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Ecology

Results of Ecological Monitoring of Citrus Fruits Grown in the Coastal Subtropical Zone of Ajara

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Considering the present-day ecology of the environment the quality indicators of agricultural products from various agrocenoses should be given particular attention and harmful effects of environmental pollutants on vegetable food products should be identified. Heavy metals are highly toxic, and their high concentrations in living organisms, soil, fruit trees, vegetables, and greens harm the chemical and physical properties of the organism. The object of the study was the fruits of lemon, mandarin and orange trees growing in the coastal subtropical zone of Ajara, namely, the citrus plantations in Akhalsopeli, Gonio, and Makhvilauri (Khelvachauri Municipality), as well as the citrus fruits (citron (*Citrus medica* L.), kumquat (*Fortunella japonica* Sw.) and pomelo (*Citrus grandis* L.)) from the collection plot in Batumi Botanical Garden. Concentrations of different microelements (lead, zinc, iron, aluminum, copper, and cadmium) were measured in them. The concentrations of microelements in the test fruits were identified by the analytical method of atomic absorption spectroscopy (AAS), which commonly uses element light absorption to measure the element concentration in a sample. The analysis of the sample aims to determine whether a sample contains particular element. As the obtained results show the content of heavy metals in citrus fruits citron (*Citrus medica* L.), kumquat (*Fortunella japonica* Sw.) and pomelo (*Citrus grandis* L.) sampled from the collection plot in the Botanical Garden is within the admissible limits. The contents of heavy metals in lemon (*Citrus limon* L.), orange (*Citrus sinensis* L.), and satsuma mandarin (*Citrus unshiu* Marcow) sampled from Khelvachauri Municipality (villages Makhvilauri, Akhalsopeli and Gonio) were higher than those in the samples from the collection plot of the Batumi Botanical Garden, but were still within the admissible limits. *© 2022 Bull. Georg. Natl. Acad. Sci.*

citrus plants, fruits, microelements, spectroscope

It is estimated that 70% of the toxic substances that regularly enter the human body come from food, 20% from air, and 10% from water. The problem of having high-quality food can be solved by ecologizing agricultural systems [1].

Today, safe food is a necessary living condition in any society, allowing for the demographic, economic, political, cultural, and intellectual development. As a result, one of the most important objectives for any state is to ensure that its population has access to safe food on a consistent basis as a necessary condition for the healthy nation [2].

Citrus plants are a large group of evergreen plants in *Rutaceae* family, *Citrus* genus. The genus incorporates 39 species and varieties. Orange is the most industrially produced citrus fruit, followed by grapefruit, mandarin, lemon, lime, and others. Citrus crops account for approximately 30% of global fruit consumption. Most citrus plants are native to India, China or Indochina. Currently, none of the species are wild, and the cultivated forms and species historically evolved in the tropics and subtropics.

Citrus fruits have been known to the Georgians since the 12^{th} century, where they were first introduced in the late 19^{th} and early 20^{th} centuries. In the former Soviet Union, more than 90% of citrus plants were cultivated in West Georgia, where mandarin, orange, lemon, grapefruit, etc. were grown in the open fields [3].

Small quantitiess of heavy metals are found in living organisms as a part of biologically active substances and regulate vital functions of the human body, while the increased concentrations of heavy metals lead to negative and destructive consequences. Heavy metals that enter the human body accumulate in the liver, and are removed from the body very slowly.

Heavy metals are chemical elements with an atomic number greater than 40. Lead, cadmium, zinc, mercury, nickel, and other heavy metals are most dangerous. Approximately 90% of heavy metals accumulate in the soil, migrate to natural waters, are absorbed by plants, and enter the food chain. Lead, mercury, cadmium, arsenic and zinc are considered to be major pollutants, owing to their technogenic accumulation in the environment. These elements inhibit metabolic processes, plant growth and development [4].

One of the causes of the environmental pollution is anthropogenic impact: industrial waste, pesticides used in agriculture, various radiation substances, sewage, vehicles, etc. Heavy metals, along with other sources of environmental pollution, are a major issue because many of them are highly toxic. Their half-life is tens, hundreds or even thousands of years. Their excess amounts in living organisms, soil, fruit trees, vegetables, and greens harm the chemical and physical properties of the organism and reduce fertility [5, 6].

