Analysis of some Heavy metals and Organic Acids in *Punica granatum* (Punicaceae) growing Adjacent to the Serpentine Soil in Kunjrin Village of Iraqi Kurdistan

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Summary

Plant samples of *Punica granatum* (Punicaceae) were collected in Kunjirin which is located at north eastern of Sulaimani governorate, during Augest to September 2008.

Punica granatum (Punicaceae) were collected, and the distribution of heavy metal content (Nickel, Cobalt,Zinc, and Manganese) in different organs (leaves, stems, and fruits) of this species analyzed .Also organic acid content(Citric acid, Fumaric acid, Malic acid, and Oxalic acid) in the plant were analyzed.

Generally the results showed that there were significant differences of heavy metals among plant organs except (Zinc) . The highest total of heavy metal content were found in the leaves within the concentration 92.61 µg g^{-1} dry weight. The lowest level were found in the fruits with concentration of 50.84 µg g^{-1} of dry weight.

The medium amount were found in the stem with 80.24 µg g^{-1} dry weight.

The results showed that the amount of selected metals in the plant fruits were lower than in the leaves, and they were found in legally admitted limits and they can be consumed by human.

Significant different distribution of organic acids were found among the plant organs.

The highest total of organic acid was found in the fruit of the plant, with the concentration of 307.25 µg g^{-1} dry weight.

The lowest level of organic acid was found in the stem (195.36 µg g^{-1} of the dry weight), the medium amount of the organic acid was in the leaves with the concentration 444.44 µg g^{-1} of the dry weight.

Introduction

Punica granatum (pomegranate) is native from Iran to the Himalaya, in the northern India , cultivated and neutralized over the whole Mediterranean region since ancient times(Sheidai *et al*, 2007). Today the pomegranate is cultivated in Iraq, Mediterranean countries, Afghanistan, India, China, Pakistan, and some parts of the United states.

It has significant economic importance since the fruits are either consumed fresh or used commercially in the juice and jam industries (Aguilar *et al* 2008, and Al.Qallaf 2009).

The constituents of pomegranate juice are anthocyanins, ascorbic acid, ellagic acid, gallic acid, and amino acids, but pomegranate leaves are composed of tannic, and flavone glycosides (Jurenka, 2008).

The fresh juice contains 85% water,10% total sugars and 1.5% pectin, ascorbic acid and polyphenolic flavonoids, in pomegranate juice, fructose and glucose are present in similar quantities, calcium is 50% of its ash content, and the principal amino acids are glutamic and aspartic acid (lauba,2007).

Pomegranate sugars are fructose (3.5 to 5.6 g/100gm) and glucose (3.4 to 6.4 g/100gm).

Contents of K, Na, Ca, and Mg are highest among minerals in fruit juice; the average concentration of vitamin C and pH are 0.09 – 0.40 mg/100gm, 2.9 - 4.21 respectively (Fadari *et al* 2005).

The increased color is associated with increased anthocyanin concentration, moderate CO_2 atmospheres prolong the storage life and maintain quality of pomegranates, including adequate red color intensity(Holeroff *et al* 1998).

The aim of the study: is to:

1- Identify and measurement some heavy metals and organic acids in different plant organs.

2- Clarifying the amount of heavy metals in the plant fruits and their relation to legally admitted limits to human consumption.

3- Comparing the heavy metal contents in the soil and different plant organs

Materials and Methods

The selected area Kunjirin village is located in the north western extension of Zagros-fold-thrust belt.

This area is located 25 km northeast of Sulaimany city, 20 km southeast of Mawat town with an altitude of approximately 1090 masl, and the studied orchards were adjacent to serpentine area.

1- Sample collection:

During August and September of 2008, six samples of organs (leaves, stem, and fruits) of Pomegranate were collected.

The specimens had one-year growth and were collected during the period of fruit maturation.

2- Drying, Grinding, and Storage of the samples,

Water were removed from plant tissues to stop enzymatic reaction and stabilize the sample.

Fresh plant tissues were dried in a dust free oven at temperature of 80 C˚ for 24 hours.

The dried tissue were stored in a moisture free atmosphere (Purpose , 2001).

Plant tissue samples were reduced be grinder to 0.5 – 1.0 mm particle size to ensure homogenity and to facilitate organic matter destruction (Purpose , 2001).

After a particle size reduction and homogenization, samples were stored in conditions that maintained sample integrity , the samples were stored in plastic containers.

3- Dry ashing and heavy metal determination:

Dry ashing (high temperature combustion) were performed by weighting 0.5 g of dried sample on sensitive balance in borosilicate crucible: the samples were heated at 500 C° in muffle furnace for 8 hours. the remainder (Ash) was dissolved with 25 ml of 2 M HCL. The prepared solution was filtered. The heavy metals were analyzed in the solution by atomatic absorption spectrophotometer, deionized water was used to dilute standards and samples. Nitric acid was used for standards preparation **(**Cobalt(Co), Nickel (Ni), Manganese(Mn), and Zinc(Zn) **)** (Brooks 1998).

