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# Application of foliar sprays containing copper, zinc and boron to mature clonal tea (*Camellia sinensis*): effect on yield and quality

**by**

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**Submitted in partial fulfilment of the requirements for the degree in M.Sc. (Agric) Agronomy**
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**June 2012**

# DEDICATION

To my dear husband Fexter

**DECLARATION**

I, undersigned, hereby declare that the dissertation submitted herewith for the degree MSc (Agric) Agronomy to the University of Pretoria, contains my own independent work and has not been submitted for any degree at any other university.

Signed______________________________________________

Chikondi Njoloma (28469242)

**ACKNOWLEDGEMENTS**

I thank the almighty God for enabling me to accomplish this work. Thanks to Tea Research Foundation of Central Africa (TRFCA) board of management for seeing the need for me to undergo this study and for providing funding for this study without which this study would be a non-starter. I would like to thank TRFCA technical staff, Mr. M.H. Mabvimba and Ms T. Malema who helped with data collection. I also would like to thank the Director and all scientists of the TRFCA for critiquing this document.

I am indebted to my supervisors Dr. Nicolette Taylor and Prof. Zeno Apostolides for their constructive comments and their contribution towards this work.I would like to express my gratitude to Eastern Produce Malawi for allowing me to use their fields at Mianga and Glenorchy estates for the experiments and for all the support that they provided during the entire period of my study.

Finally, I would like to thank my parents, relatives and friends for their support as well as prayers. My special thanks should go to my dear husband Fexter and my children Brian, Florence and Chisomo for their patience and understanding during the time when I could not be available for them.

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# ABSTRACT

Most of the fields planted with clonal tea in Malawi are deficient in micronutrients. This was evident in leaf analysis data collected in 2007 from 170 mature clonal tea fields of some of the tea estates in Malawi, which showed very high incidences of B, Zn and Cu deficiencies. Current fertiliser recommendations have emphasised much on macronutrients, such as N, Pand K, but little attention has been paid to micronutrient elements despite continuous removal through harvesting. A study was therefore conducted to assess the effect of foliar applications of Cu, Zn and B containing fertilisers on yield and quality of mature clonal tea plants. Field experiments were laid out in randomised blocks in two fields planted with cultivars PC 105 and 108 at Mianga and Glenorchy estates in the Mulanje district in Malawi and were replicated four times at each site. The treatments were T1 (control, no spray), T2 [ 0.1% boric acid (190.8g B/ha)], T3 [1% copper sulphate solution (4.35kg Cu/ha)], T4 [1.25kg/ha zinc oxide (1kg Zn /ha)], T5 [0.1% boric acid, 1% copper sulphate solution, 1.25kg/ha zinc oxide], T6 [1% of Commercial micronutrient mix ( N 1.7%, P 2.3%, K 1.6%, Mg 0.25%, Fe 1288 mg/kg, Mn 1005 mg/kg, Zn 2182 mg/kg, Cu 732 mg /kg, B 8202 mg/kg, Mo 3681 mg/kg, kelp extract 75 mg/kg, amino acids 50 g/kg, phytofulvate 50 g/kg)], and T7 [2.48kg/ha zinc sulphate (1kg Zn /ha)].

Micronutrient foliar sprays affected yield significantly at Glenorchy tea estate, but had no significant effect on yield at Mianga estate. Copper sulphate solution applied at 1% concentration decreased yield, but the other foliar applications did not impact yield relative to the control at Glenorchy. Tea quality by taster's scores was not affected at Mianga estate, whilst at Glenorchy quality was affected, with the commercial micronutrient mix giving the lowest total score. Individual parameters that contributed to the differences in total scores at Glenorchy estate included brightness, briskness, colour of liquor, colour of infusion and colour with milk. Thearubigin (TR) concentration was the only biochemical quality parameter that was affected by the micronutrient foliar sprays. The level of TR was increased in all treatments that received the foliar sprays and the control treatment gave the lowest amount of TRs at both sites. An increase in the Thearubigin/Theaflavin (TR/TF) ratio, obtained in all treatments that received the foliar sprays, provides evidence that more catechins were being converted to TRs than TFs during fermentation.

Foliar levels of B, Zn and Cu were raised significantly by the application of the respective foliar sprays, except for the commercial micronutrient mix which did not significantly increase B and Zn levels at both sites, but raised Cu levels to the recommended level only at Mianga, but not at Glenorchy estate. Concentration of Cu was extremely high in tea leaves treated with copper sulphate either alone or in combination with boric acid and zinc oxide. Levels of other nutrient elements, namely N, P, K, S, Zn, Cu, Mg, Mn, Mo, Ca, Fe, B, Al and Na in the soils where different foliar sprays were applied were similar at both sites. Likewise, foliar levels of N, P, K, S, Zn, Cu, Mg, Mn, Mo, Ca, Fe, B, Al and Na were not different in all plots at Mianga, but at Glenorchy differences in foliar levels of Al and S were noted. High concentrations of Al and S in the leaves were observed in plots that received copper sulphate applied alone. High foliar concentrations of Al and Cu in leaves treated with copper sulphate could be the reason for the low yields obtained in the plots treated only with copper sulphate at Glenorchy estate.

Foliar application of Cu, Zn and B in forms of 0.1% boric acid (190.8g B/ha)], 1% copper sulphate solution (4.35kg Cu/ha)], [1.25kg/ha zinc oxide (1kg Zn /ha)], 1% of a Commercial micronutrient mix (N 1.7%, P 2.3%, K 1.6%, Mg 0.25%, Fe 1288 mg/kg, Mn 1005 mg/kg, Zn 2182 mg/kg, Cu 732 mg /kg, B 8202 mg/kg, Mo 3681 mg/kg, kelp extract 75 mg/kg, amino acids 50 g/kg, phytofulvate 50 g/kg), and 2.48kg/ha zincsulphate (1kg Zn /ha) to mature clonal tea did not significantly increase yields and tea tasters scores, therefore their application to clonal tea with the aim of improving yield and quality may not be necessary. Results from this study indicated that clonal tea, specifically cultivars (PC 108 and PC 105), could not give positive results in terms of yield and quality of tea due to foliar application of Cu, Zn and B within the first season of application. However, if application is aimed at raising concentrations of Cu, Zn, and B, then boric acid, zinc sulphate, zinc oxide and the commercial micronutrient mix may be used without negatively affecting yield and quality of tea significantly. Copper sulphate however, at the application concentration and frequency used, should not be done because of the observed yield decline in copper sulphate treated plots. More prolonged research is required to determine if long term applications can correct perceived deficiencies and increase yield.

# CHAPTER 1: GENERAL INTRODUCTION

Zinc, B and Cu are among the important micronutrient elements in tea production (Sultana *et al.,* 1978, Malenga 1979, Mitini-Nkhoma 1987, Barbora *et al*., 1993, Malenga 1994, Gohian *et al.,* 2000). It becomes a matter of concern when deficiencies of these are evident because either yield or quality of tea is adversely affected. Although these micronutrient elements are required in minute quantities, for a continuously cropped plant like tea, there is always a drain on these elements which eventually leads to deficiencies if not replaced (Dale, 1971).

High incidences of Cu, B and Zn deficiencies in leaf analyses of these elements were shown from approximately 170 mature clonal tea fields in 2007, from some of the estates in Malawi. Unfortunately, current fertiliser recommendations in tea, especially in Malawi and Zimbabwe, emphasise the application of major nutrients, particularly N, P and K, whilst micronutrients are not regularly applied in tea bushes, hence their removal from the soil continues without an organized replacement plan (Verma &Ranade 2010).

There are numerous commercial micronutrient products available for use by the growers. These range from single elemental products to multi-micronutrient pre-mixes. These multi-micronutrient pre-mixes have the potential to serve as a remedy to micronutrient deficiencies, especially in cases where multiple micronutrient deficiencies occur. If proven more effective,they can serve as better alternatives to those that are currently recommended, such as zinc oxide and copper sulphate.

As most of the tea fields in Malawi are deficient in micronutrients and little attention is given to the application of these micronutrients, foliar applications may be able to improve both tea yield and quality in deficient plantations.A study was therefore conducted in order to evaluate the effect of different formulations of foliar applied Cu, Zn and B on yield and quality of deficient clonal tea plants. Specifically, the study aimed at evaluating the effects of foliar sprays,including one commercial micronutrient mix, on yield and yield components, contents of polyphenolic compounds in made tea, as well as on tea taster's scores. It also aimed at establishing the effect of foliar sprays on concentrations of B, Cu, Zn and other nutrient elements in the leaves of treated tea bushes. It was hypothesised that foliar applied fertilisers containing Cu, Zn and

Bwould have significant effects on yield and quality of deficient tea plants, and couldsignificantly raise nutrient levels in deficient plants.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Origin of tea

Tea (*Camelliasinensis*) is an evergreen perennial crop, of the family *Theaceae* or *Camellia* that is grown in tropical, subtropical and temperate climates of the world. Its young leaves are plucked and processed into a beverage that is consumed worldwide. China was the first country to use it as a drink (Paul *et al.,* 1997). There are two main varieties of tea namely: *Camellia sinensis* var *assamica*, which has relatively large leaves and *Camellia sinensis* var *sinensis*, with small, semi-erect leaves. The *assamica* variety originated from the forests of Assam in north-eastern India and *sinensis* tea from Sichuan province in south-western China (Kamau, 2008). These areas are found at the point of intersection between latitude 29° N and longitude 98° E (Mondal *et al*., 2004) and are characterised by a monsoon climate, with high rainfall and high humidity during warm, wet summers and cool, dry winters (Kamau, 2008). Due to extensive hybridisation, most of the tea cultivars that are commercially grown at present exhibit characteristics intermediate between *assamica* and *sinensis* (Mondal *et al.,* 2004). The crop is successfully grown in areas that are located between latitudes 45° N and 34° S, covering about 52 countries (Mondal *et al*., 2004). The main tea producing countries are Bangladesh, China, India, Indonesia, Sri-Lanka, Vietnam in Asia, Burundi, Kenya, Malawi, Rwanda, Tanzania, Uganda, Zimbabwe in Africa, Argentina and Brazil in South America, and Iran and Turkey in the Middle East (Hicks, 2009). Figure 2.1 shows some of the major tea producing countries in the world.

Tea cultivation in Malawi started in 1896 (Ellis & Nyirenda, 1995) and until today, tea has been cultivated on a commercial basis. Tea is the second largest export crop in Malawi, contributing 7.9% to Malawi's total export earnings (MCCI, 2008) and contributes approximately 4% to the world's tea exports (Dharmasena, 2003). The Malawian tea industry produces black tea that is exported to European, Asian and American markets.In Malawi, tea is only grown in Thyolo, Mulanje and Nkhatabay districts (Munthali, 2007) (Figure 2.2).


**Figure 2.1** **Major worldwide tea producing countries (http://www.palaisdesthes.com/en/tea/tea-producing-countries.htm, accessed online on 13 May, 2011)**


**Figure 2.2 Tea growing districts in Malawi (Munthali, 2007)**

## 2.2 Tea Production: Agronomic requirements and management practices

### 2.2.1 Rainfall

Tea is grown in a wide range of climatic conditions from equatorial to humid temperate climates (Kamau, 2008). A minimum annual rainfall of 1200mm is a requirement, however,for optimum growth and yield an annual rainfall amount ranging between 2500 mm and 3000 mm is ideal

(Anandacoomaraswamy *et al*., 2000). Annual rainfall of less than 1150 mm reduces tea yields and irrigation is recommended for such areas (Kamau, 2008).

### 2.2.2 Temperature and solar radiation

Atmospheric temperature, soil temperature and solar radiation are important factors that influence tea shoot development and extension (Matthews & Stephens 1998, Carr & Stephens, 1992). Tea grows very well within a temperature range of 18-25 °C. Temperatures below 13 °C and above 30 °C are detrimental to shoot growth (De Costa *et al*., 2007). Kamau, (2008) also indicated that daytime temperatures above 30 °C and night temperatures below 10 °C cause a decline in the extension and development of shoots and growth of leaves. Base temperature for tea shoot extension has been reported to vary from 7 °C to 15 °C, with an average of 12.5 °C (De Costa *et al.,* 2007). Night and day soil temperatures also influence flowering of the tea bushes. According to De Costa *et al.,* (2007), high soil temperatures during the day and low night temperatures induce flowering of the tea bushes, implying a reduction in vegetative growth (De Costa *et al.,* 2007).

Temperature and photoperiod trigger axillary buds, which remain on plucked shoots, to enter a dormant state (Matthews & Stephens, 1998). A decline in day length and temperature leads to bud dormancy and this mostly occurs between the months of January and June in Tanzania and Malawi (Matthews & Stephens, 1998). Tanton (1982) stated that “day length does not affect shoot extension when nights are cool (10 °C), but growth rate is depressed by short days (11 h) when nights are warm (20 °C)”. Tea requires an average of five sunshine hours per day (17 MJ $d^{-1}$) for optimum growth. Solar radiation levels above 350 W $m^{-2}$ saturate the single top leaves of a tea bush, while the whole canopy of tea plants become light saturated at 700-800 W $m^{-2}$ (Kamau, 2008).

### 2.2.3 Soil

Tea is generally cultivated in a wide range of soil types, but there are specific soil characteristics that are required for successful tea cultivation. These include soil pH and some physical characteristics such as depth, structure and texture (Kamau, 2008). Tea requiresdeep, well drained, permeable, acidic soils, with pH within the range of 4.5 to 5.5 for its optimal growth and development (De Silva, 2007). Other reports have indicated pH ranges of 4.0-6.0 to be ideal for tea (Othieno, 1992). Due to changes in the chemistry of the soils, pH of the soils in tea gardens may diverge from the normal levels and in such situations amendment chemicals, such as lime, are used to correct it.

A soil depth of at least 2 m and a crumbly, structured soil with about 50% pore space is required for optimum growth of tea (Dey, 1969). Tea thrives well in soils of texture ranging from sandy loam to clays, silts and loams of all kinds, however, due to their low field capacity, sandy soils are not desirable unless mechanisms are put in place to ensure a good distribution of water and nutrients (Kamau, 2008). High organic matter levels in tea gardens have been reported compared to other agro-ecosystems (Solomon *et al*., 2002).

In Malawi, tea is grown on soil types that have been washed from a granite massif and on sedimentary gneissic soil types (Willson & Clifford, 1992). In some parts of the Mulanje district, the soils are deep, well drained latosols within the textural classes of clays or sandy clays (Cloughley *et al.,* 1983). The soils are characterised by low pH, as well as high organic matter content.

### 2.2.4 Planting

Planting is done when rains have started and there is sufficient rain to wet the soil at least twice the planting depth and when there is a high probability that rainfall will continue (Grice, 1990). In Malawi, planting is done between November and mid-February (Grice, 1989). Conservation measures are a pre-requisite in all areas to be planted with tea especially on sloping grounds. Contour planting either in single or double hedged rows, is suitable in hilly terrains. Rooted plants raised in nurseries either from seeds or from cuttings are used for planting in the fields.

Different plant spacings have been recommended for both rain-fed and irrigated tea. For rain-fed tea the recommended spacings are: 120 by 90 cm, 120 by 85 cm, 120 by 80 cm, 120 by 80 cm, 120 by 75 cm, 120 by 70 cm, 150 by 90 cm and they give population densities (plants $ha^{-1}$) of 9259, 9804, 10417, 111111, 11905, and 7692 respectively. Under irrigation 120 by 65 cm or 120 by 60 cm, giving population densities of 12821, 13889 respectively are recommended (Grice, 1989, Kamau, 2008). A double hedge plant layout of 120 by 60 by 90 cm, which gives a plant population of 12345 plants $ha^{-1}$, recommended for irrigated tea has been followed by other growers (Grice, 1990). Most tea growing countries have adopted the spacing of 120 by 60 cm, which has been found to produce superior yields compared to the other plant spacings (Bore *et al.,* 1998).

### 2.2.5 Mulching

It is recommended to mulch tea with grass immediately after planting and the practice needs to be maintained for the first two seasons (Kayange & Grice, 1991). Other materials such as plastic sheets can be used for mulching in areas where grass is scarce (Kayange, 1986). Mulching reduces loss of soil moisture due to evaporation, protects the soil from the severe destructive action of raindrop impact and the burning of soil organic matter by sunshine, and maintains a high degree of permeability to incident rain and irrigation. Mulching also modulates soil temperature within the upper layers of the soil (Grice, 1984). Plant survival, bush development and yields are better in mulched tea plants than in unmulched tea plants. Yield improvement due to mulching young tea for the first two years was noticeable in the fifth and sixth seasons (Kayange & Grice, 1991).

### 2.2.6 Fertiliser application

Like all other plants, tea requires various nutrients for normal growth and productivity. Under normal circumstances, plants get the required nutrients from the soil, except for small quantities of N and other elements that are obtainable from air and rainwater through absorption by the leaves (Kamau, 2008). However, most soils cannot supply adequate quantities of most nutrient elements and growers supplement the soil nutrients with fertilizers. Furthermore, tea harvesting involves removal of young tender shoots which contain considerable amounts of nutrients and the main elements that are removed from tea fields through harvesting include N, P and K, with quantities varying depending on tea producing area, cultivar and type of plucking (Kamau, 2008). For instance, N removal by plucking ranges from 40 to 160 kg N $ha^{-1}$ assuming made tea yields of 1-4 t $ha^{-1}$ (Kamau, 2008). According to Sedaghathoor *et al.* (2009), the content of N in the flushing tea shoot is the highest followed by K, Ca, P, S, Mg and Zn, with the harvestable shoot containing 3.5-5% nitrogen on a dry matter basis.

Fertilization rates for N, P, K depends largely on genotype and age of the bush and they also vary from region to region (Kamau, 2008). For example, in Malawi during the planting year, 60 g of single super phosphate per plant needs to be applied in a planting pit at planting and 30 g of ammonium sulphate applied three weeks after planting (Grice, 1990). Thereafter, annual N, P and K applications are done in N:P:K ratio of 15:3:5 at rates ranging from 50 kg ha$^{-1}$ N, 10 kg ha$^{-1}$ P and 17 kg ha$^{-1}$ K to 300 kg ha$^{-1}$ N, 60 kg ha$^{-1}$ P and 100 kg ha$^{-1}$ K for vegetatively propagated cultivars and from 20 kg ha$^{-1}$ N, 0 kg ha$^{-1}$ P and 0 kg ha$^{-1}$ K to 250 kg ha$^{-1}$ N, 50 kg ha$^{-1}$ P and 83 kg ha$^{-1}$ K for seedling tea (Grice, 1990). Other nutrient elements that are removed from the fields in smaller amounts include Mg, S, Ca, Fe, Mn, B, Cu and Zn (Kamau, 2008).

### 2.2.7 Pruning

Pruning in tea basically involves the artificial removal of all or most of the leaf bearing branches of the plant (Nissanka *et al.,* 2004). Although pruning of the tea bush results in no productivity and suddenly exposes the pruned branches to the hot sun, thereby making them prone to sun scorch, it is a necessary operation in any tea plantation because unpruned tea would outgrow the height at which plucking (harvesting) is possible (Grice 1990). Normally, the height of tea plants under cultivation is maintained between 60 and 100 cm in order to facilitate easy plucking (Grice & Clowes, 1990). In addition to maintaining the plucking table height, pruning helps renew maintenance foliage and keep yields at high levels, (Kamau, 2008), improves bush hygiene and reduces incidents of pest and diseases (Dutta, 2011).

During the pruning year, yield is greatly reduced but a year after pruning there is an increase in yield up to the second year at which maximum yields are obtainedand thereafter, there is a gradual decline in yield (Malenga, 1997, Nissanka *et al*., 2004, Dutta 2011). Recovery from pruning is dependent upon the health of the plant, amount of energy reserves present and the process of ageing (Kamau, 2008). Different pruning rounds have been recommended for different areas. For example, in Malawi, tea can be pruned every two to four years (Grice, 1990). However, skiffing can be done to prolong the pruning rounds (Nissanka *et al*., 2004). Skiffing is light pruning, which involves removing the green wood in mature tea at about 15 cm above the normal pruning height (Kamau, 2008).

In young tea, pruning operations are done in order to achieve a proper bush frame formation by suppressing the centrally dominant apical growth and ensure an even distribution of number and thickness of branches (TTRA, 2008a). The prunings are left within the field in order to protect fields from erosion and maintain soil fertility, since considerable amounts of N, P, and K are returned to the soil from the prunings (Kamau, 2008).

### 2.2.8 Plucking

Harvesting in tea involves nipping off of tender apical portions of shoots consisting of a terminal bud and 2 to 3 leaves above the plucking table and is referred to as plucking (Kamau, 2008, TTRA, 2008b). A collection of individual shoots containing two leaves and a bud (2+b) or three leaves and a bud (3+b) (Matthews & Stephens 1998) comprise the economic yield of the tea plant. Plucking is a vital aspect of tea production. The quality of plucking determines the quality of tea to be manufactured, whilst the cost of plucking has a major influence on the profitability of the enterprise (Wilkie, 1993). Tea is normally harvested by hand, however, due to scarcity and high costs of labour, mechanical plucking using either machines or shears has become inevitable in most tea growing areas (Grice & Clowes, 1990).

The determination of plucking rounds takes into consideration the standard of leaf on the crop and value of made tea. These are connected to the need to pluck the tea at a time that coincides with the majority of shoots that are due for plucking (Grice & Clowes, 1990). For example, during the rainy season in Malawi, the 2+b and 3+b standards are attained when the shoots are 42 days old.

Yield in tea is mainly affected by mean individual shoot dry mass, basal shoot population density (number of shoots $m^{-2}$) and time taken for an axillary bud to grow into a shoot suitable for plucking, which is also known as the shoot replacement cycle (Grice & Clowes, 1990, TTRA, 2008b). Shoot mass is dependent on shoot size, and dry matter content which varies with season and management practices, such as fertiliser application and irrigation (Carr, 2000). Shoot population is not only affected by management factors, but also varies with cultivar (Carr, 2000). Shoot replacement cycle depends upon growth and development, which mainly depends on climate, although irrigation and misting can help increase the number of shoot replacement cycles in a year (Grice & Clowes, 1990).

Shoot growth rate, which is affected by cultivar, climate and type or standard of plucking, is an important factor to consider in plucking. Initially, the bud is very small, with a size of about 2 mm, and grows into a 6 cm long shoot with only a leaf and a bud (1+b) at 5 weeks and thereafter, its size increases rapidly between the fifth and the eighth week, when it grows into three leaves and a bud shoot (Ellis & Grice 1976). If shoots are allowed to grow freely, the terminal bud eventually becomes dormant after a specified number of leaves, normally up to 12, have unfolded (Ellis & Grice 1976, Carr 2000). A tea shoot that has a dormant terminal bud is known as a *banjhi* shoot (Carr, 2000). Tea growers in Malawi and the other parts of central Africa get most of the yield (over 80%) in the hot, wet season from mid November to April and low yields in the cool, dry season (May to August) and hot, dry season (September to mid November) (Squire**,** 1977). This is due to changes in shoot growth rates between seasons.

Plucking is performed at regular intervals, also known as plucking rounds, and they vary from 4-21 days depending on the growing conditions and harvesting policy (TTRA, 2008b, Cloughley *et al.,* 1983). In Malawi and Zimbabwe, 10/11 day plucking rounds are recommended for hand and shear plucking, while a 14 day round is recommended for machine plucking (Grice & Clowes, 1990,Nyasulu, 2000). For optimum productivity, there is a need to harmonise plucking operations with pruning, as both stimulate growth (Kamau, 2008).

### 2.2.9 Tea Manufacturing

Different types of teas are produced from the green tea shoots of *Camelliasinensis* depending on the manufacturing process they undergo. The common tea types are white, green, oolong and black teas (Hilal & Engelhardt, 2007). The major process that differentiates black and oolong tea from the other types is fermentation. The different processes are summarised in Figure 2.3.

