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Research Article

Study and Determination of As, Cr, and Pb in Amaranth Seeds

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The concentration of As, Cr, and Pb toxic elements was determined in three species of amaranth seeds: *A. hypochondriacus*, *A. cruentus*, and *A. dubius*. The determinations were carried out by inductively coupled plasma optical atomic spectroscopy (ICP-OES). The result shows that Cr and As were found in minor concentrations than allowed by the World Health Organization (WHO; 120 and 4 mg kg^{-1} resp.); As (mg kg^{-1}): *A. dubius* (0.76) *A. cruentus* (<0.50) and *A. hypochondriacus* (<0.50); Cr (mg kg^{-1}): *A. dubius* (8.19) *A. cruentus* (1.15) and *A. hypochondriacus* (1.20). However, the concentration of Pb was high: *A. dubius* (19.04) *A. cruentus* (30.20) and *A. hypochondriacus* (35.56) more than the maximum of WHO (10 mg kg^{-1}). To avoid systematic error, recovery and validation studies were performed: recovery test: 102.3%; validation (by standard addition): 96.0–103.1%. Due to the fact that amaranth had been proposed as new food due to its nutraceutical properties, the high concentration of Pb found in this study indicates that Pb should be evaluated in these amaranth species to avoid the intake of toxic element by human beings.

1. Introduction

Amaranth is a plant species that was frequently used in America before the Spanish conquest. Then, this crop was no longer consumed and amaranth was forgotten. From a total of 70 species belonging to the *Amaranthus* genus, 40 are from America. At present, amaranth is studied as food due to its high protein content, its excellent amino acid profile, and nutraceutical properties. It is known that amaranth can concentrate heavy metals and there are numerous reports that show this ability: a previous work reported the accumulation of Cd in *Amaranthus* in both shoot and root of *A. hybridus*, *A. spinosus*, *A. viridis*, and *A. rudis* [1]. Other authors reported the effect and accumulation of heavy metals by amaranth in saline soils [2]. Also, the ability of *Amaranthus dubius* and *Amaranthus hybridus* to bioconcentrate several mineral elements was proved [3]. Iron, lead, and zinc concentrations were studied in amaranth plants from Kenya [4]. The content of several metals, such as Al, Fe, Ni, Cr, and Pb, was determined in amaranth [5]. Both the effect of As in the growth of amaranth plants and a new method for the extraction of As from the plants were reported [6]. In Nigeria, both, the bioconcentration of trace elements in leaves of *Amaranthus caudatus* [7] as well as the study of trace elements in *Amaranthus hybridus* [8] were carried out. On the other hand, trace elements were analyzed in leaves of *Amaranthus hybridus* from Africa [9]. The uptake of As and heavy metals by native *Amaranthus blitoides* was studied in Spain [10]; as a result, the use of *Amaranthus albus* and *Amaranthus blitoides* was proposed for bioremediation in contaminated zones [11]. *Amaranthus hybridus* was studied due to its potential danger of bioaccumulation of toxic trace metals under biosolid amended irrigated soils if plants are used as edible vegetables [12]. A recent report [13] reveals that the intake of *Amaranthus lividus*, *hybridus*, and *dubius* harvested from contaminated soils may be dangerous to human health due to their ability to accumulate metals. The Cd accumulation ability of *A. hybridus*, showing high bioconcentration factors in soil culture and hydroponic solution [14] was also reported.

Amaranth seeds have very important nutritional properties and they are very frequently used to obtain flours. The previously mentioned reports studied the concentration of several transition elements in leaves and roots of different species of *Amaranthus* although toxic elements have not been previously studied in their seeds.

Due to the toxic effect of this plant for intake and also to its negative effect for human health, this paper discusses the concentration of the toxic elements: As, Cr, and Pb found in *A. cruentus*, *A. dubius*, and *A. hypochondriacus*.

2. Materials and Methods

2.1. Sampling. Twenty seed samples of 50 g every one were collected for each plant population (*A. hypochondriacus*, *A. cruentus*, and *A. dubuis*) from south west of San Luis and north east of La Pampa provinces (Argentina), during the summer of 2011-2012. Every complete seed sample was dried, grinded, sieved, and saved into a flask in a dark and fresh place until analysis.

2.2. Reagents. Ultrapure nitric acid was purchased from Sigma (St. Louis, MO, USA). All standard solutions were prepared using spectroscopic grade (>99.98%) Merck (Darmstadt, Germany) reagents. Ultrapure water (18.2 MΩ cm) was obtained using a Barnstead Easy-Pure RF compact water system (Dubuque, IA, USA).

2.3. Sample Preparation. The seed samples of the three plants were individually ground using a Wiley 3379 series grinder (Swedesboro, NJ, USA) and passed through a sieve (0.50 mm diameter). A portion of powder (2.0 g) was transferred to a porcelain crucible and 5 mL of indium solution (500 mg L^{-1}) were added as internal standard to evaluate the degree of recovery. The mixture was covered with a cap and reduced to ashes in a furnace Dalvo (Buenos Aires, Argentina) at 500°C for 6 h. The ashes were dissolved with 5.0 mL of concentrated nitric acid until the evolution of gases had stopped. Then, the solution was transferred to a 50 mL volumetric flask and completed to mark with deionized water [15, 16].

2.4. Instrumental. As, Cr, and Pb elements and the internal standard were determined using a Varian ICP-OES model ICP-OES Vista Pro (Palo Alto, CA, USA), with a Czerny-Turner monochromator, holographic diffraction grid, and a VistaChip charge coupled device (CCD) array detector. The operation parameters of the system ICP-OES are listed in Table 1, which were selected based on signal/noise ratio, time of analysis, emission intensity levels, and presence of a possible background effect.

