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MINERAL COMPOSITION OF AMARANTH (*AMARANTHUS* L.) SEEDS OF VEGETABLE AND GRAIN USAGE BY ARHIVBSP SELECTION

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ABSTRACT
The questions of the practical usage of the analytical scanning electron microscope JSM 600 LA by JEOL company (Japan) with EDS system – microanalysis for the studying of the ash elemental composition of seeds 9 breeds (Vegetable and Grain application) 4 species genus *Amaranthus* L. – *A. hypochondriacus, A. cruentus, A. hybridus, A. caudatus, A. tricolor*. Plant seeds by Federal center of vegetable production selection were envisaged. We studied the concentration of 14 basic elements (in weight %) contained in the mineral part of amaranth seeds. In the amaranth seeds of vegetable forms the accumulation order of the elements is the following: Ca >K >P >Mg >Si >Se >Fe >Mo ≈ S ≈ Cl ≈ Zn >Na >Al. In the seeds of the grain forms the order is different: K >P >Ca >Si >Se >Mg >Fe >Na >Mo >Cl ≈ S ≈ Mn ≈ Zn ≈ Al. The amaranth seeds of the grain forms are rich in macro – and microelements. P, K, Cl and S in the seeds of the grain forms are accumulated on 50, 37, 15 and 5% more and Si, Fe and Al in 2.6 and 1.8 times more than in the vegetable forms seeds. The breeds with the high concentration of the elements are recommended for using in the selection process. The elevated level of the essential macro- and microelements such as Ca, K, P, Mg, Mo, S and Cl stipulates the perspective of the functional products creation on the base of the studied amaranth seeds for the enrichment of the food stuffs.

Keywords: amaranth; seeds; analytical scanning electron microscopy; Energy Dispersive X-ray Analysis (EDS); ash elements

INTRODUCTION
The unique feature of the plants is their ability to extract and metabolize the mineral elements of the soil and water mediums.

It is known that for the acrospires growth and development the following elements are necessary: macroelements N, P, S, K, Ca, Mg and Fe, microelements Cu, Mn, Mo, Zn, B. However, besides these elements, a group of useful elements was distinguished, to this group such elements as Na, Cl, Si were included, they are included in the metabolic processes and with their absence in the medium the plant cannot go through all the development cycle. All the amaranth plant organs as the primary producers are the source of the mineral elements which get into the human being organism in the form of organic molecules and complexes and in the forms of ions in the balanced concentrations. Besides the plant hereditary characters the soil and other multifactorial conditions that accompany the process of amaranth growth influence on the mineral composition of the amaranth seeds **(Jamriška, 1996; Zheleznov et. al., 1997)**.

The seeds of the amaranth engineered breeds of the species – *A. hypochondriacus*, *A. cruentus*, *A. hybridus*, *A. caudatus*, *A. tricolor* are useful for the human nutrition as they have energetic, nutritive, dietary and medical values **(Rastogi and Shukla, 2013; Bojňanská and Šmitalová, 2015)**. The high dietary value of the amaranth seeds is due to the presence of organic and inorganic biochemical components such as carbohydrates (mono- and disaccharides, pectin, dietary fibers) **(Kamysheva, 2010)**, antioxidants – phenol acids, flavonoids, vitamins, proteins, fats and so on **(Tharun et.al., 2012, Gumul et.al, 2017)**.

In the amaranth seeds the essential – nonreplaceable elements (mineral substances) are 0.7 – 1.5 %; they do not possess energetic value as proteins, fats and carbohydrates, however, the human life is impossible without them.

The research up-to-date new technologies in physiological and medical researches confirm an important role of microelements on the level of metabolic reactions and submolecular processes, the activity of which depends on the presence of the certain macro- and microelements in our daily diet **(Avtsyn et al., 1991; Peter and Gandhi, 2017, Motyleva et.al., 2017)**.

The purpose of our work was to research the special features of mineral substances accumulation in the seeds of seven amaranth breeds of vegetable and grain usage created in Federal Research Center for Vegetable Growing (FRCVG).

