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RESEARCHES ON THE INFLUENCE OF THE ATMOSPHERIC POLLUTANTS IN SATU MARE AREA ON CERTAIN PHYSIOLOGICAL PROCESSES IN *ZEA MAYS*

DOCTORAL THESIS
Abstract

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Contents

The doctoral thesis includes 420 pages; it is structured into 6 chapters, 40 tables, 233 figures and 470 references

Key words: environmental quality, heavy metals, water status, respiration, mineral nutrition

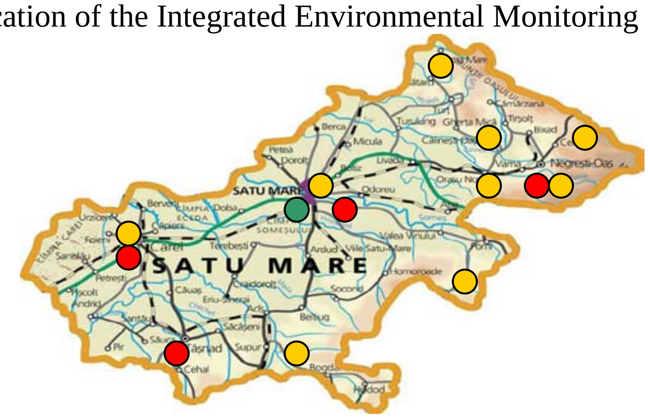
THE AIM AND THE OBJECTIVES OF THE RESEARCH

The influence of different pollutants released by means of air in the surrounding environment – the heavy metals: lead, zinc and copper – on one of the main agricultural cultures in our country, maize has been selected, as subject of research with the aim to improve the knowledge ont his subject.. The general research objectives are as follows:

- To determine some physiological parameters for a general evaluation of the heavy metals' influence on the physiological processes of maize;
- To assess the manner in which the physiological parameters proposed for analysis reflect the heavy metals influence;
- To use the obtained results to monitorise the environmental quality.

ASPECTS RELATED TO THE ENVIRONMENTAL STATUS IN SATU MARE COUNTY

The monitoring of the environmental quality in Romania is achieved through the application of the Integrated Environmental Monitoring System.



Gaseous pollutants analysis
Settling powders
Precipitations

Figure 1. The network for monitoring the air quality in Satu Mare County (www.apmsm.ro)

Toxic substances dispersed in the atmosphere and diluted in precipitation cause damages especially to assimilatory tissues of plants. They impair the protective layer on leaves and penetrate into the tissues from which they leach Mg contained in chlorophylls, and other biogenic elements, primarily K, Ca and P (Taiz et al., 2002). The air quality monitoring (Figure 1) provided by Satu Mare Environment Protection Agency underlines that the average concentrations of heavy metals in the rainfalls water were, in year 2007, as can be seen Table 1.

*Table 1.*Average concentration of Cu, Zn, Pb, Mn, Cr, Cd in the rainfalls water in Satu Mare County (2007) (www.apmsm.ro)

Sampling location	Cu µg/l	Zn µg/l	Pb µg/l	Mn µg/l	Cr µg/l	Cd µg/l
Supur	0.192	10.06	0.470	0.476	0.11	0.088
Livada	0.132	16.846	0.501	0.813	0.14	0.152
Călineşti	0.113	8.379	0.494	0.501	0.097	0.067
Berveni	0.548	14.432	0.707	0.536	0.181	0.072
Huta	0.122	12.058	0.571	0.378	0.102	0.082
Negreşti	0.098	9.87	0.548	0.422	0.119	0.061
Tarna	0.181	13.248	0.520	4.264	0.17	0.088
ValeaVinului	0.5	13.226	0.510	0.596	0.241	0.083
pH	5.54-7.65	5.54-7.65	5.54-7.65	5.54-7.65	5.54-7.65	5.54-7.65

The effects of pollutants over the vegetation

Heavy metals are found in the atmosphere primarily as aerosols - systems consisting of fine solid or liquid particles, below 100 microns, dispersed in the air. Air pollution with heavy metals is due to geochemical sources (natural emissions of particles and gases from volcanic eruptions, fires) but especially to anthropogenic sources: iron ore mining industry, metallurgy, electronics, chemical industries, transports, use of herbicids, combustion processes, etc. (Alloway, 1990, Ross, 1994). A third source of atmospheric pollution with heavy metals is the biotic environment, through vegetation, waste disposal, dejections (Table 2).

Table 2. Total evaluation of the quantities of heavy metals spread in the atmosphere as a result of the entropic action as compared to the natural sources (according to Thorsteinssin, 1989, quoted by Ross, 1994*)*

Emission	Heavy Metals (x 10^3 tone)			
	Cd	Cu	Ni	Zn
Natural	0.83	18	26	44
Entropic	316	2160	1000	14.000

The increase of toxic effects on the plants' metabolism is subject to atmospheric deposits on ground, respectively on vegetation, beyond the tolerance limits of plants. Bathory (2003), when studying the accumulation of heavy metals transferred by air to forest vegetation, concluded that it depends on the species and tolerance degree of the plant, on the age of the vegetative body, on the type of metal: heavy metals are taken up in the order: Pb> Zn> Cu.

The leaves' capacity to uptake metals from the air is different. It depends on the air humidity (high humidity promotes leaf absorption), on the type of metal: Zn and Cu are taken up faster in the leaves than Pb which is more adsorbed on the surface of leaves (Little and Martin, quoted by Greger et al, 2004), on the pH (this factor is very important for the penetrations through humid means - Greger, 2004), on the moderate oxidation state of the environmental compartments (Ernst, 1998).

Ross (1994) indicates that heavy metal toxicity transferred in plants by air depends on: the levels (quantity) of the metal in the environment, the vector - the exposure type (ingestion, absorption through the roots after metals deposit from the atmosphere on the ground), the distribution of dose / exposure time, type and severity of the effect, the time required for the effect to manifest. Ochiai (1987), quoted by Ross (1994) specifies the mechanisms by which heavy metals manifest their toxicity:

- blocking the functional groups of molecules with important biological role: enzymes, polynucleotide or the nutrients conveying systems;
- substitution of essential metal ions in bio-molecules or other functional cell units;
- distortion and inactivation of bio-molecules, especially the enzymes;
- destroying the cell membranes integrity (through direct effect on the sulphydryl groups of the membrane constituents and by direct or indirect induction of membrane lipids over-oxidation through free toxic radicals and cell-organite – the oxidative stress (Vangronsveld and Clijsters, 1994, quoted by Onac, 2005).

