High Concentration of Heavy Metal and Metalloid Levels in Edible Campomanesia adamantium Pulp from Anthropic Areas

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Abstract: This study aimed to quantify the extent of heavy metal, non-metal and metalloid level of Campomanesia adamantium pulp obtained from area crossed by road of the high large vehicle traffic and intensive agriculture modern farm, and for monitoring the health risks associated with pulp human consumption. For this purpose, three spots located within this area, ripe fruits were collected in roadside, bush and margin-farm. Pulp samples were digested by microwave-assisted equipment, and mineral elements were quantified by ICP OES. The mineral elements average demonstrated no statistical difference observed between this pulp (p > 0.05). The heavy metals and metalloid concentrations that exceeded FAO/WHO standards are ordered Pb > As > Mo > Co > Ni > Mn > Cr. Therefore, among these metalloid and heavy metals, As, Pb and Cr were found higher in farm-margin > roadside > bush (1.5 × 10^5, 1.1 × 10^3 and 6.2 × 10^4) respectively. Therefore, As is the most important metalloid with higher levels in farm-margin, roadside and bush (1.5 × 10^5, 1.1 × 10^3 and 6.2 × 10^4 > 10^{3-10^4} and 3.38, 2.30 and 1.34 > 1) respectively, to total cancer risk and hazard quotient, if 100 g daily of pulp are consumed.

Keywords: Cerrado; Myrtaceae; edible fruit; farm-margin; roadside; macro- and microelements; health risk.

1. Introduction

Native fruits contribute to food security. Heavy metal contamination of edible plants has been regarded as an environmental and public health hazard. Due to the severe anthropogenic activities, as high large-vehicle traffic and intensive modern agriculture, the environment becomes prone to high toxicity and bioaccumulation of heavy metal in plants used for food or medicines [1–3]. Among several species of plants, Campomanesia adamantium (Cambess.) O. Berg (Myrtaceae), popularly known as “Guavira or Guabiruba”, stands out for its wide occurrence in the Cerrado and other biomes, as Atlantic Forest and Pampa in Brazil with intensive and intense anthropogenic activities [4]. Also, roots, leaves and fruits of this species are popularly used as antirheumatic, anti-diarrheal, hypcholesterolemic, anti-inflammation, urethritis, cystitis and others [5–7]. Moreover, several studies reported the potential activities of C. adamantium fruits as antibacterial and antifungal [8,9], anti-hyperalgesic, antidepressive [10,11], antiproliferative against cancer cells [12,13], hepatoprotective [14], an inhibitor of leukocyte mobility, neurogenic pain and oedema [6]. The genus Campomanesia includes 37 species, and 26 are endemic in Brazil.
The *C. adamantium* fruits, characterized by a citrus aroma and sweet flavor, are consumed fresh or used to produce homemade liqueurs, juices, ice creams, jellies, backer products, and others [15]. Additionally, they are natural sources of a considerable amount of ascorbic acid, fibers, vegetable oil, polyphenols, and monoterpenic substances [6,13,16].

To date, there are only studies that have quantified minerals in the peel, pulp and seed of *C. adamantium* collected near urban areas [17,18]. However, no studies have been carried out on assessing chemical elements in fruits collected close to roads with high vehicle traffic in agricultural regions. Fertilizers, pesticides, and vehicle fumes contain heavy metals and metalloids, such as potassium (K), arsenic (As), iron (Fe), lead (Pb), chromium (Cr), manganese (Mn), molybdenum (Mo), Nickel (Ni), and other elements, which in high amount contaminate the environment, edible plants and consequently humans [1,3,19,20].

In view, this study aimed to quantify Fe, Ni, Co, Cr, As, Pb, Mo, Mn, P, K, Zn, Se in the *C. adamantium* fruit pulp collected in three spots from the roadside (500 m) to bush (1000 m) and farm-margin (3000 m).

2. Materials and Methods

2.1. Fruit collection and sample preparation

We collected ripe fruits in three different spots from the roadside (500 m) to the bush (1000 m) and farm-margin (3000 m) in Campo Grande, Mato Grosso do Sul state, Brazil, 20°46′34.208″S, 54°10′28.567″W (Fig. 1), in November 2019. Manually, the pulp was separated from the peel and seed, immediately dried in an air circulation oven at 40 °C for 48 hours. The dried pulp was milled using mortar and pestle and sieved to obtain the refined powder, placed into amber and hermetic glass bottle and frozen at −20 °C for further analyses.

