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SPECIES VARIATION OF *Aegilops* GENUS AND HEAVY METAL CONTENT IN PLANT HABITAT SOIL AT SOUTHERN ADRIATIC LOCALITIES

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The *Aegilops* genus is a wild relative to the bread wheat, having chromosomes homologous to wheat chromosomes. That genus could be the source of many usefull abiotic stress tolerance genes. Facing a global climate changes, as well as, environmental erosion, it is important to create a desirable genetic variability that could correspond to environmental challenges. Heavy metals in soil could cause soil pollution, could lead to different phenotypic changes in plants, and could enter food chain. Assessment of *Aegilops sp*. population variation, as well as, heavy metal content in their habitat was the main goal in this research. *Aegilops* population composition was examined and samples were taken from 55 localities of South Adriatic coastal and littoral areas. Topsoil samples from all the localities were taken and heavy metal content, namely Cr, Pb, Zn, Ni, Cd and Cu, was analyzed,. Manganese content was measured, as well. Value of pH was established.

Key words: biodiversity, wild relatives, *Aegilops,* soil, heavy metal

INTRODUCTION

According to some estimates by the 2020, the world need for wheat is going to increase by 40%. The suggestion for breeders is to enhance the grain yield and to diminish the impact of agriculture on the environment. However, there are some more factors to be taken into account – global climate change, which puts more challenges to agricultural production, and the existing erosion of the environment, as a serious obstacle for the wheat grain yield increment. Climate change and environmental erosion have abiotic stress as a common consequence. The presence of abiotic stress in wheat production, as well as, food production in a whole, should be taken into account and prevailed. Broadening of genetic variability is needed in order to meet new requirements in agricultural production. Wild relatives, landraces, local populations are recognized as a prospective source of desirable genes for abiotic stress tolerance, which could be utilized in wheat breeding programs.

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Heavy metals are heterogeneous group of chemical elements with various and sometimes deleterious biological effects on plants and soil microorganisms. Since heavy metals classification is based on physical, rather than their joint chemical characteristics, their toxic effect differs in intensity. Some of heavy metals are of the physiological importance for plants and humans (Fe – iron, Cu – cuprum, Zn – zinc), others are very toxic (Pb – lead, Hg – mercury, Ni – nickel, Cd – cadmium, Mn – manganese) (NENOVA *et al*., 2009; CI *et al*., 2010). By the increment of concentration in soil and plants the toxicity of heavy metals rises. Heavy metals in soil are originated out of parent rocks in lithosphere, or by human activities - industrial pollution, extensive use of agro chemicals (mineral fertilizers, plant protection chemicals), sewage sludge, city dumps etc. Heavy metals from soil could migrate into plants by adsorption. Through resorption, heavy metals could enter human organism and cause all king of health problems (FLORA, 2009). Heavy metal pollution in soil commonly appears near industrial sites, mines, roads, or in agricultural areas due to long term fertilization (PALANIAPPAN *et al*., 2002; LIAO *et al*., 2008).

Soil pH is a very important soil characteristic. Most soil processes depends on the value of soil pH. The determination of soil pH is usually conducted in a suspension or filtrate, by colorimetric or electrometric method. Determination in a suspension can be conducted in three media: H_2O suspension - for pedogenetic and pedodinamic conclusions, and in the KCl or $CaCl_2$ suspension, for the ecological purposes (ČAPKA *et al*., 2009).

The cultivars that belong to *Poaceae* family are durable and low input. That makes them convenient for growing in abiotic stress soil conditions for yield, as well as, for phytoremediation. For both aspects of utilization, target breeding is required. However, conducting selection, particularly in main crops, under the same criteria for a long period of time, cereal breeders have faced the problem of gradual genepool narrowing. In order to improve that situation, the extensive work has been done in collecting, documenting and utilizing useful genetic variability of wild relatives, landraces and local populations. As very close wheat relative, giving 2/3 of genetic material to *Triticum*, *Aegilops* genus is of interest not only for evolutionary research, but also for broadening genetic variability reasons (ZAHARIEVA *et al*., 2001; ZAHARIEVA *et al*., 2003; LOSKUTOV, 2004). In a past two decades that genus has been extensively collected and investigated (KIMBER and FELDMAN, 1987; HODGKIN *et al*., 2008; AGHAEI *et al*., 2008; MAXTED *et al*., 2008). The evaluation and utilization of *Aegilops* and *Triticum* species in obtaining gene sources for heavy metal tolerance improvement is an ongoing task (LANDJEVA *et al*., 2003; CHHUNEJA *et al.,* 2006; RAWAT *et al.,* 2009, ROY *et al*., 2011). Efforts of connecting heavy metal content to Quantitative trait loci (QTLs) are of interest, as well, since QTL are of great importance in yield and yield component traits formation (BÁLINT *et al*., 2007). According to "List of priorities for *Triticum* and *Aegilops* genepools" issued by CIAT IRRI Biodiversity International (gisweb.ciat.cgiar.org), there is high requirement for *Aegilops geniculata* (syn. *Aegilops ovata*), and a requirement for *Aegilops kotschy*, as well. All *Aegilops sp*. samples were collected for Serbian National Genebank (DIMITRIJEVIĆ *et al*., 2011; PETROVIĆ and DIMITRIJEVIĆ, 2012).

The aim of this article is to study *Aegilops sp.* variability in coastal and littoral areas of South Adriatic, and to investigate soil type and heavy metal content at finding spots.

MATERIALS AND METHODS

The area in study is located in the Southern Adriatic, covering coastal, littoral and inland area of Montenegro. The position of localities varied from secluded areas to areas affected by human activities (fig. 1).

Figure 1. Map of localities in coastal, littoral, and inland part of South Adriatic

The samples of *Aegilops* genus were collected from localities, previously visualy screened, photographed, audio described, as well as, in writting, and positioned using GPS (Global Positioning System Garmin 12 Channel Receiver Personal Navigator). The state of populations was recorded with the emphasis to population homogenity (one species or mixed population), fig. 2.

Soil samples, 300-400g, were taken from 55 localities from about 30cm surface layer, for further chemical analysis. pH value was obtained using Kappen method in two different suspensions: H_2O, and KCl. The soil samples were divided in three groups according to their acidity determined in KCl: acid, neutral and alkaline soils. All the soil samples with pH≤ 6.5

were classified as acid soil, pH= 6.5-7.2 are neutral, and pH≥ 7.2 are considered as alkaline (ČAPKA *et al*., 2009). Heavy metals analyzed were chromium (Cr), lead (Pb), zinc (Zn), nickel (Ni), cadmium (Cd) and cuprum (Cu). Manganese (Mg) content was measured, as well. Microelement and heavy metal content (mg/kg) was established in ccHNO_3+H_2O_2 using atomic absorption spectrophotometer Varian 600. As an orientation, controll values were MAC (Maximum Allowed Content) according to Serbian regulative.

