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Risk elements, antioxidant activity and polyphenols in pseudocereal grains

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Pseudocereals, with their irreplaceable nutritional composition and amounts of bioactive substances with a positive effect on human health, are becoming a trend in human nutrition. In this work, we compared the safety of individual types of pseudocereals, namely buckwheat (*Fagopyrum esculentum*, var. Zita), quinoa (*Chenopodium quinoa*, var. Carmen), amaranth (*Amaranthus cruentus*, var. Pribina), and sorghum (*Sorghum bicolor*, var. Ruzrok) in terms of the content of hazardous metals. We assessed the ability of individual species of pseudocereals to accumulate hazardous metals from the soil in the consumable parts of the plant. The ability of heavy metals to accumulate was calculated using a bioaccumulation factor. We also evaluated the influence of the content of selected hazardous metals on the antioxidant capacity of grains of individual types of pseudocereals. We determined the total polyphenol content and the total antioxidant content using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical spectrophotometrically. We determined the content of hazardous metals by the AAS method (Atomic Absorption Spectrometry). In the soil from all plots with cultivated species of pseudocereals, we recorded an increased content of Cd, Pb, Co in comparison with the limit value set by Law no. 220/2004. The content of risk elements was not exceeded in the consumption parts of plants and the content of Cd and Pb was below the detection limit. From a safety point of view, it is possible to prefer the *Chenopodium quinoa*, which had the lowest content of heavy metals in the grains. Buckwheat follow, and at about the same level are amaranth and sorghum bicolor. The safest or the most resistant plant species with the lowest ability to accumulate hazardous metals from soil to grains, from the group of crops we monitor, is the *Chenopodium quinoa*.

Keywords: pseudocereals, DPPH, polyphenols, risk elements

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Introduction

The definition of pseudocereals is that they are like the fruits or seeds of non-digestive species that are consumed in a very similar way to cereals (Das, 2016). From a botanical point of view, they belong to dicotyledonous plants, therefore they do not belong to cereals that are monocotyledonous, but since they produce starch-rich seeds, they are grown and used similarly as cereals. According to most researchers, they are termed "pseudocereals", due to their related properties to cereal grains: starch content, texture, palatability, and method of preparation (Ciudad-Mulero et al., 2019).

Native Americans already fed pseudocereals, which were able to improve their endurance and mental development, thanks to their positive qualities. The Mayans and Incas considered these grains sacred (Rollán et al., 2019). The origin of the amaranth and the quinoa is in Latin America. Buckwheat originally spread from Asia (Cai et al., 2016). Hermuth et al. (2012) state that sorghum is one of the longest-grown crops currently grown on all continents. Its origin is from Egypt, where it was used as a crop. He returned to Europe in the 15th and 16th centuries through the Arabs.

Pseudocereals are a relatively common ingredient in human nutrition. They are a staple food as they are the primary suppliers of carbohydrates and proteins to the world‘s population. They also provide significant amounts of energy and trace elements for human nutrition (Margitanová et al., 2009).

Pseudocereals are important gluten-free crops, including buckwheat, amaranth, quinoa, and sorghum. Their nutritional properties and suitability for the preparation of gluten-free foods predetermine their use as functional foods (Martínez-Villaluenga et al., 2020). They are known to have good nutritional value, thanks to proteins with high biological value. Amaranth, quinoa, and buckwheat have a much higher protein content than cereals, and also the quality of the proteins is better because the amount of lysine found in cereals is limited (Kocková and Valík, 2011; Das, 2016).

Pseudocereals are promising crops of the future due to their high genetic variability, which can adapt to different climatic conditions, from tropical to temperate climates (Joshi et al., 2018). Pseudocereal grains have a high content of starch, fiber, and protein with a quality, balanced composition of essential sulfur-rich amino acids. They are also a good source of minerals (Ca, Fe, and Zn), vitamins, and phytochemicals such as saponins, polyphenols, phytosterols, phytosteroids, and betalains with potential health benefits (Martínez-Villaluenga et al., 2020).

