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EFFECTS OF SOME HEAVY METALS IN SOME MORPHO-PHYSIOLOGICAL PARAMETERS IN MAIZE SEEDLINGS

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ABSTRACT

Nowadays the heavy-metal pollution of the soil is causing ever greater problems, exacerbated by the fact that the heavy metals accumulated in plants may, either directly or indirectly, find their way into animals and human beings. Maize is one of the world's most important crops, ranking third after wheat and rice, so the changes induced by one of the most toxic heavy metals. The experiment design was randomly with repetition. Prepared seeds are placed on the germinator for germination which was added 10 mL H_2O. Cultivation lasted 15-20 days at temperature 25°C in vegetative room. During the experiment in growth period are prepared the concentrations of heavy metals (600 mL/1 Kg compost); for lead (Pb^{2+}): 200 μM (T1), 400 μM (T2) and 400 μM (T3); for cadmium (Cd^{2+}): 60 μM (T4), 120 μM (T5) and 180 μM (T6); for Mercury (Hg^{2+}): 33 μM (T7), 66 μM (T8) dhe 100 μM (T9). Elements concentrations of heavy metals (HM's) solutions include Pb, Cd and Hg, were examined in all (except control) treatments, because their intoxication was at higher doses compare to control and a part of them of residual od HM's in the substrate was transport in different parts of plants. The plants of maize populations for leaf area was characterized on higher values 40.48 cm^2 $plant^{-1}$ or expressed in percentage the variation was 82.01%. The exposure of maize seedlings to Pb^{2+}, Cd^{2+} and Hg^{2+} resulted in a reduction of chlorophyll and carotenoids content in leaves compare to control. From our research with different treatments with various genotype and heavy metals the differences in the content of chlorophyll effects and carotenoides was different and significantly higher at level of probability of LSD p = 0.01.

Keywords: Maize, Cadmium, Lead, Mercury, Photosynthetic Pigment

1. INTRODUCTION

Heavy Metals (HM's) make a significant contribution to environmental pollution as a result of human activities such as mining, smelting, power transmission, intensive agriculture (Nedelkoska and Doran, 2000) or heavy metal contamination affects the biosphere in many places worldwide (Meagher, 2000). HM's can affect plant growth and production in a multiple way by inhibiting a number of physiological processes in plants. They were shown to cause disturbance in plant ion (Wallace *et al*., 1992; Barcelo *et al*., 1986) and water balance to interfere with protein metabolism through influencing nitrate and sulphate reduction (Nussbaum *et al*., 1988; Hernandez *et al*., 1997). A major environmental concern due to dispersal of urban and industrial waste generated by human activities is the contamination of soil with heavy metals. Polluted soil poses a severe problem to both health and land problem (Akhionbare *et al*., 2010). The discharge of heavy metals as a by product of various human activities has been accompanied by large scale soil pollution (Shivhare and Sharma, 2012).They present a risk for primary and secondary consumers and ultimately humans (Zeller and Feller, 1999). Among toxic metals, Lead (Pb) and Cadmium (Cd) appear to be the most dangerous to the environment

