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Characterization of Manganese Toxicity and its Influence on Nutrient Uptake, Antioxidant Enzymes and Biochemical Parameters in Tea*

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Abstract: Soil acidification enhances the solubility of metals such as Mn and Al, resulting in phytotoxicity including suppression in growth and yield. A potted plant experiment was conducted by adding manganese externally to the soil. There were seven treatments and three replications. Irregular brown spots all over the leaf surface right from the petiole up to the leaf tip between the marginal veins was found to be specific toxicity symptoms. The surviving plants were uprooted and separated into leaves, stem, root and soil on which the chemical analyses were carried out. At any given treatment, the accumulation of manganese was higher in leaf than that of root and stem. The critical toxic limit of manganese in root, stem and leaf was found to be 584, 892 and 4784 mg kg⁻¹, respectively. The phosphorus content decreased with increase in manganese concentration in soil and all plant parts. High manganese levels in soils led to plant nutrient imbalances especially in relation to other divalent cations such as Mg²⁺, Ca²⁺, Zn²⁺ and Fe²⁺. Significant decrease in amino acid, chlorophyll and carotenoid content of leaves was noted due to external addition of manganese, while catechin and polyphenol contents increased significantly. The activity of catalase was significantly higher till 1000 mg Mn kg⁻¹ of soil. The peroxidase activity increased significantly due to the increase in manganese addition.

Key words: Critical toxicity limit, nutrients, chlorophyll, carotenoids, catalase, peroxidase

INTRODUCTION

Manganese toxicity is often more common than manganese deficiency in acid soils for a number of plant species (Foy, 1984; Hue *et al.*, 2001). Mn is known to play an important role in the biosynthesis of chlorophyll and several enzymes along the isoprenoid biosynthetic pathway (Macfie and Taylor, 1992). Thus, excess manganese inhibits chlorophyll synthesis and induces a decline in photosynthetic rate (Macfie and Taylor, 1992). Long term and heavy dose applications of fertilizers or other organic amendments to agricultural soils and soil anaerobic conditions such as water logging or poor drainage may lead to an increase in the content or availability of Mn and other heavy metals (Paschke *et al.*, 2005). Even though the toxic effects of manganese is not commonly reported in tea gardens, reduction in soil pH of mature tea fields due to continual synthetic fertilizer application (Duangpatra *et al.*, 1979) and preheating of nursery soils to kill eelworm could result in manganese toxicity and hence it is worthwhile to generate data on this aspect. Excess manganese supply causes formation of visible brown depositions in the cell walls of leaves of cowpea (*Vigna unguiculata*), which consisted of oxidized manganese and oxidized phenols (Fecht-Christoffers *et al.*, 2003). Oxidation of manganese and phenolic compounds in the leaf apoplast was proposed to be catalysed by apoplastic peroxidases. The main aim of the present study was to 1) monitor the toxicity symptoms and fix the critical toxicity limit, 2) to determine the distribution and accumulation of soil applied manganese,

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3) to understand the interaction of Mn with other essential elements in plant and soil and 4) to find out its influence on antioxidant enzymes like catalase and peroxidase and biochemical parameters of green leaves.

MATERIALS AND METHODS

Potted tea plants (one year old) of the clone UPASI-9 were used for this study with seven treatments including an untreated control. The soil used for this study was sandy loam in texture having 71% sand, 10% silt and 19% clay. Mn was added externally to make the soil having 0, 100, 500, 1000, 3000, 5000 and 7000 mg Mn²⁺ per kg of soil. The experiment was conducted in triplicate. A moisture meter (theta meter type HH1) was used to maintain the soil moisture at 20%. The plants were monitored every day and the visual symptoms were recorded when noticed. The experiment was continued for a period of 90 days and the plants were uprooted on 90th day and were separated into root, stem and leaf.