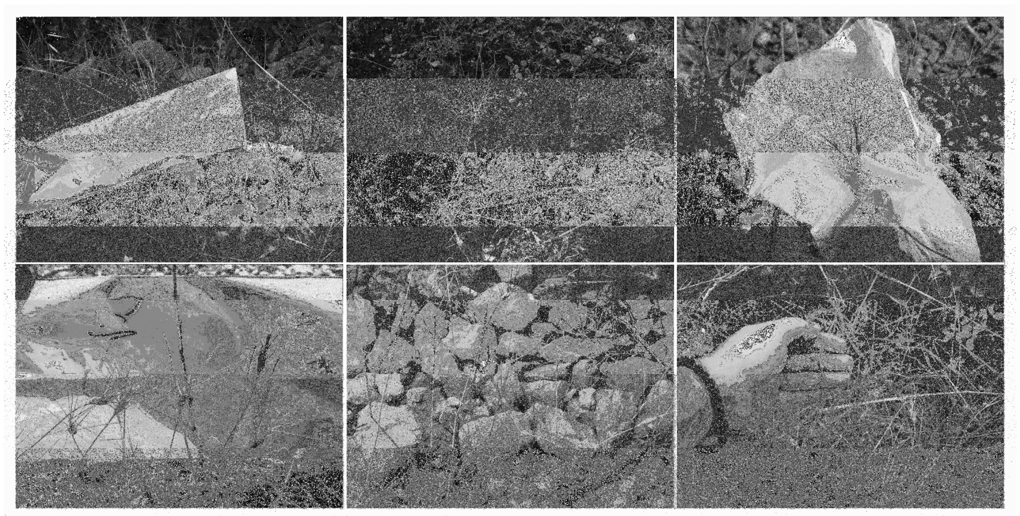

Figure 2. Plants of *Aegilops sp*., in their natural surroundings, on different grounds, and in different stages of maturity (Photo: Dimitrijevic & Petrovic)

Univariate statistics, one-way Analysis of variance (ANOVA) and Pearson's correlation analysis were applied in order to follow the variation and relationships. Linear regression was used for following trends. The data were processed using Statistica 10 (StatSoft Inc.) and Microsoft Excel.

RESULTS AND DISCUSSION

Field and laboratory analisys. Aegilops, a very close wheat relative, has vast genetic variability in Mediterranean region (KIMBER and FELDMAN, 1987; PETROVIĆ and DIMITRIJEVIĆ, 2005; DIMITRIJEVIĆ *et al*., 2011). Considerable variability of *Aegilops* genus has been noted and collected, in about 200 samples, in inland, littoral and coastal Montenegro. During several years (2000-2006), extensive investigations of *Aegilops sp.* had been done, not only by collecting samples, but also in close following of the variation of populations in consecutive years. Within that period of inventory, samples of *A. ovata* L., *A. triaristata* Willd, *A. kotschy* Boiss., *A. biuncialis* Vis., *A. cilindrica* Host., has been gathered. Species of *Aegilops* genus, that has been denoted and collected, commonly settled sunny slopes and terrain, mostly on shallow, poor land base, near the road, being exposed to air and soil pollution. All the soil of lead, cadmium and chrome. A higher heavy metal content could be found coming from stratified parent material, industrial pollution, misusing of plant protection chemicals in agriculture, sewer mud dissipation or city waste depots. The toxicuty of heavy metals (copper, zinc, lead, nickel, chromium and cadmium) affect changes in multiple forms of peroxidases leading to shoot and root growth depression (BÁLINT *et al.*, 2007). There is considerable usable genetic variability harbored in *Aegilops sp*., drought and heat good responses, salt tolerance, zinc efficiency, copper tolerance etc. (LANDJEVA *et al*., 2003; MOLNÁR *et al*., 2008; RAWAT *et al.*, 2009; LEONOVA and CHIKIDA, 2011). In our investigations the presence of *Aegilops sp.* populations at the localities with higher heavy metal content indicate more tolerant genotypes, having in mind possible utilization of that biodiversity in wheat breeding programmes for improving abiotic stress tolerance, that is in accordance to MONNEVEUX *et al.*(2000). That material is to be preserved and further genetically analysed. At 55 representative localities, soil samples has been taken and analysed for microelement and heavy metal content (fig. 1, fig. 3).

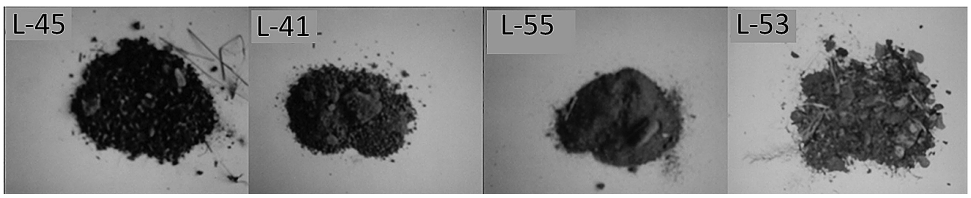

Figure 3. Some samples of different soil types where *Aegilops sp*. was collected from. From left to right: L-41 Road from Bar to Virpazar, calcareous light red soil; L-45Krnjice village, Rumija mountain above the Skadar lake, skeletal calcareous soil, powdered with brown soil; L-53 Valley by the Bojana river, road from Darza village to Sveti Đorđe (Saint George) village, brown, skeletal soil with organic matter; L-55 Sveti Nikola (Saint Nicholas) village, by the outfall of Bojana river, near to the Albanian border, calcareous sand (Photo: Dimitrijevic & Petrovic)

At the all collecting sites pH measured in soil samples in KCl varied between pH=6.15 at L-42 (village Limljani, 42.195^{o}N, 19.091^{o}E), and pH=8.11at L-55 (village Sveti Nikola, 41.876^{o}N, 19.369^{o}E), with pH= 7.16 on average. Results of soil pH measured in the suspensions of H_2O varied between pH=7.04 at L-7 (42.552^{o}N, 19.158^{o}E) to pH=8.44 at L-55 (41.877^{o}N, 19.365^{o}E), with the mean value of pH= 7.66 (tab. 1). Generally, all the soil samples exhibited neutral to moderately alkaline reaction (fig. 3, tab. 1). The only slightly acid reaction was observed at L-42 (42.198^{o}N, 19.101^{o}E) showing pH_{KCl}= 6.15. Predominant neutral and moderate alkaline reaction made possible to use Serbian MAC values as a control, since they were highly compatible to tolerant levels of heavy metal content in soil solution given by BRINNKMANN and PLASS (1984).