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(Malkowski *et al*., 2005). Pb^{2+} and Cd^{2+}, are growth inhibition, ion uptake and transport disturbances, enzyme activation or inhibition photosynthesis (Fargasova, 2001; Geebelen *et al*., 2002). Mercury (Hg) poisoning has become a problem of current interest as a result of environmental pollution on a global scale. The availability of soil Hg to plants is low and there is a tendency for Hg accumulation in the roots. Indicating that roots serve as a barrier to Hg uptake (Tripathi and Tripathi, 1999). Cereals in this case maize are known to be good accumulators of contaminants (Malgorzata and Andzej, 2005). The mechanisms of heavy metal toxicity on photosynthesis is still a matter of speculations, this may be partly due to the differences in experimental design, but some evidence points to the involvement of electron transport in lights reactions (Giardi *et al*., 1997). The lack of cultivable and productive land has been attributed to soil contamination from high rate of heavy metals (USEPA, 1997). Land contamination/degradation is a threat to sustainable agricultural development and food security in developing countries (Adejumo *et al*., 2011). Many researchers have investigated the uptake and accumulation of Lead (Pb) and Cadmium (Cd) in different plant species. However, the mechanism of accumulation of heavy metals is still not completely understood (Malkowski *et al*., 2005). Pb and Cd are the most widespread no nutrient heavy metals (Mihailovic, 2010). Also, here no exception as Kosovo, through the thermal power plant in Obiliq, ferronickel Factory, or foundry in Zvecan, through released very large amount of heavy metals, which not only contaminate the land, but have negative effects on the growth and development of plants during vegetation. The aim of the experiments was to evaluate the effects of some heavy metals in maize seedlings for different physiological traits.

2. MATERIALS AND METHODS

2.1. Plant Material and Growth Conditions

The plant material which was include in our study were four different maize (*Zea mays* L.) genotypes; Local maize population (LMP's) originating from Kosova, while Bodrog (H-4), MV277 (H-6) and Miranda (H-12) were from Agricultural Institute in Martonvasar-Hungary. The seeds were disinfected with $HgCl_2$ 0.01% for 20 sec and 70% ethanol for 5 min, to avoid fungal contamination and then rinsed three times with distilled water and after were sterilized. Maize seeds were germinated on moistened filter paper. Prepared seeds are placed on the germinator for germination which was added 10 mL H_2O for ten days in temperature 25°C. During these periods are prepared compost (minimum 1 kg/replicates) pots for cultivars and each treatment. In totally are 9 pots for heavy metals include $PbCl_2$, $CdCl_2$, $HgCl_2$ and 1 control. The maize seedlings were transferred to compost in 1 kg weight of pots in controlled environment cabinets or controlled rooms with a 12 photoperiod and temperature 25/19°C day/night and 75% relative humidity. The compost consisted of pH ($CaCl_2$) = 5.8; salt concentration (g L^{-1} KCl = 0.9; Nitrogen (NH_4+NO_3) mg L^{-1} $CaCl_2$ = 120; Phosphorus (P_2O_5) mg L^{-1} CAL=150 and potassium (K_2O) mg L^{-1} CAL = 200. During the experiment in growth period are prepared the concentrations of heavy metals (600 mL/1 Kg compost); For lead (Pb^{2+}): 200 μM (T1), 400 μM (T2) and 400 μM (T3); for cadmium (Cd^{2+}): 60 μM (T4), 120 μM (T5) dhe 180 μM (T6); for Mercury (Hg^{2+}): 33 μM (T7), 66 μM (T8) dhe 100 μM (T9). These concentration of heavy metals corresponding with concentration of substrate in mg kg^{-1}: T1 – Pb^{2+}, 25 mg kg^{-1}; T2-Pb^{2+}, 50 mg kg^{-1}; T3 - Pb^{2+}, 25 mg kg^{-1}; T4 - Cd^{2+}, 4 mg kg^{-1}; T5 - Cd^{2+}, 8 mg kg^{-1}; T6 - Cd^{2+}, 12 mg kg^{-1}; T7 - Hg^{2+}, 4 mg kg^{-1}; T8 - Hg^{2+}, 8 mg kg^{-1}; T9 - Hg^{2+}, 12 mg kg^{-1}. Pigments were extracted by grinding 60-80 mg freshly sampled leaves in 80% (v/v) acetone/water containing $MgCO_3$ (0.5%w/v) at room temperature for 24 h in the dark. Photosynthetic pigments of all the samples were extracted in triplicate to minimize experimental errors. Concentration of chlorophyll and carotenoid contents were measured by using absorbance recorded at 662, 644 and 440 nm for maximum absorption of chlorophyll 'a' (*Chl a*), chlorophyll 'b' (*Chl b*) and Carotenoids, respectively. The extinction coefficients were determined by a UV-Vis spectrophotometer (SECOMAM, Anthelie Advanced 5). Pigment contents were calculated in mg g^{-1} Fresh leaf Weight (FW) by applying the absorption coefficient equations described by Lichtenthaler (1986):