Scientific hypothesis

The comparative data on the mineral composition of the seeds of the different species *Amaranthus* L. breeds, being grown in Moscow region, do not exist. We checked whether there are differences in the content of macro- and microelements in species of the genus *Amaranthus sp.* vegetable and Grain forms.

MATERIAL AND METHODOLOGY

Place and objects of research

The objects of the research are the seeds of the vegetable and grain amaranth breeds by FRCVG (Moscow region, Odintsovo) (Table. 1). The plants were grown in the open on the experimental fields of FRCVG. The seeds samples for the analysis were prepared on the stage of biological ripeness.

Soil growing

The soils of the FRCVG experimental and production base are sod-podzolic medium loamy. The agrochemical characteristics of the arable (0 – 20 cm) soil layer before sowing and planting amaranth plants were as follows: humus content according to Tyurin – 1.6 – 2.3%, reaction medium pH KCl 5.9 – 6.1, hydrolytic acidity 1.30 – 1.55 meq.100 g^{-1} of soil, the sum of the absorbed bases is 18.7 – 19.2 meq.100 g^{-1} of soil, the degree of saturation with bases is 88 – 94%, the content of mobile phosphorus is 400 – 550 mg.kg^{-1} of soil, Potassium 150 – 210 mg.kg^{-1} soil, mineral nitrogen 7.0 – 10.0 mg.kg^{-1}.

Amaranth plants were grown on clean (background) soils that are not contaminated with heavy metals (within the permitted sanitary standards adopted in Russia).

Sample preparation

The data of the quantitative elemental composition, given in the present paper, are taken in the laboratory of physiology and biochemistry of the Centre of the plants genofond and bioresources of Federal State Budgetary Scientific Institution All-Russian Horticultural Institute for Breeding, Agrotechnology and Nursery, Moscow. The researches are original and are fulfilled with the usage of the modern analytical equipment. The average seeds weighing with the mass of 10 g was mineralized in the muffle furnace Naberterm (Germany) at T = 400ºC. The received ash was dispergated by ultrasound at 18 kHz frequency for 15 minutes. The dispergate even layer was applied on the object table covered with carbonic scotch.

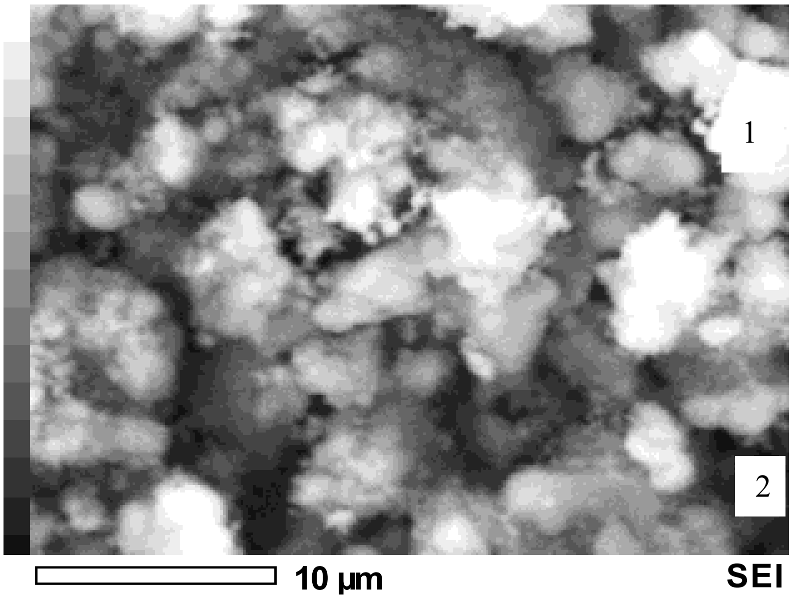
EDS - analysis

The chemical composition of the basic ash components (Na, P, S, K, Mn, Fe, Mg, Ca, Al, Si, Cl, Zn, Se, Mo) was determined by the method of energy dispersive spectrometry (ESD) on the analytical raster electron microscope JEOL JSM 6090 LA. The microscope resolution is 4 nm at accelerating voltage 20 kV (secondary electrons image), zooming is from x 10 till x 10 000. While performing the elemental analysis the working distance (WD) is 10mm. Energy-dispersive spectrometer allows to carry out the quantitative X-ray microanalysis with the desired analyzing area: in a point or areally, and to receive the maps of elements allocation.