Screening all the localities at the examined South Adriatic coastal and littoral area for the heavy metal content reveals, vast variation between localities and regions was found. However, nickel was the only heavy metal exceeding MAC even three to four times at a number of locations. Lead, cadmium and chromium exceeded MAC at some locations, as well (tab. 1).

The inland part was covered with 17 probing sites in total. For easier handling, the inland part was arbitrary divided in two – the area roughly north of Podgorica, the Capital of Montenegro, and the area south of the Capital. The first appearence of *Aegilops* genus could be collected in Morača canyon about 3 km before Bioče village, about 11 km north of Podgorica, the capital of Montenegro, site L-1 (42.542°N, 19.339°E, about 120m alt.), fig. 1. That is the site near to the exit of a mountain region and where the first sign of littoral climate could be noted throuh surrounding vegetation, air scent and higher air temperature. Pure populations of *Aegilops triaristata* were collected at yellow-brown calcareous, skeletal alluvium soil, at two localities. A higher content of nickel and quite high content of lead was found in soil samples due to the presence of the road traffic. Alkaline soil pH reaction was denoted at both sites (pH_{KCl}= 7.68, or pH_{H2O}= 7.95 at L-1 and pH_{KCl}= 7.75 or pH_{H2O}= 8.31 at L-2), tab. 1.

Table 1. Microelement and heavy metal content at 55 representative localities, in the Montenegrian littoral area, where Aegilops sp. were collected from. The values above Maximal Allowed Content (MAC) according to the regulations of Republic of Serbia are bolded

Area	Locality	Cu mg/kg	Zn mg/kg	Mn mg/kg	Pb mg/kg	Cd mg/kg	Ni mg/kg	Cr mg/kg	pH KCl	pH H_20
Inland part	1	25,27	119,30	374,00	68,07	1,933	**97,43**	37,23	7,68	7,95
	2	27,80	96,43	569,33	95,70	1,233	**148,07**	52,77	7,75	8,31
	3	45,63	109,67	747,00	86,70	1,267	**154,33**	46,40	7,16	7,29
	4	25,23	97,93	939,00	63,73	1,033	**121,80**	23,77	7,22	7,79
	5	31,53	73,60	1677,00	34,27	1,333	**170,17**	31,70	7,13	7,50
	6	26,97	81,83	1149,33	57,50	1,967	**101,47**	34,40	7,15	7,36
	7	31,07	93,43	1892,67	46,93	1,100	**131,17**	42,33	6,59	7,04
	8	19,00	77,40	464,00	51,67	1,533	**75,10**	16,50	7,02	7,15
	9	39,70	127,30	1251,00	60,67	**3,033**	**84,73**	49,53	6,57	7,28
	10	20,23	43,37	395,33	24,63	1,533	**172,57**	41,67	7,59	8,26
	11	17,27	83,23	289,00	24,70	1,167	**77,27**	27,83	7,14	8,06
	12	24,80	85,57	882,33	52,13	1,067	**108,17**	27,17	7,33	7,85
	13	28,73	68,90	1312,33	35,10	0,167	**182,23**	34,77	7,23	7,78
	14	37,30	96,07	887,33	36,37	0,633	**190,47**	81,77	6,81	7,43
	15	9,73	42,87	136,13	56,10	2,300	33,73	7,43	7,05	7,41
	16	6,67	22,67	66,60	36,80	2,400	27,33	11,10	7,73	8,1
	17	23,37	184,67	271,33	**171,17**	2,800	**59,03**	25,37	7,53	8,04
Coastal part - North	18	81,93	174,33	430,00	**120,13**	2,200	**61,50**	14,07	7,33	7,79
	19	52,27	126,17	424,00	76,43	1,333	46,73	1,27	7,06	7,54
	20	36,53	84,47	878,00	33,97	0,967	**213,20**	24,17	7,08	7,62
	21	44,03	150,07	739,67	**109,37**	1,200	**188,33**	26,67	6,84	7,19
	22	48,50	174,00	661,00	**216,33**	2,533	**171,00**	53,63	7,21	7,48
	23	26,30	69,83	961,00	49,73	1,400	**91,70**	73,07	7,08	7,85
	24	30,20	83,50	884,00	52,10	2,567	**111,70**	72,77	7,14	7,83
	25	9,07	30,90	89,40	34,17	**3,467**	**54,67**	14,27	7,6	7,71
	26	30,40	104,27	946,67	55,03	2,667	**88,07**	93,33	7,15	7,85
	27	37,87	120,83	1108,67	64,10	**4,833**	**99,10**	**115,83**	6,99	7,75
	28	15,43	51,60	187,50	39,60	**3,700**	**52,27**	15,50	7,12	7,61
	29	27,27	75,20	847,67	44,50	1,567	**87,80**	42,57	7,28	7,95
	30	33,77	86,70	362,67	**195,00**	2,733	**88,23**	30,70	7,46	7,63
	31	31,57	95,90	349,33	**113,43**	2,733	**100,90**	33,63	7,35	7,62
	32	17,07	64,80	535,67	49,77	**3,700**	**76,17**	29,87	7,29	7,79
	33	35,37	112,73	990,00	55,67	**5,533**	**92,40**	**145,33**	7,02	7,57
	34	26,87	88,67	1038,00	50,07	**3,967**	**75,10**	**160,42**	6,99	7,71

