MICRONUTRIENT DEFICIENCIES IN TEAK (*TECTONA GRANDIS*) SEEDLINGS: FOLIAR SYMPTOMS, GROWTH PERFORMANCE AND REMEDIAL MEASURES

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SUJATHA, M. P. 2008. Micronutrient deficiencies in teak (*Tectona grandis*) seedlings: foliar symptoms, growth performance and remedial measures. Considering the constraints in diagnosing nutrient disorders encountered in teak seedlings, a study was conducted to induce deficiency symptoms of Fe, Cu, Zn, Mn, Mo and B and to find out its impact on growth performance and nutrient concentration by adopting the sand culture technique. According to the results, deficiency of nutrients caused the development of certain symptoms which were specific to each nutrient. Deficiencies of Mo and Cu caused considerable reduction in plant height whereas development of healthy new leaves was seriously affected by Fe and B deficiencies. The order of abundance of micronutrients in teak seedlings was Fe > Mn > Zn > Cu and in general there was no uniform and definite relation between the concentration of nutrients (except Cu) and the expression of deficiency symptoms. In the case of Cu, there was a gradual decreasing trend in its concentration with the expression of deficiency symptoms. Results of the study suggest that total foliar concentration cannot be used as a reliable indicator in the deficiencies of Fe, Zn and Mn. Trials to rectify the deficiency symptoms revealed that recovery was fast and easy if the respective nutrients were applied at the early stage of deficiency. Foliar spray of 0.3% $FeSO_4$, 1% Bordeaux mixture, 0.1% $ZnSO_4$, 0.1% $MnSO_4$, 0.1% MoO_3 and 0.05% boric acid were found to be effective in rectifying the deficiency of Fe, Cu, Zn, Mn, Mo and B respectively.

Keywords: Deficiency, foliar symptoms, growth, micronutrients, teak seedlings

SUJATHA, M. P. 2008. Kekurangan mikronutrien dalam anak pokok jati (*Tectona grandis*): gejala daun, prestasi pertumbuhan dan langkah-langkah pemulihan. Satu kajian teknik kultur tanah telah dijalankan untuk melihat kesan kekurangan Fe, Cu, Zn, Mn, Mo dan B terhadap prestasi pertumbuhan anak benih pokok jati dan kepekatan nutriennya. Keputusan menunjukkan bahawa kekurangan nutrien mengakibatkan sesetengah gejala yang spesifik kepada setiap nutrien. Kekurangan Mo dan Cu menyebabkan kekurangan ketara dalam ketinggian anak pokok sementara pembentukan daun baru yang sihat terjejas teruk akibat kekurangan Fe dan B. Turutan jumlah mikronutrien dalam anak benih pokok jati ialah Fe > Mn > Zn > Cu dan secara umumnya, tidak terdapat hubungan seragam dan tertentu antara kepekatan nutrien (kecuali Cu) dengan gejala kekurangan. Dalam kes Cu, kepekatan nutrien menunjukkan trend pengurangan yang beransur-ansur dengan kewujudan gejala. Keputusan kajian menunjukkan bahawa jumlah kepekatan daun bukanlah petunjuk kekurangan Fe, Zn dan Mn yang baik. Ujian untuk memulihkan gejala kekurangan menunjukkan bahawa pemulihan adalah cepat dan mudah jika nutrien yang berkenaan diberi pada peringkat awal kekurangan. Semburan daun yang mengandungi 0.3% $FeSO_4$, 1% campuran Bordeaux, 0.1% $ZnSO_4$, 0.1% $MnSO_4$, 0.1% MoO_3 dan 0.05% asid borik masing-masing didapati berkesan untuk memulihkan kekurangan Fe, Cu, Zn, Mn, Mo dan B.

INTRODUCTION

Production of healthy and vigorous seedlings in forest nurseries demands balanced supply of both macro- and micronutrients. As a valued timber in Kerala, India a large number of teak seedlings are produced and supplied every year for the establishment of plantations. However, producing quality seedlings has not been without problems. The recent practice of growing seedlings in root trainers containing compost as potting media has resulted in poor growth due to micronutrient deficiencies (Chacko *et al.* 2002). Usually most of the nursery managers fail to diagnose these nutrient disorders for appropriate remedial measures. There is a lot of literature on foliar symptoms and associated growth changes caused by micronutrient deficiencies. However, such studies are scarce in forestry especially for tropical species (Gopikumar & Varghese 2004, Swaminathan & Srinivasan 2004). Thus, we undertook this study to understand micronutrient deficiency symptoms in teak seedlings and suggest appropriate remedial measures to overcome them.