Nutrient Analysis

The soil samples were air dried and passed through 2 mm sieve (Klose and Tabatabai, 2000). The separated vegetative parts were oven-dried at 60°C and homogenized. About one gram from every plant part was digested with HNO₃/HClO₄ mixture (Haunter *et al.*, 1987) and analysed for Fe, Zn, Mn, Ca, Mg, Na and P (Bhargava and Raghupathi, 2001) using Atomic Absorption Spectrophotometer (GBC 908AA). The metal standards used in this study were traceable to NIST (National Institute for Standards and Technology).

Antioxidant Enzyme Assay and Estimations

Peroxidase (EC 1.11.1.7) activity was estimated as per the procedure described by Hammerschmidt *et al.* (1982). Crude enzyme was prepared by grinding 0.5 g of leaf sample with 0.1 M phosphate buffer, centrifuged at 18,000 g at 5°C for about 15 min. The reaction medium consisted of 0.25% guaiacol in 0.01 M sodium phosphate buffer (pH 6.0) and 0.1 M hydrogen peroxide. Enzyme extract (0.1 mL) was added to initiate the reaction, which was estimated colorimetrically at 470 nm. Crude enzyme was diluted so as to increase the absorbance by 0.1 to 0.2 unit per minute at 470 nm. Activity was expressed as change in absorbance min⁻¹ g⁻¹ fresh weight of tissue.

Catalase activity (EC 1.11.1.6) was assayed spectrophotometrically as described by Chaparro-Giraldo *et al.* (2000) using 3 mL assay mixture containing 10 mM potassium phosphate buffer (pH 7.5) and 2.5 mM H₂O₂ prepared fresh before use and 100 µL enzyme extract. The activity was measured by monitoring the degradation of H₂O₂ at 240 nm for 1 min against a blank. The activity was expressed in µmol of H₂O₂ degraded min⁻¹ g⁻¹ fresh weight of tissue.

Biochemical Parameters

Leaf samples of known quantity weighed in a mortar was ground with sufficient quantities of chilled methanol. It was filtered and made up to 50 mL in a volumetric flask using methanol. This solution was diluted five times with methanol and used for the estimation of chlorophyll A, B and carotenoids (Wellburn, 1994) and the absorbance was recorded at 470, 653 and 666 nm using UV-VIS spectrophotometer (GBC 918).

About 0.5 g of leaf sample was weighed and ground with ethyl alcohol. The contents were filtered and the filtrate was made up to 50 mL with ethyl alcohol. This alcoholic extract was used for the estimation of polyphenols (Dev choudhury and Goswami, 1983), catechins (Swain and Hillis, 1959) and amino acids (Moore and Stein, 1948) and the absorbance was recorded at 700, 500 and 570 nm using UV-VIS Spectrophotometer (GBC 918).

Statistical analysis was carried out by the standard method (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The plants established on the soil containing $7000 \text{ mg Mn}^{2+} \text{ kg}^{-1}$ soil showed the symptoms of toxicity on 4th day after imposing treatment. Irregular brown spots all over the leaf surface right from the petiole up to the leaf tip between the marginal veins were noted (Fig. 1). The toxicity appeared first in the upper tier of leaves. This is in line with the observation made by Kitao *et al.* (2001), in Japanese white birch in which the symptoms started first with younger leaves. The plants grown in soil containing $7000 \text{ mg Mn}^{2+} \text{ kg}^{-1}$ soil died within 13 days, while the plants supplied with 5000 and 3000 $\text{mg Mn}^{2+} \text{ kg}^{-1}$ soil died within 18 and 23 days. Even though manganese toxicity has been reported to be accompanied by chlorosis in other plants (Kitao *et al.*, 2001), no chlorosis was observed in our studies. Such chlorotic symptoms were reported mostly with Fe deficient soils, whereas tea is grown in iron-enriched soils. The brown speckles observed on the leaf marginal and interveinal area were mainly due to the accumulation of manganese (Horst, 1988) in the form of manganese oxide and (1,3)- β -glucan (Wissemeier and Horst, 1987).