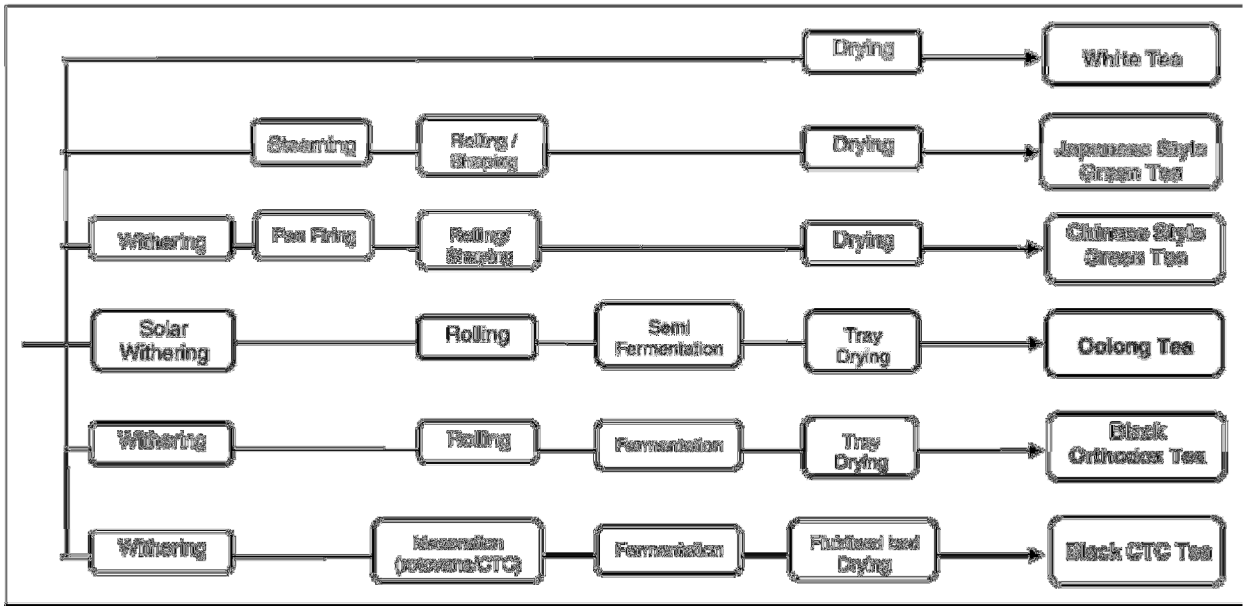


**Figure 2.3 Major steps in tea manufacturing which correspond to different tea types (Hilal & Engelhardt, 2007).**

Plucked tea shoots contain high concentrations of polyphenolic compounds, mainly the catechins and flavonoids (Forrest & Bendall, 1969), as well as the enzyme polyphenol oxidase (Hilal & Engelhardt, 2007). These chemicals are separated by cellular structures in a growing shoot and cannot react. However, during black tea manufacturing, maceration of the shoots disrupts the intracellular compartments and exposes the polyphenolic compounds that are present in the cell vacuole. These compounds are then oxidised by the endogenous oxidative enzyme, polyphenol oxidase, leading to the formation of theaflavins (TFs) and thearubigins (TRs) during the process known as fermentation (Ellis & Nyirenda 1995, Subramanian & Venkatesh, 1999).During the drying process of tea, these compounds are fixed within and on the surfaces of the black tea. When an infusion of tea is made after adding boiling water, these compounds are brought back into solution (Ellis & Nyirenda, 1995).

#### 2.2.9.1 Tea Quality

Tea quality is determined based on sensory evaluation by professional tea tasters who test the processed tea for brightness, briskness, colour, aroma, taste and strength of liquor, which form some of the important parameters in sensory evaluation (Wright *et al.,* 2000, Liang *et al.,* 2002). Tea quality can also be measured by chemical analysis for the flavouring components according to Willson & Clifford (1992). These components have been found to show significant correlations with individual sensory quality parameters (Liang *et al.,* 2002), for example, the quantities of TFsdetermine brightness and briskness, whilst strength and colour are due to TRs(Grice, 1990).

Tea containing high levels of antioxidant/pro-oxidant phenolic molecules, mainly catechins, flavanols, TFs and TRs, is deemed to be of good quality (Obanda *et al.,* 1997) and is preferred because of the beneficial effects of these polyphenolic compounds on human health. The catechins and flavonoids found in plucked leaves have also been found to contain anti-cancer properties (Azam *et al.,* 2004). The harvestable shoots of tea contain high concentrations of these polyphenolic compounds,which contribute more than 20% to the dry mass of the shoots (Ellis & Nyirenda, 1995).It is for this reason that tea is considered a healthy drink, on top of being a source of mineral elements such as Zn, Mn, Fe, Cu, Mg, Ti, Al, Sr, Br, Na, K, P, I and F (Seenivasan *et al.,* 2008).

### 2.2.10 Pest, disease and weed control

Pest and disease attack poses the biggest challenge in tea production in most tea growing areas because it leads to a significant reduction in crop. The common pests of tea include *Helopeltis schouteden*,popularly knownasmosquito bugs, *Teragra quadrangula* (carpenter moth), and termites (*Ancistrotermes sp*.) (Rattan, 1990). Insect pest attack in tea generally leads to yield loss between 11 and 55% if left uncontrolled (Hazarika *et al.,* 2009). According to Rattan (1984), yield losses of up to 55% and 30% due to mosquito bug attack and spider mites have been recorded in Malawi and Kenya respectively (Rattan, 1984 & Sudoi, 1995). Mosquito bugs attack both young and old tea and termites and carpenter moths cause serious damage to young tea. Other pests include jelly grubs, caterpillars, aphids, tea leaf weevils, mites (red spider mites (*Oligonychus coffeae*), yellow tea mite (*Polyphagotarsonemus latus* Banks), scarlet mites (*Blevipalpusphoenicis, B. obovatus and B. californicus*), scale insects and tea thrips*(Scirtothrips aurantii*) (Lightfoot, 2009). The most common weed species in tea gardens are black jack (*Bidens pilosa*), wandering jew (*Commelina benghalensis*), couch grass (*Cynodondactylon*) and nut grass (*Cypericearotundus*) (Lightfoot, 2009).

In the past, a number of organosynthetic pesticides have been used to control pest, disease and weed attack. According to Hazarika *et al.,* (2009), this has led to rapid conversion of harmless species into pests, development of resistance and presence of undesirable pesticide residues in made tea, which has become a major area of concern among most tea consumers. Whilst chemical control is recommended in situations of severe attack, tea growers are encouraged to use Integrated Pest Management (IPM) techniques and monitor the use of chemicals in order to prevent overuse of pesticides and subsequent residues in made tea (Lightfoot, 2009, Hazarika *et al.,* 2009). For this reason only eight chemicals are recommended for use by growers in Malawi and Zimbabwe for the control of pests, diseases and weeds (Lightfoot, 2009). For the control of red spider mites, yellow tea mite and scarlet mites, sulphur products such as polysulphide sulphur, calcium polysulphide and sulphur are recommended. For the control of fungal diseases, copper products such as cupric hydroxide and copper oxychloride are recommended. For control of post-emergent and pre-emergent weeds, glyphosate and S-metolachlor are recommended respectively (Lightfoot, 2009).

**2.3 Acidification of tea soils**

Lower soil pH levels than the optimum is not uncommon in most tea gardens. Tea soils become acidified by the tea plants themselves due to rhizodeposition of large quantities of organic acids, such as oxalic acid, citric acid and malate (Shi *et.al.,* 1999). Fertilisation with nitrogenous fertilisers, such as ammonium sulphate and urea,has also contributed to soil acidification in tea soils (Barak *et al*., 1997, Shi *et al.,* 1999, Dang, 2005).

Nitrogen is the most important major nutrient element required by tea plants followed by K and P. Like most plants where vegetative parts (young shoots) constitute the economic yield, tea requires high levels of N for sustainable yields (Dang, 2005). This is why N application rates of as high as 1000 kg $ha^{-1}$ per year have been applied to tea plants in order to increase yield (Okano & Matsuo, 1996, Owuor *et al.,* 2000). Reports have indicated that N fertilisation significantly increases the levels of extractable aluminium, whilst decreasing soil pH and the levels of exchangeable base cations, such as K, Ca and Mg (Ruan *et al.,* 2006).

At low pH, nutrient deficiencies may arise because soil conditions do not allow for efficient uptake of nutrients, mainly due to Al toxicity, which has been recognized as a major factor limiting plant growth in acidic soils (Marschner, 1986). Ahsan (1994) also indicated that the dominance of exchangeable Al in low pH soils causes Al toxicity, which is responsible for low crop yield in tea fields. The exchangeable $Al^{3+}$,and $H^{+}$cations replace other polyvalent cations on the clay minerals in the soil renderingthem unavailable to plants. This phenomenon was demonstrated by Fung *et al.* (2008) in studies on growth and nutrient uptake of tea under different Al concentrations. It was shown that the uptake of Cu, Zn and Fe were significantly reduced in Al treatments, with Cu and Zn uptake severely restricted.

Furthermore, due to prunings that are left in the tea fields, high levels of organic litter are maintained in tea fields. Upon decomposition of these residues organic acids are released, which acidify the rhizosphere thereby increasing the availability of Al and its complexes (Ruan *et al.,* 2003, Ruan *et.al*., 2006, Jin *et. al.,* 2008,). Excess levels of Al in the rhizosphere leads to restricted growth of plant roots, because cell division in root apical meristematic cells ceases when exposed to Al (Marschner, 1986). Certain nutrient elements, such as Cu, are also reportedly less available due to immobilisation in the organic matter (Marcos *et al*., 1998).

A soil pH survey performed in tea fields in Malawi by Nyasulu (2006) confirmed the low pH status of Malawian tea soils, in which a majority of fields registered pH readings below the optimum range of 4.5 to 5.5. Latosolic soils,on which tea is mostly grown in Malawi, are usually characterised by a high capacity to release Fe and Al sesquioxides, increased loss of silica and high dominance of new clay minerals such as smectites, allophone, halloysite and increased weathering kaolinite (Bell, 1992). As these minerals are highly weathered and leached, they are inherently low in Zn and other micronutrients (Alloway, 2008). This may explain why deficiencies of Cu, Zn and other micronutrients were observed in a majority of tea fields in Malawi (Figure2.4).

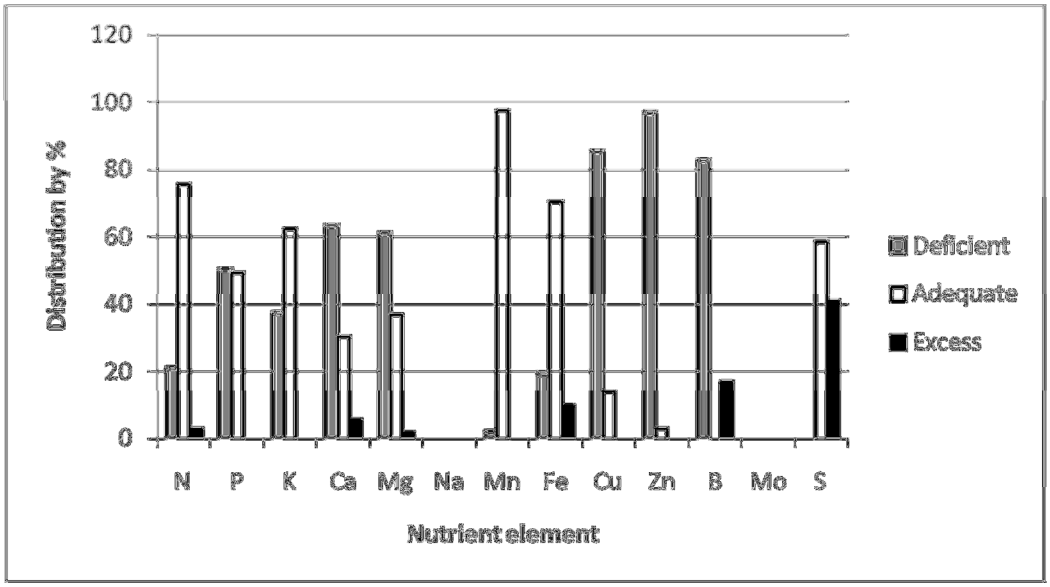


**Figure 2.4 Distribution of nutrient levels in leaves of *Camelliasinensis* collected from 170 mature clonal tea fields of Eastern Produce Malawi in 2007 (2007 leaf analysis data collected by the C.Njoloma from the estates (Eastern Produce Malawi)).**

More than 80% of 170 tea fields under vegetatively propagated cultivars in some of the tea estates in Malawi were deficient in Cu, Zn and B. According to Willson & Gunther (1981), the critical level of Cu, Zn and B in tea leaves is >15 mg/kg, >30 mg/kg and 30 mg/kg respectively and 3-10 mg/kg, 2-15 mg/kg and >1.0 mg/kg for Cu, Zn and B in the soil respectively.

## 2.4 Copper, Zinc and Boron in crop production

In a naturally balanced agro-ecosystem, crop plants would find all the nutrients they require from the soil. It is, however, widely acknowledged that most agro-ecosystems are far from balanced and cultivated soils cannot supply the nutrients in sufficient quantities. This is because of the disturbance in the nutrient cycle that has been aggravated by high population density, which has led to continuous cultivation, in order to get maximum harvestable product, resulting in the nutrient cycle incurring huge nutrient losses through the harvestable yield (Nandwa & Bekunda 1998). This is why growershave to supplement nutrients through application of organic or inorganic fertilizers either to the soil on which the plant is growing or to its aerial parts.

Copper, Zn and B are among the essential micronutrient elements in plant nutrition. Although required in very small quantities, they play very important physiological roles in plants by taking part in important biochemical reactions within the plant systems and forming part of important enzymes, some of which aid in metabolism of photosynthetic products and reduction in oxidative stress (Fernandes &Henriques, 1991). For instance, Cu is associated with a number of enzymes such as cytochrome oxidase, phenolase, polyphenol oxidase and Cu aids in chlorophyll synthesis and metabolism of carbohydrates and proteins (Alam & Raza, 2001, Alam *et al.,* 2007). Zinc is necessary for production of growth regulators such as indole acetic acid (IAA) and acts as an activator of a number of enzymes (Alam & Raza, 2001). Boron is associated with meristematic activity, membrane functions and is also necessary for the synthesis of proteins, lignin and metabolism of pectin (Alam & Raza, 2001). Boron also helps in maintaining osmotic potential within the plant cells and aids in the translocation of sugars across membranes (Alam & Raza, 2001).

### 2.4.1 Copper

Copper is one of the essential elements in plant nutrition. Copper exists in two oxidation states and these are $Cu^{+}$ and $Cu^{2+}$ (Yruela, 2005). Copper acts as a structural element in many regulatory proteins. It also takes part in photosynthetic electron transfer, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signalling (Fernandes & Henriques, 1991, Yruela, 2005).

Most Cu containing metalloenzymes are involved in catalysing redox reactions (Fernandes & Henriques, 1991). Copper has a high redox potential which favours reaction with oxidants. As reported by Fernandes &Henriquess (1991), Cu forms part of superoxide dismutase, which is a Cu and Zn containing enzyme that is associated with chloroplasts in higher plants. Different compartments of plant cells such as chloroplasts, mitochondria, microsomes, glyoxysomes, peroxisomes, apoplasts and the cytosol produce reactive oxygen species (ROS) and, according to Alscher *et al*., (2002), superoxide dismutase presents a plant cell's first line of defence against these ROS.

Both deficient and excessive levels of Cu are detrimental to plant growth. Copper deficiencies are mostly seen on young leaves and reproductive organs (Yruela, 2005). Copper deficient plants exhibit an alteration in the expression of a number of genes and activation of morphological changes, such as root and leaf pose. According to Loustalot *et al.* (1945), metabolism of carbohydrates is impaired due to Cu deficiencies. Typical Cu deficiencies are seen on young leaves and extend downward along the leaf margins where malformation of leaves, chlorosis and necrosis occur (Yruela, 2005). Copper deficiencies also decrease the formation of plastocyanin leading to a reduction in photosystem I electron transport (Yruela, 2005).

Copper is an inherently toxic element by virtue of its redox properties. According to Yruela (2005), redox cycling between $Cu^{2+}$ and $Cu^{+}$ catalyses the production of highly toxic hydroxyl radicals which subsequently damages DNA, lipids, proteins and other biomolecules. Inhibition of photosynthesis and suppression of enzyme activities are some of the effects of excess levels of Cu (Loustalot *et al.,* 1945, Alaoui-Sosse *et al.,* 2004). This is why excess Cu leads to inhibition of plant growth and impairment of important cellular processes (Yruela, 2005).

#### 2.4.1.1 Factors affecting Cu availability and uptake

Total soil Cu concentration is not related to plant uptake and application of Cu to the soil is not always followed by an increase in plant Cu levels (Fernandes & Henriques 1991). Copper in the soil is available in various forms and its bioavailability is closely related to its distribution in different chemical forms i.e. water soluble, exchangeable, organically bound, associated with carbonates and hydrous oxides of Fe, Mn and Al, and it is also available in residual forms (Zhang *et al.,* 2006). It has been reported that Cu exists predominantly in soil as organically bound (Stevenson, 1991) and residual forms or as acid soluble forms (Alva *et al.,* 2000). It was observed that soil pH is the main factor, besides organic matter, clay content, CEC and total Cu, controlling Cu uptake as shown in Table 2.1 where a highly significant negative correlation between soil pH and exchangeable Cu is shown.

**Table 2.1 Correlations between fractions of Cu and soil properties (adopted from Zhang *et al.,* 2006)**

| **Property** | **Exchangeable Cu** | **Carbonate bound Cu** | **Organic matter bound Cu** | **Oxide bound Cu** | **Residual Cu** |
|---|---|---|---|---|---|
| pH | -0.903*** | 0.821*** | -0.349* | -0.331 | -0.352 |
| Organic matter | -0.315 | 0.112 | 0.017 | -0.247 | -0.511 |
| Clay | -0.503** | 0.384* | -0.159 | 0.007 | -0.059 |
| CEC | -0.181 | 0.596*** | 0.005 | -0.010 | -0.101 |

Key: * significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$

**2.4.1.2 Effect of Cu on yield and quality of tea**

Effects of Cu on yield and quality of tea have been reported by a number of authors (Barua & Dutta, 1972, Gartrell, 1981, Willson & Clifford, 1992, Barooah *et al.,* 2005, Saikh, 2007). In some cases an increase in tea yield as a result of Cu applications has been observed, whilst in other cases application of Cu has caused negative yield responses. In terms of tea quality, Cu is a very essential element for the formation of polyphenol oxidase, an essential Cu containing enzyme/catalyst in the fermentation process of black tea manufacturing (Seenivasan *et al.,* 2008). Copper deficiencies therefore lead to poor fermentation and subsequently poor quality tea.

Other reports have indicated that Cu plays a vital role in enhancing processes involved in the pro-oxidant actions of tea polyphenols, which is an important mechanism for their anti-cancer properties (Azam *et al.,* 2004). The high redox potential of Cu indicates its ability to react with oxidants (Fernandes & Henriquess, 1991). According to Azam *et al.* (2004), Cu mediates oxidation of epicatechins and epigallocatechin gallate leading to formation of polymerised polyphenols and it was observed that Cu oxidised catechins were more efficient anti-cancer pro-oxidants. This makes Cu an important element, especially nowadays when the market is focussing on tea with beneficial health properties.

Studies performed in Malawi showed that foliar application of Cu had no effect on yield, but a lasting improvement in fermentation was observed and a foliar spray of a 0.5-1% solution of

$CuSO_4$ rectified deficiencies (Grice, 1990). According to Squire (1977), spraying a 1% $CuSO_4$ solution to Cu deficient tea bushes improved the activity of polyphenol oxidase and subsequently improved tea quality. Barooah *et al.* (2005) found that the average content of Cu and Mn in tea soils strongly correlated with productivity of the tea field. Gartrell (1981) also reported the long lasting effectiveness of Cu fertilisers, when applied to soil and in some instances, foliar application of Cu compounds, effectively counteracted deficiencies (Willson & Clifford, 1992).

Copper has been applied as fungicides to tea plants mostly in the form of Cu hydroxide, Cu oxychloride and Cu oxide and reports by Barua & Dutta (1972), and Saikh (2007) pointed out that these applications led to a decline inyield. Alaoui-Sosse *et al.* (2004) also observed that the growth of cucumber was inhibited as Cu content increased after adding 20 mg $g^{-1}$ of Cu chloride in the growing medium. This inhibition was mainly due to a decrease in leaf expansion, which implies reduced photosynthesis. This was attributed to an altered source-sink relationship when there was an accumulation of non-structural carbohydrates in the source leaves, which in turn induced a feedback inhibition on photosynthesis (Alaoui-Sosse *et al*., 2004). It was suggested that because only expanding leaves showed reduced growth, leaves became weaker sinks of photoassimilates as their expansion declined, thereby accounting for the accumulation of non-structural carbohydrates in the source leaves (Alaoui-Sosse *et al.* 2004).

### 2.4.2 Zinc

Zinc is an essential micronutrient, with a particular physiological function in all living systems. Its essentiality is demonstrated by its role as a cofactor in a number of enzymes in biochemical pathways that are primarily concerned with carbohydrate metabolism in photosynthesis and transformation of sugars to starch, protein metabolism, auxin growth regulators, pollen formation and maintenance of membrane integrity (Alloway, 2008).Zinc is involved in N metabolism of plants. Spraying of 1-2%$ZnSO_4$ increased nitrate reductase activity and also resulted in a 15-20% increase in N and protein content of tea shoots (Barbora *et al.,* 1993). Zincis required for the synthesis of IAA, which is responsible for active shoot growth in tea (Alam & Raza, 2001,Sedaghathoor *et al.,* 2009).

Zinc plays an important role in photosynthesis and mobilisation of assimilates and has been shown to mobilise photosynthates towards pluckable shoots in tea (Barbora *et al,* 1993).Approximately 9.0% of assimilates were partitioned towards pluckable shoots in tea plants treated with 1% $ZnSO_4$compared to 5.4% in untreated plants.Caffeine content in tea shoots also increased following foliar application of Zn(Barbora *et al.,* 1993). Leaf chlorophyll content, stomatal conductance and net photosynthesis are adversely affected by inadequate supply of Zn(Barbora *et al.,* 1993).

Stunted growth and reduced leaf size are the most distinctive visible symptoms of a Zn deficiency, which is a result of disturbances in auxin metabolism (Alloway, 2008). In tea, typical Zn deficiency symptoms include sickle shaped leaves due to unequal growth of the two halves of the leaf blade, wavy leaf margins due to more rapid growth of the leaf edge compared to the leaf blade, greenish-yellow chlorosis spreading from the edge of the leaf to the middle and rosetting of flushing shoots due to shortened internodes and lack of growth vigour and poor flushing of the shoots (Barbora *et al.,*1993).

**2.4.2.1 Factors affecting Zn availability and uptake**

Zn availability to plants is affected by a number of factors, among which are the presence of other nutrient elements, growing conditions and physical and chemical properties of the soil (Barbora *et al*., 1993). Most tea soils are deficient in Zn and an annual Zn application has been recommended to promote shoot growth, which is necessary for yield (Verma, 1999). According to Alloway (2008), conditions that induce Zn deficiencies include low concentration of Zn, which is typical of sandy soils; low pH; highly weathered parent materials with low Zn content, a condition which is common in tropical regions; high calcium carbonate content (calcareous soils); salinity, peat and muck organic soils; high phosphate status; prolonged water logging conditions and a high concentration of magnesium and bicarbonates in the soil or irrigation water.

Zinc has a tendency of being strongly bound to organic matter and oxides. According to results obtained by Zhang *et al*., (2006) in Table 2.2, it was also reported that Zn has a tendency of being bound to carbonates in high soil pH conditions when they observed a significant positive correlations between carbonate bound Zn and soil pH.

**Table 2.2 Correlations between fractions of Zn and soil properties (adopted from Zhang *et.al.,* 2006)**

| Property | Exchangeable Zn | Carbonate bound Zn | Organic matter bound Zn | Oxide bound Zn | Residual Zn |
|---|---|---|---|---|---|
| pH | -0.918*** | 0.752*** | -0.016 | -0.200 | 0.129 |
| Organic matter | -0.272 | -0.128 | 0.395* | -0.092 | -0.081 |
| Clay | -0.478** | 0.153 | 0.096 | 0.122 | 0.329 |
| CEC | -0.239 | 0.350* | 0.052 | -0.160 | -0.114 |
| Total Zn | 0.290 | -0.080 | 0.860*** | 0.984*** | 0.836*** |

* significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$

Soil acidification and high levels of organic matter are common features in most of the soils where tea is grown, hence Zn deficiencies are expectedly common in such soils. Deficiencies of Zn in tea have also been reported to be pronounced during periods of prolonged dry spells and in pruning years and are masked during periods of rapid growth (Rahman & Sharma, 1974). This is also an indication that soil moisture content has a role to play in Zn availability, as Sud *et al*. (1995) reported that higher rainfall aided in the uptake of Zn due to the higher capacity of the roots to exchange cations. Investigations conducted in tea shoots of china tea showed a decreased level of Zn with high temperature, which was confirmed by the increased uptake of Mn at these higher temperatures, since Zn and Mn are antagonistic with respect to their uptake by plants (Sud *et al*., 1995).

Negative interaction between Zn and P has been widely reported (Loneragan & Webb, 1993, Alam *et al*., 2007, Alloway, 2008). Increased levels of available P in the soil decrease the concentration of Zn in the plant shoots i.e. uptake of Zn is adversely affected by increased concentration of P (Loneragan & Webb, 1993, Alam *et al.,* 2007). Addition of phosphate fertilisers promotes growth, thereby causing a dilution in the Zn concentration in plant tissues to levels which induce Zn deficiency (Alloway, 2008). Zinc is not readily absorbed by the tea plants from the soil and its deficiency is not readily corrected by soil application of Zn compounds. Foliar application is the most effective method of increasing Zn concentrations in tea plants (Sedaghathoor *et al.,* 2009).

### 2.4.2.2 Effect of Zn on yield and quality of tea

Effects of Zn on tea yields and quality have been reported by several authors (Barua & Dutta 1972, Malenga 1979, Malenga 1986, Dev Chaudhury *et al.,* 1989, Fung & Wong 2001). Experiments that were conducted at Tocklai in India by Barua & Dutta (1972) indicated a 20% yield increase in tea due to the application of Zn alone, but when it was applied in combination with other elements such as B, Mn, Mo and Mg (Table 2.3), no further yield increase was observed and a reduction in yield relative to the control for some combinations occurred.