![Figure 1. Collection spots of *Camponanesia adamantium* fruits located between the state road MS-040 with high large-vehicle traffic and intensive modern agriculture in Campo Grande – Mato Grosso do Sul State, Brazil. 1. Roadside = 500 m; 2. Bush = 1000 m; and 3. Farm-margin = 3000 m.](image)

2.2. Microwave assisted digestion procedure

The pulp samples were weighed according to Lima et al. [18], and prepared as described: 0.5 g sample plus 5 mL HNO₃ (65% Merck, Darmstadt, Germany) and 3 mL H₂O₂ (35% Merck, Darmstadt, Germany) were individually placed into PTFE bottles of the DAP 60 type (Berghof). The mixture was allowed to remain in the open air for 10 min predigestion and then digested using a microwave digestion system (Speedwave four®, Berghof, Germany). After digestion, samples were diluted to 100 mL with ultrapure water (conductivity 18.2 MΩcm produced by Millipore, Water Purification System Milli-Q Bi-
We conducted the sample digestion steps according to the schedule shown in Table 1. All the digestion analysis was performed in triplicate.

Table 1. Microwave digestion parameters.

| Steps | 1 | 2 | 3 | 4 | 5 |
|-------|---|---|---|---|---|
| Power (W) | 1305 | 1305 | 0 | 0 | 0 |
| Temperature (°C) | 170 | 200 | 50 | 50 | 50 |
| Ramp time (min) | 1 | 1 | 1 | 1 | 1 |
| Hold time (min) | 10 | 15 | 10 | 10 | 1 |
| Pressure (Bar) | 35 | 35 | 0 | 0 | 0 |

2.3. ICP OES elemental analysis

After the microwave system’s digestion procedure, we transferred the samples from the vessels to 50 mL Falcon vessels and then filled them to 30 mL with ultrapure water. Using inductively coupled plasma optical emission spectroscopy (ICP OES, Thermo Fischer Scientific, Bremen, Germany, iCAP 6300 Duo) technique, we determined the sample chemical elements. The selected emission lines (wavelength in nm) for determining elements in pulp and operating conditions of ICP OES are summarized in Table 2.

Table 2. Instrumental analytical conditions for ICP OES of element analyses.

| Parameters | Setting |
|------------|---------|
| RF power (W) | 1250 |
| Sample flow rate (L min⁻¹) | 0.45 |
| Plasma gas flow rate (L min⁻¹) | 12 |
| Integration time (s) | 5 |
| Stabilization time (s) | 20 |
| Pressure of nebulization (p si) | 20 |
| Plasm view | Axial |
| Gas view | Air |
| Analytical wavelength (nm) | Fe (259.940), Ni (231.604), Co (228.616), Cr (267.716), As (193.759), Pb (214.441), Mo (202.030), Mn (257.610), P (177.595), K (766.490), Zn (213.856), Se (196.090). |

2.4. Calibration curves

For the ICP OES method, we obtained multiple-element stock solutions containing 1000 mg/L of the Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, P, S, V, Se, and Zn from SpecSol (SpecSol, Quimlab, Brazil), and analytical calibration standards prepared. For each element detected, a limit of detection (LOD) was 0.0002–0.003 (mg/L), limit of quantification (LOQ) 0.006–0.01 (mg/L) and correlation coefficient (R²) 0.9995–0.9998 were determined. A blank and seven calibration curves were generated using the
following concentrations: 0.01, 0.02, 0.05, 0.2, 1.0, 2.0 and 5.0 mg/L of the element standard. All experiments were carried out in triplicates.

An addition/recovery test for the elements under study was performed in a pulp sample by spiking of 0.5 mg/L of each analyte. The method had a recovery interval of 80–110% for the spike 0.5 mg/L.

2.5. Human health risk assessment

We compared our results with recommended intake standards of RDA/AI, UL, FAO/WHO, US. EPA and hazard quotient. The human risk for non-carcinogenic was calculated following the equation adopted by Liang et al. [20]. Cancer risk is a probability of an individual developing any cancer over a lifetime, during the daily doses exposure to 70 years; chronic daily intake dose (CDI) of carcinogenic elements (mg/kg/day); and slope factor (SF) is the carcinogenicity (mg/kg/day). The SF of As, Cr and Pb are 1.5, 0.5 and 0.0085 mg/kg/day, respectively, following equation 1:

Cancer Risk = CDI × SF

The cancer risk is a sum of individual variety carcinogenic elements risk in different exposure pathways, which is the total cancer risk (R). In agreement with US EPA [22], the value of acceptable or tolerable cancer risk ranges from 10\(^{-6}\) to 10\(^{-4}\), while > 10\(^{-4}\) is considered unacceptable.

