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RECURRING TRANSFORMATION OF MINERAL NUTRIENTS AND PHENOLICS IN POMEGRANATE (*Punica Granatum* L.) FRUIT

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ABSTRACT

Pomegranate fruit is an important source of potentially healthy bioactive compounds and mineral nutrients. Changes in total phenolic compound, concentrations, and levels of macronutrients (P, K, N, Mg, Ca and Na) and micronutrients (Zn, Cu, Mn, Fe and B) in arils and peel of pomegranate fruit were recorded from 10 days after full bloom until harvest. Total phenolics levels increased at early stage of growth both in peel and arils of fruit, but thereafter generally decreased during maturation and reached to 3.70 and 50.22 mg g-1 of dry weight in arils and peel, respectively, at harvest. The amount of total phenolics in peel was markedly higher than arils of pomegranate fruit. The concentration of most elements in arils and peel decreased during fruit growth and development. At harvest the relative order of concentration of macronutrients both in arils and peel was K > N > Ca > P > Mg > Na. The concentration of most micronutrients was greater in the arils than in the peel especially in early season. The relative order of concentration of micronutrients in arils was B > Fe > Zn > Cu > Mn. The accumulation of all the macro- and microelement within the fruit also increased during fruit growth and development. These results provide important data on total phenolics and macro- and micronutrient changes during fruit growth and development, emphasizing that pomegranate fruit can be a good source of bioactive compounds and minerals.

Key words: Pomegranate (*Punica granatum* L.), Mineral nutrients, Total phenolic compound, Fruit growth and development, Macro- and micronutrients.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an important tree of the tropical and subtropical regions of the world which is valued for its delicious edible fruit [1]. The edible part of the fruit is called arils which are eaten fresh and can be preserved as syrup or used for making jam. In addition, the tree is also valued for its pharmaceutical properties. The fruit peel, stem, root bark and leaves are a good source of secondary products such as tannins, dyes and alkaloids.

Fruit and vegetable are an important source of essential elements. Mineral nutrients and phenolics are natural component of many fruit and play an important role in maintaining fruit quality and determining nutritive value. Pomegranate is a rich source of polyphenols. And especially arils contain substantial amount of polyphenols such as gallic Acid, protocatechinuie acid, chlorogenic acid, caffeic acid, Ferulic acid, coumaric acids and catechin. Moreover, other properties of arils and husk of pomegranate fruit such as antioxidant, anti-inflammatory, and ant atherosclerotic against some diseases (osteoarthritis, prostate cancer, heart disease, HIV-1) have been reported [2,3].

The pomegranate has been of recent interest for its nutritional and antioxidant characteristics. First time Singh et al [4] reported antioxidant properties of the extracts from pomegranate peel and seeds. The methanol extract of pomegranate peel showed the highest antioxidant activity among all the extracts.

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Kulkarni and Aradhya [5] have analyzed antioxidant activity and chemical changes in pomegranate arils during fruit development. There was also an interest on antimutagenic activity and antioxidant activity of peel, pith and carpellary membrane of pomegranate fruit [6,7]. Recently, the physical and chemical changes of pomegranate fruit have been reported. Al-Maiman and Ahmad [8] showed that composition of minerals varied markedly among the three ripening stages. The amounts of potassium, calcium and sodium were highest in both juice and seeds followed by magnesium, phosphorous, zinc, iron and copper.

They demonstrated that pomegranate fruit can be a good source of nutrients and variation could originate from the pomegranate cultivar, and agro-climatic as well [9]. Besides these studies, data on changes of minerals and phenolics of pomegranate fruit from fruit set to ripening and minerals in peel are still scarce. In addition, the chemical composition of fruit differs depending on cultivar, growing region, climate, maturity, cultural practice. Significant variations in organic acids, phenolic compounds, sugars, water-soluble vitamins and minerals composition of pomegranates have been reported over the years by various reports [10,11]. There is an important consumption of fruit and vegetable in Indian populations.

