EFFECT OF ZINC FERTILIZATION ON IRON, MANGANESE AND COPPER CONTENT IN CHENOPODIUM (CHENOPODIUM ALBUM L.) GROWN ON ACID AND ALKALINE SOILS AMENDED WITH ORGANICS

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ABSTRACT
A greenhouse pot experiment was undertaken to study the effect of applied zinc (Zn) on iron (Fe), manganese (Mn) and copper (Cu) content in edible portion of Chenopodium (Chenopodium album L. var. Pusa bathua no. 1). Four levels of Zn (0, 5, 50 and 100 mg kg⁻¹) and three levels of organics (control, 3% FYM and 3% sludge) were applied to assess the Fe, Mn and Cu content in shoot of Chenopodium grown on acid and alkaline soils. Results indicated that Fe content in shoot reduced to the tune of 13.3, 32.9 and 43.9% at 5, 50, and 100 mg of applied Zn kg⁻¹, respectively over control. More or less, similar extent of reduction in Mn content in shoot was recorded at different levels of applied Zn. However, effect of Zn application on Cu content was statistically non-significant. On an average, Fe and Mn content in Chenopodium were found to be reduced significantly due to the application of both FYM and sludge over control (no organic), but significant reduction in Cu content was found to be associated with sludge application.

Key words: Leafy vegetable, Micronutrients, Organics, Zinc fertilization.

INTRODUCTION
Generally the regions in the world with Zn deficient soils are also characterized by widespread zinc (Zn) deficiency in humans (Cakmak, 2008). Recent estimates indicate that nearly half of the world population suffers from Zn deficiency. Hence, enrichment of edible portion of crops with micronutrients, particularly Zn, is considered as one of the priority areas of agricultural research. At the same time, assessing the effect of application of particular micronutrient in enriching the edible portion of crops on the plant content of other micronutrient cations is also important. Usually, excessive uptake of one micronutrient or metallic cation inhibits the absorption and translocation of other metallic cations in plants (Yizong et al., 2009). Hence, application of Zn, particularly at higher levels, may compete with the absorption of other micronutrient cations by plants and thereby reducing their content in plants. Along with Zn, other trace metals such as Fe, Mn and Cu are also very important for sustenance of various physiological and metabolic processes in humans. Hence, it is imperative to study the effect of high rates of Zn application on the plant content of other micronutrient cations.

Long term use of sewage-sludge and industrial effluents on agricultural lands often results in heavy buildup of organic carbon and elevated level of metals in soil. Accumulation of substantial amount of Zn in such soil has been reported in various studies (Rattan et al., 2005; Datta et al., 2000). One can imagine that high build up of Zn in soil receiving sewage-sludge and industrial effluents may restrict the absorption of other essential micronutrients like Fe, Mn and Cu by plants grown thereon. Besides, application of Zn along with organics is expected to enhance its use efficiency by crops (Datta et al., 2007; Rattan et al., 2008). However, information on interactive effect of applied Zn and organics on the absorption of other micronutrients by crops, particularly leafy vegetables, is scanty.

Soil pH is the most important factor governing the availability of native as well as applied metal in soil. For example, solubility of Zn decreases
by 100 folds due to each unit increase in soil pH
and vice-versa (Lindsay, 1979). One can envisage
that the availability of micronutrient cations is far
higher in acid soil as compared to that in alkaline
soil. Hence, availability of applied Zn is expected to
vary with soil pH which in turn affects the uptake or
content of other micronutrient cations in plant.
The inverse relationship of the phytoavailability of
micronutrient cations with pH across soil types is far
more consistent than that with soil organic
carbon. In this regard, the finding of Robertson et
al. (1982) may be mentioned, where they suggested
that decrease in soil pH following sludge application
was the major reason for the higher mobility and
availability of heavy metals in sludge-treated soils.
Usually, the availability of applied Zn in acid and
alkaline soil is assessed by the plant uptake data.
However, chemical extractants such as strong
chelating agents (Datta et al., 2007; Paulose et al.,
2007) can be used for assessing the metal availability
to plants. In the present investigation, an attempt
has been made to study the Fe, Mn and Cu content
in Chenopodium as affected by Zn application along
with FYM and sewage-sludge in acid and alkaline
soils.