No visual symptoms of toxicity appeared on the plants planted in soil upto $500 \text{ mg Mn kg}^{-1}$ of soil, while it was noticeable at 1000 mg treatments. Hence, the manganese content observed in leaf, stem and root of the particular treatment (1000 mg) was taken as the limits of toxicity. Accordingly, $584, 892$ and $4784 \text{ mg kg}^{-1} \text{ Mn}$ were considered to be the levels of toxicity in root, stem and leaf of the tea plant. After 90 days the surviving plants were uprooted and separated into leaves, stem, root and soil on which chemical analyses were carried out. The manganese content estimated in soil and plant parts are given in Fig. 2. At any given treatment, the accumulation of manganese was higher in leaf than in root and stem (Loneragan, 1988). Statistical analysis carried out between the externally added manganese and manganese content of soil ($r = 0.905$; $p = 0.01$), root ($r = 0.997$; $p = 0.01$), stem ($r = 0.986$; $p = 0.01$) and leaves ($r = 0.820$; $p = 0.05$, $df = 6$) exhibited a positive and significant correlation co-efficient.

The phosphorus content decreased with increase in manganese concentration in soil, root, stem and leaf (Fig. 3). This could be because of the formation of less soluble complexes between phosphorus and manganese ions (Nogueira *et al.*, 2004; Sarkar *et al.*, 2004). It also reflected on the negative correlation coefficient existed between content of P in soil and Mn in leaf ($r = -0.85$; $p = 0.05$ and $df = 6$).

With increase in the concentration of the manganese in leaf and soil, the contents of Ca, Mg, Zn and Fe decreased (Fig. 4-7). Manganese uptake by plants mainly occurs in the reduced bivalent form. High manganese levels in soil may lead to plant nutrient imbalances especially in relation to other divalent cations such as Mg^{2+} , Ca^{2+} , Zn^{2+} and Fe^{2+} (Marschner, 1995; Cenni *et al.*, 1998;



Fig. 1: Manganese toxicity symptoms

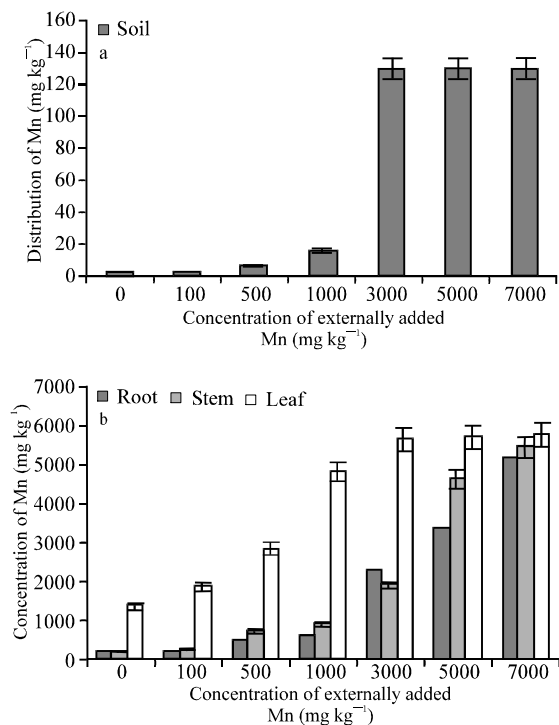


Fig. 2: a) Levels of available Mn in soil after 90 days of external addition b) absorption and distribution of Mn to various plant parts. The error bars represent the relative standard deviation

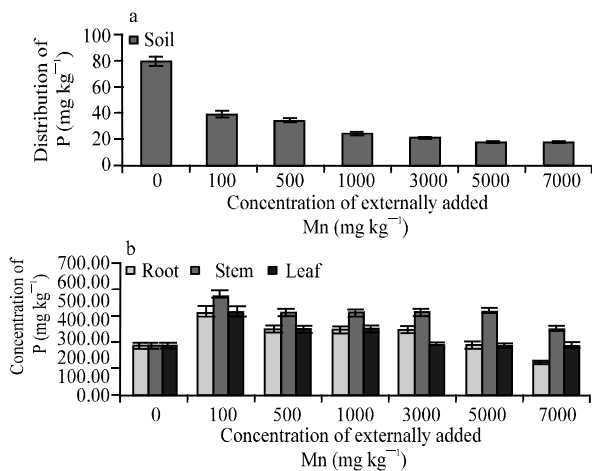


Fig. 3: Influence of externally added Mn (a) distribution of P in soils and b) P uptake by various plant parts. The error bars represent the relative standard deviation

