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Macro and micro nutrients in *Holy basil* (Tulsi): A possible supplement for natural medicine

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Abstract

This study has been performed to analyze macro and micro-nutrients in different parts such as leaves and stem of Tulsi plant collected from Rajshahi University Campus and one the villages of Natore District, Bangladesh. The heavy metal content in the corresponding soil samples has also been checked in order to clarify the possible accumulation of trace metals to the plant. It was seen from the results that a plenty of the macro-nutrient Ca was present (89.6 to 431.55 ppm) in both leaf and stem of the Tulsi. Similarly, the content of micro-nutrient Fe was also significant (3.34 to 49.53 ppm). Content of the other nutrients such as Zn, Mn, Ni and Cu was found to be within the permissible level suggested by the World Health Organization (WHO) and significant difference observed in the quantity of the trace metal contents from one place to another. Harmful metals content such as Pb, Cd and as was analyzed and was found to be within the permissible limit. Finally, the leaf and stem of the Tulsi is being recommended to be a great source of Ca, Fe deficient patients of the rural as well as the urban people.

Keywords: Tulsi, essential micro nutrients, heavy metal, atomic absorption spectroscopy

1. Introduction

Ocimum tenuiflorum, commonly known as holy basil in English, belongs to the plant family Lamiaceae. Its cultivation originally started from Indian sub-continent but now grows in different parts of the world like Australia, West Africa, and some Middle Eastern countries (Upadhyay, 2017) [1]. This plant has a large history within Ayurvedic medicine for more than 4000 years because of its multitude uses as curative purposes (Sai *et al*. 2014) [2]. In the Ayurveda, tulsi is often considered as “Mother medicine of nature”, “The Queen of Herbs” and an “Elixir of Life” because of its outstanding medication powers (Mohan *et al*. 2011) [3]. Its different parts like leaves, stems, root, seed, flowers etc. are known to possess therapeutic potentials and used as analgesic, anticancer, antiasthamatic, antiemetic agents. Some recent investigations using tulsi extracts show outstanding anti-aging, immune modulatory, antibacterial and anti-inflammatory qualities (Hassanpouraghdam *et al*. 2010 and Jordan *et al*. 2010) [4-5]. In addition, tulsi is widely used for the treatment of coughs, anxiety, asthma, diarrhea, fever, dysentery, headaches, kidney malfunctions, arthritis, eye diseases, vomiting, cardiac and genitourinary disorders, back pain, skin diseases etc. (Pattanayak *et al*. 2010 and Mondal *et al*. 2009) [6-7]. In the Indian Materia Medica, tulsi leaf extracts are considered for treatment of bronchitis, rheumatism, and pyrexia (Nadkarni *et al*. 1982) [8]. The roots and stems have been traditionally used against mosquito and snake bites and for malaria (Chopra *et al*. 1992) [9]. Although, modern medicinal process is too developed nowadays even then almost 75% people are still relying on the conventional medicinal plant treatment. This treatment is becoming popular because of low cure cost and ease of availability of these plants with significantly negligible toxic side effect. However, some heavy metals like arsenic (As), lead (Pb), cadmium (Cd) chromium (Cr) etc. are extremely toxic to human body and environment as well as much hazardous for sound health. The World Health Organization (WHO) recommended that raw materials come from the medicinal plant for making final products will be checked in presence of heavy metals, pesticides, bacterial or fungal concentration (WHO. 2005) [10]. Although trace elements are very small in quantities but has a dangerous role in several biological reaction occurs in living cells. People think that medicinal plant do not have adverse side effect but this is not true at all. If these trace elements are higher than that of permissible level then it affects negatively in the body reactions (Calixto *et al*. 2000) [11]. Tulsi has been subjected for various researchers because of its pharmacological and wide range of therapeutic applications. Calcium (Ca), iron (Fe), zinc (Zn), manganese (Mn), nickel (Ni), copper (Cu) etc. are nutritionally essential as micro-nutrients and required in trace quantities for maintaining proper body functions and metabolic activities (Goyer *et al*. 2001) [12]. A lot of scientific reports regarding this have been published during the last decade. To the best of our knowledge, there are no available researches have been carried out comparing the macro and micronutrition values of tulsi plant.