The human health risk of heavy metal intake was evaluated based on the chronic daily intake dose (CDI, mg/kg/day) for a chemical contaminant in the pulp over the exposure period and the pulp intake quantity. We calculated CDI using the following equation 2:

\[
CDI_{\text{pulp}} = \frac{C_{\text{pulp}} \times IR_{\text{pulp}} \times EF \times ED}{BW \times AT}
\]

Where, CDI\(_{\text{pulp}}\) – chronic daily pulp intake dose; \(C_{\text{pulp}}\) – concentration of mineral content in the pulp; \(IR_{\text{pulp}}\) – ingestion rate (mg/day); \(EF\) – exposure frequency (day/year); \(ED\) – exposure duration (life expectancy); \(BW\) – body weight; and \(AT\) – average time (ED × 365 days). The adult’s body weight, approximately 70 kg, and the average daily pulp consumption is 100000 mg/day. The risk to human health by intake of heavy metal contaminated pulp was measured using a hazard quotient (HQ), which is a ratio of CDI and chronic oral reference dose (RfD), determined by the following equation 3:

\[
HQ = \frac{CDI}{RfD}
\]

The RfD values for the risk calculation were established by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives [23] and the United States Environmental Protection Agency [24]. The RfD values for the elements were established: K = not available, Pb = 0.004 mg/kg bw/day, P = not available, As = 0.0003 mg/kg bw/day, Se = not available, Fe = 0.7 mg/kg bw/day, Mo = not available, Cu = not available, Mn = 0.14 mg/kg bw/day, Mo = 0.005 mg/kg bw/day, Zn = 0.3 mg/kg bw/day, Co= 0.03 mg/kg bw/day, Ni = 0.02 mg/kg bw/day, Mn = not available, and Cr =
0.003 mg/kg bw/day [24]. Equation (3), toxic risk is considered to occur if HQ > 1, whereas HQ < 1 represents negligible hazard (adverse non-carcinogenic effects).

2.6. Statistical analysis

We analysed data by one-way ANOVA using the GraphPad Prism software version 8.0 for Mac (GraphPad Software, San Diego, CA, USA). The significance of the differences between means for individual element level was considered at \( p < 0.05 \).

3. Results and Discussion

In this section, the article is composed of two subsections: 3.1 present data on the concentration of the minerals obtained in pulp collected in roadside, bush and farm-margin, and the comparison of these concentrations with other published studies. In subsection 3.2, data of the type of chemical elements quantified for each site was used to calculate EDI and HQ values.

3.1. The mineral concentration in pulp collected in three different sites

Twelve mineral elements were found in C. adamantium pulp collected in three different sites from the road: roadside (500 m), bush (1000 m) and farm-margin (3000 m) (Table 3).
Table 3. *Campomanesia adamantium* pulp collected in three different sites from the road: roadside (500 m), bush (1000 m) and farm-margin (3000 m) quantified by ICP-OES (mg/100 g ± SD) compared with nutritional recommendations for adult, pregnancy and children by RDA/AI, UL and FAO/WHO.

| Elements | Roadside (mg/100 g) | Bush (mg/100 g) | Farm-margin (mg/100 g) | Male 31–50 y RDA/AI* (mg/day) | Female 31–50 y RDA/AI* (mg/day) | Male/female 31–50 y UL (mg/day) | Pregnancy 31–50 y RDA/AI* (mg/day) | UL (mg/day) | Children 4–8y RDA/AI* (mg/day) | UL (mg/day) | FAO/WHO (mg/day) |
|----------|---------------------|----------------|------------------------|-------------------------------|-------------------------------|---------------------------------|---------------------------------|------------|-------------------------|------------|------------------|
| K        | 33.02±0.01          | 31.02±0.01     | 58.01±1.34             | 4700                          | 4700                          | ND                              | 4700                           | ND         | 4700                    | ND         | 3510 [25]        |
| Pb       | 5.36±0.02           | 7.02±0.01      | 6.85±1.05              | ND                            | ND                            | 4000                            | ND                              | ND         | ND                      | ND         | 0.02 [26]        |
| P        | 3.24±0.02           | 3.04±0.02      | 5.24±0.80              | 700                           | 700                           | ND                              | 700                             | 3500       | 500                     | 3000       | 700 [25,27]      |
| As       | 1.96±0.04           | 1.14±0.03      | 2.84±0.52              | ND                            | ND                            | 400                             | ND                              | ND         | ND                      | ND         | 0.01 [26]        |
| Se       | 0.20±0.01           | 0.22±0.02      | 0.40±0.10              | 55                            | 55                            | 400                             | 400                             | 60         | 30                      | 150        | 0.06 [27]        |
| Fe       | 0.23±0.02           | 0.12±0.01      | 0.40±0.10              | 8                             | 18                            | 45                              | 27                              | 45         | 10                      | 40         | 2 [28]           |
| Mo       | 0.10±0.02           | 0.09±0.02      | 0.19±0.01              | 150                           | 150                           | 1100                            | 50                              | 2000       | 22                      | 600        | 0.045 [27]       |
| Zn       | 0.08±0.01           | 0.07±0.01      | 0.13±0.02              | 11                            | 8                             | 40                              | 11                              | 40         | 5                       | 12         | 3 [27]           |
| Co       | 0.07±0.01           | 0.02±0.00      | 0.08±0.02              | ND c                          | ND c                          | ND c                            | ND c                            | ND c       | ND c                    | ND c       | 0.0014 [29]     |
| Ni       | 0.06±0.01           | 0.04±0.01      | 0.10±0.02              | ND                            | ND                            | 1                               | ND                              | 1          | ND                      | 0.3        | 0.2 [28]        |
| Mn       | 0.05±0.01           | 0.03±0.01      | 0.07±0.01              | 2.30                          | 1.80                          | 11                              | 2.60                            | 11         | 1.50                     | 3          | 3 [27]           |
| Cr       | 0.03±0.01           | 0.01±0.00      | 0.05±0.01              | 0.035*                        | 0.025*                        | ND                              | 0.030*                          | ND         | 0.015*                   | ND         | 0.002 [28]      |