However, there appears to be rather limited data on nutritional values of pomegranate fruit and its role in human health. As far as we know, there are no data in the literature about changes in phenolics and minerals composition during growth and development of pomegranate fruit in India and changes of mineral nutrients of peel in literature. Knowledge of the seasonal accumulation of nutrient is necessary to develop criteria for describing the optimum nutrient status for both yield and quality of tree crops. Macro- and micronutrient deficiencies and toxicities result in a wide assortment undesirable alteration in the appearance of horticultural products. In general, these alterations in macro- and micronutrients are in correlations to shape, and size of the product. Bar-Akiva et al [12] showed that high percentage of nitrogen in the ammoniac form results in smaller orange fruit. On the other hand Daane et al [13] found that nitrogen deficiency reduces stone fruit size. Phosphorus deficiency results in large 'Valencia' orange [14,15], while zinc and iron deficiency decreases citrus fruit size [16].

In pomegranate, Mirdehghan and Rahemi [17] have studied physical changes of fruit during fruit growth and development. They showed that the main changes in fruit size have occurred within 60 days after full bloom. Therefore, this study was undertaken to complement the information available on changes in phenolics and minerals component of peel and arils of pomegranate fruit from fruit set to ripening.

MATERIALS AND METHODS

Plant material and experimental design

Pomegranate fruits cv. 'Malas Yazdi' was obtained from mature trees (13-year-old) from the Agricultural Research Center of Malegaon province. The trees were spaced 6 and 3 m between and along the rows, respectively. Trees received routine cultural practices suitable for commercial fruit production including winter and summer pruning, fertilization and irrigation.

Since flowering occurs in about 3 distinct waves, about 500 flowers were marked at full bloom to provide fruit samples. Then, growth and development were followed by sampling 20 single fruits every 10 days. The fruits were transferred to the Laboratory soon after harvest in plastic bags. Then, the fruit were peeled manually and after 4 days at 65°C in hot air oven, dry weight of peel and arils (containing seed) were measured and powdered to get 60 mesh size. Four replicates were maintained for each analysis and each replicate indicating five pomegranate fruits. All reagents, solvents and standards were of analytical reagent grade [18].

Extraction and determination of total phenolics

The peel and arils powder were extracted by stirring using a magnetic stirrer with 600 ml of MeOH at room temperature (25°C) for 4 h. The extract was filtered through Whatman no. 41 filter paper for removal of particles. The residue was re- extracted with 500 ml of MeOH and filtered. The extracts were pooled and concentrated under vacuum at 40°C. The concentration of total phenolics in the methanolic extract was determined according to the method of Kotamballi and Murthy [19] and results were expressed as (+)-catechin equivalents. Five milligrams of each dried pomegranate extract was dissolved in a 10-ml mixture of acetone and water (6:4 v/v). Samples (1 ml) were mixed with 5.0 ml of 7.5% sodium carbonate solution. After standing for 30 min at room temperature the absorbance was measured at 765nm using a Spectronic spectrophotometer. The estimation of phenol compound in the extract was carried out in triplicate and averaged.

Nutritional analysis

The powder was ashed in a muffle oven at 550 ± 25°C. The Resulting white ash was then dissolved in 10 ml of 2N HCL and adjusted to a volume of 100 ml for determination of macro- and microelements [20,21]. Suitable dilutions were subsequently made for determination of various minerals. The Mg, Ca, Cu, Zn, Fe, and Mn contents were measured using a Perkin-Elmer (model 3110) atomic absorption spectrophotometer. Sodium and potassium content were determined by PFP flame photometer. The spectrophotometric method was used for phosphorous determination. Boron in samples was measured by dry ashing and subsequent measurement

of B by colorimetry using azomethane-H [22]. For nitrogen analysis, samples were digested according to the method of Chapman and Pratt and total nitrogen content was determined using Kjeldhal method [23].

Data were analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance using LSD test using the Statistical Analysis System (SAS) software Version 6.12.