X-ray microanalysis data are presented in the form of standard protocols which contain the microstructure picture of the sample under study, the table of the data in weighting and atomic correlation, spectra and histograms. The spectrum example is shown in Figure 1.

Table 1 Amaranth (*Amaranthus* L.) seeds of vegetable and grain application by Federal center of vegetable production selection.

Elements	**Vegetable forms**				**Grain forms**				
	Valentina	**Don-Pedro**	**Nezhenka**	**Pamyati Kovasa**	**Kizlyarets**	**Krepysh**	**Syuita**	**Shuntuk**	**Variation coefficient (%)**
Na	0.092	0.053	0.906	0.088	0.118	0.121	0.978	0.093	
K	8.991	7.804	13.774	17.673	18.768	15.574	16.598	15.471	13.78
P	9.126	8.851	7.914	13.793	14.351	14.115	13.632	12.611	6.35
Ca	17.234	20.361	14.262	13.171	10.501	11.574	10.788	13.528	10.04
Mo	2.556	2.771	2.962	3.122	3.387	3.345	2.534	2.881	0.09
Mg	6.207	6.871	4.326	6.425	6.591	5.741	4.776	5.232	0.74
S	1.770	1.728	2.054	2.071	2.021	2.098	1.718	2.242	0.03
Si	0.423	0.091	1.526	0.255	0.218	0.191	2.542	3.106	1.26
Cl	0.859	1.044	3.651	1.718	2.023	1.993	4.084	0.684	1.36
Mn	0.155	0.451	0.241	0.288	0.063	0.194	0.171	0.396	0.02
Fe	0.170	0.318	0.341	0.187	0.326	0.263	0.405	0.886	0.04
Al	0.193	0.082	0.202	0.083	0.127	0.091	0.134	0.678	0.04
Zn	0.204	0.236	0.214	0.213	0.221	0.257	0.232	0.156	0.01
Se	0.405	0.482	0.322	0.303	0.359	0.345	0.354	0.198	0.99
∑	**48.037**	**51.143**	**52.695**	**59.391**	**59.047**	**55.902**	**58.946**	**58.162**	



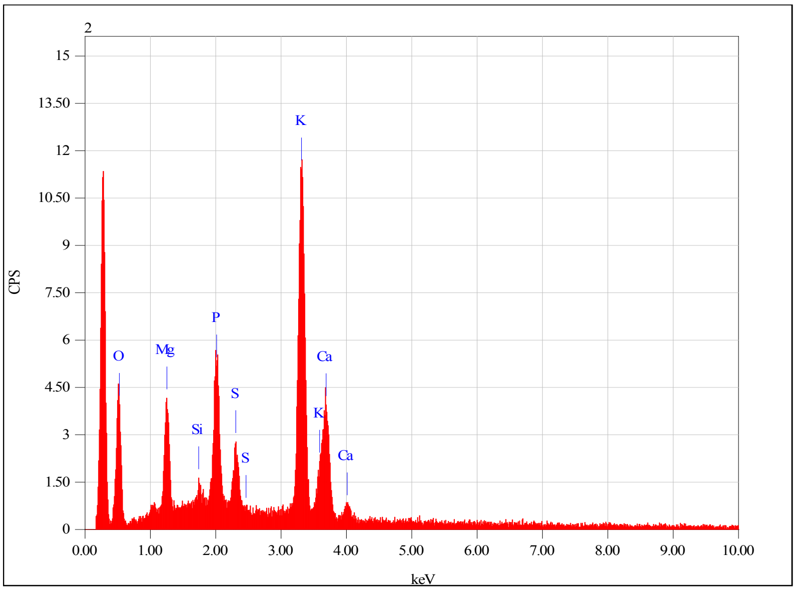


Figure 1 The microstructure picture of the sample under study (1) and the general view of the X-ray spectrum lines that show the elements presence in the analyzing area (2).