Note. ND – not determined; ∗The value for AI is used when there are no calculated values for the RDA.
Concentration of minerals quantified in *C. adamantium* pulp samples are depicted in decreased order in Table 3. The average concentration of minerals in pulp collected in roadside followed in decreased order K > Pb > P > As > Fe > Se > Mo > Zn > Co > Ni > Mn > Cr; bush: K > Pb > P > As > Se > Fe > Mo > Zn > Ni > Mn > Co > Cr, and farm-margin: K > Pb > P > As > Se > Fe > Mo > Zn > Co > Ni > Mn > Cr. The concentration of Pb, As and Cr in the present study are higher compared with the average reported for fruits and pulp by previous studies [17,18,30], which concentrations exceeded the FAO/WHO permissible limit recommended for edible berries and small fruits [25–29]. On the other hand, high concentrations of Mo, Co, Ni and Mn are reported in *C. adamantium* fruits compared with the present study [30], which can be correlated with the occurrence of these minerals in natural environments [31,32].

In general, the average of all minerals quantified in *C. adamantium* pulp followed a decreasing order K > Pb > P > As > Se > Fe > Mo > Zn > Co > Ni > Mn > Cr. The results demonstrated no statistical difference observed between the average of minerals quantified in this pulp collected in three spots located between the road of high large-vehicle traffic and intensive farm modern agriculture ($p > 0.05$). However, a statistical difference was observed between average values of the elements found in three spots: K, Pb, P, As, Se, Fe, Mo, Zn, Co, Ni, Mn and Cr ($p < 0.0001$).

Thus, we observed that the concentration behavior of minerals decreased from roadside (500 m) to bush (1000 m) and increased to farm-margin (3000 m). However, the concentration of Pb and Se increased from roadside toward bush and toward farm-margin, as illustrated in Fig. 2.

![Figure 2](image.png)

Figure 2. Behavior of mineral quantities distribution in *Campananesia adamantium* pulp collected in three different sites. A. Chemical element content > 1 mg/100 g; B. Chemical element content ≤ 0.4 mg/100 g.

Table 3 listed the levels of minerals quantified (mg/100 g ± SD) in the *C. adamantium* pulp compared with the limit specification of RDAs/AI and UL values for males and females (31–50 y), pregnant women (31–40 y) and children (4–8 y) [33], and FAO/WHO and WHO [25–29] to permissible level for fruits and food.

We calculated the pulp mineral percentages from mean values (Table 3) based on RDA, AI, UL, and FAO/WHO and WHO limits [25–29], while based on the FDA...
parameters (10–19% for “good source” of nutrition, and ≤ 20% for “excellent source”), the studied minerals were qualified [34].

Potassium (K) contents in the pulp of roadside (33.02 ± 0.01 mg/100 g), bush (31.02 ± 0.01 mg/100 g) and farm-margin (58.01 ± 1.34 mg/100 g) correspond to proportions ≤ 1% for males, females, pregnant women, and children to 4700 mg/day by RDA parameters. The UL of K has no established values for males, females, pregnant women and children. The K concentration in this pulp is the lowest (3510 mg/day) FAO/WHO limit [25]. According to FDA parameters, this pulp is not a good source of K [34]. The K concentrations in this pulp are lower than 130–253 mg/100 g reported in previous studies for C. adamantium fruits and pulp [17,18,30]. However, K concentrations in this pulp are near blueberry and alfalfa (39 mg/100 g) [34]. The health benefit of K in the body is associated with blood pressure regulation, stroke risk reduction, preventing renal system dysfunction, decreasing urinary calcium excretion, reducing kidney stone formation and osteoporosis disease [35], regulating blood lipid concentrations [36] and maintaining bone and cardiovascular health [37,38].

Lead (Pb) quantities in the pulp of roadside (5.36 ± 0.02 mg/100 g), bush (7.02 ± 0.01 mg/100 g) and farm-margin (7.88 ± 1.05 mg/100 g) correspond to 26800%, 35100 % and 39480% by 0.02 mg/day FAO/WHO parameters [26]. The RDA and UL parameters for Pb have no established values for adults and children. Based on the FDA parameters, this pulp is an excellent source of Pb [34]. In this pulp, Pb concentrations are lower than 0.06 mg/100 g, reported in previous studies for fruits of C. adamantium [17]. On the other hand, Pb concentrations in this pulp are near other edible fruits such as apple (2.35 mg/100 g), mango (6.72 mg/100 g) [39] and tomato (5.41–11.73 mg/100 g) [40]. The risk of consumption of food with a high amount of Pb is correlated with intelligence reduction, bone joint weakness, accelerated bone maturation, increased blood pressure, spontaneous abortion, renal dysfunction, allergic diseases [41], respiratory and cardiovascular diseases [42].