**Table 2.3 Green leaf yield for different nutrient elements and the estate control (adopted from Barua & Dutta, 1972)**

| **Nutrient element** | **Application rate (kg ha$^{-1}$)** | **Yield per plot of 238 bushes (kg)** | **% gain (+) or loss (-) over the control** |
|---|---|---|---|
| Magnesium (Mg) | 13.6 | 134.8 | -8.8 |
| Zinc (Zn) | 11.2 | 177.3 | +19.9 |
| Boron (B) | 5.6 | 143.5 | -2.9 |
| Manganese (Mn) | 5.6 | 161.1 | +8.9 |
| Molybdenum (Mo) | 5.6 | 151.9 | +2.8 |
| Zn+B+Mg | 11.2Zn+5.6B +13.6Mg | 166.4 | +12.5 |
| Zn+B+Mn | 11.2 Zn+5.6 B+5.6Mn | 176.6 | +19.4 |
| Zn+B+Mo | 11.2 Zn+5.6 B+5.6Mo | 164.8 | +11.5 |
| Zn+B | 11.2 Zn+5.6 B | 175.6 | +18.7 |
| Control | - | 147.9 | - |

### 2.4.3 Boron

Boron is another essential nutrient element in the physiology of plants. Its roles are closely linked to the primary cell wall structure, membrane functions and reproductive growth of plants (Blevins & Lukaszewski, 1998). Boron is involved in the activities related to the development and strengthening of the cell wall, cell division, fruit and seed development, and sugar and phosphate transport (Power & Woods, 1997). Boron has also been reported to be involved in the movement of Ca within plants. Boron forms complexes with constituents of cell wallsand plasma membranes, and it also forms complexes with phenolic compounds (Cakmak & Romheld, 1997). Boron is therefore crucial in maintaining the integrity of plasma membranes (Cakmak & Romheld, 1997).

Deficiencies of B are common in soils that are inherently low in B, such as those derived from acid granite and other igneous rocks and fresh water sedimentary deposits, light textured sandy and gravely soils, alkaline calcareous soils and those soils that are low in organic matter (Ho, 2000). High rainfall areas also experience B deficiencies due to leaching of $[H(BO)]_3$, which is the most available form for plant uptake (Camacho-Cristobal *et.al.*, 2008). Both vegetative and reproductive stages of plant development are affected due to B deficiencies (Dordas & Brown, 2005), especially during periods of rapid growth and meristematic development (Blaser *et al.*, 1967).

Boron deficiencies induce destruction of the apical meristems of plant roots, leading to development of swollen root tips (Blaser *et al.*, 1967, Dordas & Brown, 2005). Cell differentiation is adversely affected under conditions of B deficiency and reports by Blaser *et al.* (1967) indicated that it is the xylem cells that are more affected than phloem cells. Generally, cells of plants growing under severe B deficient conditions die (Dordas & Brown, 2005). As a result plants growing under B stress may show limited growth in growing tissues (Brown *et al.*, 2002), conditions of necrosis in growing buds, cracking and breaking of stems and petioles, whitish upper surfaces of leaves, crinkling of leaf blades, abortion of flowers and shedding of fruits (Ho, 2000, Brown *et al*., 2002).

Boron toxicity, just like B deficiencies, has detrimental effects on plant growth. Prasad & Dey (1979) demonstrated that B application to tea plants at a concentration of 0.6% was toxic to the plants, as severe scorching and defoliation of leaves was observed. These authors found that symptoms of B toxicity include the appearance of brown patches of dead tissue from interveinal regions extending towards leaf margins and curling of leaf blades.

### 2.4.3.1 Effects of B on yield and quality of tea

Information on effects of B application on tea quality is scarce, however, its effects on improving tea yield have been reported. Foliar application of 0.25% (w/v) boric acid at an application rate of 1 kg $ha^{-1}$ $yr^{-1}$ split in three to four applications increased yield significantly (Gohian *et al.,* 2000). This increase in yield was mainly attributed to an increase in leaf area index (LAI), as seen in Table 2.4 (Gohian *et al.,* 2000). As is the case with any nutrient element, yield will not increase indefinitely as its application increases, as Barua & Dutta (1972) observed a yield loss when B was applied at the rate of 5.6 kg $ha^{-1}$ $yr^{-1}$, an indication that the rate at which it was applied was high enough to cause toxic effects on tea.

**Table 2.4 Impact of various concentrations of foliar applied boric acid on yield and yield attributing characters in tea (adopted from Gohian *et al.*, 2000)**

| B kg $ha^{-1}$ | Yield kg $ha^{-1}$ | Shoot/100g | Inter-nodal length (cm) | LAI |
|---|---|---|---|---|
| 0.0 | 2763 | 117 | 2.43 | 3.58 |
| 0.5 | 3110 | 127 | 2.47 | 3.88 |
| 1.0 | 3212 | 135 | 2.43 | 4.42 |
| 1.5 | 3154 | 125 | 2.48 | 3.95 |
| CD (P=0.05) | 156 | Ns | Ns | 0.39 |

Correction of B deficiencies can be done through soil or foliar applications. When Prasad & Dey (1979) compared these two methods of B application in young tea, it was found that foliar applications were as effective as soil application in terms of B uptake and that lower rates used in foliar applications were as effective as higher rates used in soil applications. It was also observed that uptake of B from foliar applied boric acid at 0.1%, 0.2%, 0.4%, 0.6% concentrations, supplying 1.0kg B $ha^{-1}$, 2.0kg B $ha^{-1}$, 4.0kg B $ha^{-1}$, 4.2 kg B $ha^{-1}$ progressively increased with increased levels of application (Prasad & Dey,1979).

## 2.5 Application of plant nutrients through foliar sprays

Foliar fertilisation is the feeding of plants with nutrients through leaves (Wallace & Wallace, 1983) and has gained importance in the growing of cropsand has been the most effective way of supplying trace elements to plants, such as Fe, Zn, Cu, Mn and B, which are required in very small quantities (Pulschen, 2004). It is advantageous over soil applications in the sense that the application rates are much lower than for soil applications, and a uniform application is easily achieved. Furthermore, foliar application allows for the correction of deficiencies in less time than would be required for soil applications (Fageria *et al*., 2009). According to Majid &Ballard (1990), foliar application of nutrient elements is a rapid process, which is applicable for those elements that are not readily mobile in both plants and in the soil, such as Cu (Mengel & Kirkby, 1982) and Zn (Durzan, 1995). However, there is a risk of leaf burn as a result of foliar sprays, especially when high concentrations are applied, which may lead to yield losses. Ellis (1971) reported incidences of leaf scorch when $CuSO_4$ was applied at doses higher than 1%.

Correction of deficiencies needs to be done before visual deficiency symptoms are seen. If spraying is delayed until deficiency symptoms appear, maximum yields may not be possible. In addition, there is little residual effect from foliar sprays, which implies that more than one spray is required per season,especially when deficiencies are severe (Fageria *et al.,*2009). This results in higher application costs unless they can be combined with pesticide spray applications (Mortvedt, unknown). The effects of soil applied nutrients are, on the other hand, long lived when compared to those of foliar applied nutrients, but there is reduced risk of contamination of ground water systems with foliar applications and they can be a used to complement soil applications, especially during periods of critically restricted nutrient supply (Amiri *et al.,* 2008).

There are numerous processes taking place within soils and nutrients applied to the soil are victimised by a number of reactions such as mineralisation, leaching and organic complex formation, which results in a decrease in their plant-available fractions. Foliar applications are effective in raising the concentration of a particular nutrient, especially in the leaves, and the level of uptake of other nutrients, as reported by Amiri *et al*. (2008) who studied the influence of foliar and soil fertilization of N and Zn on yield and fruit quality, and soil, leaf, and fruit mineral nutrient levels in apples. Other nutrient elements that were affected by foliar Zn and N application included Mg, Fe and Cu, with a combination of soil and foliar Zn and N application treatment registering relatively higher levels of these nutrients than the soil treatments alone. Foliar application of N (10g $l^{-1}$) and Zn (8g $l^{-1}$) showed an improvement in apple yield compared to the soil application. When the application methods were combined, the highest yield was obtained. The fraction of N taken up by the apple trees was greater in foliar treatments than in soil application, which led to elevated levels of N in the leaves and hence high vegetative growth (Amiri *et al.,* 2008).

The effectiveness of foliar nutrient application lies in the rate at which the applied nutrients are absorbed by the leaf and transported to other parts of the plant including the roots (Bukovak & Wittwer, 1957, Haslett *et al.,* 2001). Transportation of foliar applied nutrient elements is mainly dependent upon their ability to be transported via the phloem. Some elements are phloem mobile while others are not. Generally Zn and Cu are reportedly partially mobile (Wooldridge, 2002, Newett, 2005), while B mobility is species dependent favouring those plant species that produce simple sugars known as polyols, which tend to complex with B to form polyol-B-polyol compounds that are transported to meristematic regions (Brown & Hu, 1996, Brown & Shelp, 1997, Power & Woods, 1997, Blevins & Lukaszewski, 1998, Boarretto *et.al.,* 2008). Such sugars include sorbitol, mannitol or ducitol, however, no evidence is available whether the tea plant is a producer of such polyols.

### 2.5.1 Mechanisms for foliar uptake of nutrients

Mechanisms governing the uptake of foliar applied nutrients involve the entry of the applied nutrients into the leaf tissues and translocation to other parts of the plants. Foliar absorption, like root absorption, involves both active and passive mechanisms (Fageria *et al.,* 2009). Entry of nutrients through the leaves starts with entry through aqueous pores that are found on the cuticle of leaves, then the cell walls of epidermal cells and finally through the plasma membrane by active transport (Cheristensen, 2005).

The leaf's ability to absorb nutrients from the surface depends upon the degree of permeability of the leaf epidermis (outer layer) and the presence and density of stomata, (Newett, 2005). This is controlled by a number of factors including the structure of the leaf surface (the cuticle, stomata aperture and stomatal density); number, permeability and arrangement of ectodesmata; degree of surface wetting (adhesion); concentration of micronutrients in the aqueous blend; and humidity (Ferrandon & Chamel, 1988, Wojcik, 2004, Durstberger *et al.,* 2008,). The number of ectodesmata is controlled by the environmental conditions such as air temperature, solar radiation, pathogenic infections and the physiological state of the leaves which also varies with genotype (Wojcik, 2004).

### 2.5.1.1 Penetration through the cuticle

Foliar applied elements enter the plants through the cuticular membrane by diffusion, a process which does not involve expenditure of energy (Cheristensen, 2005). At first the leaf cuticle, which is composed of cutin, lignin, pectin and wax, was considered a barrier to absorption of foliar applied nutrients (Yamada *et al.,* 1964, Kannan, 1980, Haslett *et al*., 2001, Wojcik, 2004, Schreiber, 2005) and the stomata were thought to be the only pathway through which foliar applied elements could enter the plant tissues (Kannan, 1980). It is generally accepted that stomata play a significant role in the uptake of foliar applied elements, however, the mechanisms by which stomata assist in the penetration of foliar applied elements are not clear, although recent studies have provided strong supporting evidence of the possibility of stomatal entry of solutes by diffusion along pore surfaces (Fernandez & Eichert, 2009). Different authors have given conflicting results regarding significance of stomata on penetration of foliar applied elements (Yamada *et al.,* 1964, Kannan, 1980, Burkhardt &Schroth 2000, Wojcik, 2004, Cheristensen 2005, Schreiber, 2005). Furthermore, even the stomata themselves, as well as trichomes, are known to be covered by a cuticle (Kannan, 1980, Schreiber, 2005).According to Yamada *etal.* (1964) in his studies on cuticles of tomato fruit (astomatous) and onion leaves (stomatous) in order to assess the effect of stomata on the rate of penetration of ions, it was discovered that penetration rates of ions were not significantly affected by the different types of cuticles, meaning the presence or absence of stomatal pores does not affect the entry of ions. However, Kannan (1980) argued that based on the fact that permeability of air and water molecules through the stomata is greater than through other parts of the leaves, stomatal penetration of solutes in intact leaves is possible. Burkhardt &Schroth (2000) also conducted a study on the role of stomatal opening for the uptake of foliar fertilisers by tree crops. This study showed that stomatal penetration of $^{15}NO_3^-$was possible and it exceeded that of cuticular uptake. Cheristensen (2005) mentioned that the cuticle within the substomatal openings is thinner than the rest of the leaf surface providing evidence for the observed high $^{15}NO_3^-$penetration rate.

It has been shown in many instances that foliar applied nutrients find their way into the inner tissues through the cuticle (Kannan, 1980, Wojcik, 2004). The cuticle contains epicuticular and intracuticular waxes (Wojcik, 2004). Epicuticular waxes are the outermost hydrophobic component on the leaf surface consisting of ketones, esters and long chain fatty acids and alcohols (Wojcik, 2004). The intracuticular waxes are found within the cuticular membrane and are considered more polar than epicuticular waxes (Wojcik, 2004). According to Kannan (1980), absorption of nutrients through the leaves among plant species is dependent upon the characteristics of the cuticle, such as thickness, surface wax and wettability, as well as mass of solute. Cutin found in the cuticular membrane contains many free hydroxyl groups which weaken the hydrophobic interactions and facilitate entry of nutrients through the cuticular membrane (Wojcik, 2004). The pectin layer is comprised of negatively charged galacturonic acids. There is a gradual increase in negative charge from the epicuticular waxes to the pectic layer, creating an electro-chemical gradient that facilitates movement of cations and water molecules through the cuticular membrane (Wojcik, 2004).

The applied element then progresses through to the plasma membrane by active transport, which involves energy expenditure. Penetration or entry of foliar applied nutrient elements from the outside to the interior of plant tissues was discovered to be possible with the aid of connecting tissues known as the ectodesmata (Franke, 1961, Wojcik, 2004). Ectodesmata are microscopic structures (diameter < 1 nm) found mostly in the outer walls of the epidermal cells of leaves and other above ground plant organs (Kannan, 1980) (Figure 2.5). They occur adjacent to the cuticular spaces to the plasmalemma membrane and they facilitate cuticular entry of solutes (Kannan, 1980). Ectodesmata are permeable to solutes with smaller radii such as urea, however, permeability to larger molecules such as synthetic chelates is limited (Wojcik, 2004). Unlikethe plasmodesmata, ectodesmata do not communicate with plasmic strings of neighbouring cells, they terminate on the surface of the outer epidermal cells thereby having a direct protoplasmic connection between the epidermal cells and the outer surface (Franke, 1961).

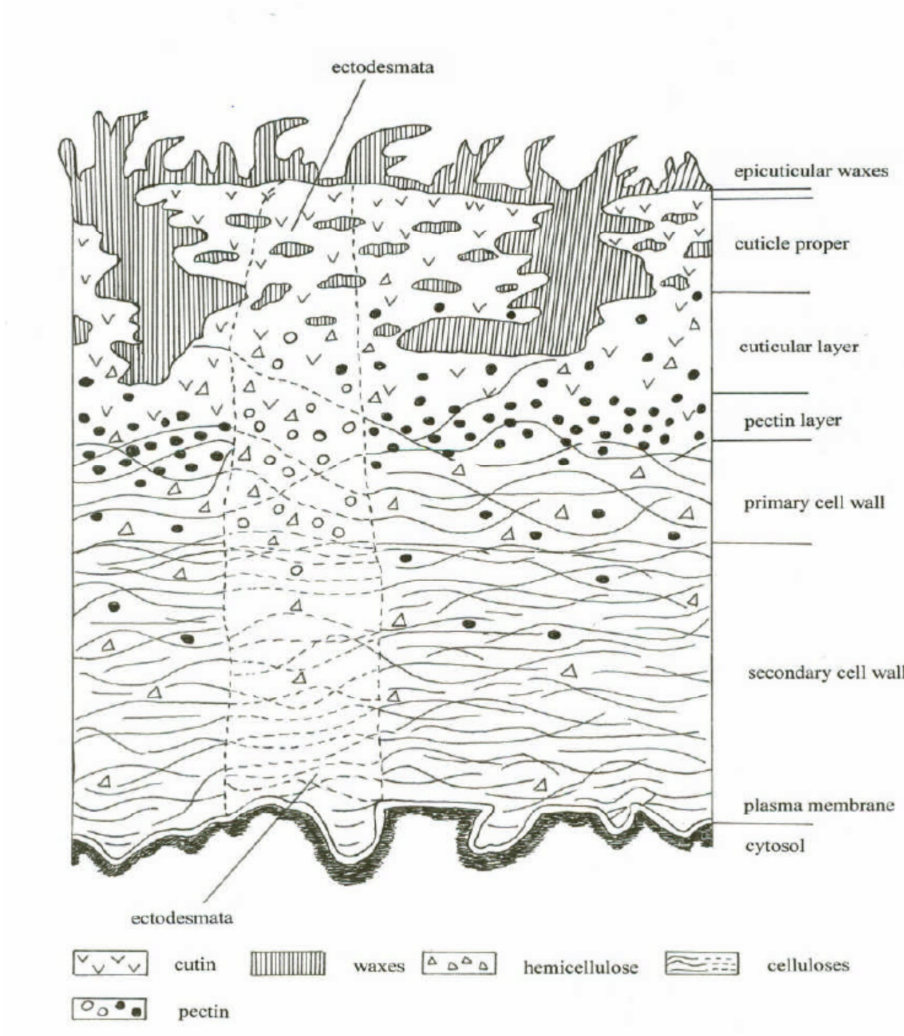


**Figure 2.5 Ultrastructure of the outer wall of an epidermal leaf cell showing ectodesmata(Wojcik, 2004).**

In broad leaved crops and trees, the lower surface (abaxial) of the leaf has a higher stomatal density and higher ectodemata density than the upper surface (adaxial) of the leaf (Fritz, 1978, Wojcik, 2004). The tea crop however, has stomata only on the abaxial surface of the leaf (Squire, 1977, Olyslaegers *et al.,* 2002). Furthermore, cuticles of the abaxial leaf surface are thinner than those on the adaxial surface. Unfortunately, application techniques available for foliar fertilisers allows for more of the applied nutrient on the upper side (adaxial) of the leaf than the lower (abaxial) surface of the leaf. According to Fritz (1978), the uptake ability of the leaf is not fully utilised because the side of leaf which receives more of the applied nutrients has a lower absorbability.

### 2.5.2 Effect of chemical form on foliar absorption

Both positive and negative responses have been observed on the effect of the chemical form in which the ion is applied on uptake rate of foliar applied nutrients. Metal ions such as Zn and Cu are applied in different forms namely: inorganic forms such as sulphates, oxides and chlorides; organic chelates such as EDTA; and organic non chelates (Pulschen, 2004). These possess different chemical properties and as a result their solubility and mobility differ (Pulschen, 2004, Wojcik, 2004).

### 2.5.3 Solubility of fertiliser in water

Suitability of a compound to be used as a foliar spray depends on its solubility and as water is the main carrier of foliar applied nutrient fertilisers, such substances have to be soluble in water in order to facilitate uptake (Fageria *et al*., 2009). Certain compounds, especially oxides, are not soluble in water and are therefore not suitable for foliar application (Table 2.5).

**Table 2.5 Solubility of different foliar fertilisers in water (adopted from Fageria *et al*., 2009)**

| Nutrient | Common name | Formula | Element % | Solubility |
|---|---|---|---|---|
| Boron | Boric acid | $H_3BO_3$ [B $(OH)_3$] | 17 | Soluble |
| | Borax | $Na_2B_4O_7.10H_2O$ | 11 | Soluble |
| | Naborat (anhydrous) | $Na_2B_4O_7$ | 20 | Soluble |
| | Na pentaborate | $Na_2B_{10}O_{16}.10H_2O$ | 18 | Soluble |
| | Na tetraborate | $Na_2B_4O_7.5H_2O$ | 14 | Soluble |
| | Boron frits | | 1.5-2.5 | Slightly soluble |
| Zinc | Zinc sulphate (Monohydrate) | $ZnSO_4.H_2O$ | 36 | Soluble |
| | Zinc sulphate (Heptahydrate) | $ZnSO_4.7H_2O$ | 23 | Soluble |
| | Zinc chloride | $ZnCl_2$ | 48-50 | Soluble |
| | Zinc Oxide | ZnO | 50-80 | Insoluble |
| | Zinc chelate | $Na_2ZnEDTA$ | 9-14 | Soluble |
| | Zinc frits | | 4-9 | Slightly soluble |
| Copper | Copper sulphate (monohydrate) | $CuSO_4.H_2O$ | 35 | Soluble |
| | Copper sulphate (pentahydrate) | $CuSO_4.5H_2O$ | 25 | Soluble |
| | Copper chloride | $CuCl_2$ | 47 | Soluble |
| | Cuprous oxide | $Cu_2O$ | 89 | Insoluble |
| | Cupric oxide | CuO | 75 | Insoluble |
| | Copper chelate | $Na_2CuEDTA$ | 13 | Soluble |
| | Copper chelate | NaCuHEDTA | 9 | Soluble |

### 2.5.4 Chelatisation

Organic chelates are formed when metallic micronutrients such as Fe, Mn, Zn and Cu combine with certain organic compounds. In the process the organic molecule encircles the metal cation forming an outwardly electronically neutral compound (Figure 2.6) (Fritz, 1985). Some chelates are derived from synthetic products and have high stability while others are derived from natural products and have a low stability (Lucena *etal.,* 2010). According to Lucena *et al*. (2010), complexes are intended for mild deficiencies, non-susceptible crops, fertigation, or foliar sprays because of their low stability.

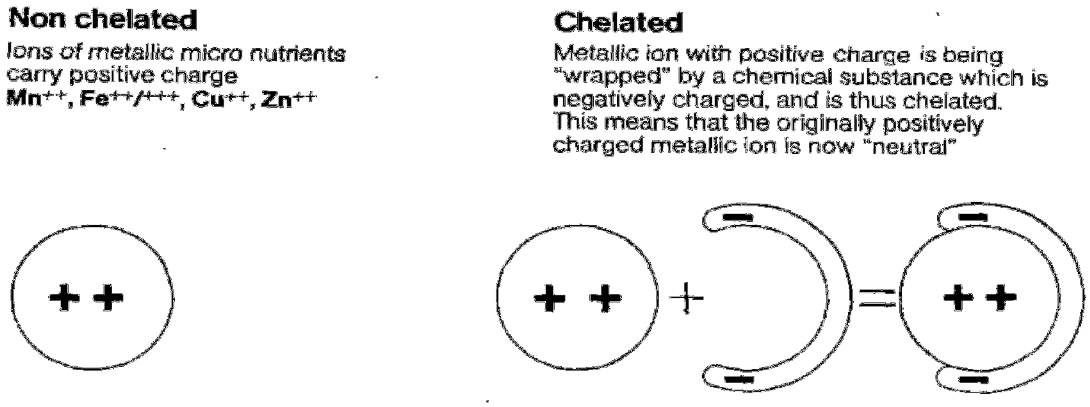


**Figure 2.6 Chelatisation, as explained by Fritz (1985)**

Elements applied to plants as foliar sprays need to have a high capacity of being retained on the cuticle of the leaf so that they are not easily washed away (Ferrandon & Chamel, 1988) and allow time for the elements to move into the inner tissues of the leaf. The effect of chelatisation on the cuticular retention of micronutrients was demonstrated by Ferrandon & Chamel (1988). It was observed that retention of Zn by the cuticle was lower when supplied as a chelate than as sulphate, as a lower Zn concentration ratio (cuticle:radioactive solution ratio) was found when Zn was supplied as a chelate rather than an organic salt (Tables 2.6 and 2.7). Additionally, more Zn was found in the washing solution when $ZnCl_2$ and Zn EDTA were supplied than when $ZnSO_4$ was supplied, further explaining low absorption of Zn EDTA by the leaf cuticle. The cuticle is negatively charged and the lower retention of Zn chelate was attributed to the limited number of metal cations available for retention by the negative sites of the cuticle and the large size of the complex, which decreased the rate of binding on the leaf surface and hence access to inner sites (Ferrandon & Chamel, 1988).

**Table 2.6 Ratios of the final concentration of Zn between the cuticle and the radioactive solution as a function of time and chemical form (Ferrandon & Chamel, 1988)**

| Time | $ZnSO_4$ | Zn EDTA | $ZnCl_2$ |
|---|---|---|---|
| 30 minutes | 1046 | 153 | - |
| 1 hour | 1386 | 188 | 1231 |
| 4 hours | 2382 | 281 | 2656 |
| 24 hours | 3316 | 288 | 3718 |
| 48 hours | 3880 | - | 3598 |
| 72 hours | 4823 | 277 | 5642 |

Molecular mass of the solute has a significant effect on its penetration through the leaves(Kannan, 1980). Substances with higher molecular masses penetrate the leaf more slowly as compared to those with lower masses(Wojcik, 2004). Ferrandon & Chamel (1988) determined that foliar uptake of Zn was better when it was applied in an inorganic form than in organic forms (Tables 2.6 and 2.7). According to Wittwer *et al*. (2006), "chelatisation of metallic elements such as Fe, Mn and Zn reduces the rate of absorption but increases the translocation of the absorbed nutrients". Schönherr (2002) also found that because the aqueous pores of the cuticle are very narrow, smaller ions should penetrate faster than the bigger ones.