Taking into consideration the spectrum lines intensity the concentration of the desired element can be determined. The fractional accuracy of the chemical analysis is spread in the following way: at the element concentration from 1 to 5% the accuracy is less than 10%; from 5 till 10% the accuracy is less than 5%; at the element concentration more than 10% the accuracy is less than 2%. 100 ash areas of each sample were studied. The local analysis is 3 mm, the scanned area is not less than 12 µm.

Statisic analysis

For statistical evaluation were used standard metods using statisniral software Statgraphics Centurion XVII (StatPoint Inc.USA).

RESULTS AND DISCUSSION

The concentration of 14 basic elements (in mass %) contained in the amaranth seeds mineral part was studied (Table 2).

Herewith the main proportion of the ash elements in the seeds belongs to Ca. Ca takes part in the processes of living organisms growth and development, goes into the composition of coenzymes and cells nucleuses, it also takes part in the most important processes for the organism such as metabolism, immunity, regeneration and others **(Gusev, 1998; Gins and Gins, 2011)**.

The proportion of Ca in the amaranth seeds of the vegetable breeds fluctuates from 13.171 to 20.361; while in the grain cultures it varies from 10.501 to 13.598 mass %.

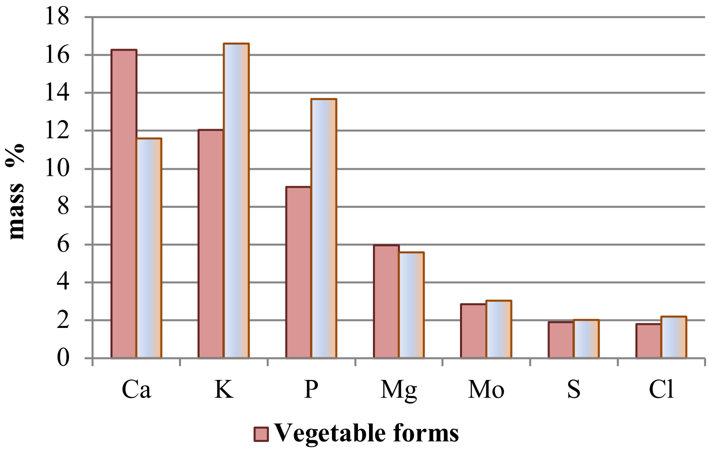


Figure 2 The comparative concentration of macroelements in the ash of the amaranth seeds of vegetable and grain forms.

Table 2 The elemental composition of amaranth seeds, mass % in the ash.

