Comparative Study of Mineral Content in Different Varieties of Green Tea

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Abstract. *Camellia sinensis* L. leaves composed of different concentrations of mineral contents play a vital role in human nutrition and health. In this study, locally processed three different green tea varieties i.e. P3, P5 and P9 were used for mineral content determination at National Tea and High Value Crops Research Institute (NTHRI), Shinkiari, Mansehra, Pakistan. Atomic absorption spectrophotometer, flame photometer and Kjehldal apparatus were used for the determination of mineral concentration in all the collected tea samples. Mineral composition of tea samples were identified in the following quantity order: high level of nitrogen (37300 to 41380 mg/L), calcium (515.6 to 522.1 mg/L) and phosphorus (742 to 1220 mg/L) were observed in all tea samples compared to other minerals. Cobalt (Co), molybdenum (Mo), sodium (Na), zinc (Zn), nickel (Ni) and copper (Cu) were highest in P3, while least amount was identified in P5, variety. On the contrary, calcium (Ca), potassium (K) and lead (Pb) contents were maximum in P5, while minimum were in P3. This study revealed that the levels of mineral elements in different varieties of green tea vary from the permissible level but the monitoring of their levels in tea plant is obligatory for future risk measurements.

Keywords: *Camellia sinensis* L.*,* varieties, samples, minerals, atomic absorption spectrophotometer

Introduction

Tea is the infused leaves extract of *C. sinensis* L. and one of the most consumable drinks worldwide after water (Xiao *et al*., 2008). Statistical figures showed that average tea consumption throughout the world is 4 fluid ounces per day per capita (Zhu *et al*., 2006). However, per capita consumption of tea in Pakistan is more than one kilogram after the United Kingdom and all of its tea is being imported from the other tea producing countries, proved Pakistan the second largest tea importing country in the world (Latif *et al*., 2008).

Tea minerals are the most important ingredients due to their nutritional and physiological roles. All types of minerals such as sodium (Na$^+$), calcium (Ca$^+$), potassium (K$^+$), manganese (Mn$^+$), magnesium (Mg$^+$), zinc (Zn$^+$), phosphorus (P$^+$) and Iron (Fe$^+$) are present in Tea (Han and Li, 2002). Macro minerals of tea are Mg, Ca, Na, K, P, S, Mn and Al which generally can be found in mg/g level (Herrador *et al*., 2001). On the other hand, micro-minerals of tea are Fe, Cu, Zn, B, Sr, Co, Ni, Rb, Cr, Ba and Ti and generally can be found both in μg/g and mg/g level (Fernandez *et al*., 2002). Most abundant

of these micro-minerals in tea are Zn, Fe and Cu (Seenivasan *et al*., 2008; Shen *et al*., 2008). Research has shown that besides essentially required secondary metabolites and minerals, some toxic elements such as As, Cd, Hg and Pb may also be present in tea leaves (Han *et al*., 2006).

To sustain proper function of human body mineral intake is essential in daily diet. Micro mineral like manganese is required as an essential nutrient for several enzymes to function properly and play a key role for normal bone development, brain function and nervous system (Liang *et al*., 2006). Copper is associated with impaired health in deficiencies as well as in excess. Copper deficiency results bone disorders and anemia (Uauy *et al*., 1998). Zinc is a key element in the majority of the human metabolic pathways. Deficiency of zinc may be responsible for growth retardation, loss of appetite, skin changes and immunological defects (Reddy *et al*., 2007).

When teas are brewed, different chemical compositions including minerals are extracted differentially into infusions, making the infused beverage a vital source of macro and micro mineral constituents (Chen *et al*., 2009). Some nutritionally important minerals are

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essentially required in daily intake on the basis of RDA and tea may be the best source of these important minerals but if minerals levels exceeds from its RDA they may also be toxic to human body, especially in case of accumulation (Han *et al*., 2006). Habitual use of such tea may have adverse effect on human health. Therefore, a detailed study on mineral content of the local tea varieties could be helpful to understand the levels of minerals in these teas and to recommend for daily intake. Unfortunately, no such research on locally grown tea varieties has been conducted before. Hence, the present study was designed with three locally grown tea varieties in Fig. 1 i.e. P3, P5 and P9 to understand the mineral contents and their nutritional and physiological importance in daily human nutrition.

