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Changes in the Composition of Elements in Non-Aerated Green Teas Processed from Seedling Tea (*Camellia sinensis*) Due to Variations in Nitrogenous Fertilizer Rates and Seasons.

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ABSTRACT

Soil nutrients are lost to the plant with harvested crop in tea production with most ending up as minerals and contribute to human nutrition upon tea consumption. Production of non-aerated green tea in Kenya has been low. The changes in the mineral profiles of these teas due to nitrogenous fertilizer rates and seasons is not been documented. Several elements were profiled from non-aerated green teas processed from seedling tea grown under varying fertilizer rates of up to 800kg N/ha/year in different seasons. They included N, P, Ca, Mg, Mn, Zn, Cu and Fe which registered 3.32-3.79%, 0.22-0.29 %, 1.47-2.13%, 0.42-0.87%, 0.14-0.38%, 0.11-0.28%, 19.29-41.88ppm, 11.68-25.53ppm and 175.83-206.28ppm respectively with nitrogenous fertilizer rates. The elements were significantly (pd”0.05) higher during the wet and warm, and wet and cold seasons than in the warm and dry, and cold and dry seasons. For the seasons, N, P, K, Ca, Mg, Mn, Zn, Cu and Fe registered 3.19-4.22%, 0.16-0.35%, 0.51-2.10%, 0.42-0.92%, 0.12-0.44%, 0.13-0.35%, 8.74-84.94ppm, 6.67-84.67ppm and 66.07-363.39ppm respectively. The values changed with rainfall distribution, although the response patterns differed between individual elements. Inceasing NPKS fertilzer rate caused a rise in N, P and K values especially, from October to June. However, there was a shift in the months of July to September between 400 and 800 kg N/ha/year, in the first year of study. During the second year, the observed shift in year one of the study was revesred. Consequently throughout the year the increments in nutrients corresponded to that of fertilzer rates. The results demonstrate that soil nutrients removed with crop vary with seasons and nitrogenous fertilizer rates. High rate of fertilizer contributed to more N, P and K and other micro-elements such as Mn.and Fe to the finished products. The results also demonstrate that in non-aerated green tea production, application of high rates of nitrogen is necessary to maintain plant nutritional requirements and fresh leaf quality.

INTRODUCTION

African countries including Kenya produce mainly aerated black teas. These teas are predominantly of Cut, Tear and Curl (CTC) formation, and very little orthodox teas. About 95% of the tea produced in Kenya is for the export market (*1*). There is a serious lack of product diversification leading to the over-supply of the CTC black teas which causes price stagnation/decline leading to unstable earnings for the African tea industry. As a result, returns to tea famers have been low causing dissatisfaction with the commodity as a cash crop (*2*). Consequently, the Kenyan tea industry has embarked on research to develop alternative teas and tea products to expand the Kenyan tea market and enhance revenue from tea enterprises (*3*). Non-aerated green tea is an alternative tea product with potential in some new markets for Kenyan teas including China, Japan and the health conscious consumers. Research on tea quality in Kenya in the past concentrated on technologies to manage (*4, 5*) and improve (*6*) CTC black tea quality. Studies on other tea products, especially the development of other types of tea including non-aerated green tea production technologies are rare. Nitrogenous fertilizers (*7-10*) and seasons (*11-13*) of production have a major influence on the black tea quality precursors and CTC black tea in Kenya. The influence of seasons and nitrogenous fertilizers on the nutrients and mineral values of non-aerated green tea have not been assessed.

Studies have demonstrated the medical benefits from tea consumption. The medicinal claims include remedy to ailments such as controlling bleeding, healing wounds, regulating body temperature and blood sugar, promoting digestion among others (*14*). Non-aerated green tea has become the row material for extracts used in various beverages, health foods, dietary supplements, and cosmetic items (*15*). Consumption of green tea is on an upward trend worldwide due to its associated health benefits (*16-21*). These health benefits include reduction of risks of heart disease (*17*), boosting heart rate (*16*), anti-cancer, anti-hypertensive, anti-inflammatory, anti-arthritis, anti-microbial properties (*19-21*), photo-protection, increased microcirculation and modulation of skin properties of women (*22*). But there are also undesirable effects. Consumption of large volumes of non-aerated green tea, and green tea extracts, may cause oxidative stress and liver toxicity (*23*), interfere with the anti-cancer drug bortezomib (Velcade) and other boronic acid-based proteasome inhibitors (*17*).

The cultivation of a healthy and high yielding tea plant that produces desirable good quality leaves for processing into tea beverages requires implementation of the recommended agronomic practices. Nitrogenous fertilizer use is mandatory in tea production to produce such leaves (*24*). Plants require 17 nutrients for growth and development (*25*). The major nutrient include carbon, hydrogen and oxygen obtained from the air and water. The primary macronutrients include nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and secondary macronutrients sulphur (S), magnesium (Mg) while micronutrients or trace minerals include boron (B), chlorine (Cl), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), molybdenum (Mo) and nickel (Ni) (*26-29*). Plants obtain nutrients from the soil but when these become limited, fertilizers and foliar feeds are used to supplement nutrition. The plants use specific nutrients in various parts for different essential functions (*30*). When inadequately supplied by the soil, low plant growth and yields and poor quality leaves and fruits result. Adequate supplementation through the addition of fertilizers then becomes mandatory to reverse the negative effects (*28, 31, 32*). Understanding the factors that influence nutrient levels is necessary to enable timely corrective measures. The influence of seasons and nitrogen fertilizers on the plant nutrient contents were investigated. Macro and micro-elements of processed non-aerated green teas were carried out and documented to improve the processing technologies of non-aerated green tea.

