability in Zn uptake could be attributed to water-soluble Zn.

Another factor must have influenced Zn uptake, possibly placement or distribution of the granules. Of the effective sources, F12 and Ruffin had the greatest number of granules per pot (Table 2). Because of the actual Zn content of the sources used and their different densities, it was impossible to apply the same number of granules to each pot (Table 3). The sources that provided a significant increase in uptake were manufactured using $ZnSO_4$ as the source of Zn. Those that did not increase uptake significantly
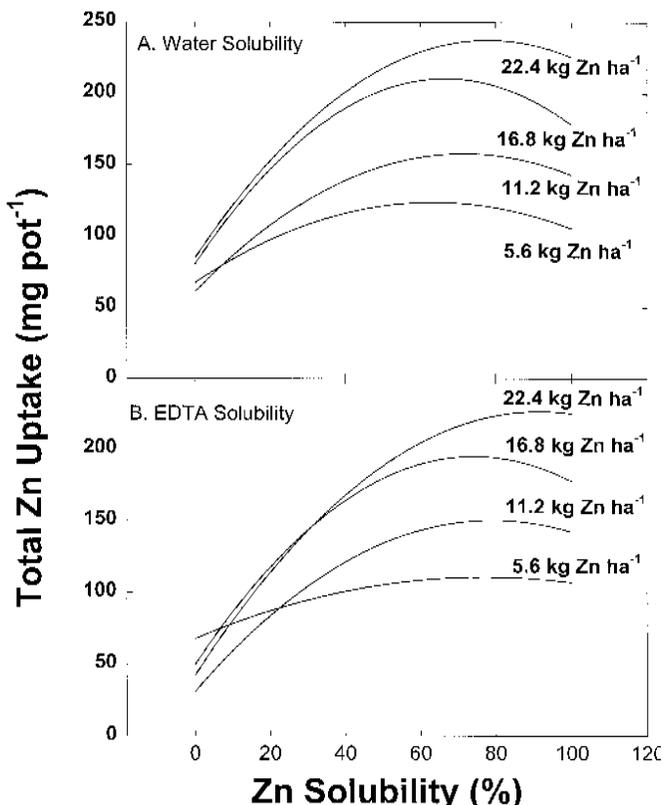

Figure 4. Total Zn uptake by rice 20 d after flooding as influenced by Zn fertilizer rate and the amount of water-soluble Zn (A) *or 2% EDTA-soluble Zn* (B) *in the fertilizer in Experiment 1.*

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were manufactured with ZnO as the primary source of Zn.

Experiment 2

Occurrence of Symptoms. When the degree of bronzing was assessed 7 d after flooding the rice, at least some degree of bronzing was observed in 80% of the pots (Table 7). Powdered Zn oxysulfate was effective in reducing the occurrence of bronzing. Plants receiving the powder form showed bronzing in only 25% of the pots. Bronzing in cotton has been associated with iron toxicity (Ponnamperuma et al., 1955) and with excess P, causing an accentuation of Zn deficiency due to a large P/Zn ratio (Cakmak and Marschner, 1986). In this study, plants showing bronzing appeared to have higher P and also higher iron concentrations than plants not showing bronzing (Table 8).

Dry Matter Yield. Significantly greater DMY (22.5 g per pot) was ob-

Table 5. Statistical parameters associated with regression analysis of uptake and percentage of water solubility (WS) for Experiment 1.

Rate			*P*-value	
(kg Zn ha^{-1})	Equation	R^2	WS[†]	WS^2
5.6	$U = 67 + 1.78WS - 0.014WS^2$	0.45	0.0007	0.0015
11.2	$U = 61 + 2.71WS - 0.019WS^2$	0.64	0.0001	0.0006
16.8	$U = 80 + 3.91WS - 0.029WS^2$	0.55	0.0001	0.0007
22.4	$U = 84 + 3.92WS - 0.025WS^2$	0.61	0.0001	0.0063

[†] WS = percentage of total Zn that is water-soluble.

Table 6. Statistical parameters associated with regression analysis of uptake and percentage of EDTA solubility (ES) for Experiment 1.

Rate			*P*-value	
(kg Zn ha^{-1})	Equation	R^2	ES[†]	ES^2
5.6	$U = 68 + 1.13ES - 0.007ES^2$	0.12	0.0986	0.2499
11.2	$U = 31 + 3.02ES - 0.019ES^2$	0.38	0.009	0.0209
16.8	$U = 49 + 3.91ES - 0.026ES^2$	0.26	0.0177	0.0355
22.4	$U = 42 + 4.02ES - 0.021ES^2$	0.42	0.0001	0.0981

[†] ES = percentage of total Zn that is EDTA-soluble.

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tained with application of powdered Zn oxysulfate compared with the no-Zn and the 2.5-mm treatments (Table 7). Plants receiving 1.5-mm granules also had significantly higher DMY than plants receiving no Zn and 2.5-mm granules. When compared with all other treatments, the DMY of plants receiving 2-mm granules was not significantly different.