These mechanisms of action through which heavy metals manifest their toxicity are possible due to the ability of the metal ions to strongly bond with the atoms of oxygen, nitrogen, sulphur, which can be found in large quantity in the biological systems and which can serve as binders for the ions of all essential metals (Ross, 1994).

The effects of heavy metal toxicity are manifested at cellular level through: the modification of plasma membrane permeability, changes in ultrastructure of the organites, influencing the metabolic processes from cytosol. At physiological level, the effects of heavy metal toxicity is manifested through their influence on plants nutrition - plants growth, (Dobrota, 1999, Onac 2005), reduction of the intensity of photosynthesis and transpiration increase of the respiration, disruption of the fluid system of the plants - one of the first and most important effects that heavy metals have on plants' metabolism, this being the main cause of disturbance of other physiological and metabolic processes.

Material and Methods

The field experiment

Field experiments were conducted at Livada Research and Development Station, Northern Romania. The study took place over a 2-yr period (2006 and 2007). Sixteen experimental plots, each of 21 m^2, were installed and submitted to different treatments. All types of plots were fertilized with 300 g NPK 1:1:1 (300 kg × ha^{-1}) and 200 g NH_4NO_3 (200 kg × ha^{-1}). All the plots were surrounded by a border of 1m. Maize was sown directly into permanent raised beds at a distance of 60cm between rows and 35-40 cm between plants (Mereuta and Dobrota, 2008).

The average multiannual temperature recorded in Livada Station in the latest 40 years was of 9.5^{0}C (Figure 2), the variation amplitude being of 4.2^{0}C. The sum of the effective temperatures (T>10°C) in the interval April – October has a multiannual average value of 1380°C, which corresponds to the thermal requirements of the early and semi-early maize hybrids.

The average amount of annual precipitations over the last 47 years was 728 mm with an uneven distribution during the growing season and average annual oscillations between 374 mm (2004) and 1581.9 mm (1966). During the active growing season (IV-VII) about 305 mm rainfalls are registered, with oscillations between 161 mm (1972) and 569 mm (1965), with a gradual downward trend on decades (Figure 3). The volume of rainfalls is below the potential vapour-transpiration.

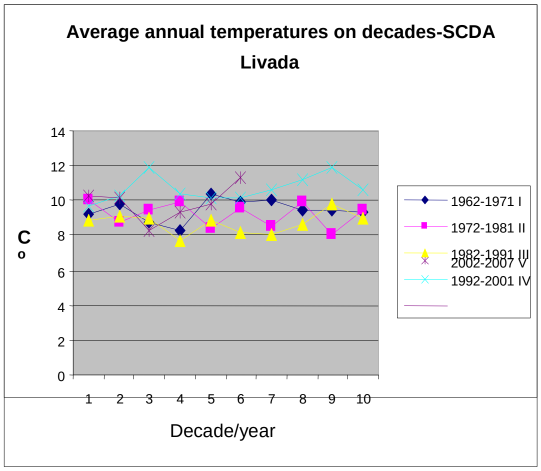


Figure 2. Air annual average temperature (Livada, 1962 - 2007)

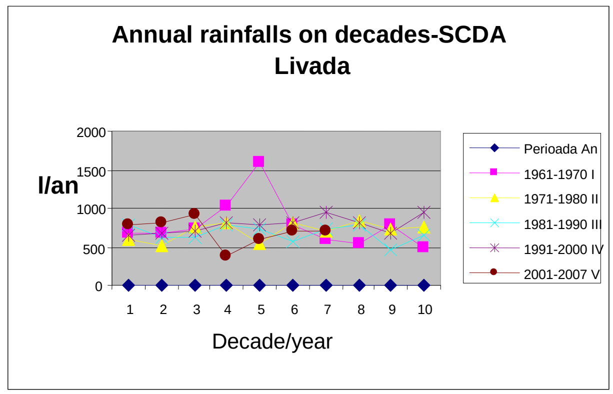


Figure 3. Annual rainfalls on decades (Livada, 1962 - 2007)

Vegetal Material

Two varieties of maize (Zea *mays L)* –Ribera (Pioneer group) and Turda 200, indigenous hybrid were selected for the experiments considering that the heavy metals uptake by maize and their accumulation in plant, in the supra-terrain organs, especially in the leaves but also in the seeds – used as food animals and / or people – represent one of the means of bio-accumulation and contamination of the human population with heavy metals (Kabata-Pendias A, 2000, Cumpătă, Beceanu D, 2007).

The study of the physiologic effects induced by the heavy metals Pb, Cu, Zn transferred through air over the maize plants was performed in the period 2006-2007. The land preparation was realized in accordance with the requirements of agricultural technology, through deep autumn ploughing and spring discing and levelling. The experiments were located using the arrangement method: the Latin rectangle, in three variants and a control variant, for each hybrid, respectively type of heavy metal evaluated, with four repetitions for each.

The two sorts of maize were seeded with a SPC 6 seeding machine, with a 60 cm distance between rows. The seeding was made towards the end of May of every year, when the soil temperature was of minimum 10^0C. Three manual hoeing were performed, the distance between the plants on row being in the end of approximately 30 – 40 cm. throughout the growing period, the weeds were removed through weeding.

The solutions containing heavy metals – Pb (lead acetate), Cu (copper sulphate), Zn (zinc sulphate) in concentrations of 0,2 x10^4 ppm Cu, 0,2 x10^4 ppm Zn and 0,32 x10^4 ppm Pb; 2 x10^4 ppm Cu, 2 x10^4 ppm Zn and 3,2 x10^4ppm Pb and 4 x10^4 ppm Cu, 4 x10^4 ppm Zn and 6,4 x10^4 ppm Pb were applied 10 days after germination. The climatic characteristics have been recorded for the entire period of the experiment.

The analysis and comments on physiological processes were performed on leaves harvested in 4-6 leaves phase and on 8-10 leaves phase. The harvesting and preparation of plant samples for analysis were performed as recommended in "Working Methodologies of Soil and Agrochemical Research Institute"

Methods

1. *Determining the influence of air pollutants - Cu, Zn, Pb - on the Zea mays water status* was made by:
   • Measuring the water uptake intensity by determining the total humidity in the leaves
   • Measuring the transpiration intensity using a porometer.
2. *Evaluating the influence of the air pollutants - Cu, Zn, Pb - on the Zea mays mineral nutrition* was made by:
   • *Determining the dry weight content* –the analyzed vegetal mass (leaves) was found after the sample was dried at 105^0C for 4 hours.
   • *Determining the total nitrogen*: the total nitrogen determination was made using the Kjeldahl method.
   The *determination of total phosphorusus* was done by colorimetric determination with ammonium molybdate solution.
   • *Determination of potassium*: was done through flam photometric determination.

