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Effects of Nitrogenous Fertilizer Rates, Plucking Intervals and Location of Production on the Micronutrient Levels of Clonal Black Tea

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ABSTRACT: Micronutrients are important to both animal and plant life. In plants, the levels can vary widely due to biotic and abiotic factors. Tea is widely grown and its beverages are claimed to be the most widely consumed fluids after water. Some factors causing changes in tea growth and yields, and hence nutrients assimilation include location of production, fertilizer application and harvesting policies. Influence of location of production, nitrogenous fertilizer rates, and plucking intervals on clone TRFK 6/8 black tea micronutrients were investigated. The micronutrients levels significantly ($P \leq 0.05$) varied with location of production. Although Mn and Se levels were not influenced by nitrogenous fertilizer rates, Fe and Zn ($P \leq 0.05$) increased while Cu ($P \leq 0.05$) decreased with an increase in nitrogen fertilizer rates. Plucking intervals had no influence on clonal black teas micronutrients levels. Overall, the levels were within the range that had been noted in a previous study to supply inadequate daily requirements of micronutrients. Thus biotic and abiotic factors in this study cannot be used to cause the micronutrients levels to be high enough to adequately supply the micronutrients daily demands of human requirement. Tea cannot therefore be considered a suitable source for the daily requirement of micronutrients for human beings despite its high consumption.

KEYWORDS: *Black tea;* Camellia sinensis; *Nitrogenous fertilizer; Plucking interval; Micronutrients*

Introduction

Elements, including manganese, iron, zinc, copper and selenium are beneficial to animal and plant health, although at curtain levels they may be classified as toxic. The deficiency or excess of any of these elements may cause diseases and/or be deleterious to human health.[1] The world understands the nature, magnitude and problems caused by micronutrient deficiency often called "hidden hunger", the situation is still prevalent. Hidden hunger is a situation where quantities of foods are adequate but lack vital micronutrients.[2] The consumption of foods rich in micronutrients is one way of alleviating the hidden hunger. Tea beverages, infusions of dried young tender shoots of *Camellia sinensis* L. O. Kuntze[3, 4] are the most popular, non-alcoholic beverages in the world,[5] and most widely consumed fluids after water.[6, 7] In a recent survey of East African black teas, it was observed that generally the infusions had relatively low levels of micronutrients, consequently it was concluded black tea could not be used to alleviate the hidden hunger even with consumption of over ten cups per day.[8] The black teas used in the study were, however, randomly selected and it was not known if the levels of the micronutrients could be positively changed by varying some biotic and abiotic factors.

In Kenya, tea grows at different rates in different locations. The effect of region on growth is demonstrated to change with locations in India.[9] In recent studies, tea quality parameters[10–13] and quality precursors[14–17] change with geographical area of production. Indeed even the yields[12, 15, 16, 18, 19] and quality[13, 20–24] vary with geographical area of production, the variation occurring in unpredictable patterns. Such changes were attributed to many factors including non-use of single cultivar and different agronomic inputs. However, when the studies on single cultivar were used under same agronomic inputs, quality[10–12, 23] and yield[11, 12, 18] variations persisted. It is not known if the micronutrients levels in black tea of one cultivar grown in different regions vary, such that production in specific geographical locations could be a way of enhancing levels on micronutrients in black tea for human benefit.

Agronomic inputs influence the soil chemical parameters[25] leading to changes in the absorption and hence variations in the chemical composition of the harvested leaf.[26] Nitrogen, phosphorous and potassium are the main nutrients for tea. They are usually supplied in compound nitrogenous fertilizers.[26] Nitrogenous fertilizer application is the second most expensive agronom-

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ic input in tea production[27] after harvesting. Although nitrogenous fertilizer plays a major role in plant growth, it alters the absorption of micronutrients by the plants from the soil as increasing rates of nitrogenous fertilizer generally increase soil acidity.[26, 28] This may cause variations in availability of some of the micronutrients. The recommended rate of fertilizer application in Kenya, that is also widely used in East African countries is 100–250 kg N^{-1} ha^{-1} yr^{-1} as NPKS 25:5:5:5 or NPK 20:10:10 29, with the actual rate being dependent on level of production. But, the optimal rates for different regions vary.[11, 12] Nitrogenous fertilizer application influences the yield through variations in rate of shoot extension, individual shoot weight and density.[30, 31] Appropriate use of nitrogenous fertilizers leads to increase in tea production[32–34] but the high rates of fertilizer application reduce black tea quality[31, 35] and increase the fatty acids in tea leaf.[15–17] These variations may be indicators that micronutrients could also be changing due to rates of nitrogenous fertilizer application. Thus, application of nitrogenous fertilizer could be one avenue of improving the ability of black tea to supply micronutrients to human diets. This study was conducted to establish if variations of nitrogen fertilizer rates could enhance availability of the micronutrients in different tea-growing areas in Kenya.

