



RESEARCH ARTICLE

The effects of fertilization on the green tea elements

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ABSTRACT

Camellia sinensis, which is widely used as a beverage in our country and in the world, has various beneficial effects on human health due to its various components. Farmers use chemical fertilizers to get more products. However, the use of more chemical manure may cause some problems in terms of environmental pollution and human health. Due to the lack of some nutritious minerals in the soil, various manure ingredients are used for better cultivation and growth. In this study, in order to investigate the effect of use of manurate on tea plant on 5 different soils in Rize, the concentrations of 18 elements in the leaves were analyzed using the ICP-MS device. When manure was used in tea, the concentration of Li, Mg, K, Al, Ca, Cr, Mn, Fe, Cu, Zn, As, Se, Cd, Pb elements increased and Na, Co, Ni, Hg values decreased.

Keywords: *Camelia sinensis*, element, environmental pollution, green tea, ICP MS, manure

1. INTRODUCTION

The tea plant is a perennial plant of the genus *Camellia* of the Theaceae family, which is green in all seasons, usually grown in high regions. Tea is the second most commonly drank liquid on earth after water. It is being consumed socially and habitually by people since 3000 BC [1]. The tea plant is grown in at least 30 countries, in various parts of Asia, Africa and the Middle East. There are 2 varieties of *Camellia sinensis*, *Camellia sinensis* variety tea (Chinese tea) grown in China, Japan and Taiwan and *Camellia sinensis* variety *assamica* (Assam tea) which is common in south and southeast Asia. Chinese tea varieties are grown in Turkey as well [1, 2].

Green tea contains many ingredients; such as enzymes, polyphenols, alkaloids, nitrogen compounds, caffeine, essential oils, carbohydrates, pigments, vitamins, organic acids, aroma-forming substances, and minerals [3-5]. Epigallocatechin gallate (EGCG), a group of catechins, is a very effective antioxidant, believed to be a crucial substance in the therapeutic properties of green tea [6, 7]. In addition to green tea is a medical plant widely used by local people in India, China, Japan and Thailand for a long time [8, 9]. From this point on; some of the ingredients obtained from the tea are said to be beneficial to human health [1].

It was determined that tea plants have antioxidant, anticancer, antiaging, and anti-inflammatory effects [10-12]. Several studies have tried to prove that regular use of green tea is preventive against some chronic diseases. Green tea is protective against coronary heart diseases, diabetes [13], various types of cancer such as stomach and colorectal [8, 14], breast, throat, prostate [15]. Also, green tea has been shown to be effective in regulating bone density, preventing obesity, protecting against UV rays, preventing hemolysis, aging and cognitive disorders [8, 14].

In this study, it is aimed to determine the effect of the fertilization on concentration of 18 elements in the green tea leaves grown at 5 different region in Rize, Turkey. This study further investigate decreasing the amount of chemical fertilizers or searching alternative fertilizer methods.

2. MATERIALS & METHOD

2.1. Supply of plant samples

Rize; in the highest part of the Eastern Black Sea region 400 20' east and 410 20' between the northern latitudes, is located in northeastern Turkey. The pH of

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the soil where green tea is collected is around 3.14-5.88. The average annual rainfall over 2000 mm, the dispersion of rain by month should be regular, at least 70 % relative humidity and soil. Tea is collected three times in a year. First collection is in May, second is in July, and the third is in August-September. Green tea with and without manure in the soil were collected in five separate regions in an area of 2 kilometer from-Rize, in Turkey (5 fertilized and 5 unfertilized tea leaves were taken from this region (Table 1)). Manure content is that: N, NH₄-N, P, K, in 50 kg package. 60-70 kg of manure per hectare is applied at the end of March.