Phosphorus (P) contents in the pulp of roadside (3.24 ± 0.02 mg/100 g), bush (3.04 ± 0.02 mg/100 g) and farm-margin (5.24 ± 0.8 mg/100 g) correspond to proportions ≤ 1% for males, females and pregnant women (700 mg/day) and children (500 mg/day) by RDA parameters. The P quantities correspond to values ≤ 0.2% for males/females (4000 mg/day), pregnant women (3500 mg/day) and children (3000 mg/day) by UL limits. The P concentrations roadside, bush and farm-margin pulp correspond to proportions < 1% to 700 mg/day by FAO/WHO limits [25,27]. In agreement with FDA parameters, this pulp is not a good source of P [27]. Indeed, P concentrations in this pulp are lower than 17–196 mg/100 g reported in previous studies on fruits and pulp of C. adamantium [17,18,30]. However, P concentrations in this pulp are near blackberry and watermelon (5–11 mg/100 g) [34]. The health benefit of P consumption is related to bone mineralization, cell energy generation, cardiovascular regulation and neuromuscular function [43], and modulation of short-chain fatty acid gut bacteria producers [44].

Arsenic (As) quantities in roadside (1.96 ± 0.04 mg/100 g), bush (1.14 ± 0.03 mg/100 g) and farm-margin (2.84 ± 0.52 mg/100 g), correspond to 19600%, 11400% and 28400% by 0.01 mg/day FAO/WHO limits [26]. The RDA and UL parameters for As have no
established values for adults and children. By FDA parameters, this pulp is an excellent source of As [34]. The As concentrations in this pulp are higher than 0.07 mg/100 g reported in previous studies on C. adamantium fruits [17], and are near edible vegetables such as lettuce (2.73 mg/100g) [7], Colocasia antiquorum (0.6–12.5 mg/100 g), gourd leaf (0.8–15.8 mg/100 g) [45], fish, seafood and seafood products (0.16–0.56 mg/100 g) [46]. The risk of consumption of food with a high amount of As is associated with cancers (skin, lung and bladder) [45], respiratory disease, gastrointestinal disorder, liver malfunction, neuro-cardiovascular dysfunction, anaemia disorder, leucopenia and thrombocytopenia effects, diabetes [47], cytotoxicity and genotoxicity effects [48].

Selenium (Se) concentrations to the pulp of roadside (0.20 ± 0.01 mg/100 g), bush (0.22 ± 0.02 mg/100 g) and farm-margin (0.40 ± 0.1 mg/100 g) correspond to values < 1% for males and females (55 mg/day), pregnant women (400 mg/day) and children (30 mg/day) by RDA parameters. The Se quantities in pulp of roadside, bush and farm-margin correspond to proportions < 1% for males and females (400 mg/day), pregnant women (60 mg/day) and children (150 mg/day) by UL limits. The Se concentrations in roadside, bush and farm-margin pulps correspond, respectively, to 333.33%, 366.67% and 500% by 0.06 mg/day FAO/WHO limits [27]. According to FDA parameters, this pulp is an excellent source of Se [34]. The Se concentrations in this pulp are lower than 0.88 mg/100 g reported in previous studies on C. adamantium fruits [17], and higher than grapes, apricot, kiwi, litchi, macadamia and pistachio (0.0001–0.007 mg/100 g) and near cashew nut (0.02 mg/100 g) [34]. Other studies have recommended 0.018 mg/day of Se quantity intake for children (4–6 y), 0.023 mg/day for adolescent males 10–18 y and 0.021 mg/day for adult females (19–65 y), 0.027 mg/ day for males and 0.0204 mg/day [49]. The benefit of consumption of Se is correlated with preventing and decreasing diabetes mellitus, cancers [50], ameliorate men fertility [51,52], human neuropathies [53] and hepatic steatosis [54].

Iron (Fe) concentrations to the pulp of roadside (0.23 ± 0.02 mg/100 g), bush (0.12 ± 0.01 mg/100 g) and farm-margin (0.40 ± 0.10 mg/100 g) correspond to values < 4% by RDA parameters for males (8 mg/day), females (18 mg/day), pregnant women (27 mg/day) and children (10 mg/day). The Fe quantity in the pulp of roadside, bush and farm-margin correspond to < 1% by UL parameters for males, females and pregnant women (45 mg/day) and children (40 mg/day). In concordance with FDA parameters, this pulp is not a good source of Fe [34]. The Fe concentrations in this pulp are lower than 1–2.6 mg/100 g reported in previous studies on fruits and pulp of C. adamantium [17,18,30]. However, the Fe contents of this pulp are between apple, guava and pineapple (0.12–0.29 mg/100 g) [34]. The health benefits of food consumption with Fe are improving maximal oxygen respiration and exercise performance, hemoglobin synthesis, electron transport, anemia prevention, deoxyribonucleic acid synthesis, gut microbiota modulation, neurodevelopment, immunity, pregnancy development [55–57].