4- Organic acid analysis:

The HPLC was used to separate and identify common organic acids in studied plant species. One gram of plant sample powder was extracted with 50:50 (v/v) methanol water for 20 minutes, the extract was filtered, then 20 µL of filtered sample were analyzed by using Supelco gel C-60 1H column, with a mobile phase 0.15% phosphoric acid and detected at 210 nm UV (Hussain, 2007 and Ribeiro *et al* 2007).

5- Statistical analysis:

Statistical significance evaluation was determined by one way analysis of variance ANOVA, SPSS version 15, data were shown as mean ± (stander error), Duncan test was used to clarify significant differences among mean values at probability level of ($P<0.05$) .

Results and Discussions

Results in figure (1) showed different distribution of heavy metals in the plant organs.

Cobalt contents showed no significances between leaves and stems (5.82 µg g^{-1}, 5.53 µg g^{-1}; but Cobalt contents were decreased significantly in the fruits 0.0 µg g^{-1} .

The amount of Nickel showed statistical difference among different organs. The Nickel were decreased significantly in leaves, stems, and fruits (19.7 µg g^{-1} ;11.75 µg g^{-1},and 0.333 µg g^{-1}) respectively , also amount of Manganese were decreased significantly in leaves, stems, and fruits, 38.48 µg g^{-1} , 27.71 µg g^{-1} , and 19.63 µg g^{-1} . however amount of Zinc showed no significant differences between leaves, stems, and fruits,(28.61 µg g^{-1} , 35.25 µg g^{-1}) and 30.88µg g^{-1}) respectively

Results in figure (2) showed different distribution of organic acid in the pomegranate organs. Citric acid contents showed no significant differences between leaves (105.25 µg g^{-1}) and fruits, (111.25 µg g^{-1}) however citric acid decreased significantly in the stem (50.30 µg g^{-1}).

The amount of Fumaric acid showed statistical differences among different organs, they were increased significantly in fruits, leaves and decreased in stems, (117.77 µg g^{-1},80.42 µg g^{-1}, and 42.10 µg g^{-1} respectively).

Also the amount of Oxalic acid were increased significantly in the leaves (104.63 µg g^{-1})and decreased in the stems(53.76 µg g^{-1}) and fruits, 28.0 µg g^{-1}.

Malik acid contents showed no significant differences between stems (49.20 µg g^{-1}) and fruits; (50.23 µg g^{-1}) ut Malik acid contents were increased significantly in leaves (154.13 µg g^{-1})

The results showed that the cobalt contents in the leaves were higher than the sufficient ranges needed for plant growth and development. However the cobalt contents were lower than the toxicity range , but the cobalt contents in the leaves of pomegranate were 5.82 µg g^{-1},it was higher than the sufficient range (0.02 µg g^{-1}), the high nickel contents in the analyzed plant leaves were in agreement with Eboh and Thomas, (2005) who clarified that nickel level in cannabis leaves were (10.40 µg g^{-1})dry weight . this high nickel level might be related to the soil being contaminated with numerous amount of nickel from serpentine soil, it might also be related to easy mobility of nickel in plants (Kabata-Pendias 2001).

Zinc contents in the leaves of pomegranate 28.61 µg g^{-1}, they were in the sufficient ranges (20-150 µg g^{-1}).

In addition, manganese contents in leaves of pomegranate were 38.48 µg g^{-1} ,and were in sufficient ranges (20-300 µg g^{-1}).

Organic acids have important role in the detoxification of heavy metals. The kind of organic acids differ among plant species, Citric and malic acids are supposed to be responsible for chelating and transportation of nickel in to the plant cell vacuoles; however citric acid seems to be most likely the nickel chelator (Jocsak *et al* 2005).

The organic acid concentration appears to be different between cultivars. The content of organic acid is highly variable during plant development, probably under the influence of external factors like temperature (Univer *et al* 2004)

The results show unequal distribution of organic acids among the different plant organs and also among plant species. Thus results are agreement with (Falade *et al* 2003)who indicate that orange juice contains highest level of ascorbic acid but low in citric acid, lime juice is very rich in citric acid, and pin apple juice contains a low level of the organic acids.