Material and methodology

Biological material

We took samples of individual pseudocereals in the full ripening phase: amaranth (*Amaranthus cruentus*, var. Pribina), quinoa (*Chenopodium quinoa*, var. Carmen), buckwheat (*Fagopyrum esculentum*, var. Zita), and sorghum (*Sorghum bicolor*, var. Ruzrok). Subsequently, the treatment of the samples was continued by mechanical cleaning, water jet cleaning, and drying to constant weight. Finally, the individual samples were ground. The ground samples were stored in paper bags for further analysis. The pseudocereal seeds were homogenized with a mixer to a final fine powder. Subsequently, we prepared extracts. We weighed 10 g of the homogenized sample on analytical balances, added it to the extraction cartridges, and extracted it in 100 mL of 80% methanol for 8 h in Twisselmann. Upon completion, the resulting extract was filtered into 50 mL centrifuge tubes using filter paper.

Determination of soil exchange reaction in KCl (pH/KCl)

We poured 20 g of fine soil I. over with KCl solution (c = 1 mol.dm^{-3}) and left for 24 h at room temperature. The prepared suspension was shaken by Heidolph Promax 1020 shaker at a frequency of 180 oscillations per minute for 10 minutes. After shaking and gradually settling the suspension and subsequent filtration through FILTRAK 390 filter paper, pH/KCL in the filtrate was measured.

Determination of total heavy metal content in the soil

1 g fine soil II. Was weighed into a boiling flask and added 2–3 cm^3 of distilled water, 2.5 cm^3 of concentrated HNO_3 and 7.5 cm^3 of concentrated HCl. The suspension was left over night. The suspension was extracted at reflux for 2 h. Suspension was filtered through FILTRAK 390 filter paper into a dry volumetric flask (V = 100 cm^3). Before the filtration the filter was moistened by 10% HNO_3. We determine the content of heavy metals by the VARIAN AA 240FS using the AAS method.

Soil extraction with NH_4NO_3 solution

20 g of fine soil I. was weighed into containers (100 cm^3). Subsequently, we added 50 cm^3 of NH_4NO_3 (c = 1 mol.dm^{-3}). Content was mixed and closed into the container. Extract the suspension on a Heidolph

Promax 1020 was shaken for 2 h at 180 oscillations per minute. We used FILTRAK 390 filter paper. The first part of the filtrate was discarded. We also performed a blank experiment with the samples. Later, 0.5 cm^3 of concentrated HNO_3 was added to the filtrate. The content of hazardous metals was determined by the AAS method using the VARIAN 240 FS instrument.

Determination of the content of macroelements in the grains of the examined pseudocereals

2 g of the homogenized sample poured with 10 cm^3 of HNO_3 and 5 cm^3 of concentrated $HClO_4$. Let stand for 24 hours. The sample was mineralized in a sand bath to form white $HClO_4$ vapors, then filtered into a volumetric flask (100 cm^3), which we made up to the mark with distilled water. We pipetted 2 cm^3 from the extract into a volumetric flask (50 cm^3) and made up to the mark with distilled water. We determined the content of macroelements on a Varian AA 240 FS instrument by atomic absorption spectrometry. In the determination of P, we diluted 1 cm^3 of the extract into a 50 cm^3 volumetric flask, while we also added 8 cm^3 of the mixed solution. Subsequently, we replenished the bank with distilled water to the mark. The sample prepared in this way was allowed to stain for 2 hours and the intensity of the staining was measured at a wavelength of 666 nm on a Shimadzu UV/VIS-1800 spectrophotometer.

Determination of heavy metal content in pseudocereal grains

1 g of homogenized sample was poured into 5 cm^3 of HNO_3 and 5 cm^3 of redistilled water in a mineralization cartridge. We performed the mineralization on a MARS X-press. After the mineralization was completed, the minerals were filtered into a 50 cm^3 volumetric flask. We filled the contents of the bank to the mark with distilled water. We determined the content of risk elements in the seeds of individual pseudocereals by the AAS method using the VARIAN 240 FS device.