Molybdenum (Mo) concentrations to the pulp of roadside (0.10 ± 0.02 mg/100 g), bush (0.09 ± 0.02 mg/100 g) and margin-farm (0.19 ± 0.01 mg/100 g) correspond to proportions ≤ 1% by RDA parameters for males and females (150 mg/day), pregnant women (50 mg/day) and children (22 mg/day). The Mo contents in the pulp of roadside,
bus and farm-margin correspond to values ≤ 0.2% by UL parameters for males and females (1100 mg/day), pregnant women (2000 mg/day) and children (600 mg/day). The Mo concentrations in pulp to roadside, bush and farm-margin correspond respectively to 222.22%, 177.78% and 422.22% by 0.045 mg/day FAO/WHO parameters [27]. In agreement with FDA parameters, this pulp is an excellent source of Mo [34]. However, the Mo concentrations in this pulp are lower than 0.4 – 30 mg/100 g reported in previous studies on fruits of C. adamantium [18,30]. The Mo food consumption is recommended for infants (0.015–0.04 mg/day) and all individuals ≥ 10 years old (0.025–0.15 mg/day) [58]. The health benefit of Mo is correlated with toxicity prevention by several metabolites, reduction of aerosol organs irritability, night blindness, neurological damage, aches and pain [59–61]. The Mo concentrations of this pulp are between pea seeds and tomato (0.10–0.20 mg/100 g) [62].

Zinc (Zn) concentrations to the pulp of roadside (0.08 ± 0.01 mg/100 g), bush (0.07 ± 0.01 mg/100 g) and farm-margin (0.13 ± 0.02 mg/100 g) correspond to values < 2% by RDA limits for males and pregnant women (11 mg/day), females (8 mg/day) and children (5 mg/day). The Zn quantity in pulps of roadside, bush and farm-margin corresponds to 1% by UL parameters for males, females, pregnant women (40 mg/day) and children (12 mg/day). The Zn concentrations in this pulp correspond to 2.67%, 2.27% and 3.4% by 3 mg/day FAO/WHO limits [27]. Based on FDA parameters, this pulp is not a good source of Zn [27]. The Zn concentrations in this pulp are lower compared with 0.2–0.5 mg/100 g reported in previous studies on fruits and pulp of C. adamantium [17,18,30]. However, the Zn amounts are between apple, grapes and tomato (0.04–0.17 mg/100 g) [34]. The health benefit of consumption of Zn food is associated with preventing or reducing oxidative stress, infections (malaria, pneumonia and diarrhea), cell ageing, atherosclerosis, neuropsychological diseases, autoimmune and degenerative diseases, Alzheimer’s disease, inflammation cytokine storms, cancers, diabetes mellitus, obesity, depression, gastrointestinal and reproductive organ dysfunction, retina disease, and improving fetal and childhood skeletal growth and development [63–65].

Cobalt (Co) concentrations to the pulp of roadside (0.07 ± 0.01 mg/100 g), bush (0.02 ± 0.00 mg/100 g) and farm-margin (0.08 ± 0.02 mg/100 g) correspond to 5000%, 1428.57% and 5714.29% by 0.0014 mg/day WHO limits [29]. The RDA and UL parameters for Co have no established value for adults and children. Conforming to FDA parameters, this pulp is an excellent source of Co [34]. The Co concentrations in this pulp are lower than 8 mg/100 g reported in previous studies on C. adamantium pulp [30]. The Co concentrations are between strawberries, apple, grapes, mango, tomato and orange (0.03–0.08 mg/100 g) [39]. The risk of consumption of food with a high amount of Co is correlated with inflammation and hypersensitivity reactions [66], neurological, cardiovascular and endocrine deficiency [67].

Nickel (Ni) concentrations to the pulp of roadside (0.06 ± 0.01 mg/100 g), bush (0.04 ± 0.01 mg/100 g) and (0.1 ± 0.02 mg/100 g) correspond 6%, 4% and 10% for male, female, pregnant women, and 20%, 13.33% and 33.33% for children, respectively by 1 mg/day, 1 mg/day and 0.3 mg/day UL limits. The Ni concentrations of the roadside, bush and farm-margin correspond 30%, 20% and 50% by 0.2 mg/day FAO/WHO limits [28]. The
RDA parameters for Ni has no established value for adults and children. According to FDA parameters, this pulp is an excellent source of Ni [34]. The Ni concentrations in this pulp are lower than 4.2 mg/100 g reported in previous studies on fruits of C. adamantium [17]. The Ni concentrations are between paw-paw, mango, watermelon and banana fruits (0.023–0.089 mg/100 g) [68]. Some articles reported the health benefit of Ni food consumption is correlated with gut microbiota balance, welfare [69]. However, other studies correlated Ni with hazardous conditions for human health such as cardiovascular, kidney and lung dysfunctions, oxidative stress and others [70].