MATERIALS AND METHODS

Site and materials

Fresh green leaves for the trial was obtained from seedling tea planted in 1959 at Timbilil (0° 22'S; 35°21'E; 2,180 m above mean sea level (amsl)). The long term fertilizer trial was set up to establish the effects of high rates and splitting annual application of nitrogenous fertilizer on tea in 1983 (*33*) at Timbilil Estate of the Kenya Agricultural and Livestock Research Organization-Tea Research Institute (KALRO-TRI) Kericho, Kenya (Formerly the Tea Research Foundation of Kenya). The experiment was originally set as a factorial two trial with rates and splitting annual applications as the treatments. However, after 15 years of experimentation without response to splitting annual nitrogen application, it was converted to long term trial on high rates of nitrogen downgraded to test long term effects of high rates of nitrogen only in randomized complete block design (RCBD), replicated three times. The fertilizer rates were 0, 50, 100, 200, 400, and 800 kg N/ha/year applied as NPKS 25:5:5:5 in October every year. Leaves for non-aerated green tea processing were obtained from October to September for 2 years (2011-2012 and 2012-2013). The trial was set up in a field adjacent to the KALRO-TRI weather station and all weather data were recorded on a daily basis. Plucking was done in 4 seasons each year in a 10-14 day interval per plucking round. The seasons were divided into cold and dry (July-September), warm and wet (October-December), warm and dry (January-March) and cold and wet (April-June) which was a modification from the work by (*34*).

Non-aerated green tea processing

About 500g per plot of two leaves and a bud of fresh green leaves, were hand plucked from the seedling tea bushes in the four seasons each year for processing. The leaves were kept at ambient temperature and transported to a miniature processing factory at KALRO-TRI, where they were processed into non-aerated green tea as outlined in an earlier study (*3*). The leaves were steamed for 1 min (Philips model HD 9120, China), macerated using a CTC machine, 3 cuts (Tea Craft limited, Bedford, UK) and dried at 120ºC in a mini fluidized bed drier (Tea Craft limited, Bedford UK) to a moisture content (MC) of 4%. Processed teas were stored in sealed silver lined sachets at room temperature (20-24°C) to await the analysis of elements.

Nitrogen determination

Nitrogen was determined using the Kjeldal method (*35*).

Mineral determination

Elements including P, K, Ca, Mg, Mn, Zn, Cu and Fe

Table 1: Effects of fertilizer rates (kg N/ha/year) on elements assayed from non-aerated green tea from seedling teas for 2 years (2011/2012 and 2012/13)

Year	Rate (kg N)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (%)	Zn (ppm)	Cu (ppm)	Fe (ppm)
2011-2012	0-800	3.65±0.53	0.28±0.06	1.94±0.73	0.50±0.11	0.16±0.04	0.18±0.08	39.05±28.18	18.94±25.85	188.63±110.57
2012-2013	0-800	3.61±0.37	0.23±0.04	1.58±0.27	0.70±0.24	0.31±0.14	0.19±0.17	24.40±14.76	13.24±4.65	132.42±82.42
	LSD, p=0.05	0.08	0.01	0.1	0.02	0.01	0.02	3.81	2.88	13.93
	CV (%)	11.49	17.51	31.43	20.22	31.63	62.47	63.55	94.54	45.86
2011-2012	0	3.48±0.36	0.28±0.04	1.96±0.14	0.55±0.10	0.17±0.02	0.13±0.04	41.88±31.92	20.23±20.93	206.28±136.57
	50	3.54±0.51	0.28±0.05	1.93±0.16	0.59±0.07	0.17±0.03	0.15±0.10	37.59±25.69	25.53±47.34	196.24±112.56
	100	3.58±0.49	0.28±0.07	2.13±1.69	0.56±0.07	0.17±0.03	0.13±0.03	39.69±28.61	17.29±20.05	192.02±111.28
	200	3.76±0.51	0.28±0.05	1.79±0.22	0.44±0.04	0.15±0.02	0.19±0.04	36.37±27.18	15.70±15.77	178.43±95.30
	400	3.75±0.59	0.27±0.05	1.95±0.51	0.44±0.06	0.14±0.02	0.23±0.04	39.35±31.27	16.62±16.28	175.83±100.16
	800	3.79±0.65	0.29±0.08	1.86±0.21	0.42±0.13	0.14±0.06	0.26±0.06	39.41±25.39	18.28±21.04	182.96±107.62
	Mean	3.65	0.28	1.94	0.5	0.16	0.18	39.05	18.94	188.63
	LSD, p=0.05	0.18	0.02	0.33	0.03	0.01	0.02	8.74	7.24	14.15
	CV (%)	10.44	13.12	37.21	14.52	14.47	27.65	48.15	82.27	16.14
2012-2013	0	3.32±0.19	0.23±0.05	1.70±0.15	0.87±0.23	0.38±0.19	0.12±0.05	27.00±13.19	14.36±3.88	151.28±88.87
	50	3.61±0.57	0.23±0.05	1.69±0.13	0.78±0.30	0.31±0.12	0.11±0.06	26.22±15.38	14.24±3.21	138.53±79.14
	100	3.64±0.26	0.22±0.04	1.47±0.40	0.71±0.20	0.31±0.12	0.22±0.37	19.29±9.61	12.19±1.48	130.25±87.22
	200	3.67±0.39	0.22±0.05	1.55±0.15	0.68±0.22	0.30±0.12	0.16±0.06	27.77±23.62	14.11±6.74	130.26±75.61
	400	3.74±0.25	0.22±0.03	1.58±0.12	0.59±0.14	0.28±0.11	0.25±0.09	24.89±10.97	12.83±6.95	126.24±86.71
	800	3.70±0.32	0.23±0.04	1.48±0.41	0.56±0.15	0.26±0.11	0.28±0.09	21.19±9.83	11.68±2.07	117.94±77.84
	Mean	3.61	0.23	1.58	0.7	0.31	0.19	24.4	13.24	132.42
	LSD, p=0.05	0.15	0.01	0.12	0.04	0.03	0.07	5.09	2.04	9.98
	CV (%)	8.87	13.3	16.4	13.24	23.43	79.1	44.92	33.19	16.22

S.D=Standard deviation.

were determined by the method of AOAC (*36*) using an inductive couple plasma emission spectrophotometer (ICPE) (ICPE 9000 Series, Kyoto, Japan).