**Table 2.7 Amount of Zn found in washing solution (three changes) after retention experiments (72 hours) with zinc sulphate, zinc chloride and zinc EDTA (Ferrandon & Chamel, 1988)**

| Change | $ZnSO_4$ (nmol) | Zn EDTA (nmol) | $ZnCl_2/Cl_3$(nmol) |
|---|---|---|---|
| **First** | $4.0 \pm 0.0$ | $5.0 \pm 0.1$ | $7.0 \pm 0.1$ |
| **Second** | $1.0 \pm 0.2$ | $4.0 \pm 0.0$ | 0 |
| **Third** | $1.0 \pm 0.2$ | $2.0 \pm 0.3$ | 0 |

Uptake of anions through the cuticle is poor compared to cations (Fritz, 1985, Burkhardt &Schroth, 2000). This is attributed to the negative charges that are found within the cuticular membrane which tend to attract the positively charged ions and bind them within the membrane for uptake. However, Schönherr (2002) argued that cations and anions penetrate in equivalent amounts because electrical neutrality must be maintained within the tissues. Grigg (1999) also mentioned that it is the penetration of cations that occurs first when they are attracted to the negative charge on the tissue surfaces and move passively according to concentration gradient. After a certain period, there is a change in the electrical balance within the tissues causing it to be more positive, thereby enabling the anions to start moving into the tissues in order to maintain the electrical balance (Grigg, 1999).

Figure 2.7 illustrates concentrations of Zn in shoot tissues of wheat as affected by method of application and formulation. The plants were grown without any Zn (-Zn control), with Zn supplied to the root environment (Zn control), and in the other four treatments Zn was applied as a foliar spray as ZnO, $ZnSO_4$, Zn EDTA and glycine-chelated biomin Zn. It was discovered that Zn concentration in the shoot tissue was highest when applied as glycine-chelated biomin zinc (chelated form) followed by zinc sulphate and zinc EDTA and lowest in treatments where zinc was supplied to the roots (Zn control), foliar ZnO and –Zn control. This may to some extent be explained by solubility issues, especially in case of ZnO, which is insoluble in water.

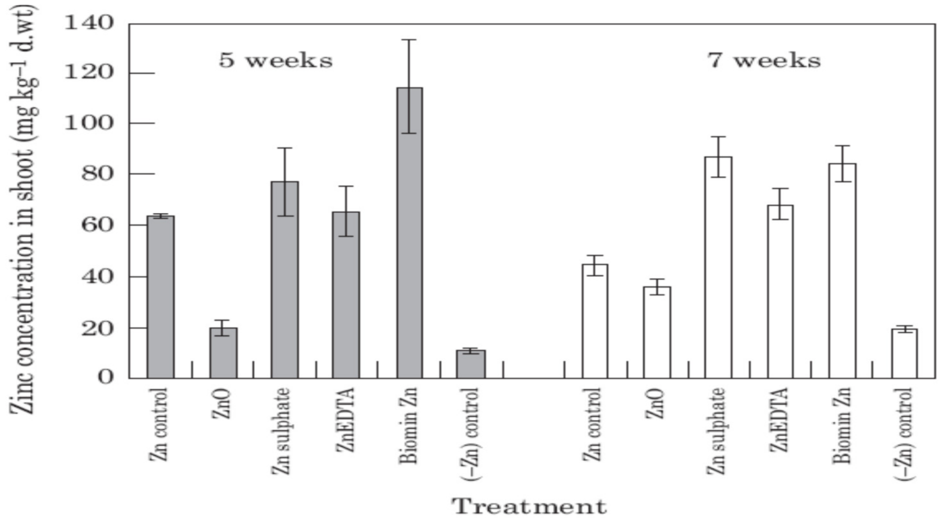


**Figure 2.7 Zinc concentration in shoot tissue of wheat grown in nutrient solutions for various periods of time (5 and 7 weeks), either without Zn supply (-Zn control), or Zn supplied in the root environment (Zn control), or as foliar sprays (ZnO, Zn sulphate, Zn EDTA and Biomin Zn) (from Haslett *et al.*, 2001).**

There was limited Zn transportation from the roots to the shoots of the wheat plants. Evidence for this was obtained in the greater root to shoot Zn concentration ratios observed in –Zn and Zn controls. The most commonly used Zn (inorganic and chelated forms) in foliar fertilisation are $ZnSO_4$ and Zn EDTA respectively, and in the studies involving wheat plants, it was shown that the overall difference between these two forms is very minimal, hence no justification for preferential use of expensive chelated forms as opposed to cheap inorganic Zn forms (Haslett *et al.*, 2001).

### 2.5.5 Environmental factors related to foliar uptake

Environmental factors that affect foliar absorption are indirect in nature (Tukey & Marczynski, 1984). These include relative humidity, light and/or temperature and pH of the nutrient solution to be applied which affect the development of the epicuticular waxes, which in turn comprise the entry points of foliar applied nutrients (Tukey & Marczynski, 1984). Environmental factors may also affect the functionality of the nutrient solution to be applied.

#### 2.5.5.1 Effect of Relative Humidity (RH) on absorption

Relative humidity is one of the major factors affecting penetration of salts through cuticular membranes (Schönherr, 2002). Salts applied on the leaf surfaces have to dissolve for them to penetrate the cuticle. This is determined by point of deliquescence (POD) and humidity over the salt residue (Schönherr, 2002). Point of deliquescence is the humidity over a saturated solution containing a solid salt (Schönherr, 2002). According to Schönherr (2002)when humidity is above POD, the salt residue on the cuticle dissolves and when it is below POD a salt residue is formed and penetration ceases.

High humidity favours entry of salts across the cuticle, as it allows for the maintenance of a moist environment on the applied surface, ensuring the applied substances remain in solution and are available for absorption for longer periods of time (Tukey & Marczynski, 1984). Additionally, high air humidity causes swelling of the cuticular membranes, loosening its components and due to this change in configuration, absorption of hydrophilic compounds increases (Wojcik, 2004).

#### 2.5.5.2 Effect of solar radiation and temperature on absorption of foliar applied nutrients

The effects of solar radiation on absorption of foliar applied nutrients are related to temperature effects. In active uptake, accumulation of ions is against a concentration gradient and requires the use of energy which is derived from respiratory metabolism in the case of roots, and photosynthesis in the case of leaves (Fageria *et al*., 2009).In enzymatically isolated tobacco leaf cells and corn leaf tissues, light enhanced the uptake of ions, which indicates a role for metabolic energy, supplied through photosynthesis, in ion uptake through the leaves (Kannan, 1980). A similar observation was made in barley leaf tissues where uptake of salts and organic acid synthesis was consistently higher in the light than in the dark. This light enhanced uptake was attributed to the additional source of ATP that was available from oxidative phosphorylation (Kannan, 1980).

A different view was presented by Pulschen (2004) who expressed concern about negative indirect effects that solar radiation may have on foliar uptake, however, these effects are mainly temperature related. As noon is approached, there is an increase in ambient temperature and a decrease in relative humidity which leads to rapid evaporation of foliar sprays. This may cause rapid drying of sprays fromfoliar surfaces and accumulation of salts on the leaf surfaces, subsequently causing leaf scorching and burning (Pulschen, 2004). On the other hand, Tukey and Marczynski (1984) have reported beneficial effects of temperature on foliar absorption, attributing this to temperature effects on the orientation of the epicuticular waxes. Plants that were grown at warm temperatures (25 °C) and a relatively high irradiance of 440 $Wm^{-2}$ had their epicuticular waxes arranged in an upright manner, leaving small openings between the wax platelets, thereby increasing the surface area for entry of the foliar nutrients (Tukey & Marczynski, 1984). On the other hand, in plants that were grown at low temperature of 15 °C and a low irradiance of 145$Wm^{-2}$, the waxes looked small and densely packed, preventing contact with the cell surface underneath(Tukey & Marczynski, 1984).

### 2.5.6 Additional factors related to foliar uptake

#### 2.5.6.1 Leaf Area

Leaf Area is one of the factors required for efficient uptake of foliar applied nutrients (Fageria *et al.,* 2009). Applying foliar fertilizers to a large LAI means a large area for interception of spray material. In certain crops such as wheat, a LAI of 2-4 $m^2$ $m^{-2}$ is adequate for efficient uptake (Fageria *et al.,*2009). The spray material has to be uniformly distributed over the leaf area and spraying during windy days may cause variability in spray deposition and therefore uptake of nutrients (Fageria *et al.,* 2009).

#### 2.5.6.2 Effect of pH of nutrient solution on foliar uptake

The pH of the nutrient solution to be used in foliar application plays a role in nutrient uptake. It has been pointed out by Tukey & Marczynski (1984) that a pH between 2 and 3 is not desirable because severe injury to leaves is caused at such levels and this negatively affects foliar uptake. According to Wojcik (2004) the ideal solution pH for maximum uptake of most nutrients is between 3.0 and 5.5, however, higher pH values have been reported for different elements and compounds.

#### 2.5.6.3 Concentration of spray solution

Passive transportation of foliar nutrients is positively correlated with the concentration of the applied solution on the leaf surface. However, this is only true up to certain concentration levels.

In certain circumstances, especially when the concentration of the spray material is too high, injury occurs to the leaf surfaces largely due to malfunctioning of the ectodesmata (Wojcik, 2004). This is mostly accelerated by high day temperatures. According to Fageria *et al*. (2009), too high day temperatures cause burning of plant foliage and these authors recommend that best spray applications are achieved when the plant is not water stressed and when tissues are cool and turgid. Such conditions are best achieved in the morning, late afternoon or on days when temperatures are low. This minimises the problem of leaf scorching that is associated with foliar applications. Maximum concentrations of particular mineral nutrients in foliar applications are affected by factors including plant species, plant developmental stage, plant nutritional status, health of the plant and weather conditions (Wojcik., 2004).

**2.5.6.4 Effect of surfactants on penetration**

Surface-active agents, also known as surfactants, are chemicals that lower surface tension of liquids (Cheristensen, 2005). Surfactants have been used in a number of plants in which foliar applications of nutrients, pesticides and fungicides have been made (Crowley *et al.,* 1994, Gaskin & Murray 1997, Wojcik, 2004,). Surfactants have the ability to increase the area of contact and time of retention on foliage surfaces and its use has produced variable results on penetration of foliar applied nutrients. Surfactants mainly influence the spray retention and leaf wettability (Fernandez & Ebert 2005) by their action on the surface tension between the liquid and the leaf surface and may either reduce or improve retention (Cheristensen, 2005). They also reduce the air layer between the liquid and the leaf surfaces thereby increasing penetration of solutes through stomata and cuticular membranes and limiting the drying of spray droplets (Wojcik, 2004).

Surfactants possess both lipophilic and hydrophilic groups which decrease the surface tension between the liquid and the leaf leading to an increase in the leaf wetting (Wojcik, 2004). The most frequently used surfactants in agriculture are ethoxylated alcohols, alkylphenols, sorbitan, organosiliconeand alkyl amines (Wojcik, 2004). Schönherr (2002) reported that addition of the wetting agent alkyl-polyglucoside glucopon 215 CSUP at 0.2 g $l^{-1}$ reduced the half time of penetration of $CaCl_2$ from 204 hours to 17 hours, thereby increasing the rate of $CaCl_2$ uptake. Surfactants fall into two major categories i.e. ionic and non-ionic (Wojcik, 2004, Fernández

&Ebert, 2005).According to Wojcik (2004), estimation of the efficiency of non-ionic surfactants in improving mineral nutrient absorption by leaves is done using the value of hydrophilic-lipophilic balance (HLB). Surfactants with higher HLB value induce a better cuticular penetration than those with a lower value (Wojcik, 2004).

Effectiveness of surfactants depends upon species, composition and concentration of surfactants (Gaskin & Murray, 1997, Fernández & Ebert, 2005). Non-ionic surfactants are more effective than ionic surfactants because of the inertness and compatibility with most organic ions of non-ionic surfactants. Non-ionic surfactants also do not form insoluble salts in the presence of hard water (Cheristensen, 2005). On the other hand, Fernández & Ebert (2005) speculated that anionic surfactant molecules can be expected to interact with the electrolysed cations forming large molecules that may block cuticular pores and interfere with leaf permeability. On the contrary, Reed & Tukey (1987) found no correlation between the degree of absorption of phosphate and ionic forms of surfactants.

The target leaf surface characteristics also have a bearing on the effectiveness of surfactants and this varies with species. Surfactants are more effective in species whose leaf surfaces are water repellent or “hard to wet” species, contrary to easily wetted species where application of surfactants tend to promote spray run off (Gaskin & Murray, 1997, Reed & Tukey, 1987).

Although there are numerous reports on the beneficial effects of surfactants there have been reports on the detrimental effects of surfactants on the absorption of foliar applied nutrients. Reports by Wojcik (2004) have indicated that surfactants such as organosilicone can decrease leaf nutrient uptake capability due to damage of cellular membranes and/or precipitation of inorganic salts on the leaf surface. The choice of which surfactant to use is therefore important in foliar sprays involving use of surfactants.

### 2.5.7 Translocation of foliar applied nutrients to other parts of a plant

Long distance translocation of nutrient elements occurs in the vascular system, namely the phloem and xylem, with water as the translocating agent (Brown & Shelp, 1997). Foliar nutrient uptake mechanisms and pathways are similar to those of plant growth substances, which are applied to leaves in order to control plant growth, and systemic insecticides to prevent insect infestation (Kannan, 1980). Effectiveness of application of foliar applied nutrient elements depends highly upon how efficiently the nutrient in question can be transported via the phloem (Bukovak & Wittwer, 1957). Transportation of substances in the phloem is independent of transpiration (Brown & Shelp, 1997).

**2.5.7.1 Phloem mobility of copper, zinc and boron**

The efficiency of foliar applied nutrients depends upon their mobility within a plant and foliar nutrient application is more successful for those elements that are phloem mobile (Haslett *et al.,* 2001, Wojcik, 2004. Different nutrient elements are absorbed at different rates, for instance, studies done in bean plants showed that foliar applied nutrients were absorbed at different rates and B, Cu and Zn were classified as partially mobile (Wooldridge, 2002, Newett, 2005). Wooldridge (2002) studied the effect of trace element sprays and fritted trace elements on growth and Mn, Zn, Cu and B contents of young apple trees. In this study, only the parts of the plant, such as leaves, shoots and bark that were directly exposed to the Cu sprays had increased Cu levels compared to the other non exposed parts. However, high B concentrations were observed in the wood in plants treated with B. It was therefore concluded that Cu is not readily taken into the tree structures, whereas B is readily exported to the other parts of the plants. Newett (2005) described Zn and B as nutrients with limited phloem mobility in avocados.

Boron transportation through the phloem depends on the amount of simple sugars, known as polyols, produced. Sugars such as sorbitol, mannitol or ducitols, tend to complex with B to form polyol-B-polyol compounds that may be transported by the phloem to meristematic regions (Brown & Hu, 1996, Brown & Shelp, 1997, Boaretto *et al*., 2008). Thus even though boron has been considered phloem immobile, studies have shown that boron is mobile in plant species which produce sorbitol as a major sugar (Power & Woods, 1997, Blevins & Lukaszewski, 1998). Movement of foliar applied B has been observed in woody Rosaceae plants such as apples, Prunus *spp*. and pear (Brown & Hu 1996). Positive response of foliar applied B has also been observed in soybeans, which contain large quantities of a polyol known as pinnitol (Blevins & Lukaszewski, 1998). In some fruit trees, foliar application of B temporarily increased B concentration in leaves during fall (autumn), but during the late fall and winter B moved from the leaves to the bark. This provides evidence of phloem mobility of B and that it is aided by B-complexing sugars.

In Zn transport experiments done on wheat, movement of foliar applied Zn was directed mainly towards the stem, which contained 34% of the applied Zn ($^{65}Zn$), then the youngest leaf (8%), followed by the roots (6%) and very little (0.1%) in the older leaves (Haslett *et al.,* 2001). This provides evidence that foliar applied Zn was effectively absorbed and translocated to other parts of the wheat plant from the leaves. The presence of Zn in the root tips, especially in the foliar application treatments, is an indication of the possibility of its movement in the phloem and its presence in the young leaves is an indication of its movement through the xylem (Haslett *et al*., 2001).

A different observation was made in 'Hass' avocados (Newett, 2005). Absorption of mineral elements through the leaves proved difficult in 'Hass' avocados, to such an extent that application was followed by reduced response or no response at all. Evaluation of different application methods of Zn in 'Hass' avocados i.e. soil/irrigation-applied $ZnSO_4$, irrigation applied Zn chelate (Zn-EDTA), trunk injection of $ZnNO_3$, and foliar application of $ZnSO_4$, ZNO or Zn metalosates, showed that soil/irrigation supply of $ZnSO_4$ at 3.2 kg tree$^{-1}$ either annually or quarterly was the most effective in correcting Zn deficiency by increasing the Zn concentration in leaf tissues. In foliar applied treatments, it was shown that less than 1% of Zn was actually absorbed by leaf tissues, with very little translocated to the neighbouring tissues from the application points (Newett, 2005).

## 2.6 Conclusion

Fertilization remains a major determinant of yield in tea production. Although fertilization with micronutrients has been given less consideration in the Malawian tea industry, fertilization with micronutrients is as beneficial as fertilization with macronutrients. Copper, B and Zn are required by tea plants in minute quantities, but they play very vital physiological roles and contribute significantly to the healthy development of the tea crop. Their effects have been directly linked to yield and quality, but in some instances indirect effects have been observed. Applications of these elements, depending on the concentration, have contributed to improvements or declines or no effect at all on the quality, yield, and/or yield components, such as number of shoots, shoot dry mass and leaf area index. However, the levels of these elements at which optimum yields are attained is what is of interest to most agriculturalists.

Despite the complexity in the interaction of Cu, Zn and B with the various factors affecting their availability in the tea soils, a holistic approach to determining the optimum supply levels of these nutrient elements, taking into account all the factors contributing to availability and uptake of these by the tea plant will result in their optimized use. Because certain concentrations of Cu and Zn cause yield losses, it is strongly advised to maintain supply of these elements within the optimum levels to avoid such toxicities which can also have subsequent detrimental effects on human health.

Foliar application of micronutrients is beneficial in plant production and is more effective, particularly for the supply of micronutrients such as Zn, Cu and B that are required only in minute quantities. It is convenient during critical growth periods when demand for nutrients is high and it is a faster way of correcting deficiencies, since the uptake process is rapid. Foliar application is preferred especially when soil conditions do not allow for efficient uptake of soil applied nutrients and it can be used to supplement soil applied nutrients. Furthermore, in a crop such as tea, whose economic yield comprises young tender shoots that are removed from the plant regularly, the replenishment of nutrient elements that are lost through this plucked leaf is a necessity.

# CHAPTER 3: MATERIALS AND METHODS

## 3.1 Study Sites

Two similar field experiments were conducted in Malawi in order to achieve the study objectives. The experiments were performed in Cu, Zn and B deficient tea fields at Mianga and Glenorchy tea estates in the Thyolo and Mulanje districts of Malawi respectively. The trial at Mianga was located at an altitude of 960 metres above sea level, latitude16° 5.648'S and longitude 35° 4.733'E. The trial at Glenorchy was located at an altitude of 650 metres above sea level, latitude 15° 59.417'S, longitude 35° 28.943'E.

Deficiencies of Cu, B and Zn in these fields were detected following leaf analyses performed in 2007 by various estates in Malawi. Levels of Cu, Zn and B in leaves collected from these fields were below the recommended levels of 15 mg/kg, 30 mg/kg and 30 mg/kg respectively (Willson & Gunther, 1981).

The bushes at Glenorchy were planted with a mixture of cultivars PC 108 and 105 in 1996 and are irrigated. Tea bushes at Mianga were planted in 1986 with PC 105 under rain-fed conditions. Table 3.1 shows the amount of water (both irrigation and rainfall) received by the plants from January to June 2010 during the course of the experiments at Glenorchy and Mianga.

**Table 3.1 Amount of water (rainfall and irrigation) received by the plants at Glenorchy and Mianga estates from January to July 2010.**

| **Month** | **Glenorchy** | | **Mianga** |
|---|---|---|---|
| | **Rainfall (mm)** | **Irrigation (mm)** | **Rainfall (mm)** |
| January, 2010 | 207.0 | - | 139.4 |
| February, 2010 | 308.0 | - | 314.7 |
| March, 2010 | 269.4 | - | 113.9 |
| April, 2010 | 364.3 | - | 177.7 |
| May, 2010 | 67.5 | 36.0 | 45.1 |
| June, 2010 | 111.0 | 72.0 | 47.0 |
| **Sub total** | **1327.2** | **108.0** | **837.8** |
| **Grand total** | **1435.2** | | **837.8** |

## 3.2 Experimental design and Treatments

There were a total of seven treatments and details are provided as follows:

Treatment 1 (T1) : No spray (control)

Treatment 2 (T2) :0.1% boric acid (776 g B $ha^{-1}$ $yr^{-1}$)

Treatment 3 (T3) :1% copper sulphate (17.7 kg $Cuha^{-1}$ $yr^{-1}$)

Treatment 4 (T4) :1.25kg/ha zinc oxide (4.0 kg Zn $ha^{-1}$ $yr^{-1}$)

Treatment 5 (T5) :0.1% boric acid, 1% copper sulphate solution, 1.25kg/ha zinc oxide (776g $Bha^{-1}$ $yr^{-1}$), 17.7kg $Cuha^{-1}$ $yr^{-1}$, 4.0 kg $Znha^{-1}$ $yr^{-1}$)

Treatment 6 (T6) :1 % of Agrilibrium micronutrient mix (32.5 g $Cuha^{-1}$ $yr^{-1}$, 97.0 g $Znha^{-1}$ $yr^{-1}$, 364.2 g $Bha^{-1}$ $yr^{-1}$)

Treatment 7 (T7) :2.48kg/ha zinc sulphate (4.0 kg Zn $ha^{-1}$ $yr^{-1}$)

The treatments were arranged in a Randomised Complete Block Design with four replicates. The arrangement of treatment plots in the field is provided in Figures 3.1 and 3.2. Plots at Mianga had a total of 30 bushes each spaced at 1.20m by 0.75m with a plant population of 11111 plants $ha^{-1}$. Plots at Glenorchy had 40 bushes with a spacing of 1.2m x 0.6m and a plant population of 13889 plants $ha^{-1}$.

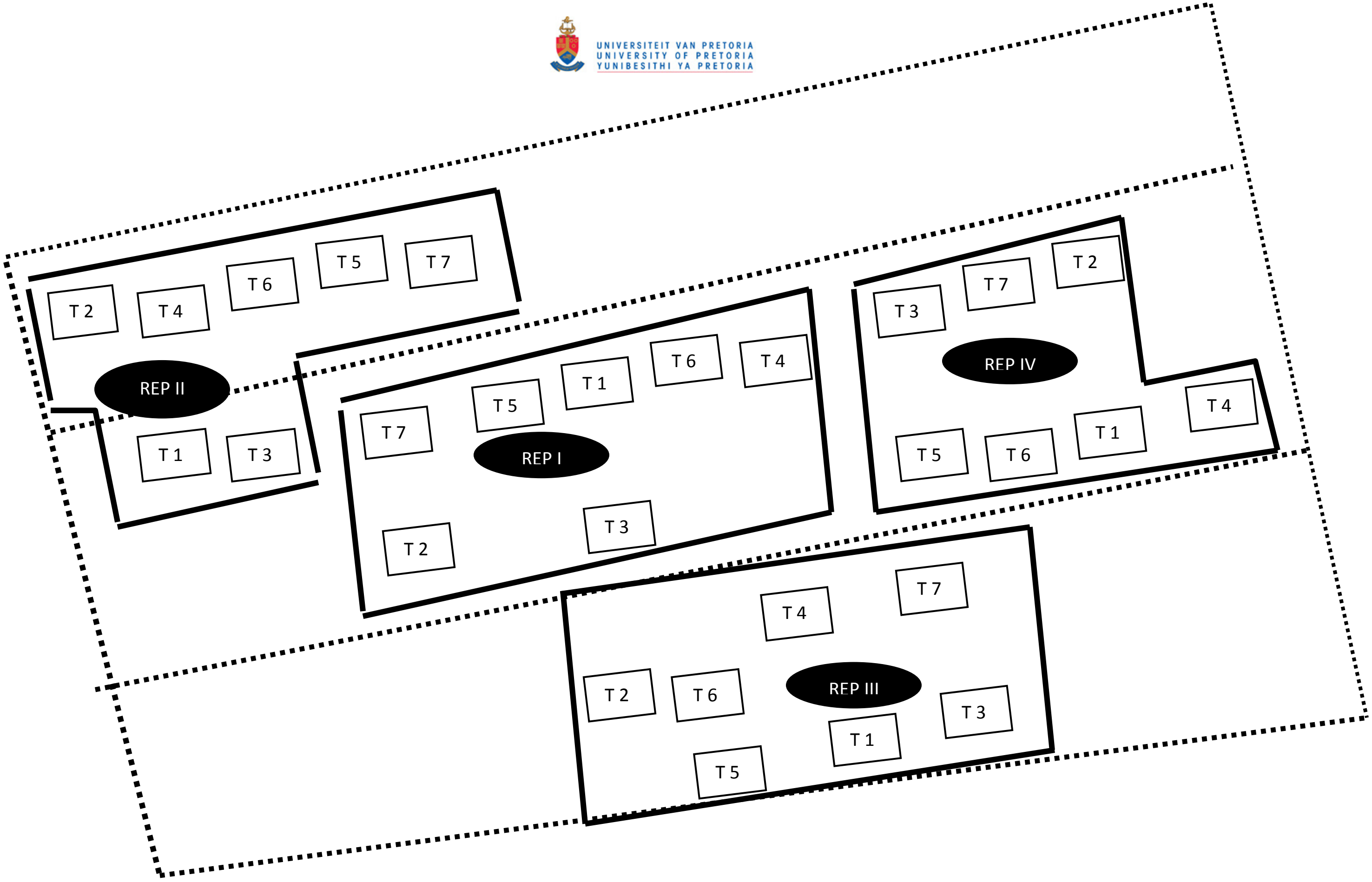


**Figure 3.1 Layout of treatment plots at Mianga estate (Figure not drawn to scale)**

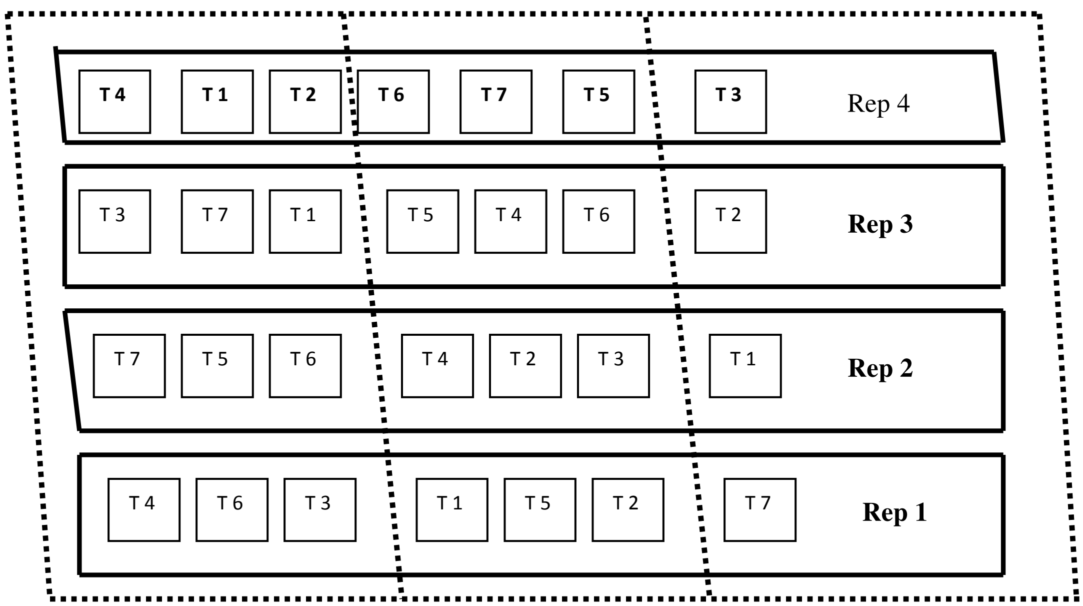


**Figure 3.2 Layout of treatment plots at Glenorchy estate (Figure not drawn to scale)**

### 3.3 Selection of chemicals and their application concentrations

Selection of chemicals was based on their solubility in water considering the fact that the chemicals were to be applied as foliar spray applications. Boric acid is highly soluble in water and is more suitable for foliar application than other forms of B such as borax and borates (Zekri & Obreza, 2009). The application concentration of 0.1% boric acid was chosen based on findings by Prasad & Dey (1979) who observed a significant increase in uptake of B even at 0.1% concentration of boric acid in young tea.