In this present study, black basil (*Ocimum sanctum*) from two different places (Rajshahi University Campus and Natore Osudhi Village, Bangladesh) has been collected and then assessed nine trace elements (Ca, Fe, Zn, Mn, Ni, Cu, Pb, Cd, As) concentration of different parts (leaves and stem) of the Tulsi by Atomic Absorption Spectroscopic (AAS) method. At the same time, concentration of trace elements in soil samples from the same place has also been assessed as well.

2. Materials and Methods

Location, Collection of Tulsi Plant and Soil Samples

Samples (Tulsi plant and soil) are collected from two different locations. One is from Uttar Para of Rajshahi

University Campus and other is from Natore Piprul village (locally know as Osudhy village) of Natore District, Bangladesh. Distance between the two places are of about 60 Km from each other. The geographical location of the study points is shown in Fig. 1. Soil samples were collected from the surrounding area of the plants roots at about 5-8 cm depth. The soil samples are then mixed thoroughly and then dried, powdered, sieving and put in the polyethylene sampling bags and then placed in cool place until analysis. The plants were washed with fresh running water to remove dirt, dust, and other foreign agents and divided into two parts such as leaf and steam. The leaf and steam samples were dried in a shed at room temperature and then heated in a heating block in a muffle furnace for complete drying. The dried samples are then crushed by a mortar and pestle for making finely powdered. The powdered sample was then kept in polyethylene sampling bags and put in a cool place until next processing.

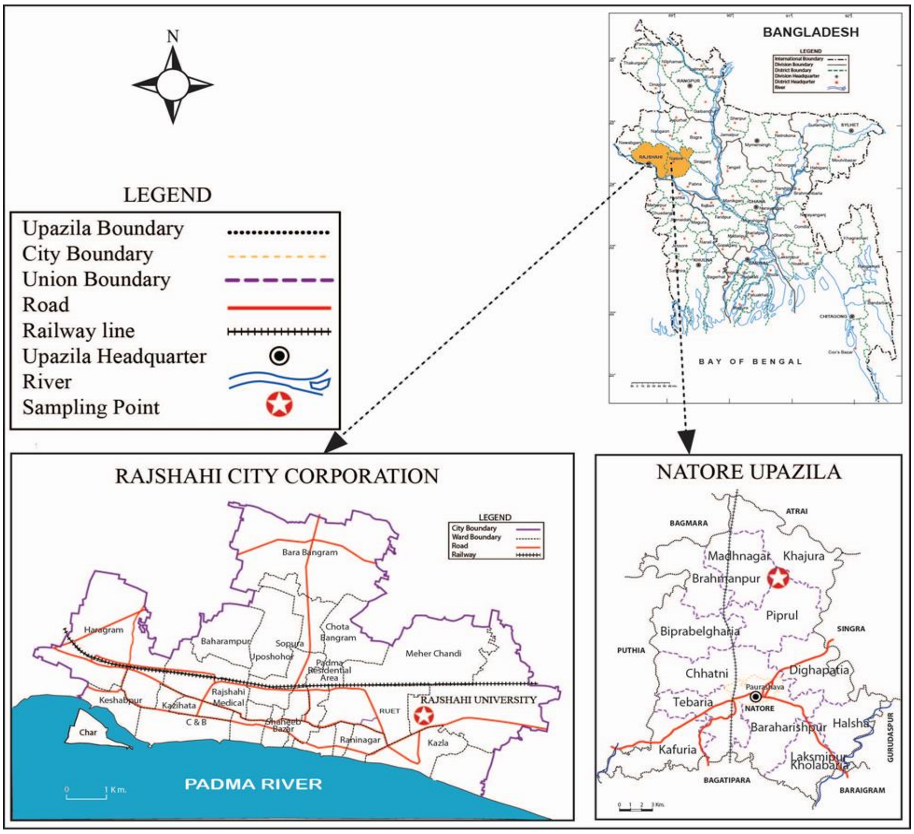

Fig 1: Location of the sampling points. Sampling points are shown by star sign in the Map

Chemicals and Reagent

Analytical grade chemicals were purchased from E. Marck, Germany. Ultra-purified deionized water containing no ions other than H^+ and OH^- was used throughout the study. 65% nitric acid (HNO_3), 30% hydrogen peroxide (H_2O_2), and 70% perchloric acid ($HClO_4$) were used for digestion purpose, while multielement standard solution was used as a reference material.