Manganese (Mn) contents to pulps of roadside (0.05 ± 0.01 mg/100 g), bush (0.03 ± 0.01 mg/100 g) and farm-margin (0.07 ± 0.01 mg/100 g) correspond to values ≤ 4% for males (2.3 mg/day), females (1.8 mg/day), pregnant women (2.6 mg/day) and children (1.5 mg/day) by RDA parameters. The Mn quantities correspond to proportions < 2.5% for males/females and pregnant women (11 mg/day), and children (3 mg/day) by UL limits. The Mn concentrations in pulps of roadside, bush and farm-margin correspond to 1.33%, 1.00% and 2.33%, respectively, by 3 mg/day FAO/WHO limits [27]. By FDA parameters, this pulp is not a good source of Mn [34]. The Mn concentrations in this pulp are lower than 0.09–0.21 mg/100 g reported to previous studies on fruits and pulp of C. adamantium [17,18,30]. However, the Mn contents are near paw-paw and wheat (0.08–1.0 mg/100 g) [71]. The health benefit of consumption of Mn food is associated with gut microbiota balance, regulating oxygen species and anemia conditions between mother and fetus, and neurodevelopment [72–74].

Chromium (Cr) concentration to the pulp of roadside was 0.03 ± 0.01 mg/100 g corresponds to 116.67%, 83.33%, 100% and 50% for males (0.035 mg/day), females (0.025 mg/day), pregnant women (0.03 mg/day) and children (0.015 mg/day) by AI parameters. The bush Cr quantity of 0.01 ± 0.00 mg/100 g in pulp corresponds to 350%, 250%, 300%, and 150% for males, females, pregnant women and children, respectively, by AI limits. The farm-margin Cr content was 0.05 ± 0.01 mg/100 g corresponds to 70%, 50%, 60%, and 30% for males, females, pregnant women and children, respectively, by AI standard. The Cr quantities in the pulp of roadside, bush and farm-margin correspond to 6.67%, 20% and 4%, respectively, by 0.002 mg/day FAO/WHO limits [28]. The RDA and UL parameters for Cr have no established values for adults and children. According to FDA parameters, this pulp is a good source of Cr [34]. However, the Cr concentrations in this pulp are lower than 0.1–1.14 mg/100 g reported by previous studies on C. adamantium pulp [18,30]. The Cr contents are near edible fruits such as strawberry and melon (0.3 mg/100 g) [75].

3.2. Health risk assessment

We calculated the carcinogenic risk (CR) of three chemical elements Pb, As and Cr in pulp obtained from fruits collected in roadside, bush and farm-margin (Table 4). The values of As and Cr were farm-margin > roadside > bush, while the Pb values differed (farm-margin > bush > roadside). The total cancer risk (R) values for farm-margin, roadside and bush were 1.5 × 10⁻³, 1.1 × 10⁻³ and 6.2 × 10⁻⁴, respectively, which were higher compared with the acceptable parameters (10⁻⁴ to 10⁻²), showing the importance of these.
values to carcinogenic risk for the pulp consumers [22]. The total cancer risk is presented in decreased order As > Pb > Cr, demonstrating that As is the main pollutant chemical element that can be correlated with several cancer incidences among all heavy metals found in this pulp. Furthermore, the total cancer risk incidence can be higher for the consumers intake 400 g/day pulp as recommended [76], from farm-margin, roadside and bush (6.0 \times 10^{-3}, 4.1 \times 10^{-3} and 2.4 \times 10^{-3}, respectively), in the region crossed by a road of high large-vehicle traffic and intensive modern agriculture. However, the total cancer risk for consumption of 10 g/kg/day of pulp from the roadside, bush and farm-margin was estimated to 1.1 \times 10^{-4}, 6.3 \times 10^{-5} and 1.5 \times 10^{-4}, respectively, which are within acceptable parameters [22].

The non-carcinogenic risks for mineral elements are summarized in Table 4. The CDI values of the minerals in fruit pulp are presented in decreased order for three collection sites: K > Pb > P > As > Fe > Se > Mo > Zn > Co > Ni > Mn > Cr for roadside, K > Pb > P > As > Se > Fe > Mo > Zn > Co > Ni > Mn > Cr for bush and K > Pb > P > As > Se = Fe > Mo > Zn > Ni > Co > Mn > Cr for farm-margin. The ordered quantities of mineral elements are different for Fe and Se from roadside, while Se, Fe, Ni and Co for farm-margin compared with bush areas. The major chemical elements in the pulp in decreased order are farm-margin > roadside > bush, which signifies that the farm and road have spread these minerals to pollute fruits. In contrast, Pb and Se are ordered from farm-margin > bush > roadside, which explains that the highest amount of these chemical elements have spread from the farm.

