MANGANESE SOLUBILITY AND PHYTOTOXICITY AFFECTED BY SOIL MOISTURE, OXYGEN LEVELS, AND GREEN MANURE ADDITIONS

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ABSTRACT

Manganese (Mn) toxicity is common in tropical acid soils, second only to aluminum toxicity. Changes in soil pH and redox potential ($E_h$) caused by soil moisture conditions and organic amendments certainly affect Mn solubility and toxicity to plants grown in soils with high Mn reserves. Laboratory incubation and greenhouse experiments were conducted to quantify such effects using a high-Mn Oxisol and a moderate-Mn Mollisol from Hawaii, with soybean (*Glycine max* L. cv. Kahala) being a test crop. The soils were mixed with a green manure (leucaena leaf) at 0, 10, and 20 g kg$^{-1}$, and were incubated at 24 ± 1 °C at 80% of field water holding capacity -- equilibrated with air (FC), or N$_2$ gas (N$_2$) -- or submerged under 8 cm of water. Soil pH, $E_h$, and soluble Mn as measured in saturated paste extract, Mehlich-3 and hydroxylamine hydrochloride (NH$_2$OH) solutions were periodically determined over 56 days of incubation. The submergence and N$_2$ treatments increased soil pH towards 7.0 and decreased soil $E_h$ from 600 mV to between 300 and 400 mV, indicating a more reducing environment, which seemed to be controlled by the MnO$_2$-Mn$^{2+}$ and/or FeOOH-Fe$^{2+}$ redox couples. Green manure additions had similar effects, but to a lesser extent. Consequently, soluble soil Mn concentrations increased by 100 – 1000 fold
relative to those of the controls (no manure, at FC). There was a marked decrease in soybean
growth and a marked increase in leaf Mn concentration in the treatments of submergence and
green manure additions. A 10% reduction in dry matter yield was expected when leaf Mn exceeded 200 mg kg\(^{-1}\), leaf Ca/Mn (weight-to-weight) < 80, saturated paste Mn > 0.50 mg L\(^{-1}\), Mehlisch-3 Mn > 200 mg kg\(^{-1}\), and NH\(_2\)OH Mn > 1100 mg kg\(^{-1}\). The effects were most evident at 14 – 28 days after incubation.

INTRODUCTION

In acid soils, Mn phytotoxicity is common, second only to aluminum toxicity. Concentrations of
soluble manganese are dependent on amounts of readily reducible Mn in the soil, soil pH, and
the availability of electrons (e\(^{-}\)), as shown below (1):

\[
\text{MnO}_2 + 4\text{H}^+ + 2\text{e}^{-} \leftrightarrow \text{Mn}^{2+} + 2\text{H}_2\text{O} \quad [1]
\]

Although MnO\(_2\) is generally assumed to be the most stable Mn oxide in soils, Mn\(_2\)O\(_3\) and Mn\(_3\)O\(_4\)
are also common (2; 3). Sparrow and Uren (4) claimed that soluble Mn (II) is formed by the
reduction of both Mn (III) and Mn (IV) oxides. Hue et al. (5) stated that soils with “high” Mn
levels became phytotoxic at pH < 6.0. But even at pH > 6.0, Mn toxicity can occur when
conditions favor a reducing environment, such as flooding or poor drainage, or in the presence
of reducing/chelating agents (6; 7). More e\(^{-}\) is available by the addition of organic materials (e.g.,
green or animal manures) and/or by the removal of Oxygen (O\(_2\)) from the soil environment.
Oxygen is a strong e\(^{-}\) acceptor, thus its decreased concentrations allows more e\(^{-}\) to be available
and to reduce and dissolve solid Mn oxides as shown in reaction [1]. Different plant species or
even varieties within a species have different degrees of tolerance to Mn (8; 9). For example, Mn
toxicity was observed when leaf Mn (in mg kg\(^{-1}\)) exceeded 150 in bean (Phaseolus vulgaris), 650
in clover (Trifolium subterraneum), 1000 in watermelon (Citrullus lanatus), and 5000 in lowland
rice (*Oryza sativa*) (10; 11). Marschner (12) reported that soybean cv. T203 had shoot dry weight increase by 30% when shoot Mn concentrations increased from 208 to 527 mg kg\(^{-1}\), whereas soybean cv. Bragg had shoot dry weight decrease by 21% when shoot Mn increased from 297 to 532 mg kg\(^{-1}\). Furthermore, high levels of other nutrients alleviated Mn, particularly Ca (13; 15), Mg (14; 15), and Si (16). In fact, Hue et al. (5) reported that leaf Ca/Mn ratio is a better predictor of Mn toxicity in soybean cv. Kahala than leaf Mn concentration alone. The objectives of this study were (i) to characterize the solublization (reduction) of solid soil Mn over time in two Hawaiian acid soils when the e’ availability was modified by submergence or incubation under a N\(_2\) atmosphere with the addition of various rates of green manure; and (ii) to quantify the toxic effect of Mn on soybean growth.