Materials and Methods

All the research work pertaining to field, processing and laboratory for investigations was carried out at National Tea and High Value Crops Research Institute (NTHRI), Shinkiari, Mansehra and COMSATS University of Information and Technology Abbottabad, Pakistan.

Morphological features of selected tea varieties for the study. *P3*. P3 belongs to *Camellia sinensis* and best for green tea. It has minimum inter-nodal distance and numerous plucking points with good canopy. Leaves are narrow, early flusher and the variety is cold and drought tolerant.

***P5*.** P5 belongs to *Camellia sinensis* and best for both black and green tea. Plants possess large shiny leaves with inter-nodal space having 3-6 cm. Physical appearance is very attractive. Good plant frame/canopy and high yielder. Late flusher but sufficient plucking points.

***P9*.** P9 belongs to *Camellia sinensis* and best for both black and green tea. Leaf less shiny than P5 and leaf size intermediate between P3 and P5. It is low yielder compared to P5.

Sample collection and preparation. Fresh leaves of all the 03 varieties were collected and processed for green tea at NTHRI processing factory. Samples were collected randomly for further procedure.

Determination of nitrogen by Kjeldahl apparatus. Nitrogen content was determined by kjeldahl apparatus (VELP UDK 159). First step of nitrogen determination was digestion of samples. 0.25g of each tea was treated

Fig. 1. Tea varieties P3, P5 and P9.

with conc. H_2SO_4 for 3 h until transparent solution was obtained. Followed by distillation, all material was diluted with 250 mL of distilled water and then treated with 10 mL of 40% NaOH in distillation apparatus. Finally titration took place, 2% boric acid obtained in a flask and methyl red added as an indicator, thus ammonia was released. Collected solution was titrated

against 0.1 normal H_2SO_4. Gentle reading was noted manually on Kjeldahl apparatus (AACC (2000)'s method 46-13).

Sample preparation for mineral analysis. *Samples of different varieties of green tea.* Processed green tea leaves of all the 03 varieties (P3, P5 and P9) obtained from NTHRI Shinkiari, Mansehra for making powder were placed in desiccators to prevent gaining moisture.

Digestion of samples for mineral analysis. Dried powdered samples of all the 03 varieties of green tea were digested. One gram of each tea sample obtained in a 100 mL of digestion flask and treated with 20 mL mixture of HCL, H_2SO_4, and HNO_3 in 10:4:1 ration by volume. Initially heated gently and then vigorously.

a. Standard potassium dihydrogen phosphate (KH_2PO_4) solution. A quantity of 2.19 g KH_2PO_4 dried at 105 °C and 1000 mL volumetric flask filled with distilled water +2.19 g + KH_2PO_4 to prepare 500 ppm stock solution.

Sodium and potassium determination by flame photometry. Flame photometer works on the principle that excited atoms of certain element emits a characteristic wave-length, when return to their ground state. This type of emitted radiation is related to kind and amount of the element.

Reagents. *Ammonium acetate.* Glacial acetic acid 1N: 60 mL and 75 mL of ammonium hydroxide (app. 25% NH_3) were diluted to one liter. The pH adjusted to 7.0 by addition of either ammonium hydroxide or acetic acid.

Potassium standard 1000 ppm. Dried KCl 1.9069 g by weigh was dissolved and diluted to 01 L with ammonium acetate.

Sodium standard 1000 ppm. Dried NaCl 2.5419 g by weigh was dissolved and diluted to 01 L with ammonium acetate.

Mineral analysis by atomic absorption spectroscopy. Selected minerals were analyzed by digested sample solution. The mineral elements were magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), calcium (Ca), cobalt (Co), copper (Cu), lead (Pb), nickel (Ni), aluminum (Al) and molybdenum (Mo) were analyzed by atomic absorption spectrophotometer (AAS).