Plant heights and number of tillers per plant measured at harvest are shown in Table 7. Although height and tillering differed among treatments, the granule size did not significantly affect plant height or tillering. A positive relationship could be observed between plant height and Zn uptake (R^2 = 0.68), and between the number of tillers per plant and Zn uptake (R^2 = 0.49) (Fig. 5). The Zn concentration in the rice shoots was not correlated with plant height or number of tillers per plant.

Tissue Zn Concentration. Plant Zn concentration was also influenced by the particle size of the Zn fertilizer (Table 7). The mean concentration of plants receiving powder Zn oxysulfate was 23.4 mg kg^{-1}. This value is in the range of sufficiency as described by Tanaka and Yoshida (1970). Plants receiving granular Zn oxysulfate or no Zn had a significantly lower Zn concentration than plants receiving powdered Zn. The mean concentration for these plants ranged from 17.1 to 17.6 mg kg^{-1}, which is in the "likely Zn-deficient range" of 15-20 mg kg^{-1} (Tanaka and Yoshida, 1970). Since differences in DMY were significant among plants treated with no Zn or granular Zn oxysulfate, the observation of similar concentrations among these treatments can be attributed to a dilution effect (Table 7).

Total Zn Uptake. Plants treated with powdered Zn oxysulfate had

Table 7. Plant height, number of tillers per plant, DMY, Zn concentration, Zn uptake, and percentage of pots showing bronzing in rice treated with different granule size of 36% Zn oxysulfate in Experiment 2.

Granule Size	Height (cm)	Tillers per Plant	DMY (g)	Zn (mg kg^{-1})	Zn Uptake (mg per pot)	Bronzing (%)
No Zn	63.08[a]	3.75[a]	12.02[a]	17.1[a]	0.20[a]	100
Powder	69.23[b]	5.25[b]	22.49[b]	23.4[b]	0.52[b]	25
1.5 mm	65.78[a]	5.25[b]	21.17[b]	17.2[a]	0.37[ab]	75
2.0 mm	69.98[b]	5.50[b]	16.96[ab]	17.6[a]	0.31[ab]	100
2.5 mm	64.58[a]	4.25[ab]	13.66[a]	17.6[a]	0.24[a]	100
LSD[†]	2.72	1.34	5.61	3.87		
P-value	0.0002	0.0596	0.0044	0.0138		

[†] Means in the same column followed by the same letter were not statistically significant at α = 0.05 confidence level.

Table 8. Mean nutrient concentration and ratios of selected nutrients of rice shoots treated with 36 % Zn oxysulfate of different granule size in Experiment 2.

Granule size	Ca	K	Mg	Na	P	S	Cu	Fe	Mn	Zn	P/Zn	K/Na	Mg/Na	Ca/Na
	(g kg⁻¹)									(mg kg⁻¹)				
NoZn	1.76[a]	37.0[b]	2.18[c]	1.12[a]	4.02[c]	2.79[a]	5.95[a]	132[a]	899[a]	17.08[a]	0.024[c]	33.59[b]	1.98[c]	1.596[b]
Powder	2.07[c]	31.3[a]	1.54[a]	2.16[b]	2.98[b]	2.83[a]	6.45[ab]	90[a]	1036[ab]	23.40[b]	0.013[a]	14.65[a]	0.73[a]	0.971[a]
1.5 mm	1.90[ab]	33.8[ab]	1.58[ab]	1.50[a]	2.78[c]	2.77[a]	7.33[abc]	109[a]	1169[bc]	17.18[a]	0.016[b]	24.14[ab]	1.12[ab]	1.353[ab]
2.0 mm	1.95[bc]	36.0[b]	1.77[b]	1.36[a]	2.90[ab]	3.19[b]	8.75[bc]	99[a]	1340[c]	17.55[a]	0.017[b]	28.39[b]	1.38[b]	1.518[b]
2.5 mm	1.83[ab]	34.4[ab]	1.65[ab]	1.47[a]	2.93[ab]	3.08[b]	8.15[bc]	134[a]	1181[bc]	17.55[a]	0.017[b]	25.68[ab]	1.23[ab]	1.340[ab]
LSD[†]	0.1	3.56	0.21	0.5	0.174	0.237	1.8	44.41	181.8	3.87	0.0032	11.86	0.55	0.5234
P-value	0.004	0.0332	0.0001	0.007	0.0001	0.0044	0.0268	0.1879	0.0015	0.0138	0.0001	0.0469	0.004	0.1579

† LSDs were calculated at α = 0.05. Means in the same column followed by the same letter are not significantly different.

Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; N = sodium; P = phosphorus; S = sulfur.

significantly greater Zn uptake than plants receiving no Zn or 2.5-mm granules (Table 7). Plants treated with 1.5- and 2-mm granules did not have a significantly lower uptake than plants treated with the powdered Zn, but they also were not significantly different from the control.

Even when a gradual increase in Zn uptake could be observed with decreasing granule size (Table 7), only the powder form produced plants with Zn concentrations in the sufficient range. This lack of efficiency of the granular form can be attributed to the low water solubility of the fertilizer (Table 1, Fig. 4) and to the distribution of the granules throughout the soil. Since granules of 1.5 mm weighed less than granules of 2.0 or 2.5 mm,
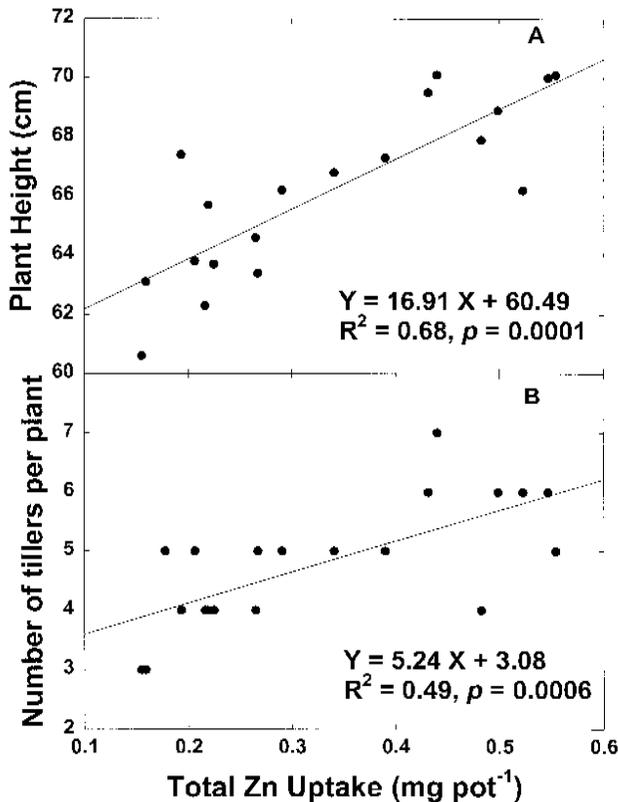
Figure 5. Relationship between rice plant height (A) *or tiller production* (B) *and total Zn uptake by rice 20 d after flooding in Experiment 2.*

more smaller granules were used, resulting in a better distribution of the Zn throughout the soil, higher surface area of contact of Zn fertilizer with the soil, and subsequently better Zn uptake. The effect of distribution was also evidenced by the large variation in Zn uptake by plants treated with granular Zn oxysulfate. The standard deviations in Zn uptake for plants treated with granules of 1.5, 2.0, and 2.5 mm were 0.12, 0.14, and 0.08 mg, respectively, whereas for the no-Zn and powdered Zn treatments the standard deviations were only 0.03 and 0.04 mg, respectively. Wells (1980) and Wells et al. (1973) recommended 11.2 kg of Zn ha^{-1} (10 lb acre^{-1}) for rice, although only 1 to 3 kg of Zn ha^{-1} (1 to 3 lb acre^{-1}) was actually needed. The excess Zn was recommended to overcome the low mobility of Zn in the soil and the distribution problem associated with broadcasting 5 to 6 kg ha^{-1} of granular material.

Effect of Zn Sufficiency on Other Nutrients. The preferential uptake of divalent cations over monovalent cations by Zn-deficient rice was reported by Moore and Patrick (1988). Their observations were corroborated in this study (Table 8). Plants receiving powdered Zn oxysulfate showed less bronzing and had the highest DMY, Zn concentration, and Zn uptake (Table 7). These plants also had lower potassium-to-sodium, magnesium-to-sodium, and P/Zn ratios and a lower magnesium concentration than plants treated with granular Zn oxysulfate (Table 8). This observation is of particular interest because the correlation coefficients associated with DMY and these ratios and nutrient concentrations are larger than the correlation coefficient for the DMY and Zn concentration (Table 9). Katyal and Ponnamperuma (1974) also observed that the magnesium concentration of Zn-deficient plants was always higher than that of healthy plants.

Welch at al. (1982) related Zn deficiency to leakage of low-molecular-weight solutes from the cell membrane of roots of Zn-deficient plants. The application of powdered Zn oxysulfate seemed to have brought about a different yet beneficial nutrient balance that resulted in a significant increase in DMY. The rice plants showed improved growth with higher tissue concentration of sodium (Fig. 6), attributable to the addition of Zn. Although sodium is considered an essential micronutrient (Brownell, 1979) and some plants show an increase in DMY due to the uptake of sodium (Lehr, 1953), a beneficial effect of sodium on rice has not been documented. Marschner (1971) described the existence of plants that exhibit a positive response to substitution of sodium for potassium in the tissue. The enhancement in growth is primarily due to cell enlargement rather than an increase in photosynthetic efficiency. A larger photosynthetic area results in increased DMY. In this experiment, when Zn was supplied to the plants, the tissue sodium concentration increased and the potassium concentration decreased (Figs. 6 and 7). This apparent substitution of sodium for

potassium was higher for plants receiving powdered Zn oxysulfate. Dry matter yield was negatively correlated (R^2 = 0.39, P = 0.0033) to tissue potassium concentration (Fig. 6).

