TRACE ELEMENTS IN BITTER MELON (Momordica charantia L.) AND THEIR DISTRIBUTION IN DIFFERENT PLANT PARTS

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ABSTRACT
This study was conducted to determine some trace metals quantities in the different plant parts of bitter melon (Momordica charantia L.) grown in Pazar district of Rize Province in the Black Sea Region of Turkey. The experiment was established in the trial field belonging to the Faculty of Agriculture and Natural Science in Recep Tayyip Erdogan University. The plants were uprooted on 9th of August in 2015, separated in their stem, root, branch, unripe fruit flesh, ripe fruit flesh, leaf and seed parts, and analyzed by ICP-OES to determine the level of Cd, Pb, Ni, Fe, Zn, Cu, Al, Cr and Mn. Concentrations of Al, Fe, Mn and Zn were found higher than other elements in all of the plant parts. The roots had the highest concentration values of all trace elements examined except Zn and Mn compared to the other parts of the plant. Mn was found highest in leaves and branches. In consideration of 100 g fresh weight for each part of plant, the highest total trace element amount was determined in roots of bitter melon followed by seeds, leaves, branches, stems, unripe fruits (flesh) and ripe fruits (flesh) respectively. The levels of trace elements investigated in all parts of the bitter melon were below the maximum permissible limits except roots contained the high levels of Fe and Al concentration.

Keywords: bitter melon, ICP-OES, trace elements.

1. INTRODUCTION
Bitter melon (Momordica charantia L.) from Cucurbitaceae family commonly known as “bitter gourd” and “bitter melon”, cultivated in many tropical and subtropical regions of the world, is an annual and climber plant [1]. Bitter melon plant which is grown in many provinces of Turkey and used widely in traditional medicine is a valuable cultural plant and easily adaptable to the ecological conditions of Rize province in Black Sea Region [2].

The fruits of bitter melon, which have a potential to use in food systems to increase the value of various food because of rich fenolic compounds, are used as medicinal vegetable in different countries [3, 4]. In addition, bitter melon has valuable pharmacological activities for many diseases. The fruits and leaves of bitter melon possess also laxative, antibilious, emetic and stomachic effects [5]. Furthermore, unripe fruits, seeds and aerial parts of bitter melon have been used to treat diabetes in the world.

Verma [6] reported that all parts of the fruit and tissues of bitter melon are safe and useful for human consumption and there is a quite good variation of the concentration of the elements in different parts of the plant. Similar research results were reported by Kosanovic et al. [7]. The results obtained from their study indicated that bitter melon could be a good source of essential elements such as Ca, Mg, Mn, Cu and Zn. However, if trace elements exceed the standards, human health could be under risk. Bitter melon leaves are a rich source of nutrients and phytochemicals. Zhang et al. [8] reported that bitter melon leaves are a rich source of nutrient and phytochemicals. They have found high amount of K, Ca, Mg and P, range from 1850.8 to 2811.8, 837.4 to 4978.2, 317.3 to 512.4, 329.6 to 314.2 respectively, and low amount of Fe, Mn, Zn, Cu and Al, range from 8.4 to 16.7, 5.5 to 11.7, 4.1 to 5.9, 1.1 to 1.4 and 6.2 to 23.1 mg/100 g DW basis, respectively in the leaves.

Essential and toxic elements in an agroecosystem can be inherited from soil parent material or introduced through human activities such as industrialization or fuel combustion. The effects of metals on pollution problems create serious concern because their physiological effects can be substantial even at extremely low concentrations. On the other hand, some plants could concentrate both essential and toxic trace elements. Most of marsh plant species concentrate metals primarily in roots while some species distribute a greater proportion of metals into above ground tissues, especially leaves [9].

In the present study, various parts of bitter melon grown in Pazar/Rize conditions were examined to determine if the present quantities of trace elements create a risk for human health and to compare the parts regarding the examined elements.

2. MATERIALS AND METHODS

2.1. Materials
In this research, the plants were grown in the practice field in Faculty of Agriculture and Natural Sciences in Pazar district of Rize Province in the Black Sea Region of Turkey. The used seeds were bitter melon (Momordica charantia L.) populations belonging to the faculty inventory.