RESULTS AND DISCUSSION

Fruit growth

The dry weight of peel and arils increased regularly throughout the season (Fig. 1). Early in the season the weight of peel was dominant per fruit while around middle of season, the weight of arils became dominant and continued increasing until September to a mean dry weight of 35.03 and 22.33 g fruit 1 in arils and peel, respectively. The seasonal increase in fruit dry weight is typical of the pattern of fruit growth established for many other fruiting crops [24,25] of 20 fruits. Vertical bar represents + S.E.

Total phenolic content

Changes in concentration of total phenolics of peel and arils are shown in Fig. 2. The amount of total phenolics in peel was markedly higher than arils of pomegranate fruit (nearly eight- fold). Total phenolics levels increased at the early stage of growth both in peel and arils of fruit, but thereafter generally decreased during maturation and reached to 3.70 and 50.22 mg g-1 of dry weight in arils and peel, respectively, at harvest. There were some Fluctuations in the phenolics content of peel from 60 to 100 days after full bloom. Some of these phenolics in pomegranate have previously been separated and identified by comparison with authentic standards using reversed-phase high-performance liquid chromatography (HPLC). Phenolic acids have repeatedly been implicated as natural antioxidants in fruit, vegetable especially pomegranate fruit. For example, gallic acid, caffeic acid, catechin and ferulic acid had identified in freshly prepared pomegranate juices. Kulkarni et al. have been isolated and identified a radical scavenging antioxidant punicalagin from pith and carpellary membrane of pomegranate fruit. They have suggested that the mechanism of antioxidant action of punicalagin and methanol extract is by donating electrons to free radicals. They also suggested that pith and carpellary membrane waste of pomegranate fruit is an economically viable source of punicalagin, a natural and potent antioxidant. Li et al [26] recently reported that pomegranate peel extract had markedly higher antioxidant capacity than the pulp extract in scavenging or preventive capacity against superoxidase anion, hydroxyl and proxyl radicals. They showed that the contents of total phenolics, flavonoids and proathocyanidins were also higher in peel extract than in pulp extract. The large amount of phenolics contained in peel extract may cause its strong antioxidant ability. Also, pomegranate peel exhibited higher antioxidant activity than seeds. Therefore, pomegranate peel extract appeared to have more potential as a health supplement rich in natural antioxidants than the pulp extract and merits further intensive study.

Macronutrients concentration

For most of the macronutrients, there were strong differences in the seasonal changes of nutrient concentration for peel and arils of pomegranate fruit. Most nutrient concentrations in arils and peel of fruit declined (Figs. 3 and 4) at two rates, very sharply during the 40 days after full bloom and then gradually in the following days after full bloom and prior to harvest. The overall decline in nutrient concentration during early growth is largely the result of the rate of nutrient accumulation being less than of the growth of the fruit. Exceptions to this general pattern was sodium in which concentrations fluctuated in arils (Fig. 4c) and seasonal concentration remained static at harvest, but tended to increase in peel (Fig. 4f). Calcium concentration in peel decreased rapidly during 30 days after full bloom (Fig. 4e) but the concentration in arils increased during 20 days after full bloom and remained constant until day 40 and then decreased rapidly (Fig. 4b). This may be related to formation of seed inside the arils. Our results agree with the study on 'Navel' orange fruit that suggested Ca concentrations increased in first stage of development in pulp and then decreased until maturity. Similar to our results, Garcia-Martinez et al [27] reported an initial decrease in Ca concentration of peel followed by an increase in Ca concentration of 'Washington Navel' and 'Valencia Late' orange fruit 4 months after the first harvest date. The pattern of decline of macronutrients concentration in arils and peel of pomegranate fruit during fruit development is similar to trends found in other fruit, e.g. tamarillo [28,29], Japanese pear [30] and 'Navel' orange. At harvest the relative order of concentration of macronutrients both in arils and peel was K > N > Ca > P > Mg > Na. This trend in arils and peel of pomegranate fruit at harvest time is similar to trends found in 'Navel' orange. The content of sodium is greater in 'Taifi' cultivar of pomegranate while in our sample was less than other elements.

