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Risk Assessment of Toxic Elements in Fruits and Vegetables Grown in Ararat Region, Armenia

D.A. Pipoyan, M.R. Beglaryan, G.H. Tepanosyan, L.V. Sahakyan
Center for Ecological-Noopshere Studies, NAS RA
david.pipoyan@cens.am, meline.beglaryan@cens.am, gevorg.tepanosyan@cens.am, lilit.sahakyan@cens.am

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ABSTRACT

This study aims to assess toxic elements' contents in fresh fruit and vegetable, as well as related potential health risks to the adult population in the Ararat region. In the frame of the research, the transfer of toxic elements from soil to plants was evaluated and non-carcinogenic risks from food consumption were assessed. The results indicated that only in the case of apple consumption there is a low level of non-carcinogenic risk to *As* exposure. Meanwhile, in multi-food consumption and multi-element ingestion cases, the low level of risk was reported both for *As* and *Pb* exposure. Further comprehensive assessments considering more elements and additional routes of exposure are needed in the region.

Introduction

Fruits and vegetables are important nutritious components of the population's diet (FAO, 2020). Intake data available for 162 countries, indicated that the weighted mean vegetable intake was 186 g/day (Kalmpourtzidou, et al., 2020). Global fruit intake was 114 g/day (1.1 servings (100 g/day)), with highest intakes reported in Latin America (146 g/day) and lowest in the Middle East and North Africa (MENA) region (Micha, et al., 2015). According to the published data on national food balances, the per capita consumption of fruits and vegetables in the Republic of Armenia is more than 800 grams/day (Statistical Committee of the Republic of Armenia, 2020a). Therefore, continuous monitoring of these products' safety is of high priority for the population health risk assessment (Pajević, et al., 2018).

The issues related to the safety of fruits and vegetables were the subject of investigations worldwide. Numerous studies were directed to assess contamination of plants with toxic elements and associated potential risks to human health (Alam, et al., 2020, Filippini, et al., 2020, Gupta, et al., 2021, Pajević, et al., 2018, Raj and Maiti, 2020, Sanaei, et al., 2021). The contamination can occur due to natural or anthropogenic activities, which contribute to elevated levels of toxic elements in the agro-ecosystem (Pajević, et al., 2018). Investigations carried out in different countries (Chang, et al., 2014, Gupta, et al., 2021, Hu, et al., 2020, Raj and Maiti, 2020, Vrhovnik, et al., 2016) as well as in Armenia (Pipoyan, et al., 2019, 2018), revealed that fruits and vegetables have been contaminated with trace elements, particularly with lead *(Pb)*, cadmium *(Cd)*, arsenic *(As)* and mercury *(Hg)*. These are known as toxic elements causing serious adverse health effects (e.g. neurodevelopmental effects, lung damage, kidney damage, cancer, etc.) even at low levels of exposure (Dorne, et al., 2011, Raj and Maiti, 2020). With the growing concern associated with dietary exposure to toxic elements, regulatory requirements have been set for the safety of food products including fruits and vegetables. In the Republic of Armenia, product safety is regulated by the Eurasian Economic Union's technical regulation setting the maximum allowable levels of the abovementioned toxic elements (Eurasian Economic Community, 2011).

The production of fruits and vegetables is more developed in the Ararat region of Armenia. Ararat is one of the economically developed regions of the country. The current basis of the region's economy is agriculture. As of 2019, the share of agriculture in the region's economy was 14.9 %. It is mainly specialized in viticulture, fruit-growing and vegetable-growing. The manufacture of food products (processing and canning of fruits and vegetables) and drinks is well-developed (Statistical Committee of the Republic of Armenia, 2020b). It is worth mentioning that the produced fresh fruits and vegetables, as well as processed foods, are not only consumed locally but are also being exported (e.g. to countries of the Eurasian Economic Union).

Despite the fact, that Ararat is one of the important agrarian regions and main providers of fruits and vegetables in the country, there is a lack of investigation data on food contamination with toxic elements and associated health risks, as well as the possible sources of pollution. Therefore, the goal of the present study is to assess the contents of toxic elements *(Pb, As, Cd, Hg)* in fresh fruits and vegetables, as well as associated potential health risks to the adult population of the Ararat region.