Plucking is an important agronomic undertaking and the most expensive agronomic input in tea production. During the plucking operation, young leaves are removed[36] for processing into various tea beverages. The recommended plucking standard is two leaves and a bud that gives desirable good black quality teas[31, 35, 37] and acceptable yields.[29, 36] Tea grows at different rates in different locations,[38–40] leading to the achievement of recommended two leaves and a bud[29] after different time lengths. Recommended plucking interval in Kenya varies from 7 to 14 days.[29] Short plucking intervals remove the leaves when the pluckable shoots are still young and are mostly two leaves and a bud.[30, 41] Short plucking intervals reduce "breaking-back",[42] a process that reduces yields, but improves quality.[35] Aroma quality precursors especially fatty acids, whose degradation products reduce tea quality, were demonstrated to increase with longer plucking intervals 15–17. The changes in the various quality attributes may also suggest there may be variations in the micronutrient content of tea with varying plucking intervals and that such variations may change at different locations of production and nitrogen fertilizer rates. The influence of plucking intervals on micronutrient levels in different regions and rates of nitrogen were studied.

Materials and Methods

Sites and Sample Collection

Trials were set on clone 6/8 plantations that are uniformly managed and with known past cultivation history planted at Changoi in lower Kericho, Timbilil Estate (Tea Research Foundation of Kenya) in upper Kericho, and Sotik Highlands (Arrocket) Tea Estate in Sotik whose altitude, latitude, longitude and year of plantation are given in Table 1.

At each site, the experiment was set as a factorial two arrangement laid out in a randomized complete block design with five fertilizer rates (0, 75, 150, 225 and 300 kg N^{-1} ha^{-1} yr^{-1}) and three plucking frequencies (7, 14 and 21 day rounds) and treatments replicated three times. Each effective plot comprised of 48 plants surrounded by a line of tea bushes that served as guard rows. On the day the three plucking intervals coincided in each experiment, 1 kg of tea leaves were harvested from each plot and miniature black tea processed.[43] Unsorted black tea samples were subjected to analysis of the micronutrients.

Analysis of the Micronutrients

A modified standard procedure of AOAC[44] was followed in the preparation of samples of the unsorted black teas. Accurately weighed (Mettler Toledo, Switzerland) 1,0000 g black tea for analysing Mn, Fe, Zn and Cu while

Table 1: Site Locality and Hisotry Clone 6/8 Cultivar

Site Locality/history	Changoi	TRFK Timbilil	Sotik Highlands (Arrocket)
Altitude (m)	1872	2178	2100
Latitude Longitude	00 31′S 350 16′E	00 22′S 350 21′E	00 36′S 350 4′E
Year planted	1989	1986	1974
Plantation age*	22	25	37

*As at year 2011.
Source: Individual/estates records.

2,0000g black tea for Se analysis were transferred into ashing tubes and kept in a muffle furnace for ashing at 460°C for 12 hr. The ashed samples were digested using double acid (concentrated hydrochloric and nitric acids in a 1:1 ratio) and hydrogen peroxide in the ratio of 2:3. Care was taken to ensure that all ash came into contact with the acid. All the chemicals used were of analytical grade (Sigma Aldrich). Further the crucible containing acid solution was kept on a hot plate and evaporated to dryness under a hood. The final residue was dissolved in 0.05 M hydrochloric acid solution for extraction and made up to 25 ml for Mn, Fe, Zn and Cu analysis and to 10 ml for Se analysis. Standard solutions were prepared by diluting the stock solution with 0.05 M hydrochloric acid. The micronutrients were analysed using atomic absorption spectrophotometer (Shimadzu AA-6200 Model, Japan) under standard instrumental conditions.