Table 1. Situation of manure in the tea fields

Field	Situation of fertilization
1	10 years fertilized
2	10 years fertilized
3	10 years fertilized
4	10 years fertilized
5	10 years fertilized
6	10 years unfertilized
7	10 years unfertilized
8	10 years unfertilized
9	10 years unfertilized
10	10 years unfertilized

Green tea samples (five) used in our tests were collected from a tea garden at the first collection periods in May 2018. Leaves on top branches of tea plants were collected, placed in a sealed plastic bag and immediately transferred to laboratory. Before extraction, an extensive literature search was carried out to find out the most effective and suitable extraction procedure. Green tea leaves were liofilised by Telstar LyoQuest (P: 0.2 milibar, 24 hours) and stored -20°C. Situation of manure in the tea fields are detailed in Table 1.

2.2. Preparation of ICP-MS Standards and Solutions

Firstly, for the measurement of Co, Se, Na, Mg, K, Ca, Mn, Fe, Cu, Zn, Al in tea leaves standard solutions were prepared. For the measurement of alkali metals (Ca, Mg, Na, K) to be healthy, standards should be prepared as ppm (mg/L) and others (Cd, Pb, Hg, As, Fe, Mn, Co, Ni, Cu, Zn, Al, Cr, Ag, Se, V, Be, Ba, Tl, U, Ga, Li, Rb, Cs, Sr, Sb) should be prepared in ppb ($\mu\text{g/L}$) level. The standards used are as follows: 1000 ppm ($\text{mg L}^{-1} = \mu\text{g mL}^{-1}$) Ca, Mg, Zn, Sb :10 ppm Mix Cd, Pb, As, Fe, Mn, Co, Ni, Cu, Zn, Al, Cr, Ag, Se, V, Be, Ba, Tl, U, Ga, Li, Na, Ca, Mg, K, Rb, Cs, Sr : 10ppm Hg.

HNO₃ %1: Ultra-pure water is filled in half of a 250 ml glass tube. 2.4 ml HNO₃ added to the line up to 220 ml with pure water. Preparation of 1ppm stock solution containing 27 elements and 100 ppb Hg: 1mL 10 ppm Agilent 8500-6940 2A mixture and 0.1mL 10 ppm Agilent 8500-69400-Hg is added to the test tube. Then 8ml pure water is added on it. Preparation of 100 ppm Ca+Mg stock solution: 1mL Ca 1000 ppm ($\mu\text{g mL}^{-1}$, mg

L⁻¹) (Merck170308) mixture and 1mL 1000 ppm Mg (Merck170331) is added to the test tube. Then 8ml pure water is added on it.

In this study, green tea samples were collected from an area of throughly 600-650 meters above sea level in Rize. Green tea with and without manure in the soil were collected in five separate regions in an area of 2 kilometer.

2.3. ICP MS test procedure

Fresh tea leaves were dried for 24 hours and then placed in a microwave. Microwave acid digestion procedure was as follows: Place a TFM vessel on the balance plate, tare it and weight of the sample. Introduce the TFM vessel into the HTC safety shield. Add the acids; if part of the sample stays on the inner wall of the TFM vessel, wet it by adding acids drop by drop, then gently swirl the solution to homogenize the sample with the acids. Close the vessel and introduce it into the rotor segment, then tighten by using the torque wrench. Insert the segment into the microwave cavity and connect the temperature sensor. Run the microwave program to completion. Cool the rotary by air or by water until the solution reaches room temperature. Open the vessel and transfer the solution to a marked flask.

Measurements of multi elements in the tea leaves were performed using the Agilent 7700x ICP-MS. The samples were directly put into the ICP-MS system using a standard peristaltic pump with Tygon pump tube (internal diameter of 1.02 mm) and ASX-520 automatic sampler. Analyses were executed in time resolve analysis mode using an integration time of 3 ms for all stages. A rinse solution containing 1% nitric acid was used to provide sample washing during each stage. The general settings of the Agilent 7700x system are detailed in Table 2. Our analysis conforms to the EPA 6020 standard.