Materials and Methods

The goal of the study was to determine the content of microelements in the fruits of citrus plants grown in Ajara’s coastal humid subtropical zone. Lemon, mandarin, and orange fruits from citrus plantations in the villages of Akhalsopeli, Gonio, and Makhvilauri of Khelvachauri Municipality, as well as citrus fruits (citron (*Citrus medica* L.), kumquat (*Fortunella japonica* Sw.), and pomelo (*Citrus grandis* L.)) grown on a collection plot in Batumi Botanical Garden were sampled for analysis, and content of microelements was measured in them.

Lemon (*Citrus limon* L.) is a subtropical perennial evergreen plant in *Rutaceae* family. The lemon tree grows to a height of 3-7 m. A mandarin tree has prickly branches and pale green, serrated, leathery oblong-lanceolate or oblong-ovate leaves. The flowers are androgyne, white, and aromatic. Lemon fruit is many-seed berry. The flesh is greenish-yellow in color, fine-grained, juicy, and very sour.

Lemon fruit contains 3.5-8.1% acids (primarily citric), 1.9-3% sugars, 87.5% water, 45-140 mg vitamin C per 100 g, as well as vitamins P and B, pectin substances, phosphorus, potassium, calcium, and magnesium.

Lemon is a heat-, light- and the damp-loving citrus species. It is native to Southeast Asia. The main regions where lemons are grown as open fields are the Black Sea coast of the Caucasus, Azerbaijan and Central Asia.

Orange (*Citrus sinensis* L.) is a subtropical evergreen plant in *Rutaceae* family.

It can grow to 12 m on a strong stock and 4 to 6 m otherwise. An orange tree has dark green leathery leaves that are oval in shape, androgyne, white, solitary or clustered in inflorescence, and fruit that is a many-seeded berry. The flesh of the fruit is juicy, sweet or sour-sweet. Orange fruit contains 78-89% water, 5-14% sugars, 0.1-1.5% organic acids, 0.8-0.36% fiber, and 0.6-0.7% nitrogenous substances. It contains vitamins B1, B, C, and provitamin A. The vitamin B content of orange is the same as in milk. The fruit peels contain a variety of essential oils. Orange fruit is used to treat hypo- and avitaminosis, as well as to aid digestion.

The fruit's peels, leaves, and flowers contain 1.2-2.1% essential oil, which is used in perfumery and food industry. Orange is damaged at -7°C, and it freezes completely at -10°C [7].

Satsuma mandarin (*Citrus unshiu* Marcow) is the most commonly spread mandarin variety. The tree can reach a height of 3m and has a trunk diameter of 3-3.5m. It has rather large androgyne flowers. The petals of the crown contain glands that contain a large amount of essential oil. The fruit is seedless and weighs 60-80g. The peel is orange, and the flesh is light orange and juicy.

Heavy metals in the fruits samples were studied with an atomic absorption spectroscope by applying graphite cuvettes at LEPL Laboratory Research Center of the Autonomous Republic of Ajara.

Atomic Absorption Spectroscopy (AAS) is an analytical technique to measure the concentration of elements. It uses the principle of light absorption by the elements to calculate their concentrations. The absorption of a light beam by free atoms in the ground electronic state in gaseous phase is measured quantitatively. The atoms absorb ultraviolet or visible light and are shift to a higher electronic energy level.

The concentration of the analyzed element is determined by absorption. Concentrations are typically measured by calibrating the instruments according to the working curve, with standard solutions of the known concentration. Atomic absorption is the most common method for detecting metals and non-metals. It can be used to measure the concentrations of up to 70 different chemical elements in environmental media.

The cathode with the shape of a cylindrical cavity concentrates the emitted radiation into a beam that passes through the quartz window and is directed to the sample vapor. The atoms of different elements absorb the wavelengths of light typical to them.