Statistical Analysis

The data were analysed using a 3×5×3 factorial design in a randomized complete block design, with location as main treatment, nitrogen NPKS 25:5:5:5 fertilizer rates as sub-treatments and harvesting intervals as sub-sub treatments. MSTAT-C statistical package (Michigan State University, MI) was used for ANOVA, while linear regressions were performed using MS-Excel statistical package.

Results and Discussion

There were significant ($P \leq 0.05$) changes in the level of the five micronutrients in clone TRFK 6/8 black tea due to location of production (Tables 2). It was therefore not possible to make black teas with similar micronutrient contents in all the three locations even with the use of one cultivar, possibly due to differences in the soil pH at the three sites.[45] Some of the micronutrients like Fe, Zn and Cu are more bioavailable in strongly acidic soils,[46] thus the levels of the micronutrients inversely followed that of pH.[47] Equally mature leaf Fe and Zn levels were high in locations with lower pH.[48] The changes in the levels these micronutrients could be also due to several factors including temperature,[49] rainfall and rainfall distribution,[50] altitude[39, 40] and sporadic hail damage experienced in the tea-growing locations[38, 50] observed in different locations in Kenya. Although these factors were not monitored in the present study, the extents of their variations may be large at the various geographical locations. Equally past crop husbandry and management may also play a bigger role in micronutrient variations in the different geographical locations. However, it was noted that even with uniform management, in one cultivar, micronutrients in black tea are different.

The changes in micronutrient levels in black tea due to application of nitrogenous fertilizer rates are presented in Tables 2 and 3. Mn and Se levels did not vary with an increase in nitrogenous fertilizer rates. These results were similar Mn in the first mature leaf[48] where increasing nitrogenous fertilizer rates did not significant ($P \leq$ 0.05) difference. This was possibly due to the highly acidic nature of the tea soils which made Mn to be available in excess and thus luxurious uptake was expected ending up with no specific pattern.[48]

Mn levels are high in strongly acidic soils and that the nutrient can be accumulated highly in the leaves to an extend that application of fertilizer or manure might not affect the levels.[51] Irrespective of location of production, nitrogenous fertilizer rates did not affect the Mn content of black teas. Thus for optimal Mn in black tea for alleviation of hidden hunger, nitrogenous fertilizer rates cannot dictate the levels of Mn in black teas. The results were, however, within the same magnitude as those observed in an earlier study in which the East African black teas could supply adequate amounts of Mn to alleviate human needs just by consuming only 3 to 5 cups of black tea per day.[8]

The Se levels in clonal black tea did not follow any order and nitrogenous fertilizer rates had no effect on these levels (Table 2 and 3). The Se levels were also very low compared to other micronutrients. It is possible Se levels in the soils are very low or the plant absorbs very little Se. To increase Se concentrations in tea, Se-enriched fertilizer like fertilizer of sodium selenite or sodium selenate had been used.[52] To enhance the Se levels, application of Se-enriched fertilizers could be recommended.

Fe levels significantly ($P \leq 0.05$) increased with the increase in nitrogenous fertilizer rates in the three locations (Tables 2 and 3). Generally, increasing the rates of nitrogenous fertilizer reduce the soil pH[45, 47, 53] and thus increasing the bioavailability of Fe. This change in pH may also cause variations in the metal content of teas. There was linear increase with rates with very strong association (high magnitudes of R^2) between rates of nitrogen and Fe levels (Fig. 1). Increase in rates of nitrogen is, therefore, one method of enhancing the Fe levels in black tea. However, the levels were within the same levels observed in a previous study[8] that did not meet the daily micronutrient requirement, even if bioavailability was maximum.

Zn levels significantly ($P \leq 0.05$) increased with the

Table 2: Effects of Location of Production and Nitrogen Fertilizer Rates on Micronutrients