Coastal/Littoral part - South	35	27,77	85,60	1090,67	54,63	**3,400**	**85,87**	**148,30**	7,02	7,57
	36	26,97	96,07	619,00	59,57	**7,619**	**75,70**	38,77	6,85	7,36
	37	33,97	115,57	561,67	88,77	1,667	**170,17**	36,50	7,09	7,66
	38	43,40	64,73	689,33	25,23	0,800	**61,67**	6,73	6,88	7,21
	39	24,43	116,60	332,40	**100,40**	1,633	48,63	8,30	6,9	7,27
	40	16,47	106,10	308,43	73,97	**3,067**	**52,93**	10,43	7,37	7,64
	41	19,50	58,10	756,67	22,00	1,200	44,97	7,83	7,2	8,26
	42	24,83	61,33	545,00	13,77	0,567	33,63	75,27	6,15	7,88
	43	13,90	47,43	482,33	27,77	1,467	46,80	10,10	7,23	7,72
	44	26,17	173,33	488,33	**126,40**	**3,500**	**54,70**	14,67	6,95	7,42
	45	14,80	30,33	245,77	33,10	2,800	**88,67**	14,33	7,3	7,76
	46	25,63	141,17	618,33	59,77	**4,267**	**67,37**	15,67	7,2	7,66
	47	26,97	76,63	704,67	86,10	1,367	**219,47**	47,27	7,14	7,83
	48	28,60	69,47	717,33	48,00	1,167	**212,20**	36,27	7,16	7,63
	49	24,57	100,07	341,33	**103,63**	2,833	**93,53**	24,03	7,11	7,52
	50	20,37	71,33	361,00	83,27	1,800	**88,93**	27,30	7,16	7,56
	51	16,77	46,07	519,00	35,77	1,533	**119,20**	41,83	7,21	7,64
	52	29,73	81,10	770,33	37,73	2,733	**182,83**	**136,37**	7,12	7,67
	53	19,47	69,37	467,00	41,47	2,433	**130,87**	43,93	7,04	7,46
	54	43,07	72,10	937,33	33,53	1,000	**438,00**	**184,21**	6,79	7,28
	55	11,07	35,30	472,33	15,63	0,833	**278,90**	58,57	8,11	8,44
	MAC	100,00	300,00		100,00	3,000	50,00	100,00		

The valley of Zeta river west of Podgorica was probed with 9 collection sites, from suburbs of Podgorica, Vranjske njive (site L-3, 42.470°N, 19.251°E), along the left bank of the river Zeta spring, up to the town of Danilovgrad (L-8, 42.525°N, 19.133°E), and along the right Zeta's bank to L-11 (42.469°N, 19.189°E). The soil at all localities, except L-9 appeared to be brown on clays, loams or sand, lessive or calcareous, skeletal with gravels, and shallow to middle shallow. Pure populations of *Ae. triaristata* in different stages of maturity, were predominant. The localities showed higher content of nickel. *Ae. ovata* was collected from two sites L-5 (42.532°N, 19.181°E), and L-9 (42.532°N, 19.094°E). At L-9, red, non-skeletal, weak calcareous soil with developed vegetation expressed higher content not only of nickel, but also of cadmium, and within MAC limits, but the highest content of Zn of all Zeta valley examined sites. *Ae. kotschy* was found at one site, L-8 (42.519°N, 19.122°E), only (fig. 1). That locality had Ni content that was above MAC upper limit, but the lowest of all Zeta valley sites, as well as, lowest Cr content. Most of the examined sites were within pH neutral limits, but the two of them inclined to acidity (L-7 and L-9 with pH_{KCl}= 6.59 and pH_{KCl}= 6.57, respectively). Alkaline soil reaction sites were L-4 reaching pH_{KCl}= 7.22 or pH_{H_2O}= 7.79, and L-10 with pH_{KCl}= 7.59 or pH_{H_2O}= 8.26 (tab. 1).

The southern inland area lied within the limits of a triangular territory between three cities Podgorica, Cetinje and Virpazar (fig. 1). At these 6 localities a predominant were mixed populations of *Ae. ovata* and *Ae. triaristata*. Pure populations of *Ae. ovata* were collected at two sites L-16, and L-17. Generally, it could be noted that popularions of *Ae. ovata* appeared remarkable more frequently south of Podgorica (latitude 42.438), approaching littoral/coastal area. The first three localities were with red browned skeletal soil on limestone with gravels, quite shallow. The last three localities were characterized by light to dark brown skeletal calcareous soil with gravels. The most of the examined sites in that area expressed the high content of nickel, higher than MAC. The exceptions are two localities, L-15 and L-16 that were considerably distant from the highway motorways. The locality L-17 by the highway Podgorica-Cetinje had the lead content higher than MAC, as well (fig. 1). At that site the highest zinc and cadmium content, among all six sites in that area was found (184.67mg/kg and 2.800mg/kg, respectively), but under MAC value (300.00mg/kg and 3.000mg/kg, respectively), tab. 1.

The coastal and the littoral part of Southern Adriatic in Montenegro were divided in Northern and Southern part. Northern part was studied through 19 probing sites, territorially divided again in three subareas. At the localities of the first area covering west bank of Boka Kotorska Bay from village Bijela (42.451°N, 18.651°E) to village Njivice (42.448°N, 18.503°E), by the border to Croatia, coded L-18 to L-23, *Ae. ovata*, *Ae. triaristata* and *Ae. kotschy* were collected. In that area *Ae. kotchy* was observed in a large population for the first time. Two types of *Ae. kotchy* were denoted, one with white, and the other with red awns. *Ae. ovata* was collected at one site L-20 (42.438°N, 18.602°E), only, being in mixed population with *Ae. triaristata* (fig. 1). The high content of lead and nickel was found in soil samples. On average the soil at the goat grass collection sites could be classified as skeletal terra rossa or calcocambisol, with numerous scattered peaces of limestone, presumable due to closeness of busy road and a number of waste depots in the area. According to pH_{KCl}= 7.33, L-18 was the only one showing a weak alkaline reaction, all the other probing sites were within the limits of neutral reaction (pH_{KCl} between 6.84 and 7.21), tab. 1. The second subarea lies between collection sites L-24 to L-29, covering Luštica peninsula, from village Radovići (42,398°N, 18.670°E) to the village Begovići L-29 (42.384°N, 18.608°E). The general sampling problem in that subarea was undeveloped road network, and very shallow, stony terrain in the lower parts of Peninsula. Tivat to town Budva crossroad (42.378°N, 18.749°E). Luštica peninsula shelters large populations of *Aegilops sp*., holding almost all biodiversity of goat grass denoted through out Montenegro. Populations of *Ae. ovata*, *Ae. triaristata*, *Ae. biuncialis* and *Ae. kotschy* are partly pure, consisting of one species and partly mixed. The peninsula has been largely environmentally intact till the beginning of the previous decade, but during the last half of the decade a remarkable erosion of biodiversity took place due to population and construction. The soil is skeletal terra rossa, non-calcareous to low calcareous with hihger level of nickel, or/and cadmium, possibly because of the closely settled Tivat airport. The locality L-27 (42.379°N, 18.609°E), by the road on the upper ridge of the peninsula, had three elements above MAC namely, cadmium (4.833mg/kg), nickel (99.10mg/kg), chromium (115.83mg/kg), as well as the highest manganese content recorded in that area (1108.67mg/kg). L-27 was covered by a large mixed goat grass population consisting of *Ae. ovata*, *Ae. triaristata* and *Ae. biuncialis* (tab. 1, fig. 1). Most of the probing sites were within the limits of neutral or very weak alkaline reaction (pH_{KCl} between 6.99 and 7.60), tab. 1. Area on Bigovo peninsula was arbitrary marked as the third subarea of the Northern part of total screened coastal and littoral zone. Bigovo was probed at seven collecting sites. From the L-30 (42.378°N, 18.749°E) crossroad on the road from town Tivat to town Budva to L-36 (42.316°N, 18.769°E) near the village Kubasi. The most of the examined sites harbored mixed populations consisting of *Ae. ovata*, Ae. *triaristata* and *Ae. biuncialis* or *Ae. ovata* and *Ae. triaristata*. All the localities were on red brownest soil (terra rossa), low calcareous, calcocambisol on hard limestone and quite shallow, except L-30 that was on alluvial calcareous loamy deep soil. Cuprum and zinc varied within MAC permitted values. The manganese content varied from 362.67mg/kg (L-30) to 1038mg/kg (L-34). Lead exceeded MAC at two locations L-30 (195.00mg/kg), and L-31 (113.43mg/kg), near a busy touristic route from Budva to Tivat. Cadmium was above MAC limits at all the localities except the first two, while nickel was high above MAC allowed limit at all probed saites (fig. 1, tab. 1). The first three localities in the subarea showed a weak alkaline soil reaction (pH_{KCl} between 7.29 and 7.46). At the other four localities soil pH_{KCl} reaction was neutral (tab. 1).