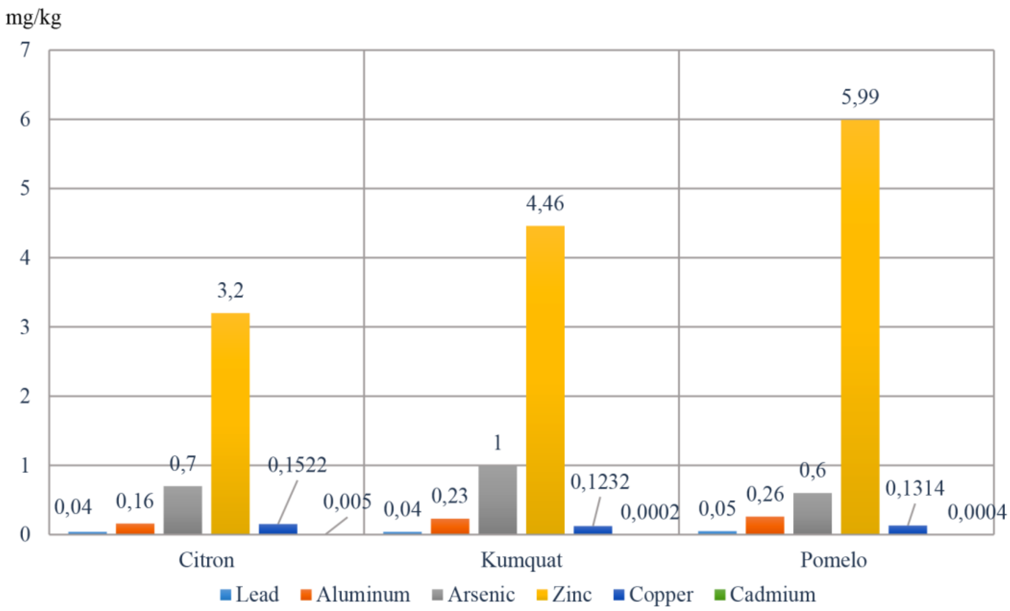

Fig. 1. Results of the biochemical study of citrus fruits from the Botanical Garden collection plot.

Based on the light emission by the element, the analysis of a sample determines whether a sample contains the element. A beam of electromagnetic radiation generated by excited lead atoms will pass through the vaporized sample. Part of the radiation is absorbed by the lead atoms in the sample. The more atoms in the vapor, the more radiation is absorbed.

An atomic absorption spectrometer consists of four major parts: 1 – light source (usually a hollow cathode lamp or electrodeless discharge lamp), 2 – atomic cell (atomizer), 3 – monochromator, and 4 – detector and data transfer unit. The atomizer and the radiation source – the cathode lamp – are the two main components of an atomic absorption spectrometer [8].

appropriate wavelengths (lead: 283.5 nm; aluminum: 309.3 nm; iron: 248.3 nm; zinc: 307.6 nm, copper: 327.4 nm, and cadmium: 288.8 nm) was placed in the spectrometer to identify each type of heavy metal, and the amounts of heavy metals were determined using the developed technique. We prepared solutions of different concentrations according to the reference solution as follows; 1 ml of basic standard solution (C = 1000 mg/L) was added by 0.03 normality HNO_3 in 100 ml in a volumetric flask to obtain intermediate solution, which was used to make standard solutions of different concentrations. Specifically, for lead, standard solutions of the following concentrations were prepared: 5 µg/L, 10 µg/L, 15 µg/L, 20 µg/L, 25 µg/L, and 30 µg/L. 5 µg/L concentration standard