Data analysis

The data obtained were subjected to analysis of variance (ANOVA) as RCBD using a SAS programme (*37-39*).

RESULTS AND DISCUSSION

There were significant variations in the nutrients in non-aerated green teas due to nitrogen fertilizer rates and season (Table 1 and Figures 1 and 2). The levels of nitrogen in non-aerated green tea increased with the increase in rates of nitrogenous fertilizer regardless of the season or month. This pattern was displayed from the months of October to June but there was a shift in the months of July to September at 400 and 800kg N/ha/year (Table 1). A similar increase had been observed in mature leaf nitrogen contents for clonal (*40-43*) and seedling teas (*44, 45*). The results demonstrate that there is high removal of nitrogen nutrient when high rates of nitrogen is applied. However, such high removal peak was registered at about 400 kg N/ha/year. The K and P in non-aerated green tea content increased at low rates of nitrogen. At high rates, the pattern was less clear. In mature leaf a general decline in K (*40-42, 44, 46*) and P (*40-42, 44*)

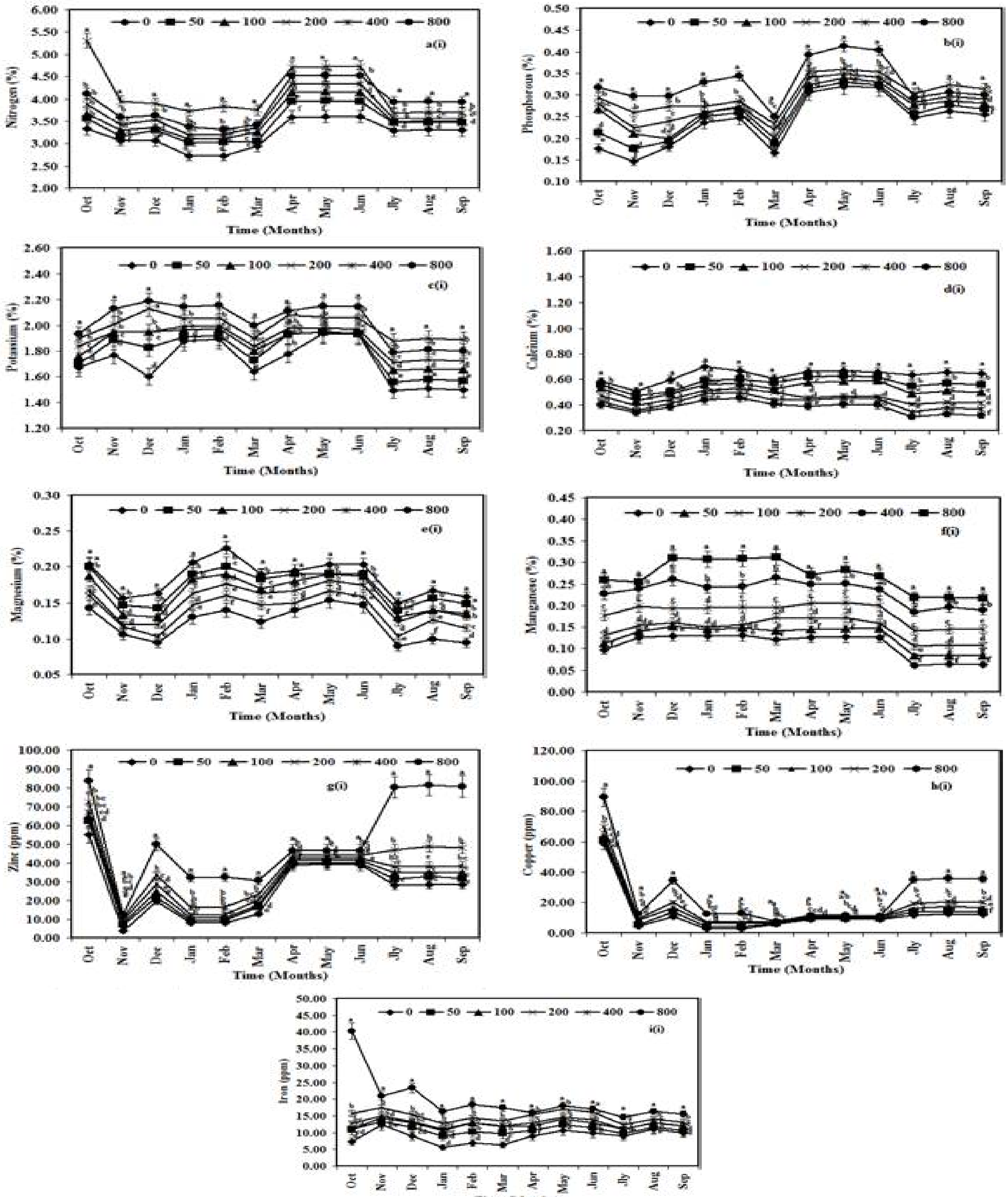

Fig. 1: Elements profiles of non-aerated green tea processed from seedling tea cultivated under varying rates of nitrogen fertilizer (kg N) in year 1 (2011-2012) October to September. The vertical bars represent Standard deviation (S.D) of the mean of 3 replicates.