Determination of Trace Elements

Nine trace metal concentration such as: Ca, Fe, Zn, Mn, Ni, Cu, Pb, and Cd in the selected plant materials and in the soil samples were determined by atomic absorption spectrophotometer (SHIMADZU, AA-6800) equipped with flame and graphite furnace at the Central Science Laboratory of Rajshahi University. Detailed instrumental measurement conditions are outlined in Table-1. All the analyses were performed using an air-acetylene flame for the atomization of metal content. Arsenic (As) was determined by atomic absorption spectrophotometer (model GBC 932) at Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh. Calibration was accomplished using a linear fit for all the elements. The data were rounded off suitably according to the value of standard deviation from measurements in triplicate.

Table 1: Operating conditions for the determination of the heavy metals and minerals.

Elements flow	Wavelength (nm)	Current (mA)	Burner height (mm)	Burner angle (degree)	Slit width (nm)	Fuel gas flow (L/min)	Lighting mode	Type of oxidant
Ca	422.7	10	7	0	0.5	2.0	BGC-D2	Air-acetylene
Fe	248.3	10	7	0	0.2	2.0	BGC-D2	Air-acetylene
Mn	279.5	10	7	0	0.2	2.0	BGC-D2	Air-acetylene
Ni	232.0	12	7	0	0.2	2.2	BGC-D2	Air-acetylene
Zn	213.9	8	7	0	0.5	2.0	BGC-D2	Air-acetylene
Cu	324.8	6	7	0	0.5	1.8	BGC-D2	Air-acetylene
Cd	228.8	8	7	0	0.5	1.8	BGC-D2	Air-acetylene
Pb	217.0	12	7	0	0.5	2.0	BGC-D2	Air-acetylene

Sample Digestion
A homogeneous solution was prepared by mixing HNO_3 and H_2O_2 in the ratio of 2:1. 1.0 g sample is then dissolving in the solution for digestion. To complete this process the solution was heated at about 150°C until the final volume turns to 5 mL. The solutions was then cooled and filtered. The filtrate was diluted up to the mark (Khan *et al*. 2008) [13]. 1.0 g soil sample was dissolved in 10 mL HNO_3 and kept for 24 hrs. Then, 5 mL of $HClO_4$ was added to the soil sample solution. The mixture was then heated until the volume was reduced to 5 mL. Then, the sample solution was cooled and filtered using Whatman filter paper (7 cm. No. 41). The filtrate was stored in refrigerator as stock solution until analysis (Soylak *et al*. 2004) [14].

3. Results and Discussion
Trace Metal Contents in the Soil Sample
Trace heavy metal concentrations in the tested soil samples and plant samples collected from Rajshahi University (RU) Campus and Natore Piprul (NP) village study points were analyzed and the mean concentrations of Ca, Fe, Zn, Mn, Ni, Cu, Cd, Pb and As are summarized in Table 2. It is seen from the Table that the metal contents varied significantly from RU campus soil sample to Natore soil sample. The amount of metal contents in Natore soil sample is much higher than that of RU campus sample except Cd. The fraction of metals in RU campus soil and Natore soil were found to be present are in order like: As (62.00 and 82.85 ppm), Pb (0.18 and 0.45 ppm), Cd (0.022 and 0.018), Ca (55.45 and 215.77 ppm), Ni (0.288 and 1,018 ppm), Cu (0.303 and 0.895), Zn (0.6837 and 2.745), Fe (346. 45 and 503.45) and Mg (5.45 and 12.55 ppm). The order of micro and macro nutrients in the soil samples in both places are in order Ni~Cu<Zn<Mn<Ca<Fe. A comparison study about the soil composition of the study area is shown in Fig. 2.