Table 9. Correlation coefficients associated with DMY and selected nutrients and ratios of nutrients in rice shoots in Experiment 2.

Nutrient	r	P-value
Mg/Na	−0.81	0.0001
Na	0.79	0.0001
K/Na	−0.77	0.0001
Mg/Zn	−0.72	0.0003
Ca/Na	−0.72	0.0003
P/Zn	−0.70	0.0005
Mg	−0.65	0.0016
Zn	0.51	0.0205

Ca = calcium; K = Potassium; Mg = magnesium; Na = sodium; P = phosphorus.
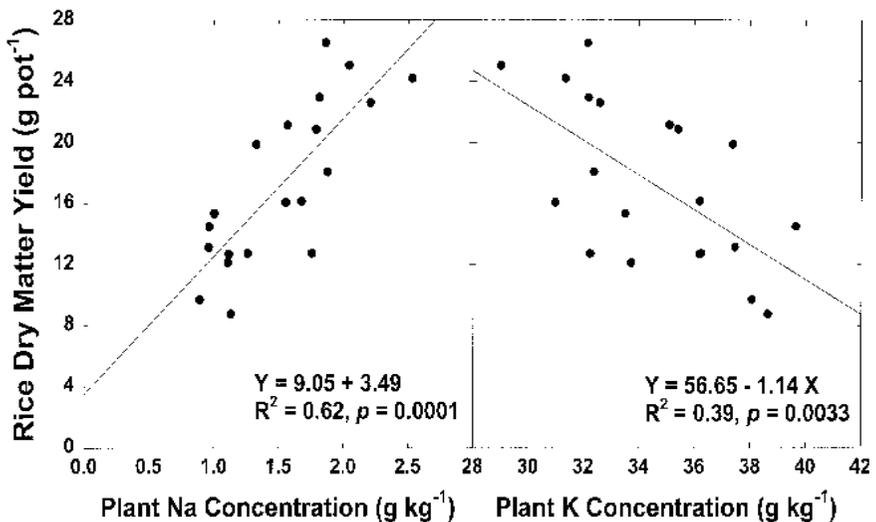
Figure 6. Relationship between rice dry matter production 20 d after flooding and sodium (Na) (A) or potassium (K) (B) uptake by rice in Experiment 2.

Experiment 3

Dry Matter Yield. DMY was significantly different from the DMYs of the control pots only for plants receiving 11.5 kg of Zn ha⁻¹ (Table 10). This was in contrast to prior experiments in which significant increases in DMY were obtained for treatments receiving more Zn (Fig. 3). The relationship between the three different soil test methods and the DMY of the rice shoots

Table 10. Plant Zn concentration, uptake, and DMY of rice shoots grown at different levels of Zn fertilization in Experiment 3.

Zn Rate (kg ha⁻¹)	Plant Zn (mg kg⁻¹)	Zn Uptake (mg per pot)	DMY (g per pot)
0	12.9ᵃ	21ᵃ	1.56ᵃ
5.6	49.2ᵇ	98ᵇ	1.97ᵃᵇ
11.2	55.4ᶜ	139ᶜ	2.49ᵇ
16.8	60.0ᵈ	135ᶜ	2.25ᵃᵇ
22.4	61.3ᵈ	135ᶜ	2.21ᵃᵇ
LSD†	3.2	34	0.76
P-value	0.0001	0.0001	0.1094

† Means followed by the same letter were not significantly different at $\alpha = 0.05$.

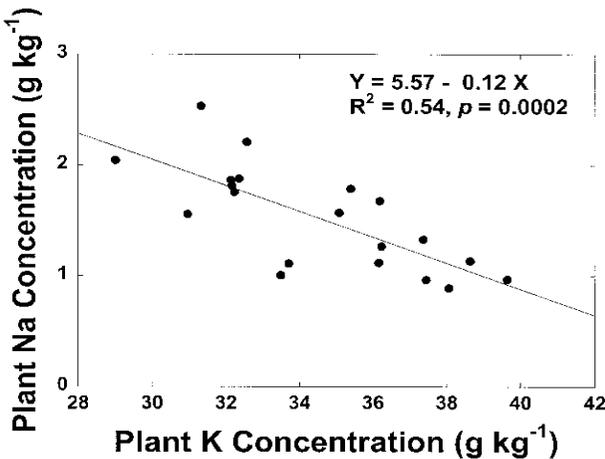
$$Y = 5.57 - 0.12\ X$$
$$R^2 = 0.54,\ p = 0.0002$$

Figure 7. Relationship between sodium (Na) uptake and potassium (K) uptake by rice 20 d after flooding in Experiment 2.

is illustrated in Figure 8. In each case, a significant quadratic response was observed. The DTPA extractant, considered the preferred micronutrient extractant (Jones et al., 1982), was slightly better at predicting rice DMY (R^2 = 0.58) than the Mehlich 3 (R^2 = 0.54) or HCl (R^2 = 0.51) extractants. However, all the methods were highly significant.