**MATERIALS AND METHODS**

**Soil Properties And Treatments In The Laboratory Incubation Experiment**

A Mollisol (clayey, mixed, isohyperthermic, Pachic Haplustolls, Waialua series) and an Oxisol (clayey, kaolinitic, isohyperthermic, Rhodic Eutrustox, Wahiawa series) were used in this study. Total Mn concentration was 2000 mg kg\(^{-1}\) in the Mollisol, and 15 000 mg kg\(^{-1}\) in the Oxisol. These Mn levels are considered moderate and very high, respectively, as compared to the average Mn level of 1000 mg kg\(^{-1}\) worldwide as reported by Kabata-Pendias and Adriano (17). Leaves of leucaena (*Leucaena leucocephala*), a tropical tree legume, were sun dried to constant moisture (~10%) and used as green manure. The green manure was thoroughly mixed with the soils at 0, 10 and 20 g kg\(^{-1}\) in tall 1-L beakers. The soils were moistened to either 80 % field water holding capacity (250 g water kg\(^{-1}\) soil), or submerged in 8 cm of standing water. Another set of soils at 80% moisture of field capacity was treated with green manure placed in tall beakers, which were then fitted with plexiglass lids and rubber stoppers with inlet and outlet glass tubing to facilitate gas entry and exit. This set of treatments were flushed with N\(_2\) gas for 30 min to remove O\(_2\) then sealed from the ambient atmosphere. The N\(_2\) atmosphere was
maintained by flushing with N$_2$ gas every two days for 15 min. This experiment was a 2 (soil series) X 3 (green manure levels) X 3 (water/O$_2$ levels) factorial in a completely randomized design with 3 replications.

The treated soils were incubated for 56 days at 24 ± 1 °C. Soil subsamples were taken at 7, 14, 28 and 56 days for pH, Eh and Mn measurements. Soil pH and Eh were measured in a saturated paste extract (18). Soil Mn was extracted from a saturated paste, with the Modified Mehlich-3 solution (19), in which soil:solution ratio was 1:12.5 on a weight basis, and with an NH$_2$OH-HCl solution (20). Mn in the filtrates was analyzed with an inductively coupled plasma spectrometer (ICP). Oxygen levels in soil-water interface of the submergence and N$_2$ treatments were measured with a dissolved O$_2$ electrode (Orion Model 97-08) before every soil sampling.

Greenhouse Experiment Evaluating Mn Phytotoxicity

The leucaena green manure at 0, 10 and 20 g kg$^{-1}$ was thoroughly mixed with 3 kg of soil in plastic pots. The soils were wetted to FC and incubated for 21 days. Four pre-germinated soybean cv. Kahala seeds were planted in each pot, which were thinned to 2 seedlings after 5 days. In the FC treatment, the soil moisture was adjusted to field capacity everyday. In the submergence treatment, the pot was placed in a pan containing about 8 cm of standing water. All treatments received as basal nutrients (in mg kg$^{-1}$): 140 N, 200 Ca, 48 Mg, 160 S, 5 Cu, 5 Zn, 2 B and 0.5 Mo. The soybeans were grown for 5 weeks, and then harvested and leaf and stem dry weights measured. Leaf Mn concentration was determined by dry ashing 0.25 g material at 500 °C for 4 hours, the residue was dissolved in 5 mL 1 $M$ HNO$_3$ and heated at 120 °C to dryness to ensure a complete dissolution of MnO$_2$; this residue was subsequently dissolved in 10 mL 0.1 $M$ HCl for chemical analysis. Leaf nutrients, including Mn and Ca, were measured using ICP.
Statistical Analysis

Analysis of variance and least significant mean comparisons were performed using the SAS program (SAS Institute, Cary, NC). Regression analysis was performed with the SIGMA PLOT 2000 program (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Effects Of Moisture/O₂ Levels And Green Manure On Soil pH, Eh, And Extractable Mn Concentrations

Submerging under 8 cm of water increased soil pH (mean of 4 readings taken at 7, 14, 28, and 56 days after incubation) between 0.6 to 1.1 units towards neutral (Figs. 1A and 2A). The pH increases were more pronounced in the high-Mn Oxisol, from 5.5 in the aerated no manure to 6.8 in the submerged high manure treatments (Fig. 1A), than in the moderate-Mn Mollisol. The effects were most striking in submerged soils with green manure amendments.