The work was supported by the Science Committee of MESCS RA, in the frames of the research project "Development of Geochemical Maps to Ensure Sustainable Agricultural Development and Food Safety" and State Program on "Environmental GeoEcological Studies".

Materials and methods

Study site, sampling and analyses

The investigated fruits and vegetables were sampled from the Ararat region which is one of the 11 administrative regions (marzes) of the Republic of Armenia. It has an area of 2 090 km^2 (7 % of total area of Armenia). The region is located in the southwest of Armenia. From the north, it has borders with Armavir region, Yerevan and Kotayk region. From the east, it is bordered by Gegharkunik and Vayots Dzor regions. Turkey and Nakhchivan Autonomous Republic respectively form the western and southern borders of the region. Ararat is the largest region with its rural population (population de jure number is 11754) (Statistical Committee of the Republic of Armenia, 2020b).

The sampling of fruits and vegetables was done in the frame of the state monitoring program (Goverment of the Republic of Armenia, 2018) from June to October, 2019. It was implemented according to the standard operational procedures (SOPs) developed in the Center for Ecological-Noosphere Studies (CENS) using methodological guidelines of Codex Alimentarius Commission (CAC, 1993) and ISO standard on sampling (ISO 874-1980, 2017). The subsamples (at least 3) of the major cultivated and available fresh fruits and vegetables were randomly taken from agricultural plots in 13 rural communities of the Ararat region. The subsamples were mixed in a polyethylene bag to form composite samples for each fruit and vegetable. In total, 33 composite samples of 11 species of major fruits (cherry, peach, plum, grape and apple) and vegetables (watermelon, melon, green beans, tomato, cucumber and eggplant) were formed and investigated.

The sample preparation and subsequent chemical analysis of the edible parts of fruits and vegetables were done in the Republican Veterinary-Sanitary and Phytosanitary Laboratory Services Center (RVSPCLS) SNCO accredited following ISO 17025 standard. The contents of *Pb, Cd, Hg* and *As* in fruits and vegetables were determined using the atomic-absorption spectrometry (AAS, Thermo Fisher iCE-3500). The replicate analysis of each composite sample of fruit and vegetable was conducted. For the quality assurance of analysis, the Multi-Element Aqueous CRM US EPA 23 standard solution was used. The recovery ratios ranged from 95 to 98.8 % throughout the analytical procedures. The limit of detection (LOD) and limit of quantification (LOQ) are presented in Table 1.

Table 1. Limit of detection and limit of quantification*

Toxic elements	LOD	LOQ
Pb	$1 * 10^{-3}$	$5 * 10^{-4}$
As	$2 * 10^{-3}$	$1 * 10^{-3}$
Cd	$5 * 10^{-4}$	$2.5 * 10^{-5}$
Hg	$2 * 10^{-3}$	$1 * 10^{-3}$

*Composed by the authors.

Among the investigated toxic elements, Hg was not detected in all studied samples of fruit and vegetable. Besides, Cd contents were below the limit of quantification (<LOQ). Therefore, these two elements were excluded from further discussions.

Transfer factor (TF)

TF characterizing the transfer of toxic elements from soil to edible parts of fruits and vegetables was calculated with the following equation (Tasrina, et al., 2015):

$$TF = C_{plant} \times C_{soil}, \quad (1)$$

where C_{plant} and C_{soil} are the contents of a toxic element in the plant extraction and soil, respectively.

The TFs were evaluated based on the contents of toxic elements in studied plant species and adjacent soil samples. It should be mentioned that *Cd* and *Hg* were not detected in soil samples. The data on contents of other toxic elements *(Pb, As)* in soil samples were provided by the Environmental Geochemistry Department of CENS. The soil samples were taken from the same agricultural plots where the investigated fruits and vegetables were cultivated and sampled. The contents of toxic elements in soil samples were detected using Innov X-5000 X-ray fluorescence spectrometer in compliance with the US EPA 6200 (US EPA, 2007).