2.2. Soil characteristics
The physical and chemical characteristics of the experimental soils are presented in Table-1. The organic
matter content of the experimental soils was 1.27% (low), lime content 0.10% (limless), salinity 0.62 Ds/m (saltless) and pH 4.71 (acid reaction). Available phosphorus and changeable potassium levels were 2.18 mg/kg (very low) and 0.45 cmol/kg (enough), respectively.

2.3. Methods

Five plants were selected as casual in fruiting period on 9th of August in 2015 and were uprooted. Each plant was divided into its parts using plastic knife and washed with deionized water to avoid potential contamination.

The plants were divided into their parts as the following:

1. Ripe fruit flesh
2. Seeds of ripe fruits
3. Unripe fruit flesh
4. Stem
5. Branch
6. Root

Same parts were combined and kept in an oven at 80 °C to dry completely. 10.0 g of each dried material was divided into small pieces and then pounded in a mortar to pulverize. Afterwards, it was maintained at +4°C until the analysis.

Elemental analysis was realized using inductively coupled plasma-optical emission spectrometry (ICP-OES) in Central Research Laboratories Application and Research Center in Recep Tayyip Erdogan University. Each sample weighted as 0.3 g and was digested with a mixture of 5 ml nitric acid and 3 ml hydrogen peroxide and the analysis was conducted using the ICP-OES. The concentration of heavy metals such as Aluminium (Al), Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Nickel (Ni), Lead (Pb), Chromium (Cr) and Cadmium (Cd) were analyzed. The reading of each sample was calculated by taking the average readings of triplicate sets. The values found as µg/ml were converted into µg/g dry weight (DW) and µg/100 g fresh weight (FW).

3. RESULTS

Trace elements play an important role in the metabolic pathways during the growth and development of plants, when available in the required concentration. In addition, the results recorded by Srinivasu et al. [10] showed that medicinal plants could be used in the treatment of diabetes mellitus as they contain the elements Cr, Zn, Cu, Ni, Mn and Se which play vital roles in blood glucose reduction. On the other hand, plants are affected by the surrounding pollution. Moreover, the plants are exposed to a high level of pollution by industrial effluents or emissions rich in heavy metals which cause a risk to humans who consume them. Therefore, herbal products are also the most important sources of toxic elements potentially. Akram et al. [11] recorded permissible limits of some heavy metals as the follows: 20 ppm for Fe, 10 ppm for Cu, 50 ppm for Zn, 1.5 ppm for Ni, 200 ppm for Mn, 0.3 ppm for Cd. In addition, WHO [12] reported that the acceptable limits for humans are 10 ppm for Pb and 2 ppm for Cr.

In this study, trace elements tested varied depending on the parts of bitter melon plant (Table-2). Al (Aluminium) had the highest concentration (307.18 µg/g dry weight) in plant and other elements were found to decrease: Fe>Mn>Zn>Cu>Pb>Ni>Cr>Cd. Al and Fe values were above the maximum permissible limits only in roots according to our findings.

The values of Fe, Zn, Al and Mn were generally abundant in all the parts of Momordica charantia L. In addition, root was the richest part of the plant for concentration values of all elements examined, except Zn and Mn. On the other hand, 100 g fresh root contained the highest of Al and Fe values. Mn was the highest element in the leaves while the left elements were higher in the seed.

Additionally, the highest value calculated based on 100 g fresh weight of any parts of the plant was 6527.45 µg for Al followed by Fe, Mn, Zn, Cu, Ni, Pb, Cr and Cd, respectively (Table-2).

3.1 Aluminium (Al)

Aluminium is abundantly present in most soils and plants. Aluminium is taken into the body in various ways such as lung, skin and gastrointestinal tract. That accumulation of aluminium in the body causes to the situations such as neurological disorders, hyper alumina, dialysis encephalopathy and anemia [13]. A minimal risk level (MRL) of Al has been suggested as 140 mg/day after oral exposure in the United States for an average person of 70kg [14]. However, the European Food Safety Authority (EFSA) has established 10 mg/day accounting the possible accumulation of Al in the organism [15].