Table 2. Agilent 7700x operating conditions

Parameter	Value
Forward power	1550 W
Carrier gas flow rate	0.8 L min ⁻¹
Make-up gas flow rate	0.32 L min ⁻¹
Spray chamber temperature	2 °C
Sampling depth	8.0 mm
ORS ³ helium gas flow rate	5.0 mL min ⁻¹

3. RESULTS & DISCUSSION

Environmental pollution is one of the most important factors that threaten life. Unconsciously used chemical fertilizers for more products in agriculture threaten the all living life balance. On the other hand, this chemical manure content has a negative effect on substance cycles in nature. These two basic problems cause us to think seriously about plant growth and the use of chemical manure for more products.

Black tea is a very popular drink in our country. In other words; the tea in the cup is consumed more by the people, in some parts of our country than the water in the cup in some periods of their lives. In this study, the concentrations of 18 elements in the leaves

were analyzed by using the ICP-MS device in order to investigate the effect of use of manure on tea plant on 5 different soils in Rize. The results of multi-element analysis in green tea leaf samples are given in the table below (Table 3).

Table 3. Data obtained in the ICP-MS system for tea samples (Conc. ($\mu\text{g kg}^{-1}$))

Tea Sample	Li (7) ($\mu\text{g kg}^{-1}$)	Na (23) ($\mu\text{g kg}^{-1}$)	Mg (24) ($\mu\text{g kg}^{-1}$)	Al (27) ($\mu\text{g kg}^{-1}$)	K (39) ($\mu\text{g kg}^{-1}$)	Ca (43) ($\mu\text{g kg}^{-1}$)
1	538.31	48261.53	2858044.18	1433023.60	9430681.03	4319273.87
2	696.97	365311.74	3703355.89	1196247.78	10820696	5739499.9
3	915.39	37036.43	3054146.19	2120700.14	8954240.97	9133760.17
4	631.95	56964.90	4712793.90	1832648.62	13737686.45	4759987.55
5	855.69	90178.81	7649338.47	2322422.2	13328255.74	14332095.96
6	1249.88	84986.14	6410117.56	2128400.91	OR	10181722
7	1395.75	71966.72	4832051.20	2113987.87	13097179.86	9829438.59
8	1418.62	72487.78	4668564.63	2449992.61	13626183.08	8345042.71
9	1119.53	84070.92	5324989.32	2223668.84	OR	8362212.11
10	1015.54	86106.70	5408671.66	4959969.27	15901676.71	11947958.53
Tea Sample	Cr (52) ($\mu\text{g kg}^{-1}$)	Mn (55) ($\mu\text{g kg}^{-1}$)	Fe (56) ($\mu\text{g kg}^{-1}$)	Co (59) ($\mu\text{g kg}^{-1}$)	Ni (60) ($\mu\text{g kg}^{-1}$)	Cu (63) ($\mu\text{g kg}^{-1}$)
1	239.74	662183.89	169897.82	384.45	2507.57	5734.8
2	263.42	966069.37	197261.76	1341.56	17348.78	9495.98
3	239.82	2265836.92	155366.13	1447.82	6611.97	7700.24
4	208.13	1220090.79	145927.31	600.76	4424.51	9947.87
5	459.2	2542646.86	374634.94	565.0	6777.47	14350.9
6	544.28	2210857.5	360111.3	1991.41	9153.17	12074.01
7	306.14	1401290.32	253158.02	526.02	5491.92	7651.1
8	529.83	1329223.5	295800.81	462.74	8608.98	10111.01
9	497.27	1865493.72	288011.43	442.35	7011.39	11825.69
10	483.25	1492773.55	354691.9	303.29	3335.16	8392.27
Tea Sample	Zn (66) ($\mu\text{g kg}^{-1}$)	As (75) ($\mu\text{g kg}^{-1}$)	Se (82) ($\mu\text{g kg}^{-1}$)	Cd (111) ($\mu\text{g kg}^{-1}$)	Hg (201) ($\mu\text{g kg}^{-1}$)	Pb (206) ($\mu\text{g kg}^{-1}$)
1	23143.03	47.31	112.04	40.87	266.08	242.7
2	47753.29	46.72	122.48	58.73	165.25	255.93
3	23564.54	53.48	91.15	69.95	138.08	159.78
4	39132.2	63.47	120.29	52.79	144.09	146.23
5	88585.36	78.26	121.29	47.34	218.03	281.97
6	72518.39	66.04	149.09	142.49	189.09	358.4
7	43158.06	69.32	119.88	101.0	162.74	359.72
8	32342.94	68.95	121.85	58.36	192.36	475.53
9	46319.52	84.03	127.5	49.93	147.62	363.98
10	45629.9	91.65	156.12	83.63	142.05	561.65