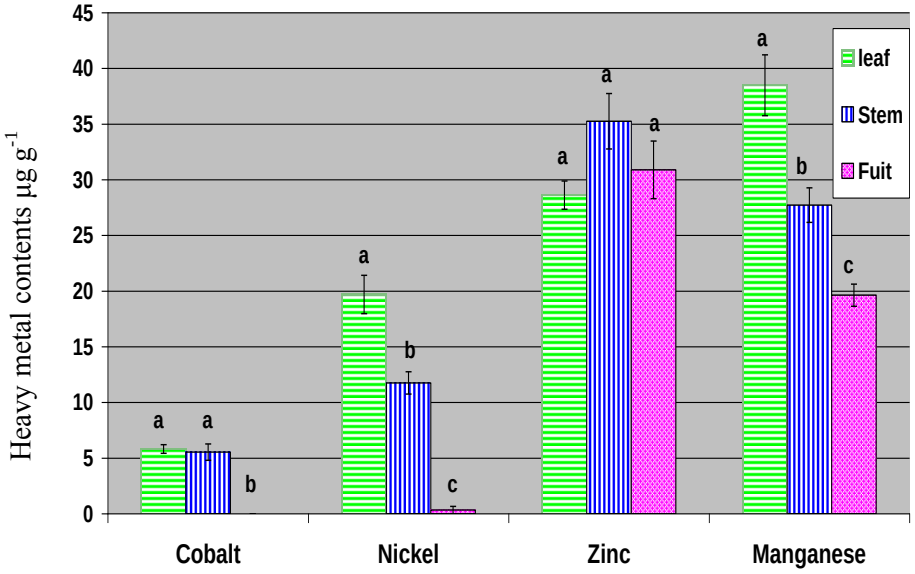

Fig.1: Heavy Metal Content (µg g^{-1} of dry matter) in Different Pomegranate Organs .
Different Letters Indicate Significant Differences Among Organs at the ($p < 0.05$) Level Probability, (Mean±SE) n=6.

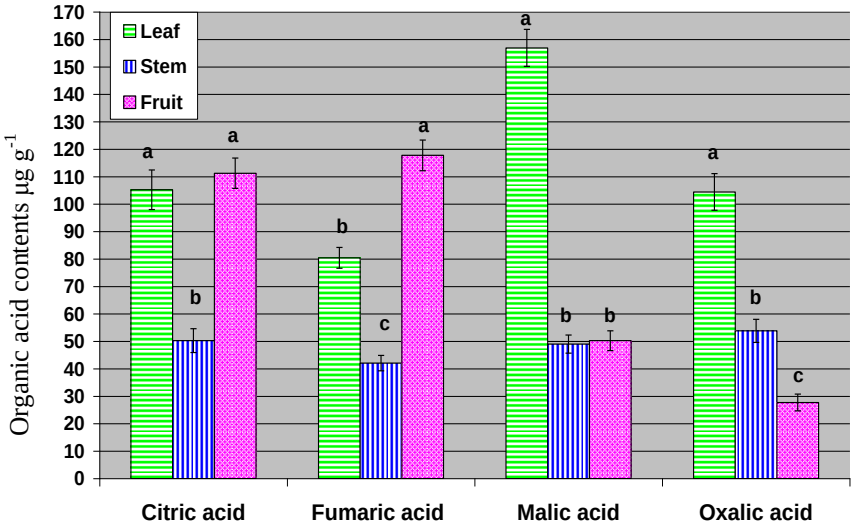

Fig. 2: Organic Acid Content (µg g^{-1} of dry matter) in Pomegranate Organs.
Different Letters Indicate Significant Differences Among the Organs at the ($p < 0.05$) Level Probability, (Mean±SE), n=

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تحليل بعض العناصر الثقيلة والاحماض العضوية في نبات الرمان المستزرعة في بساتين المتاخمة للترب السربنتينية في قرية كونجرين في كوردستان العراق

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الملخص

تم جمع نماذج الساق والاوراق والثمار من نبات الرمان في قرية كونجرين المتاخم للاراضي السربنتينية الواقع في شمال شرق محافظة السليمانية ، حيث تمت دراسة توزيع وتقدير العناصر الثقيلة (نيكل، كوبلت، زنك، منغنيز) وذلك جنبا الى جنب مع تقدير الاحماض العضوية (ستريك و فيوماريك ومماليك واوكزاليك) في الاعضاء الثلاث، حيث اظهرت النتائج بان هناك فروقات معنوية بين العناصر الثقيلة المدروسة في الاعضاء الثلاث ماعدا عنصر الزنك. وسجلت الاوراق اكبر كمية كلية من العناصر وذلك بمقدار 92.61 مايكروغرام/غرام من الوزن الجاف ، اما اقل كمية كلية من العناصر سجلت في الثمار وذلك بمقدار 50.44 مايكروغرام/غرام من الوزن الجاف، وسجلت في السيقان الكميات الكلية المتوسطة من العناصر وذلك بكمية 80.24 مايكروغرام/غرام من الوزن الجاف .خلال دراسة نتائج هذه الدراسة تمت ملاحظة اقل كمية من العناصر الثقلية تجمعت في الثمار وهذا يسمح باستهلاك الثمار من قبل الانسان حسب القيمة المسموحة دوليا للعناصر الثقيلة .

كذلك سجلت فروقات معنوية بين الاحماض العضوية في الاعضاء الثلاث حيث سجلت اكبر كمية كلية من الاحماض العضوية في الثمار وذلك بمقدار 444.44 مايكروغرام/غرام من الوزن الجاف، واقل كمية كلية من الاحماض العضوية سجلت في السيقان وذلك بكمية 195.36 مايكرغروام/ غرام وسجلت في الاوراق الكميات الكلية المتوسطة من الاحماض العضوية وذلك بمقدار 307.25 مايكروغرام/غرام من الوزن الجاف.