The hazard quotient (HQ) values of the minerals in pulp estimated in decreased order to roadside were As > Pb > Mo > Cr > Ni > Co > Fe > Mn > Zn; bush: As > Pb > Mo > Cr > Ni > Co > Fe > Zn > Mn; and farm-margin: As > Pb > Mo > Cr > Ni > Co > Mn > Fe > Zn. The quantities of Mn, Fe and Zn are irregularly distributed in farm-margin, roadside and bush areas. The majority of chemical elements are ordered to farm-margin > roadside > bush, which explains that farm and road are sources of higher amounts of these minerals. In contrast, Pb is ordered from farm-margin > bush > roadside, meaning that this mineral has spread in a higher amount from the farm. The majority of minerals presented HQ < 1, while As highest values in farm-margin, roadside and bush were 3.33, 2.30 and 1.34, respectively. Therefore, at the consumption of 100 g/kg/day of pulp, As could be the main cause of several cancer types and other chronic diseases.
| Samples        | K       | Pb      | As     | Se     | Fe     | Mo     | Zn     | Co     | Ni     | Mn     | Cr     |
|---------------|---------|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Roadside      | CR      | 0.000016| 0.001036| -      | -      | -      | -      | -      | -      | -      | 0.0000053 |
|               | CDI     | 0.011631| 0.001888| 0.001141| 0.000690| 0.000070| 0.000081| 0.000035| 0.000028| 0.000025| 0.000021| 0.000018| 0.000011 |
|               | HQ      | 0.472016| 2.301370| -      | 0.000116| 0.007045| 0.000094| 0.000822| 0.001057| 0.000126| 0.003523 |
| Bush          | CR      | 0.000021| 0.000602| -      | -      | -      | -      | -      | -      | -      | 0.000018 |
|               | CDI     | 0.010927| 0.002473| 0.001071| 0.000402| 0.000077| 0.000042| 0.000032| 0.000025| 0.000007| 0.00014 | 0.000011| 0.000004 |
|               | HQ      | 0.618200| 1.338552| -      | 0.000060| 0.006341| 0.000082| 0.000235| 0.000705| 0.000075| 0.001174 |
| Farm-margin   | CR      | 0.000024| 0.001501| -      | -      | -      | -      | -      | -      | -      | 0.000088 |
|               | CDI     | 0.020435| 0.002779| 0.001846| 0.001000| 0.000141| 0.000141| 0.000067| 0.000046| 0.000028| 0.000035| 0.000025| 0.000018 |
|               | HQ      | 0.694814| 3.334638| -      | 0.000201| 0.013386| 0.000153| 0.000939| 0.001761| 0.000176| 0.005871 |
4. Conclusions

According to RDA and UL limits, the pulp of *C. adamantium* collected in areas located between the road of high large-vehicle traffic and intensive modern agriculture farm present the lowest concentration of K, P, Se, Fe, Mo, Zn, Ni, and Mn. However, based on FAO/WHO parameters, the highest concentrations are Pb, As, Se, Mo and Ni, and the lowest for K, P, Fe, Zn and Mn. The Cr concentration is higher than FAO/WHO and AI limits. Values of Pb, As, Co and Cr are not established by RDA and UL standards, including K, not established for the last parameter. This pulp is an excellent source of Pb, As, Se, Mo, Co, Ni and Cr, while it is not a good source of K, P, Fe, Zn and Mn, based on FDA parameters. It is notable that plants that grow and develop between intensive anthropogenic and severe activities are contaminated by heavy metals such as Pb, As, Mo, Co, Ni, Mn and Cr. Additionally, the concentrations of these heavy metals increase, while K, P, Fe and Zn decrease, except Se. Thus, the consumption of plants collected in these environments can be a hazard to human health. Therefore, toxicological studies can be needed to guarantee the safe consumption of edible plants collected in areas under intensive severe anthropogenic activities.

Overall, the estimated carcinogenic risk and total cancer risk in this pulp are represented by As, Pb and Cr, which are in higher concentrations in pulp collected in farm-margin, followed by roadside and bush. Thus, the primary crucial heavy metal is As, presenting HQ > 1 (3.33, 2.30 and 1.34 in pulp collected in farm-margin, roadside and bush, respectively). However, quantities ≤ 10 g daily intake of pulp obtained in these areas can decrease the total cancer risk and are within accepted parameters and HQ < 1 for all minerals assessed in this pulp. We demonstrated that intensive agriculture modern farms and areas crossed by roads of large-vehicle traffic are sources and flow of pollutants to contaminate fruits and vegetables that grow in surrounding areas.

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