Transport of heavy metals from soil to grains

To determine the ability to absorb hazardous metals from the soil and accumulate them in the seeds of the monitored pseudocereals, we calculated a bioaccumulation factor:

$$BAF = c\ (plant)/c\ (soil)$$

We calculated bioaccumulation factors (BAF) based on the values of pseudo total risk metal content determined in soil leachate with aqua regia (AR) and heavy metal content in grains (BAF_{AR}), as well as based on values of bioavailable forms determined in soil leachate with ammonium nitrate (NH_4NO_3) and heavy metal content in pseudocereal grains (BAF_{AN}).

Determination of the total content of polyphenols in the grains of the examined pseudocereals

To determine the total content of polyphenols (TPC) in the consumables of the examined pseudocereals, we applied the commonly used spectrophotometric method according to Lachman (2003) using a Folin-Ciocalteu probe. We prepared suspension from 10 g of sample and 100 mL of 80% methanol extract. After extraction, the suspension is filtered using FILTRAK 390 filter paper. Dilute 0.1 mL of the filtrate with distilled water in a 50 mL volumetric flask. We added 2.5 mL of Folin-Ciocalteu tube to the diluted sample. After 3 minutes, we added 5 mL of 20% aqueous Na_2CO_3 solution. The sample prepared in this way was made up to the mark with distilled water with a total volume of 50 mL and the flask was mixed thoroughly. After two hours of standing, a color complex formed. The absorbance of the blue-colored solutions was measured on a Shimadzu UV/VIS-1800 spectrophotometer at a wavelength of 765 nm. Based on the equation of the calibration curve, we obtained the values of the total content of polyphenols in pseudocereals. The obtained results were recalculated and expressed as mg of gallic acid per kg of dry material (mg GAE.kg^{-1}/d.m.).

Spectrophotometric determination of total antioxidant activity

We determined the total antioxidant activity by the method of Brand-Williams (1995). The method is based on the use of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical reactions. In the free radical scavenging activity assay, 3900 µL of the DPPH solution was added to 4000 µL tubes, followed by 100 µL of the extract or its dilution. The solutions were mixed and incubated in the dark at room temperature. We measured the absorbance value that corresponds to the initial concentration at time A0. We measured dependeces after 10 minutes (A10). Absorbance was measured using a Shimadzu UV-VIS 1800 spectrophotometer at a wavelength of 515.6 nm.

Statistical analysis

We applied a one-way analysis of variance (ANOVA) to evaluate the results mathematically and statistically. We determined statistically significant differences using the LSD contrast test with a 95% confidence level, with a P-value <0.05. We used the STATHATGRAPHICS centurion XVI program for statistical processing of results. We evaluated the content of heavy metals in the soil with descriptive statistics.

Results and discussion

Land from the territory of Piešťany, from plots on which individual types of pseudocereals were grown, belongs to sandy-clayey to clayey soils. We evaluated the measured values based on the Decree of the Ministry of Agriculture and Rural Development of the Slovak Republic no. 338/2005. The soil reaction was neutral to alkaline. The pH of the soil significantly affects the balance and availability of macroelements for plants. All species studied have the best yields when grown on soil with a neutral, alkaline pH, which corresponds to our soil (Jaroszewska et al., 2019; Farooq et al., 2016; Jäger, 2016).

Macronutrients and pH of soil

Our values for the content of macroelements in the soil (Table 1), where amaranth was grown, had on average higher values than reported by Jimoh et al. (2020). In their study, they have the results of analyses of soil macronutrients and pH/KCl in the soil before and after planting. We compared our values with the measured values after planting, because our soil sample was also taken after planting amaranth. Jimoh et al. (2020) claim that some macroelement concentrations in soil have decreased after planting, while others have increased. The concentration of P and K decreased after planting, on the contrary, the content of Ca and Mg increased almost threefold compared to the original concentration. For this reason, the amaranth could serve as a phytoremediator, especially in areas with reduced macroelements. In comparison with the content of K and P in the soil of quinoa, reported by Haseeb et al. (2018) is significantly lower than ours. Janovská et al. (2008) argue that buckwheat does not use nutrients evenly from the soil during its growth. Its requirements for the amount of P received are usually higher during the flowering period and the production of sutures. Kováč (2011) states that sorghum as a crop is relatively demanding on the content of macronutrients occurring in the soil.