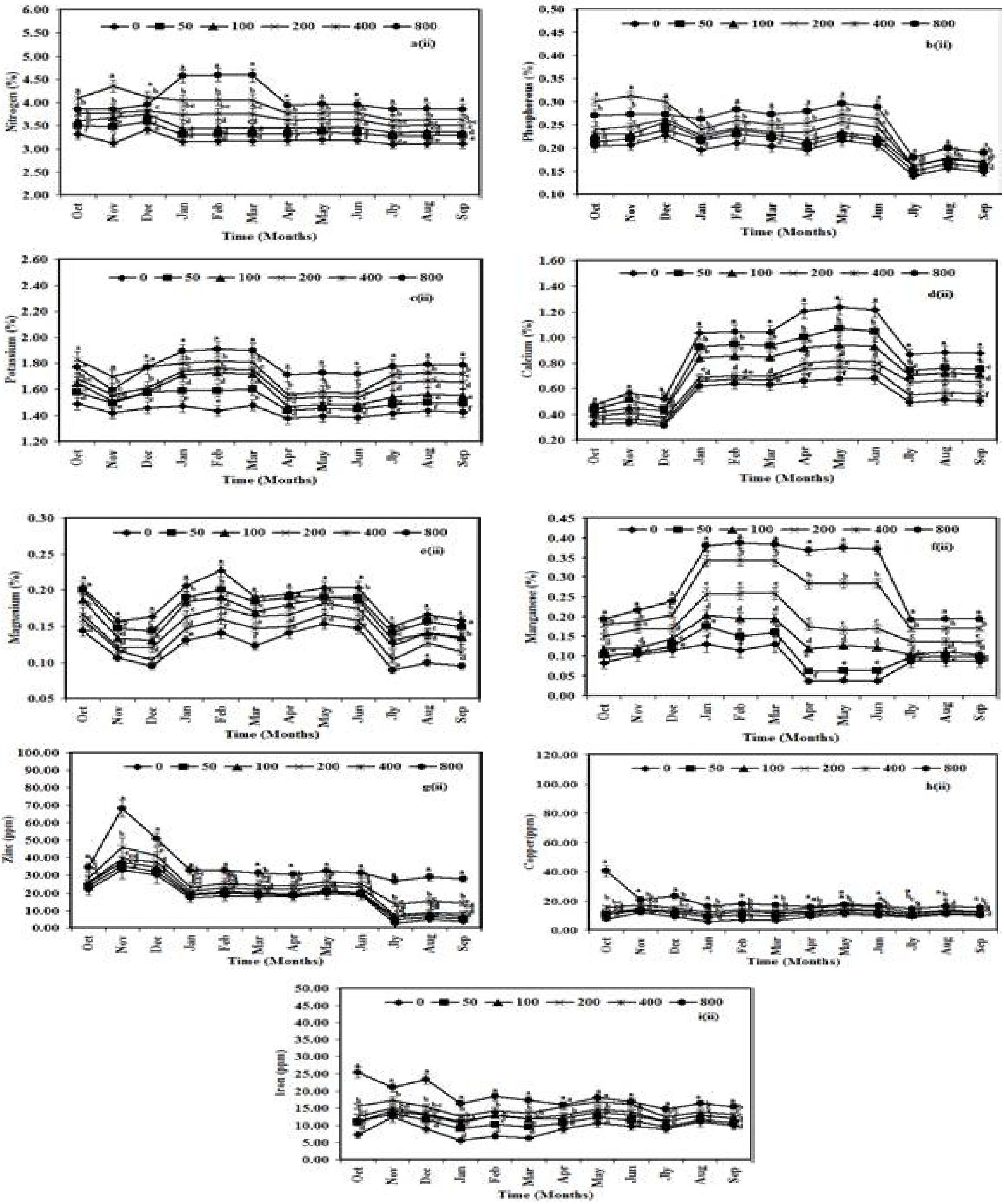

Fig. 2: Elements profiles of non-aerated green tea processed from seedling tea cultivated under varying rates of nitrogen fertilizer (kg N) in year 2 (2012-2013) October to September. The vertical bars represent Standard deviation (S.D) of the mean of 3 replicates.

Table 2: Monthly changes in elements profile of non-aerated green tea for 2 years (2011/2012 and 2012/13)