MATERIALS AND METHODS

Deficiency symptoms of various micronutrients (Fe, Cu, Zn, Mn, B and Mo) in teak seedlings were induced artificially in sand culture. For this, white quartz sand was washed thoroughly with tap water followed by 0.01 N HCl in order to make it completely free from various embodied salts. The HCl was washed away using deionized water and the purified sand was kept in plastic pots. Meanwhile, teak seedlings from the nursery were transplanted into polythene bags containing potting mixture comprising soil, sand and cow dung in the ratio of 2:1:1. After a period of two months the seedlings were uprooted and their roots were washed thoroughly with deionized water before being transplanted to the pots containing purified sand. The seedlings were kept inside a glasshouse where a mean temperature of 22–31 ^{o}C, relative humidity of 75–100% and a light intensity of 900 Lux for a period of 10 hours were maintained. The pots in the glasshouse were arranged in seven groups of 20 pots each. The plants were supplied with 50 ml deionized water twice a day during the first four days and once a day after that.

Modified Hoagland No. 2 nutrient solution containing N (16000 µM), K (6000 µM), Ca (4000 µM), P (2000 µM), S (1000 µM), Mg (1000 µM), Cl (50 µM), B (25 µM), Mn (2.0 µM), Zn (2.0 µM), Cu (0.5 µM), Mo (0.5 µM) and Fe (20 µM) were prepared separately (Epstein 1972). After a period of two weeks from when the seedlings were transferred into pots nutrient solutions free of Fe, Zn, Cu, Mn, Mo and B respectively were supplied to the first six groups at 50 ml/pot twice a week for the first 30 days and after that on alternate days while on other days they were given deionized water only. In order to avoid precipitation while mixing with other nutrient solutions, $FeSO_4$ (0.1%) was applied separately at 2 ml/plant to all plants except those labelled as -Fe for treatment without Fe. Plants of the seventh group were kept as controls and were supplied with full composition nutrient solution. All plants were observed regularly and the expression of symptoms, plant height and condition of leaves were recorded. Attempts were also made to compare the induced symptoms with those observed in nurseries at the Kerala Forest Research Institute and the State Forest Department.

Newly matured foliar samples showing deficiency symptoms were collected from 10 randomly selected pots at three and six months from the date of planting and oven dried. The plant samples were powdered and digested using a mixture of 5 ml sulphuric acid, pinch of salicylic acid and 5 ml hydrogen peroxide. Care was taken to avoid contamination during the processing of plant samples. Micronutrients in the digested samples were determined using Varian atomic absorption spectrophotometer (Spectra 200). The concentration of Mo and B could not be determined due to lack of facilities.

Recovery studies were conducted in the remaining 10 pots of each nutrient. Complete Hoagland solution was applied to the plants in five pots while foliar spray of respective nutrient solution was given to the plants in the remaining five pots and the responses were recorded.

RESULTS

Appearance of foliar symptoms

The plants, which were not supplied with specific nutrients started to develop symptoms of nutrient deficiency within a few weeks after the experiment commenced. Some of the symptoms such as retarded growth, shortening of internodes and production of long leaves were common in most cases. However, nutrient deficiency could be diagnosed according to characteristic symptoms of each nutrient (see below).

-Fe plants, i.e. without supply of Fe, were the first to develop deficiency symptoms. After 55 days of planting, yellow chlorotic spots appeared in the interveinal area of the newly emerged leaves whose intensity increased during development. Gradually, the entire leaf became yellow and the green colour was restricted only to the veins (Figure 1) leading to interveinal chlorosis, a characteristic symptom of Fe deficiency. Later, most of the leaves became completely yellow and at the acute stage, yellow leaves became necrotic and whole leaves died off. Another characteristic symptom observed was cupping of leaves (Figure 2). Rusty patches on lower leaves and multiple stems were also noticed in Fe-deficient plants.