$$Chl\ a\ (mg\ g^{-1}\ FW) = [9.784\ (OD662)-0.99\ (OD644)]\times V/FW$$

$$Chl\ b\ (mg\ g^{-1}\ FW) = [21.426\ (OD644)-4.65\ (OD662)]\times V/FW$$

$$Carotenoids\ (mg\ g^{-1}\ FW) = [4.695\ (OD440)-0.268\ (Chl\ a + Chl\ b)]\times V/FW$$

Where:
FW = Fresh leaf weight
OD = Optical Density
V = Volume of sample

After 15 days of exposure, the following parameters were determined in different part of plants include; Heavy Metal Contents (HMC), Leaf Area (LA), chlorophylls a, b and carotenoids concentrations. All analysis for determination of HM's including: Pb^{2+}, Cd^{2+} and Hg^{2+} was done by Spectrophotometer of Atomic Absorber (SAA) Thermo Elemental M, by U.S. EPA Method 245.5 Cold Vapor Atomic Absorption Spectroscopy.

LA was determined according to the formula: A = L×W×0.75, where L is the leaf length, W is the leaf width, 0.75 is the factor of recalculation for maize. The same formula has been used by Aliu *et al*. (2010).

2.2. Statistical Analyses

The experiment was performed in a randomized design with five (5) replicates. Differences among the Pb^{2+}, Cd^{2+}, Hg^{2+} and other morpho-physiological parameters were tested using MINITAB-14, statistical program. Mean separation within columns are done by Duncan's Multiple Range test.

3. RESULTS

The soil content with Pb, Cd and Hg are shown in **Table 1**. The Pb^{2+}, Cd^{2+} and Hg concentrations were significantly higher in the treatments T3. Elements concentrations of HM's solutions include Pb^{2+}, Cd^{2+} and Hg^{2+}, were examined in all (except control) treatments, because their intoxication was at higher doses compare to control and a part of them of residual HM's in the substrate was transport in different parts of plants. The differences for the concentration of Pb^{2+} in the substrate and content of plant were highly significant between genotypes. Higher content was found in the third treatment T3 (even in the substrate in plants) with the average values of 64.54 mg kg^{-1} (H-4) and 101.32 mg kg^{-1} (LMP's). While with very low absorption values are determined for the substrate at H-6 (26.31 mg kg^{-1}) and in plant content with lead was H-12 (9.23 mg kg^{-1}. The differences for the content of Pb in the substrate compared to the control were very high significant at all genotypes, while compare the differences between the two extreme values between genotypes were +39.23 mg kg^{-1} or with variation on 86.37% and the content of heavy metals in plants was higher significant differences, these differences were extreme values +92.09 mg kg^{-1} or with variation of 166.61%. Result are Presented in **Table 1**. Also the significant differences were found between treatments for HM's include Cd^{2+} and Hg^{2+} at all genotypes that were in the research. The presence of cadmium in the soil substrate was with different concentrations. A higher value of Cd is determined to T3 in the genotype H-6 (34.38 mg kg^{-1}) and compared with the control value (0.28) differences were 34.1 mg kg^{-1}. And in plant residues for the content of Cd^{2+}, the higher value is found to T3 in the genotype H-12 with average values of 35.31 mg kg^{-1}. The higher presence of Hg in soil substrate was determined in T3 respectively at H-6 genotypes on average value 109.72 mg kg^{-1}, relatively higher concentration. Also the content of Hg in plant residues was evident with a presence of 83.43 mg kg^{-1}. Results presented in **Table 1**. In our results for LA, the experimental average values μ were 22.24 cm^2 plants $^{-1}$. The plants of LMP's for LA was characterized on higher values 40.48 cm^2 $plant^{-1}$. Compare to experimental value μ the differences were significantly higher +18.24 cm^2 $plant^{-1}$ or expressed in percentage the variation was 82.01%, presented in **Fig. 1**.