In the present study, root of bitter melon was on the first rank for the concentration of Aluminium as 307.18 µg/g DW which was calculated in fresh weight of the roots as 6527.45 µg/100 g FW that can not be considered up to the minimal risk level even according to the EFSA [15]. The lowest levels of Al were found in unripe and ripe fruit flesh as 43.67 and 21.09 µg/100 g FW respectively (Table-2 and Figure-2).

3.2. Iron (Fe)

Iron is an essential element for almost all living organisms. It participates in some metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport [16]. On the other hand, iron can form free radicals. Moreover, It can lead to tissue damage depending on its uptake in excessive amounts.

Fe is present in large quantities as Al in plants and soils with low pH. Amount of Fe was found quite high in the range of 21.85-3272.23 µg/100g FW which was the second element after Al in the present study (Table-2, Figure-1 and 2). Roots (153.99 µg/g DW) were the richest part of the plant for Fe while the least concentration of Fe (4.08 µg/g DW) was found in ripe fruit flesh.
3.3. Manganese (Mn)
Manganese is an activator and also a constituent of several enzymes, and often considered to be among the least toxic of the trace elements when administered orally [17].

In the present study, the next element which was abundant in the plant, was found in much more amount in leaves with 31.37 µg/g DW (Table-2 and Figure-1) and 831.77 µg/100 g FW. Mn concentration decreased in leaf, branch, root, stem, unripe fruit flesh, ripe fruit flesh and seed, respectively, regarding its amounts in the different parts of the plant (Figure-2). On the other hand, Mn had the lowest values in flesh of fresh fruits.

3.4. Zinc (Zn)
Zinc has an essential role in the processes of genetic expression. The biochemical roles of zinc show its relation with great numbers of enzymes. Its involvement in such constitutional activities presumably accounts for the essentiality of zinc [17].

The highest value in branch was obtained from Zinc concentration (16.79 µg/g DW) among the trace elements researched in the study (Table-2 and Figure-1). Furthermore, quantity of Zn (412.34 µg/100 g) in fresh seeds was higher than the other ones (Figure-2). The values obtained from branch, leaves, stem, root, unripe fruit flesh and ripe fruit flesh of the plant followed it respectively. The lowest values determined in unripe fruit flesh and ripe fruit flesh were 56.96 and 37.46 µg/100 g FW, respectively.

3.5. Copper (Cu)
Copper is an essential element for human. Therefore, its uptake is recommended. However, when Cu exceeds its safe level concentration, it causes various illness such as hypertension, sporadic fever, uremias, coma etc. [17].

In this study, roots were at the top regarding Cu concentration while stems were the last in the present investigation. The values of experiment showed that Cu varied from 0.71 to 3.73 µg/g DW (Table-2 and Figure-1), which fallen below the safe limits for human health. However, campared to the parts of plant regarding amounts in fresh weight, the highest Cu value belonged to seeds while ripe fruit flesh was the last for the Cu value, 127.43 and 4.53 µg/100 g FW, respectively (Figure-2).

3.6. Nickel (Ni)
Nickel is one of the essential elements for plant growth. It has widespread distribution in the environment and many industrial uses. Living creatures in nature can be exposed to nickel in the air, water and food. The large part of nickel in the human body originates from drinking water and food [18].

Its concentration in agricultural lands is generally very low. In the present study, Ni was found low amounts in the plant. Roots of bitter melon were on the first rank while leaves were the last for the concentration of Nickel (Table-2 and Figure-1). In the same way, the highest Ni value also calculated in fresh seed weight while the lowest values were found in ripe fruit flesh (Figure-2). The present investigation revealed that Ni varied from 2.20 to 31.26 µg/100 g FW.