Spectrophotometer determination was based on reaction with a suitable reagent, certain element develop specific colour and intensity. Organic phosphorus treated with ammonium molybdate $((NH_4)_6Mo_7O_{24}\ 4H_2O)$ formed ammonium phosphor-molybdate, which were reduced to made molybdenum blue. Absorbance of sample for the concentration of phosphate was measured at 470 nm of wave length.

Reagents. *Batrons reagent (Mixture A+B).* Solution A. Ammonium molybdate 25 g added into 400 mL of distilled water and mixed before filtered.

Solution B. 300 mL of water was boiled and 1.5 g of ammonium vanadate added in it and left for cooling, 25 mL of conc. HNO_3 was added and cooled again.

Solution (A+B). Solution A poured into B to make volume of 01 L with distill water.

Sulphuric acid. (1:6, acid + water ratio)

Statistical Analysis. The mean and Least Significant Difference (LSD) values were analyzed statistically by using STATISTIX software at 5% level of significance (Steel and Torrie, 1980).

Results and Discussion

Minerals are primary metabolites, play a vital role in growth and development of organisms and responsible for many physiological functions like nervous, skeletal system and also cellular reactions. Millions of the people throughout the world take tea as a beverage so analysis of mineral nutrients becomes critical (Olivier *et al*., 2011). In this study, different macro and micro minerals were identified in leaves of the selected tea varieties which are summarized below.
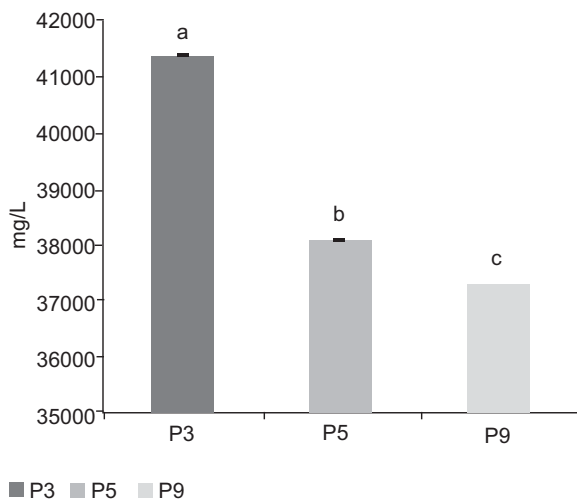
Macro mineral. The results of macro mineral composition of all the 03 different tea varieties (green tea samples) are presented below in Figs. 2-5. Tea basically grown for its vegetative produce has higher concentration of N as compared to other elements. Fig. 2 represented that the N content in different tea varieties (for green tea samples) ranged from 37300 to 41380 mg/L. According to ADI, nitrate ion value is 3.7 mg/Kg of body mass. Among all of the selected tea varieties; P3 had significantly the higher quantity of N content followed by P5 and lowest was in P9.

The level of P ranged from 742 to 1220 mg/L (Fig. 3), whereas RDA recommendation for phosphorus is 3500 mg/L. Results revealed that a higher level of phosphorus was in P3 variety samples as compared to other two varieties while the lowest was observed in P9 variety. This might be due to varietal features and leaf character. Phosphorus is a vital mineral in the human body after

calcium and possesses various functions such as bone mineralization, energy metabolism and framework of DNA and RNA structure (Golub, 2011).

The amount of Ca ranges between 515.6 to 522.1 mg/L in the samples in this study (Fig. 3), whereas comparative values of RDA for calcium is 400 mg/L. From the result it was observed that Ca content was a bit higher in the variety P5 as compared to P9 and P3 but all are comparable to each other. Fresh tea leaves being used for the processing of green tea may be the main reason of higher values of calcium content. However, this value is obtained from the dry green tea samples and in the tea infusion it should be less. Calcium plays a critical role in strengthening of bones, muscle formation and better heart functioning; therefore increased levels of calcium contents would be necessary for human body which can be obtained from tea (Obiajunwa *et al.*, 2002).