When manure was used in tea, the concentration of Li, Mg, K, Al, Ca, Cr, Mn, Fe, Cu, Zn, As, Se, Cd, Pb elements increased and Na, Co, Ni, Hg values decreased (Fig 1) (Fig 2). As a result of the use of manure required for

tea, a change was observed due to the binding of minerals.

Taskin and his colleagues (2015) stated the following results regarding a study: K; about 21. 106 ($\mu\text{g kg}^{-1}$), Ca; about 1685. 105 ($\mu\text{g kg}^{-1}$), Mg; about 22. 106 ($\mu\text{g kg}^{-1}$)

kg⁻¹). In our study; concentration of K was measured at lower than this value in tea with manured, concentration of Ca was measured in some places less than this value and concentrations of Magnesium measured at lower than this value (Fig 3) (Taskin et al. 2015). The graph of our study of Ca, Mg and K values is shown below.

The comparison of the average values of the 18 elements in the green tea leaf with and without manured is given in the graph below (Fig 4). Decrease in concentration of elements; it can be attributed to the inability of the tea plant to get them enough from the soil.

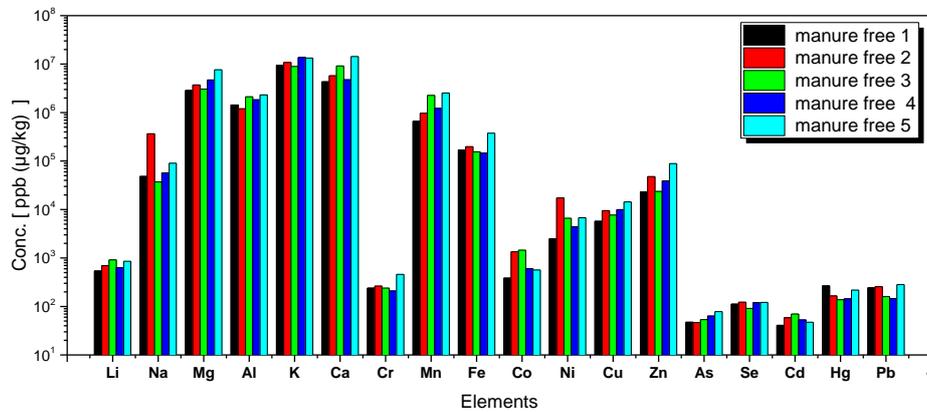