3.7. Lead (Pb)
Lead is a cumulative poison and can affect living organisms when consumed even in little amounts in the long term. The effects of lead toxicity on environment has caused an extensive concern in recent decades. Plants generally have much lower levels of Pb unless they are exposed to heavy metal contamination. In this study, the highest value (1.00 µg/g DW) for Pb concentration was obtained from roots while the lowest Pb value (0.33 µg /g DW) was found in seeds (Table-2 and Figure-1). On the other hand, high values were calculated from both roots and seeds regarding 100 g FW (Figure-2). The lowest values were determined in unripe and ripe fruit flesh, as 2.66 and 2.24 µg/100 g, respectively.

3.8. Chromium (Cr)
Chromium is a trace element which has been known to be an essential nutrient for humans and animals for a long time. Its main role is maintenance of normal glucose tolerance in the body [19, 20].

The highest Cr concentration at 0.35 µg /g DW was determined in the roots of the plant while unripe fruit flesh of the plant had the lowest one (0.13 µg/g DW) (Table-2 and Figure-1). Fresh seeds of the plant had a higher Cr value with 10.82 µg/100 g than the other parts (Figure-2). Low values were obtained from both fresh unripe and ripe fruit flesh as 0.95 and 1.05 µg/100 g, respectively.

3.9. Cadmium (Cd)
Cadmium is heavy metals of which consumption of high concentration can cause to reduce growth like chromium and lead. Moreover, it induces hepatic and renal impairment [21].

Plants generally contain much lower amounts of Cd like Pb unless they are exposed to heavy metal contamination. In this research, among the parts of plant, roots had the highest Cd concentration with 0.08 µg/g DW followed by the values obtained from leaves, stems, seeds and the others, respectively (Table-2 and Figure-1). In addition, while 100 g fresh weight of seeds had the highest Cd values, fruit flesh had the lowest values (Figure-2).

4. DISCUSSIONS
Essential trace elements, which are required in amounts ranging from 50 µg to 18 mg per day, are important nutrients for humans [22]. High intakes in the consumption of essential trace elements cause toxicity while low intakes lead to nutritional deficiencies. In the present study, the values of trace elements examined are found in safe limits for each analyzed part of bitter melon, except root. Our results show that the analyzed edible parts of bitter melon plants can be considered as potential sources to provide reasonable amount of the required elements.
Although Pb was found highest amount in the roots at the concentration of 1.00 µg/g DW (1.00 ppm), it is not over the permissible limit of 10 ppm. Oseni et al. [23] previously reported similar findings that roots of bitter melon had the highest deposition of this heavy element and 40% higher than concentration in the shoots.

Cr, Mn, Cu, Zn and Pb values measured in the present samples are not in agreement with those published by Kosanovic et al. [7] who reported around 33.3, 2540.0, 790341.0, 5035.0 and 10.95 µg /kg FW respectively. The measured Pb values is 26.64 and 22.24 µg/kg fresh unripe and ripe fruit flesh respectively which are around two times higher while the others are lower than those reported by the same authors.

Our results on fruits for Cu, Zn, Cd, Pb and Ni concentrations are higher, but lower for Fe than the values reported by Nirmal Kumar et al. [24] who found 0.270 mg/gr of Cu, 2.740 mg/gr of Zn, 0.008 mg/gr of Cd, 0.136 mg/gr of Pb and 0.221 mg/gr of Ni in fruit flesh samples.

Our results on seeds for Fe, Mn, Zn and Cu concentrations are lower than the values reported by Horax, et al. [25]. These researchers determined 88±9 mg/gr of Fe, 12±2 mg/gr of Mn, 55±5 mg/gr of Zn and 23±5 mg/gr of Cu. On the other hand, the results belonging to Mn concentrations in leaves confirm previously published data, and are in line with the values reported by Verma [26] who determined 36.29 ppm of Mn.

The concentration values of Zn, Cu, Cd and Fe in fruit, except Ni, determined by Aslam et al. [27] were higher than the results in the present study. Considering the trace element levels of unripe fruit flesh and ripe fruit flesh, only Cd was increased while Fe, Zn, Al and Mn decreased as the fruit matured in the present study. Zhang et al. [8] found the higher levels of Fe, Mn, Zn, Cu and Al in the leaves of the plant based on the three maturation stages than our findings. Fe, Mn, and Al increased as the leaves matured while the Zn level did not vary significantly among maturity stages in their study [8].