Micronutrient	Location	N-Rates (kg N/ha/year) 0	75	150	225	300	Mean Locations
Mn (µg/g)	Timbilil	652.33	685.89	654.78	660.56	678.11	666.33
	Sotik Highlands	557.56	554.78	586.11	576.56	572.22	569.44
	Changoi	662.22	648.56	655.89	631.44	655.11	650.64
	Mean N-Rate	624.04	629.74	632.26	622.85	635.14	—
	C.V (%)			5.12			
	L.S.D ($P\leq0.05$)			NS			29.22
Fe (µg/g)	Timbilil	110.33	121.89	121.78	148.22	128.44	126.13
	Sotik Highlands	69.89	75.22	71.89	75.33	83.56	75.17
	Changoi	60.67	71.56	70.22	69.67	70.11	68.44
	Mean N-Rate	80.3	89.56	87.96	97.74	94.04	—
	C.V (%)			16.10			
	L.S.D ($P\leq0.05$)			10.93			13.13
	Interactions (SxN)			15.73			
Zn (µg/g)	Timbilil	18.11	25.11	29.00	31.11	29.89	26.64
	Sotik Highlands	19.44	20.78	21.00	22.78	29.22	22.64
	Changoi	20.33	22.22	22.44	24.89	25.22	23.02
	Mean N-Rate	19.30	22.70	24.15	26.26	28.11	—
	C.V (%)			20.60			
	L.S.D ($P\leq0.05$)			3.75			3.51
	Interactions (SxN)			5.40			
Cu (µg/g)	Timbilil	23.67	22.78	21.11	17.44	12.67	19.53
	Sotik Highlands	9.00	7.22	4.56	3.67	3.11	5.51
	Changoi	19.00	15.22	13.33	13.67	14.56	15.16
	Mean N-Rate	17.22	15.07	12.96	11.70	10.11	—
	C.V (%)			24.46			
	L.S.D ($P\leq0.05$)			2.78			2.97
	Interactions (SxN)			3.56			
Se (µg/g)	Timbilil	1.58	1.91	1.56	1.93	1.62	1.72
	Sotik Highlands	1.48	1.46	1.66	1.56	1.60	1.55
	Changoi	2.50	2.03	2.49	1.96	2.28	2.25
	Mean N-Rate	1.86	1.80	1.90	1.82	1.83	—
	C.V (%)			23.60			
	L.S.D ($P\leq0.05$)			NS			0.39

increase in nitrogenous fertilizer rates (Tables 2 and 3, Fig. 2). This was also attributed to the increased acidity of the soils when nitrogenous fertilizers rates were increased[53] which made Zn to be more bioavailable.[54] The R^2 values (Fig. 2) were also very high demonstrating strong association between nitrogen rates and Zn levels. Despite the increase, Fe levels were within the same levels observed in a previous study[8] that did not meet the daily micronutrient requirement, even if bioavailability was 100%.

Cu levels in black tea significantly ($P\leq0.05$) reduced with increased rates of nitrogenous fertilizer (Tables 2 and 3, Fig. 3). High concentrations of Zn cause reduction of Cu transport in plants.[55] Thus, the increase in Zn with increase in nitrogen rate might have reduced the Cu absorption[55] by the tea plant. Again, the Cu levels could have decreased with high rates of nitrogenous fertilizer rates due to increase in soil acidity[45, 47, 53] that inhibited Cu absorption by the tea plants. The degree of association between decline in Cu levels with increase in nitrogen fertilizer rates was also strong with high R^2 values (Fig. 3). These results demonstrate the nitrogen fertilizer use in not an option in increasing availability of Cu in tea beverages.

The changes in micronutrient levels in black tea due to plucking intervals are presented in (Tables 3 and 4). Plucking intervals did not significantly ($P\leq0.05$) influence the micronutrient content of clonal black teas.

Table 3: Effect of Plucking Intervals and Fertilizer Rates on Micronutrients in Black Tea

		N-Rates					
Micronutrient	P-Freq	0	75	150	225	300	Mean P-Freq
Mn (µg/g)	7	623.11	629.22	630.89	608.67	622.67	622.91
	14	626.44	636.44	632.44	613.33	639.11	629.56
	21	622.56	623.56	633.44	646.56	643.67	633.96
	Mean N-rate	624.04	629.74	632.26	622.85	635.14	—
	CV (%)			5.12			
	LSD ($P \leq 0.05$)			NS			NS
Fe (µg/g)	7	80.56	89.33	85.44	96.56	94.44	89.27
	14	82.44	86.78	90.33	102.00	91.89	90.69
	21	77.89	92.56	88.11	94.67	95.78	89.80
	Mean N-rate	80.30	89.56	87.96	97.74	94.04	—
	CV (%)			16.10			
	LSD ($P \leq 0.05$)			10.93			NS
Zn (µg/g)	7	19.56	19.56	24.89	26.22	30.00	24.04
	14	20.33	23.78	23.56	26.56	27.22	24.29
	21	18.00	24.78	24.00	26.00	27.11	23.98
	Mean N-rate	19.30	22.70	24.15	26.26	28.11	—
	CV (%)			20.60			
	LSD ($P \leq 0.05$)			3.75			NS
Cu (µg/g)	7	14.33	12.67	14.44	13.11	10.11	12.93
	14	17.33	17.89	12.89	13.89	10.00	14.40
	21	13.78	13.00	13.33	14.00	10.22	12.87
	Mean N-rate	15.15	14.52	13.56	13.67	10.11	—
	CV (%)			24.46			
	LSD ($P \leq 0.05$)			2.78			NS
Se (µg/g)	7	1.87	1.78	1.74	1.64	1.74	1.76
	14	1.76	1.72	1.93	2.00	1.88	1.86
	21	1.93	1.90	2.02	1.80	1.88	1.91
	Mean N-rate	1.86	1.80	1.90	1.82	1.83	—
	CV (%)			23.60			
	LSD ($P \leq 0.05$)			NS			NS