Potassium is a fundamental constituent for plant development; it is the cofactor which has functions in protein synthesis and activation of enzymes. In the current study, K concentration varied from 47.97 to 50.00 mg/L (Fig. 4) while RDA value for potassium is 5400 mg/L. Similar to the previous results of Ca, the mean values concerning potassium content (K) in P5 showed slightly higher concentration compared to the other two varieties but they were comparable to each other.

The Na concentration is ranged between 18.00 to 23.68 mg/L in the samples in this study (Fig. 4) and the RDA value is 268000 mg/L. Maximum sodium content was observed in P3 variety green tea samples followed by P5 and P9. However, P3 was comparable with P5 and P5 was comparable with P9. Sodium has vital role in enzymatic processes as cell activator, it is mainly associated with cell membrane function and also necessary for muscle functioning (Constantin and Alexandru, 2011).
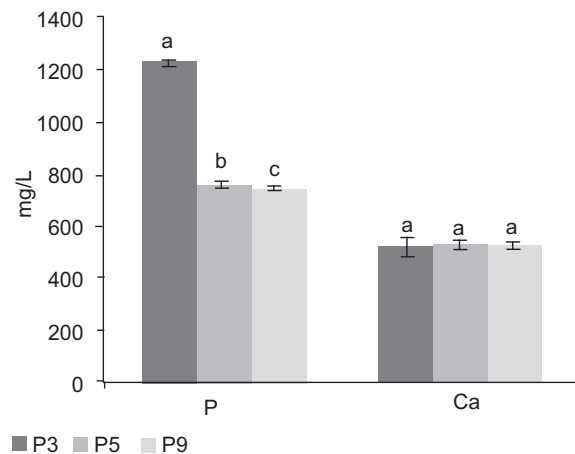
Fig. 3. Level of phosphorus and calcium in green tea samples of different varieties. Data show the mean ± SD. Means denoted by different letter are significantly different (P < 0.05).
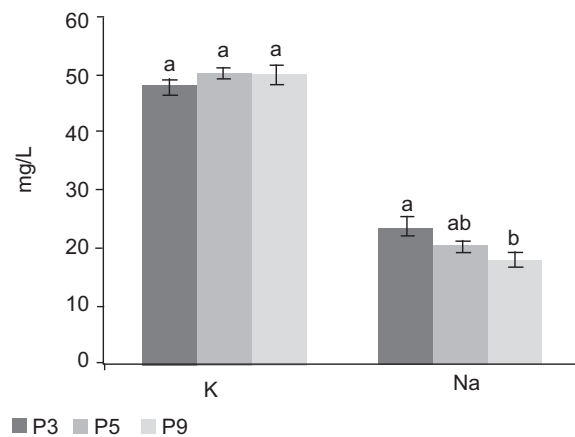

Fig. 2. Nitrogen concentration in green tea samples of different tea varieties. Data show the mean ± SD. Means denoted by different letter are significantly different (P < 0.05).

Fig. 4. Concentration of potassium and sodium in green tea samples of different varieties. Data show the mean ± SD. Means followed by different letter are significantly different (P < 0.05).

The concentration of Mg among all tea varieties varied from 1.425 to 2.080 mg/L (Fig. 5). In P9 tea sample the higher amount of Mg (2.080 mg/L) was observed that was followed by P3 and P5. Magnesium is an indispensable mineral element having role in maintenance of bones and teeth structure and also acts as more than 300 enzymes activator in human body (Romani, 2014).

Micro minerals. The results of micro mineral composition of all the 03 different tea varieties (green tea samples) are presented below in Figs. 6-7. Among the micro minerals aluminum was most abundant in the green tea samples which ranged between 51.65 to 67.50 mg/L (Fig. 6). Data clearly showed that P9 contained significantly higher amount of aluminum (67.50 mg/L) among the three varieties followed by P5 and P3. This result is more or less similar to the findings of Tadayon *et al.*, (2011). A high amount of Al content in the majority of the tea samples investigated is not unexpected because tea plant was acknowledged as high aluminum accumulator due to being acidic in nature. As aluminum is abundantly available in acidic soil, it is the most vital mineral nutrition of tea plant.