The exposure of maize seedlings to Pb^{2+}, Cd^{2+} and Hg^{2+} resulted in a reduction of chlorophyll and carotene content in leaves compare to control. From our research with different treatments with various genotype and heavy metals the differences in the content of chlorophyll effects and Carotenoides was different and significantly higher at level of probability of LSD p = 0.01. In plants of LMP's the effect with a high reduction in the content of chlorophyll *a* was found to treatment of third-content of Cd (0.696) and for the content of chlorophyll b, a + b and carotenoides effect was the content of lead in the third treatment but not excluding Cd and Hg.

Table 1. Contents of heavy metals (mg kg^{-1} soil) in soil substrates and maize seedlings

Treatment	Soil Content with HM's				Plants content with HM's			
	LMP's	H4	H-6	H-12	LMP's	H-4	H-6	H-12
Control	-4.97	-6.55	-5.70	-6.09	0.51	6.67	5.02	5.73
T1/mg Pb	6.60	6.60	1.05	10.15	13.40	20.27	10.58	7.94
T2/mg Pb	11.21	23.16	21.49	38.24	15.24	23.61	23.17	7.66
T3/mg Pb	49.52	64.54	26.31	43.85	101.32	43.64	35.83	9.23
Control	-0.71	0.07	0.28	1.12	-0.40	0.05	0.29	0.89
T1/mg Cd	4.92	8.22	4.55	5.85	10.30	14.39	7.81	20.84
T2/mg Cd	10.86	11.22	6.12	8.60	15.92	23.33	10.82	23.53
T3/mg Cd	12.01	15.44	34.38	14.52	19.09	27.13	27.65	35.31
Control	1.23	2.69	2.52	1.99	2.14	2.85	2.68	2.05
T1/mg Hg	5.38	4.37	36.51	5.79	9.17	27.10	22.33	16.04
T2/mg Hg	9.30	4.98	37.85	40.10	9.31	35.59	26.71	22.56
T3/mg Hg	11.89	6.41	109.72	44.28	9.75	83.43	32.95	37.24

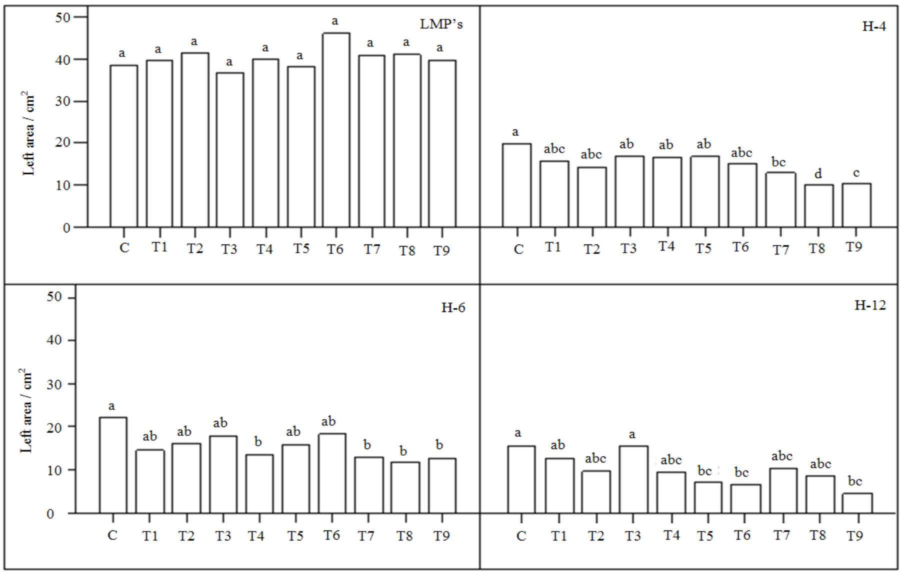

Fig. 1. The effects of different concentration of lead, cadmium and mercury on leaf area in maize seedlings