De Varennes *et al.*, 2001). Manganese shows the properties of both the alkaline earth cations (Mg^{2+} and Ca^{2+}) and heavy metals (Zn and Fe). Since Mn^{2+} participates in cation competition, the increase in number of manganese ions would depress the uptake of Ca, Mg, Zn and Fe. The nutrients

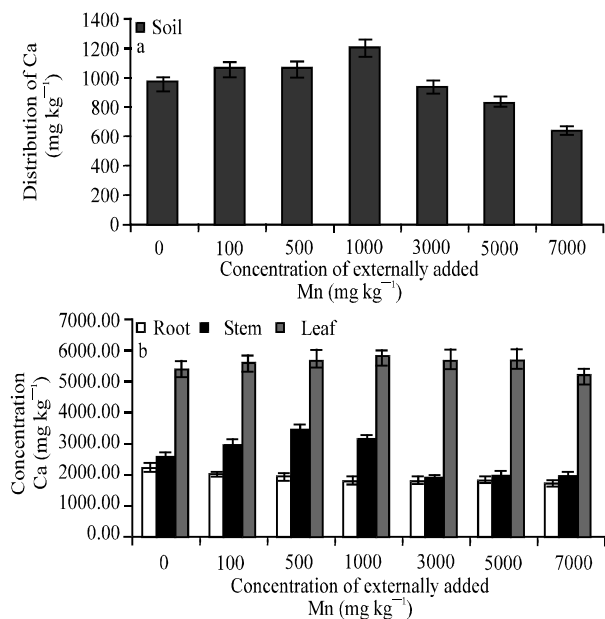


Fig. 4: Influence of externally added Mn (a) distribution of Ca in soils and b) Ca uptake by various plant parts. The error bars represent the relative standard deviation

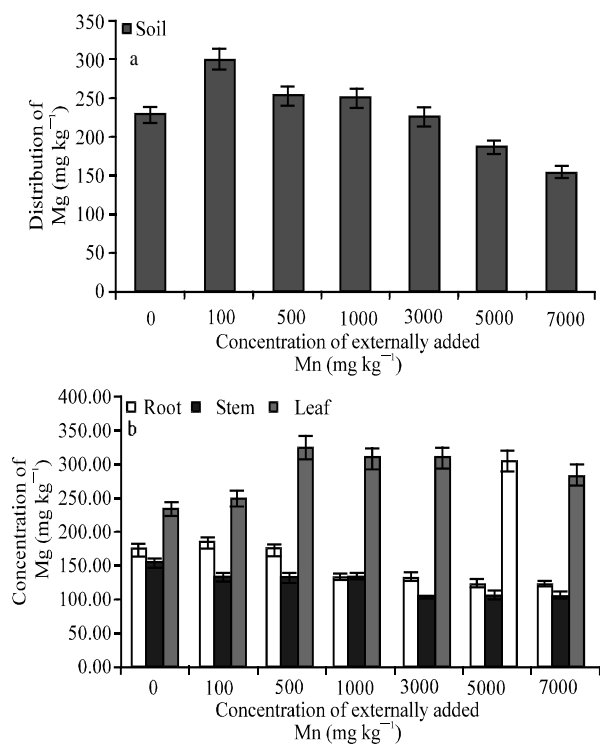


Fig. 5: Influence of externally added Mn (a) distribution of Mg in soils and b) Mg uptake by various plant parts. The error bars represent the relative standard deviation