Risk elements in the soil

In Table 2, we present the values of the determined content of hazardous metals in the soil extract of the aqua regia, the so-called pseudo total content. It represents all forms of metal present in the soil except for silicate forms. We compared the recorded values with the limit amount set by Law no. 220/2004.

Exceedance of the limit value of hazardous metals in soil was recorded for two risk elements, namely Co and Cd. The exceeding amounts of these risk elements in soil extracts were measured in all monitored pseudocereal species (Table 2).

Acidic soil pH is said to be considered the most important factor influencing the increased uptake of heavy metals by plants. In alkaline soils with pH (7.1–8.1) the risk of heavy metal leaching and their bioavailability to plants is lower and the presence of organic substances may also inhibit the absorption of metals from the soil solution. By altering these

Table 1 Characteristics of macronutrient content (mg.kg^{-1}) and pH in soil from Piešťany

Species	K	Ca	Mg	P	pH/KCl
Amaranthus cruentus	199.60	4372.30	534.60	45.40	7.42
Sorghum bicolor	188.70	4628.30	398.80	59.90	7.34
Chenopodium quinoa	222.30	4780.90	434.50	51.00	7.32
Fagopyrum esculentum	192.60	4429.90	387.40	65.90	7.25

Table 2 Pseudo total content of risk elements in the soil extract of aqua regia (mg.kg^{-1})

Species	Zn	Cu	Co	Ni	Cr	Pb	Cd	Hg
Amaranthus cruentus	55.10	22.70	15.60	46.90	34.30	37.60	1.14	0.067
Sorghum bicolor	55.60	22.30	15.70	44.30	30.50	35.80	1.02	0.046
Chenopodium quinoa	52.20	21.70	17.00	49.40	31.20	37.40	1.06	0.044
Fagopyrum esculentum	54.90	21.70	16.50	46.50	29.80	37.30	1.09	0.018
Limit value AR	150	60	15	50	70	70	0.70	0.5

Notes: AR – aqua regia

soil properties, which determine the solubility of metals in the soil, it is possible to immobilize heavy metals in the solid phase. The mobility of metals and their bioavailability can be affected by the addition of organic and inorganic substances. The basic treatment limiting the mobility of metals is deacidification of the soil by liming (Paltseva et al., 2018; Zwolak et al., 2019). Table 3 shows the content of bioacceptable forms of hazardous metals in the soil extract with ammonium nitrate.

The content of Cd and Pb was exceeded in soil extracts with ammonium nitrate in all monitored pseudocereals. Vollmannová et al. (2013) also reported an increased Pb content in the soil ammonium nitrate leachate. Their measured value was 2.3 times higher, while the our measurement was 4.5 times higher than the critical value.

Amari et al. (2017) report that cadmium and lead are very common pollutants in the environment with a long biological half-life. Muszyńska and Labudda (2019) argue that in temperate soils the half-life of selected heavy metals ranges from 75 to 380 years for Cd and from 1000 to 3000 years for Cu, Ni, Pb, Zn, and Se. For this reason, all these elements are considered non-biodegradable and persistent. However, they can be partially removed from the place where they accumulate by the natural ability of the plant species. Certain plant species can absorb heavy metals through penetration into roots or leaves. Radovanovic et al. (2020) report that cadmium is a highly toxic metal for humans and plants even in very low concentrations. The toxic effects of cadmium on plants can manifest themselves as a stress factor causing various physiological disorders. Jeddou et al. (2017) report that cadmium is found in elevated concentrations (0.1–1 mg.kg^{-1}) in soils worldwide. Harangozo (2018) states that lead is insoluble in neutral, weakly alkaline, or weakly alkaline soils with a higher content of organic matter and humus. Its content in the soil depends on the organic low molecular weight substances present in the soil. Demková et al. (2017) argue that heavy metals accumulated in the soil usually migrate into the vegetation and subsequently through the food chain into the human body.