Table 2: Trace metal concentration (ppm) in soil samples collected from two different places. R is defined as the soil of Rajshahi University Campus and N is defined as the soil of Natore Piprul village.

Sample ID	Detail	Ca ppm	Fe ppm	Zn ppm	Mn ppm	Ni ppm	Cu ppm	Pb ppm	Cd ppm	As ppm
R-1	Soil	55.45	346.45	0.683	5.450	0.288	0.303	0.180	0.022	0.0620
N-1	Soil	215.77	503.45	2.745	12.550	1.018	0.895	0.450	0.018	0.0828
R-2	Leaf	431.55	17.91	1.127	0.606	0.132	0.616	0.070	0.010	0.0062
N-2	Leaf	192.50	6.47	1.714	0.842	0.502	0.679	0.430	0.034	0.0045
R-3	Stem	239.00	3.34	1.931	0.247	0.066	0.313	0.110	0.011	0.0061
N-3	Stem	89.60	49.53	0.779	0.574	0.144	0.552	0.060	0.049	0.0083

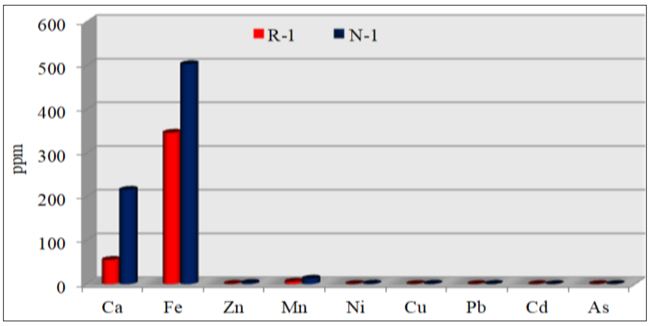

Fig 2: Comparison study of soil composition of the study area. R-1 is Rajshahi University Campus soil sample and N-1 is soil sample of Natore piprul village.

Macro and Micro-nutrients
These metals are mainly a type of food (e.g. fat, protein, carbohydrate) required in large amounts in the diet for proper metabolism and functioning of body organs. Sodium, potassium, magnesium, calcium are belonging to macro-nutrients. Some metals are required relatively smaller amount compared to macronutrients and extremely important for the proper functioning of body. They include vitamins and minerals which are necessary for energy production, immune function, blood clotting and other functions. Metals such as Ni, Cu, Zn, Fe, Mn etc. are essential and belong in the micro-nutrient group. These metals have essential biological functions in plants and animals like for immune function, brain development and many other important functions and their requirement are still under research. The macro-nutrient Ca and micro-nutrients Fe, Zn, Mn, Ni, Cu, together with some toxic metals such as Pb, Cd and As were determined in leaf and steam samples of Tulsi plant (Table 2). A comparison study of macro and micro-nutrients in leaf and steam samples of Tulsi plant is shown in Figs. (3-4).

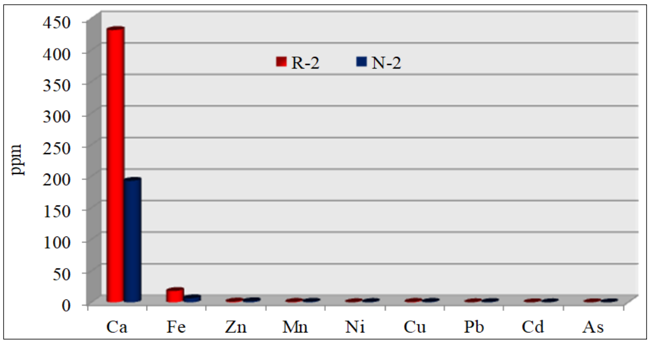

Fig 3: Comparison study of macro and micro nutritional composition of the leaf sample of Tulsi. R-2 is the Tulsi of Rajshahi University Campus and N-2 is the Tulsi of Natore piprul village.