These results were similar to the yield response to plucking intervals which did not vary significantly with plucking intervals.[10, 12] Contradicting reports on yield responses to plucking intervals have been reported, for example, in Malawi yields decreased with increased plucking rounds,[56, 57] but increased in Kenya with short plucking intervals.[41] An increase in plucking intervals reduced black tea quality[10, 12, 31, 41] partly due to increase in green leaf fatty acid content.[15–17] In other studies, quality parameters increased with shorter plucking intervals[31, 35, 41, 58] while yields increased with long plucking intervals.[10, 12] In the present study, plucking intervals do not have a significant effect on the micronutrient of black teas, implying that plucking intervals is not an option in increasing the availability of the micronutrients studied in black teas.

There were no significant ($P \leq 0.05$) interactions between location of production and nitrogenous

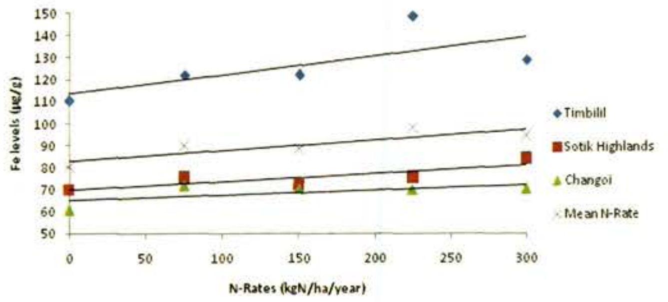

Fig. 1: Effects of nitrogen fertilizer rates on iron levels in the unsorted black teas. $Fe_{(Timbilil)} = 0.163x + 107.6$ ($R^2 = 0.916$); $Fe_{(Changoi)} = 0.049x + 63.04$ ($R^2 = 0.816$); $Fe_{(Sotik\ Highlands)} = 0.036x + 69.68$ ($R^2 = 0.890$); $Fe_{(Mean\ N\text{-}Rates)} = 0.074x + 80.78$ ($R^2 = 0.919$).

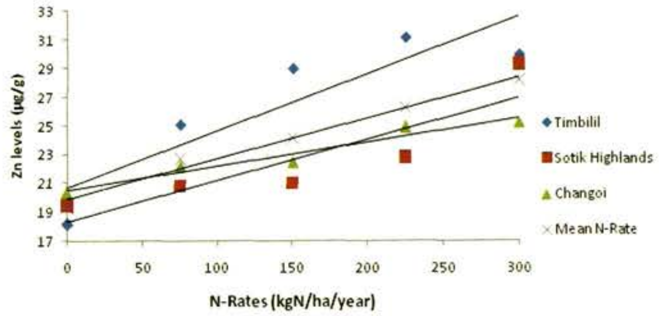

Fig. 2: Effects of nitrogen fertilizer rates on zinc levels in clonal black tea. $Zn_{(Timbilil)} = 0.52x + 19.73$ ($R^2 = 0.856$); $Zn_{(Changoi)} = 0.016x + 20.53$ ($R^2 = 0.936$); $Zn_{(Sotik\ Highlands)} = 0.028x + 18.33$ ($R^2 = 0.878$); $Zn_{(Mean\ N\text{-}Rates)} = 0.028x + 19.86$ ($R^2 = 0.980$).

fertilizer application for the micronutrients Mn and Se (Table 2) meaning that the response patterns for these micronutrients occurred in a similar manner. For the micronutrients Fe, Zn and Cu, there were significant ($P \leq 0.05$) interactions between location of production and nitrogenous fertilizer application rates (Table 2) meaning that the response patterns were different at each region. This is also evident from Figures 3, 4 and 5 where the gradients of the liner relationships varied widely. On the other hand, there were no significant ($P \leq 0.05$) interactions between nitrogenous fertilizer application rates and plucking intervals for all the micronutrients (Table 3) meaning suggesting the responses were in similar patterns. There were significant ($P \leq 0.05$) interactions between location of production and plucking intervals for all the micronutrients (Table 4) indicating that the response patterns were different at each site.