Elements	Vegetable forms				Grain forms				
	Valentina	Don-Pedro	Nezhenka	Pamyati-Kovasa	Kizlyarets	Krepysh	Syuita	Shuntuk	Variation coefficient (%)
Na	0.09 ±0.01	0.05 ±0.01	0.91 ±0.08	0.09 ±0.01	0.12 ±0.02	0.12 ±0.01	0.98 ±0.10	0.09 ±0.02	
K	8.99 ±0.60	7.80 ±1.17	13.77 ±1.67	17.67 ±1.90	18.77 ±1.50	15.57 ±1.46	16.60 ±1.47	15.47 ±1.38	13.78
P	9.13 ±1.18	8.85 ±1.49	7.91 ±1.47	13.79 ±1.18	14.35 ±1.10	14.12 ±1.66	13.63 ±1.97	12.61 ±1.34	6.35
Ca	17.23 ±1.23	20.36 ±1.77	14.26 ±1.53	13.17 ±1.15	10.50 ±1.21	11.57 ±0.84	10.79 ±1.08	13.53 ±1.50	10.04
Mo	2.56 ±0.15	2.77 ±0.20	2.96 ±0.09	3.12 ±0.17	3.39 ±0.10	3.35 ±0.19	2.53 ±0.19	2.88 ±0.24	0.09
Mg	6.20 ±0.08	6.87 ±0.18	4.33 ±0.14	6.43 ±0.06	6.59 ±0.08	5.74 ±0.15	4.78 ±0.23	5.23 ±0.06	0.74
S	1.77 ±0.04	1.73 ±0.03	2.05 ±0.19	2.07 ±0.06	2.02 ±0.03	2.10 ±0.03	1.72 ±0.02	2.24 ±0.08	0.03
Si	0.42 ±0.03	1.90 ±0.20	1.53 ±0.10	0.26 ±0.03	0.22 ±0.01	0.19 ±0.02	2.54 ±0.30	3.11 ±0.25	1.26
Cl	0.86 ±0.04	1.04 ±0.18	3.65 ±0.28	1.72 ±0.16	2.02 ±0.23	1.99 ±0.07	4.08 ±0.42	0.68 ±0.04	1.36
Mn	0.16 ±0,07	0.45 ±0.09	0.24 ±0.02	0.29 ±0.05	0.06 ±0.02	0.19 ±0.06	0.17 ±0.14	0.40 ±0.05	0.02
Fe	0.17 ±0.09	0.32 ±0.18	0.34 ±0.14	0.19 ±0.07	0.33 ±0.07	0.26 ±0.07	0.41 ±0.09	0.89 ±0.12	0.04
Al	0.19 ±0.04	0.08 ±0.01	0.20 ±0.06	0.13 ±0.01	0.13 ±0.01	0.09 ±0.01	0.13 ±0.05	0.68 ±0.04	0.04
Zn	0.20 ±0,03	0.24 ±0.02	0.21 ±0.02	0.21 ±0.01	0.22 ±0.03	0.26 ±0.03	0.23 ±0.03	0.16 ±0.03	0.01
Se	0.41 ±0.03	0.48 ±0.03	0.32 ±0.03	0.30 ±0.03	0.36 ±0.02	0.35 ±0.01	0.35 ±0.04	0.20 ±0.03	0.99
∑	**48.04**	**51.14**	**52.70**	**59.39**	**59.05**	**55.90**	**58.95**	**58.16**	

K is a macroelement that is responsible for the regulation of the majority metabolic reactions that flow in living organisms. The very special role in controlling the homeostasis belongs to K. It controls osmotic pressure transmembrane potential, charges equilibrium, cathode-anion balance, pH – everything that the homeostasis of cells and tissues consists of. In the ionic form K can be found in all the organs, tissues and cell structures in the concentrations that exceed the concentration of other ions **(Meathnis et al., 1997)**. The concentration of K in the amaranth seeds of the vegetable breeds fluctuates from 7.804 (breed Don Pedro) to 17. 673 (Pamyati Kovasa) mass % relatively. In the seeds of the grain application the fluctuations are not essential – from 15.471 to 18.768 mass % at average K contains on 37% more in the breeds of grain usage than in the seeds of vegetable breeds (Figure 2). More than 50% of P is presented in tissues in the form of inorganic P (Pin). P is a part of DNA and RNA, phospholipids, phosphate esters, nucleoside phosphates – ATP, ADP, NATPH, where it fulfills the structural function (in composition of first two types of compounds), in the rest ones – metabolic. P plays a very important role in the cell energetics. For the plants the analogue of P is phytin – Ca^{2+} – Mg^{2+} - the sol of inositephosphoric acid, essential quantities of which are accumulated in the seeds. The concentration of P in the amaranth seeds of vegetable and grain application is 9.021 and 13.677 mass % at average relatively, wherein in the ash of the amaranth seeds of grain breeds the proportion of P is on 50% more than in the seeds of vegetable forms **(Schachtman et. al., 1998)**.

Mg is necessary for the processes of regeneration and renewal of cells, tissues and organs. It activates a large number of enzymes that take part in the processes of CO^2 and N assimilation. In cytosol Mg counter-balances organic compounds (sugars groups, nucleotides, organic and amino acids). Mg is necessary for the keeping up of the cathode-anion balance and pH regulation.