Tissue Zn Concentration. Plants receiving no Zn had an average tissue Zn concentration of 12.9 mg kg^{-1} (Table 10). This concentration was associated with soil-extractable Zn values of 0.37, 0.96, and 0.62 mg kg^{-1} of DTPA-extractable Zn, Mehlich 3-extractable Zn, and HCl-extractable Zn, respectively (Fig. 9). Also, it falls within the "very likely Zn-deficient range" (10 to 15 mg kg^{-1}) as defined by Tanaka and Yoshida (1970) and is below the 15-mg kg^{-1} critical level suggested by Sedberry et al. (1971). Plants from these pots were the only ones showing bronzing. Zinc concentration increased significantly with increases in the Zn application rate to 16.8 kg ha^{-1} (Table 10). The Zn concentration in the tissue of plants grown in pots treated with 5.6 kg ha^{-1} increased sharply over that of plants grown without Zn. But a more gradual increase was observed for plants growing at higher Zn rates than for those grown at 5.6 kg ha^{-1}.

Rice Zn concentration increased exponentially with increasing extractable Zn regardless of extraction method (Fig. 9). With use of the equation in Figure 9A and a critical Zn level of 20 mg kg^{-1}, the critical DTPA-extractable Zn level would be predicted to be 0.56 mg kg^{-1} (1.12 lb acre^{-1}). Mikkelsen and Brandon (1975) proposed 0.5-mg of Zn kg^{-1} (1 lb acre^{-1}) as the critical level for California soils. Given the equations in Figures 9A and 9B for Mehlich 3 and the HCl methods, the critical levels would be 1.2 and 0.82 mg of Zn kg^{-1} (2.4 and 1.64 lb acre^{-1}), respectively. Ziah et al. (1994) proposed 2.4 mg kg^{-1} of Mehlich 3-extractable Zn as the critical level for rice soils of Pakistan. Katyal and Ponnamperuma (1974) have proposed 1 mg kg^{-1} for the HCl method.

Total Zn Uptake. Total Zn uptake by plants receiving 5.6 kg ha^{-1} was significantly greater than that of plants receiving no Zn (Table 10). Plants receiving 11.2, 16.8, and 22.4 kg ha^{-1} had greater Zn uptake than plants receiving 5.6 kg ha^{-1}. This followed the pattern of the Zn concentrations in the shoots.

There was a highly significant quadratic response of Zn uptake to soil-extractable Zn (Fig. 10). The large R^2 indicated that a great proportion of the variability in Zn uptake can be ascribed to soil-extractable Zn. These coefficients were 0.82, 0.81, and 0.80 for DTPA, Mehlich 3, and 0.05M HCl, respectively. Thus each of the methods provided good estimates of total Zn uptake.

Comparison of Soil Test Procedures. The relationship between Mehlich 3-extractable Zn and DTPA-extractable Zn is shown in Figure 11. The R^2 for
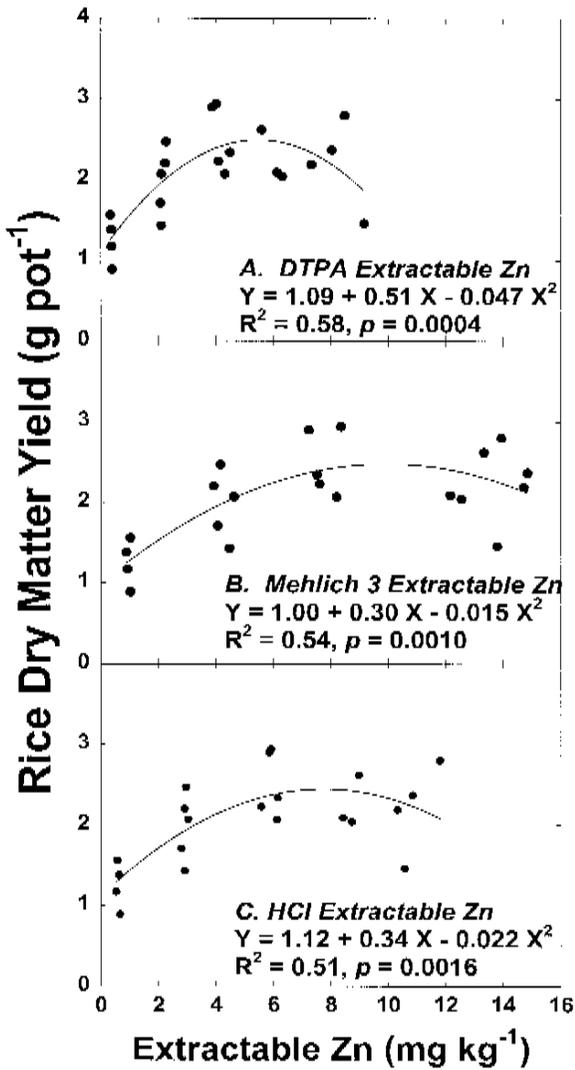
Figure 8. Relationship between rice dry matter production 20 d after flooding and soil available Zn extracted with DTPA (A), Mehlich 3 (B), or 0.05 M HCl (C) in Experiment 3.