Macroelements in pseudocereal grains

In comparison with the results of Gordillo-Bastidas et al. (2016) and Nowak et al. (2016), we can state that our measured values (Table 4) in quinoa are comparable in the content of K, P, Ca, Na. The value of Mg measured by us was lower than stated by the authors. Angeli et al. (2020) report comparably higher contents of P, Ca, Mg and similar contents K. Rodríguez et al. (2020) report the same P content in quinoa, higher Ca content, and lower K and Na content. In contrast, Palombini et al. (2013) in their study report lower values of P, Na, and K content and higher amounts of Ca and Mg in quinoa. He also states in his work significantly higher measured values of K, P, Mg, Na content in amaranth. On the contrary, the P content is halved. Comparable K and Na contents in amaranth are reported by Coelho et al. (2018) and Rodríguez et al. (2020), but they have a higher P and Ca content, but report a lower Mg content. Abdelhalim et al. (2019) report a comparable P content in sorghum, but the Ca content is several times higher than our value. Pontieri et al. (2014)

Table 3 Content of bioacceptable forms of risk elements in soil leachate NH$_4$NO$_3$

Species	Zn	Cu	Ni	Pb	Cd	Fe	Mn	Co
Amaranthus cruentus	0.08	0.09	0.28	0.46	0.12	0.25	0.33	0.25
Sorghum bicolor	0.10	0.08	0.32	0.45	0.13	0.22	0.22	0.27
Chenopodium quinoa	0.10	0.09	0.33	0.40	0.13	0.25	0.32	0.83
Fagopyrum esculentum	0.08	0.09	0.31	0.49	0.13	0.29	0.20	0.28
CV NH$_4$NO$_3$	2.0	1.0	1.5	0.1	0,1	–	–	–

Notes: CV – critical value NH$_4$NO$_3$ (c = 1 mol.dm^{-3}) set by Law no. 220/2004

Table 4 Content of macroelements in pseudocereal grains

Species	K (mg.kg^{-1})	Na (mg.kg^{-1})	Ca (mg.kg^{-1})	Mg (mg.kg^{-1})	P (mg.kg^{-1})
Amaranthus cruentus	4470.5 ±189.6^{a}	43.6±1.85^{a}	751.6±30.68^{c}	1225.1±64.05^{a}	1301.1±59.56^{a}
Sorghum bicolor	4576.0 ±186.81^{a}	85.3 ±4.46^{c}	191.6±7.82^{a}	1159.8±60.64^{a}	1461.3±118.05^{b}
Chenopodium quinoa	9759.1 ±510.2^{c}	145.6±7.61^{d}	857.10±44.81^{d}	1534.4±62.64^{b}	2100.0±109.79^{c}
Fagopyrum esculentum	5642.8 ±295.01^{b}	63.80±2.60^{b}	239.5 ±9.78^{b}	1137.4±59.46^{a}	2891.7±68.02^{d}

Notes: LSD test, mean ± standard deviation (n = 4), the coefficients (a, b, c, d) show a statistically significant difference, p <0.05

report a comparable K content, several times higher Na content, higher Mg, Ca, and P content in sorghum. Al-Snafi (2017) and Joshi et al. (2019) and Zhang and Xu (2017) report higher values of Ca, Mg, P content in edible buckwheat, but lower values of K content. In comparison with the results of macroelements reported by Rodríguez et al. (2020) in edible buckwheat, the Ca content coincides with ours, the amount of P is slightly higher, K and Na are lower.

Microelements in pseudocereal grains

Table 5 shows the values of the determined content of microelements in the investigated species of pseudocereals.

In comparison with the measured concentrations in amaranth and quinoa according to Bratovcic and Saric (2019), we can state that in the copper content our results were comparable in both crops. As for the amount of zinc, our measured values were lower; in quinoa, the value was half, and in amaranth 3 times lower. Our values also differed in the amount of iron. In quinoa and amaranth, our value was halved. Angeli et al. (2020), in turn, report comparably higher iron and zinc contents in quinoa.