Zinc oxide, although insoluble in water (Fageria *et.al.,* 2009), has been reported to have a positive impact on tea shoot growth and hence yield (Barbora *et al.,* 1993, Alam & Raza, 2001). In Malawi, studies done on seedling tea have also indicated positive yield response due to soil or foliar zinc oxide application (Malenga 1979, Malenga 1986, Grice*et al.,* 1988). Some tea growers in Malawi have been applying zinc oxide to their fields. The application concentration of 0.1% zinc oxide supplied 1.25kg $ha^{-1}$ of zinc oxide per application giving 1kg Zn $ha^{-1}$ per application or 4kg Zn $ha^{-1}$ $yr^{-1}$. The application rate of 1.25kg $ha^{-1}$ was arrived at according to studies conducted on seedling tea in Malawi (Grice *et.al.,* 1988). Zinc sulphate was included because it is soluble in water and hence suitable for foliar application (Fageria *et.al*., 2009). Zinc sulphate was applied at 0.4% concentration, a concentration that would supply the same amount of Zn as that supplied by 0.1% zinc oxide.

Copper sulphate is soluble in water (Fageria *et.al.,* 2009) and studies have shown that application of copper sulphate solution at 0.5-1% concentration has corrected Cu deficiencies in seedling tea (Squire, 1977, TPH, 1990). In this study Cu was applied as copper sulphate at 1% concentration.

The commercial micronutrient mix was included among the treatments considering that the fields were deficient in Cu, B and Zn and micronutrient mixes are convenient when it comes to correcting multiple micronutrient deficiencies. The concentration of 1% was chosen following recommendations by the manufacturer.

All plots received two applications of compound T fertiliser (N: P: K ratio of 25:5:10 +3S). The first application of compound T was done in November, 2009 at 640kg $ha^{-1}$ i.e. 160kg N $ha^{-1.}$ The second application was done in January 2010 at 160kg $ha^{-1}$ i.e. 40kg N $ha^{-1}$. The plots also received a foliar spray of Tsama EPM mix in January, 2010 containing 102g $L^{-1}$ of P, 108g $litre^{-1}$ of Cu and 319g$L^{-1}$ of Zn applied at 2L$ha^{-1}$.

## 3.4 Spraying

Application of foliar sprays was done using a 20-litre knapsack sprayer, which was calibrated according to the procedures described by Rattan (1988) in order to ensure that the exact dosage was applied and that the chemicals were evenly distributed onto the tea plants. Spraying was done in the morning once a month for four months starting in March 2010 until June 2010 at a spraying volume rate of 1111 l $ha^{-1}$. Days on which spraying was done coincided with the plucking days in such a way that spraying immediately followed plucking and the next plucking was done after 10 or 11 days at each site; this is true for the second through to the third application. In the first application, the plan was to spray on the plucking day soon after plucking (end February) but it was not possible because of rains which meant that spraying could only be done five or six days after plucking. No surfactants were used in this trial because we had difficulties in the choice of which surfactants to use as not much research has been done on efficacy of different surfactants in tea, especially in Malawi. Care was taken not to spray on rainy days. Whenever it rained or rains were expected on the date of spraying, spraying was postponed to another closest date.

The actual dates of application are provided inTable 3.2 and the quantities of Cu, Zn and B applied per plot are provided Table 3.3.

**Table 3.2Dates of fertiliser application at Mianga and Glenorchy estates**

| | **Mianga** | **Glenorchy** |
|---|---|---|
| Compound T application (25:5:10 +3S) | November, 2009 and /January 2010 | November, 2009 and /January 2010 |
| Tsama EPM micronutrient mix | January, 2010 | January, 2010 |
| First treatment application | 4 March 2010 | 5 March 2010 |
| Second treatment application | 8 April 2010 | 9 April 2010 |
| Third treatment application | 10 May 2010 | 11 May 2010 |
| Fourth treatment application | 10 June 2010 | 11 June 2010 |

**Table 3.3 Total amount of Cu, Zn and B applied per plot during the four foliar applications from March to June 2010**

| Chemical | Element | Concentration of Chemical | Amount of chemical applied $ha^{-1}$ | Amount of element applied $ha^{-1}$ | Amount of element applied $plot^{-1}$ (Mianga) | Amount of element applied $plot^{-1}$ (Glenorchy) |
|---|---|---|---|---|---|---|
| $H_3BO_3$ | Boron | 0.1 % (m/v) | 4.44 kg | 776g | 2.1g | 2.2 g |
| $CuSO_4.5H_2O$ | Copper | 1% (m/v) | 69.6 kg | 17.7 kg | 47.7g | 51.0 g |
| ZnO | Zinc | 0.1% (m/v) | 5.0 kg | 4.0 kg | 10.8g | 11.5 g |
| $ZnSO_4.7H_2O$ | Zinc | 0.4% (m/v) | 17.6 kg | 4.0 kg | 10.8g | 11.5 g |
| Commercial micronutrient mix | Copper | 1% (v/v) | 44.4 litres | 32.5g | 0.1 g | 0.1 g |
| | Zinc | 1% (v/v) | 44.4 litres | 97.0g | 0.3 g | 0.3 g |
| | Boron | 1% (v/v) | 44.4 l litres | 364.2g | 1.0 g | 1.0 g |

## 3.5 Soil analysis

At the beginning of the trials, before treatments were initiated, representative soil samples were randomly collected from the trial plots using a stainless steel soil auger. Cores were taken from 0-15 cm and 30-45 cm depths. Samples from one trial site and from similar sampling depths were mixed together to form one composite sample. The composite samples were air dried and ground using a mortar and pestle. The samples were then passed through a 2mm sieve, packed and sent to the University of Pretoria's Plant Production and Soil Science laboratory for analysis. The soil samples were analysed for pH, P, K, Mg, Ca, Na, C, CEC, N, B, Zn, Cu, Fe, Mn, Mo, Al, ash and particle size distribution.

To check the nutrient status of the soils after application of treatments from each experimental plot, soil samples were once again collected in May 2010using the same procedures as described above, except that samples from each treatment were analysed separately.

### 3.6 Foliar analysis

Leaf samples were also collected according to the procedures described by Grice (1990) in May 2010. Leaf samples comprised only the third leaf from selected shoots from each plot and were oven dried to constant mass at 85 °C (Grice, 1990). The dried leaf samples were sent to the University of Pretoria's Plant Production and Soil Science Laboratory and were analysed for N, P, K, S, Zn, Cu, Mg, Mn, Mo, Ca, Fe, B, Al and Na using standard methods of analysis adopted at the laboratory.

### 3.7 Yield and yield components (made tea yield, shoot density and shoot dry mass)

Harvesting was done by hand plucking of tender shoots from the tea bushes on every 10$^{th}$ or 11$^{th}$ day at both sites from March 2010 to June 2010. At Mianga yield recording started on 8 March 2010 and ended on 21 June 2010 while at Glenorchy, it started on 9 March and finished on 22 June 2010. On each plucking day, mass of plucked leaf from each plot was recorded and expressed as made tea yield by multiplying the green leaf yield by a factor of 0.22. There were a total of 11 plucking days (harvests) at each site and yield for all the 11 harvests was summed to get total yield for the period (March to June 2010).

Shoot density was determined by using a 0.75 m by 1.2 m square grid, which was thrown randomly on the plucking table at three positions in each plot. Different shoot categories were counted at each position and the number of shoots per m$^2$ was calculated. This was performed once a month.

Shoot dry mass was determined from a 200g sample of green leaf from the plucked leaf once a month. The leaf sample was separated into different categories of shoots, which were: 1L+b, 2L+b, 3L+b and 4L+b shoots. The samples were dried in the oven at 85°C to constant mass. Data on shoot dry mass was expressed as average shoot mass for each shoot category.

### 3.8 Made tea Quality

#### 3.8.1 Determination of black tea quality by sensory evaluation and biochemical analysis

In this study, a sample weighing 300 g of green leaf plucked from each of the trial plots was collected once a month from March to June 2010 from the seven treatments and four replicates.

Each sample was processed separately into black tea in the Mini Processing Unit at TRFCA. From each of the black tea samples, two 50 g samples were weighed and packed separately. One of the 50 g black tea samples from each plot was sent to Tea Brockers of Central Africa (TBCA) for sensory evaluation by professional tea tasters while the other 50 g black tea sample was sent to University of Pretoria for biochemical analysis.

Black tea quality parameters assessed by tea tasters included: brightness, briskness, colour of liquor, colour of infusion, colour with milk and strength of liquor. A scoring scale of 1 to 10 is used by the tea tasters for each parameter, 1 being the lowest score and 10 the highest (Clowes & Mitini–Nkhoma, 1987).

**3.8.1.1 Determination of Caffeine, Theaflavin and Thearubigin contents in black tea**

Biochemical compounds that were determined included: caffeine by spectrophotometry as described by Yao *et.al.* (2006).Thearubigins (TRs) and theaflavins (TFs) by extraction and spectrophotometry as described by Roberts & Smith (1963) The TR/TF ratio was also determined.

**3.8.1.2 Determination of Caffeine in black tea**

Lead acetate solution was prepared by dissolving 25g $(CH_3COO)_2$ Pb in 50ml of triple distilled water (ddd$H_2O$). A 0.0324% solution of hydrochloric acid was prepared by diluting 90ul 32% hydrochloric acid in 100ml of ddd$H_2O$. Another solution of sulphuric acid was also prepared by adding 16.7ml of 98% $H_2SO_4$ to 80 ml of ddd$H_2O$ first and then the solution was made up to 100ml with ddd$H_2O$. A 1mg $ml^{-1}$ stock solution of caffeine was also prepared by transferring 0.1g ± 0.001g caffeine into 100ml of ddd$H_2O$ in a volumetric flask. Standard solutions of (0, 0.005, 0.01, 0.15, 0.02 and 0.025) mg $ml^{-1}$ of caffeine were prepared.

**3.8.1.3 Preparation of black tea sample for caffeine determination**

The black tea sample was prepared by turning on the water bath set at a temperature of 90$^{o}$C. A bottle containing sufficient ddd$H_2O$ was placed in a water bath and was allowed to equilibrate for 20 minutes. 0.1g ± 0.001g ground black tea leaf was weighed in a 10ml glass tube placed and was placed in a water bath. 5ml of ddd$H_2O$ was added onto the tube and was closed with a rubber stopper. The mixture in the glass tube was incubated for 10 minutes and vortexed after 0, 5 and 10 minutes. The solution was centrifuged for 10 minutes at 3000 x g. The supernatant was transferred into a 10ml volumetric flask and 5.5ml of hot ddd$H_2O$ was added. The volumentric flask was filled with cold ddd$H_2O$ to a 10 ml mark.

### 3.8.1.4 Caffeine measurement

The prepared tea samples were diluted with water at a ratio of 50:50. 100 ul of diluted tea sample was added to 50ul HCl solution and 10ul of lead acetate solution and vortexed. The mixture was allowed to stand for 10 minutes after which 840ul of ddd$H_2O$ was added to two Eppendorph tubes of 1ml each and vortexed. The solution was centrifuged for 30 seconds. The supernatant was transferred into a 1ml Eppendorph tube and 10ul of $H_2SO4$ was added and vortexed. The solution was allowed to stand for 5 minutes and later 490ul of ddd$H_2O$ was added and vortexed. The solution was centrifuged again for 30 seconds. Absorbance was measured on the supernatant solution at 274nm with UV/visible spectrophotometer. Caffeine concentrations of the standard solutions were determined and a standard curve was plotted. Caffeine content in the tea sample was then calculated using the following formula:

Caffeine (%) =(E/1000) x $V_0$ x (100/$V_1$) x (1/0.5)/W = (E/1000) x 10ml x (100/0.1ml) x (1ml/0.5ml)/0.1g-moisture content)

Where

E =mg caffeine from standard curve (1000 conversion to g)

$V_0$ =total volume of tea solution

$V_1$ =volume of tea used for measurement (100/= dilution of tea sample solution)

1/0.5 =dilution factor (500ul tea solution diluted to 1ml)

W =dry weight of tea sample used.

### 3.8.1.5 Determination of Theaflavin and Thearubigin content in black tea samples

2.5% Sodium hydrogen carbonate ($NaHCO_3$) solution was prepared. A saturated aqueous oxalic acid solution was prepared by mixing 10g of oxalic acid with 100ml of ddd$H_2O$ in a volumetric flask. 60% methanol solution was also prepared.

**3.8.1.6 Preparation of black tea sample for TR and TF analysis**

A water bath was set at a temperature of 90$^o$C. 0.002±0.001g black tea sample was weighed in a 10ml glass extraction tube. 1litre of ddd$H_2O$ was heated in 90$^o$C water bath for a minimum of 30minutes for equilibration. Glass extraction tube containing tea sample was then placed in water bath and 5ml of ddd$H_2O$ was added into the glass extraction tube. A stopper was put and the contents were mixed on vortex. The contents were kept in water bath for 10 minutes and were vortexed for 5 and 10 minutes. Tubes were removed and the contents were left for 5 minutes to cool down to room temperature. Stopper was removed and the contents were centrifuged for 10minutes at 3500rpm. The supernatant was decanted into a 10ml volumetric flask and was set aside. Extracted supernatant was filtered with 0.22µM filter coupled to a 10ml syringe.

**3.8.1.7 Extraction of TFs and TRs from tea sample**

One ml of prepared black tea sample was mixed with 1ml of Isobutyl Methyl Ketone (IBMK) and the mixture was vortexed for 30 seconds for four times. Two bottom layers of IBMK were put separately (solutions B and D). One of the two top layers of IBMK was mixed with 168µl of methanol (solution A) and the other layer was mixed with 500µl of $NaHCO_3$ and vortexed for another 30 seconds resulting into two layers. The bottom layer was discarded and the top layer was then mixed with 168µl of methanol (solution C). Solutions A, B, C and D were transferred into ELISA plates. The optical densities of the solutions were then measured at 375nm and 450nm. Theaflavin and Thearubigin contents in percentage were calculated using the following formulae:

% theaflavins = $6.25 \times Ec \times f_1$

% thearubigins = $[(12.5E_D + 6.25 (E_A-E_C)] \times f_2$

Where

$f_1 = 0.36$ for readings at 380nm or $f_1 = 1.07$ for readings at 460nm

$f_2 = 1.13$ for readings at 380nm or $f_2 = 4.40$ for readings at 460nm

## 3.9 Statistical analysis

All data,except for data on foliar Cu levels, was subjected to two-way analysis of variance, with treatment and block effects, using GENSTAT Discovery Edition 3 statistical package. Treatment means were separated using the Duncan's Multiple Range Test (DMRT) at $p<0.05$ level of significance. Data on foliar Cu did not meet requirements for ANOVA procedures for it did not follow a normal distribution, therefore non parametric analysis was done by Friedman's two way ANOVA by ranks using SAS statistical package (SAS/STAT 9.2) (SAS Institute Inc.,2008).

# CHAPTER 4: RESULTS AND DISCUSSION

## 4.1 Effect of foliar sprays of Cu, Zn and B containing fertilisers on tea yield and yield components

Productivity in tea, just like any other crop, is a function of a number of factors such as climate, soil, genotype and cultural practices. Fertilisation is one of the major determinants of yield in tea besides planting material, pruning and harvesting patterns (Drinnan, 2008). A balanced nutrition with both macronutrients and micronutrients is a requirement for tea to produce satisfactory yields and products of desired quality. Micronutrients, although they are required in minute quantities, play important roles in plant growth and development. They function either as catalysts or at least they are closely linked to catalytic processes within plants. Cu, Zn and B are among the essential elements for growth and development hence their deficiencies severely limit crop production.

Fertilisation in tea especially in Malawi has put more emphasis on macro elements such as N, P and K with little attention paid to fertilisation with micronutrient elements. As a result most fields have suffered micronutrient deficiencies, especially those of Cu, Zn and B, in recent years. Foliar applications of Cu, Zn and B containing fertilisers may therefore be able to improve tea yields. This study was conducted to evaluate the effects of foliar sprays of Cu, Zn and B containing fertilisers,including one commercial micronutrient mix, on yield and yield components of tea.

Results have shown that made tea yield was significantly affected by the treatments at Glenorchy estate (P= 0.001), however,at Mianga tea estate yield was not significantly affected. At Glenorchy, yield was significantly reduced due to application of copper sulphate alone and when copper sulphate was applied in combination with boric acid and zinc oxide. Yields from plants treated with boric acid alone, zinc oxide alone, zinc sulphate alone and the commercial micronutrient mix were statistically similar to those of the control treatment. The highest yielding plants were those treated with zinc sulphate and the commercial micronutrient mix. Results of total yield for 11 harvests (from March to June) for the seven treatments are presented in Figure 4.1.

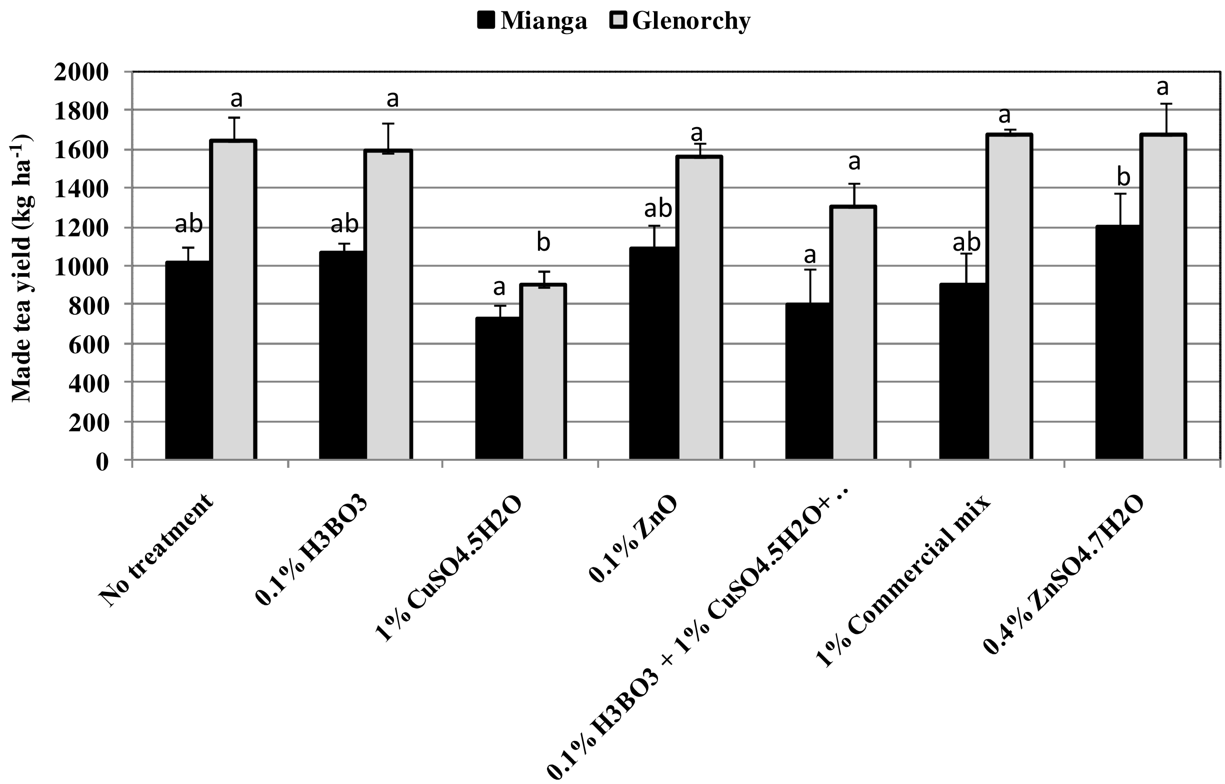


**Figure 4.1 Made tea yield for different foliar treatments of Zn, Cu and B containing fertilisers (Note: Vertical bars represent standard error of the means (n=4))**

Table 4.1 presents percent change in yield, compared to the control, due to application of various micronutrient treatments at both sites. At Glenorchy estate, results on made tea yield clearly indicated that there was a significant yield decrease of 45.2 % when copper sulphate (T3)was applied alone but when copper sulphate, boric acid and zinc oxide were applied together, yield significantly decreased by 20.7%. Boric acid applied alone and zinc oxide applied alone non-significantly reduced yields by 3.3% and 4.7% respectively while commercial micronutrient mix and zinc sulphate non-significantly increased yields by 2.2% and 2.1 % respectively.

At Mianga estate, yield was not significantly affected by the treatments. Although not significant, the lowest yield was obtained when copper sulphate was applied alone and the highest was obtained when zinc sulphate was applied.

**Table 4. 1 Percentage change in yield, relative to the control (T1), due to application of B, Zn and Cu containing fertilisers**

| Treatment | Concentration of chemicals applied | Site A# Yield (kg $ha^{-1}$) | Site B# Yield (kg $ha^{-1}$) | Site A#change in yield (kg$ha^{-1}$) | Site B#change in yield (kg$ha^{-1}$) | Site A# % change in yield | Site B# % change in yield |
|---|---|---|---|---|---|---|---|
| T1 | No treatment | $1016^{ab}$ | $1641^{b}$ | - | - | - | - |
| T 2 | 0.1% $H_3BO_3$ | $1063^{ab}$ | $1587^{b}$ | 47 | -54 | 4.6 | -3.3 |
| T 3 | 1% $CuSO_4.5H_2O$ | $728^{a}$ | $898^{a}$ | -288 | -742 | - 28.3 | - 45.2 |
| T 4 | 0.1% ZnO | $1085^{ab}$ | $1564^{b}$ | 69 | -77 | 6.8 | -4.7 |
| T 5 | 0.1% $H_3BO_3$ + 1% $CuSO_4.5H_2O$+ 0.1% ZnO | $795^{a}$ | $1301^{b}$ | -221 | -340 | - 21.8 | - 20.7 |
| T 6 | 1% Commercial mix | $907^{ab}$ | $1677^{b}$ | -109 | 36 | - 10.7 | 2.2 |
| T 7 | 0.4% ZnSO4.7H2O | $1204^{b}$ | $1675^{b}$ | 188 | 34 | 18.5 | 2.07 |
| **P value** | | **0.070** | **0.001** | | | | |
| **CV %** | | **22.5** | **15.9** | | | | |
| **LSD** | | **324.8** | **348.6** | | | | |

**Note: Means within each column with the same letters are not statistically different at 95% level of confidence.**

#Site A is Mianga estate, Site B is Glenorchy estate

A collection of individual shoots containing two leaves and a bud (2L+b) or three leaves and a bud (3L+b) comprise the economic yield of the tea plant (Matthews & Stephens 1998). Results on shoot density for 1L+b, 2L+b, 3L+b,4L+b and *bhanjhi* shoots and total number of shoots $m^{-2}$for the two sites are presented in Tables4.2 and 4.3. At Mianga estate, a significant decrease in shoot density of 2L+b, 3L+b and total number of shoots was observed when copper sulphate was applied alone. Plots that received boric acid alone, zinc sulphate, zinc oxide alone, the commercial micronutrient mix and a mixture of copper sulphate, zinc oxide and boric acid did not differ from the control, although zinc sulphate application tended to give the highest number of 2L+b, 3L+b shoots $m^{-2}$ and total number of shoots $m^{-2}$. There were no significant differences in the number of *banjhi*/dormant shoots $m^{-2}$ as a result of foliar application of Cu, Zn and B in this study.