WHO set limit for Pb is 10 μg/g, while the findings of the present study showed that the Pb content of the green tea samples ranged between 0.309 to 23.64 mg/L (Fig. 6). P5 tea samples were found with highest Pb concentration (23.64 mg/L) and this concentration is at a risky level. In the P3 variety the Pb concentration is

quite low than the P5, and lowest was identified in the P9 variety. These results are somewhat similar with the results of Milani *et al.* (2015). Jin *et al.* (2005) reported that soil media could be the main source of provision of Pb in tea. Growth of tea plants takes place mostly in high acidic soil where level of Pb is higher, hence soil and water pollution can lead to high Pb concentration in tea leaves (Han *et al.*, 2006).

Iron is considered a necessary micronutrient in all type of plants particularly in tea plant. According to the findings of this study the Fe contents of all green tea samples were in between 0.570 to 1.031 mg/L (Fig. 7) as comparable with the results of Tadayon *et al.* (2011). Maximum level of Fe was observed in P9 (1.031 mg/L) tea sample that was followed by P3 and P5. The RDA value for iron is 2600 mg/L. Iron is a key element for all living organisms. It plays a momentous role in different metabolic process such as electron and oxygen transportation.

Manganese is necessary for proper functioning of several enzymes as it acts as activator for more than 35 different enzymes (Liang *et al.*, 2006). In this study, the concentration of Mn in green tea samples of different varieties was in between 0.0012 to 0.211 mg/L (Fig. 7). Significantly the highest was in the P9 and that was followed by P5 and P3. Present findings are quite concord with the results reported by Tadayon *et al.* (2011).

Likewise other minerals zinc is also a portentous mineral element here and in this study it ranged from 0.655 to
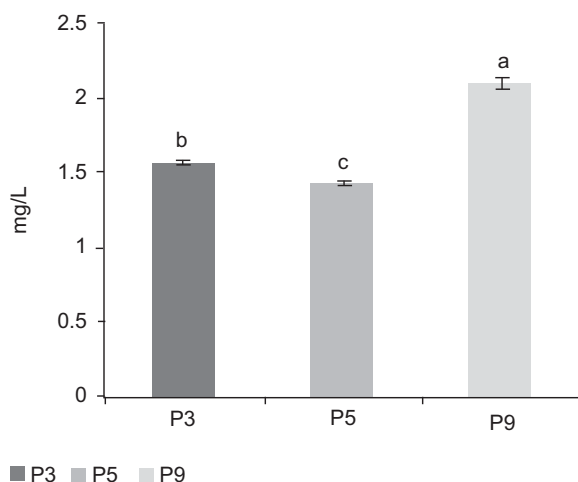
Fig. 5. Magnesium concentration in green tea samples of different varieties. Data show the mean ± SD. Means denoted by different letter are significantly different (P < 0.05).
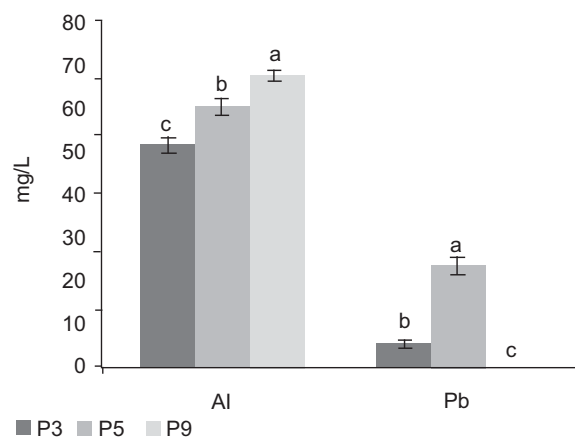
Fig. 6. Aluminum and phosphorus concentration in green tea samples of different varieties. Data show the mean ± SD. Means denoted by different letter are significantly different (P < 0.05).

1.316 mg/L (Fig. 7). It was found that P3 sample has highest amount of zinc content (1.316 mg/L) than that of other green tea varieties and the concentrations in P5 and P9 are comparable to each other. Metabolic activity of 300 enzymes depends upon the zinc in human body as reported by Deshpande *et al.* (2013) that it is very crucial for protein, DNA synthesis and cell division.