Chronic non-carcinogenic risk

The potential non-carcinogenic health risk associated with chronic exposure of toxic elements was assessed calculating the hazard quotient (HQ):

$$HQ_{plant} = EDI/RfD, \quad (2)$$

where *RfD* is the oral reference dose of toxic elements. For *As*, RfD is equal to 0.0003 mg/kg/day, respectively (US EPA, Integrated Risk Information System, 1991). For *Pb* the provisional tolerable intake values (PTWI) were used as RfD. Before using PTWI (0.004 mg/kg/day) in calculations (EFSA, 2010), the values were divided into 7.

To assess HQs, the estimated daily intake (EDI) of each toxic element was calculated with the following equation (WHO/FAO, 2008):

$$EDI = (C \times IR)/(BW), \quad (3)$$

where *C* is the average content (mg/kg) of toxic elements in fruits and vegetables, *BW* is the body weight (70 kg) and the *IR* is the mean daily consumption of each fruit or vegetable. The data on IR was taken from the food consumption database (Figure 1) provided by the Informational-Analytical Center for Risk Assessment of Food Chain of CENS (www.cens.am). The consumption database has been developed using food frequency questionnaire (FFQ) methodology (FAO, 2018) and the statistical treatment of the data were done using SPSS (IBM SPSS, V.22) software (Ares, 2014).

The assessed HQs were summed to calculate hazard indexes (HI) characterizing the potential non-carcinogenic risk due to the multi-element exposure and combined consumption (multi-food consumption) of investigated fruits and vegetables:

$$HI = \sum_{i=1}^{n} HQ, \quad (4)$$

where *n* is the number of fruit and vegetable samples.

For non-carcinogenic risk characterization, the risk level description adopted by Tepanosyan et al. was used (Tepanosyan, et al., 2021). The following levels were considered: no risk (HQ < 0.1), low risk (0.1-1), medium risk (1-4) and high risk (HQ > 4).

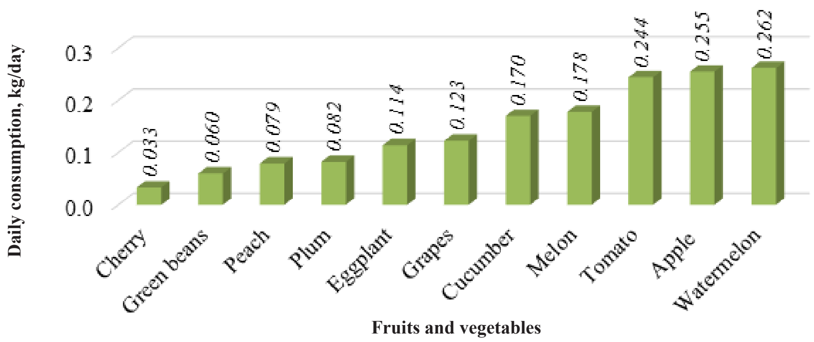

Figure 1. Daily consumption of investigated fruits and vegetables *(composed by the authors)*.

Results and discussions

Toxic elements in fruits and vegetables

The contents of *Pb* and *As* in studied fruits and vegetables are presented in Table 2.

Table 2. Contents of toxic elements in selected fruits and vegetables***

<table>
<tr><th rowspan="4">Fruits and vegetables</th><th colspan="3">Mean content of toxic elements (Mean ± SD, mg/kg)</th></tr>
<tr><th colspan="2">Pb</th><th>As</th></tr>
<tr><th colspan="3">Allowable level (mg/kg) according to technical regulation (Eurasian Economic Community, 2011)</th></tr>
<tr><th>0.4*</th><th>0.5**</th><th>0.2</th></tr>
<tr><td>Tomato</td><td colspan="2">0.034 ± 0.008</td><td>-</td></tr>
<tr><td>Cucumber</td><td colspan="2">0.032 ± 0.008</td><td>-</td></tr>
<tr><td>Green bean</td><td colspan="2">0.023 ± 0.001</td><td>-</td></tr>
<tr><td>Eggplant</td><td colspan="2">0.039 ± 0.004</td><td>-</td></tr>
<tr><td>Watermelon</td><td colspan="2">0.035 ± 0.003</td><td>-</td></tr>
<tr><td>Melon</td><td colspan="2">0.042 ± 0.011</td><td>-</td></tr>
<tr><td>Cherry</td><td colspan="2">-</td><td>0.022 ± 0.013</td></tr>
<tr><td>Apple</td><td colspan="2">0.039 ± 0.023</td><td>0.019 ± 0.01</td></tr>
<tr><td>Peach</td><td colspan="2">0.033 ± 0.007</td><td>-</td></tr>
<tr><td>Plum</td><td colspan="2">0.041 ± 0.008</td><td>0.017 ± 0.01</td></tr>
<tr><td>Grape</td><td colspan="2">-</td><td>-</td></tr>
</table>