Year	Month	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (%)	Zn (ppm)	Cu (ppm)	Fe (ppm)
2011-2012	Oct	3.86±0.88	0.26±0.05	1.80±0.10	0.50±0.07	0.18±0.02	0.17±0.06	84.94±32.54	84.67±52.10	73.28±18.05
	Nov	3.41±0.30	0.22±0.05	2.51±2.36	0.42±0.06	0.13±0.02	0.19±0.05	8.72±3.21	9.06±5.73	284.11±34.73
	Jan	3.46±0.28	0.23±0.05	1.94±0.21	0.47±0.08	0.12±0.03	0.20±0.07	29.78±10.62	20.89±9.41	104.56±19.27
	Feb	3.19±0.36	0.27±0.04	2.09±0.46	0.55±0.09	0.17±0.04	0.20±0.08	14.83±10.33	6.83±3.38	363.39±55.98
	Mar	3.20±0.37	0.28±0.04	2.10±0.46	0.56±0.09	0.19±0.05	0.20±0.08	15.01±10.33	6.99±3.38	363.52±55.98
	Apr	3.30±0.29	0.21±0.03	1.82±0.13	0.51±0.07	0.16±0.02	0.20±0.07	20.30±6.11	6.76±0.65	324.37±59.01
	May	4.21±0.38	0.34±0.03	1.97±0.15	0.53±0.11	0.17±0.02	0.21±0.11	42.31±2.70	10.25±0.95	128.14±19.37
	Jun	4.22±0.39	0.35±0.03	1.98±0.15	0.54±0.11	0.18±0.02	0.20±0.07	42.48±2.60	10.36±0.93	128.26±19.36
	July	4.21±0.38	0.34±0.03	1.98±0.15	0.53±0.11	0.18±0.02	0.19±0.05	42.39±2.64	10.31±0.93	128.21±19.35
	Aug	3.57±0.22	0.28±0.02	1.68±0.14	0.45±0.12	0.12±0.02	0.13±0.06	55.44±30.28	19.94±8.13	121.39±14.77
	Sep	3.59±0.22	0.29±0.02	1.70±0.14	0.47±0.12	0.14±0.02	0.14±0.06	56.44±30.22	20.84±8.11	122.42±14.37
	Oct	3.58±0.22	0.28±0.02	1.69±0.14	0.46±0.12	0.13±0.02	0.13±0.06	55.94±30.25	20.39±8.11	121.90±14.56
	Mean	3.65	0.28	1.94	0.5	0.16	0.18	39.05	18.94	188.63
	LSD, p=0.05	0.25	0.03	0.47	0.05	0.02	0.03	12.36	10.24	20.01
	CV (%)	10.44	13.12	37.21	14.52	14.47	27.65	48.15	82.27	16.14
S.D=Standard deviation.										
2012-2013	Oct	3.69±0.26	0.24±0.04	1.67±0.12	0.40±0.05	0.13±0.02	0.14±0.05	37.00±27.29	16.33±11.59	67.06±12.34
	Nov	3.71±0.40	0.25±0.04	1.54±0.10	0.42±0.11	0.16±0.02	0.15±0.05	43.22±12.4	15.56±3.09	77.61±10.81
	Dec	3.77±0.24	0.26±0.03	1.63±0.12	0.40±0.08	0.16±0.02	0.17±0.05	37.67±7.17	14.28±4.90	66.00±6.94
	Jan	3.71±0.54	0.23±0.02	1.56±0.51	0.79±0.16	0.38±0.04	0.25±0.09	22.06±5.83	10.89±3.57	135.44±38.62
	Feb	3.72±0.54	0.24±0.03	1.57±0.51	0.81±0.16	0.40±0.04	0.35±0.44	23.78±5.93	12.67±3.79	137.39±38.61
	Mar	3.71±0.54	0.24±0.02	1.57±0.51	0.80±0.16	0.39±0.04	0.30±0.23	22.91±5.88	11.78±3.68	136.42±38.62
	Apr	3.54±0.28	0.23±0.03	1.51±0.11	0.89±0.19	0.42±0.03	0.17±0.12	22.05±4.53	12.67±2.61	63.22±14.84
	May	3.57±0.27	0.25±0.03	1.53±0.11	0.92±0.20	0.44±0.03	0.18±0.12	24.11±4.60	14.44±2.68	65.22±14.84
	Jun	3.56±0.28	0.24±0.03	1.52±0.11	0.91±0.19	0.43±0.03	0.18±0.12	23.08±4.56	13.56±2.64	64.22±14.84
	July	3.45±0.27	0.16±0.03	1.60±0.13	0.67±0.13	0.25±0.16	0.13±0.05	11.39±11.75	11.28±1.93	260.56±22.09
	Aug	3.47±0.27	0.18±0.03	1.62±0.13	0.69±0.13	0.27±0.16	0.13±0.05	13.18±11.95	13.17±1.82	257.06±35.83
	Sep	3.46±0.27	0.17±0.03	1.61±0.13	0.68±0.13	0.26±0.16	0.13±0.05	12.28±11.85	12.22±1.87	258.81±27.33
	Mean	3.61	0.23	1.58	0.7	0.31	0.19	24.4	13.24	132.42
	LSD, p=0.05	0.21	0.02	0.17	0.06	0.05	0.1	7.2	2.89	14.12
	CV (%)	8.87	13.3	16.4	13.24	23.43	79.1	44.92	33.19	16.22

had been observed. This was despite the concurrent rise in the nutrients with increase in rates of nitrogen in the NPKS fertilizer formulation used in the studies. The decline in non-aerated green tea K levels at high rates of nitrogen fertilizer arose from leaching of the nutrients (*24, 46*), while P levels declined due to fixation arising from increased soil acidity (*24, 42*). Although high rates of nitrogenous fertilizer improves non-aerated green tea quality (*47*), this may change soil chemical properties making long term tea production unsustainable. Increase in the rate of nitrogenous fertilizer also significantly increased (pd”0.05) Mn and Fe in non-aerated green tea (Table 2). Similar increase in Mn had been observed in mature leaf (*48*) and were attributed to increased availability of the nutrient with increase in soil acidity. However, as observed in mature leaf, Ca and Mg levels declined (*48, 49*) in non-aerated green tea due to high rates of nitrogenous fertilizer. While Ca levels decreased due to fixation caused by increased acidity, Mg levels decreased