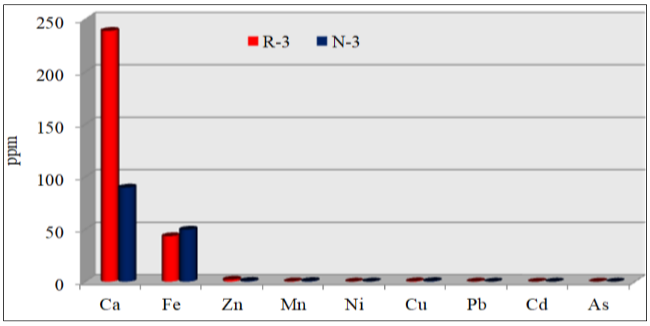

Fig 4: Comparison study of macro and micro nutritional composition of the stem of Tulsi. R-3 is the stem of tulsi of Rajshahi University Campus and N-3 is the stem of Ttulsi of Natore piprul village.

Calcium

Calcium is an essential plant nutrient and also crucial parts of bones and teeth. The heart, nerves, and blood-clotting systems also need calcium to work properly. Calcium carbonate is used as an antacid for “heartburn”. The amount of Ca is in leaf and steam in both R-2 and R-3 samples (431.55 and 239.0 ppm, respectively) and N-2, N-3 samples (192.50 and 89.60 ppm, respectively) are found in significant quantity and the amount is lower in Natore sample than RU campus sample Figs (3~4). However, both the leaf and steam of tulsi plant could be a natural source of Ca that could be used for the treatment of osteoporosis, rickets, and osteomalacia.

Iron

It is the most abundant and an essential element for all plants and animals. Most of the iron in the body is found in the hemoglobin of red blood cells and in the hemoglobin of muscle cells. Iron is needed for transporting oxygen and carbon dioxide (National Academy Press, Washington, DC, 2000) [15]. At high concentration, it causes tissues damage and some other diseases in humans. Deficiency of it leads to myocardial infarction, gastrointestinal infection and nose bleeding. The WHO recommended level of iron in medicinal plants is 20.0 mg/kg, while its dietary intake is 10–28 $mgday^{-1}$ (WHO 1998) [16]. It is seen from the experiment that the presence of Fe in leafs and stem in R-2, R-3 samples are (17.91 and 3.344 ppm) while in N-2, N-3 samples are (6.47 and 49.53 ppm). So the change in location from RU to Natore leads to the lowering of Fe content in leaves and tremendous increase of it in stem. The higher amount of Fe in stem of Natore sample is may be because of the presence of higher concentration of Fe in soil (503.45 ppm).

Zinc

Zinc is a mineral. It is called an "essential trace element" because very small amounts of zinc are necessary for human health. Since the human body does not store excess zinc, it must be consumed regularly as part of the diet. Zinc has very important role in DNA synthesis, normal cell growth, brain development, bone formation, wound healing and behavioral response (Adelekan *et al*. 2011) [17]. The amount of Zn present in R-2 and R-3 sample (1.1275 and 1.931 ppm) and Natore sample (1.714 and 0.7795 ppm) are much lower than the permissible limit. According to WHO, the quantity of Zn needed for edible plants was 27.4 ppm but not specific range was mentioned to the medicinal plants.

Manganese

It is considered an essential nutrient, because the body requires it to function properly. People use manganese as medicine. It plays vital role in normal reproduction and functioning of the nervous system. Its deficiency produces severe skeletal and reproductive abnormalities in mammals. High concentration of Mn causes hazardous effects on lungs and brains of humans. The Mn concentration in both leaves and stems in RU campus (0.606 and 0.247 ppm) and Natore sample (0.842 and 0.574 ppm) are significantly lower in quantity. WHO’s maximum permissible limit of Mn in medicinal plants is 200 ppm, while its daily intake is 11mg. In 2010 Jaben *et al* showed that Mn permissible ranges from 44.6 to 339 ppm (J¨arup, 2003) [18]. So from our results it is seen that the Mn levels are much lower than the permissible range thus it is important to take necessary steps so that the Mn content would be in the normal range.