-Cu plants expressed deficiency symptoms 84 days after planting. During the early stages of deficiency, yellow patches appeared on the margins of lower leaves. Later, the veins and veinlets of matured leaves became green and very distinct (vein clearing) while the interveinal area remained yellow (Figure 3). As the plant growth advanced, the entire leaf became chlorotic and gradually necrosis developed beginning from the tip of the leaves. In between, white chlorotic mottling appeared on younger leaves and the affected leaves lost their softness and became crispy. Some leaves were oblong in shape. New leaves formed in the advanced stage of deficiency were wrinkled and their growth, arrested (Figure 4).

-Zn plants expressed deficiency symptoms 64 days after planting. Young leaves became crispy as in Cu-deficient plants. Veins and veinlets of newly developed leaves became more prominent. Gradually, white chlorotic spots appeared on the midrib and veins of the leaves (Figure 5), unlike in Fe-deficient leaves where the interveinal area became discoloured. At a later stage, the leaf lamina became chlorotic and necrosis developed from the tip of the leaf. Appearance of bronze coloured spots, which progressively merged to form patches, was also observed on lower leaves. At the acute stage, margins of older leaves became necrotic and premature leaf shedding occurred. In some cases, newly formed leaves were extraordinarily large and leaf drooping was observed (Figure 6).

After 65 days of planting chlorosis of newly matured leaves occurred in plants which were not supplied with Mn. Later these leaves became completely chlorotic. As the deficiency progressed whole leaves of the plant turned chlorotic (Figure 7). Nevertheless, at later stage, lower leaves remained chlorotic and the upper leaves became green.

-Mo plants developed yellow patches on the tip and upper margin of older leaves 65 days after planting. These patches gradually spread to the entire margins and finally became necrosis. In some plants leaves became bluish-green in colour. As in Cu-deficient plants newly matured leaves expressed vein-clearing symptoms but remained green in the interveinal area. The characteristic symptom observed in Mo-deficient plants was the

Figure 1 Interveinal yellowing of new leaves due to Fe deficiency

Figure 2 Cupping and inward curling of leaves due to Fe deficiency

Figure 3 Chlorosis followed by leaf tip necrosis due to the deficiency of Cu

Figure 4 Small and wrinkled new leaves due the deficiency of Cu

Figure 5 Chlorosis of midrib and veins due to the deficiency of Zn

Figure 6 Large and drooping new leaves due to the deficiency of Zn

Figure 7 Chlorotic spots on leaves due to Mn deficiency

absence of leaf tip and the reduction in the size of leaf lamina towards the base, resembling the cauliflower whiptail (Figure 8).

-B plants developed symptoms only six months after planting. The new leaves developed were small in size and were closely clustered (Figure 9). Both upper and lower leaves were very brittle. All leaves were yellow and at the acute stage they became necrotic leading to premature leaf fall. B-deficient plants also produced multiple stems and in some plants new leaves were narrow and tapered.

Induced symptoms vs. observed symptoms

Foliar symptoms observed in the forest nurseries expressed by the root trainer-grown teak seedlings included yellowing of younger leaves, curling and cupping of leaves, vein clearing with the appearance of reticulate pattern and wrinkling of new leaves; these were similar to the induced symptoms of Fe and Cu deficiencies. In root trainer nurseries foliar symptoms were observed even at the two- to four-leaf stage. In clonal

Figure 8 Shortening of leaf lamina and absence of leaf tip due to the deficiency of Mo

Figure 9 Clustering of new leaves due to the deficiency of B

nurseries, in addition to the deficiencies of Fe and Cu, reduction in the size of leaf lamina and absence of leaf tip were also observed, indicating Mo deficiency. The observed symptoms of Fe, Cu and Mo were confirmed by the application of respective nutrients as described later in the paper.

Growth performance

Growth of plants in all treatments was monitored regularly and the height and total number of healthy leaves were recorded at three and six months after planting. We observed drastic retardation in the growth of plants when they were deficient in micronutrients. The deficiency of Fe, Cu, Mn and Mo led to the shortening of internodes and clustering of branches. Similarly, development of long leaves was observed under Cu, Zn, Mn and Mo deficiencies.

There was considerable reduction in the height of plants (Table 1) due to the deficiency of micronutrients. The reduction in height compared with control varied from 18–45% at three months after planting while at six months, 20–52%. Reduction in height was least in B-deficient plants compared with other nutrient deficiencies.