2.5. Statistical Analysis. Calibration curves for each element were constructed in triplicate using six different concentrations, giving regression coefficient (r^2) values which ranged from 0.9957 to 0.9992. The analysis of amaranth seed samples was carried out by 20 replicate measurements, and the results were expressed as mean ± standard deviation (SD) [2].

Table 1: The operating parameters of the system ICP-OES.

Parameter instrument operating conditions		
As	Cr	Pb
λ (nm) As: 188.980	λ (nm) Cr: 205.560	λ (nm) Pb: 220.353
Power: 1.2 kW		
Plasma gas flow: 15 L/min		
Auxiliary gas flow: 1.5 L/min		
Nebulizer pressure: 250 kPa		
Pump rate: 15 rpm		
Sample uptake rate: 1.2 mL/min		
Replicate read time: 30 s		
Background correction Fitted		
Replicate read time: 10 s		
Replicate readings: 3		

Table 2: Concentration of As, Cr, and Pb in *A. dubius*, *A. cruentus*, and *A. hypochondriacus*.

	A. dubius		*A. cruentus*		*A. hypochondriacus*	
	Mean[a]	SD[b]	Mean[a]	SD[b]	Mean[a]	SD[b]
As	0.76	0.08	<0.5	—	<0.5	—
Cr	8.19	0.12	1.15	0.08	1.20	0.07
Pb	19.04	1.03	30.20	1.25	35.56	1.52

[a]Expressed in mg kg^{-1} of dried seed.
[b]Standard deviation ($n = 20$).

3. Results and Discussion

3.1. Toxic Element Analysis. Table 2 shows the results of the analysis obtained for the three *Amaranthus* species. For Pb, high concentrations were found in the 3 species, overcoming the allowed limit in foods by the World Health Organization (WHO) [17] whose value is 10 mg kg^{-1}. Pb concentration is higher in *A. cruentus* and *A. hypochondriacus*, being more than three times the WHO allowed limit; for *A. dubius*, the Pb concentration is twice this limit. Cr was found in the 3 species, but its values are below the WHO allowed limit of 120 mg kg^{-1} of vegetable. The concentration of As for *A. cruentus*, *A. hypochondriacus*, and *A. dubius* were below 4 mg kg^{-1}, which is the WHO allowed maximum.

Toxic elements are present in species of amaranth seeds at different concentrations, which, in some cases, exceeded the permissible levels, such as the case of Pb. This could be attributed to the use of contaminated irrigation water, soil, and the addition of some fertilizers and herbicides. Toxic elements can accumulate in plants through both foliage and root systems.

Laidlaw et al. [18] state that urban agriculture presents challenges in terms of the health of farmers and consumers. Studies have shown that urban soils can receive large inputs of trace metals from different anthropogenic sources but especially from automobile emissions. Plants growing in contaminated environments can accumulate trace elements

Table 3: Results of the validation by standard addition carried out in *A. dubius*.

	Initial value[a]	SD[b]	Added[a]	Found[a]	Recovered[c]
As	0.76	0.08	0.50	1.24	96.0
Cr	8.19	0.12	1.15	9.32	98.3
Pb	19.04	1.03	3.80	22.96	103.1

[a]Mean value and concentration in $\mathrm{mg\,kg^{-1}}$.
[b]Standard deviation ($n = 20$).
[c][(found-initial)/added] × 100.

in high concentrations, causing a serious health risk to consumers.

Lead is of particular concern because there is increasing evidence that relatively low concentrations of lead in the blood can affect children's mental development, an effect that persists into adulthood. Lead poisoning causes permanent neurological, developmental, and behavioral disorders, particularly in children [18].

3.2. Recovery Analysis. To avoid the presence of systematic error due to sample pretreatment, validation, and recovery, assays were performed. The method of standard addition is considered as a validation method [19, 20]. Standard addition was carried out for every toxic element in *A. dubius*. Table 3 presents the results of standard addition, which shows high recuperation from 96.0–103.1%.

On the other hand, an addition of indium as internal standard serves for evaluating the recovery percentage as well as the losses in the ashing step [21].

The addition of indium was performed prior to the mineralization step and the mean recovery value was $102.3 \pm 1.3\%$ ($n = 10$), which is in agreement with data from a previous report [22].

To guarantee the absence of systematic errors into the laboratory (by operator, instruments, etc.), blind quality control (QC) test samples were intercalated to the real samples, which were prepared with known concentrations of As, Cr, Pb, and In but unknown for the operator.

One QC sample was included every 10 real samples. The results showed recoveries from 95 to 102% for blind QC samples, indicating absence of systematic error into de laboratory. The limits of detection (LOD) obtained for As, Cr, and Pb were 0.15, 0.23, and 0.57 $\mathrm{mg\,kg^{-1}}$ of dried seed, respectively.

4. Conclusions

This paper discussed the presence of toxic elements in three amaranth species. Based on results obtained and since the bioconcentration ability of amaranth for several metals is well known, as well as the fact that if amaranth is consumed it can be harmful for the human health, it is advisable to analyze the presence of Pb in amaranth seeds previous to consumption. These levels of Pb indicate that amaranth seed are toxic for the human beings. Regarding other toxic elements present in these species, As and Cr were found in low concentrations; their analysis is also advised, mainly in polluted zones or regions under risk of metal contamination, to ensure that they are under the allowed limits.

The results presented here can be seen as a basis for further study in relation to phyto-remediation of contaminated soils and should make known on the need to use non-contaminated areas for the cultivation of these species of amaranth.

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