Nickel

It is an essential element for plants and animals. The body needs nickel, but in very small amounts. Nickel is a common trace element in multiple vitamins. In small quantity, is used for increasing iron absorption, preventing iron-poor blood (anemia), and treating weak bones (osteoporosis). High at high level, it is responsible for depression, cancer, heart attacks, loss of vision, skin problems, rash, itching and vomiting (National Academy of Sciences, Washington, DC. 1999) [19]. The leaves and stem of RU campus sample had the concentration (0.132 and 0.066 ppm) which is much lower than the concentration found in Natore sample (0.502 and 0.144 ppm). WHO’s permissible limit of nickel in medicinal plant is 1.5 ppm, while its routine requirement for mankind is 1mg day^{-1} (WHO, 2005) [20].

Copper

Copper is essential for all living things. It is not formed in the body and must be obtained from food and drinking water each day as part of a balanced diet and, occasionally, through the use of dietary supplements. It has a great role for the normal growth and development. High concentration of Cu causes metal fumes fever, acne, depression, hair and skin decolorations, hyperactivity, dermatitis, respiratory tract diseases, and some other fatal diseases in human beings. The presence of Cu in leaves and stem of RU campus sample has lower in amount than Natore sample but both concentrations are much smaller than the permissible limit provided by WHO. Whose permissible limit of copper in medicinal plants is 10 $mgkg^{-1}$, while its intake in food is 2-3 $mgday^{-1}$.

Lead

It is a non-essential heavy metal. It is a serious cumulative body poison, which can affect every organ and system in the body. High level accumulation of it leads to anemia, colic, headache, brain damage, convulsions and chronic nephritis of the kidneys, and central nervous system disorder (Rehman *et al*. 2013) [21]. WHO sets the permissible limit for medicinal plant is 10 ppm. In our study the amount of Pb in plant parts are in the range of 0.06 to 0.43 ppm which is even less than 1 ppm.

Cadmium

It is also a non-essential heavy metal. It is extremely toxic even at low concentration. It causes acute poising and show adverse effects on kidney, liver, lung, bones and vascular immune systems. It causes learning disabilities and hyperactivity in children (Hunt, 2003) [22]. The experimental results of Cd concentrations among the plant parts in both locations are in the range 0.010 to 0.049 ppm. The WHO recommended level of cadmium in medicinal plants is 0.3 ppm (National Academy of Sciences, Washington, DC, 1999) [19] which shows our results is not a problem yet.

Arsenic

Arsenic is extremely toxic for human health and has no beneficial metabolic functions for human [19]. Its low level exposure cause nausea and vomiting decreased production of RBCs and WBCs. The Food and Drug Administration says that long-term exposure to high levels of arsenic is associated with higher rates of skin cancer, bladder cancer and lung cancer, as well as heart disease. Other affect includes abnormal ECG, headache, hair loss, liver damage, sore throat, weakness etc [19]. The amount of As present in RU campus leaves and stem are nearly equal (6.25 and 6.15 ppm) while in Natore sample leaves and stem contain (4.50 and 8.375 ppm).

4. Conclusion

The experimental results obtained from the present research showed that the macro, micro nutrients and trace elements are in significantly higher quantity in soil sample of "Natore Piprul Village" in comparison to that of the soil sample of "Rajshahi University Campus". It might be due to the different geographical environment or the bioaccumulation process of the soil composition of the studied area. Macronutrient Ca and micronutrient Fe content in the leaf of tulsi are found higher in the sample collected from RU campus than that of the sample collected from the Natore Piprul village. Ca and Zn contents in the stem are also higher in the sample of RU campus. All plants absorb water and mineral from soil and thus trace metals are distributed or accumulated in the plants likewise. The assays of these metals can differ from location to location, plant to plant and even from part to part of a plant, depending upon the chemical composition of soil and absorption rate by plants. Thus, it is essentially required to check every medicinal plant for contaminant load before processing it for further pharmaceutical purposes or for local human consumption. Finally, it is recommended that both leaf and stem of tulsi are of a great source of macro and micronutrients for the metabolic action for human body.

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