MATERIALS AND METHODS
A greenhouse pot experiment was
conducted to assess the impact of soil applied Zn on
Fe, Mn and Cu content in Chenopodium grown on
acid and alkaline soils amended with FYM and
sewage-sludge. For each soil, the treatments consist
of four levels of Zn, i.e. 0, 5, 50 and 100 mg kg⁻¹ soil
and three levels of organics, i.e. control, 3% FYM
and 3% sludge. For this purpose, two bulk surface
(0-15 cm) soil samples were collected from Cooch
Behar district (26°19' N, 89°23' E), West Bengal
(acid soil) and experimental farm of Indian
Agricultural Research Institute (28°30' N, 77°10' E),
New Delhi (alkaline soil). Soil samples were
processed and soil pH, electrical conductivity (EC),
cation exchange capacity (CEC) and organic carbon
were determined using standard procedures
(Jackson, 1973). For available micronutrient cations,
soils were extracted with 0.005 M DTPA – 0.01 M
CaCl₂ (Lindsay and Norvell, 1978) and
concentration of Zn, Cu, Fe and Mn in the extracts
was determined by atomic absorption
spectrophotometer. The soil pH (1:2 soil:water) for
acid and alkaline soils were 5.65 and 8.25,
respectively. The values of EC in supernatant liquid
of same soil-water suspension were determined to
be 0.50 and 0.26 dS m⁻¹ for acid and alkaline soil,
respectively. Texture of acid soil was sandy clay loam
and that of alkaline soil was sandy loam. Organic
carbon and CEC for acid soil were 0.94% and 13.5
cmol kg⁻¹, respectively; the corresponding values for
alkaline soil were 0.45% and 11.4 cmol kg⁻¹. DTPA
extractable Zn, Cu, Fe and Mn in acid soil were 1.81,
2.08, 51.2 and 20.7 mg kg⁻¹, respectively; coreresponding values for alkaline soil were 1.32, 1.50,
3.80 and 8.50 mg kg⁻¹. Sludge and FYM were
analysed for total nitrogen (N), phosphorus (P),
potassium (K) and metal (Zn, Cu, Fe, Mn, Ni and
Pb) content using standard procedures (Jackson,
1973). Total organic carbon in FYM and sludge was
determined by wet oxidation method (Snyder and
in FYM were 40.9, 1.10, 0.55 and 0.90%,
respectively; corresponding values for sewage-sludge
were 43.1, 3.45, 0.36 and 0.45%. Total Zn, Cu, Fe,
Mn, Ni and Pb in FYM were 154, 62.0, 5200, 264,
30.0 and 10.0 mg kg⁻¹, respectively. Total Zn, Cu,
Fe, Mn, Ni and Pb in sludge were 1024, 600, 16400,
310, 150 and 140 mg kg⁻¹, respectively.

Plastic pots of 4 kg capacity were filled with
3 kg of each soil and a basal dose of N : P₂O₅ : K₂O
@ 11.1 : 11.1 : 22.2 mg kg⁻¹ soil was added in solution
form to the soil of each pot through urea,
diammonium phosphate and muriate of potash,
respectively. Sewage-sludge and FYM @ 3% on
weight basis were added in powder form and
thoroughly mixed with soil; Zn was applied as
ZnSO₄.7H₂O in solution form. All twenty-four
treatment combinations (2 soils × 3 organics × 4
levels of Zn) were replicated thrice and experiments
were laid out in completely randomized design. The
soil in each pot was then irrigated with tap water
and the pots were maintained at field capacity
moisture for one month. After one month, seeds of
Chenopodium were sown and a uniform plant
population of 8 plants per pot was maintained after
a fortnight of germination. Pots were irrigated daily
with required amount of water on weight basis to
maintain field capacity. The crop (above ground
edible portion) was harvested at 30 days after sowing
(DAS). Plant samples were washed with tap water
followed by dilute HCl (0.1 N) and finally rinsed with
distilled water. The samples were first air-dried and
then oven-dried in hot air oven at 60±5°C until the attainment of constant weight. Plant samples were ground and digested in a di-acid mixture of HNO₃/ HClO₄ (9:4) on an electric hot plate and analysed for Fe, Mn and Cu content using atomic absorption spectrophotometer. Post-harvest soil was taken out of each pot, air-dried, ground and passed through 2 mm sieve for determination of DTPA (0.005 M) extractable Zn following standard procedure.