In the cell wall approximately 2.5% of the general concentration of Mg can be found. In the cell wall and in the seeds membrane Mg^{2+} is coordinately connected with carboxylic groups of pectin substances and takes part in the creation of the inner physiological environment of plants. Mg, Ca and N are localized in the seed membrane. ATP, phosphoinositol (phytin) in combination with Mg are accumulated in the seeds in the form which is comfortable for storage **(Nechaev et.al., 2007)**. In the amaranth seeds of

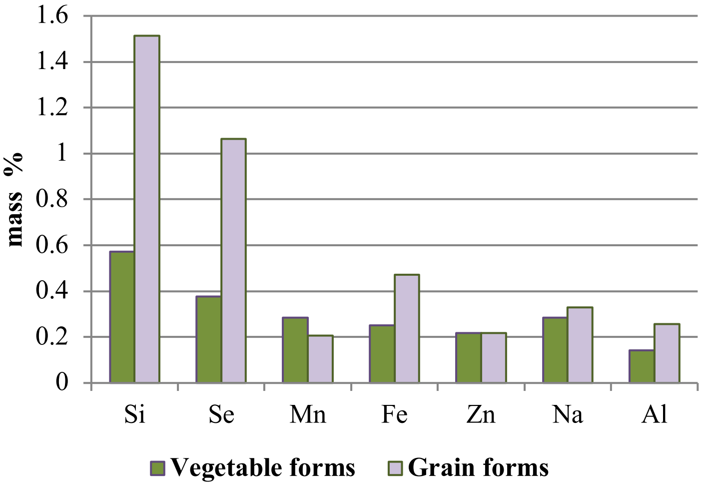


Figure 3 The comparative concentration of macroelements in the ash of the amaranth seeds of vegetable and grain forms.

vegetable and grain forms the concentration of Mg has close values 5.592 and 5.585 mass % relatively.

Mo fulfills a number of useful functions for the organism: it is a cofactor and an activator of oxidases (xanthine oxidase and serine oxidase), takes part in the amino acids synthesis, in the exchange of the vitamins C, E and B12 inside an organism **(Avtsyn et al., 1991)**. The concentration of Mo in the amaranth seeds of the breeds under study fluctuates from 2.556 till 3.387 mass %, wherein, in the seeds of grain application Mo is accumulated on 6% more than in the seeds of vegetable breeds (Figure 2).

Firstly S is necessary for the synthesis and regulation of the plant produced protein quantity and quality. S is a biogenous element as a part of proteins and glutathione, it possesses antioixidant activity, provides the process of the energy transfer in the cell by transporting electrons, takes part in the methyl groups transportation and fixation, covalent, hydrogen and mercaptide connections production, enables the genetic information transfer. In the amaranth seeds of the grain breeds S is contained on 5% more than in the seeds of the vegetable application breeds (Table 2, Figure 2).

At the present time Cl belongs to the microelements. It is the most important biogenous element of living organisms. Cl ions together with Na and K ones take part in the support of the osmotic equilibrium and salt- water exchange regulation. The transportation of Cl contributes to the realization of the following functions: electrical and Ca^{2+} – signalization, membrane potential and pH gradient control. Cl together with Ca is included in the mechanism of the stomatal movements **(Avtsyn et al., 1991)**. In the amaranth seeds of the grain breeds Cl is contained on 15% (2.196 mass %) more than in the seeds of the vegetable application breeds – 1.818 mass % (Table 1, Figure 2).

Si is an obligatory element for the plants **(Kolesnikov and Gins, 2001)**. It is accumulated in large amount in the leaves cell walls, especially in the ecderonic tissues of the scape leaves and the roots. Si is not only the base of the tissues framed element, but it also controls a number of biological and chemical processes in the living organism. It influences the seeds emergence and the seedlings growth it quickens the scapes growth and the dry biomass accumulation. Si has a protective effect toward the harmful influence of Al ions, creating alumosilicates which are included in the composition of phytoliths. Si enlarges the plants resistivity to abiogenous stresses.

The concentration of the biogenous eminent – Si in the amaranth seeds of grain breeds is in 2.6 times more than in the seeds of the vegetable forms (Table 1, Figure3).