RESULTS AND DISCUSSIONS

Experimental design was a randomized complete block with four replications. Annual data for each parameter over the whole 2-yr period were subjected to analysis of variance (ANOVA), the limits of differences, the correlations' analysis –the correlation coefficient (r), the mathematical regression and the curve response. The interpretation of the significance of the differences, was performed using the limit differences DL 5%, DL 1%, DL 0,5%.

Analysis of the influence of copper, zinc, on the water regime by determining the total moisture in the leaves of maize

Although it has a low-specific consumption - only 300 units of water are required to achieve a unit of dry substance - maize has high requirements concerning humidity, rewarding the water surplus from rainfalls or irrigations with increased productions. It is not affected by the drought occurred during the first phases of vegetation when the requirements of humidity are low; the highest water consumption (i.e. 500% of the total consumption during the vegetation season) is recorded in the first 1 - 2 weeks from the male inflorescence emergence and the milk – wax maturity.

ANOVA test showed the strong correlation of other factors as well (environmental factors), along with the heavy metals' effect on plants, manifested through the variation of the total humidity (F calculated = 2.101697-Turda 200, F calculated = 1.95146-Ribera, F critical = 2.466266); the synthetic results for the 2 years of study are presented in Table 3 and Table 4.

In ***Turda 200*** hybrid (Table 3, Figure 5.6) the effect of heavy metals Cu, Zn, Pb on total humidity of the leaves is manifested through its decrease with the increasing concentration of copper, zinc, respectively lead applied, from the lowest concentration to the average concentration of the metal, but the overall humidity is maintained at values higher than those of the control in the variants in which the lowest concentration of copper and lead were applied. At the highest concentrations, the metals Cu, Zn, Pb induce a different effect on the total moisture of the leaves: zinc and lead bring about the further reducing of the leaves' humidity, stronger in the case of zinc than of lead, while at its highest concentration, copper brings about a slight increase of total moisture concentration of maize leaves.

*Table 3.*Influence of Cu, Zn, Pb on total humidity of the leaves of maize (cv. **Turda 200)**

Variant	Total humidity, (g water/kg leaf) Multiannual averages			Difference as to the control	% as to the control	Significance	r	R^2	Equation
	2006	2007	general average						
control	85.85	77.58	81.71	0	100				
V_1: .2x10^4ppmCu	84.60	83.21	83.90	2.19	102.678	**			
V_2: 2x10^4ppmCu	84.47	74.78	79.62	-2.09	97.442	o			$y=82.9 -3.05x +0.76x^2$
V_3: 4x10^4ppmCu	85.64	80.24	82.94	1.22	101.497	-	-0.085	0.614	
V_4:0.2x10^4ppmZn	86.15	75.95	81.05	-0.67	99.185	-			
V_5: 2x10^4ppmZn	84.80	73.04	78.92	-2.80	96.577	oo			$y=81.1 -0.99x -0.16x^2$
V_6: 4x10^4ppmZn	84.05	65.80	74.93	-6.79	91.693	ooo	-0.993	0.996	
V_7: .32x10^4ppmPb	84.00	80.62	82.31	0.60	100.731	-			
V_8: 3.2x10^4ppmPb	78.85	74.96	76.91	-4.81	94.117	ooo			$y=82.4 -2.92x +0.39x^2$
V_9: 6.4x10^4ppmPb	83.68	75.89	79.79	-1.93	97.640	o	-0.564	0.935	

The highest correlation between metal concentration and low humidity was showed for the variants treated with *zinc*; the calculated simple linear regression analysis ($y = 81.68-1.63 x$), shows that one unit increase in the analyzed metal concentration brings about a total humidity decrease by 1.63 units. For *copper*, there is no significant statistic correlation between metal concentration and humidity of the leaves, the value of the determination / correlation coefficient being reduced. In the case of the variants where *lead* was applied, it is noticed that there is a significant statistical correlation between the metal concentration and the total moisture, but its effect on the physiological index analyzed depends largely on other factors as well. Overall, the lead effect is similar to that of zinc, except that the reduction of humidity in the maize leaves occurs later in the when using zinc as a treatment.

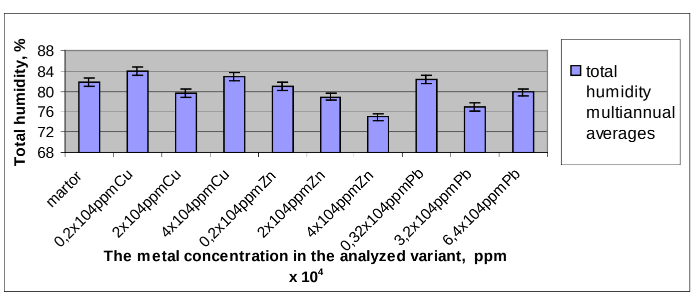


Figure 5. Influence of Cu, Zn, Pb on total humidity of the leaves of maize (cv.**Turda 200)**

The effect of heavy metals copper, zinc, lead on the total humidity of the Ribera cultivar occurs similarly to that of Turda 200, with the specification that, in the case of Ribera hybrid, the correlation coefficients between the applied metal concentration and the value of the total humidity of maize leaves, are lower compared to those determined for hybrid Turda 200, thus confirming Ribera’s feature as a hybrid more resistant to drought.

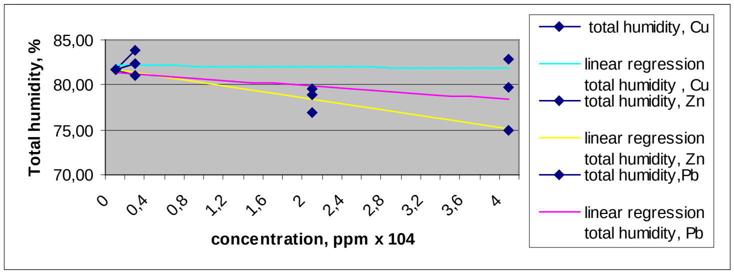


Figure 6. The correlation between the Cu, Zn, Pb concentration and the total humidity of maize – Turda 200

The results obtained in the two years of study are consistent with the observations mentioned in the bibliographic sources: the effect of heavy metals over the total leaves’ humidity depends on the capacity to absorb metals from the air. All external factors that affect water evaporation, changing the total moisture of the leaves (the leaf water content), including the concentration of metal ions in the air, acts by altering the cuticle permeability by changing the stomatal opening (Atanasiu, 1984). It is possible that the stomatal movement not to be directly affected by the action of heavy metals but rather by their strong interference with the transport of K^+, Ca^{2+} and of the abscissic acid into cells (Sanita di Troppi and Gabrielli, 1999).