Analysis of variance (ANOVA) method was followed to assess the effect of applied Zn, organics and soil type on DTPA extractable Zn in soil, content of Fe, Mn and Cu in edible portion of crop adopting factorial concept through completely randomized design (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

DTPA extractable Zn in post-harvest soil: On an average, DTPA extractable Zn increased by 1.56, 6.58 and 14.2 folds over control (without Zn) due to application of Zn @ 5, 50 and 100 mg kg⁻¹, respectively (Table 1). As expected, mean DTPA extractable Zn was higher in acid soil as compared to alkaline soil. Application of FYM, on an average, marginally reduced (14.5 mg kg⁻¹) the DTPA extractable Zn with respect to control (15.6 mg kg⁻¹) without organics, whereas sludge was ineffective in changing the extractable Zn. The reduction in DTPA extractable Zn in FYM amended soil contradicts the findings of Chaudhary and Narwal (2005). The ineffectiveness of sludge in altering DTPA extractable Zn may be related to higher inherent Zn content of sludge as compared to that of FYM. However, perusal of data revealed that application of both FYM and sludge significantly reduced the DTPA extractable Zn in acid soil with respect to control (no organic). Whereas, the organic amendments could not alter the DTPA extractable Zn in alkaline soil. The results indicate that the effect of organics on Zn availability varies with soil pH. The interactive effect of soil type and organics on the pH of post-harvest soils was found to be statistically significant (data not shown). Both of these organic amendments significantly reduced the pH of alkaline soil. However, application of these amendments significantly increased the pH of acid soil. This may be the reason behind the reduced extractable Zn in acid soil due to application of organics. It is difficult to explain such a differential effect of organics on the pH and

<table>
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<th>Applied Zn (mg kg⁻¹)</th>
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<th>5</th>
<th>50</th>
<th>100</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1.61</td>
<td>2.69</td>
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<td>5.0</td>
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<td>Sludge (SN)</td>
<td>1.99</td>
<td>2.44</td>
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<td>5.12</td>
<td>3.84</td>
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<tr>
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<td>4.95</td>
<td>5.27</td>
<td>4.56</td>
</tr>
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<td>4.45</td>
<td>5.01</td>
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<td>4.91</td>
</tr>
<tr>
<td>Sludge (SN)</td>
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<td>6.82</td>
<td>7.53</td>
<td>6.08</td>
</tr>
<tr>
<td><strong>Organics (O)</strong></td>
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<td>4.84</td>
<td>5.01</td>
<td>5.68</td>
<td>4.56</td>
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<tbody>
<tr>
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<td>CD (P = 0.05)</td>
<td>Zn : 0.74, S = 0.52, O = 0.60, Zn × O = 1.29, S × O = 0.60, S × Zn × O = NS</td>
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</table>
available Zn in diverse soil types. The interactive effect of Zn and organics on DTPA extractable Zn revealed that at the highest level of Zn application (100 mg kg\(^{-1}\)), both the organics could significantly reduce the extractable Zn with respect to control soil (no organic) at the same level of applied Zn. One can infer that application of Zn at such high level might have been toxic to microorganisms in soil which in turn reduced the mineralization of added organic materials. As a result, sludge and FYM might have formed more stable complex with Zn in soil where high level of Zn was applied. Lal (1990) also reported that growth of bacteria and fungi was progressively inhibited in the growth medium supplemented with increasing amount of metal including Zn.