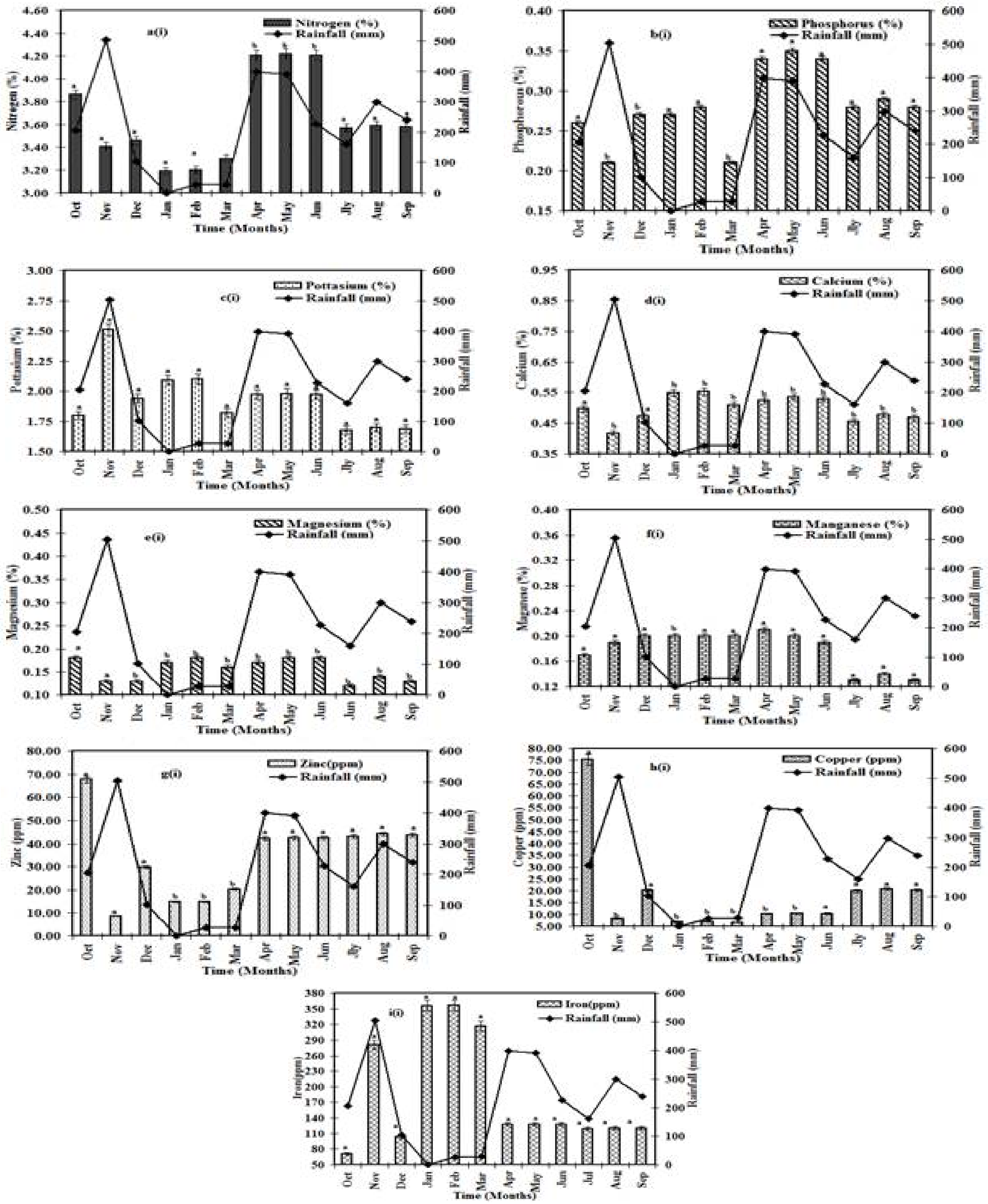

Fig. 3: Elements profiles of non-aerated green tea processed from seedling tea cultivated under varying rainfall patterns in year 1 (2011-2012) October to September. The vertical bars represent standard deviation (S.D) of the mean of 3 replicates.

Table 3: Summary of rainfall and temperature recordings (October 2011-September 2013) at Timbilil Estate, Kericho.

Month	Year 2011		Year 2012		Year 2013	
	Rainfall (mm)	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)	Temperature (°C)
January			0	16.7	114.9	16.7
February			26.8	17.7	12.4	16.5
March			27.7	18.0	217.0	17.0
April			398.4	15.3	449.9	16.1
May			391.1	16.4	349.0	15.4
June			226.9	16.0	237.2	15.1
July			160.9	15.7	105.1	16.2
August			298.9	16.1	111.9	15.2
September			239.1	15.7	130.6	15.8
October	204.8	16.8	269.4	16.9		
November	503.7	16.4	227.6	16.9		
December	103.3	17.1	172.3	16.4		

as a result of enhanced leaching triggered by excess ammonium ions in the high rates of N (*24*). Although Zn and Cu also significantly varied due to nitrogenous fertilizer rates, the patterns were not systematic. This aspect requires further experimentation to create a good understanding of the response. The data presented here, demonstrate that nitrogenous fertilizer application rates is a major factor that influence the amounts of these nutrients removed in non-aerated green tea.

There were monthly and seasonal variations in the levels of all nutrients monitored (Tables 1 and 2). The monthly changes in the pattern of responses to nitrogenous fertilizers rates for the individual nutrients followed the same patterns. Thus, although the annual fertilizer was applied in October, the effect was felt throughout the year. Similar effects had been observed on the yields of seedling tea (*33, 50*). The results suggest that annual fertilizer application may not be necessary in Kenya. Indeed, longer intervals of application of nitrogenous fertilizer of up to 24 months did not cause significant differences (pd”0.05) in soil chemical properties (*44*) and yields.

There are four distinct seasons in Kenya tea growing areas modified from (*34*). The recently distinguished recurring seasons from weather include warm and wet in October to December, warm and dry in January to March, cold and wet in April to June and cold and dry from July to September. The changes in the nutrient contents of non-aerated green teas with time of the year are presented in Table 2 and figures 2 to 4. Significant (pd┐ 0.05) monthly and seasonal variations in the nutrients were observed (Table 2). The different seasons were also characterized with varying amounts of rainfall and temperature patterns (Table 3). Generally high rainfall resulted in increments of non-aerated green leaf nutrient contents. Similar results had been observed in studies carried out in different countries (*51-54*). Soil moisture status also influences polyphenol contents of tea (*55*). The rainfall pattern followed a similar pattern as that followed by some macro and micro-nutrients and polyphenols (*56*). These results suggest that these nutrients play important roles in the biosynthesis of the polyphenols and especially catechins in tea.

The rainfall and temperature values are means from the days of individual months recorded during the research period.

Apart from the use of these nutrients to promote tea growth and quality, the macro and micronutrients in tea are also sources of dietary minerals to tea consumers. The tea bush is known to accumulate trace elements. Aerated black tea from different countries have varying levels of Ca, Na, Mg, K, Mn, Cr, Fe, Co, Ni, Cu, Zn and Cd (*57*). Some of the nutrients are beneficial to human health while a number can be toxic at high levels. Results presented in this study show that non-aerated green teas from seedling tea contains some of these minerals. Indeed drinking tea can be a major source of Mn and Zn (*58, 59*). Regular consumption of non-aerated