Copper is one of the indigenous metals found in tea followed by Zn and Al (Seenivasan *et al.*, 2008). Copper content varied greatly in the investigated green tea samples and the contents were ranged from 0.040 to 0.448 mg/L (Fig. 7). Maximum level of copper content (0.448 mg/L) was observed in P3 sample followed by P9 and P5. Copper is an obligatory mineral element having critical function in connective tissue, bone maintenance, proper growth and development in human body.

Molybdenum contents of green tea samples in this study varied between 0.947 to 1.102 mg/L (Fig. 7). Variety P3 showed significantly the highest Mo content (1.102 mg/L) compared to the other green tea varieties. The discrepancy in mineral contents of various type of tea leaves may be due to numerous factors such as different plant species, fertilizer application, soil property, climatic condition and generic differences.

Among the micro nutrients, Ni was also identified in this study but at a very low level and only in the green tea samples of the P3 tea variety at a concentration of 0.004 mg/L. No other tea variety possessed Ni. It was suggested that fertilizers are the major cause of nickel

contamination (Marcos *et al.*, 1998). Since Ni is a contaminated element, therefore its acceptable limit in tea is not recommended.

Results indicated that the concentrations of cobalt (Co) in all green tea samples varied from 0.201 to 0.451 in mg/L (Fig. 7). The WHO acceptable values for Co concentration is 0.015 mg/Kg. P3 sample showed maximum level of Co content (0.451 mg/L), which was followed by P5 and P9 samples. Tea have been found to be sensitive to many inorganic ions including Na, Pb and Co when applied to plants in excessive quantities a resulting their concentration being increased in the final product (Ranganthan *et al.*, 1981).

Conclusion

All the selected tea varieties under study differed to each other considering the mineral contents. Particularly resulted for high content of ash of nitrogen which is quite clear evidence that tea have a richest source of mineral elements. Highest amount of nitrogen (N), phosphorus (P), sodium (Na), zinc (Zn), copper (Cu), molybdenum (Mo), nickel (Ni) and cobalt (Co) were identified in the green tea samples of P3 variety whereas the least amount recorded in P5 variety. It was also observed that P5 showed maximum level of calcium (Ca), potassium (K) and lead (Pb) contents while low level of these mineral elements were present in P3 variety. On the other hand, the concentration of magnesium (Mg), aluminum (Al), iron (Fe) and manganese (Mn) observed higher in P9 samples. Green tea has demonstrated to be a rich source of Na, P, N, K, Ca, Al and Mg. The observed level of mineral elements in different varieties of green tea are below the permitted level but regular monitoring of their levels in tea is obligatory for future risk measurements. The study provides a better knowledge regarding the quality of selected tea varieties of NTHRI, Shinkiari. Similarly, it provides a solid foundation for consumer's preference. P5 tea variety was found with higher Pb concentration i.e. 23.64 mg/L which is above the threshold level of human body. Further study can be carried out to investigate the main cause of lead contamination in P5 variety and precautionary measurements should be carried out to avoid its hazardous effects.
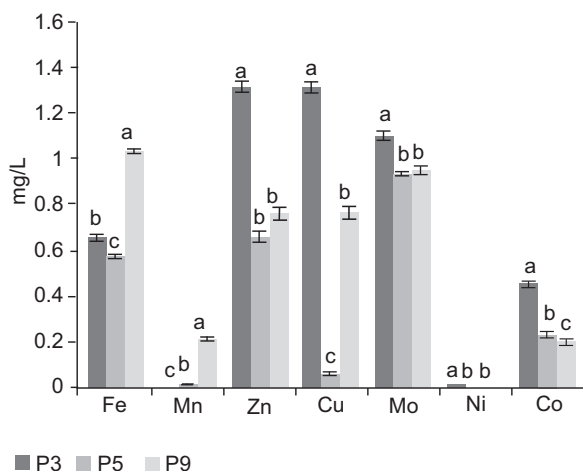
Fig. 7. Micro mineral concentration in green tea samples of different varieties. Data show the mean ± SD. Means denoted by different letter are significantly different (P < 0.05).

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