Deficiencies of all micronutrients except B caused significant reduction in the number of healthy leaves compared with control at three and six months of growth. At three months the healthy leaves in Fe-deficient plants were 69% less than the control. At severe deficiency, reduction in the number of healthy leaves was 87.5% in Fe-, Mn- and B-deficient plants and 81.3% in Cu-, Zn- and Mo-deficient plants compared with control.

Root growth was considerably affected when all the micronutrients were absent (Figure 10). However, among single nutrient deficiencies, only Cu deficiency resulted in retardation of root growth.

Figure 10 Root growth as influenced by the deficiencies of micronutrients

Nutrient concentration in leaves

The concentration of Fe, Cu, Zn and Mn in newly matured leaves of plants showing deficiency symptoms and of healthy plants were compared

Table 1 Growth of teak seedlings due to the deficiency of micronutrients

Growth parameter	Age of plant	Control	-Fe	-Cu	Treatment -Zn	-Mn	-Mo	-B
Height (cm)	3 months	42.2 *(0.102)	28.4 (0.091)	24.3 (0.087)	26.1 (0.092)	27.3 (0.108)	23.1 (0.114)	34.8 (0.088)
	6 months	62.3 (0.124)	33.6 (0.109)	35.4 (0.116)	32.8 (0.098)	35.9 (0.092)	29.6 (0.080)	49.6 (0.078)
Reduction in height (%)	3 months	-	33	42	38	35	45	18
	6 months	-	46	43	47	42	52	20
Healthy leaves	3 months	13 (0.002)	4 (0.005)	8 (0.003)	8 (0.002)	10 (0.003)	10 (0.002)	13 (0.003)
	6 months	16 (0.005)	2 (0.001)	3 (0.001)	3 (0.001)	2 (0.001)	3 (0.002)	2 (0.002)
Reduction in healthy leaves (%)	3 months	-	69.2	38.5	38.5	23.1	23.1	-
	6 months	-	87.5	81.3	81.3	87.5	81.3	87.5
Dry root weight (g)	6 months	12.64 (0.022)	13.82 (0.029)	7.79 (0.009)	14.45 (0.031)	10.92 (0.018)	12.92 (0.012)	10.22 (0.024)

n = 10,*Figures in parentheses indicate standard errors.

at both early and severe stages of deficiencies (Table 2). At the early stage of Fe deficiency, mean leaf concentration was 422 ppm while similar leaves in control plants contained 417 ppm. After a period of six months Fe concentrations were 328 and 287 ppm in healthy and deficient plants respectively. The concentration of Cu in plants was very low. At three months, healthy plants contained 18 ppm Cu compared with only 10 ppm in deficient plants. An apparent decreasing trend from 12 to 8 ppm was observed in Cu-deficient plants at six month after planting. Zn concentration in leaves was higher than those of Cu, but far below than Fe. The concentration of Zn dropped from 65 ppm in healthy plants to 54 ppm in deficient plants at three months after planting but after a period of six months it was similar in both plants. After three months of growth Mn content in healthy and deficient plants were about the same, i.e. 185 and 180 ppm respectively. However, at six months the difference was bigger at 148 and 126 ppm respectively.

Results obtained showed that the order of abundance of micronutrients in teak seedlings was Fe > Mn > Zn > Cu. In general it was also observed that there was no uniform and definite relation between the concentration of nutrients (except Cu) and the expression of deficiency symptoms compared with control plants.

Recovery studies

In order to determine the measures to rectify symptoms caused by the deficiency of micronutrients, respective nutrients were supplied through soil as well as foliar spray during early and later stages of the deficiency.

Application of complete Hoagland solution through soil to the Fe- deficient plants imparted green colour to the foliage within one month. Trials on foliar application of $FeSO_4$ at different concentrations (1, 0.55 and 0.3%) were also conducted considering the necessity in cases where soil application was not possible. Foliar spray of 1% $FeSO_4$ caused the death of whole leaves indicating phytotoxicity. Some necrotic spots developed on the leaves when 0.5% $FeSO_4$ was applied. However, foliar spray of 0.3% $FeSO_4$ two times a week resulted in the rectification of symptom. At severe stage of deficiency the affected leaves were not able to recoup but new green leaves emerged without any symptom of phytotoxicity. Thus, as a remedial measure, foliar spray of 0.3% $FeSO_4$ twice a week is suggested when soil application is not possible.