The coastal/littoral area South was arbitrary divided in three subareas. The first subarea covers the direction from town Bar to town Virpazar. Seven probing sites represent that subarea, going from L-37 (42.097°N, 19.109°E) to L-43 just before the village Boljevići (42.224°N, 19.092°E), fig. 1. Four Aegilops species were collected on the probing sites: *Ae. ovata*, *Ae. triaristata*, *Ae. biuncialis* and *Ae. Kotsch* in mixed populations, mostly. *Ae. ovata* was populating lower, and higher altitude, but at higher altitudes (L-40, L-41) that species could be found in pure populations only. *Ae. kotschy* and *Ae. biuncialis* were found near to the coastal area, while *Ae. triaristata* was collected at lower altitudes on the both side of the Rumija mountain that separates Bar and Virpazar. The soil where plant samples were gathered was light to deep brown, calcareous to low calcareous, partly skeletal and partly rich with organic matter. At the higher altitude the localities were on brownish forest soil on rocky limestone. On the Virpazar side (L-43) terra rossa rocky and eroded was the predominant type. Generally, localities closer to Bar town had higher content of heavy metals due to the fact that Bar is well known South Adriatic harbour with the industrial zone in the neighborhood. Lead content was higher than MAC at one locality (L-39), while nickel level was higher than MAC at L-37, L-38, and L40. Cadmium was above the MAC limit at L-40. The soil samples exhibited neutral pH values at the most, except L-42 (pH_{KCl}= 6.15) having weak acid reaction in according to pH determined in KCl, and L-40 having slight alkaline reaction (pH_{KCl}= 7.37), tab. 1. Second area covers six probing sites on the both sides of Rumija Mountain. Three localities are toward the Skadar lake (L-44 to L-46), and three are at the other side toward the coast in a region called Možura (L-47 to L-49), fig. 1. The populations of *Ae. ovata* were predominant and in mixed populations with *Ae. triaristata*, mostly. At the locality L-44 (42.222°N, 19.115°E) *Ae. kotshy* was collected, as well as, *Ae. biuncialis* at L-47 (42.018°N, 19.313°E). High content of nickel (from 54.70mg/kg at L-44 to 219.47mg/kg at L-47), higher than MAC, was denoted at all the probing sites in that area. Cadmium appeared to be quite high, higher than MAC at L-44 (3.500mg/kg) and L-46 (4.267mg/kg) at the localities to the north of the Rumija Mountain, toward the Skadar Lake, were the soil was terra rossa eroded and rocky. The soil type of the three probing sites that remains was brown soil on eocenic flysh, mostly. The content of all the other examined elements varied but under the MAC border values (fig. 1, tab. 3). According to pH measured in H_2O, the soil reaction was weakly alkaline (pH from 7.42 at L-44 to 7.83 at L-47), while pH measured in KCl showed neutral reaction (pH from 6.95 at L-44 to 7.2 at L-46) with the exception of L-45 having weak alkaline reaction (pH 7.3), tab. 3, fig. 2.

The third subarea at the south was between the town of Ulcinj and Albanian border. Six probing sites were screened and tested at that area, from L-50 (41.939°N, 19.257°E) to L-55 (41.877°N, 19.365°E), fig. 1. *Ae. ovata* was collected at all localities being in mixed populations, mostly. *Ae. triaristata* grew at localities north of the town of Ulcinj, while *Ae. biuncialis* appeared in mixed populations as we were moving towards the south, reaching southernmost point L-55. The first two localities were on brown skeletal soil on eocenic flysh, while L-52, and L-53 were on terra rossa (red soil) humic and very rocky. L-54 was on brown reddish humic skeletal soil, and L-55 on calcareous sand. Nickel appeared to be above MAC at all examined localities in the area. L-54 (41.938°N, 19.338°E) exhibited the highest content of manganese (937.33mg/kg) and had chromium content (184.21mg/kg) above MAC, as well as, L-

52 (41.947°N, 19.324°E) with chromium content of 136.37mg/kg. At the most localities soil reaction was neutral according to pH measured in KCl (pH_{KCl} from 6.79 to 7.21). The only exception was L-55 showing moderate alkaline reaction (pH_{KCl}= 7.37, and pH_{H2O}= 8.44), tab. 1.

Statistical analisys. According to univariate statistical parameters a notable variation in heavy metal and manganese concentration between 55 examined probing sites could be observed. Overall mean of 113.25mg/kg that exceded MAC showed nickel polution, in general, at the studied area. Maximum mean values of lead, cadmium, nickel and chromium (216.33mg/kg, 7.619mg/kg, 438.00mg/kg and 184.21mg/kg, respectively) exciding MAC are signs about the existence of sites having heavy metal concentration in soli higher than allowed (detailed view in table 1). Those heavy metals varied more than cuprum and zinc, particularly chromium having coefficient of variation of 91.28% (tab. 2).