Analysis of the micro-elements copper, zinc and lead influence on the water status of maize leaves by determining the intensity of transpiration

Anova tests indicated the lack of variants’ homogeneity (F critical = 2.466266; for cv. Turda 200, F = 3.0197, and for cv. Ribera, F = 3.0776). The synthetic results of the effect of heavy metals: copper, zinc, lead on the transpiration intensity in the maize leaves, Turda 200 hybrid (Table 4, Figure 7.8) reveal that the effect of all metals on the physiological indicator examined is manifested through the increase of transpiration intensity as the metal concentration increases. The response curve of maize leaves transpiration intensity under the influence of growing concentrations of metals applied is most correctly approximated through a graphical representation of the quadratic regression type, which highlights tight correlation coefficients between the metal concentrations and the intensity of the physiological indicator analyzed.

At high concentrations, exceeding $2x10^4$ ppm Cu and Zn respectively over 3.2×10^4 ppm Pb, it is found that metals induce the decrease of foliar transpiration intensity, but its value remains higher than the one of the control, because of the plant defence reactions.

Ribera cultivar behaves similarly to Turda 200 hybrid. In all the variants analysed, the values of transpiration intensity in the Ribera hybrid leaves are higher as compared to the ones recorded in the Turda 200 one.

Similar results are reported by Bartok (2005) who indicates that the action of heavy metals onhe plants water status results in increased transpiration. Copper and zinc have the most significant effects on the increase of transpiration intensity, which is explained by their influence on the composition of cytoplasm by altering the cellular osmotic pressure, the pH and by the action over phosphorusylase, enzyme involved in starch hydrolysis, which stimulates the stomatal opening.

Table 4. Influence Cu, Zn, Pb on the transpiration intensity in Turda 200 cultivar leaves

Variant	transpiration intensity, (g water/kg leaf) – Multiannual averages 2006	2007	general average	Difference as to the control	% as to the control	Significance	r	R^2	Equation
control	11.14	8.21	9.68	0	100				
V_1: .2x10^4ppmCu	17.32	19.41	18.36	8.68	189.669	**			
V_2: 2x10^4ppmCu	21.39	29.47	25.43	15.75	262.707	***			
V_3: 4x10^4ppmCu	20.48	28.90	24.69	15.01	255.062	***	0.925	0.857	$y=12.8+10.4x-1.87x^2$
V_4:0.2x10^4ppmZn	18.50	14.52	16.51	6.83	170.558	*			
V_5: 2x10^4ppmZn	22.50	25.69	24.10	14.42	248.967	***			
V_6: 4x10^4ppmZn	12.69	21.90	17.30	7.62	178.719	**	0.944	0.893	$y=11.8+11.4x-2.52x^2$
V_7:0.32x10^4ppmPb	10.73	12.09	11.41	1.73	117.872	-			
V_8: 3.2x10^4ppmPb	12.46	17.01	14.74	5.06	152.273	-			
V_9: 6.4x10^4ppmPb	12.65	15.72	14.18	4.05	146.488	-	0.984	0.969	$y=10.15+2.31x-0.26x^2$

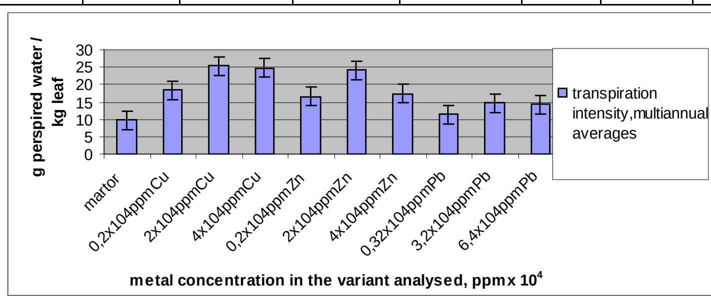


*Figure 7. I*nfluence of Cu, Zn, Pb on the transpiration intensity in **Turda 200** cultivar leaves

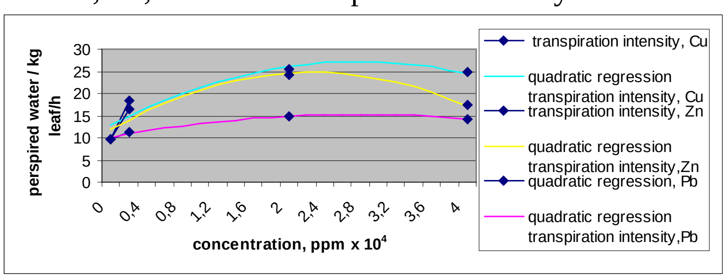


Figure 8. The correlation between Cu, Zn Pb concentration and the transpiration intensity in Turda 200 cultivar

2. THE INFLUENCE OF ATMOSPHERIC POLLUTANTS – HEAVY METALS: Cu, Zn, Pb – ON THE ZEA MAYS MINERAL NUTRITION

An analysis of the influence of copper, zinc, lead microelements on the dry weight content

Anova testing highlighted the powerful effect of other factors as well (environment factors) together with the effect of the heavy metals analysed on the plants manifested through the dry weight accumulation variation (F = 1.919531 in Turda 200 cultivar, F = 1.951176 in Ribera cultivar as compared to the critical F = 2.466266).

The analysis of the obtained results (Table 5, Figure 9.10) indicates that in **Turda 200** hybrid the effect of heavy metals Cu, Zn, Pb over the accumulation of dry weight in the maize leaves occurs as follows: at the lowest Cu, Pb concentrations occurs a decrease of the dry weight accumulation in the maize leaves, as compared to the control, the most intense in the variants being the treatment with copper in concentration of $0.2x10^4$ ppm; the variants that used minimum lead concentration, the reducing of dry substance accumulation as compared with the control is smaller, insignificant in statistical terms.

The variants using average concentrations of copper, zinc and lead highlight the fact that the dry weight accumulation increases as compared to the control, significantly in the case of copper, respectively distinctly significant and very significant in the case of the variants using zinc and lead. The highest concentration of zinc and lead induce the stimulation of dry weight accumulation, a very significant effect in the case of the variants using a concentration of $4x10^4$ ppm zinc. At its highest concentration, copper bring about an statistically insignificant reduction of the dry substance accumulation.