In the case of Cu-deficient plants soil application of Hoagland solution resulted in the recovery of deficient plants. Foliar application of $CuSO_4$ even at 0.3% led to phytotoxicity. However, the Cu-deficient plants recovered by spraying 1% Bordeaux mixture.

As in the case of Fe and Cu, application of complete Hoagland solution through soil rectified the deficiency of Zn, Mn, Mo and B. With regard to foliar spray, application of 0.2% $ZnSO_4$, 0.2% $MnSO_4$ or 0.2% H_2MoO_4 twice a week managed to eliminate deficiency symptoms of respective nutrients but new leaves failed to emerge. The same nutrients when applied at 0.1% resulted in the emergence of new flushes in all cases. Among the above nutrients, recovery

Table 2 Concentration of micronutrients in teak seedlings at different stages of deficiency

Condition of the plant	Age of plant	Concentration (ppm)			
		Fe	Cu	Zn	Mn
Healthy	3 months	417 *(7.93)	18 (0.001)	65 (0.41)	185 (1.29)
Deficient	3 months	422 (10.04)	10 (0.001)	54 (0.72)	180 (1.25)
Healthy	6 months	328 (3.71)	12 (0.001)	38 (0.42)	148 (0.54)
Deficient	6 months	287 (6.37)	8 (0.001)	39 (0.72)	126 (4.74)

n = 10, *Figures in parentheses indicate standard errors.

through foliar spray at severe stage of deficiency was possible only in the case of Mn. The B-deficient plants developed phytotoxicity upon spraying 0.1% boric acid and the recovery was possible only at 0.05% H_3BO_3.

Results indicated that plants under early stage of nutrient deficiency could recover their original vigour within a few days of nutrient supply while those under severe stage of deficiency could recoup only in certain cases.

DISCUSSION

Switching over to root trainer technology from traditional stump method is a recent trend observed in the production of teak seedlings throughout Kerala. However, development of abnormal shoots and leaves and the consequent growth decline in teak seedlings are some of the major problems encountered in this technology. Most of these abnormalities are caused mainly by improper nutrition of plants especially the deficiency of micronutrients (Chacko *et al.* 2002) and the measures to rectify these abnormalities assume great concern.

Interveinal chlorosis of young emerging leaves as reported in various agricultural crops (Chapman 1975) and *Eucalyptus* (Dell *et al.* 1995) and inward curling and cupping of leaves were also the characteristic symptoms observed due to the deficiency of Fe in teak seedlings. The decrease of chlorophyll concentration in young leaves is attributed mainly to the role of Fe in the biosynthesis of chlorophyll coupled with its immobile nature (Epstein 1972). Fe acts as a metal component in redox reactions or as a bridging element between enzyme and substrate and hence various changes in the metabolic processes also occur due to the deficiency of Fe. These cause various problems including retardation in plant height and healthy leaves, shortening of internodes and production of multistems (Hewitt 1959). Even though a reduction of Fe concentration was observed in deficient plants it cannot be considered as a reliable indicator of Fe deficiency in teak seedlings. This is because in some cases samples whose visual symptoms indicated Fe deficiency had comparable Fe concentrations with those of controls. This might be due to the fact that the deficient level of a nutrient is decided by its metabolically active fractions and not its total concentration. Similar observations were made by Mohammed *et al.* (1998) during the development of Fe chlorosis in citrus lemon trees.

Development of yellow patches on the lower leaves, vein clearing, chlorosis of entire leaf followed by necrosis and development of small and wrinkled new leaves were symptoms expressed due to the deficiency of Cu. This nutrient acts as a metal activator of several enzymes such as tyrosinase, laccase, ascorbic acid oxidase and butyryl Co-A dehydrogenase and is also important in light reaction (Hewitt 1959). Kamala *et al.*(1986) also observed the development of yellow patches on the leaves of teak seedlings due to Cu deficiency. Retardation of terminal growth, shortening of internodes and clustering of branches due to Cu deficiency have also been reported (Chapman 1975). Development of long leaves has been attributed to Cu, Zn, Mn and Mo deficiencies. As in the case of Fe, deficiency of Cu caused significant reduction in plant height and the number of healthy leaves within three to six months after planting. Root development was also seriously affected by the lack of sufficient concentration of Cu compared with other nutrients. Unlike Fe, there was a gradual decrease in the total concentration of Cu with the development of deficiency symptoms.