the regression analysis was 0.95, and the *P*-value associated with the model was 0.0001. This R^2 value is equal to the 0.95 reported by Vocasek and Friedericks (1994) for several soils from the U. S., and greater than the 0.64 value reported by Ziah et al. (1994) for calcareous soils of Pakistan. García et al. (1997) reported an R^2 of 0.88 for Argentina soils. The equation determined from the current study was:

$$\text{M3-Zn} = 1.84(\text{DTPA-Zn}) + 0.61 \qquad \text{[Eq. 2]}$$

where, M3-Zn = Mehlich 3-extractable Zn and DTPA-Zn = DTPA-extractable Zn.

The slope and intercept obtained were similar to those reported by Vocasek and Friedericks (1994) (Eq. 1). If the equation for the model developed from this study applies, the normally reported critical range for DTPA-extractable Zn for rice soils, 0.5 to 0.8 mg kg^{-1} (Jones et al., 1982) would be equivalent to 0.92 to 1.47 mg of Mehlich 3-extractable Zn per kg of soil. The value calculated on the basis ofplant Zn concentration (1.2 mg kg^{-1}) also falls within this range (Fig. 9).

The relationship between 0.05M HCl-extractable Zn and DTPA-extractable Zn was highly significant, with an R^2 of 0.99 (*P*-value = 0.0001) (Fig. 11B). This R^2 value is similar to the one Katyal and Ponnamperuma (1974) obtained for soils in the Philippines (R^2 = 0.93). Using the equation developed for this method in relation to DTPA-extractable Zn, the critical values for HCl-extractable Zn would be 0.68 and 1.09 mg kg^{-1}. The critical HCl-extractable Zn level for rice suggested by Katyal and Ponnamperuma (1974) was 1.0 mg kg^{-1}, which is within the calculated range. The critical value determined in this study based on tissue Zn concentration, 0.82 mg kg^{-1}, also falls within this range (Table 4). If a quick assessment of soil Zn is necessary, this would be a very convenient method because of its ease of use. However, it does not lend itself to multi-element analysis and routine soil testing laboratory procedures. Also, it has been shown that free calcium carbonate ($CaCO_3$) may reduce the effectiveness of this extractant (Nelson et al., 1959). Therefore, the results of this method should be interpreted in light of other soil properties, such as pH or titratable alkalinity (Cox, 1987).