*Table 5.*Influence of Cu, Zn, Pb on the dry weight accumulation in the Turda 200 maize leaves

Variant	g DW./kg fresh substance – Multiannual averages 2006	2007	General average	The difference as to the control	% as to the control	Significance	r	R^2	Equation
control	14.15	22.42	18.29	0.00	100.00				
V_1: $0.2x10^4$ppmCu	15.40	16.79	16.10	-2.19	88.02	oo			
V_2: $2x10^4$ppmCu	15.55	25.22	20.39	2.10	111.48	x			
V_3: $4x10^4$ppmCu	14.35	19.77	17.06	-1.23	93.29	-	0.086	0.616	$y=17.01 +3.06x – 0.76x^2$
V_4:$0.2x10^4$ppmZn	13.85	24.05	18.95	0.66	103.64	-			
V_5: $2x10^4$ppmZn	15.20	26.95	21.08	2.79	115.26	xx			
V_6: $4x10^4$ppmZn	15.95	34.20	25.07	6.79	137.12	xxx	0.993	0.996	$y=18.5+ 0.99 x + 0.16 x^2$
V_7: $0.32x10^4$ppmPb	16.00	19.38	17.69	-0.60	96.73	-			
V_8: $3.2x10^4$ppmPb	21.15	25.04	23.09	4.81	126.29	xxx			
V_9: $6.4x10^4$ppmPb	16.32	24.11	20.22	1.93	110.56	x	0.566	0.935	$y=17.6 + 3x – 0.39x^2$

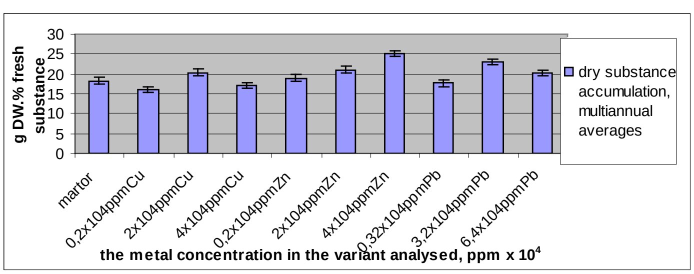


Figure 9. Influence of Cu, Zn, Pb on the dry weight accumulation in the Turda 200 cultivar leaves

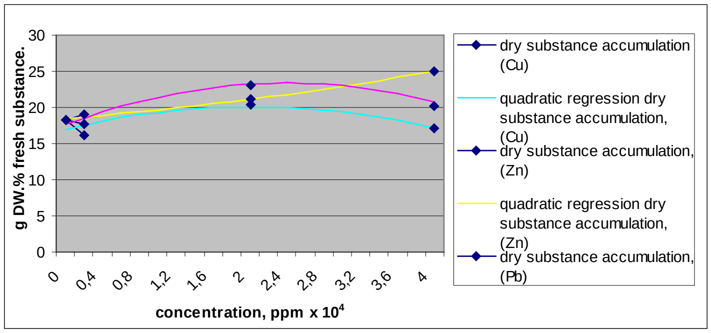


Figure 10. The correlation between the Cu, Zn, Pb concentration and the accumulation of dry weight in the Turda 200 maize leaves

The highest correlation between metal concentration and increased dry weight accumulation in leaves was determined for the variants treated with zinc. The calculated linear regression analysis (y=18.32+1.63x), shows that one unit increase in metal concentration analyzed brings about a 1.63 units increase of the dry substance in Turda 200 maize leaves.

Ribera Hybrid has a behaviour of dry weight accumulation in leaves similar to the one of Turda 200 hybrid, excepting that, in the variants using lead at its highest concentration, the increase of dry weight accumulation in leaves is not statistically significant The highest correlation between metal concentration and increased dry weight accumulation in leaves was determined for all variants treated with *zinc* (R^2 = 0.994, linear r = 0.993), the difference from the control being very significantly positive, at the average and maximum zinc concentration applied. The analysis of the calculated simple linear regression (y = 17.13+1.64x) shows that one unit increase in metal concentration analyzed brings about the 1.64 units increase of the dry weight n the Ribera hybrid maize leaves, like in the case of Turda 200 hybrid.

The results obtained in the two years of study are consistent with the observations mentioned in the bibliographic sources: the effect of heavy metals on the accumulation of dry weight in leaves suggests a sigmoid curve, characteristic to the growth parameters of the annual plants (Dornescu D Ţigănaş L, Băjescu I, 1983). The process of dry weight accumulation is characterized by very slow growths in the first developmental phases (up to 6-7 leaves, which corresponds to the first series of determinations), followed by a period of intense accumulation of the dry weight (the second series of determination). Experiments made have revealed the effect of stimulating the accumulation of dry weight for variants that applied zinc and lead, but increasing the concentration causes different responses, suggesting the physiological individual sensitivity, signalled also by Bathory D et al, 2000.

The fact that the varinats using zinc determined the most significant increases of the dry weight is explained by the increased mobility of this element, compared to Pb and Cu (Alloway, 1990, Ross, 1994) both in plants and in soil as well as the increased accessibility of this microelement for plants (Onac, 2005). In the case of the variants treated with copper, at the highest concentration applied, the reducing of its effect on the dry weight increase in leaves can be explained by the accumulation of the microelements in roots, the favorite accumulation place of Cu, fact found at the maize plants exposed to excessive Cu (Marschner, 1995). Bibliographical sources indicate that the impact analysis of Cu on cells ultrastructure underline that it is less affected compared to plant physiological processes, which indicates that *Zea mays* developed a strategy of resistance to copper toxicity (Ouzounidou et al., 1995).

Analysis of the copper, zinc, lead microelements influence on the total nitrogen content of maize leaves

The global analysis of the results obtained in the two years of study, Anova testing highlighted the lack of variants' homogeneity (F = 3.499472 in cv.Turda 200, respectively F = 3.57618 in cv.Ribera as to the critical F = 2.466266).

In **Turda 200** hybrid (Table 6, Fig. 11,12) one notices the fact the effect of heavy metals Cu, Zn, Pb over the total nitrogen content in the maize leaves is manifested through the decrease of the total nitrogen content in leaf by reducing the absorption of the ammonium ion due to the heavy metals interaction with the ATP-asis and oxidoreductase of the plasmatic membrane responsible with the preserving of the electrochemical gradient that has a role in the active absorption of NH_4^+ and the decrease of ammonium ion assimilation by reducing the activity of glutamine-synthetaze and glutamate de-hydrogenation (Burzynski and Buczek,1998, quoted by Onac, 2005).