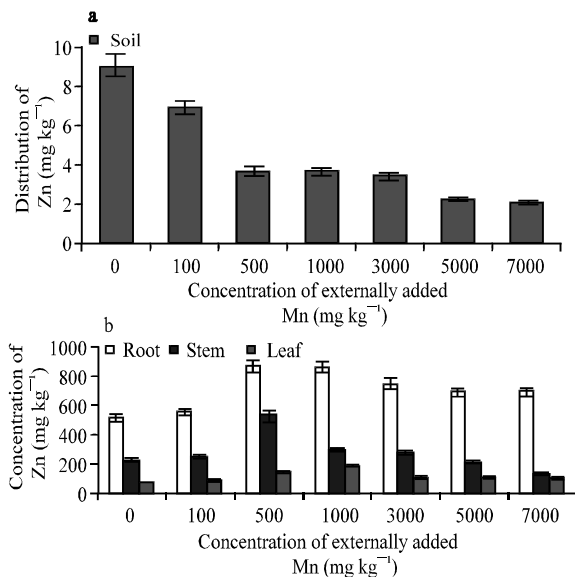


Fig. 6: Influence of externally added Mn (a) distribution of Zn in soils and b) Zn uptake by various plant parts. The error bars represent the relative standard deviation

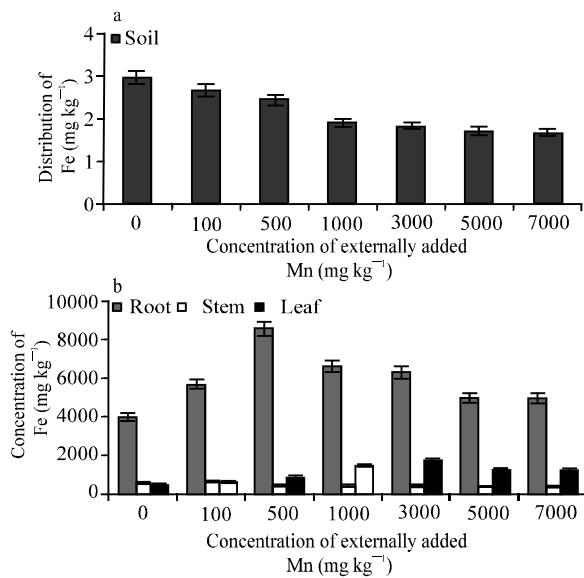


Fig. 7: Influence of externally added Mn (a) distribution of Fe in soils and b) Fe uptake by various plant parts. The error bars represent the relative standard deviation

entering the roots by diffusion may be hampered by manganese due to inhibition of root hair production and reduction of stomata dimensions (Lidon, 2002). The rate of soil applied manganese has shown negative and significant correlation with Mg content of soil ($r = -0.86$; $p = 0.05$) and stem ($r = -0.81$; $p = 0.05$ and $df = 6$).

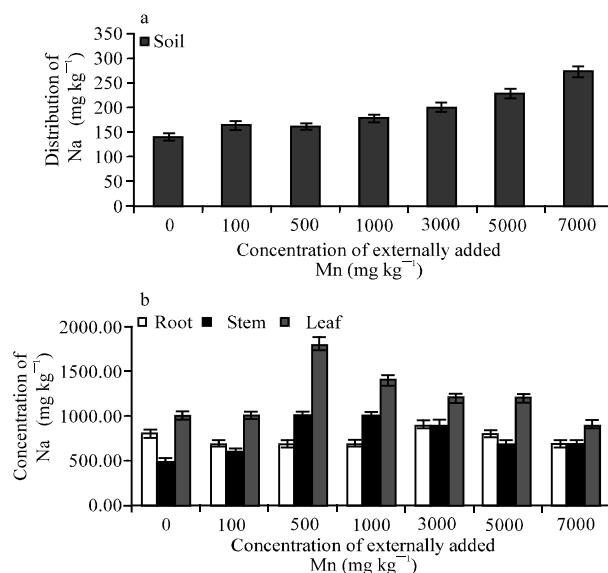


Fig. 8: Influence of externally added Mn (a) distribution of Na in soils and b) Na uptake by various plant parts. The error bars represent the relative standard deviation