The correlating Fe and Zn content in quinoa is reported by Nowak et al. (2016), but the amount of measured copper is higher in comparison of our results. In contrast, Rodríguez et al. (2020) report corresponding copper and manganese contents in quinoa, but have lower iron and zinc contents. Martinez-Lopez et al. (2019) and Rodríguez et al. (2020) state in their publication the amount of iron and copper in amaranth is comparable to our results, but the content of manganese and zinc is higher, even zinc 2.3 times. Coelho et al. (2018) report similar iron and cobalt contents, but zinc and manganese contents are higher than our measurements. Al-Snafi (2017) states a comparable content of zinc in buckwheat, a higher content of manganese, copper compared to our measured values, but on the contrary a lower content of iron. Joshi et al. (2019) and Zhang and Xu (2017) report a comparable content of iron and copper in buckwheat, but a higher content of manganese. In contrast, Rodríguez et al. (2020) in their study states that the content of manganese in buckwheat is identical to our values, the registered content of copper and zinc is higher, on the contrary, iron indicates less. Pontieri et al. (2014) report a similar amount of iron in sorghum, but higher manganese, nickel, and 2.3 times higher zinc.

Heavy metals in pseudocereal grains

Table 6 has shown the values in the heavy metal content of the monitored pseudocereal species. From the point of view of safety, based on the results, we can state that the content of Cd and Pb in the grains of the monitored pseudocereal species was below the detection limit. In this respect, the monitored pseudocereals are safe for the consumer.

Table 6 Heavy metal content in pseudocereal grains

Species	Hg (mg.kg^{-1})	Cd	Pb
Amaranthus cruentus	0.0099 ±0.0004^{c}	ND	ND
Sorghum bicolor	0.0065 ±0.0002^{b}	ND	ND
Chenopodium quinoa	0.0040 ±0.0001^{a}	ND	ND
Fagopyrum esculentum	0.0416 ±0.0017^{d}	ND	ND
Limit value (FC SR)	0.05	0.1	0.2

Notes: LSD test, mean ± standard deviation (n = 4), the coefficients (a, b, c, d) show a statistically significant difference, p <0.05; FC SR – Food Code of the Slovak Republic; ND – not detected

The basis for increasing the safety of the food chain is a correct understanding of the mechanisms, regulation of storage, and distribution of heavy metals in plants (Aprille and Bellis, 2020). Zhang and Xu (2017) state that plant-derived products can often be influenced by environmental and geological factors such as soil type and pH, climatic conditions, and others. Elemental analysis is usually considered an effective tool because plants can absorb macro-and micro-elements as well

Table 5 Content of microelements in pseudocereal grains

Species	Cu (mg.kg^{-1})	Zn (mg.kg^{-1})	Mn (mg.kg^{-1})	Fe (mg.kg^{-1})	Cr (mg.kg^{-1})	Ni (mg.kg^{-1})	Co (mg.kg^{-1})
Amaranthus cruentus	5.5 ±0.04^{c}	11.5 ±0.60^{c}	19.9 ±0.81^{c}	91.9 ±3.75^{c}	0.9 ±0.037^{b}	0.3 ±0.01^{c}	0.3 ±0.01^{b}
Sorghum bicolor	3.8 ±0.05^{a}	9.8 ±0.51^{b}	10.8 ±0.56^{a}	53.1 ±2.78^{a}	3.60 ±0.20^{c}	0.3 ±0.02^{c}	0.6 ±0.03^{c}
Chenopodium quinoa	4.0 ±0.20^{a}	10.0 ±0.52^{b}	16.20 ±0.65^{b}	60.3 ±2.46^{b}	0.5 ±0.03^{a}	0.1 ±0.004^{a}	0.3 ±0.012^{b}
Fagopyrum esculentum	4.6 ±0.24^{b}	7.3 ±0.38^{a}	11.5 ±0.47^{a}	56.3 ±2.28$^{a, b}$	0.9 ±0.05^{b}	0.2 ±0.01^{b}	0.1 ±0.005^{a}
FC SR	10	50	–	–	4	3	–

Notes: LSD test, mean ± standard deviation (n = 4), the coefficients (a, b, c, d) show a statistically significant difference, p <0.05; FC SR – the hygienic limit set by the Food Code of the Slovak Republic as heavy metals from the soil, and therefore there is a link between the content of elements in the soil and the degree of their accumulation in crops.