*Table 6.*Influence of Cu, Zn, Pb over the total nitrogen content of maize leaves, **Turda 200 hybrid**

Variant	Total nitrogen Nt,(% în DW) – Multiannual averages 2006	2007	general average	Difference as to the control	% as to the control	significance	r	R^2	Equation	
control	3.17	2.33	2.75	0	100					
V_1: 0,2x10^4ppmCu	3.02	2.29	2.65	-0.10	96.539	-	-0.978	0.997	**y=2.73-0.3x+0.03x²**	**y=2.69 -0.17x**
V_2: 2x10^4ppmCu	2.55	2.00	2.27	-0.48	82.696	oo				
V_3: 4x10^4ppmCu	2.33	1.80	2.07	-0.68	75.228	ooo				
V_4:0,2x10^4ppmZn	3.27	2.16	2.71	-0.03	98.816	-	-0.949	0.997	**y= 2.77-0.47x+0.07x²**	**y=2.7 -0.21x**
V_5: 2x10^4ppmZn	2.62	1.59	2.10	-0.65	76.503	ooo				
V_6: 4x10^4ppmZn	2.51	1.44	1.97	-0.77	71.858	ooo				
V_7: 0,32x10^4ppmPb	2.98	2.07	2.53	-0.22	91.985	-	-0.967	0.970	**y= 2.67-0.21x+0.02x²**	**y=2.64 -0.1x**
V_8: 3,2x10^4ppmPb	2.65	1.69	2.17	-0.58	78.962	ooo				
V_9: 6,4x10^4ppmPb	2.42	1.42	1.92	-0.71	69.945	ooo				

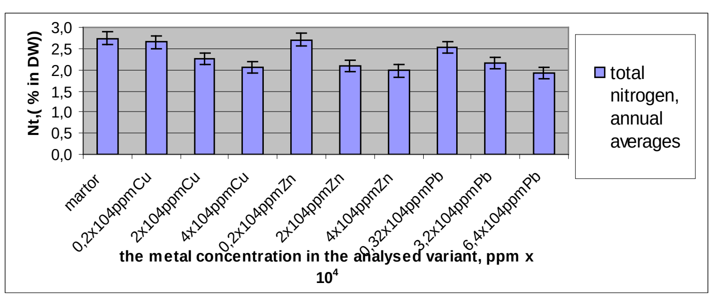


*Figure 11.*Influence of Cu, Zn, Pb on the total nitrogen content of maize leaves, Turda 200 hybrid

The lowering of the total nitrogen accumulation in the maize leaves, as compared to the control, is noticed from the lowest concentrations of metals Cu, Zn, Pb, the highest decrease being recorded at the variants using a concentration of 0.32 x10^4 ppm lead. However, the reduction of the nitrogen content in leaves at the lowest concentration of copper, zinc, lead applied is not statistically significant in comparison with the control. At an average concentration of metals (0.2 x 10^4 ppm Cu, Zn and 0.32 x 10^4 ppm Pb), the decrease of the total nitrogen content in leaves is very significant compared to the control in the variants that used zinc and lead and distinctly significant in the variants using copper. At a maximum concentration of metals, the decrease of the total nitrogen content in leaf is very significant compared to control, in all variants examined.

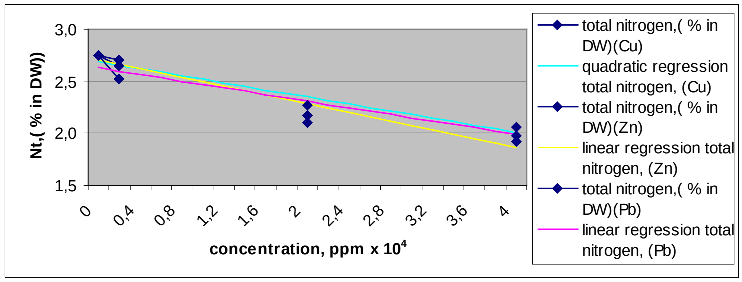


Figure 12. The correlation between the Cu, Zn, Pb concentration and the content of total nitrogen in the maize leaves, Turda 200 hybrid

The highest correlation between the metal concentration and the decrease of the total nitrogen content in the leaves of Turda 200 hybrid maize was determined for the variants treated with copper followed by zinc and lead. The one unit increase in the concentration of the metals analyzed brings about the 0.1 - 0.17 units decrease of total nitrogen content in the leaves of Turda 200 hybrid.

From the point of view of the total nitrogen content in leaves, ***Ribera*** hybrid has a similar behaviour to Turda 200 hybrid. For this hybrid, the one unit increase in the concentration of the metals analyzed brings about the 0.11 - 0.1725 units decrease of total nitrogen content in the maize leaves, more than in the Turda 200 hybrid case.

For the variants using lead, the more reduced correlation between Pb concentration and total leaf nitrogen content, compared to that determined in the case of Zn, is possibly caused by the installation of a process of partial exclusion of Pb from the leaves or from the whole plant, the cellular wall having a decisive role in this process (Verkleij and Schat, 1990, quoted by Onac, 2005).

The analysis of the microelements copper, zinc, lead influence on the phosphorusus content in the maize leaves

In the global analysis of the results obtained in the two years of study, Anova testing highlighted the lack of variants' homogeneity (F = 2.22493 in Turda 200 hybrid, respectively F = 11.09653 in Ribera hybrid, as to the critical F = 2.466266). In ***Turda 200*** hybrid (Table 7, Figures 13,14) the effect of the heavy metals Cu, Zn, Pb applied at foliar level in a concentration of 0.2; 2.4 x 10^4ppm Zn and Cu, respectively 0.31, 3.2 and 6.4 x 10^4 ppm Pb over the phosphorus content in leaf is manifested through its gradual decrease as the metal concentration increases.

The reducing of phosphorus accumulation in the maize leaves, as compared to the control, is noticed starting from the average concentrations of the zinc and lead applied (2 x 10^4ppm Zn and 3.2 x 10^4ppm Pb), the most accentuated decrease being noticed in the variants using maximum concentrations of the analysed metals. At an average concentration (2 x 10^4ppm) of copper applied, the phosphorus content in leaf is comparable to the one existing in the control. At the smallest metals concentration, it is noticed an effect of stimulating the phosphorus accumulation in leaves but it is not statistically significant compared to the control in the case of zinc and lead effect and distinctly significant in the case of copper effect.