Bathing the soils in the N₂ atmosphere caused soil pH to increase by about 0.5 units, in all treatments but the no manure treatment of the Mollisol (Figs. 1A & 2A). The differential increases in soil pH suggest that there were additional soil processes active in the presence of ample water and low O₂ than in the absence of O₂ alone.

With the depletion of O₂ by submergence, Eh of the high-Mn Oxisol decreased from ~600 mV in the no manure treatment to 510 and 420 mV in the 10 and 20 g kg⁻¹ manure treatments, respectively (Fig. 1B). These redox potentials seem to be controlled by the MnO₂ – Mn²⁺ couple as shown below.

\[ \text{MnO}_2 \text{ (solid)} + 4\text{H}^+ + 2\text{e}^- \leftrightarrow \text{Mn}^{2+} + 2\text{H}_2\text{O}, \text{E}_{oh} = 1.23 \text{ V} \] \[ \text{[1]} \]

Using the Nernst equation, \( E_{h} \) (at 25 °C) of reaction [1] can be expressed as:

\[ E_{h} = E_{oh}^0 - (0.059/2) \log [(\text{Mn}^{2+})/(\text{H}^+)^4] \]
or \[ E_h = 1.23 - 0.0295 \log (\text{Mn}^{2+}) - 0.118 \text{pH} \] [2]

Equation [2] allows us to calculate \( E_h \) of the soil system based on the measured pH and the estimated Mn \(^{2+}\). The activity of Mn \(^{2+}\) were derived from the following assumptions: (i) Mn \(^{2+}\) concentration in the soil solution is about 3 times Mn \(^{2+}\) concentration extracted by the saturated paste (based on the soils’ moisture content of approximately 30% field capacity), and (ii) Mn \(^{2+}\) activity is about 0.5 times the total soluble concentration of all Mn species. These calculations yielded 630, 526, and 490 mV respectively for the 0, 10, and 20 g kg\(^{-1}\) green manure treatments of the Oxisol. These values are similar to those measured and plotted in Fig. 1B. In contrast, similar calculations produced \( E_h \) values much more positive (580, 510, and 510 mV) than those shown in Fig. 2B for the moderate-Mn Mollisol, containing 0, 10, and 20 g kg\(^{-1}\) green manure. Thus, it seems logical to conclude that the MnO\(_2\) – Mn \(^{2+}\) couple did not control \( E_h \) of the submerged Mollisol, perhaps because all solid Mn had been dissolved. On the other hand, if we consider the FeOOH – Fe \(^{2+}\) couple as shown below.

\[
\text{FeOOH (solid)} + 3 \text{H}^+ + e^- \rightleftharpoons \text{Fe}^{2+} + 2\text{H}_2\text{O}, \, \text{E}_{oh} = 1.057 \text{V} \] [3]

Using similar calculations, \( E_h \) was estimated to be 230 mV for the submerged Mollisol with 20 g kg\(^{-1}\) manure and 300 mV for the treatment with 10 g kg\(^{-1}\) manure. These values agree with those in Fig. 2B. (Activities of Fe \(^{2+}\) were assumed to be \( 10^{-6.5} \) and \( 10^{-7.0} \) M, which are not unreasonable for a submerged high-Fe soil like this Mollisol.)