Table 2. Univariate statistics for elements (mg/kg) examined at 55 probing sites in South Adriatic. Values that are above MAC (Maximum Allowed Content) are bolded

Statistical parameters	Cu	Zn	Mn	Pb	Cd	Ni	Cr
Mean	28,06	89,38	668,46	64,22	2,21	**113,25**	45,92
Standard Error	1,68	5,01	50,42	5,62	0,18	9,68	5,65
Minimum	6,67	22,67	66,6	13,77	0,167	27,33	1,27
Maximum	81,93	184,67	1892,67	**216,33**	**7,619**	**438,00**	**184,21**
Range	75,260	162,00	1826,07	202,56	7,452	410,67	182,94
Standard Deviation	12,48	37,18	373,93	41,71	1,36	71,77	41,91
Variance	155,90	1382,50	139824,50	1739,50	1,80	5151,40	1756,70
Coefficient of variation (%)	44,50	41,60	55,94	64,94	61,67	63,37	91,28
MAC	100,00	300,00		100,00	3,000	50,00	100,00

Further analysis of observed heavy metal and manganese variation was conducted using one-way ANOVA. For easier handling, 55 probing sites were divided into three areas according to geographical position. Namely, “Inland part” covered localities deeper in the continent separated from the sea by the mountain wreath. That area was arbitrary divided in two subareas, roughly one subarea North-West of Podgorica (the Capital of Montenegro), and the second subarea to the south of the town. Second area covered “Coastal part- North), geographically divided into three subareas, and the third area “Coastal/Littoral part South” was arbitrary divided into three subareas (tab. 1, fig. 1). The ANOVA results showed that area appeared to be the source of variation for cuprum and cadmium. The rest of heavy metals and manganese showed no significant variation due to rough geographical position. However, at the level of subareas, significant differences were observed not only for cuprum and cadmium, but also in case of nickel and chromium (tab. 3).

Interrelationship between heavy metals, manganese and soil reaction estimated by pH_{KCl} and pH_{H2O} values revealed that cuprum was positively correlated with zinc, manganese and lead content, as well as, negatively with pH soil value. Highly significant positive correlations were observed for zinc and lead content, chromium with nickel and manganese and nickel and manganese content in soil samples. Manganese was significantly negative correlated with pH values, while a highly significant positive relationship between pH values obtained in KCl and H_2O was expected (tab. 4).

Table 3. Summarized results of one-way ANOVA for 3 areas and 8 subareas arbitrary assigned at South Adriatic for heavy metal and manganese examination of 55 probing sites

Sources of variation	Element	df	SS	MS	F	$F_{tab\ 0.05}$
Area	Cu	2	924,880	462,440	*3,21	
	Zn	2	3053,154	1526,577	1,11	
	Mn	2	530321,520	265160,760	1,96	
	Pb	2	5264,6160	2632,308	1,54	3,18
	Cd	2	22,392	11,196	*7,51	
	Ni	2	8649,233	4324,617	0,83	
	Cr	2	5982,786	2991,393	1,75	
Subarea	Cu	7	3006,097	429,442	*3,73	
	Zn	7	16140,275	2305,754	1,85	
	Mn	7	887083,503	126726,215	0,89	
	Pb	7	75768,498	2594,666	1,61	2,20
	Cd	7	49,676	7,097	*6,65	
	Ni	7	83145,136	11877,88	*2,86	
	Cr	7	28771,750	4110,250	*2,92	

Table 4. Pearson correlations among elements in soil samples of the studied area at 55 localities

	Cu	Zn	Mn	Pb	Cd	Ni	Cr	pH_{KCl}	pH_{H2O}
Cu	1,000	**0,637	*0,331	**0,391	-0,074	0,226	0,186	*-0,298	*-0,323
Zn		1,000	0,118	**0,710	0,214	-0,064	0,016	-0,174	-0,247
Mn			1,000	-0,201	-0,121	*0,331	**0,435	**-0,420	*-0,277
Pb				1,000	0,180	-0,083	-0,147	0,128	-0.159
Cd					1,000	-0,360	0,208	-0,026	-0.119
Ni						1,000	**0,435	0,041	-0.038
Cr							1,000	-0,248	-0.016
pH_{KCl}								1,000	**0,656
pH_{H2O}									1,000

$t_{0,05(53)}$=0,273
$t_{0,01(53)}$=0,354

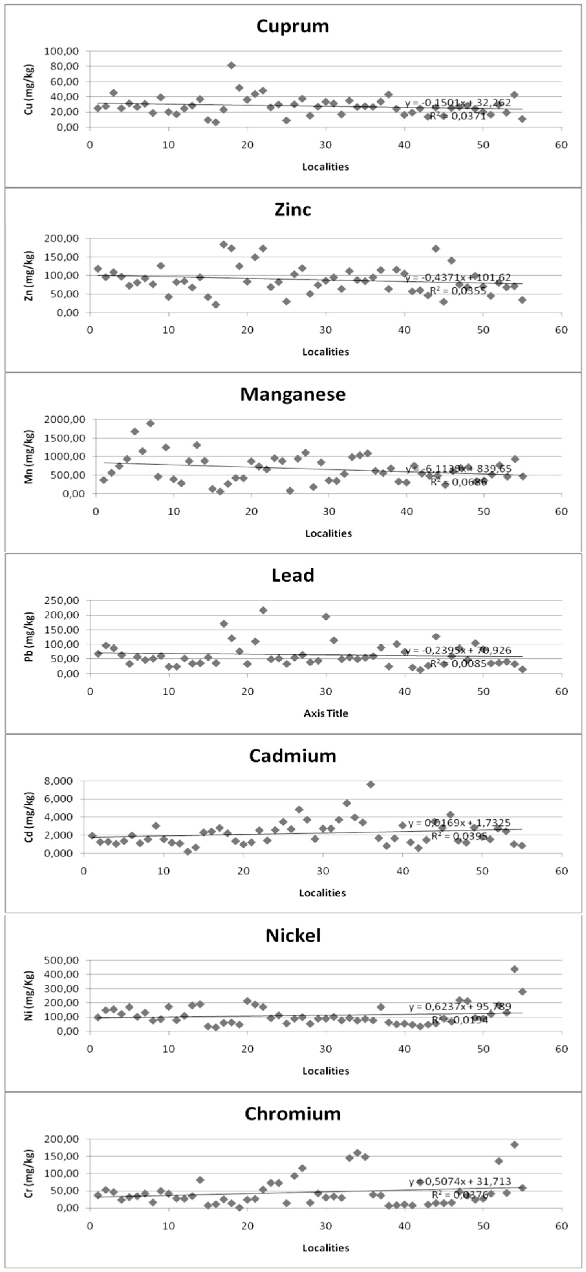

Figure 4. The array and a trenline of heavy metal content and manganese in soil at 55 plant habitat localities of South Adriatic

According to the array and a trenline of heavy metal content and manganese it could be observed that a content of cuprum, zinc, manganese and lead decreased from inland via northern coastal to coastal and littoral area to the south. That was quite opposite to trends calculated for cadmium, nickel and chromium. Variation around the trend line reveals a higher deviation of cuprum content in soil samples from localities L-18 and L-19, otherwise the rest of the localities was grouped along the trend line. Zinc content deviated more at the localities on Luštica Penninsula and localities by the Skadar Lake. Manganese content varied more in inland area and generally the deviation from the trend line grew smaller going south. Lead content deviation was higher at some localities of the northern coastal area, highly depending of the locality position in respect to a highway network. Cadmium and nickel followed trend line quite close, except some localities on Luštica and Bigovo peninsulas for cadmium content in soil, and localities at the southern localities near to Albanian border for nickel content. Chromium showed greater deviation from a trend line at several localities at Bigovo peninsula, and localities at a very Albanian border to the south (fig. 1, tab. 1, fig. 4).