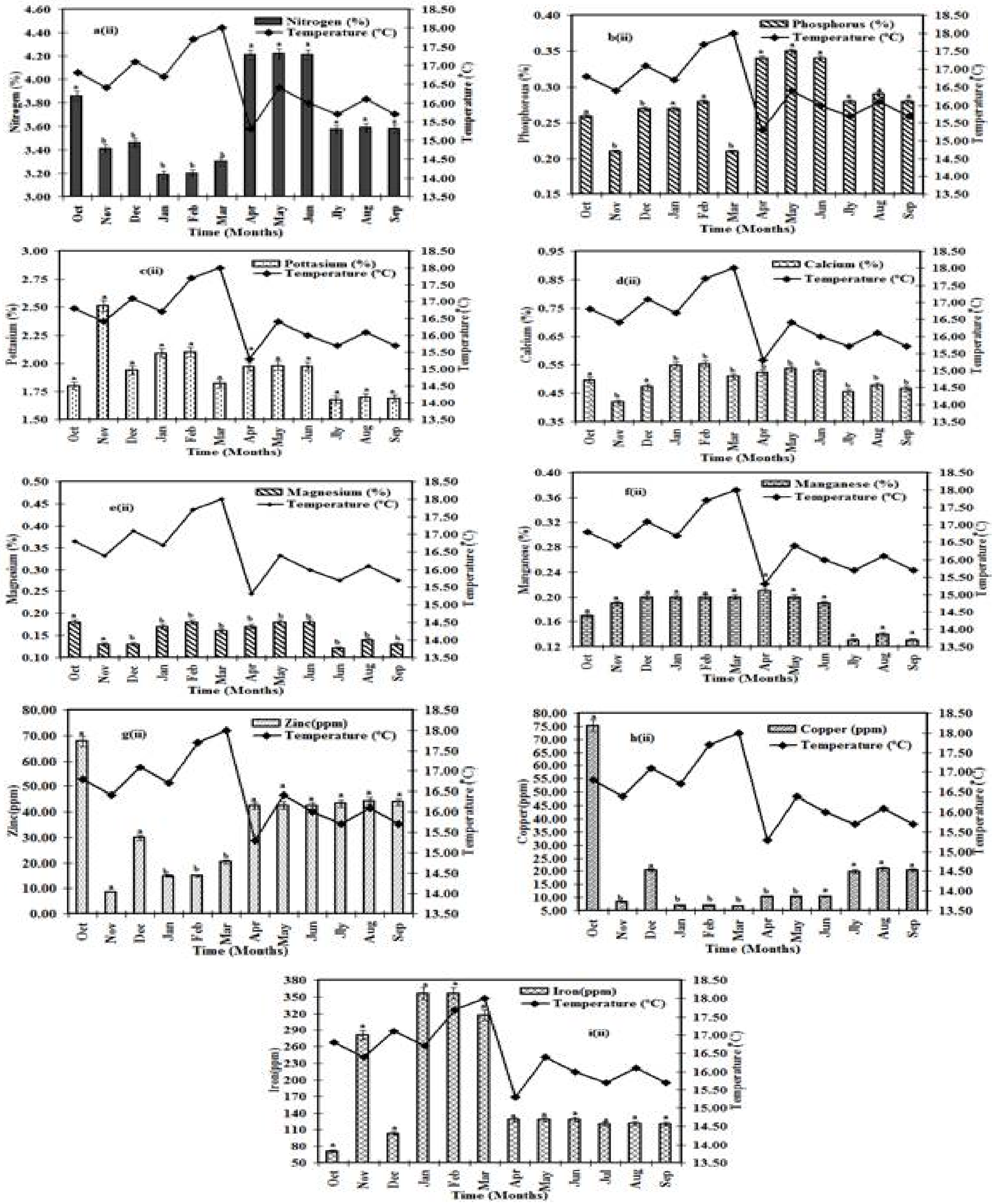

Fig. 4: Elements profiles of non-aerated green tea processed from seedling tea cultivated under varying temperature patterns in year 1 (2011-2012) October to September. The vertical bars represent Standard deviation (S.D) of the mean of 3 replicates.