Table 7. ***The influence of Cu, Zn, Pb on the phosphorusus content in the leaves of Turda 200 hybrid maize***

Variant	phosphorus P, (% în DW.) Multiannual averages 2006	2007	General average	Difference as to the control	% as to the control	Significance	r	R^2	Equation	
control	0.40	0.27	0.33	0	100.00					
V_1: $0.2x10^4$ppmCu	0.43	0.28	0.35	0.02	105.53	xx	- 0.871	0.846	y=0.34+ 0.003x $-0.003x^2$	y=0.34 - 0.01x
V_2: $2x10^4$ppmCu	0.42	0.24	0.33	-0.01	98.13	-				
V_3: $4x10^4$ppmCu	0.40	0.20	0.30	-0.04	88.27	ooo				
V_4:$0.2x10^4$ppmZn	0.42	0.27	0.34	0.01	102.76	-	- 0.928	0.863	y=0.335- 0.008x+ $0.0003x^2$	y=0.34 - 0.006x
V_5: $2x10^4$ppmZn	0.40	0.24	0.32	-0.01	96.19	-				
V_6: $4x10^4$ppmZn	0.39	0.22	0.31	-0.03	91.26	ooo				
V_7:$0.32x10^4$ppmPb	0.39	0.28	0.33	0.00	99.48	-	- 0.983	0.995	y=0.33- 0.008x+ $0.0005x^2$	y=0.33 - 0.005x
V_8:$3.2x10^4$ppmPb	0.38	0.25	0.31	-0.02	93.73	oo				
V_9:$6.4x10^4$ppmPb	0.36	0.23	0.30	-0.04	88.20	ooo				

The highest correlation between the metal concentration and the decrease of phosphorus content in the Turda 200 hybrid maize leaves was determined, for the variants treated with lead and zinc, which induce an average decrease by 0.005 (for lead) and 0.006 units (if zinc) of the phosphorus content in the leaves of Turda 200 hybrid maize. There is a significantly negative correlation also between the copper concentration and the phosphorus content in leaves, the value of the correlation coefficient being lower compared with lead and zinc.

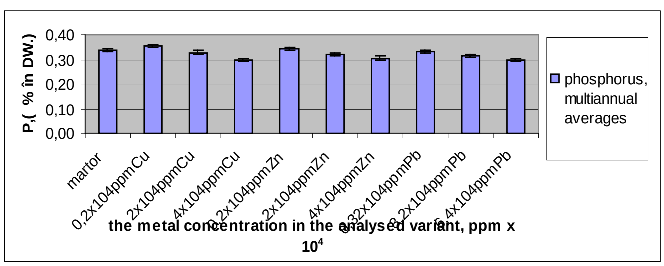


Figure 13. The influence of Cu, Zn, Pb on the phosphorus content in the leaves of **Turda 200** hybrid maize

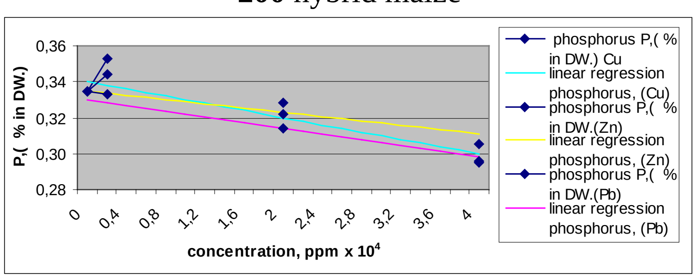


Figure 14. The correlation between the Cu, Zn, Pb concentration and the phosphorusus content in the maize leaves of Turda 200 hybrid

As far as the content of phosphorus in leaves is concerned, ***Ribera*** hybrid has a similar behaviour to Turda 200 hybrid, meaning that the increase of the applied metals concentration brings about the decrease of phosphorus content in leaves with an average of 0.008 units, the differences as compared to the control being higher than the ones recorded in the case of Turda 200 hybrid.

When evaluating the phosphorus content in leaves comparatively for the two years of study, it was noticed that it was smaller in 2007 than in 2006, for both the control (0,42 % P in dry weight in 2006 and 0,35% P in dry weight in 2007) as well as for the experimental variant. The decrease of phosphorus content in leaves was higher in 2007 and the determinations performed to the second series (18 days after applying the metals, the 6-8 leaves phase) showed smaller phosphorus content in leaves than in the first series of determination.

The results obtained confirm the observations mentioned in other researches (Dornescu D, Ţigănaş L, Băjescu I.-1983, Dragoş M., 2001). The phosphorus absorption rhythms are different, depending on the development phases, being more intense in the early phases characterized through vegetative growth with accelerated rhythms; however, the quantities of phosphorus accumulated in the plant are generally 10 times smaller than the ones of nitrogen (Dornescu D, Ţigănaş L, Băjescu I.-1983). The decrease of phosphorus quantity in the aerial organs of the maize along the vegetation period is owed to the fact that, in the first stages of vegetation, the cellular metabolism is much more intense (Radoviciu E.M., Tomulescu I.M., Merca V., 2004). The climatic conditions influence the phosphorus accumulation: it can occur a nutritional misbalance (especially in the case of zinc nutrition) in the cold and wet springs if the phosphorus status of the soil is increased.

Analysis of the microelements copper, zinc, lead influence on the mineral nutrition by determining the potassium content in the maize leaves

When analysing the global results of the two years of study, the Anove testing highlighted the lack of variants' homogeneity (F = 4.428103 in Turda 200 hybrid, respectively F = 9.384714 in Ribera hybrid, as compared to the critical F = 2466266).

When analysing the results obtained, (Table 8, Figures 15, 16) one notices that in **Turda 200** hybrid, the effect of heavy metals Cu, Zn, Pb applied in concentrations of 0.2; 2; 4 x 10^4ppm for Zn and Cu, respectively 0.32, 3.2 and 6.4 x 10^4ppm for Pb, on the potassium content of leaf is manifested through its decrease as the metal concentration increases.

At the smallest copper and lead concentrations, one notices an increase of the potassium content in leaves, significantly positive as to the control only in the case of copper. The diminishing of the potassium content in leaf is distinct and very significant as to the control starting with the average concentration of the metals applied – except for zinc for which the difference is not significant and it is accentuated when the metals concentration is maximum.

The significances previously set out concerning the effect of Cu, Zn, Pb in the three concentrations are supported by the correlation coefficients illustrating the relationship between the concentrations of these metals and the potassium content of leaves. The highest correlation between metal concentration and decreased potassium content in the Turda 200 hybrid maize leaves was determined for the variants treated with lead and copper. The linear regression analysis reveals that one unit increase in the metals concentration analyzed brings about an average decrease of 0.10 (for lead) and 0.16 units (for copper) of potassium content in the Turda 200 leaves. There is a significantly negative correlation between the concentration of zinc and the potassium content in leaves, the value of the correlation coefficient being, however, much smaller, this showing that the decrease of the potassium content in leaf is much influenced by other factors, different from the concentration of zinc applied.