The variation could originate from the pomegranate variety, and agro-climatic and environmental condition. The macronutrients concentration in arils are higher than the peel except calcium and sodium, where Ca concentration is higher in peel than arils especially early in the season and sodium concentration is relatively equal to arils and peel.

The high concentration of Ca in the peel and the low concentration in the pulp can be attributed to the low mobility of Ca in phloem [31].

Macronutrient accumulation

The quantity of a nutrient present in fruit was calculated as the product of the elemental concentration by dry weight yield. The relationships between time and macro and micronutrients accumulation in arils and peel are fitted according to regression equation shown in each figure. For each nutrient, the quantity accumulated at successive harvests increased throughout the season (Figs. 3 and 4). The magnitude of the increase diminishing with time in Mg and Ca of peel. During the season considerable accumulation occurred during early fruit development and continues until harvest for most of macronutrients especially in arils. For a number of elements including K and P, there was a strong linear relationship between elemental and dry matter accumulation throughout growth. This is usually implied to be indicative of nutrient supply via phloem. In contrast, approximately 70% of Ca requirements by fruit may be taken up during the first stages of growth and development, when supply by xylem is likely to predominate. Significant accumulation during early fruit growth identifies this as a period when adequate supply of Ca to the plant is crucial, and when the Ca nutrition of fruit might most easily influence those properties where fruit quality is known to suffer because of Ca imbalance later in the season. This association may be more important in peel where causing some problem such as fruit cracking that leads to loss of quality of fruit. The patterns of increasing most of nutrient accumulations by fruit with age are similar to those described for tamarillo, Japanese pear and apple.

Micronutrients concentration

Compared to the macronutrients, seasonal trends were, generally, the same both in arils and peel for all micronutrients. Concentration of all micronutrients both in arils and peel decreased from a maximum just after fruit set to a minimum at fruit harvest (Figs. 5 and 6). Exception to this general pattern was boron where the minimum concentration was 60 and 130 days after full bloom for arils and peel, respectively (Fig. 6b and d). By harvest, the micronutrients present at highest concentrations in arils was B (22.2 mg kg -1) followed by Fe (14.5 mg kg -1).

Minors were Zn, Cu and Mn with concentrations of 11.75, 8 and 6 mg kg-1, respectively. In comparison, the content of Zn, Cu and B in arils of pomegranate are higher than edible part of persimmon that is 4, 2 and 6 µg g-1, respectively [32], while the amount of Mn and Fe in flesh part of persimmon is higher than pomegranate arils. The relative order of concentration of micronutrients in peel of fruit was B > Fe > Zn = Mn > Cu. These results are in accordance with those obtained by other authors in similar studies. Storey and Treeby found the same trends for micronutrient in 'Navel' orange. The concentration of micronutrients was greater in the arils than in the peel especially in early of the season with the exception of element B where this difference was not very much. In general, the average concentration of Zn, Cu and B found in this paper, in the arils of pomegranate, were greater than those reported in 'Navel' orange, while the concentration of Mn and Fe were similar. A comparison of the results obtained by us with those found in different fruit and vegetable. Armu and Udoessien [33] reveal a trend similar to that described above. These results emphasizing that pomegranate fruit can be a good source of nutrients.

Microelements accumulation

The accumulations of all the microelement within the fruit also increased during fruit growth and development (Figs. 5 and 6). There was a strong linear correlation between dry weight yield and the amount accumulated in the arils of fruit. In peel this association among micronutrients was weaker. Most microelements especially Cu, in peel of pomegranate, where this association was weaker, accumulated during early stages of growth and development (Fig. 5e). Most changes in fruit size of pomegranate have occurred during 60 days after full bloom and it seems that there is a high demands for minerals in this period for fruit growth and development. High concentration of macro and microelements in peel and arils of pomegranate in early stage of growth show that it is necessary to perform a good balance of macro and micronutrients before growth and fruit set of pomegranate fruit to satisfy their requirement to minerals.