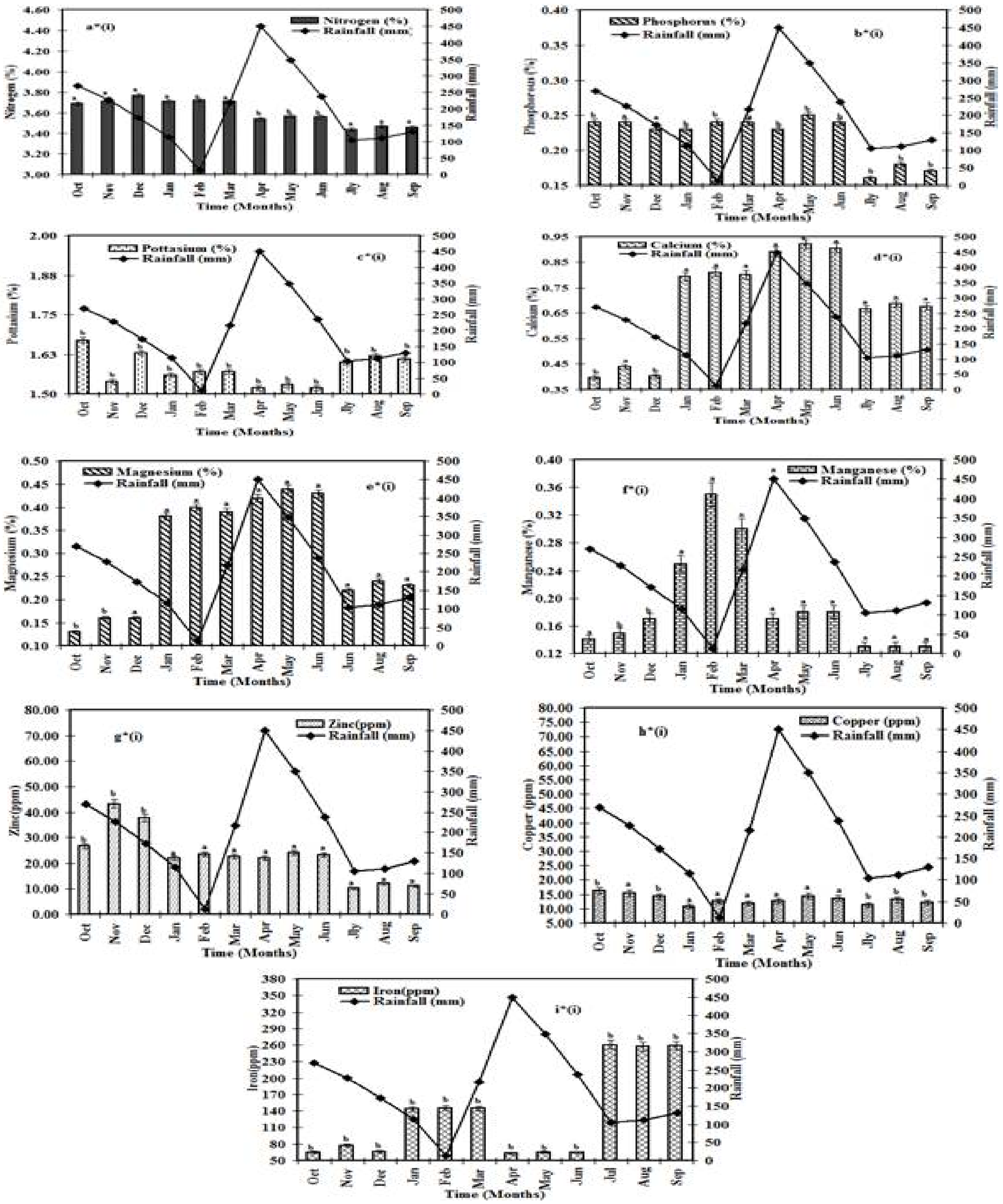

Fig. 5: Elements profiles of non-aerated green tea processed from seedling tea cultivated under varying rainfall patterns in year 2 (2012-2013) October to September. The vertical bars represent Standard deviation (S.D) of the mean of 3 replicates.

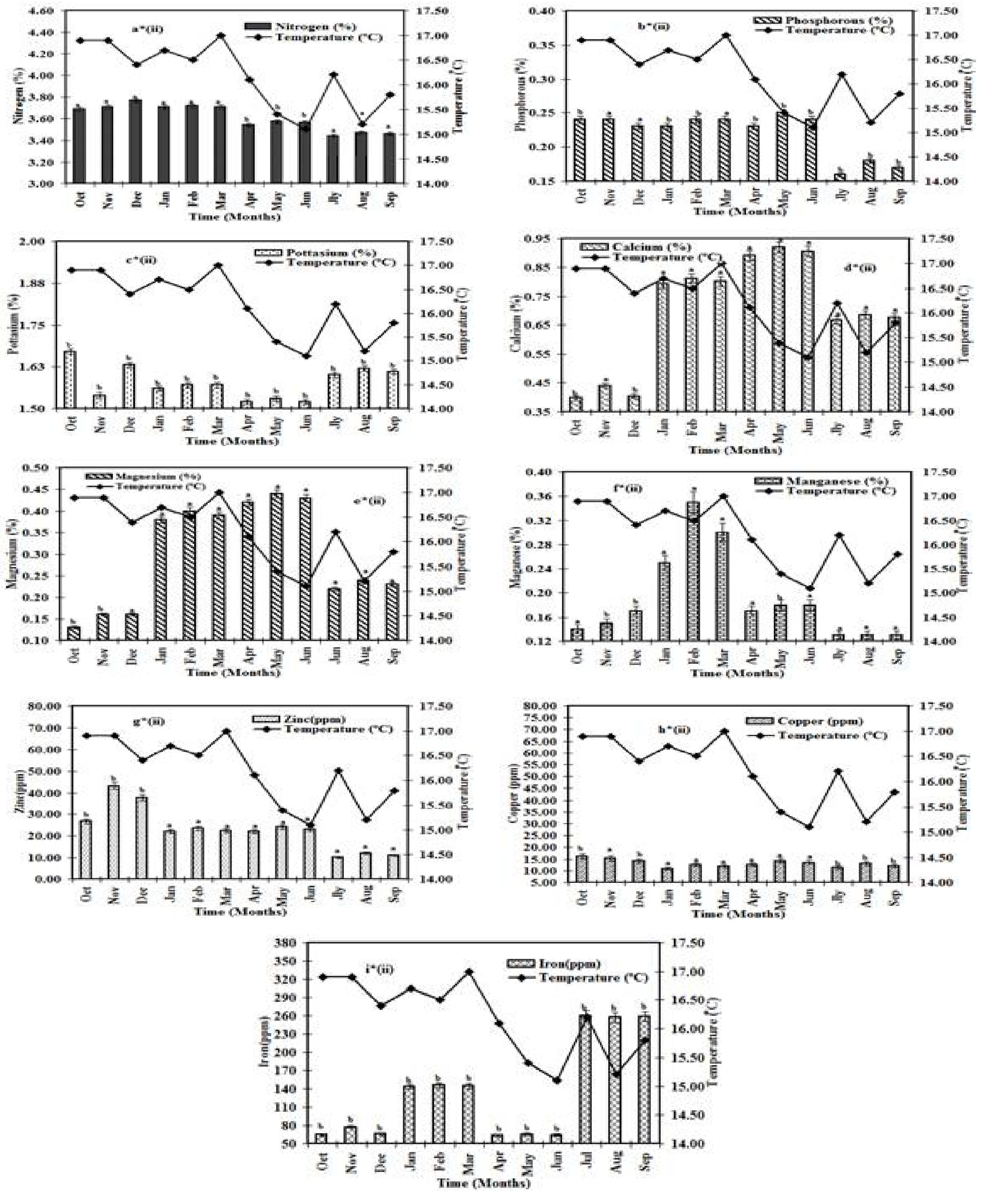

Fig. 6: Elements profiles of non-aerated tea processed from seedling tea cultivated under varying temperature in year 2 (2012-2013) October to September. The vertical bars represent Standard deviation (S.D) of the mean of 3 replicates.

green tea can therefore be a significant source of the minerals.

CONCLUSION

In conclusion, levels on nutrients in non-aerated green teas from seedling tea plantations vary with increasing rates of nitrogenous fertilizer. While N and Mn increased with high rates of nitrogen fertilizer, Ca and Mg declined. K and P levels increased at low fertilizer rates. However, the levels reached a peak after which the nutrients declined with higher rates of nitrogen fertilizer. Such variations persist throughout the year and are influenced by changes in seasonal weather parameters. Availability of adequate soil moisture, through rainfall increased the minerals uptake and levels in non-aerated green tea. For sustainable green tea production, replenishment of these minerals are necessary.

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