**Table 4. 2 Mean tea shoot density as a result of different foliar treatments of Zn, Cu and B at Mianga**

| Trt | Concentrations of chemicals applied | 1L+b (number of shoots $m^{-2}$) | 2L+b (number of shoots $m^{-2}$) | 3L+b (number of shoots $m^{-2}$) | 4L+b (number of shoots $m^{-2}$) | Bhanjhi (number of shoots $m^{-2}$) | Total (number of shoots $m^{-2}$) |
|---|---|---|---|---|---|---|---|
| T 1 | No treatment | $5.09^{a}$ | $7.52^{b}$ | $2.87^{b}$ | $1.644^{a}$ | $4.074^{a}$ | $21.20^{b}$ |
| T2 | 0.1% $H_3BO_3$ | $5.30^{ab}$ | $7.43^{b}$ | $2.801^{b}$ | $1.273^{a}$ | $3.750^{a}$ | $20.56^{b}$ |
| T3 | 1% $CuSO_4.5H_2O$ | $4.12^{a}$ | $4.91^{a}$ | $1.852^{a}$ | $0.856^{a}$ | $3.796^{a}$ | $15.53^{a}$ |
| T4 | 0.1% ZnO | $5.05^{a}$ | $6.99^{b}$ | $2.315^{ab}$ | $1.134^{a}$ | $4.074^{a}$ | $19.56^{b}$ |
| T5 | 0.1% $H_3BO_3$ +1% $CuSO_4.5H_2O$+ 0.1% ZnO | $4.68^{a}$ | $6.85^{b}$ | $2.593^{b}$ | $1.157^{a}$ | $4.144^{a}$ | $19.42^{b}$ |
| T6 | 1% Commercial mix | $5.16^{a}$ | $7.04^{b}$ | $2.546^{ab}$ | $1.042^{a}$ | $3.819^{a}$ | $19.61^{b}$ |
| T7 | 0.4% $ZnSO_4.7H_2O$ | $5.30^{a}$ | $7.87^{b}$ | $2.917^{b}$ | $1.505^{a}$ | $4.028^{a}$ | $21.62^{b}$ |
| **$P_{0.05}$** | | **0.089** | **0.002** | **0.041** | **0.119** | **0.630** | **0.001** |
| **LSD** | | **0.847** | **1.230** | **0.665** | **0.567** | **0.558** | **2.397** |
| **CV %** | | **11.5** | **11.9** | **17.5** | **31.0** | **9.5** | **8.2** |

**Note: Means within each column with the same letters are not statistically different at 95% level of confidence.**

There were highly significant differences in total number of shoots $m^{-2}$ at Glenorchy. As at Mianga, shoot density for 2L+b and 3L+b shoots also significantly differed at Glenorchy. Application of copper sulphate alone or in combination with boric acid and zinc oxide significantly reduced shoot density for 2L+b and 3L+b shoots and total number of shoots $m^{-2}$. Densities of 2L+b and 3L+b shoots and total number of shoots $m^{-2}$ were similar in plots treated with boric acid alone, zinc oxide alone, zinc sulphate and the commercial micronutrient mix. Shoot density of 1L+b differed significantly between T5 (boric acid + copper sulphate + zinc oxide) and T6 (commercial mix).

**Table 4. 3 Mean tea shoot density as a result of different foliar treatments of Zn, Cu and B at Glenorchy**

| Trt | Concentrations of chemicals applied | 1L+b (number of shoots $m^{-2}$) | 2L+b (number of shoots $m^{-2}$) | 3L+b (number of shoots $m^{-2}$) | 4L+b (number of shoots $m^{-2}$) | Bhanjhi (number of shoots $m^{-2}$) | Total (number of shoots $m^{-2}$) |
|---|---|---|---|---|---|---|---|
| T 1 | No treatment | 6.25[a] | 9.21[b] | 3.056[b] | 1.250[c] | 3.588[a] | 23.36 [b] |
| T2 | 0.1% $H_3BO_3$ | 6.16[a] | 8.26[b] | 2.292[ab] | 0.880 [abc] | 4.120[a] | 21.71 [b] |
| T3 | 1% $CuSO_4.5H_2O$ | 5.35[a] | 5.93[a] | 1.667[a] | 0.556 [a] | 4.375[a] | 17.87 [a] |
| T4 | 0.1% ZnO | 5.95[a] | 8.91[b] | 2.894[b] | 0.949[abc] | 4.005[a] | 22.71 [b] |
| T5 | 0.1% $H_3BO_3$ +1% $CuSO_4.5H_2O$+ 0.1% ZnO | 5.02[a] | 5.75[a] | 1.944[a] | 0.764 [ab] | 4.699 [a] | 18.18 [a] |
| T6 | 1% Commercial mix | 6.67[a] | 9.49[b] | 3.009[b] | 1.019 [bc] | 4.074[a] | 24.26 [b] |
| T7 | 0.4% $ZnSO_4.7H_2O$ | 6.48[a] | 9.72[b] | 2.986[b] | 1.042[bc] | 3.981 [a] | 24.21 [b] |
| | **$P_{0.05}$** | **0.147** | **< 0.001** | **0.003** | **0.045** | **0.078** | **< 0.001** |
| | **LSD** | **1.308** | **1.748** | **0.750** | **0.397** | **0.676** | **3.119** |
| | **CV %** | **14.7** | **14.4** | **19.8** | **28.9** | **11.0** | **9.6** |

**Note: Means within each column with the same letters are not statistically different at 95% level of confidence.**

Yield in tea is affected by mean individual shoot dry mass (Grice & Clowes, 1990). In the current study, there were no significant differences in average shoot dry mass among the treatments as shown in Table 4.4.The differences in yield across the treatments at Glenorchy tended to arise from differences in densities of 2L+b and 3L+b shoots which comprise the economic yield of tea (Tables4.2 and 4.3), which eventually contributed to differences in total number of shoots $m^{-2}$. Copper sulphate applied either alone or as a mixture with boric acid and zinc oxide resulted into a significant decrease in shoot population density while shoot population densities for tea plants that received boric acid alone, zinc oxide alone, zinc sulphate alone and the commercial micronutrient mix were similar to those of the control, a trend which was similar to that observed in made tea yield. Similar observations in tea were reported by Grice (1990) where an increase in yield due to zinc application was due to shoot number and not shoot size. Micronutrients therefore appear to be important for shoot growth and development, which is a vital component of yield in tea.

**Table 4. 4 Average shoot dry mass for different categories of shoots at Mianga and Glenorchy**

| Trt. | Concentrations of chemicals applied | Mianga (g per shoot) | | | | Glenorchy (g per shoot) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **1L+b** | **2L+b** | **3L+b** | **4L+b** | **1L+b** | **2L+b** | **3L+b** | **4L+b** |
| T 1 | No treatment | 0.192[a] | 0.429[a] | 0.715[a] | 1.095[a] | 0.244[a] | 0.446[a] | 0.715[a] | 1.058[a] |
| T2 | 0.1% $H_3BO_3$ | 0.305[a] | 0.485[a] | 0.805[a] | 1.350[a] | 0.204[a] | 0.365[a] | 0.615[a] | 1.321[a] |
| T3 | 1% $CuSO_4.5H_2O$ | 0.206[a] | 0.461[a] | 0.719[a] | 1.089[a] | 0.199[a] | 0.343[a] | 0.688[a] | 1.003[a] |
| T4 | 0.1% ZnO | 0.226[a] | 0.493[a] | 0.795[a] | 1.213[a] | 0.233[a] | 0.424a | 0.643[a] | 0.906[a] |
| T5 | 0.1% $H_3BO_3$ +1% $CuSO_4.5H_2O$+ 0.1% ZnO | 0.250[a] | 0.452[a] | 0.673[a] | 0.987[a] | 0.148[a] | 0.349[a] | 0.672[a] | 1.232[a] |
| T6 | 1% Commercial mix | 0.297[a] | 0.470[a] | 0.724[a] | 1.075[a] | 0.219[a] | 0.439[a] | 0.679[a] | 1.108[a] |
| T7 | 0.4% $ZnSO_4.7H_2O$ | 0.250[a] | 0.487[a] | 0.775[a] | 1.227[a] | 0.204[a] | 0.459[a] | 0.871[a] | 1.309[a] |
| **$P_{0.05}$** | | **0.095** | **0.952** | **0.618** | **0.182** | **0.597** | **0.129** | **0.261** | **0.448** |
| **LSD** | | **0.086** | **0.133** | **0.167** | **0.278** | **0.104** | **0.106** | **0.207** | **0.467** |
| **CV %** | | **23.5** | **19.1** | **15.1** | **16.3** | **33.8** | **17.6** | **20.0** | **27.7** |

**Note: Means within each column with the same letters are not statistically different at 95% level of confidence.**

Copper sulphate foliar treatments tended to have an inhibitory effect on the development of shoots, which eventually led to the decline in shoot density and eventually yield in plots where copper sulphate was applied either alone or in combination with boric acid and zinc sulphate. Copper is reported by Fernandes & Henriques, (1991)to be a potent inhibitor of photosynthesis, influencing photosynthetic electron transport, photophosphorylation and the dark reactions of photosynthesis. The inhibitory effect on photosynthesis wasalso observed on the activity of several enzymes that are responsible for the dark reactions of photosynthesis, namely ribulose 1, 5 biphosphate carboxylase in barley and phosphoenolpyruvate carboxylase in *Zeamays* (Stiborová *et.al.*, 1986, Iglesias & Andreo 1984). Barua & Dutta (1972) observed a reduction in yield of tea following copper application and a 20% yield increase due to zinc application. Majid & Ballard (1990) observed severe scorching and leaf burn when a 1% copper sulphate solution was applied either alone or in combination with urea in pine plantations, but application of copper sulphate at 0.1% concentration and urea did not scorch the pine leaves.

In the current study, some tea bushes suffered leaf scorching in treatments where copper sulphate solution was applied either singly or in combination with boric acid and zinc oxide. This was not the case in any of the other treatments. Copper was the common element in the treatments that showed leaf scorch, therefore this indicates that copper sulphate was responsible for the leaf scorch and that the concentration of copper sulphate was too high, leading to low yields in copper sulphate treated tea bushes. Excessive copper accumulation triggers the production of reactive oxygen species (Wang *et al.,* 2004). These reactive oxygen species react with cellular components resulting in oxidation of nucleic acids, as well as peroxidation of lipids, which leads to enzyme inactivation, membrane disruption, mutation and untimely cell death (Halliwell & Gutteridge, 1999). This may further explain the scorching of leaves and yield reduction observed in copper sulphate treated plots.

Zinc is indispensable for the healthy development of the tea shoot that is plucked for manufacture (Gondwe, 1969). Zinc is important for synthesis of RNA, protein synthesis in plants and tryptophan which is a precursor of indole acetic acid (IAA) thereby contributing to the growth of plants (Sharma *et. al.* 1992). A number of reports have indicated positive yield response due to Zn application (Dootson, 1976, Malenga 1979, Barbora *et al*., 1992, Sinha *et al*., 1992, Fung & Wong, 2001). Foliar applications of Zn have produced positive yield results when Zn was applied to mature seedling tea in Malawi (Malenga, 1979, Malenga, 1986). Fung & Wong (2001) also found a positive correlation between Zn and relative dry mass of tea plants. Reports by Dootson (1976) indicated that Zn application led to significant yield increase in mature Indian seedling tea. In the current study, application of Zn either as zinc oxide or as zinc sulphate did not significantly increase yield, suggesting that the concentrations at which Zn was applied in the study did not meet the requirements of the clonal tea bushes under study or there was another factor limiting yield.

Gohian *et al.* (2000) reported an increase in yield due to increased level of foliar applied B of up to 1 kg B $ha^{-1}year^{-1}$ applied in the form of boric acid at 0.25 % concentration. In the current study, B applied at 776 g $ha^{-1}year^{-1}$ as boric acid at 0.1% concentration did not have an effect on tea yields. Boric acid applied in combination with copper sulphate and zinc oxide decreased yields significantly at Glenorchy estate and non-significantly at Mianga estate however, the decrease in yield in plants treated with boric acid in combination with copper sulphate and zinc oxide was unlikely to be as a result of boric acid.

### 4.1.2 Conclusion

From the results on yield, it can be concluded that application of foliar sprays containing Cu, Zn and B used in this study significantly affected yield at Glenorchy estate but not at Mianga tea estate. Differences in factors such as water regimes between the two sites could account for differences in response to the foliar sprays. For instance, tea plants at Glenorchy estate which responded to the different treatments were under irrigation, while those at Mianga which did not give any significant response to foliar sprays were under rain-fed conditions. Under rain-fed conditions, water may be most limiting for growth rather than nutrients.

This study has provided evidence that four applications of copper sulphate at 1% concentration significantly reduced made tea yield in mature clonal tea. It is likely that the four applications, applied monthly, of 1% copper sulphate solution caused an accumulation of Cu to toxic levels in the tea plants, thereby negatively affecting yield. Excess Cu levels in plants inhibit photosynthesis in such a way that there is an altered source-sink relationship when non-structural carbohydrates accumulate in the source leaves due to excess Cu levels in leaves. This in turn induces a feedback inhibition on photosynthesis (Alaoui-Sosse *et al*. 2004). The impact on yield observed in the present study was a result of reduced shoot number or shoot population density and not shoot mass. This may arise from production of insufficient photosynthates for new growth leading to yield reduction.

### 4.2. Effect of foliar sprays of Cu, Zn and B containing fertilisers on tea quality

Quality of tea is defined based on the inherent biochemical characteristics which are reflected in the sensory evaluation by professional tea tasters (Paul, 2008). Teas from the central and southern parts of Africa fall within the low to medium organoleptic quality rating by international standards (Grice, 1990, Wright *et al*., 2000). Unlike teas from other growing areas, especially high altitude areas such as Kenya and other parts of India which are sold mainly for their aroma, teas from central Africa are sold for their briskness (astringency), brightness, colour and strength characteristics, which are mainly controlled by chemical compounds known as theaflavins (TFs) and thearubigins (TRs) (Grice, 1990, Wright *et al.*, 2000). Total TF content of black tea correlates strongly with the value of the tea and brightness of black tea infusion(Hilton & Ellis, 1972, Wright *et al*., 2000). Other reports have also indicated the importance of caffeine content in the prediction of black tea quality (Cloughley, 1982, Obanda *et al*., 1997).

There are a number of factors that affect the quality of made tea and these include type of plant material, climatic factors, nitrogen application levels, plucking standards, and manufacturing practices (Cloughley*et al.,* 1983; Hilton & Ellis 1972). For instance, high nitrogen fertilizer rates and poor plucking standards reduce the quality of black tea (Cloughley *et al.*, 1983; Hilton & Ellis 1972). Micronutrient elements, particularly Cu, have been reported to have a positive contribution to the quality of black tea (Squire, 1977, Azam *et al.,* 2004, Seenivasan *et al.,* 2008). Copper is essential for the formation of polyphenol oxidase, an important Cu and Zn containing enzyme/catalyst in the fermentation process of black tea manufacturing (Ogunmoyela, *et al*., 1994, Seenivasan *et al.,* 2008) and its deficiencies lead to poor fermentation and subsequently poor quality tea. In the current study, black teas from plots treated with Cu, B and Zn containing foliar sprays were organoleptically assessed by professional tea tasters from Tea Brokers of Central Africa and chemically evaluated for contents of TRs, TFs and caffeine.

#### 4.2.1 Effect of foliar sprays of Cu, Zn and B on taster’s scores and valuation

Results of the tea taster’s scores obtained from Glenorchy and Mianga tea estates are presented in Tables 4.5 and 4.6 as averages of the values obtained from each harvest between March and June 2010.

**Table 4. 5 Tea taster's scores as affected by different micronutrient foliar treatments at Glenorchy estate**

| Parameter | T1 | T2 | T3 | T4 | T5 | T6 | T7 | Mean | $P_{0.05}$ | CV% |
|---|---|---|---|---|---|---|---|---|---|---|
| **Brightness** | 2.500$^{b}$ | 1.583$^{a}$ | 1.625$^{a}$ | 1.500$^{a}$ | 1.58$^{a}$ | 1.33$^{a}$ | 1.75$^{a}$ | 1.7 | **0.003** | **19.6** |
| **Briskness** | 2.583$^{c}$ | 2.25$^{bc}$ | 2.125$^{bc}$ | 2.00$^{b}$ | 1.75$^{ab}$ | 1.583$^{a}$ | 2.083$^{b}$ | 2.1 | **0.002** | **13.4** |
| **Colour of liquor** | 4.917$^{b}$ | 4.667$^{b}$ | 4.125$^{a}$ | 4.167$^{a}$ | 4.16$^{a}$ | 3.91$^{a}$ | 4.16$^{a}$ | 4.3 | **0.001** | **6.3** |
| **Colour ofinfusion** | 4.333$^{b}$ | 4.417$^{b}$ | 4.00$^{a}$ | 4.500$^{b}$ | 4.20$^{ab}$ | 4.33$^{b}$ | 4.50$^{b}$ | 4.3 | **0.02** | **4.4** |
| **Colour with milk** | 4.667$^{bc}$ | 4.5$^{abc}$ | 4.75$^{c}$ | 4.083$^{a}$ | 4.20$^{ab}$ | 4.08$^{a}$ | 4.25$^{abc}$ | 4.4 | **0.042** | **7.5** |
| **Strength of Liquor** | 4.083$^{ab}$ | 4.00$^{ab}$ | 4.00$^{ab}$ | 4.167$^{b}$ | 4.00$^{ab}$ | 3.83$^{a}$ | 4.08$^{ab}$ | 4.0 | **0.299** | **4.5** |
| **Total Score** | 23.08$^{b}$ | 21.42$^{ab}$ | 20.62$^{ab}$ | 20.25$^{ab}$ | 19.9$^{ab}$ | 19.0$^{a}$ | 20.8$^{ab}$ | 20.7 | **0.002** | **5.1** |
| **Valuation (U$c/Kg)** | 230.8$^{c}$ | 214.2$^{b}$ | 206.21$^{ab}$ | 202.5$^{ab}$ | 199.2$^{ab}$ | 190.8$^{a}$ | 208.3$^{b}$ | 207.4 | **0.002** | **5.1** |

**Note: Means accompanied by the same letters within each row are not statistically different at the 95% level of confidence.**

**Key for table 4.5**
T1 means no spray (control)
T2 means 0.1% $H_3BO_3$,
T3 means 1% $CuSO_4.5H_2O$
T4 means 0.1% ZnO
T5 means 0.1% $H_3BO_3$ +1% $CuSO_4.5H_2O$+ 0.1% ZnO
T6 means 1% Commercial mix
T7 means 0.4% $ZnSO_4.7H_2O$

Treatments showed significant differences in the total taster's scores at Glenorchy estate. Black tea from the control treatment (T1) was given the highest total score and valued higher (23.08 and U$c 230.8/kg) compared to the rest of the treatments at Glenorchy, while the commercial micronutrient mix gave the lowest total score and valuation (19.0 and U$c 190.8/kg respectively). This may be due to the complexity of the mix, which apart from the micronutrient elements also contained macronutrients such as N, P, K and Mg (1.7% N, 2.3% P, 1.6% K and 0.25% Mg). Reports have indicated that N is negatively correlated with tea quality (Wright, 2005, Owuor *et.al.*, 2000).

Individual parameters that contributed to the difference in the total score included colour with milk, colour of infusion, colour of liquor, briskness and brightness, while strength of liquor remained unaffected. The control treatment scored the highest in terms of brightness and briskness, whilst all the micronutrient treatments did not differ in brightness. The commercial micronutrient mix treatment had the lowest score for briskness. The Zn treatments also differed significantly from the control in terms of briskness.

The application of zinc oxide alone, zinc sulphate alone, copper sulphate alone and the combination of boric acid, zinc oxide and copper sulphate reduced the score of colour of liquor relative to the control treatment (T1), while boric acid maintained the colour of liquor. With respect to colour of infusion, the highest score was obtained from tea plants receiving applications of zinc oxide alone or zinc sulphate alone, but was not significantly different to the control and boric acid, and commercial micronutrient mix treatments. The lowest score was obtained from application of copper sulphate alone. The scores on how well the processed leaf takes up milk (colour with milk) were significantly lower than the control in plots which received zinc oxide alone and the commercial micronutrient mix.

**Table 4. 6 Tea taster's scores as affected by different micronutrient foliar treatments at Mianga estate**

| Parameter | T1 | T2 | T3 | T4 | T5 | T6 | T7 | Mean | $P_{0.05}$ | CV% |
|---|---|---|---|---|---|---|---|---|---|---|
| **Brightness** | 3.000 [b] | 2.688 [b] | 2.188 [ab] | 2.14 [ab] | 1.93 [a] | 2.75 [b] | 2.60 [b] | 2.5 | **0.016** | **16.5** |
| **Briskness** | 3.15 [a] | 3.00 [a] | 2.67 [a] | 2.46 [a] | 2.15 [a] | 2.85 [a] | 2.75 [a] | 2.7 | **0.394** | **23.5** |
| **Colour of liquor** | 4.583 [ab] | 4.750 [ab] | 4.833 [b] | 4.58 [ab] | 4.31 [a] | 4.45 [ab] | 4.37 [ab] | 4.6 | **0.169** | **6.3** |
| **Colour of infusion** | 4.729 [a] | 4.812 [a] | 4.438 [a] | 4.72 [a] | 4.54 [a] | 4.60 [a] | 4.66 [a] | 4.6 | **0.625** | **6.4** |
| **Colour with milk** | 5.000 [b] | 4.875 [ab] | 4.667 [ab] | 4.58 [ab] | 4.31 [a] | 4.64 [ab] | 4.56 [ab] | 4.7 | **0.291** | **8.3** |
| **Strength of Liquor** | 4.208 [a] | 4.375 [a] | 4.125 [a] | 4.20 [a] | 4.22 [a] | 4.45 [a] | 4.25 [a] | 4.3 | **0.638** | **6.3** |
| **Total Score** | 24.67 [b] | 24.50 [b] | 22.92 [ab] | 22.7 [ab] | 21.4 [a] | 23.7 [ab] | 23.2 [ab] | 23.3 | **0.109** | **6.6** |
| **Valuation (U$c/Kg)** | 246.7 [b] | 245.0 [b] | 229.2 [ab] | 227.1 [ab] | 214.8 [a] | 236.0 [ab] | 232.1 [ab] | 233.3 | **0.119** | **6.7** |

**Note: Means accompanied by the same letters within each row are not statisticallydifferent at the 95% level of confidence.**

**Key for table 4.6**

T1 means no spray (control),
T2 means 0.1% $H_3BO_3$,
T3 means 1% $CuSO_4.5H_2O$
T4 means 0.1% ZnO
T5 means 0.1% $H_3BO_3$ +1% $CuSO_4.5H_2O$+ 0.1% ZnO
T6 means 1% Commercial mix
T7 means 0.4% $ZnSO_4.7H_2O$

At Mianga estate, the treatment combination of boric acid, copper sulphate and zinc oxide resulted in a significant lower total score and valuation of made tea quality than the control treatment.This lower total score can be attributed to significantly lower brightness and colour with milk scores as compared to the control treatment. No other significant differences in total scores were noted for the various treatments at Mianga estate when compared with the control.

Generally, foliar applications of Cu, Zn and B significantly reduced the tea taster's scores and valuation at Glenorchy tea estate but did not significantly affect these parameters at Mianga estate. This implies that there was no quality benefit in applying sprays of Cu, Zn and B to mature clonal tea in this study. In many instances both a reduction in yield and quality were observed which suggests that in the short term micronutrient applications were detrimental for tea production.

Only brightness varied significantly across treatments. Brightest tea was obtained from the control treatment(T1), boric acid applied alone(T2), zinc sulphate applied alone(T6) and from the plots treated with the commercial micronutrient mix, (T7). A combined application of copper sulphate, boric acid and zinc oxide (T5) produced the least bright tea while copper sulphate (T3) and zinc oxide(T4) applied separately produced medium bright tea.

### 4.2.2 Effect of foliar sprays of Cu, Zn and B on contents of theaflavin, thearubigin and caffeine

Results on biochemical parameters of made black tea as affected by foliar micronutrient treatments at Glenorchy and Mianga estates are presented in tables 4.7 and 4.8 as averages of the values obtained from each harvest between March and June 2010.