CONCLUSION

In conclusion, a comparison of the results obtained by us with those found in other studies reveals that pomegranate fruit contains important amounts of phenolics and high amount of nutrients both in arils and peel, especially microelements in edible parts that play a valuable role in daily requirement of minerals. It can be recommended that a harvest date for pomegranate fruit will give appropriate levels of phenolics and minerals for people who consume the fruit in their diets. However, since there are more than 200 cultivars in Iran which are used as fresh, Jam and concentrated juices, more studies of chemical analysis are required among different cultivars. The peel of pomegranate has been used in folk medicine for many centuries. The large amount of phenolics in peel provides a good potential as a health supplement rich in natural antioxidants and more studies need to be undertaken to identify other bioactive compounds and their useful role in medicine industry.

Figure 1. Seasonal changes in dry weight of peel (0) and arils (.) of pome- granate fruit during growth and development. Each mean is an average of 20 fruits. Vertical bar represents + S.E.

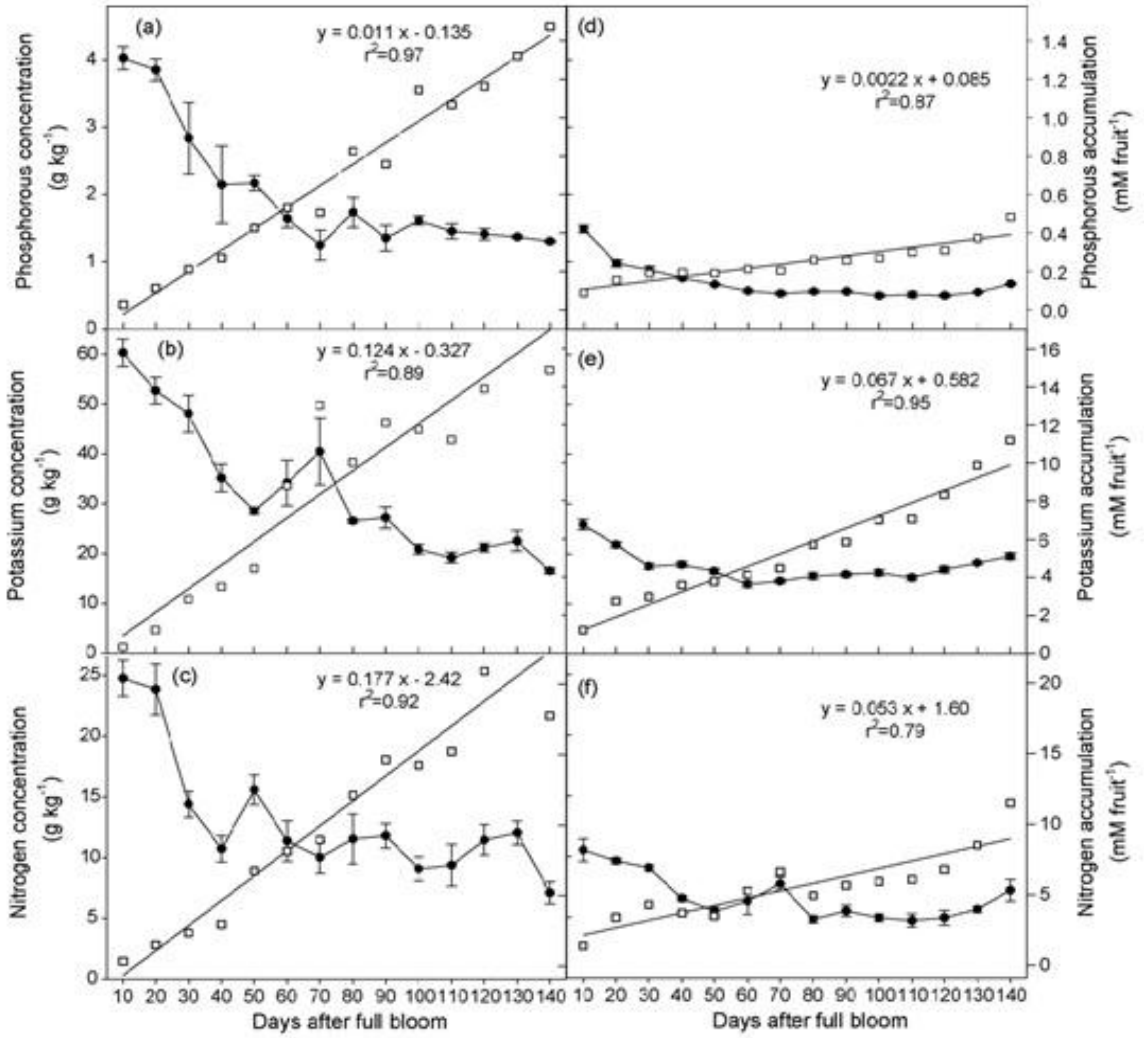


Figure 2. Seasonal changes in total phenolic compounds of peel (0) and arils (.) of pomegranate fruit during growth and development. Each mean is an average

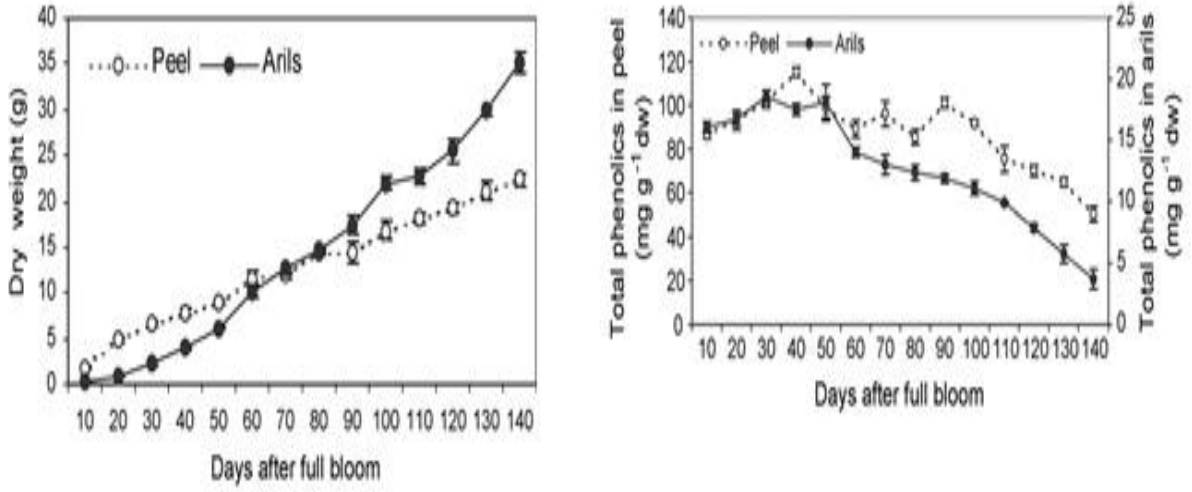


Figure 3. Seasonal changes in macronutrient (P, K and N) concentration (.) and accumulation (white square are fitted according to regression equations shown) of arils (a-c) and peel (d-f) of pomegranate fruit during growth and development. Each mean is an average of 20 fruits. Vertical bar represents + S.E.