Transport of heavy metals from soil to grains

The resulting values are given in Tables 7 and 8. As the content of lead and cadmium in the NH_4NO_3 soil extract was exceeded in all monitored pseudocereal species and their amount was below the detection limit in grains, we can state that the investigated plant species have a low ability to absorb these hazardous metals from the soil as well as a low ability to subsequently accumulate in grains.

Ogunkunle et al. (2015) report bioaccumulation factor values in amaranth in zinc and cadmium levels higher than 1. They argue that amaranth has the potential to accumulate these hazardous metals from the soil. In comparison with the reported values of Memoli et al. (2017) determined bioaccumulation factor values in *Sorghum bicolor* for chromium content higher, but for nickel and copper content comparable to our BAF_{AR} values. Bhargava et al. (2008) report bioaccumulation factor values in quinoa comparable for chromium and nickel content, but lower for copper and zinc content compared to our BAF_{AR} values. The bioaccumulation factor values according to Fu et al. (2015) in zinc and chrome buckwheat are compatible with our BAF_{AN} values. The pseudocereals we research are characterized by the significant formation of above-ground biomass, which can accumulate hazardous metals. It is plants that can absorb heavy metals from the soil and accumulate them in large volumes of biomass, which are considered to be hyperaccumulators of heavy metals and are suitable as phytoremediation crops.

Total polyphenol content and antioxidant activity of grains

Roccheti et al. (2017, 2019) measured the total content of polyphenols in amaranth (570 mg $GAE.kg^{-1}$), which is much lower than us (1251.1 mg $GAE.kg^{-1}$). Roccheti et al. (2019) and Liu et al. (2019) state the total content of polyphenols in buckwheat (2750–5320 mg $GAE.kg^{-1}$), in this range is also our measured value (3560 mg $GAE.kg^{-1}$). Han et al. (2019) measured the total content of polyphenols in quinoa (1672–3083 mg $GAE.kg^{-1}$), we can say that our contents correlate with each other. Our results for TPC in *Sorghum bicolor* are much higher, as reported by Roccheti et al. (2019). Shen et al. (2018) in their study recorded the total content of polyphenols in different cultivars of *Sorghum bicolor*

Table 7 BAF_{AR} values in pseudocereal seeds

Species	Cu	Zn	Cr	Ni	Co	Pb	Cd
Amaranthus cruentus	0.209	0.242	0.019	0.006	0.026	0.145	–
Sorghum bicolor	0.177	0.170	0.045	0.007	0.118	0.137	–
Chenopodium quinoa	0.192	0.184	0.018	0.002	0.016	0.089	–
Fagopyrum esculentum	0.133	0.212	0.006	0.004	0.030	2.271	–

Table 8 BAF_{AN} values in pseudocereal seeds

Species	Cu	Zn	Cr	Ni	Co	Pb	Cd
Amaranthus cruentus	61.11	143.75	9.0	1.07	1.20	–	–
Sorghum bicolor	47.50	98.0	32.73	0.93	2.59	–	–
Chenopodium quinoa	44.45	100.0	4.55	0.30	0.36	–	–
Fagopyrum esculentum	51.11	91.25	6.92	0.65	0.35	–	–

Table 9 Total polyphenol content (TPC) and antioxidant activity values (TAC) in the grains of the examined pseudocereals

Species	TPC (mg $GAE.kg^{-1}$)	TAC (%)	TAC (μmol $TE.g^{-1}$)*
Amaranthus cruentus	1251.1^{a}	11.175^{a}	1.184^{a}
Sorghum bicolor	11495.8^{d}	77.35^{d}	8.196^{d}
Chenopodium quinoa	2952.5^{b}	33.05^{b}	3.502^{b}
Fagopyrum esculentum	3560.3^{c}	74.0^{c}	7.840^{c}

Notes: LSD test, mean (n = 4), the coefficients (a, b, c, d) show a statistically significant difference, $p < 0.05$; * for a better comparison of the results, we also stated the values of μmol $TE.g^{-1}$

(1744–12388.3 mg GAE.kg^{-1}). Based on the knowledge from the literature, it can be stated that the values of TPC in *Sorghum bicolor* are in a wide range.