**Table 4. 7 Biochemical parameters of made tea as affected by different treatments at Glenorchy estate**

| Parameter | T1 | T2 | T3 | T4 | T5 | T6 | T7 | Mean | P | CV% |
|---|---|---|---|---|---|---|---|---|---|---|
| **Caffeine (% w/w)** | 3.58[a] | 3.46[a] | 3.75[a] | 3.64[a] | 3.85[a] | 3.70[a] | 3.58a | **3.65** | **0.755** | **9.4** |
| **Theaflavins (% w/w)** | 0.63 [a] | 0.59 [a] | 0.55 [a] | 0.59 [a] | 0.54[a] | 0.59[a] | 0.63a | **0.59** | **0.162** | **8.6** |
| **Thearubigins (% w/w)** | 7.76 [a] | 8.23 [abc] | 8.15 [ab] | 8.10[ab] | 8.30 [abc] | 8.36[bc] | 8.73c | **8.23** | **0.027** | **4.0** |
| **TR/TF ratio** | 12.45 [a] | 14.06 [ab] | 15.05 [b] | 14.50[b] | 15.38 [b] | 14.31[b] | 14.38[b] | **14.30** | **0.052** | **8.1** |

**Table 4. 8 Biochemical parameters of made tea as affected by different treatments at Mianga estate**

| Parameter | T1 | T2 | T3 | T4 | T5 | T6 | T7 | Mean | P | CV% |
|---|---|---|---|---|---|---|---|---|---|---|
| **Caffeine (% w/w)** | 3.43[a] | 3.44[a] | 3.22[a] | 3.26[a] | 3.45[a] | 3.25[a] | 3.24[a] | **3.33** | **0.602** | **7.1** |
| **Theaflavins (%w/w)** | 0.62[a] | 0.62[a] | 0.59[a] | 0.57[a] | 0.57[a] | 0.61[a] | 0.60[a] | **0.60** | **0.369** | **6.4** |
| **Thearubigins (%w/w)** | 7.95[a] | 8.51[bc] | 8.23[ab] | 8.69[bc] | 8.43[bc] | 8.86[c] | 8.48[bc] | **8.45** | **0.010** | **3.5** |
| **TR/TF ratio** | 12.81[a] | 13.65[ab] | 14.05[bc] | 15.12[c] | 14.85[c] | 14.55[bc] | 14.28[bc] | **14.19** | **0.006** | **5.2** |

**Note: Means accompanied by the same letters within each row are not statistically different at 95% level of confidence.**

**Key for tables 4.7 and 4.8**
T1 means no spray (control)
T2 means 0.1% $H_3BO_3$,
T3 means 1% $CuSO_4.5H_2O$
T4 means 0.1% ZnO
T5 means 0.1% $H_3BO_3$ +1% $CuSO_4.5H_2O$+ 0.1% ZnO
T6 means 1% Commercial mix
T7 means 0.4% $ZnSO_4.7H_2O$

Among the biochemical parameters i.e. caffeine, theaflavins (TFs) and thearubigins (TR), only levels of TRs varied significantly with treatments at both sites. At Mianga, the control treatment resulted in teas with the lowest level of TRs (7.95%), which was significantly different to the plots receiving a combined application of boric acid, copper sulphate and zinc oxide (T5); the commercial micronutrient mix (T6); zinc sulphate applied alone (T7); boric acid applied alone (T2) and zinc oxide applied alone (T4). Tea produced from plots which received an application of copper sulphate alone (T3) did not differ from the control (T1) with respect to TRs. Similarly, at Glenorchy, the control treatment (T1) produced tea with the lowest TR level (7.76%), while zinc sulphate (T7) gave the highest TR levels(8.73%). Zinc sulphate applied alone (T7) and the commercial micronutrient mix (T6) had significantly higher TR levels than the control (T1).

Although the biochemical properties of the tea obtained from the trials varied slightly between sites, with regards TR content, it was obvious that the lowest level of TRs was obtained in the control treatment at both sites. The amount of TFs in made tea is responsible for the brightness (the quality aspect responsible for a clear appearance of liquor and coppery colour as opposed to dull colour) and briskness (the degree of pungency in liquor), while TRs are responsible for colour of the infusion (Owuor & Reeves 1986). The level of TRs is inversely related to the brightness of the liquor (Owuor *et al.,* 2006) and results obtained from this study confirm these findings, whereby a higher score on brightness was obtained in the control treatments at both sites, where TR levels were the lowest. The ratio of TRs: TFs was subsequently lower in the control treatments than where micronutrient foliar sprays were applied. This suggests that application of the micronutrient sprays, especially zinc oxide and copper sulphate aided in the conversionof more TRs than TFs from catechins and flavanols present in the processed green shoots during the fermentation process. According to Owuor & Reeves (1986), at the beginning of fermentation, the rate at which TRs and TFs are formed is similar but at later stages of fermentation, the rate of TR formation surpasses that of TFs and possibly some TFs are converted to TRs. This further indicates that micronutrients applied in this study aided in prolonging the process of TR formation which was reflected in tasters score on brightness.

Application of Cu to tea plants helps in improving the fermentation of the plucked leaf during manufacture, more especially for slow fermenting cultivars (Dootson 1976, Clowes & Mitini-Nkhoma 1987). Work done by Dootson (1976) indicated that Zn and Cu application to seedling tea increased the levels of total catechins, total flavins, as well as polyphenol oxidase levels in tea leaves. Polyphenol oxidase is the enzyme which catalyses the transformation of polyphenols (catechins and flavanols) to TFs and TRs during the fermentation process of black tea (Owuor & Reeves, 1986). The availability and activity of polyphenol oxidase, of which Zn and Cu form vital components, is therefore an important factor in the formation of TRs and TFs (Ogunmoyela, *et al*., 1994). In the current study, levels of TRs were significantly increased when micronutrient foliar sprays of Cu, Zn as well as B were applied, with greater increases observed when Zn and Cu were applied as zinc oxide and copper sulphate respectively. Both cultivars investigated in the current study (PC 108 and PC 105) are inherently fast fermenting cultivars (TRFCA,2000) and addition of Zn and Cu could have sped up the fermentation process, leading to further TFs being converted to TRs. This could explain why less TRs were observed in the control treatments than in treatments where micronutrients were applied.

Leaf Cu was found to correlate positively with total phenol and tannin content of tea samples of Nigerian tea, while the relationship between foliar Zn and total phenol content was non-significant (Ogunmoyela *et al.,* 1994). Dev Chaudhury *et al.* (1989) reported no significant effects of Zn on TF content in tea and the current study's results are in agreement with these findings. A positive correlation was found between Zn and catechins, and Zn and caffeine contents in tea leaves (Ramrakrishna *et al.,* 1987), however, results from the present study show that caffeine and TF contents in black tea were not affected by the application of Cu, Zn and B containing sprays used. It is possible that some of the TFs were being converted to TRs which could explain why differences in the level of TRs were observed, with no difference in TF levels (Owuor & Reeves (1986). Rahman & Sharma (1974) also reported no significant effect of Zn foliar sprays on strength, briskness and valuation of orthodox teas.

Altitude is another important factor that determines the quality of made tea. According to Owuor *et.al.* (1990), as altitude increases, the quality of made tea improves. This is attributed to the slow growth rate of teas grown in high altitude areas which is mainly associated with low temperatures leading to increases the accumulation of catechins and flavanols in the shoots. This agrees well with what was observed in this study whereby the two sites which have different altitudes performed differently in their responses to application of micronutrients. Additionally, long term temperature data indicates that temperatures are lower in Thyolo district where Mianga is located than in Mulanje district where Glenorchy is located. In this study, Mianga which is at an altitude of 960 metres above sea levelwith lower temperature generally produced black tea of higher quality in terms of concentration of TFs and TRs, total score and valuation than Glenorchy, which is at 650 metres above sea level and with higher temperatures. It is probable that no treatment differences were observed at Mianga because the altitude had a greater impact on the black tea quality than the micronutrient elements that were applied.

### 4.2.3 Conclusion

Although a number of researchers have indicated a positive impact due to application of Cu and Zn on the quality of tea (Squire, 1977, Malenga, 1986, Grice, 1990, Seenivasan *et al.,* 2008), results obtained in this study have shown a reduction in total taster's scores and valuation as a result of Zn and Cu application at Glenorchy estate, while having no significant effect on the taster's scores and valuation at Mianga estate.

Zinc and Cu form vital components of the enzyme, polyphenol oxidase, which catalyses the fermentation process during black tea manufacture which leads to formation of TRs and TFs, important chemical parameters that determine the quality of black tea (Ogunmoyela, *et al*., 1994). Application of Cu, Zn and B in the current study significantly increased levels of TRs with greater increases observed when Zn and Cu were applied as zinc oxide and copper sulphate respectively. The study has also shown that micronutrient sprays containing B, Cu and Zn possibly aided in the conversion of catechins and flavanols present in green leaf to more TRs than TFs leading to less bright tea and a lower valuation. Levels of caffeine and TFs remained unaffected by the micronutrient foliar sprays.

The two sites responded differently in terms of taster's scores and valuation to the application of Cu, Zn and B micronutrient sprays. This may be due to site differences especially due to the fact that tea plants at Glenorchy were irrigated and those at Mianga were growing under rainfed conditions. In addition, the two sites differ in altitude and the higher taster's scores obtained from Mianga may to some extent be attributed to the effect of higher altitude and lower temperatures as compared to low taster's scores that were obtained at Glenorchy whose altitude is lower and temperatures possibly higher. It may be for this reason that no significant effect of application of Cu, Zn and B on black tea quality was observed at Mianga because the altitude effect was so great that it obscured the effect that the micronutrient elements would have. In addition, it may take an additional season of micronutrient applications before significant differences will be observed in the metabolites.

## 4.3 Effect of foliar sprays of Cu, Zn and B containing fertilisers on tea foliar nutrient levels

The major avenue of nutrient loss from tea fields is plucking, which is a continuous process. Nitrogen, P and K are the major elements that are significantly removed from the tea plantations through continuous cropping and B, Cu and Zn are among the micronutrient elements that are removed in smaller quantities (Kamau, 2008). In Malawi, removal of micronutrient elements through plucking is not followed by an organised replacement plan, hence the likelihood of occurrence of micronutrient deficiencies in Malawian tea fields is great.

Application of nutrients through foliar sprays is one way of supplying plants with elements to ensure good plant health, especially in the case of micronutrient elements such as B, Zn and Cu that are required in very small quantities. Foliar applicationsare more applicable and efficient than soil applications, especially when soil factors are prohibitive to efficient uptake of soil applied nutrient elements. It is suggested to be the fastest way of raising nutrient element levels in deficient plants (Majid &Ballard, 1990).

Copper, Zn and B interact with some of the macronutrients, as well as micronutrient elements. Nutrient interaction has been reported between Cu and N. Zekri & Obreza (2009) observed Cu deficiencies in citrus plants where heavy applications of ammonium fertilisers have been made. The concentration of Fe increased in leaves treated with 50 µM Cu by 1.4 fold, while Zn concentration was lowered by 1.2 fold in soybeans at the same Cu concentration (Bernal *et al.,* 2007), indicating that its levels of application can induce toxicities or deficiencies of other elements within the plant systems. Labanauskas *et al.* (1958) observed low levels of Zn, B and Cu in leaves of avocados when N was applied at high rates as ammonium nitrate. Zn interacts negatively with P, but positively with Fe, such that increasing Zn supply increases the Fe status of the plants (Alloway, 2008).

In the present study, elemental analysis of leaf samples was performed to ascertain if the application of different Cu, Zn and B containing foliar sprays to deficient tea bushes could raise the levels of Cu, B and Zn in tea leaves to recommended levels and also to establish the effect of applying the foliar sprays on leaf concentrations of Al, S, N, P, K, Ca, Mg and Fe to elucidate any possibility of Cu, Zn and B interactions with any of these elements. In order to assess the nutrient status of soils in the experimental plots, laboratory analysis of soil samples collected from the plots was also performed. Results of the initial soil nutrient analyses are presented in Table 4.9.

**Table 4. 9 Nutrient status and other soil characteristics for the two sites at the beginning of the trials before foliar spraying (Mean values of soil samples collected at 0-15cm and 30-45cm depths)**

| Soil characteristic | Mianga | Glenorchy |
|---|---|---|
| pH | 4.9 ± 0.1 | 4.9 ± 0.1 |
| Carbon (%) | 2.0 ± 0.1 | 2.6 ± 0.1 |
| Ash (%) | 87.8 ± 0.5 | 83.2 ± 1.2 |
| N (%) | 0.1 ± 0 | 0.1 ± 0 |
| CEC (cmol(+)$kg^{-1}$) | 17.3 ± 4.3 | 23.2 ± 10.7 |
| P (mg $kg^{-1}$) | 51.1 ± 0.6 | 84.6 ± 7.8 |
| K (mg $kg^{-1}$) | 243 ± 7.1 | 187.0 ± 1.4 |
| Ca (mg $kg^{-1}$) | 502.0 ± 12.7 | 373.0 ± 5.7 |
| Mg (mg $kg^{-1}$) | 123.0 ± 2.8 | 76.5 ± 0.7 |
| Na (mg $kg^{-1}$) | 13.5 ± 0.7 | 61.5 ± 3.5 |
| Al (mg $kg^{-1}$) | 432.3 ± 19.7 | 463.8 ± 13.4 |
| B (mg $kg^{-1}$) | 0.2 ± 0 | 0.8 ± 0.1 |
| Zn (mg $kg^{-1}$) | 2.3 ± 0.2 | 3.7 ± 0.2 |
| Cu (mg $kg^{-1}$) | 17.1 ± 0.4 | 39.4 ± 0.2 |
| Fe (mg $kg^{-1}$) | 93.0 + 5.2 | 64.1 ± 3.3 |
| Mn (mg $kg^{-1}$) | 217 ± 9.4 | 112.1 ± 9.4 |

At the beginning of the trials, the soils at both sites had a pH of 4.9, which is within the recommended pH range for tea production (Willson& Gunther, 1981,De Silva 2007). The concentration of B at both sites was at deficient levels, below the threshold value of 1.0 mg $kg^{-1}$(Willson& Gunther, 1981). Deficiencies of B revealed by the leaf analysis conducted by Estates as reported in Chapter 2.3 might originate from the fact that the soil was already B deficient and could not supply enough B to the tea plants. There was sufficient Zn in the soil at both sites, within the range (2-15 mg $kg^{-1}$)(Willson& Gunther, 1981), while Cu levels were above the sufficient range of 3-10 mg $kg^{-1}$(Willson& Gunther, 1981). Cu and Zn deficiencies observed in the leaves, as reported in Chapter 2, might be due to soil factors, such as organic matter content in the soil, that reduced the plants' ability to absorb Cu and Zn from the soil. The tendency of Cu and Zn to bind to organic matter fractions has been widely reported and this may explain the reported deficiencies in the tea leaves (Fernandes & Henriques, 1991, Saha *et al.,* 1999, Alva *et* *al*., 2000, Zhang *et.al.,* 2006). Tea soils are known to contain high levels of organic matter, but unfortunately the organic matter content of the soils from the experimental plots could not be analysed.

### 4.3.1 Levels of Cu, Zn and B in leaves of tea bushes treated with different foliar sprays

Changes in leaf micronutrient concentrations were noticeable and significant differences were found after three applications at both sites. Results of concentration of Cu, Zn and B in tea leaves treated with foliar sprays at Mianga and Glenorchy tea estates are presented in Table 4.10.

**Table 4. 10 Foliar levels of Cu, Zn and B as affected by the foliar sprays at Mianga and Glenorchy estates**

| Treatment | | B (mg/kg) | | Cu (mg/kg) | | Zn (mg/kg) | |
|---|---|---|---|---|---|---|---|
| | | **Mianga** | **Glenorchy** | **Mianga** | **Glenorchy** | **Mianga** | **Glenorchy** |
| T 1 | No treatment | $7.6^{a}$ | $9.34^{b}$ | $10.4^{b}$ | $9.5^{b}$ | $23.3^{c}$ | $21.9^{d}$ |
| T 2 | 0.1% $H_3BO_3$ | $23.3^{a}$ | $20.9^{a}$ | $10.7^{b}$ | $10.2^{b}$ | $20.7^{c}$ | $18.0^{d}$ |
| T 3 | 1% $CuSO_4.5H_2O$ | $14.4^{a}$ | $16.3^{ab}$ | $215.8^{a}$ | $217.8^{a}$ | $22.3^{c}$ | $18.7^{d}$ |
| T 4 | 0.1% ZnO | $8.7^{a}$ | $11.5^{b}$ | $36.6^{b}$ | $12.9^{b}$ | $54.7^{b}$ | $64.3^{b}$ |
| T 5 | 0.1% $H_3BO_3$ +1% $CuSO_4.5H_2O$+ 0.1% ZnO | $16.0^{a}$ | $16.7^{ab}$ | $196.7^{a}$ | $207.2^{a}$ | $77.3^{a}$ | $39.9^{c}$ |
| T 6 | 1% Commercial mix | $19.3^{a}$ | $15.9^{ab}$ | $16.5^{b}$ | $11.8^{b}$ | $27.7^{c}$ | $21.0^{d}$ |
| T 7 | 0.4% $ZnSO_4.7H_2O$ | $14.2^{a}$ | $11.9^{b}$ | $13.4^{b}$ | $10.9^{b}$ | $85.8^{a}$ | $100.4^{a}$ |
| **P value** | | **0.2789** | **<0.0001** | **0.009** | **<0.0001** | **<0.0001** | **<0.0001** |
| **CV (%)** | | **65.5** | **36.6** | **24.9** | **68.4** | **32.7** | **18.1** |

**NOTE: Means with the same letters within each column are not statistically different at 95% level of confidence**

Concentration of B in leaves was not significantly different among the treatments at Mianga estate, although levels tended to be higher in plants treated with B, either alone or mixed with other micronutrients. Concentration of foliar B ranged from 7.6 to 23.3 mg $kg^{-1}$ at Mianga. At Glenorchy, leaf B concentration was significantly affected by the treatments (P= <0.0001). Plots treated with boric acid alone gave the highest B concentration (20.9 mg $kg^{-1}$) compared to the control. At both experimental sites, B levels in all treatments, including those containing B, were lower than the threshold value of 30 mg $kg^{-1}$. It is therefore likely that the foliar concentration of B attained by the tea bushes treated with boric acid and the commercial micronutrient mix was too low to cause a significant yield increase. Further foliar applications of B or higher B application rates than 776 g $ha^{-1}yr^{-1}$ would thus be required to cause a significant yield increase.

Concentration of Cu varied significantly across treatments at both sites. Application of copper sulphate at 17.7 kg Cu $ha^{-1}$ $yr^{-1}$ alone (T3) and a combined application of copper sulphate, boric acid and zinc oxide which supplied 17.7 kg Cu $ha^{-1}$ $yr^{-1}$(T5) raised the foliar concentration of Cu to a greater extent than the other treatments at both sites. At Mianga estate, concentration of Cu in leaves from T3 (1% copper sulphate, 17.7 kg Cu $ha^{-1}$ $yr^{-1}$)and T5 [(0.1% boric acid, 1% copper sulphate solution, 1.25kg$ha^{-1}$ zinc oxide[(17.7kg Cu$ha^{-1}$ $yr^{-1}$) plots was 215.8mg $kg^{-1}$ and 196.7 mg $kg^{-1}$respectively and at Glenorchy, Cu concentration in leaves from T3 [(1% copper sulphate (17.7 kg Cu $ha^{-1}$ $yr^{-1}$) and T5 [(0.1% boric acid, 1% copper sulphate solution, 1.25kg$ha^{-1}$ zinc oxide (17.7kg Cu$ha^{-1}$ $yr^{-1}$) plots was 217.8mg $kg^{-1}$ and 207.2mg $kg^{-1}$ respectively. These Cu levels in T3 and T5 plots seem to be too high and there was probably too much Cu in these treatments which caused toxicity problems which led to reduced yields in T3 and T5 plots. The recommended levels for Cu in tea is >15 mg $kg^{-1}$ but leaves from T3 and T5 registered far higher levels of Cu compared to the rest of the treatments. According to Fageria (2001), excess Cu has destructive effects on the integrity of the chloroplast membrane thereby decreasing the plant's photosynthetic activity.

Concentration of Cu in leaves from plots that were treated with the commercial micronutrient mix, which supplied 32.5 g Cu $ha^{-1}$ $yr^{-1}$ did not differ significantly from the plots which were not treated with any Cu containing spray at both sites. Cu concentration of leaves sampled from plots treated with the commercial micronutrient mix at Mianga was 16.5 mg $kg^{-1}$i.e. slightly above the recommended minimum level of 15 mg $kg^{-1}$ for tea and 11.8 mg $kg^{-1}$ at Glenorchy which was slightly below the recommended minimum Cu concentration. Leaf samples collected from treatments at both sites which did not receive any Cu generally had Cu levels at or just below the recommended level. It was generally observed that the concentration of Cu applied in T3 (Copper sulphate) and T5 (Copper sulphate + Zinc oxide +boric acid) was far too high compared to that applied in T6 (commercial micronutrient mix) which was closer to the optimal concentration for tea.

Foliar sprays had a significant effect on concentration of Zn in leaves that received the different foliar sprays. At Mianga estate, concentration of Zn was highest (85.8 mg Zn kg$^{-1}$) in plots supplied with zinc sulphate (T 7), followed by those treated with a combination of zinc oxide, copper sulphate and boric acid (T 5) with a concentration of 77.3 mg Zn kg$^{-1}$ and those treated with zinc oxide only (T 4) which gave a foliar Zn concentration of 54.7 mg kg$^{-1}$. However, T6 (commercial micronutrient mix) was not significantly different from the treatments not receiving any Zn and had a Zn concentration below 30 mg kg$^{-1}$, the recommended foliar Zn concentration for tea. At Glenorchy estate, zinc sulphate treatment (T7) gave the highest foliar Zn concentration (100.4 mg kg$^{-1}$), followed by zinc oxide applied alone (T 4) which gave a foliar concentration of 64.3 mg kg$^{-1}$ and then a combination of zinc oxide, copper sulphate and boric acid which gave a foliar Zn concentration of 39.9 mg kg$^{-1}$. The control treatment, T2 (boric acid), T3 (copper sulphate) and T6 (commercial micronutrient mix) had Zn concentrations below the recommended Zn level. The commercial micronutrient spray supplied 97 g of Zn ha$^{-1}$ yr$^{-1}$ and its application did not raise the foliar Zn content to the recommended level at both sites. Amongst the Zn containing sprays used in the study, zinc sulphate and zinc oxide were effective at raising the foliar Zn concentration. As with Cu it is likely that the concentration at which the commercial micronutrient mix was applied was too low to supply the right amount of Zn, although at Mianga foliar Zn concentration was close to the recommended norm.

### 4.3.2 Foliar concentration of other nutrient elements

Analytical results of leaf samples for nutrient elements, other than B, Zn and Cu, which included N, P, K, S, Ca, Mg, Al, Fe and Mn, revealed that concentration of Al and S varied significantly among treatments, while the rest of the elements did not vary significantly at Glenorchy estate, (Table 4.11), while at Mianga estate, none of these were significantly affected by the treatments (Table 4.12).

**Table 4. 11 Concentrations of Al , S, N, P, K, Ca, Mg and Fe in tea leaves treated with various foliar sprays at Glenorchy**

| Treatment | | Al (%) w/w | S (%) w/w | N (%) w/w | P (%) w/w | K (%) w/w | Ca (%) w/w | Mg (%) w/w | Mn (mg kg$^{-1}$) | Fe ( mg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| T 1 | No treatment | $0.12^a$ | $0.25^a$ | $2.12^a$ | $0.37^a$ | $1.17^a$ | $0.45^a$ | $0.22^a$ | $696^a$ | $100^a$ |
| T 2 | 0.1% $H_3BO_3$ | $0.12^a$ | $0.26^a$ | $2.20^a$ | $0.38^a$ | $1.18^a$ | $0.47^a$ | $0.23^a$ | $690^a$ | $76.7^a$ |
| T 3 | 1% $CuSO_4.5H_2O$ | $0.19^b$ | $0.30^b$ | $2.25^a$ | $0.37^a$ | $1.24^a$ | $0.57^a$ | $0.25^a$ | $832^a$ | $89.5^a$ |
| T 4 | 0.1% ZnO | $0.12^a$ | $0.26^a$ | $2.31^a$ | $0.39^a$ | $1.15^a$ | $0.46^a$ | $0.22^a$ | $764^a$ | $110.1^a$ |
| T 5 | 0.1% $H_3BO_3$ +1% $CuSO_4.5H_2O$+ 0.1% ZnO | $0.14^a$ | $0.27^a$ | $2.23^a$ | $0.38^a$ | $1.28^a$ | $0.46^a$ | $0.22^a$ | $810^a$ | $86.5^a$ |
| T 6 | 1% Commercial mix | $0.12^a$ | $0.26^a$ | $2.16^a$ | $0.37^a$ | $1.16^a$ | $0.42^a$ | $0.22^a$ | $714^a$ | $72.5^a$ |
| T 7 | 0.4% $ZnSO_4.7H_2O$ | $0.12^a$ | $0.27^a$ | $2.18^a$ | $0.39^a$ | $1.19^a$ | $0.46^a$ | $0.22^a$ | $726^a$ | $70.3^a$ |
| **P value** | | **0.012** | **0.009** | **0.974** | **0.965** | **0.726** | **0.1** | **0.131** | **0.572** | **0.404** |
| **CV (%)** | | **20.4** | **5.9** | **12.9** | **10.6** | **10.0** | **13.2** | **7.8** | **16.6** | **32.6** |

**NOTE: Means with the same letters within each column are not statistically different at 95% level of confidence**

Concentration of Al at Glenorchy Estate was highest (0.19%) in leaves from plots treated with copper sulphate alone (T3), compared with the other treatments. Tea is one of the plant species that has a great tolerance to high levels of Al (Konishi, 1992, Ruan & Wong, 2001). Aluminium levels ranging from 1790 to 4381 mg kg$^{-1}$ and from 873 to 3637 mg kg$^{-1}$ have been observed in one-year old and two-months old leaves in some parts of China (Dong, *et al*., 2001). Although tea has a great tolerance to high levels of Al, it has been reported by Fung *et al.*, (2008) that Al concentrations in the rooting medium higher than 1 mmol L$^{-1}$ have been reported to retard growth of tea seedlings and that the concentration of Al in tissues of tea seedlings increased in direct proportion to the Al added to the nutrient solution. Excessive levels of Al in leaves observed in T3 plots could be the reason for the observed reduction in yield at Glenorchy estate, as the recommended concentration of Al in tea leaves is 400ppm (0.04%) (Wilson & Gunther, 1983). Although all treatments showed Al levels higher thanthe recommended value in this study, it is likely thatAl level in the other treatments were within the tolerable levels by the tea plants compared to T3 (Copper sulphate alone),where Al levels were possibly high enough to cause a decline in yield. The reason for the elevated Al level in leaves treated with copper sulphate is unclear.