Summarizing to the results it could be observed that a different levels of pollution were observed, generally, at the localities samples were taken from. At 52.7% (29 in absolute values) of the localities at least one of heavy metal in study exceeded MAC, at 27.3% (15) two heavy metals exhibited content in soil higher than MAC, and the highest pollution level according to the criteria oh the number of heavy metals being above allowed content, was observed in 5 cases where three heavy metals exceeded MAC (9.1%). The samples from L17 to L40 showed that in Coastal part – North area the localities having two or three elements with the content in excess were predominated. Coastal/littoral part to the south showed somewhat better results, while inland part, particularly north-west from Podgorica had one element in soil above MAC (tab. 1, fig. 5).

Figure 5.

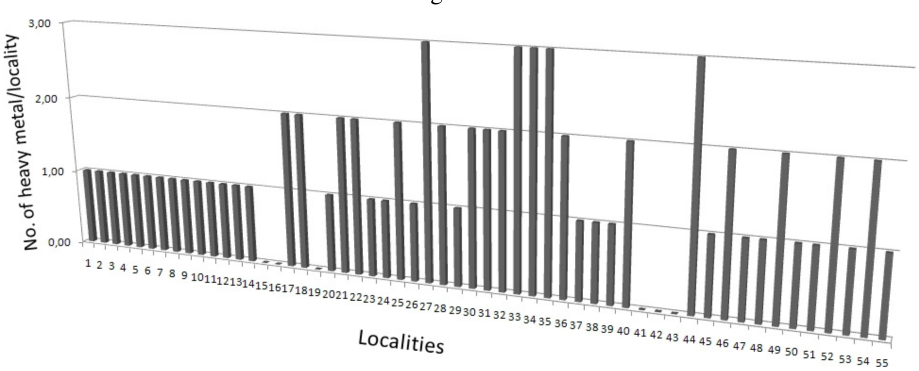

Figure 5. The number of heavy metals per probing site that exceede Maximum Allowed Content at 55 plant habitat localities in South Adriatic area

CONCLUSION

The developed agriculture in a flat area of Vojvodina (Northern part of Serbia, the south part of Pannonia) could lead to heavy metal content enhancement in soil. A part of long term research conducted at South Adriatic was to collect plant samples in order of studying genetic variability among wild relatives of *Aegilops* genus usable in cereal breeding programs for improving tolerance to abiotic stress caused by heavy metals, or to select genotypes suitable for bioremediation of the affected soil. The vast area of a South Adriatic was screened and investigated taking into account inland, littoral and a coastal part. Heavy metal content in soil varied between probing sites, as well as, between examined areas. Generally, northern areas of the examined territory had higher content of cuprum, zinc, lead, as well as, microelement manganese. Southern areas had a higher content of cadmium, nickel, chromium, as a trend. For a comparison MAC (Maximum allowed Content for heavy metals in soil as an official document of the Republic of Serbia) was used. The nickel content appeared to be above the allowed level at the 87.3% of probing sites, cadmium at 20%, lead at 16.4% and chromium at 10.9%. At their maximum those elements were highly above the MAC values – lead 216%, cadmium 254%, nickel 876% and chromium 184%. Though, cuprum and zinc did not exceed allowed border, the maximum content reached 82% and 62%, respectively. Results direct to a conclusion that heavy metal pollution was denoted in most of the probing sites (89.1%), while only 10.9% or 6 localities showed no heavy metal content above allowed values. Though, the aim of the work at this level was not to establish the sources of pollution, it seems that anthropogenic factor is predominant – traffic, waste or agricultural pollution. Values of pH measured in KCl showed neutral reaction, mostly, while corresponding pH measured in H_2O showed neutral to weak alkaline reaction. The soil was brown or red skeletal and shallow soil, predominantly calcareous. Four species of *Aegilops* genus were collected at those localities – *Ae. ovata*, *Ae. triaristata*, *Ae. kotschy* and *Ae. biuncialis*. *Ae. ovata* and *Ae. triaristata* showed more plasticity spreading through the examined area, while *Ae. kotchy* and *Ae. biuncialis* were scateringly presented. In a part of biodiversity preservation it could be concluded that the changes in *Aegilops* population structure in Montenegro area ought to be monitored periodically, since *A. cilindrica* has been found at one locality in one year, only, and *A. kotschy* has been spreading in a past six years. Moreover, in the coastal area some localities have been destroyed by constructions, so changes occure.

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REFERENCES

AGHAEI, M.J., J. MOZAFARI, A.R. TALEEI, M.R .NAGHAVI, M.OMIDI (2008): Distribution and diversity of *Aegilops tauschii* in Iran. Genet. Res. and Crop Evol., *55*, 341-349.

BÁLINT, A.F., M.S. RÖDER, R ,HELL, G.GALIBA and A. BÖRNER (2007): Mapping of QTLs affecting copper tolerance and the Cu, Fe, Mn and Zn contents in the shoots of wheat seedlings. Biol. Plant., *51* (1): 129-134

BRINNKMANN, W.L.F., W.PLASS (1984): The spatial distribution of heavy metals in the soils of the Steinbach basin-Rhine-Main area. In: Proceed. Int. Symp. on Recent Investigations in the Zone of Aeration, Munich, October, 1984, *1*, 57–68

CHHUNEJA, P., H.S. DHALIWAL, N.S, BAINS, K.SINGH (2006): *Aegilops kotschy* and *Aegilops tauschii* as sources for higher levels of grain Iron and Zinc. Plant breeding, *125*, 529-531.

CI, D., D. JIANG, B. WOLLENWEBER, T. DAI, Q.,JING, W. CAO (2010): Genetic Variance in Cadmium Tolerance and Accumulation in Wheat Materials Differing in Ploidy and Genome at Seedling Stage. J. Agr. & Crop Sci., *196*: 302–310

ČAPKA, D., I. KISIĆ, .Z, ZGORELEC, M, MESIĆ. A. JURIŠIĆ (2009): Determination of Soil pH in Dominant Soil Types in the Republic of Croatia. Agric. Consp. Sci., *74*, 1, 13-19.