Zn-deficient plants expressed chlorosis of midrib and veins in the developed leaf. Appearance of bronze coloured spots on lower leaves, which finally merged together to form necrotic patches that led to premature shedding of leaves, was attributed to phosphorus toxicity. Chlorosis of midrib and necrosis of lower leaves have been reported in maize (Rahimi & Bussler 1979). The emergence of large and drooping new leaves in the advanced stage of deficiency is probably due to auxin metabolism caused by lack of Zn. Shortage of Zn also resulted in lower plant height and fewer leaves within three to six months of growth period. There was not a clear trend between total foliar concentration of Zn and expression of deficiency symptoms.

Loss of chloroplasts and appearance of chlorotic spots on the upper leaves were initial symptoms of Mn deficiency. As deficiency progressed whole leaves turned chlorotic. However, at later stage lower leaves remained chlorotic and the upper leaves became green. Similar observations were recorded by Kamala *et al.* (1986) in teak seedlings. Affected plants lost their vigour as evidenced by the retardation of plant height (35–42%) and discoloration in healthy leaves (23-87%). Foliar concentration of Mn showed a decreasing trend at the advanced stage of deficiency. However, only a slight decrease was observed in the unhealthy foliage during early stage.

Yellowing of tip and margins of older leaves followed by necrosis, production of bluish green new leaves, interveinal yellowing and shortening of leaf lamina with the absence of tip in new leaves similar to the whip tail in cauliflower were the characteristic symptoms of Mo deficiency. Yellowing followed by necrosis in older leaves may be due to the accumulation of nitrate, which is a direct result of lack of nitrate reductase of which Mo is a component. Loss of mesophyll of leaf lamina leaving an irregular margin of tissue along the midrib and disintegration of primordia can be a probable reason for shortening of leaf lamina and irregular margin. Shortening of leaf lamina in teak seedlings due to the deficiency of Mo was also reported by Gopikumar & Aravindakshan (1988). As in the case of other nutrients, Mo deficiency also resulted in the retardation of plant height (45–52%) and reduction in healthy leaves (23–81%) at three and six months after planting.

Clustering and brittleness of new leaves, blotching and premature shedding of lower leaves and emergence of slender and tapered leaves were characteristic symptoms of B-deficient plants. Rosetting, brittleness and die back of shoots due to the deficiency of B have also been reported in cabbage by Chapman (1975). Unlike in the case of other nutrients, plant height was not seriously affected by the deficiency of B. The decline in the number of healthy leaves occurred only at the advanced stage of deficiency (87.5%), suggesting a relatively low requirement of B by teak seedlings compared with other nutrients which cause deficiency symptoms at earlier stages.

Among the nutrients under study, Fe was highest in the foliage followed by Mn, Zn and Cu. The relationship between concentration of nutrients in the foliage and development of deficiency symptoms was not definite and uniform except in the case of Cu. Hence it is suggested that total concentration cannot be used as a reliable indicator of micronutrient deficiency in teak seedlings.

Foliar symptoms developed in the study were found to resemble those observed in forest nurseries in Kerala and remedial measures employed were very effective in improving the health of plants. In the nurseries, plants expressed symptoms much earlier than the sand-culture study. This is because the practice of dibbling germinated seeds in root trainers results in imbalanced nutrition from the potting media at an early stage of growth (Chacko *et al.* 2002). Recovery from these disorders was fast and easy if the respective nutrients were applied at the early stage of deficiency.

CONCLUSIONS

Results of this study showed that the deficiency of nutrients caused the development of certain symptoms which were specific to each nutrient. Deficiencies of Mo and Cu caused considerable reduction in plant height whereas development of healthy leaves was seriously affected by Fe and B deficiencies. Recovery from these disorders was fast and easy if the respective nutrients were applied at the early stage of deficiency. The order of abundance of micronutrients in teak seedlings was Fe > Mn > Zn > Cu. In general, except for Cu, there was no uniform and definite relationship between the concentration of nutrients and the expression of deficiency symptoms. Thus, total foliar concentration cannot be used as a reliable indicator in the deficiencies of Fe, Zn and Mn in teak.

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