The decreases in soil \( E_h \), affected by the N\(_2\) atmosphere, were not as pronounced as those by submergence (Figs. 1B & 2B). Neither the MnO\(_2\) – Mn \(^{2+}\) nor the FeOOH – Fe \(^{2+}\) couples could be used to satisfactorily predict the \( E_h \) values. We have no rational explanation for the N\(_2\) effect on soil \( E_h \). Perhaps, our experimental set-up was not as completely O\(_2\) free as we had believed. However, periodic O\(_2\) measurements always
Figure 1. Soil pH and redox potential (Eh) of a high Mn Oxisol as affected by moisture/O_2 levels and green manure additions.
Figure 2. Soil pH and redox potential (Eh) of a high Mn Oxisol as affected by moisture/O₂ levels and green manure additions.
showed levels below 0.20 mg L\(^{-1}\), compared to the average of 8.0 mg L\(^{-1}\) in the moisture-saturated ambient air. Water-soluble Mn, as extracted from the saturated paste, increased 1.0 to 2.5 orders with submergence, regardless of green manure addition (Figs. 3A & 4A). According to reaction [1], Mn\(^{2+}\) activity should be decreased by 4 orders with each pH unit increase (if \(e^-\) is kept constant) and should be increased by about 3.4 orders for each 100 mV drop in E\(_h\) (if pH is constant). Furthermore, if the system is poised (i.e., pH + E\(_h\) is constant), then Mn\(^{2+}\) activity should be decreased by 2 orders for each pH unit increase (5; 20). None of these scenarios fit our experimental data. Total, water-soluble Mn concentrations (as measured by ICP) in this experiment increased much more with submergence and green manure additions than can be explained by a combination of decreases in E\(_h\) and increases in pH. As proposed by Hue and Mai (11), chelating of Mn\(^{2+}\) by organic molecules, such as catechol and organic acids, produced during submergence and/or manure decomposition kept Mn in solution irrespective of soil pH.

Mehlich-3 extractable Mn was also affected strongly by submergence, manure addition, and to a lesser extent, the N\(_2\) treatment (Figs. 3B & 4B). Since the Mehlich-3 procedure soil:solution ratio is approximately 1:10, buffers the extracting solution to approximately pH 2.5, and contains EDTA, acetic acid, and F\(^-\) ions, we believe concentrations of chelatants in the extractant and from the treated soils played a major role extracting differential quantities of soil Mn under submergence and/or green manure addition. The same explanation is also applicable to the N\(_2\) treatments, although the increases in extractable Mn in the N\(_2\) treatments with green manure were smaller than those under submergence.

The solution of NH\(_2\)OH-HCl is a strong reducing reagent, which is thought to extract all reducible Mn from a soil (21). Thus, Mn extracted by this solution should reflect soil Mn capacity (i.e., Mn containing minerals in the soils) rather than the soils’ short-term amendments, such as green manure additions. This reasoning seems applicable to our experiment by the fact that NH\(_2\)OH-extractable Mn changed little with green manure application rates, O\(_2\) levels (FC vs. N\(_2\) atmosphere), and even with soil series. It is worth noting, however, that NH\(_2\)OH-extractable Mn was highest in the submergence treatment, and from the Mollisol (on a relative scale as compared to the total Mn). The data strongly suggest that (i) submergence under 8 cm water over 8 weeks may have transformed Mn
Figure 3. Soil Mn extracted by Saturated paste, Mehlich 3, and NH$_2$OH solutions in a high-Mn Oxisol as affected by moisture/O$_2$ levels and green manure additions.
Figure 4. Soil Mn extracted by Saturated paste, Mehlich 3, and NH$_2$OH solutions in a moderate-Mn Mollisol as affected by moisture/O$_2$ levels and green manure additions.
minerals from less reducible (more stable) to more reducible forms as in the case of the high-Mn Oxisol, and (ii) practically all solid Mn in the moderate-Mn Mollisol had become reducible/dissolvable with submergence. This supports our contention that the MnO$_2$ – Mn$^{2+}$ redox couple did not “control” E$_h$ of the Mollisol under submergence.

Effects Of Incubation Duration On Soil pH, E$_h$, And Mn Under Different Moisture/O$_2$ Conditions

Duration of incubation had varied effects on soil pH, Eh, and Mn, depending on moisture/O$_2$ levels. The effects followed the same patterns for the two soils, so only data from the Oxisol are presented (Fig. 5). Generally, 14 – 28 days after treatment initiation exhibited the largest differences (values are averaged over three green manure application rates) within the treatments (Fig. 5).