At Glenorchy, the level of S was highest (0.3%) in plots treated with copper sulphate only (T3) compared to the other six treatments which did not significantly differ in foliar S concentration. Sulphur containing treatments in the study included T3 (Copper sulphate), T5 (Zinc oxide + Copper sulphate + Boric acid) and T7 (Zinc Sulphate). Application of copper sulphate supplied 17.7 kg Cu $ha^{-1}$ $yr^{-1}$ and at the same time supplied 8.9 kg S $ha^{-1}$ $yr^{-1}$. Zinc sulphate application supplied 4 kg Zn $ha^{-1}$ $yr^{-1}$ and at the same time supplied about 2 kg S $ha^{-1}$ $yr^{-1}$; this could be the reason why foliar S was higher in leaves treated with copper sulphate alone than in those treated with zinc sulphate i.e copper sulphate supplied more S than did zinc sulphate. However, the levels of S in all treatments at both sites were within the normal range of (0.2-0.3%) for tea.

**Table 4. 12 Concentrations of Al, S, N, P, K, Ca, Mg and Fe in tea leaves treated with various foliar sprays at Mianga**

| Treatment | | Al (%) w/w | S (%) w/w | N (%) w/w | P (%) w/w | K (%) w/w | Ca(%) w/w | Mg(%) w/w | Mn (mg $kg^{-1}$) | Fe (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| T 1 | No treatment | 0.10[a] | 0.27[a] | 1.98[a] | 0.40[a] | 1.21[a] | 0.43[a] | 0.23[a] | 609[a] | 60.2[a] |
| T 2 | 0.1% $H_3BO_3$ | 0.10[a] | 0.25[a] | 1.95[a] | 0.38[a] | 1.19[a] | 0.48[a] | 0.25[a] | 759[a] | 61.2[a] |
| T 3 | 1% $CuSO_4.5H_2O$ | 0.11[a] | 0.32[a] | 2.07[a] | 0.41[a] | 1.38[a] | 0.54[a] | 0.28[a] | 748[a] | 75.3[a] |
| T 4 | 0.1% ZnO | 0.10[a] | 0.28[a] | 2.10[a] | 0.40[a] | 1.32[a] | 0.50[a] | 0.27[a] | 708[a] | 76.3[a] |
| T 5 | 0.1% $H_3BO_3$ +1% $CuSO_4.5H_2O$+ 0.1% ZnO | 0.12[a] | 0.29[a] | 1.69[a] | 0.40[a] | 1.22[a] | 0.49[a] | 0.26[a] | 713[a] | 81.3[a] |
| T 6 | 1% Commercial mix | 0.09[a] | 0.28[a] | 2.07[a] | 0.42[a] | 1.22[a] | 0.45[a] | 0.25[a] | 636[a] | 77.8[a] |
| T 7 | 0.4% $ZnSO_4.7H_2O$ | 0.09[a] | 0.28[a] | 2.25[a] | 0.43[a] | 1.20[a] | 0.44[a] | 0.24[a] | 739[a] | 79.4[a] |
| **P value** | | **0.287** | **0.077** | **0.719** | **0.755** | **0.452** | **0.478** | **0.607** | **0.367** | **0.759** |
| **CV (%)** | | **18.4** | **10.3** | **21.9** | **10.9** | **11.1** | **16.9** | **14.3** | **15.2** | **31.8** |

**NOTE: Means with the same letters within each column are not statistically different at 95% level of confidence**

### 4.3.3 Soil nutrient status in the experimental plots

In order to assess the levels of nutrient elements in the soil from the plot area following foliar applications of micronutrients, soil samples were collected and analysed for pH, carbon, ash, N, CEC, P, K, Ca, Mg, Al, B, Zn, Cu, Fe, Mn and Mo. Results indicated that all plots at both Glenorchy and Mianga estates did not differ significantly in the levels of all of the elements that were analysed (Table 4.13). Other soil factors such as ash content, pH, carbon content, clay contents and CEC did not show any significant differences among the treatments. These results show that application of foliar sprays of Cu, Zn and B did not change the properties of soil.

There were differences between the sites in some soil factors before and after treatments were applied and there were also differences in the manner in which the soil factors changed between the two sites. These changes may be responsible for the differences in the yield performance of the plants at the two sites. Considering N, P and K, which are the major nutrient elements important in tea productivity, N level in soils from both sites were similar, at a concentration of 0.1% (1000 mg $kg^{-1}$) before treatments were applied at the beginning of the trial and remained constant even after applying the treatments. Tea soils have to contain at least 50 mg $kg^{-1}$ of N in order to achieve optimum yields (Willson & Gunther, 1981) and both fields in this study had sufficient levels of N throughout the trial period. However, differences were noted in levels of P, whereby soil P levels at Mianga were far much lower than at Glenorchy. Initially, soil P level at Mianga and Glenorchy were 51.1 mg $kg^{-1}$ and 84.6mg $kg^{-1}$ respectively which were normal for tea production but after application of the treatments, the soil P level at Mianga was depleted to levels below the norm (50mg $kg^{-1}$) while at Glenorchy, the soil P level remained almost constant at 83.43 mg $kg^{-1}$. The K levels at Mianga were higher than at Glenorchy before and after applying the treatments. At both sites, soil K levels were above the recommended for tea production which is 1 meq/kg (39.1 mg K $kg^{-1}$). Differences in yields between the two sites could therefore possibly be attributed to an imbalance in the N, P and K levels in the soil. It is possible that there was an imbalance among N, P and K levels in the soils at Mianga that was leading to low yields at this site and it could be the low P level which contributed to the N, P and K imbalance.

**Table 4. 13 Soil pH and contents of N, P, K, Ca, Mg, Al, B, Zn, Cu, Fe, Mn, Mo, ash, carbon and clay in soils from the experimental plots samples collected in May 2010**

| | Glenorchy | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 | T7 | Mean | $P_{0.05}$ | CV% |
| Al (mg/kg) | 528.9 | 536.5 | 521.2 | 571.3 | 575.1 | 540.5 | 556.9 | 547.20 | 0.69 | 13.4 |
| Ash % | 80.07 | 80.71 | 80.68 | 80.63 | 79.99 | 80.28 | 81.61 | 80.57 | 0.88 | 3.00 |
| B (mg/kg) | 0.61 | 0.75 | 0.68 | 0.62 | 0.60 | 0.57 | 0.56 | 0.63 | 0.19 | 24.60 |
| CEC (cmol/kg) | 25.86 | 21.19 | 25.25 | 24.45 | 22.95 | 24.26 | 23.34 | 23.90 | 0.53 | 19.90 |
| Carbon % | 2.70 | 2.60 | 2.54 | 2.64 | 2.63 | 2.49 | 2.58 | 2.60 | 0.76 | 10.50 |
| Ca (mg/kg) | 471.00 | 377.00 | 392.00 | 333.00 | 309.00 | 353.00 | 418.00 | 379.00 | 0.26 | 35.20 |
| Clay (%) | 31.77 | 34.10 | 37.12 | 33.49 | 36.67 | 35.59 | 36.71 | 35.06 | 0.28 | 36.71 |
| Cu (mg/kg) | 33.60 | 42.90 | 26.60 | 33.30 | 38.50 | 27.70 | 39.00 | 34.51 | 0.38 | 47.00 |
| Fe (mg/kg) | 63.20 | 67.50 | 60.00 | 67.60 | 70.00 | 62.00 | 73.20 | 66.21 | 0.14 | 15.20 |
| K (mg/kg) | 152.00 | 161.80 | 174.40 | 133.50 | 149.00 | 158.90 | 162.40 | 156.00 | 0.85 | 35.10 |
| Mg (mg/kg) | 87.40 | 75.40 | 77.50 | 66.10 | 55.10 | 65.90 | 82.90 | 72.90 | 0.32 | 39.20 |
| Mn (mg/kg) | 74.70 | 63.40 | 68.90 | 62.00 | 88.90 | 63.10 | 78.80 | 71.40 | 0.18 | 31.40 |
| Mo (mg/kg) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.01 | 0.05 | 40.30 |
| N (%) | 0.13 | 0.12 | 0.13 | 0.12 | 0.13 | 0.13 | 0.14 | 0.13 | 0.90 | 26.60 |
| P (mg/kg) | 80.10 | 81.70 | 64.80 | 90.30 | 92.20 | 81.10 | 93.80 | 83.43 | 0.34 | 10.20 |
| Zn (mg/kg) | 3.47 | 4.90 | 3.11 | 4.03 | 4.84 | 3.62 | 3.73 | 3.96 | 0.53 | 52.40 |
| pH | 4.91 | 4.98 | 4.61 | 4.76 | 4.71 | 4.90 | 4.83 | 4.81 | 0.43 | 5.40 |
| | Mianga | | | | | | | | | |
| Al (mg/kg) | 288.90 | 335.40 | 347.60 | 280.40 | 298.40 | 287.50 | 372.10 | 315.76 | 0.12 | 23.60 |
| Ash % | 85.81 | 90.22 | 89.36 | 89.62 | 89.53 | 89.56 | 89.24 | 89.05 | 0.40 | 4.50 |
| B (mg/kg) | 0.44 | 0.42 | 0.41 | 0.36 | 0.41 | 0.39 | 0.38 | 0.40 | 0.87 | 31.60 |
| CEC (cmol/kg) | 19.45 | 19.10 | 19.91 | 19.15 | 16.65 | 18.75 | 19.73 | 18.96 | 0.79 | 22.70 |
| Carbon % | 1.54 | 1.59 | 1.80 | 1.43 | 1.59 | 1.48 | 1.61 | 1.58 | 0.70 | 26.40 |
| Ca (mg/kg) | 682.00 | 566.00 | 606.00 | 480.00 | 542.00 | 549.00 | 512.00 | 562.43 | 0.59 | 37.60 |
| Clay (%) | 30.16 | 28.76 | 30.39 | 33.53 | 29.91 | 32.34 | 33.31 | 31.20 | 0.55 | 18.20 |
| Cu (mg/kg) | 5.49 | 5.56 | 6.02 | 6.22 | 6.10 | 4.97 | 6.17 | 5.79 | 0.11 | 16.60 |
| Fe (mg/kg) | 95.80 | 89.90 | 102.60 | 95.20 | 108.00 | 81.10 | 105.70 | 96.90 | 0.53 | 29.70 |
| K (mg/kg) | 319.10 | 262.90 | 285.40 | 250.50 | 282.40 | 288.80 | 275.00 | 280.59 | 0.53 | 25.40 |
| Mg (mg/kg) | 157.20 | 130.40 | 151.90 | 128.00 | 128.90 | 146.10 | 123.90 | 138.06 | 0.67 | 33.20 |
| Mn (mg/kg) | 290.00 | 278.00 | 298.00 | 254.00 | 287.00 | 245.00 | 283.00 | 276.43 | 0.87 | 31.00 |
| Mo (mg/kg) | 0.02 | 0.02 | 0.03 | 0.03 | 0.03 | 0.02 | 0.02 | 0.02 | 0.67 | 30.60 |
| N (%) | 0.10 | 0.10 | 0.11 | 0.08 | 0.10 | 0.09 | 0.09 | 0.10 | 0.29 | 25.30 |
| P (mg/kg) | 20.00 | 23.80 | 23.30 | 14.80 | 25.40 | 19.90 | 23.10 | 21.47 | 0.80 | 65.90 |
| Zn (mg/kg) | 1.25 | 1.49 | 1.59 | 1.16 | 1.51 | 1.09 | 1.14 | 1.32 | 0.66 | 53.60 |
| pH | 5.18 | 5.13 | 5.05 | 5.00 | 5.01 | 5.09 | 4.94 | 5.06 | 0.89 | 7.30 |

**Key for table 4.13**

T1 means no spray (control), T2 means 0.1% $H_3BO_3$, T3 means 1% $CuSO_4.5H_2O$
T4 means 0.1% ZnO, T5 means 0.1% $H_3BO_3$ +1% $CuSO_4.5H_2O$+ 0.1% ZnO, T6 means 1% Commercial mix and T7 means 0.4% ZnSO4.7H2O

Among the micronutrient elements of interest i.e. Cu, B and Zn, the concentration of B in the soil did not change significantly before and after applying the treatments at both sites. At Mianga, initial B content was 0.2mg $kg^{-1}$ and changed to an average of 0.4 mg $kg^{-1}$ while at Glenorchy, soil B content changed from 0.8 mg $kg^{-1}$ to an average of 0.63 mg $kg^{-1}$. According to Willson & Gunther (1981), the recommended soil B level is at least 1.0 mg $kg^{-1}$ and at Glenochy soil B levels were close to the recommended level while at Mianga, the soil B levels were lower than recommended.

Copper content decreased from 17.1 mg $kg^{-1}$ to an average of 5.79 mg $kg^{-1}$ at Mianga and decreased from 39.4mg $kg^{-1}$ to an average of 34.5 mg $kg^{-1}$ at Glenorchy. Soil Cu content was generally lower at Mianga than at Glenorchy estate, but Cu levels at Mianga were within the recommended levels for tea of 3 to 10 mg $kg^{-1}$ while at Glenorchy, Cu level in the soil was generally above the recommended level. However, Cu levels in the leaves of copper sulphate treated plants was much higher than in those plants not receiving Cu, implying that foliar applied Cu was staying on the leaves.

There was a slight decrease in soil Zn level from 2.3 mg $kg^{-1}$ at Mianga to an average of 1.32mg $kg^{-1}$ and no significant increase in soil Zn level at Glenorchy estate. The Zn level at Mianga was within the recommended range of 2-15mg $kg^{-1}$ for tea only at the beginning of the trials but it reduced to levels below the recommended Zn level after the final application of the foliar sprays, while soil Zn levels at Glenorchy were within the recommended Zn level for tea. Plants at Glenorchy were probably able to utilise available Zn from the soil plus Zn supplied by the foliar applications, especially that of zinc sulphate, which could have contributed to the increased yield at Glenorchy.

Generally, soils at Glenorchy were more well balanced with regards the levels of the most important major nutrient elements N, P and K, compared to soils at Mianga which had P levels below the recommended levels for tea and very high levels of K resulting in nutrient imbalances which may have led to generally lower yields from the trial plots at Mianga compared to Glenorchy. Additionally, there was a poor yield response to application of foliar sprays containing Cu, Zn and B due to the imbalance in the nutrient levels of N, P and K, which limited the plants' capacity to utilise the applied micronutrient elements.

### 4.3.4 Conclusion

Application of foliar sprays containing Cu and Zn increased the foliar levels of Cu and Zn except for the micronutrient mix. Leaf analyses have shown that the concentration at which the micronutrient mix was applied was probably too low to raise the Cu, Zn and B levels to levels recommended for tea. Levels of Cu in leaves of plants treated with copper sulphate either alone or in combination with zinc oxide and boric acid were far too high and probably reached toxic levels. In addition to changes in the levels of Cu, B, and Zn in leaves of the treated plants, application of copper sulphate either alone or in combination with zinc oxide and boric acid led to elevated levels of Al in the treated leaves, and the high Al and Cu levels in copper sulphate treated tea plants could be the reason for low yields observed in those plots treated with copper sulphate. Although boric acid significantly increased B concentration in leaves of treated plants, the concentration of B was below the recommended level for tea. Probably, concentrations higher than 0.1% boric acid or more applications of boric acid would be able to raise the B content in tea plants to recommended levels and bring about a significant yield increase. However, there is need to confirm this by further research.

Foliar applications did not have any effect on concentrations of soil Cu, B, Zn, N, P, K, S, Ca, Mg, Al, Fe,Mn in the soil and the pH at both sites and no significant effect was observed on the concentration of other elements such as N, P, K, Ca, Mg and Mn in the third leaf of the tea shoots at both sites except for Al and S which were significantly higher in copper sulphate treated plots at Glenorchy. This implies that the differences in yield across the treatments and differences in the concentration of Cu, Zn and B in the tea leaves were not due to differences in concentrations of Cu, B, Zn, N, P, K, S, Ca, Mg, Al, Fe and Mn in the soil. However, high Al levels observed in leaves treated with copper sulphate at Glenorchy suggest that copper sulphate increased Al uptake from the soil by the treated tea plants, but this needs to be confirmed by further studies.

Generally, low yields observed in trial plots at Mianga were attributed to factors, other than micronutrient applications, such as an improper balance in the nutrition of N, P and K levels in the soil at Mianga. In soils at Mianga P was lower than the recommended concentration for tea and very high levels of K were observed which could possibly have led to poor utilisation of nutrient elements for optimum yields. This could further lead to poor uptake of the applied micronutrient elements which contributed to the lack of response in yield due to applications at Mianga estate. However, it is important to indicate that tea is a perennial crop and this study was conducted within one season therefore the impact of the micronutrient sprays may be seen in the following seasons.

# CHAPTER 5: GENERAL CONCLUSIONS AND RECOMMENDATIONS

This study was initiated as a result of high incidences of Cu, B and Zn deficiencies in leaf analyses from approximately 170 mature clonal tea fields in 2007 from some of the estates in Malawi. In addition, the current fertiliser recommendations in tea, especially in Malawi and Zimbabwe, have placed more emphasis on fertilisation with macronutrient elements, particularly N, P and K, than on micronutrient elements. Furthermore, there are numerous commercial micronutrient products available for use by the growers which range from single elemental products to multi-micronutrient pre-mixes whose effect on tea yields and quality has not been evaluated. The main objective of the study was to evaluate the effect of different formulations of foliar applied Cu, Zn and B on yield and quality of deficient clonal tea plants. Specifically, the study was conducted to evaluate the effects of foliar sprays, including one commercial micronutrient mix, on yield and yield components, contents of polyphenolic compounds in made tea andon tea taster's scores. It also aimed at establishing the effect of foliar sprays on concentrations of B, Cu, Zn and other nutrient elements in the leaves of treated tea bushes. This study was based on the hypothesis that application of these micronutrients through foliar application would be able to increase both tea yield and quality in deficient plantations and also raise the levels of Cu, Zn and B to sufficient levels for optimum growth of tea plants.

Results obtained from this study, especially for the first three applications after which the leaf samples were analysed, have shown that foliar applications of Cu, Zn and B, in forms of copper sulphate, zinc (oxide and sulphate) and boric acid respectively, significantly raised the concentration of Cu, Zn and B in the third leaves of the deficient tea bushes. However, boric acid did not manage to raise the concentration of B in leaves of treated plants to the recommended levels, whilst copper sulphate tended to raise the Cu concentrations to extremely high levels. Deficiencies of Cu, B and Zn were therefore decreased by applying copper sulphate, boric acid, zinc oxide and zinc sulphate for the respective deficient elements. Applying the micronutrient mix could not correct the deficiencies, as levels of Cu, Zn and B in the leaves were still below the norm for tea production. This also suggests the need to evaluate micronutrients mixes at different concentrations rather than relying on the recommendations of the manufacturers. Furthermore, copper sulphate application either alone or in combination with zinc oxide and boric acid led to excessive levels of Al in the third leaf of the tea shoot of the treated plants. This has implications for shoot growth and thus further investigation on the impact of Cu sprays on Al accumulation should be performed.

Although levels of Cu, Zn and B were raised to recommended levels for tea, there was a non-significant improvement in yield due to application of Cu, Zn and B containing foliar sprays and there was also a significant reduction in yield due to application of copper sulphate, regardless of whether it was applied alone or as a mixture with zinc oxide or zinc sulphate. Extremely high concentrations of Cu and Al in plants treated with copper sulphate could have led to inhibition of plant growth and impairment of important cellular processes, especially the photosynthetic apparatus (Loustalot *et al.,* 1945, Alaoui-Sosse *et al.,* 2004**,** Yruela, 2005), which possibly contributed to the significant reduction in yield in those plots. Although no significant yield losses have been reported in previous studies due to copper sulphate sprayed at 1% concentration in seedling tea (Grice, 1990), this study has shown that a 1% copper sulphate application to clonal tea was too high. It is possible that clonal tea, especially PC 105 and PC 108, require much less Cu than seedling tea or that existing levels of Cu in the leaves were higher in this study than previous studies. The non-significant yield increase observed in plots treated with zinc sulphate and the commercial micronutrient mix suggests that among the foliar sprays containing Cu, Zn and B used in this study, zinc sulphate may be recommended as an alternative source of Zn to zinc oxide, while the commercial micronutrient mix may be recommended in situations where multiple micronutrient deficiencies occur in clonal tea fields in Malawi. There is, however, need to explore the effect of spraying the tea plants with copper sulphate at concentrations lower than 1% in order to determine which copper sulphate concentration will give optimum yields while maintaining the required level of Cu in the leaves of clonal tea. When considering Cu applications, growers also need to consider their fungicide application especially when using copper oxychloride as a fungicide which is reportedto have a negative impact on tea yields (Barua & Dutta (1972), and Saikh (2007). Likewise, there is need for further assessment of the commercial micronutrient mix at higher concentrations or more frequent applications. It is likely that higher concentrations might give a significant yield increase, as well as maintain levels of Cu, Zn and B within the recommended levels for tea and probably improve the yields.

Tea taster's scores and valuation were significantly reduced by almost all Cu, Zn and B treatments at Glenorchy but not at Mianga. Foliar sprays of Cu, B and Zn, applied as copper sulphate, boric acid, zinc oxide and commercial micronutrient mix, increased the levels of thearubigins while levels of caffeine and theaflavins remained constant. This means that during fermentation in black tea manufacture, catechins and flavanols in the green leaves were being converted to more thearubigins than theaflavins in the treated leaves compared to the control leaves which resulted in less bright black teas made from the Cu, Zn and B treated leaves. This implies that the applied micronutrients especially Zn and Cu to the inherently fast fermenting cultivars (PC 105 and PC 108) (TRFCA, 2000) led to prolonged activity of polyphenol oxidase which resulted in production of more TRs than TFs (Owuor & Reeves 1986) leading to lower scores on brightness and briskness of the black tea in the Cu, Zn and B treated plants.

Generally there were differences in yield responses to application of micronutrient foliar sprays between the two sites (Mianga and Glenorchy), with lower yields being observed across all treatments at Mianga than at Glenorchy. Differences among treatments were observed in yield of tea at Glenorchy estate while at Mianga, the treatments performed similarly in terms of yield. The differences in response to foliar sprays between the two sites may be attributed to differences in water regimes between the two sites, with water possibly being limiting to growth during certain dry periods under rain-fed conditions. Tea at Mianga was grown under rain-fed conditions while tea at Glenorchy had supplementary irrigation, further than this, tea at Mianga is grown at a higher elevation of 960 metres above sea level than tea at Glenorchy which is grown at an elevation of 650 metres above sea level. Low yields observed in trial plots at Mianga were also attributed to an improper balance in the nutrition of N, P and K levels in the soil at Mianga. P levels were below the norm for tea soils, whilst K was above the norm at Mianga. This probably led to poor utilisation of nutrient elements which eventually negatively affected yields at this site.

In conclusion, results from this study indicate that clonal tea, specifically cultivars (PC 108 and PC 105) did not respond positively to Cu, Zn and B foliar applications in terms of yield and quality within the first season of application. However, if application is aimed at raising concentrations of Cu, Zn, and B in deficient plants, then boric acid, zinc sulphate, zinc oxide and the commercial micronutrient mix may be used without negatively affecting yield and quality of tea significantly. Application of copper sulphate, however, at the concentration and frequency used in this study, should not be done as it causes a significant decline in yield, which according to this study has been attributed to very high levels of Al and Cu in leaves treated with copper sulphate. Long term research is therefore required to determine if long term applications can correct perceived deficiencies and increase yield and also if other carriers of Cu, Zn and B than the ones used in this study can significantly improve yield and quality of clonal tea.

In summary, some of the observations made in this study points to the need for further investigations on (1) any possible interactions between foliar applied copper in the form of copper sulphate and Al accumulation in tea leaves, (2) the effect of different concentrations of the commercial micronutrient mix used in this study and other micronutrient mixes carriers on yield and quality of tea and (3) effect of foliar application of copper sulphate at concentrations lower than 1% on yield and quality of clonal/ vegitatively propagated tea.

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