Table 2. The effects of different concentration of lead (Pb^{2+}), cadmium (Cd^{2+}) and mercury (Hg^{2+}) on photosynthetic pigment contents of different maize seedlings

	LMP's				H4				H6				H12			
	Chl a	Chl b	Total Chl	Carot.	Chl a	Chl b	Total Chl	Carot.	Chl a	Chl b	Total Chl	Carot.	Chl a	Chl b	Total Chl	Carot.
C	0.905^{a}	0.201^{b}	1.104^{a}	0.331^{a}	0.891^{a}	0.123^{a}	1.013^{a}	0.360^{a}	0.974^{a}	0.259^{a}	1.232^{a}	0.347^{a}	0.782^{a}	0.143^{a}	0.925^{a}	0.615^{a}
T1	0.529^{d}	0.085^{d}	0.614^{e}	0.212^{de}	0.865^{ab}	0.105^{abc}	0.988^{ab}	0.328^{abc}	0.634^{b}	0.147^{f}	0.781^{de}	0.235^{d}	0.749^{a}	0.132^{a}	0.882^{ab}	0.269^{ab}
T2	0.802^{abc}	0.138^{a}	0.940^{f}	0.198^{e}	0.836^{abc}	0.102^{abcd}	0.938^{abcd}	0.340^{ab}	0.892^{a}	0.203^{cd}	1.095^{abc}	0.328^{abc}	0.759^{a}	0.156^{a}	0.915^{ab}	0.279^{ab}
T3	0.761^{abc}	0.160^{bc}	0.921^{f}	0.225^{cde}	0.855^{abc}	0.098^{abcd}	0.953^{abc}	0.345^{ab}	0.745^{ab}	0.198^{cde}	1.060^{abc}	0.312^{abc}	0.853^{a}	0.175^{a}	1.027^{a}	0.308^{ab}
T4	0.870^{ab}	0.201^{b}	1.07^{ab}	0.325^{a}	0.764^{abc}	0.074^{bcd}	0.838^{bcd}	0.316^{abc}	0.822^{ab}	0.187^{de}	1.009^{bc}	0.305^{abc}	0.743^{a}	0.140^{a}	0.883^{ab}	0.267^{ab}
T5	0.775^{abc}	0.166^{bc}	0.940^{cd}	0.278^{abc}	0.724^{c}	0.057^{d}	0.781^{d}	0.289^{c}	0.779^{ab}	0.173^{ef}	0.952^{cd}	0.292^{bc}	0.784^{a}	0.167^{a}	0.951^{ab}	0.291^{ab}
T6	0.696^{c}	0.132^{cd}	0.829^{d}	0.258^{bcde}	0.821^{abc}	0.116^{ab}	0.925^{abcd}	0.342^{ab}	0.803^{ab}	0.194^{cde}	0.996^{a}	0.296^{abc}	0.702^{a}	0.159^{a}	0.861^{ab}	0.259^{ab}
T7	0.795^{abc}	0.164^{bc}	0.960^{bc}	0.293^{ab}	0.844^{abc}	0.071^{bcd}	0.915^{abcd}	0.329^{abc}	0.617^{b}	0.146^{f}	0.763^{e}	0.227^{d}	1.651^{a}	0.355^{a}	1.029^{a}	0.289^{ab}
T8	0.731^{bc}	0.149^{bc}	0.880^{cd}	0.273^{abcd}	0.798^{abc}	0.069^{cd}	0.867^{abcd}	0.319^{abc}	0.803^{ab}	0.237^{ab}	1.040^{bc}	0.276^{cd}	0.654^{a}	0.145^{a}	0.799^{ab}	0.241^{ab}
T9	0.812^{abc}	0.163^{bc}	0.975^{bc}	0.298^{ab}	0.735^{bc}	0.06^{cd}	0.795^{cd}	0.301^{bc}	0.973^{a}	0.219^{bc}	1.193^{ab}	0.342^{ab}	0.721^{a}	0.154^{a}	0.874^{ab}	0.264^{ab}
X	0.7676	0.156	0.9233	0.2691	0.814	0.0875	0.9013	0.3269	0.8042	0.196	1.0121	0.296	0.839	0.172	1.011	0.309
SE±	0.0300	0.010	0.040	0.010	0.010	0.007	0.0200	0.0060	0.0300	0.010	0.040	0.010	0.090	0.020	0.110	0.030
CV	13.510	21.67	14.67	17.01	7.080	27.42	8.8200	14.090	15.05	18.40	15.11	14.09	34.52	37.88	34.86	35.31