As for soil pH, submergence caused a steady increase from 6.4 to 6.8; N$_2$ treatment did not change it, whereas the FC treatment decreased pH from 5.7 to 5.3 (Fig. 5A). This pH decrease was probably the result of mineralization and subsequent nitrification of the added green manure. Soil E$_h$ seemed to follow a parabolic pattern with respect to the incubation time in the submergence and N$_2$ treatments (Fig. 5B). Both treatments had a minimum E$_h$ at 28 days after incubation, suggesting that between 2 – 4 weeks were required for water or N$_2$ to diffuse to and fill up all micropores of soil aggregates/particles. The FC treatment had a minimum E$_h$ at 14 days after incubation, which was 1 week earlier than the other 2 treatments. Rapid decomposition of the green manure under favorable aerobic conditions was the likely cause.

Soluble Mn extracted by saturated paste, decreased steadily with incubation time in the submergence treatment (Fig. 5C). Perhaps, some Mn$^{2+}$ re-precipitated as MnCO$_3$ as soil pH approached 7.0 (22). Despite such a decrease, soluble Mn concentrations were still very high, ranging between 120 and 40 mg L$^{-1}$. In contrast, soluble Mn reached a minimum of 0.10 mg L$^{-1}$ in the FC treatment and a maximum of 11.0 mg L$^{-1}$ in the N$_2$ treatment after 28 days of incubation (Fig. 5C).
Figure 5. Effects of incubation time on soil pH, redox potential (Eh), and soluble Mn of a high-Mn Oxisol under different moisture/O_2 levels. Vertical bars are standard errors of the means.
Figure 6. Leaf dry weight of soybean cv. Kahal, grown on a high-Mn Oxisol and moderate-Mn Mollisol under differing moisture and green manure rates.
Soybean Responses To Soil Mn As Affected By Soil Moisture And Green Manure Addition

Submergence depressed soybean growth significantly in the high-Mn Oxisol (Fig. 6A), but only slightly in the moderate-Mn Mollisol (Fig. 6B). Green manure additions generally increased plant growth under well-aerated conditions, but decreased growth under flooded conditions (Fig. 6). Mn levels in the leaf and soil can explain the differences in plant growth. For example, by plotting leaf dry weight as a function of leaf Mn, a response curve was obtained, having an $r^2$ of 0.65, indicating that a leaf Mn concentration of 200 mg kg$^{-1}$ would be associated with a 10% reduction in growth (Fig. 7A). A slight improvement in correlation ($r^2 = 0.71$) was obtained when leaf dry weight was plotted against leaf Ca to Mn ratio (Fig. 7B). A 10% growth reduction is expected when leaf Ca/Mn is below 80, which is slightly higher than a ratio of 50 proposed by Hue et al. (5).

Plant growth was well correlated with soil Mn. Especially, saturated paste Mn (Fig. 8A), which showed a value of log (Mn) = -0.3, or a Mn concentration of 0.50 mg L$^{-1}$ for a 10% growth reduction. This value is identical to that reported by Hue et al. (5) for the same soybean cultivar grown on a similar Oxisol amended with gypsum and/or lime. We also found a 10% growth reduction corresponded with a Mehlich-3 extractable Mn > 200 mg kg$^{-1}$ (Fig. 8B), and a NH$_2$OH extractable Mn > 1100 mg kg$^{-1}$ (Fig. 8C).

SUMMARY AND CONCLUSIONS

Effects of moisture/O$_2$ levels and green manure additions on soil pH, E$_{h}$, and Mn availability were studied, using a high-Mn Oxisol and a moderate-Mn Mollisol, with soybean as a test crop. Our results show that submergence raised soil pH by about 1 unit towards 7.0, lowered E$_{h}$ from approximately 600 mV to between 300 and 400 mV, and increased soluble Mn by 2 – 3 orders. The treatment with N$_2$ atmosphere had similar, but smaller effects. The effects of green manure on the soil parameters studied were less apparent and varied with moisture and O$_2$ levels. A combination of submergence and a high manure rate dissolved large quantities of soil Mn and induce Mn toxicity in soybean. A 10% growth reduction corresponded to leaf Mn levels exceeding 200 mg kg$^{-1}$, Mn of 0.50 mg L$^{-1}$ in the saturated paste, 200 mg kg$^{-1}$ in the Mehlich-3, and 1100 mg kg$^{-1}$ in the NH$_2$OH extracts.
Figure 7. Leaf dry weight as a function of leaf Mn (A) and leaf Ca/Mn ratio (B).
Figure 8. Leaf dry weight of soybean cv. Kahala as a function of soil Mn extracted with Saturated paste (A), Mehlich 3 solution (B), and NH$_2$OH solution (C).
REFERENCES