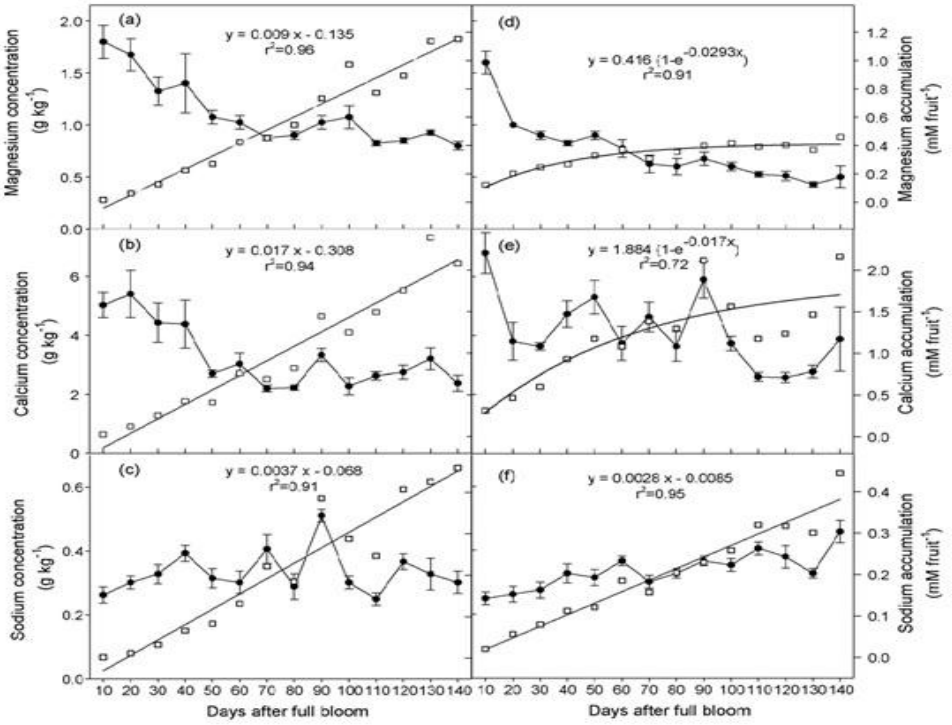


Figure 4. Seasonal changes in macronutrient (Mg, Ca and Na) concentration (.) and accumulation (white square are fitted according to regression equations shown) of arils (a-c) and peel (d-f) of pomegranate fruit during growth and development. Each mean is an average of 20 fruits. Vertical bar represents + S.E.