Note. * - for fruits, ** - for vegetables, - the content of TE in the selected samples was below the limit of detection (<LOD) or was not detected.

***Composed by the authors.

The contents of *Pb* were in the range of 0.023-0.042 mg/kg (with an average value of 0.064 mg/kg). *Pb* was not detected in samples of cherry and grape. Arsenic was detected only in 3 samples of fruits (cherry, apple and plum). Comparisons showed that the detected contents of *Pb* and *As* in all investigated fruits and vegetables were much lower than the maximum allowable levels (Table 2) set by the technical regulation on food safety (Eurasian Economic Community, 2011).

Transfer factor (TF) of toxic elements

Soil to plant transfer factor was calculated by combining the data on the content of toxic elements in plants and adjusted soils from agricultural plots in several rural communities of the Ararat region. The values are shown in Table 3.

Table 3. Soil to plant transfer factor of toxic elements*

Fruits and vegetables	TF values	
	Pb	*As*
Tomato	0.003	-
Cucumber	0.002	-
Green bean	0.001	-
Eggplant	0.003	-
Watermelon	0.003	-
Melon	0.004	-
Cherry	-	0.002
Apple	0.002	0.001
Peach	0.003	-
Plum	0.004	-
Grape	0.005	-

Note. "-" wasn't calculated, since the content of toxic element in the selected samples was below the limit of detection (<LOD) or was not detected.

*Composed by the authors.

According to some researchers (Rai, et al., 2015, Vrhovnik, et al., 2016), when TF > 1, plants undergo the bioaccumulation of trace elements from the soil. Conversely, TF < 1 indicates that the plant absorbs the element but does not accumulate it (Rai, et al., 2015, Vrhovnik, et al., 2016).

The calculated TF values are far below 1, indicating the absence of the bioaccumulation of toxic elements by the studied plant species. Similar results (TF < 1) were reported in the frame of other investigations (Jolly, et al., 2013, Pipoyan, et al., 2018, Rezaei, et al., 2019, Tasrina, et al., 2015).

Dietary exposure of toxic elements

Dietary exposure assessment was done calculating the estimated daily intake (Table 4) of toxic elements *(Pb, As)* through the consumption of fruits and vegetables grown in the Ararat region.

The obtained EDI values (Table 4) indicated that the daily intake (EDI) of toxic elements from selected fruits and vegetables didn't exceed the health based guidance values (e.g. PTWI and RfD) set by international organizations. Moreover, the same picture is observed in the case of multi-food consumption. The total EDI for *Pb* (8.26E-04 mg/kg/day) had 23.6 % contribution in the tolerable daily intake (3.50E-03 mg/kg/day). Meantime, the total EDI of *As* (9.95E-05 mg/kg/day) had the 33.2 % contribution in the oral reference dose (3.00E-04 mg/kg/day).

Table 4. Estimated daily intake (EDI) of toxic elements via fruits and vegetables consumption

Fruits and vegetables	Estimated daily intake (mg/kg/day)	
	Pb 3.50E-03 (PTWI/7)	*As* 3.00E-04 (RfD)
Tomato	1.19E-04	-
Cucumber	7.77E-05	-
Green bean	1.97E-05	-
Eggplant	6.35E-05	-
Watermelon	1.31E-04	-
Melon	1.07E-04	-
Cherry	-	1.04E-05
Apple	1.42E-04	6.92E-05
Peach	3.72E-05	
Plum	4.81E-05	1.99E-05
Grape	8.23E-05	-
Total EDIs	8.26E-04	9.95E-05

Note. PTWI/7- provisional tolerable weekly intake, RfD - oral reference dose, - wasn't calculated since the content of toxic element in the selected samples was below the limit of detection (<LOD).