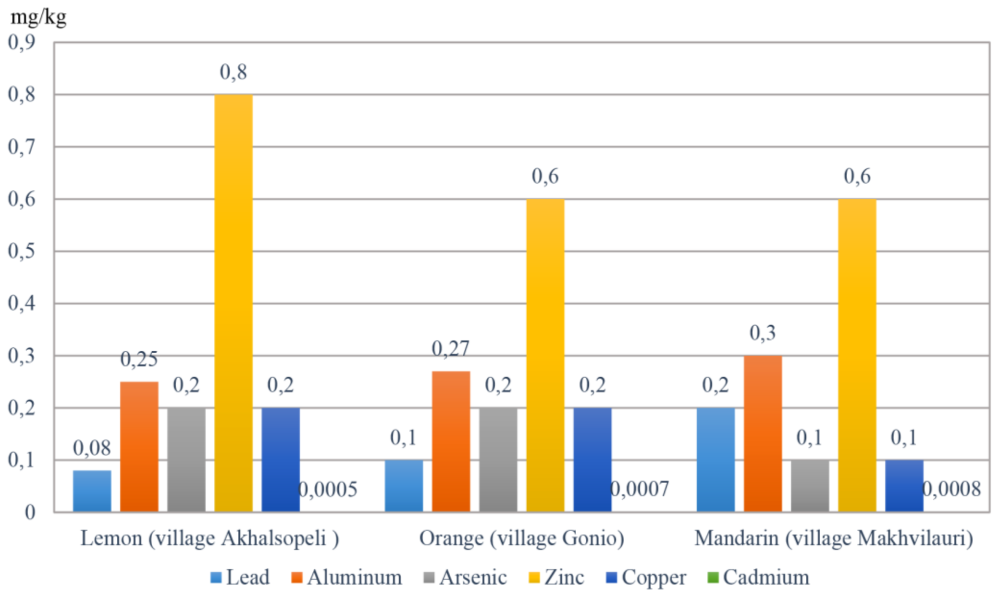

Fig. 2. Results of biochemical study of lemon, orange and mandarin fruits.

Results and Discussion

The material for research has been prepared according to the requirements for heavy metals detection in the fruits. The fruits were sampled into different types, mashed and subjected to the necessary procedures. The ready test samples were placed placed in an atomic-absorption spectrometer (KVANT.Z) with an eppendorf to determine the content of heavy metals. A special lamp with

solution was made by adding 5 µg of intermediate solution (C = 10 µg/L) to 995 µg of HNO_3 (0.03 normality); 10 µg/L concentration standard solution was made by adding 10 µg of intermediate solution to 990 µg of HNO_3; 15 µg/L standard concentration solution was made by adding 15 µg of intermediate solution to 985 µg of HNO_3; 20 µg/L concentration standard solution was made by adding 20 µg of intermediate solution to 980 µg of HNO_3; 25 µg/L standard concentration solution was made by adding 20 µg of intermediate solution to 975 µg of HNO_3; and 30 µg/L standard concentration solution was made by adding 30 µg of intermediate solution (C=10 µg/L) to 970 µg of HNO_3 ((0.03 normality). We placed the obtained solutions of different concentrations in separate eppendorfs and into the atomic absorption spectrometer.

For aluminum, the following standard solutions were prepared: 5 µg/L, 10 µg/L, 15 µg/L, 20 µg/L, 25 µg/L, and 30 µg/L by the same principle. Arsenic was identified with standard solutions: 5 µg/L, 10 µg/L, 15 µg/L, 20 µg/L; for zinc, 200 µg/L and 400 µg/L standard solutions were used; for copper, 10 µg/L, 20 µg/L, 30 µg/L standard solutions were used, and 0.5 µg/L, 1.0 µg/L, 1.5 µg/L, and 2.0 µg/L standard solutions were used for cadmium (Arsenic GOST 31266-2004, Mercury M.M. 4.1. 1472-2003) [9,10].

The results of the biochemical study of the fruits of lemon (*Citrus limon* L.), orange (*Citrus sinensis* L.), satsuma mandarin (*Citrus unshiu* Marcow) sampled from Khelvachauri Municipality (villages Makhvilauri, Akhalsopeli, and Gonio) are given in Fig. 2.

As the obtained results show, the contents of heavy metals in lemon (*Citrus limon* L.), orange (*Citrus sinensis* L.), and satsuma mandarin (*Citrus unshiu* Marcow) sampled from the villages of Khelvachauri Municipality were within the admissible limits.

Conclusion

Thus, the contents of microelements in the fruits of citron (*Citrus medica* L.), kumquat (*Fortunella japonica* Sw.) and pomelo (*Citrus grandis* L.) sampled from the collection plot in Batumi Botanical Garden were within the admissible limits indicating that the soil in the Botanical Garden is not polluted with heavy metals.

The content of microelements in the fruits of lemon (*Citrus limon* L.), orange (*Citrus sinensis* L.), and satsuma mandarin (*Citrus unshiu* Marcow) sampled from Khelvachauri Municipality (villages Makhvilauri, Akhalsopeli, and Gonio) was higher than those in the samples taken from the collection plot in Batumi Botanical Garden, still it was within the admissible limits.