Table 1: Influence of externally added manganese on certain antioxidant enzymes and biochemical parameters of green leaf

	Amino acid (g kg ⁻¹)	Catechin (g kg ⁻¹)	Polyphenols (g kg ⁻¹)	Chlorophyll (mg kg ⁻¹)	Carotenoids (mg kg ⁻¹)	Catalase*	Treatments Peroxidase \$
Control	19	120	233	3512	698	2.9	0.22
100 mg Mn kg ⁻¹ of soil	15	220	323	3553	726	3.2	0.25
500 mg Mn kg ⁻¹ of soil	14	220	350	3396	645	3.4	0.27
1000 mg Mn kg ⁻¹ of soil	14	233	353	2465	468	3.2	0.34
3000 mg Mn kg ⁻¹ of soil	11	127	287	2342	458	2.5	0.40
5000 mg Mn kg ⁻¹ of soil	11	130	247	2326	444	2.5	0.46
7000 mg Mn kg ⁻¹ of soil	11	117	220	2246	433	2.5	0.61
SEM±	1.4	4.8	9.4	97	21	0.15	0.03
CD at 5 %	3.0	10.5	20.5	210	45	0.33	0.07
CD at 1 %	4.2	14.7	28.8	295	63	0.46	0.09

*- μmole H₂O₂ degraded min⁻¹ g⁻¹ fresh weight; \$- OD min⁻¹ g⁻¹ fresh weight

Sodium was another element having positive relationship with Mn (Fig. 8). Correlation coefficient was positive and significant for sodium content of soil with Mn content of root ($r = 0.981$, $p = 0.01$), stem ($r = 0.963$; $p = 0.01$) and leaf ($r = 0.830$, $p = 0.05$ and $df = 6$). A synergism between Mn and Na has been reported already (Paivoke, 2002).

Significant decrease in amino acid content of leaves was noted due to external addition of manganese. Correlation analysis indicated that the relationship between soil available Mn and leaf amino acids was negatively significant ($r = -0.784$; $p = 0.05$ and $df = 7$). Even though the change in chlorophyll content of tea leaves was not significant due to 100 and 500 mg Mn kg⁻¹ of soil, a drastic and highly significant reduction was noted at and above the dosage rates of 1000 mg kg⁻¹. This is because excess manganese inhibits chlorophyll synthesis by blocking the Fe concerning process, (Clairmont *et al.*, 1986) which would ultimately reflect on productivity. So was the case with carotenoids. Unlike carotenoids, chlorophyll content of leaves possessed a negative correlation with the dosage of externally added Mn ($r = -0.807$; $p = 0.05$ and $df = 7$). A significant increase in catechin and polyphenol content was noted due to the addition of Mn up to 1000 mg kg⁻¹, beyond which a sharp decline was noted. This is due to the oxidation of polyphenol, which are responsible for

browning of leaves and is accompanied by formation of phenoxyradicals (Takahama and Oniki, 1992) in higher treatments (Table 1). Catechin being a phenolic compound shows a positive and significant correlation coefficient ($r = 0.926$; $p = 0.01$ and $df = 7$) with polyphenols.

The nitrate reductase, which assimilates nitrogen in higher plants, did not respond to the external addition of manganese (data not provided). The activity of catalase was significantly higher than the control due to addition of manganese at the rate of 100, 500 and 1000 mg Mn kg⁻¹ of soil. However, catalase activity showed a negative correlation with external Mn dose ($r = -0.837$; $p = 0.05$ and $df = 7$). Peroxidase activity becomes stronger and stronger due to the increase in manganese addition. It was three times higher than the control when 7000 mg Mn was added kg⁻¹ of soil (Table 1). This could be because of the stimulating effect exerted by manganese on apoplastic H₂O₂ producing enzymes like peroxidase (Fecht Christoffers *et al.*, 2003). Peroxidase shows a positive and significant correlation coefficient with soil applied manganese ($r = 0.982$; $p = 0.01$ and $df = 7$).

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