LMP's – Local Maize Population (Kosovo); H4, H6, H12-genotypes from Agricultural Institute in Martonvasar-Hungary; X-Mean of column; SE -Standard Error (±); CV-Coefficient of Variance; C-Control; T1-Pb^{2+}, 25 mg kg^{-1}; T2 – Pb^{2+}, 50 mg kg^{-1}; T3 - Pb^{2+}, 25 mg kg^{-1}; T4 - Cd^{2+}, 4 mg kg^{-1}; T5 - Cd^{2+}, 8 mg kg^{-1}; T6 - Cd^{2+}, 12 mg kg^{-1}; T7 - Hg^{2+}, 4 mg kg^{-1}; T8 - Hg^{2+}, 8 mg kg^{-1}; T9 - Hg^{2+}, 12 mg kg^{-1}. Mean in each column followed by same letters are not significantly different at the P 0.05 by one-way ANOVA with Duncan's multiple range tests

Table 3. The Correlation coefficients between traits in maize seedlings

		LMP's				H-4				H-6				H-12			
R	Controll	Chl-*a*	Chl-*b*	Chl-a+b	Carot	Chl-*a*	Chl-*b*	Chl-a+b	Carot	Chl-*a*	Chl-*b*	Chl-a+b	Carot	Chl-*a*	Chl-*b*	Chl-a+b	Carot
Cont	1.000	0.11	0.166	0.127	0.441	-0.528	-0.728(*)	-0.608	-0.653(*)	0.161	0.474	0.154	-0.041	-0.043	0.082	-0.021	-0.043
Chl-*a*	0.110	1.000	0.941(**)	0.997(**)	0.664(*)	-0.458	-0.459	-0.476	-0.536	0.629	0.417	0.595	0.639(*)	0.126	0.128	0.126	0.151
Chl-*b*	0.166	0.941(**)	1.000	0.966(**)	0.804(**)	-0.508	-0.513	-0.530	-0.585	0.491	0.324	0.479	0.515	0.121	0.11	0.119	0.143
Chl-*a*+*b*	0.127	0.997(**)	0.966(**)	1.000	0.706(*)	-0.474	-0.479	-0.494	-0.554	0.600	0.399	0.571	0.614	0.13	0.129	0.129	0.154
Carot	0.441	0.664(*)	0.804(**)	0.706(*)	1.000	-0.506	-0.51	-0.528	-0.613	0.355	0.251	0.260	0.222	0.148	0.132	0.145	0.172
Chl-*a*	-0.528	-0.458	-0.508	-0.474	-0.506	1.000	0.842(**)	0.987(**)	0.919(**)	-0.406	-0.367	-0.351	-0.325	0.191	0.172	0.189	0.186
Chl-*b*	-0.728(*)	-0.459	-0.513	-0.479	-0.510	0.842(**)	1.000	0.918(**)	0.894(**)	-0.131	-0.271	-0.132	-0.045	-0.206	-0.279	-0.219	-0.206
Chl-*a*+*b*	-0.608	-0.476	-0.53	-0.494	-0.528	0.987(**)	0.918(**)	1.000	0.944(**)	-0.339	-0.353	-0.299	-0.253	0.078	0.042	0.072	0.074
Carot	-0.653(*)	-0.536	-0.585	-0.554	-0.613	0.919(**)	0.894(**)	0.944(**)	1.000	-0.400	-0.372	-0.335	-0.303	0.062	-0.008	0.050	0.047