ეკოლოგია

აჭარის ზღვისპირა სუბტროპიკულ ზონაში გავრცელებულ ციტრუსოვანთა ნაყოფების ეკოლოგიური მონიტორინგის შედეგები

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**ქუთაისის აკ. წერეთლის სახელმწიფო უნივერსიტეტი, აგრონომიულ მეცნიერებათა დეპარტამენტი*
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გარემოს ეკოლოგიური მდგომარეობის შესაბამისად, ცალკეული აგროცენოზის პირობებში განსაკუთრებული ყურადღება უნდა ექცეოდეს სასოფლო-სამეურნეო პროდუქციის ხარისხობრივ მაჩვენებლებს. აუცილებელია, რეგიონების მიხედვით დადგინდეს თუ რა მავნეობა მოაქვს გარემოს დამაბინძურებლებს კვების რაციონში შემავალ მცენარეულ პროდუქტებზე. მძიმე მეტალები ხასიათდება მაღალი ტოქსიკურობით. ცოცხალ ორგანიზმში, ნიადაგში, ხეხილში, ბოსტნეულ კულტურებსა და მწვანილში მათი ჭარბი რაოდენობით მოხვედრა იწვევს ორგანიზმის ქიმიური და ფიზიკური თვისებების დარღვევას. კვლევის ობიექტად აღებულ იქნა აჭარის ზღვისპირა სუბტროპიკულ ზონაში გავრცელებული ციტრუსოვნების, კერძოდ, ხელვაჩაურის მუნიციპალიტეტის სოფ. ახალსოფელში, გონიოსა და მახვილაურში ციტრუსოვანთა კულტურების პლანტაციიდან აღებული ლიმონის, მანდარინის და ფორთოხლის ნაყოფის ნიმუშები და ბათუმის ბოტანიკური ბაღის საკოლექციო ნაკვეთზე გაშენებული ციტრუსოვნების (*Citrus medica* L. – ციტრონი; *Fortunella japonica* Sw. – ფორტუნელა კინკანი იაპონური; *Citrus grandis* L. ციტრუსი დიდი, შედოკი, ანუ პომპელმუსი) ნაყოფები. მათში განისაზღვრა მიკროელემენტების (ტყვია, თუთია, რკინა, ალუმინი, სპილენძი, კადმიუმი) შემცველობა. მიკროელემენტების განსაზღვრა საანალიზო ნაყოფებში განხორციელდა ატომურ-აბსორბციული სპექტროსკოპიის (AAS) ანალიზური მეთოდით. აღნიშნულ მეთოდში გამოყენებულია ელემენტების მიერ სინათლის აბსორბცია, რათა გაიზომოს მათი კონცენტრაცია ნიმუშში. ნიმუშის ანალიზი გულისხმობს ელემენტის მიერ გამოსხივებული სინათლის მიხედვით დადგინდეს, შეიცავს თუ არა ნიმუში კონკრეტულ ელემენტს. კვლევის შედეგების თანახმად, მძიმე მეტალების შემცველობა ბათუმის ბოტანიკური ბაღის საკოლექციო ნაკვეთზე აღებულ ციტრუსოვანთა (ციტრონი, ფორტუნელა კინკანი იაპონური, ციტრუსი დიდი, შედოკი ანუ პომპელმუსი) ნაყოფებში აღმოჩნდა ზღვრულად დასაშვები ნორმის ფარგლებში. ხელვაჩაურის მუნიციპალიტეტის სოფლებიდან (მახვილაური, ახალსოფელი, გონიო) აღებული ციტრუსოვნების – ლიმონის, ფორთოხლის, მანდარინი უნშიუს ნაყოფებში მიკროელემენტების შემცველობა აღმოჩნდა შედარებით მაღალი, ვიდრე ბოტანიკური ბაღის საკოლექციო ნაკვეთიდან აღებულ ციტრუსოვანთა ნიმუშებში, თუმცა ყველა მაჩვენებელი იყო ზღვრულად დასაშვები ნორმის ფარგლებში.

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