Se is a powerful antioxidant that increases the living organism resistivity to biogenous and abiogenous stressors influence is the necessary microelement that is a part of active centers in the form of animoacid selenocysteine **(Vikhreva et al., 2001)**. The concentration of Se in the amaranth seeds is 0.314 – 0.378 mass %.

Mn is a co-factor and activator of many enzymes (pyruvate kinasa. decarboxylase. syperoxide dismutase) it takes part in the synthesis of glycoproteins and proteoglycans and possesses antioxidant activity. In the amaranth seeds the concentration of Mn is 0.206 – 0.284 mass % (Table 2, Figure 3).

Fe as a part of active centers – hemoproteins and iron-sulphur proteins determines the space structure and activity and takes part in oxidation-reduction reactions. The alternative form of Fe is the molecule of protein ferritin that may accumulate up to 4,500 atoms of Fe in soluble nontoxic form. In the amaranth seeds Fe and Cu are localized in the corcule **(Shmalko and Roslyakov, 2011)**.

Organic Fe is an essential compound for the human organism. This element is a part of catalytic centres of many oxidation-reduction enzymes. Fe as a part of active centers – hemoproteins and iron-sulphur proteins determines the space structure and activity and takes part in oxidation-reduction reactions. The alternative form of Fe is the molecule of protein ferritin that may accumulate up to 4,500 atoms of Fe in soluble nontoxic form. In the amaranth seeds Fe and Cu are localized in the corcule **(Shmalko and Roslyakov, 2011)**. Organic Fe is an essential compound for the human organism this element is a part of catalytic centres of many oxidation-reduction enzymes.

Zn stabilizes the molecules structure, plays an important role in DNA and RNA metabolism, in the protein synthesis and cells fission, in the processes of the signal transmission inside the cell **(Nechaev et al., 2007; Pedersen et al., 1987)**. Zn is also an important biogenous element, its concentration in the amaranth seeds does not exceed 0.217 mass %.

Na is contained mostly in the intercellular fluid Na in the combination with K takes part in the membrane potential creation, the activation of enzymes and muscular contractions, supports osmosis, acid-alkaline and water balance, provides membrane transport **(Avtsyn et al., 1991)**. In the amaranth seeds of the grain forms the concentration of Na is on 11.5 % more than in the seeds of vegetable forms (Table 1, Figure 3).

Al is necessary for the growth and development of bone, cartilaginous and connective tissues and for their regeneration, the concentration of Al in the seeds of grain forms is in 1.8 times more than in the amaranth seeds of vegetable forms (Figure 3).

The average variation coefficient from 6.35 to 16.38% is typical for biologically significant elements of plum fruit and their accumulation limits are due to species peculiarities. The low variation coefficient from 0.74 to 1.36% indicates the accumulation stability of the elements by the culture and depends on the breed insufficiently. Pearson's linear correlation coefficient between 2 elemental compositions in the ash shows the significant correlations between Ca and Mg (r 0.84) and Ca and Mn (r 0.91).

CONCLUSION

Previously, we showed that the amaranth seeds are rich in ash substances – minerals, such as K, Na, Ca and Mg **(Gins and Gins, 2011)**. Using the method of the energy dispersive X-ray spectrometry the new data about the variety of the amaranth vegetable and grain forms mineral composition were received, the proportion of the elements in the ash was determined, the variation coefficients were calculated. The amaranth grain forms exceed the vegetable forms in the elements sum and the concentration of K, P, Si and Fe in 1.3 – 2 times. The peculiarity of the elements accumulation in the seeds depends on the genetic origin and the breeds and species special aspects. The predominant accumulation of Ca, K and P is typical for all the amaranth samples under study. The breeds with the high concentration of the elements are recommended for using in the selection process. The elevated level of the essential macro- and microelements such as Ca, K, P, Mg, Mo, S and Cl stipulates the perspective of the functional products creation on the base of the studied amaranth seeds for the enrichment of the food stuffs. Taking it into account, the studying of the composition and mineral elements concentration in different organs of the plant and their influence on the human life activity is an actual (global) problem, as the deficit of macro- and microelements in the industrial food stuff is extremely huge and dangerous for the human health, because the major part of food stuff is depleted in mineral substances.

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