**Iron (Fe) content in Chenopodium:** Iron content in shoot of test crop reduced progressively with the increasing level of Zn application. On an average, there was significant reduction in Fe content in Chenopodium by 13.3, 32.9 and 43.9% over control due to application of Zn at 5, 50 and 100 mg kg\(^{-1}\), respectively (Table 2). Much higher mean value of Fe content was recorded in Chenopodium grown on acid soil (222 mg kg\(^{-1}\)) as compared to alkaline soil (173 mg kg\(^{-1}\)). This is obviously related to both higher level and solubility of native Fe in acid soil as compared to alkaline soil. The reduction in plant Fe content with applied Zn is related to the increased availability of Zn (as evident from the DTPA extractable Zn) and inhibitory effect of Zn on the absorption and translocation of Fe (Gupta and Srivastava, 1996). In plants, Fe is chiefly translocated as a complex form with organic ligands. Un-usually high concentration of Zn in plant due to application of high levels of Zn in soil might have replaced the Fe from organic complex making Fe relatively immobile in plant (Tiffin and Brown, 1962). On an average, application of FYM and sludge significantly reduced the shoot Fe content with respect to control (no organic). However, interactive effect shows that application of organics enhanced the shoot Fe content in case of alkaline soil, while reverse trend was observed in acid soil. This may be attributed to the reduction in pH resulting from addition of organics in alkaline soil (data not shown), which in turn increased the availability of Fe in alkaline soil. Significant increase in pH due to application of

<table>
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<th>Applied Zn (mg kg(^{-1}))</th>
<th>Organic (O)</th>
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<th>Control</th>
<th>FYM</th>
<th>Sludge</th>
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<tr>
<td>mean</td>
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<td>272</td>
<td>197</td>
<td>152</td>
<td>141</td>
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</table>

**Table 2: Effect of applied Zn and organics on shoot Fe content (mg kg\(^{-1}\)) in shoot of Chenopodium grown on alkaline and acid soils.**
### TABLE 3: Effect of applied Zn and organics on total Mn content (mg kg⁻¹) in shoot of *Chenopodium* grown on alkaline and acid soils.

<table>
<thead>
<tr>
<th>Applied Zn (mg kg⁻¹)</th>
<th>Soil (S)</th>
<th>Organics (O)</th>
<th>Mean</th>
<th>Control</th>
<th>FYM (3%)</th>
<th>Sludge (3%)</th>
<th>Control</th>
<th>FYM (3%)</th>
<th>Sludge (3%)</th>
<th>Control</th>
<th>FYM (3%)</th>
<th>Sludge (3%)</th>
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<tbody>
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<td></td>
<td>Alkaline</td>
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<td></td>
<td></td>
<td>Organsics (O)</td>
<td>Mea</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>Control</td>
<td>FYM (3%)</td>
<td>Sludge (3%)</td>
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<tr>
<td></td>
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<td>122</td>
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<td>94.6</td>
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<td>118</td>
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<td>119</td>
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<td>50</td>
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<td>48.0</td>
<td>68.6</td>
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<td>75.0</td>
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<td>72.5</td>
<td>61.5</td>
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<td>100</td>
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<td>38.0</td>
<td>43.3</td>
<td>39.7</td>
<td>85.3</td>
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<td>34.3</td>
<td>57.7</td>
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<tr>
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<td>67.0</td>
<td>81.2</td>
<td>69.5</td>
<td>118</td>
<td>96.0</td>
<td>80.0</td>
<td>98.3</td>
<td>90.1</td>
<td>81.5</td>
<td>80.6</td>
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*CD (P = 0.05) Zn = 2.5, S = 1.7, O = 2.2, S × Zn = 3.5, S × O = 3.1, Zn × O = 4.4 and S × Zn × O = 6.2*

### TABLE 4: Effect of applied Zn and organics on total Cu content (mg kg⁻¹) in shoot of *Chenopodium* grown on alkaline and acid soils.