In conclusion, the micronutrients levels in black tea significantly ($P \leq 0.05$) varied with the location of production. It is, therefore, not possible to produce black tea from different locations with same micronutrient levels[8] even with the use of a single cultivar. Varying rates of nitrogenous fertilizer application changes ($P \leq 0.05$) levels of Zn, Fe, and Cu. However, with the use of the currently recommended fertilizers rates in Kenya,[29] the levels were not adequate to supply the daily minimum requirements of the micronutrients. Plucking intervals did not affect the levels of the micronutrients and should not be considered as an option in making the micronutrient available to supply the human requirements.

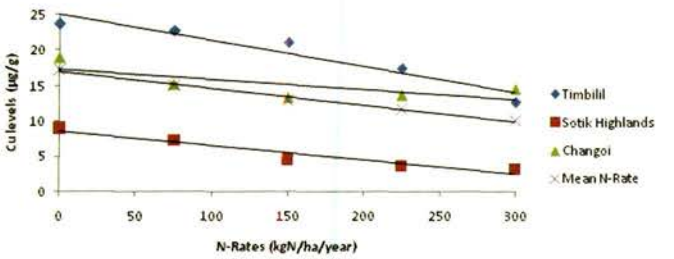

Fig. 3: Effects of N-rates on Cu levels in clonal black tea. $Cu_{(Timbilil)} = -0.036x + 25.00$ ($R^2 = 0.915$); $Cu_{(Changoi)} = -0.021x + 17.84$ ($R^2 = 0.718$); $Cu_{(Sotik\ Highlands)} = -0.020x + 8.578$ ($R^2 = 0.934$); $Cu_{(Mean\ N\text{-}Rates)} = -0.014x + 15.58$ ($R^2 = 0.988$).

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Table 4: Effects of Plucking Intervals and Location of Production on Micronutrients

		Locations			
Micronutrient	P-Freq (days)	Timbilil	Sotik Highlands	Changoi	Mean P-Freq
Mn (µg/g)	7	658.20	542.00	668.53	622.91
	14	672.80	574.33	641.53	629.55
	21	668.00	592.00	641.87	633.96
	Mean locations	666.33	569.44	650.64	—
	C.V (%)		5.12		
	LSD ($P \leq 0.05$)		29.22		NS
	Interactions (SxP)		32.65		
Fe (µg/g)	7	123.80	75.20	68.80	89.27
	14	129.13	74.47	68.47	90.69
	21	125.47	75.87	68.06	89.80
	Mean locations	126.13	75.18	68.44	—
	C.V (%)		16.10		
	LSD ($P \leq 0.05$)		13.13		NS
	Interactions (SxP)		14.67		
Zn (µg/g)	7	25.53	21.20	25.4	24.04
	14	28.67	22.20	22.00	24.29
	21	25.73	24.53	21.67	23.98
	Mean locations	26.64	22.64	23.02	—
	C.V. (%)		20.60		
	LSD ($P \leq 0.05$)		3.51		NS
	Interactions (SxP)		5.03		
Cu (µg/g)	7	18.93	4.73	15.13	12.93
	14	20.53	5.40	17.27	14.40
	21	19.13	6.40	13.06	12.87
	Mean locations	19.53	5.51	15.16	—
	C.V (%)		24.46		
	LSD ($P \leq 0.05$)		2.97		NS
	Interactions (SxP)		3.32		
Se (µg/g)	7	1.51	1.64	2.11	1.76
	14	1.92	1.30	2.36	1.86
	21	1.73	1.71	2.28	1.91
	Mean locations	1.72	1.55	2.25	—
	C.V (%)		23.60		
	LSD ($P \leq 0.05$)		0.39		NS
	Interactions (SxP)		0.44		

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