Mehlich 3-extractable Zn was very well correlated to HCl-extractable Zn (Fig. 12). If a good relationship could be established between Mehlich 3-extractable Zn and HCl-extractable Zn, then a comparison could be made between the two methods when results of an experiment were reported using HCl-extractable Zn. Using the model developed from the data in this experiment (Fig. 12), the critical level for HCl-extractable Zn reported by Katyal and Ponnamperuma (1974) would be equivalent to 1.35 mg of
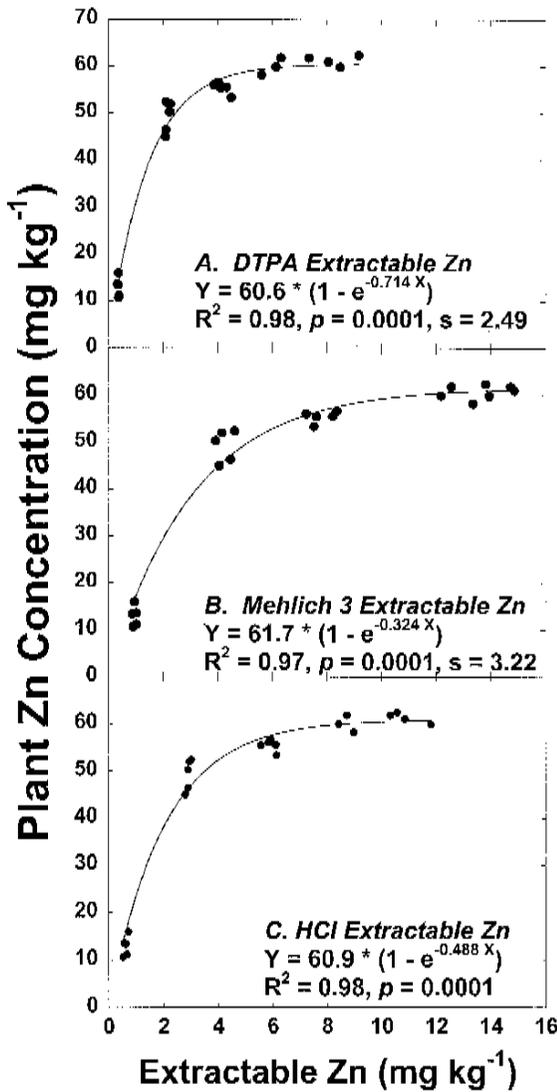
Figure 9. Relationship between Zn concentration in rice tissue 20 d after flooding and soil available Zn extracted with DTPA (A), Mehlich 3 (B), or 0.05 M HCl (C) in Experiment 3.
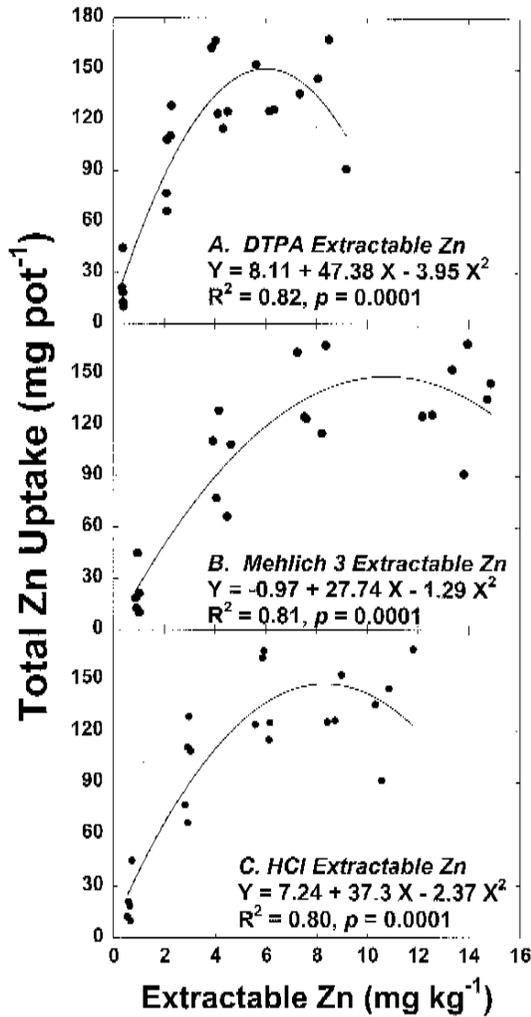
Figure 10. Relationship between total Zn uptake by rice 20 d after flooding and soil available Zn extracted with DTPA (A), Mehlich 3 (B), or 0.05 M HCl (C) in Experiment 3.

Mehlich 3-extractable Zn kg⁻¹ of soil. This value is considerably lower than the value reported by Ziah et al. (1994) for Pakistani soils. However, it is similar to the value determined from plant Zn concentration (Fig. 9) and is within the critical range determined for Mehlich 3-extractable Zn compared with DTPA-extractable Zn (Fig. 11A).
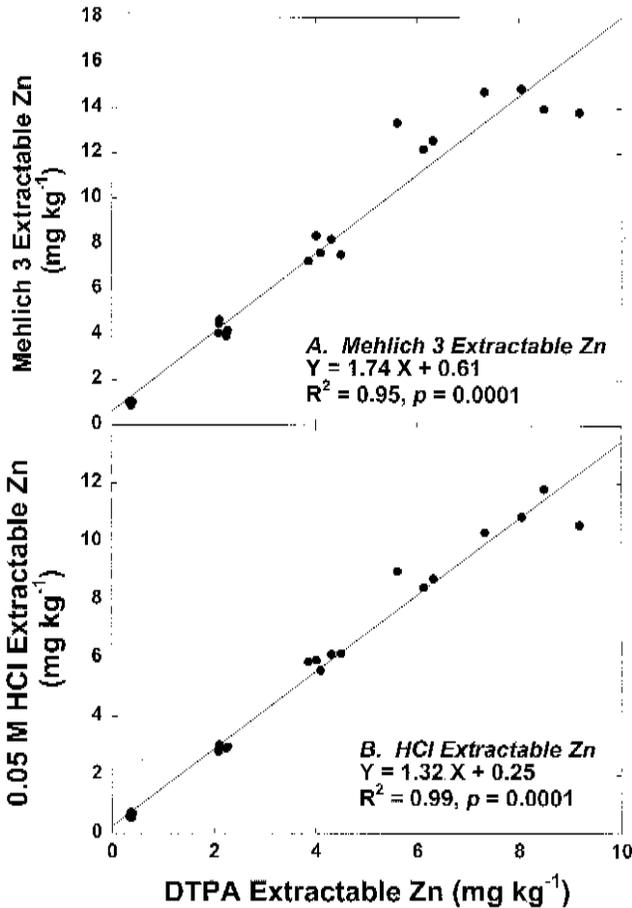