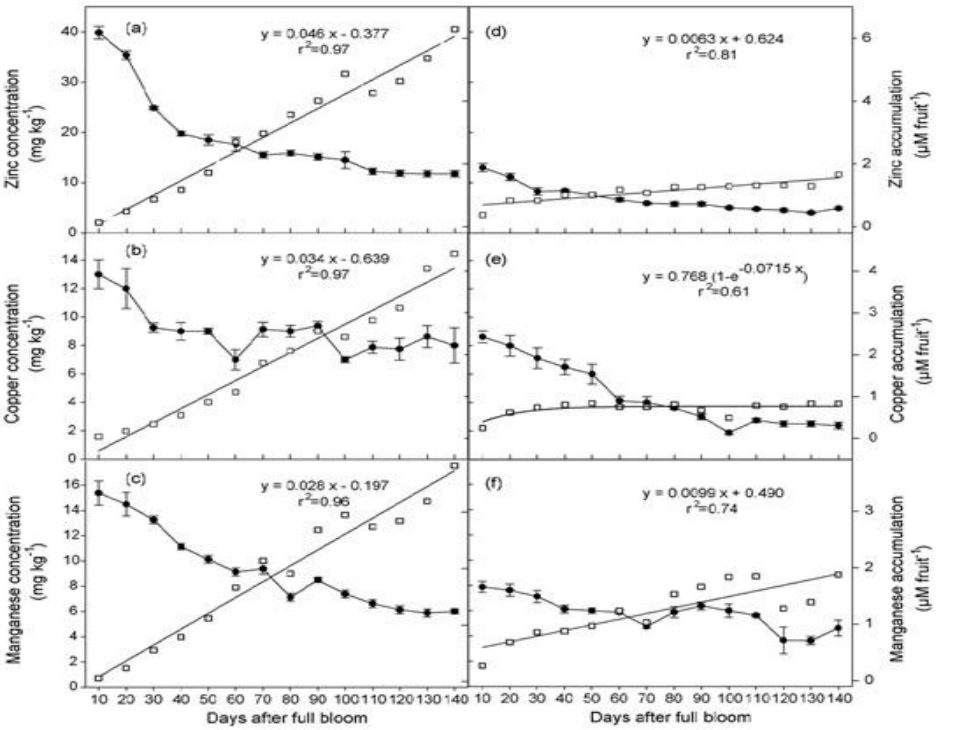
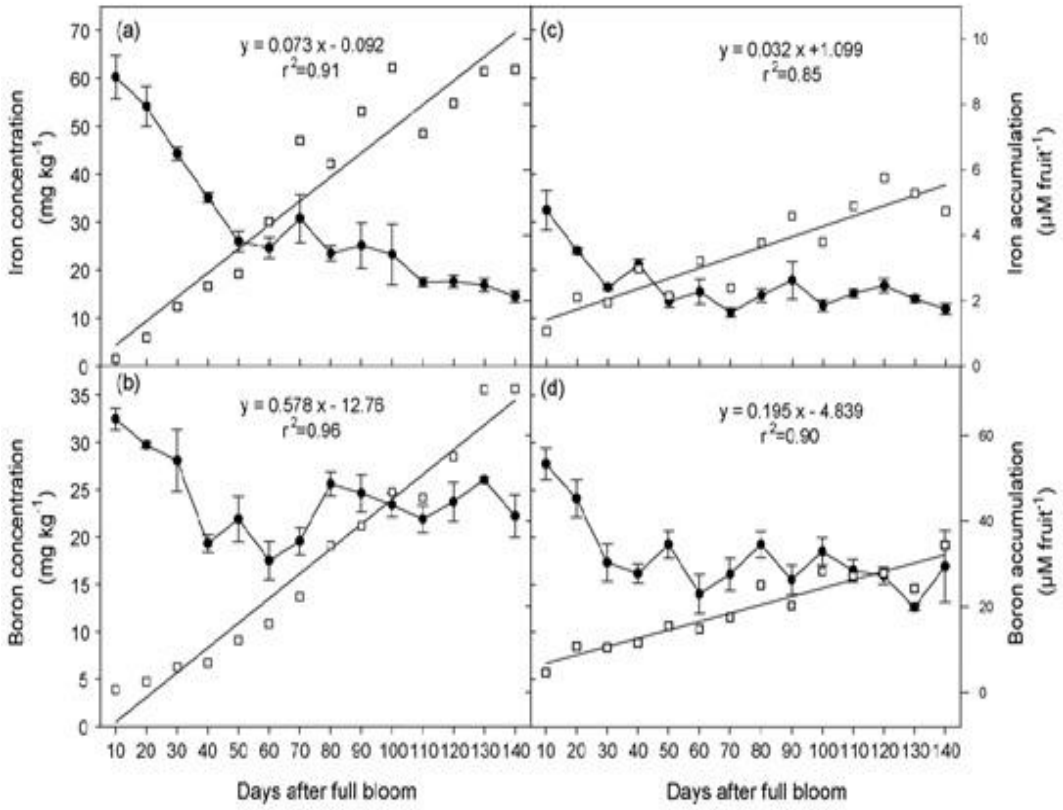


Figure 5. Seasonal changes in micronutrient (Zn, Cu and Mn) concentration (.) and accumulation ( ) (white square are fitted according to regression equations shown) of arils (a-c) and peel (d-f) of pomegranate fruit during growth and development. Each mean is an average of 20 fruits. Vertical bar represents + S.E.

Figure 6. Seasonal changes in micronutrient (Fe and B) concentration (*) and accumulation (&) (white square are fitted according to regression equations shown) of arils (a and b) and peel (c and d) of pomegranate fruit during growth and development. Each mean is an average of 20 fruits. Vertical bar represents + S.E.



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