*Composed by the authors.

Non-carcinogenic risk of toxic elements

In order to provide an assessment of potential non-carcinogenic health risks to adult population of Ararat region, the hazard quotients (HQ) of *Pb* and *As* were calculated (Figure 2). Moreover, the HI was calculated (Figure 3) to assess the possible health risks induced by toxic elements due to the total intake of all fruits and vegetables (multi-food consumption).

According to the RAIS methodology (The Risk Assessment Information System, 2020), the non-carcinogenic risk (HQ and/or HI) greater than 1 indicates that an adverse health effect could be expected, while the range of 0.1-1 indicates that some precautionary measures should be considered. If HQ/HI < 0.1, then there is no possibility of an adverse health effect. The results (Figure 2) of the current study showed that the calculated HQ values for each product (except for apple) are significantly below the precautionary level (HQ < 0.1). However, in the case of apple consumption, the low level of non-carcinogenic risk to *As* exposure was observed. When considering the multi-food consumption scenario, the hazard indexes of both *Pb* and *As* (Figure 3) exceeded the precautionary level of 0.1 indicating a low level of chronic non-carcinogenic health risk for the adult population in the Ararat region. In the case of the multi-element exposure due to the multi-food consumption, the HI is equal to 0.568. This constitutes 57 % of the safety threshold of 1.

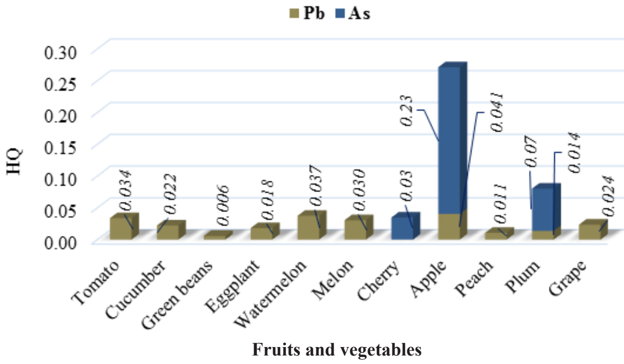

Figure 2. Daily consumption of investigated fruits and vegetables *(composed by the authors).*

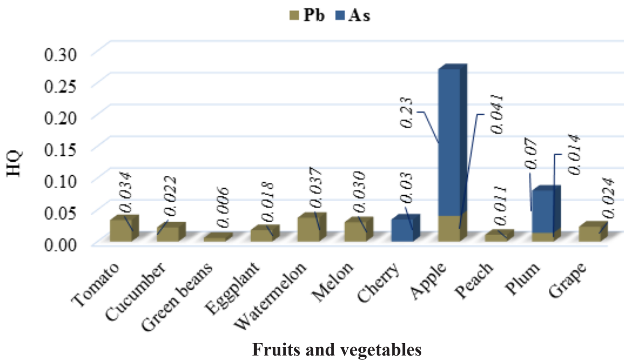

Figure 3. Hazard index (HI) of *Pb* and *As* in case of multi-food consumption *(composed by the authors).*

Conclusion

The study represents the first step to assess dietary exposure to toxic elements in one of the important agrarian regions of Armenia - Ararat. Among the studied toxic elements only for *Pb* and *As* the detected contents were reported. At the same time, it was found out that the selected fruits and vegetables grown in different rural communities in the Ararat region are not bio-accumulators of *Pb* and *As*. However, the local adult population's health risk assessment results showed some concerns associated with these toxic elements. The outcomes indicated that the total daily intakes of *Pb* and *As* resulting from the multi-food consumption, contribute a considerable proportion (23.6 % and 33.2 %, respectively) of the tolerable daily doses.

Overall, the study outcomes highlighted that even with the allowable contents of the toxic elements, the consumption of the selected fruits and vegetables can pose low level of chronic non-carcinogenic risk to the adult population in the Ararat region. To conclude, further comprehensive assessments considering more elements and additional routes of exposure are needed in the region. Moreover, the changes in the consumption of the studied fruits and vegetables can be also considered as one of the possible options for the risk mitigation.

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