Figure 11. Relationship between extractable Zn by Mehlich 3 (A) *or 0.05 M HCl* (B) *and DTPA- extractable Zn in Experiment 3.*
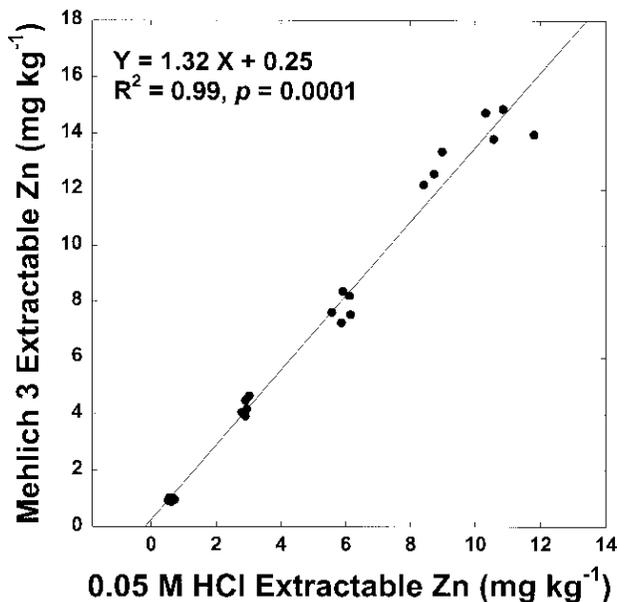
Figure 12. Relationship between Mehlich 3-extractable Zn and 0.05 M HCl-extractable Zn in Experiment 3.

CONCLUSIONS

Granular Zn sources with 54% or more of their total Zn as water-soluble were effective in decreasing bronzing and in increasing the Zn tissue concentration, Zn uptake, and DMY of rice when used at a rate of 11.2 kg ha^{-1} or greater. The sources evaluated that would be recommended on the basis of water solubility include CoZinco Zn sulfate, Ruffin Zn ligno-sulfonate, Frit 12% Zn Chelate, Frit 20% Zn oxysulfate, and Frit 36% Zn sulfate. A quadratic relationship was observed between water solubility and Zn uptake. With this relationship, the sources with approximately 70% of the Zn in water-soluble form (Frit 12% Zn and Ruffin Zn chelate) resulted in the highest Zn uptake. However, granular Zn fertilizers with 4% or less of the total Zn as water-soluble Zn were not effective in reducing the occurrence of bronzing or in increasing DMY, Zn tissue concentration, or Zn uptake by the rice crop grown immediately following application of the source. The sources that should not be applied based on water solubility include Frit 36% Zn oxysulfate and Granusol 36% Zn carboxylate.

However, all the sources should be applied at a rate of at least 11.2 kg of Zn ha^{-1}.

Uptake of Zn by the rice plant showed linear and quadratic responses to the percentage of solubility in water and EDTA, respectively. However, there was a stronger correlation between water-soluble Zn and uptake than between EDTA-soluble Zn and uptake. Thus water-soluble Zn would be a good index of plant-available Zn in fertilizer materials.

Zinc oxysulfate increased Zn concentration in the tissue of rice plants, Zn uptake, and DMY as the granule size decreased. In contrast, Zn applied in granules greater than 1.5 mm were not readily available to the plant. Zinc amendments as granules of Zn oxysulfate applied at 11.2 kg ha^{-1} and granule size greater than 1.5 mm had no effect on yields compared with a no-Zn control. If such a fertilizer were to be used for preventing Zn deficiency in rice, under the conditions of this study, granules that are 1.5 mm or smaller would be recommended.

Extractable-soil Zn by the methods tested was positively related to Zn concentration in the tissue of the rice plants and to the Zn uptake. The three methods were linearly related with each other. The mean tissue Zn concentration of Zn-deficient plants was 12 mg kg^{-1}. This concentration was associated with soil values of 0.37, 0.96, and 0.62 mg kg^{-1} of DTPA-extractable Zn, Mehlich 3-extractable Zn, and HCl-extractable Zn, respectively. Based on previously known critical values for DTPA-extractable Zn and HCl-extractable Zn and the relationship established between Mehlich 3 and the other two methods used in this study, the Mehlich 3-extractable Zn critical level for rice grown in conditions similar to this experiment is proposed to be between 0.92 and 1.5 mg kg^{-1}. More research is needed in this area to evaluate response in various soils and pH levels in both field and greenhouse studies.

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