DIMITRIJEVIĆ, M., S. PETROVIĆ, C. CÎMPEANU, D.BUCUR, and M. BELIĆ (2011): Cereals and *Aegilops* genus biodiversity survey in the west Balkans: Erosion and preservation. J. of Food, Agric. & Env., *9* (3&4): 219-225.

DIMITRIJEVIĆ, M., S.PETROVIĆ (2004): Biodiversity Problems – The Importance of Genetic Variability Preservation and Revival. International Eco- Conference Safe food, Novi Sad, Proceedings, 193-198.

FLORA, S.J.S. (2009): Metal Poisoning: Threat and Management. Al Ameen J. Med. Sci., *2* (2), Special : 4 - 2 6

HODGKIN, T., Y.J. ADHAM, K. S. POWELL (2008): A preliminary survey of wild Triticum and Aegilops species in the worl’s genebank. Hereditas, *1*, 116, 155-162.

KIMBER, G., and M.FELDMAN (1987): Wild wheat. An introduction. Special report 353. College of Agriculture, Univ. of Missouri, Columbia.

LANDJEVA, S., M. MERAKCHIJSKA-NIKOLOVA, G. GANEVA (2003): Copper toxicity tolerance in *Aegilops* and *Haynaldia* seedlings. Biol. Plant., *46* (3), 479-480.

LEONOVA, T. G. and N. N. CHIKIDA (2011): Assessment of Salt Tolerance of Aegilops L. from the VIR Collection. Russ. Agric. Sci., *37*, 4: 283–285.

LIAO, G.L., LIAO, D.X., Q.M. LI (2008): Heavy metals contamination characteristics in soil of different mining activity zones. Transactions of Nonferrous Metals Society of China, *18*, 1, 207-211

LOSKUTOV, I.G. (2004): Using of wild species genetic diversity in plant breeding. Proceedings of the 4th International Crop Science Congress, Brisbane, Australia, 26. Sep. – 01. Oct., 3,*3*, 1, 967.

MAXTED, N., K. WHITE, J. VALKOUN, J. KONOPKA, and S. HARGREAVES (2008): Towards a conservation strategy for *Aegilops* species. Plant Gen. Res., Characterization and Utilization, *6* (2), 126-141.

MOLNÁR, I., S. DULAI, M.MOLNÁR-LÁNG (2008): Can the drought tolerance traits of *Ae. biuncialis* manifest even in the wheat genetic background? Acta Biol. Szeg., *52* (1):175-178

MONNEVEUX, P., M, ZAHARIEVA, D. REKIKA, C. ROYO, M. M. NACHIT, N. D. FONZO, J. L. ARAUS (2000): The utilisation of *Triticum* and *Aegilops* species for the improvement of durum wheat. Options Mediterraneennes. Serie A, Seminaires Mediterraneens, 71-81.

NENOVA, V., M. MERAKCHIYSKA, G. GANEVA, E. ZOZIKOVA, and S. LANDJEVA (2009): Physiological Responses of Wheat (*Triticum Aestivum* L.) – *Aegilops Sharonensis* Introgression Lines to Excess Copper. J. Agr. & Crop Sci., *195*, 197–203

PALANIAPPAN, M., K. SHANUMUGAM, and S. PONNUSAMY (2002): Soil degradation due to heavy metal accumulation uder long term fertilization. 17th World Congress of Soil Science. 14-21. August, Thailand, Symp. 46, 333-337.

PETROVIĆ, S., M. DIMITRIJEVIĆ (2005): Biodiversity of *Aegilops* Genera in Montenegro. Savremena poljoprivreda [*Contemporary Agriculture*], 3-4, 460-463.

PETROVIĆ, S. and M. DIMITRIJEVIĆ (2012): Genetic erosion of diversity of cereals. Genetika, , *44,2*, 217 - 226

RAWAT, N., V.K. TIWARI, N. SINGH, G.S. RANDHAWA, K. SINGH, P. CHHUNEJA, H. S. DHALIWAL (2009): Evaluation and utilization of *Aegilops* and wild *Triticum* species for enhancing iron and zinc content in wheat. Gen. Res. and Crop Evol., *56*: 53–64

ROY, S.J., TUCKER, J. ELISE and M TESTER. (2011): Genetic analysis of abiotic stress tolerance in crops. Current Opinion in Plant Biology, 14, 232–239

ZAHARIEVA, M., E.,GAULIN, M, HAVAUX, E. ACEVEDO and P.MONNEVEUX (2001): Drought and Heat Response in Wild Wheat Relative *Aegilops geniculata* Roth. Crop Sci., *41*, 1321-1329.

ZAHARIEVA, M., A. DIMOV, P. STANKOVA, J. DAVID, P.MONNEVEUX (2003): Morphological diversity and potential interest for wheat improvement of three *Aegilops* L. species from Bulgaria. Gen. Res. and Crop Evol., *50*, 507-517.

VARIJACIJA VRSTA RODA *Aegilops* I SADRŽAJ TEŠKIH METALA U ZEMLJIŠTU BILJNIH STANIŠTA NA LOKALITETIMA JUŽNOG JADRANA

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Izvod

Rod *Aegilops* je spontani, divlji srodnik hlebne pšenice, čiji hromozomi pokazuju homologiju sa hromozomima pšenice. Ovaj rod može da bude izvor gena koji su korisni za proširenje poželjne genetičke varijabilnosti u cilju poboljšanja tolerantnosti na abiotički stres. Stvaranje ovakve genetičke varijabilnosti, kojom se odgovara izazovima globalnih klimatskih promena, kao i eroziji životne sredine, sve više dobija na značaju. Teški metali u zemljištu izazivaju njegovo zagađenje, dovode do fenotipskih promena biljaka i ulaze u lanac ishrane. Cilj rada je da se ustanovi sastav populacija i varijacija vrsta roda *Aegilops*, kao i sadržaj teških metala u zemljištu. U sklopu istraživanja, ispitano je 55 lokaliteta u obalnom i priobalnom području Južnog Jadrana. Na ovim lokalitetima je evidentiran sastav populacija roda *Aegilops* i uzimani su uzorci zemljišta na njihovim staništima. U uzorcima zemljišta je ustanovljen sadržaj teških metala Cr, Pb, Zn, Ni, Cd i Cu, sadržaj mangana (Mn) i pH vrednost.

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