Chl-*a*	0.161	0.629	0.491	0.600	0.355	-0.406	-0.131	-0.339	-0.4	1.000	0.836(**)	0.953(**)	0.936(**)	-0.548	-0.522	-0.545	-0.519
Chl-*b*	0.474	0.417	0.324	0.399	0.251	-0.367	-0.271	-0.353	-0.372	0.836(**)	1.000	0.906(**)	0.751(*)	-0.537	-0.460	-0.526	-0.520
Chl-*a*+*b*	0.154	0.595	0.479	0.571	0.260	-0.351	-0.132	-0.299	-0.335	0.953(**)	0.906(**)	1.000	0.957(**)	-0.559	-0.521	-0.555	-0.537
Carot	-0.041	0.639(*)	0.515	0.614	0.222	-0.325	-0.045	-0.253	-0.303	0.936(**)	0.751(*)	0.957(**)	1.000	-0.546	-0.529	-0.545	-0.525
Chl-*a*	-0.043	0.126	0.121	0.13	0.148	0.191	-0.206	0.078	0.062	-0.548	-0.537	-0.559	-0.546	1.000	0.981(**)	0.999(**)	0.999(**)
Chl-*b*	0.082	0.128	0.110	0.129	0.132	0.172	-0.279	0.042	-0.008	-0.522	-0.46	-0.521	-0.529	0.981(**)	1.000	0.987(**)	0.981(**)
Chl-*a*+*b*	-0.021	0.126	0.119	0.129	0.145	0.189	-0.219	0.072	0.05	-0.545	-0.526	-0.555	-0.545	0.999(**)	0.987(**)	1.000	0.998(**)
Carot	-0.043	0.151	0.143	0.154	0.172	0.186	-0.206	0.074	0.047	-0.519	-0.520	-0.537	-0.525	0.999(**)	0.981(**)	0.998(**)	1.000

**; Correlation is significant at the 0.01 level

*; Correlation is significant at the 0.05 level

While the other maize genotypes H-4 and H-6 the high effect in the reduction of physiological contents is determined by cadmium and mercury. Maize genotypes H-12 is very in our study are interesting that of all the treatments for the content of chlorophyll a and b was not found any significant difference, but for the content of chlorophyll a + b and carotenoides differences were more emphatic for probability level LSD p = 0.05. Results are presented in **Table 2**. Study of correlation coefficients between evaluated characters shows different values between the traits which was included in the research. In most cases the average control value was with strong correlative values significantly higher as chlorophyll a, b and total chlorophyll (a + b), while on carotenoid traits values the correlative values were lower and in some cases were non-significant differences as negative correlations. Results are presented in **Table 3**.