Škrovánková et al. (2020) state in their research the value of antioxidant activity in buckwheat (167–280 mg TE.100 g^{-1}), which is after recalculation (6.68–11.2 µmol TE.g^{-1}); it can be stated that our values are comparable with each other (7.840 µmol TE.g^{-1}). A similar value of antioxidant activity in buckwheat (6.2 µmol TE.g^{-1}) is published by Aleksenko (2013). In contrast, Salehi et al. (2018) report values of antioxidant activity in buckwheat (2.68–6.270 mg.g^{-1}), which is calculated (10.72–25.08 2 µmol TE.g^{-1}), that is higher compared to our values. Škrovánková et al. (2020) also measured the values of antioxidant activity in amaranth (26.4 mg.100 g^{-1}), which is calculated (1.056 µmol TE.g^{-1}), which corresponds to our results (1.184 mol TE.g^{-1}). Comparable values of the antioxidant activity of amaranth (43.2–110.7 mg.100 g^{-1}) are also reported by Park et al. (2020), which is calculated (1.728–4.428 µmol TE.g^{-1}). Antioxidant activity in quinoa in a study by Škrovánková et al. (2020) was in the range of 97.4–100.6 mg.100 g^{-1}, which is 3.896–4.024 µmol TE.g^{-1} after conversion. It can be stated that these results are consistent with our values (3.502 µmol TE.g^{-1}). Also, Valencia et al. (2018) present values of antioxidant activity in various varieties of quinoa in the range of 1.95–6.18 µmol TE.g^{-1}, which also corresponds to our values. Similar values of antioxidant activity in quinoa (4.4–4.8 µmol TE.g^{-1}) were reported by Tang et al. (2015), while its results correspond to ours (3.502 µmol TE.g^{-1}). Shen et al. (2018) determined the values of antioxidant activity in different varieties of sorghum, with an interval of 0.92–19.05 mg TE.g^{-1}, which, after recalculation (3.68–7.8 µmol TE.g^{-1}), which can be considered as comparable results with our values (8.196 µmol TE.g^{-1}).

Conclusion

We compared the soil contents of risk metals from the plots where the said crops were grown, in the aqua regia and ammonium nitrate leachate, with the limit values set by Law no. 220/2004. Exceeding the hygienic limits was recorded for three risk elements, namely Co, Cd, and Pb. Subsequently, we compared the safety of individual types of pseudocereals in terms of the content of hazardous metals, based on the maximum permissible amounts of elements given in the Food Code of the Slovak Republic (Decree No. 2/1994 of the Ministry of Health of the Slovak Republic). The content of none of the microelements exceeded the set hygienic limit in any of the monitored species of pseudocereals. The content of Cd and Pb in the grains of the monitored pseudocereal species was below the detection limit. In this respect, the monitored pseudocereals are safe for the consumer. The safest or most resistant plant species with the lowest ability to accumulate hazardous metals from soil to grains, from the group of crops which we monitored, is quinoa. BAF$_{AR}$ values have also shown that quinoa is the safest or most durable with the lowest ability to accumulate hazardous metals. The total content of polyphenols (TPC) in our pseudocereal species ranged from 1 251.1 to 11 495.8 mg GAE.kg^{-1}. The results of our research showed that the total antioxidant activity (TAC) in the monitored pseudocereal species ranged from 1.184 to 8.196 µmol TE.g^{-1}. We found that amaranth showed the lowest values of antioxidant activity and, conversely, we recorded the highest antioxidant activity in sorghum.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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