<table>
<thead>
<tr>
<th>Applied Zn (mg kg⁻¹)</th>
<th>Soil (S)</th>
<th>Organics (O)</th>
<th>Mean</th>
<th>Control</th>
<th>FYM (3%)</th>
<th>Sludge (3%)</th>
<th>Control</th>
<th>FYM (3%)</th>
<th>Sludge (3%)</th>
<th>Control</th>
<th>FYM (3%)</th>
<th>Sludge (3%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alkaline</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Organsics (O)</td>
<td>Mea</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Control</td>
<td>FYM (3%)</td>
<td>Sludge (3%)</td>
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<td></td>
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<td>14.3</td>
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<tr>
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<td>13.9</td>
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<td>19.6</td>
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*CD (P = 0.05) Zn = NS, S = 0.9, O = 1.1, S × Zn = 1.7, S × O = 1.5, Zn × O = 2.2 and S × Zn × O = 3.0*
organs might have reduced the Fe availability in acid soil (data not shown). The shoot Fe content was far less in case of Zn application with FYM than that with sludge. This may be due to the lower level of Fe content in FYM as compared to sludge.

**Manganese (Mn) content in Chenopodium:** Reduction in Mn content in Chenopodium was assessed to the tune of 15.8, 45.4 and 59.4% over control due to application of Zn @ 5, 50 and 100 mg kg⁻¹, respectively (Table 3). The mean value of Mn content in Chenopodium grown on acid soil (98.3 mg kg⁻¹) was higher than that grown on alkaline soil (69.9 mg kg⁻¹). This may be attributed to the higher Mn content of acid soil as compared to alkaline soil as evident from the initial soil properties. Application of organics (FYM and sewage-sludge) enhanced the Mn content in Chenopodium grown on alkaline soil over control (no organic), while shoot Mn content was found to be reduced due to application of organics in acid soil. These findings are in agreement with the findings of Vacha et al. (2002), where they reported that immobilization of metals was most effective in organic manure amended acid soil. However, on an average, application of organics reduced the Mn content in Chenopodium over control where no organic was added. Perusal of data revealed that at higher levels of applied Zn (50 and 100 mg kg⁻¹), application of FYM and sludge was associated with the much higher reduction in shoot Mn content over the comparable levels of applied Zn without organics. Sludge and FYM might have formed more stable complex with Mn in soil where higher levels of Zn were applied.

**Copper (Cu) content in Chenopodium:** The effect of Zn application on Cu content in Chenopodium was found to be statistically non-significant (Table 4). As expected, Cu content in Chenopodium was higher in acid soil than that in alkaline soil. On an average, application of sludge significantly reduced the Cu content in Chenopodium, while the Cu content in shoot of test crop grown on FYM amended soil was statistically at par with that grown on control soil (no organic). However, at the lowest level of applied Zn (5 mg kg⁻¹), application of FYM significantly enhanced the Cu content in shoot over the same level of applied Zn without organics. Like Fe and Mn, addition of organics (both FYM and sludge) in alkaline soil significantly increased the Cu content in Chenopodium over control (no organic), whereas reverse trend was obtained in acid soil. This may also be ascribed to the change in pH of the post-harvest soils as a result of application of organics in diverse soil types (data not shown).

It can be concluded from this study that higher level of applied Zn in both acid and alkaline soils had strong negative effect on the content of other micronutrient cations like Fe, Mn and Cu. One should be careful about the reduction of other micronutrient cations, particularly Fe in leafy vegetables while enriching the same with Zn. This will have far reaching practical implications as leafy green vegetables are considered as very good source of Fe also. By and large, both FYM and sludge had depressing effect on the content of Fe, Mn and Cu in shoot of Chenopodium.

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