4. DISCUSSION

In the present study, exposure to HM's affected different traits of maize: leaf area and chlorophyll content include Chlorophyll 'a' (*Chl a*), chlorophyll 'b' (*Chl b*) and Carotenoids. Leaf is very important photo-synthetic part, which through the green pigment chlorophyll allows that light can transform kinetic energy into potential energy. The Leaf Area (LA) plays an important role in the accumulation of organic materials (Aliu *et al*., 2010). In our study the presence of metals includes Pb, Cd and Hg at hybrid genotypes had a significant impact on reduction of LA in relation to the control and treatment concentration. This hypothesis gives us some preliminary information that local maize populations that are selected and adapted to the agro ecological conditions for centuries from our farmers, are more tolerant to environmental stress, in our case with heavy metals. Our results showed that the presence of heavy metals to plants of LMP's for LA, compared to control (38.48 cm^2 $plant^{-1}$) has caused a physiological stress, which was manifested by an increase in leafy area to the three treatment at all elements of heavy metals which was include in our results. While at hybrid genotypes this phenomenon is not manifested as LMP's with increase of LA. These results shows that with the increase of LA automatically can have also increased of organic matter per unit area. From the results of Godzik (1993) for maximum Pb^{2+} content is found in senescing leaves and least in young leaves. From the literature we learn that in most cases the presence of heavy metals causes stress and inhibits or slows the growth processes of plants, including and maize (Stiborova *et al*., 1987; Rascio *et al*., 1993).The exposure of maize seedlings to Pb^{2+}, Cd^{2+} and Hg^{2+} resulted in a reduction of chlorophyll and carotene content in leaves compare to control. The different results for total chlorophyll content were presented by Amujoyegbe *et al*. (2007) on values from 1.73 till 2.11. Also, by Jain *et al*. (2007) who investigated the effect of Cd^{+2} in different concentrations for content of maize Chlorophyll, it is concluded by them that the level of concentration 0.5 µM the chlorophyll content was higher. The Total chlorophyll content of primary leaves decreased with increasing mercury concentration, it decreased 24.9-29.2% under treatments with 0.02, 0.04 and 0.06 mM $HgCl_2$ (Zengin and Munzuroglu, 2005). The results by Ghani (2010) who investigated the effect of lead in different concentrations for Chlorophyll 'a' (*Chl a*), 'b' (*Chl b*), his results were with higher variation and depending on the increase of the concentration, for control the Chlorophyll'a' content was 0.54, while with Pb^{+2} concentration the content of *Chl a* was 0.386. Results presented by Burzynski (1987), show that effect of Pb^{2+} inhibits chlorophyll synthesis by causing impaired uptake of essential elements such as Mg and Fe by plants. Also, some results by Antosiewicz (1992) showed that the lead content tends to decrease plant organs, but this order can vary with plant species. Some plants can tolerate even concentrations of different metals without visual symptoms of toxicity (Grejtovsky *et al*., 2008; Seregin and Kozhevnikova, 2008). Results reported by Saderi and Zarinkamar (2012) for concentration of two HM's (Pb and Cd) who emphases that increased concentration to 180 µM, which shoot length was reduced by 79.30 and 83%. It is also found that the influence of Cd^{+2} can affect reducing of coleoptiles, the roots of which associated with clorosis (Nocito *et al*., 2006). Also, some results were reported by Malecka *et al*. (2012) for different treatments of lead concentration resulted on higher significance with increasing of level lead concentration. Contents of HM's in plant material not always exerted direct proportionality with content of HM's in soil (Tomas *et al*., 2012). Metal hyper accumulating species have been identified in at least 45 plant families and individual species can accumulate different metals (Mohammad *et al*., 2012).

5. CONCLUSION

The results of our investigation substantiate the following conclusions: The effect of different of concentration of heavy metals in local maize populations for leaf area has not shown any significant differences effects because these genotypes seems more are adapted to environmental stress in this case to Pb, Cd and Hg. But, for other traits the differences were significantly higher. While in other maize genotype hybrids the effects with heavy metals was with significant differences for level of probability LSD p = 0.01. The chlorophyll content in the treatment of control was the highest and significantly, while the lowest of chlorophyll content value at local maize populations in most cases is found in the third treatment with cadmium, while hybrids was not significant differences in mercury concentrations. This hypothesis gives us some preliminary information that local maize populations that are selected and adapted to the agro ecological conditions for centuries from our farmers, are more tolerant to environmental stress, in our case with heavy metals.

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