Khan et al. [28] recorded that the maximum uptake for four heavy metals (Cu, Co, Cd and Fe) was observed by the roots in M. charantia L. This result is in accordance with our results. In addition, same researchers determined that metal uptake ratios are closely associated with their concentrations in the soils.

The values of trace elements examined in this study are lower in all the parts of bitter melon plant, except roots, than the previous findings in reference. The reason of this difference may be due to the different soil structure and environmental effects. As we know, wastewater may contain various heavy metals such as Zn, Cu, Pb, Mn, Ni, Cr and Cd. Continuous irrigation of agricultural land with sewage and industrial wastewater may cause heavy metal accumulation in the soil and vegetable [29]. Our investigation field may be clearer than the others.

5. CONCLUSIONS

Information about concentrations of trace elements in different parts of bitter melon plant (Momordica charantia L.) which is a medicinal plant used also as vegetable and their dietary intake is very important to assess their risk to human health. The values of concentration vary depending on both growth conditions and parts of plant. In the study which investigated some trace elements, the elements show a large variation in all parts of the plant. In addition, all parts of bitter melon, except roots, have acceptable values for human healthy regarding trace elements investigated in the present research.

Table-1. Physical and chemical characteristics of experimental soils*.

<table>
<thead>
<tr>
<th>Texture</th>
<th>Saturation (%)</th>
<th>Lime (%)</th>
<th>Salinity (Ds/m)</th>
<th>pH</th>
<th>Organic Matter (%)</th>
<th>Available P2O5 (mg/kg)</th>
<th>Changeable K2O (cmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay</td>
<td>72.00</td>
<td>0.10</td>
<td>0.62</td>
<td>4.71</td>
<td>1.27</td>
<td>2.18</td>
<td>0.45</td>
</tr>
</tbody>
</table>

*Soil analyses performed in Ş.Şemsi BAYRAKTAR Soil Plant Analysis Laboratory in Pazar/RİZE
Table-2. Amounts of the elements with (±) standard deviations (SD) in the different parts of the bitter melon in the concentration of µg/g dry weight and µg/100 g fresh weight (n=3).

<table>
<thead>
<tr>
<th>Elements</th>
<th>Parts of the plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stem</td>
</tr>
<tr>
<td>Cd&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>Cd&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00±0.09</td>
</tr>
<tr>
<td>Pb&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.81±0.02</td>
</tr>
<tr>
<td>Pb&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.18±0.36</td>
</tr>
<tr>
<td>Ni&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.30±0.01</td>
</tr>
<tr>
<td>Ni&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.67±0.23</td>
</tr>
<tr>
<td>Fe&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.60±0.09</td>
</tr>
<tr>
<td>Fe&lt;sup&gt;b&lt;/sup&gt;</td>
<td>142.61±1.70</td>
</tr>
<tr>
<td>Zn&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.93±0.15</td>
</tr>
<tr>
<td>Zn&lt;sup&gt;b&lt;/sup&gt;</td>
<td>242.52±2.81</td>
</tr>
<tr>
<td>Cu&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71±0.01</td>
</tr>
<tr>
<td>Cu&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.34±0.22</td>
</tr>
<tr>
<td>Al&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.35±0.07</td>
</tr>
<tr>
<td>Al&lt;sup&gt;b&lt;/sup&gt;</td>
<td>156.62±1.36</td>
</tr>
<tr>
<td>Cr&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16±0.00</td>
</tr>
<tr>
<td>Cr&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.00±0.02</td>
</tr>
<tr>
<td>Mn&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.14±0.01</td>
</tr>
<tr>
<td>Mn&lt;sup&gt;b&lt;/sup&gt;</td>
<td>227.68±0.18</td>
</tr>
</tbody>
</table>

<sup>a</sup>: µg/g dry weight; <sup>b</sup>: µg/100 g fresh weight

Figure-1. Concentrations of some trace elements in different parts of the plant bitter melon (µg/g DW).
Figure-2. Amounts of all metals in different parts of the plant bitter melon (µg/100 g fresh weight).

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