Fig 1. The element concentrations in the tea grown in unfertilized soils

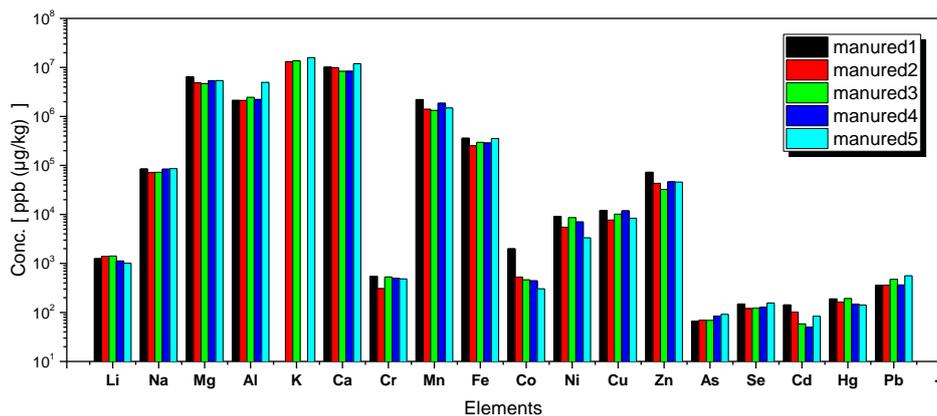


Fig 2. The element concentrations in the tea grown in fertilized soils

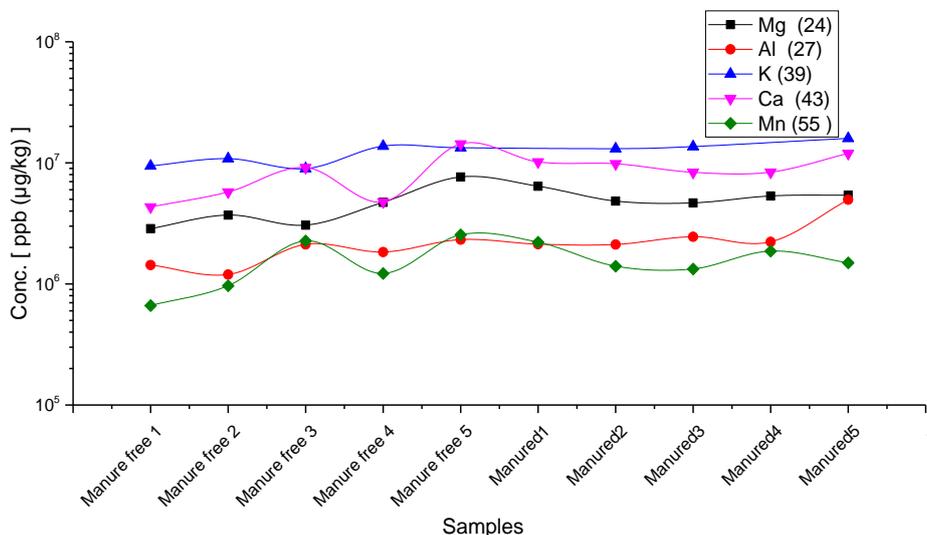


Fig 3. The changes in some elements concentrations in tea samples

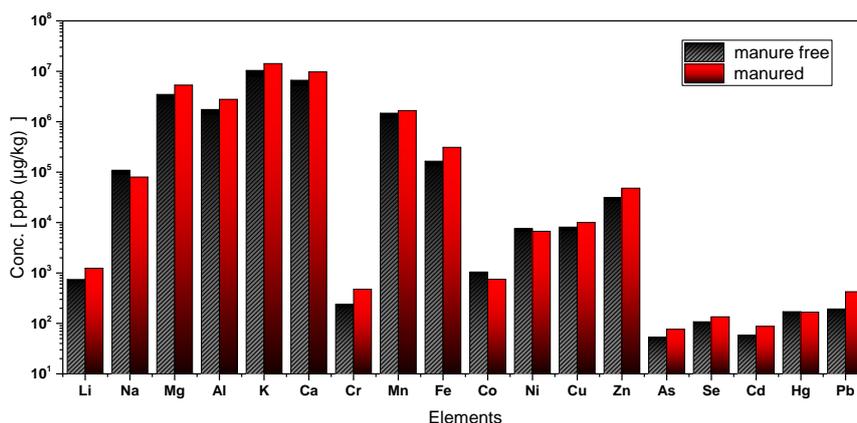


Fig 4. Comparison of element concentrations in two tea groups

4. CONCLUSIONS

The difference in the physical growth of this chemical fertilizer usage to the tea plant should be compared very well in terms of human health and benefit and harm. When this aspect of the study is examined, more and different studies are required. The increase in the concentration of elements in green tea leaves may suggest that the soil may be rich in these elements or may be due to use of manurate. After this study, a larger scale study will be planned. It is designed to include examples of the three tea cultivation periods (May, July, August-September) from other cities of the Black Sea region and the use of other manurate samples.

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