*Table 8.*The influence of Cu, Zn, Pb on the content of potassium in the **Turda 200** hybrid maize leaves

Variant	Potassium K,(% in DW) Multiannual averages			Difference as to the control	% as to the control	Significance	r	R^2	Equation	
	2006	2007	general average							
control	2.56	2.27	2.41	0	100.00					
V_1: 0.2x10^4ppmCu	2.96	2.59	2.77	0.36	114.9	xxx				
V_2: 2x10^4ppmCu	2.67	1.80	2.23	-0.18	92.54	-				
V_3: 4x10^4ppmCu	2.20	1.68	1.94	-0.47	80.41	ooo	-0.885	0.783	$y=2.59-0.16x-0.002x^2$	y= 2.59-0.16x
V_4:0.2x10^4ppmZn	2.49	1.81	2.15	-0.27	89.02	oo				
V_5: 2x10^4ppmZn	2.75	1.77	2.26	-0.15	93.58	-				
V_6: 4x10^4ppmZn	2.48	1.59	2.03	-0.38	84.25	ooo	-0.715	0.555	$y=2.29+0.001x-0.017x^2$	y= 2.31-0.06x
V_7: 0.32x10^4ppmPb	2.74	2.28	2.51	0.10	103.9	-				
V_8: 3.2x10^4ppmPb	2.36	1.89	2.12	-0.29	87.88	oo				
V_9: 6.4x10^4ppmPb	2.11	1.56	1.83	-0.58	75.96	ooo	-0.983	0.966	$y=2.47-0.11x+0.001x^2$	y= 2.47-0.10x

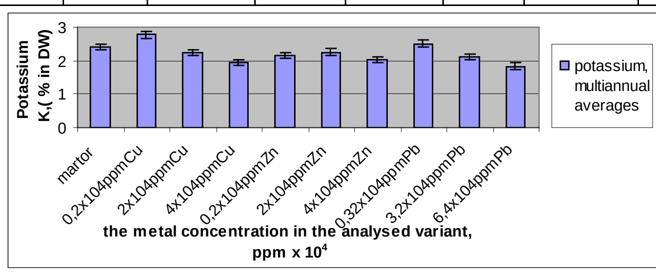


Figure 15. The influence of Cu, Zn, Pb on the content of potassium in the **Turda 200** hybrid maize leaves

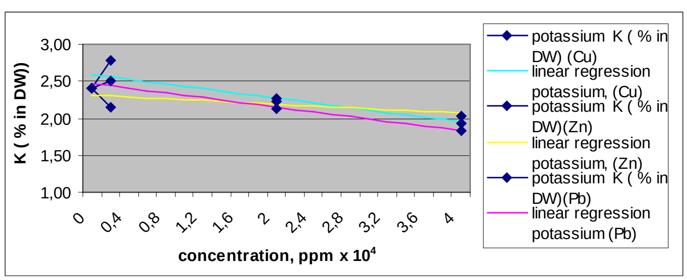


Figure 16. The correlation between the Cu, Zn, Pb concentration and the potassium content in the Turda 200 hybrid maize leaves

As far as the effect of the heavy metals Cu, Zn, Pb on the potassium content in leaf is concerned, ***Ribera*** hybrid has a similar behaviour to ***Turda 200*** hybrid, meaning that the increase of the applied metals concentration brings about the decrease of potassium content in leaves with an average of 0.085 units (for lead), 0.07 units (for zinc) and 0.12 units (for copper) respectively.

The explanation of the higher variability of the potassium content in leaf as compared to nitrogen and phosphorus can be probably owed (Sanità di Toppi, Gabbrielli, 1999) to the accumulation of phytochelatins – chelate compounds capable of bonding metallic cations as a defence mechanism of plants against the toxic concentration of the metallic ions. It is considered that phytochelatins have a role in maintaining the cellular metallic homeostasis and that they are involved in creating and/or increasing the tolerance to different metals, their synthesis being induced by a series of metals: Cu^{+}, Cu^{2+}, Zn^{2+}, Pb^{2+}.

CONCLUSIONS

The researches performed on the influence of the air pollutants - heavy-metals on certain physiological processes in Zea mays, Turda 200 and Ribera hybrids in field experiments, highlight the specific manifestations of the effect of copper, zinc, and lead translocated in plant from the air. Unlike other approaches of this subject, the herein thesis considers a research conducted in real conditions, conditions in which the plant is subjected to the action of known concentrations of pollutants. Considering this approach only a beginning for future researches, we emphasize the following conclusions:

I. Related to aspects of the environmental status in Satu Mare County – global appreciations related to the issue of the environment protection in Satu Mare County.

1. The general structure of the Integrated Environmental Monitoring System in Romania must be implemented for all subsystems, including for the integrated monitoring of the agro-ecosystems.
2. The contribution of air pollutants: heavy metals-Cu, Zn, Pb-in Satu Mare County is due to fixed sources of pollution located mainly outside the county area as well as to the road traffic.
3. The areas that are most affected by precipitations containing heavy metals: copper, zinc, lead, are as follows: Livada, Berveni, Valea Vinului.
4. The concentrations of the analysed metals in the precipitations water are as follows: Zn> Pb> Cu.

II. Related to the research results

A. The influence of the air pollutants - Cu, Zn, Pb – on the total humidity of the maize leaves

1. The increase of copper, zinc, lead concentrations applied to leaves brings about the decrease of the total humidity of the maize leaves;
2. The highest correlation (significantly negative) between the metal concentration and the maize leaves humidity was determined in the case of zinc.
3. As compared to the control, the heavy metals Cu, Pb bring about an increase of the total maize leaves humidity, at the smallest concentrations applied.
4. Zinc determines the most significant modifications of the total maize leaves humidity.
5. Ribera hybrid is more resistant to draught than Turda 200.
6. The determination / correlation coefficients between the metals concentration and the total humidity have higher values in Turda 200 hybrid as compared to Ribera, which indicates a higher sensitivity of Turda 200 hybrid as far as the heavy metals effect on the total humidity is concerned.
7. The effect of the analysed metals on the physiological indicator: the total humidity is manifested in a decreasing order, as follows: Zn>Pb>Cu.

B. The influence of the air pollutants -Cu, Zn, Pb- on the intensity of the maize leaves transpiration:

1. The foliar application of the heavy metals copper, zinc, lead, induce an increase of the transpiration intensity as compared to the control;
2. The heavy metals copper, zinc, lead bring about an increase in the intensity of the maize leaves transpiration at a higher rate as compared to the control, at the average ($2x10^4$ppm Cu, Zn; 3,2 $x10^4$ppm Pb) and maximum concentrations of the metals analysed ($4x10^4$ppm Cu, Zn; 6,4 $x10^4$ppm Pb).
3. The increase of the transpiration intensity is significant and very significant in all the variants subjected to the action of copper and zinc at minimum (0,2 x 10^4 ppm Cu, Zn) and average (2 x 10^4 ppm Cu, Zn) metals' concentrations.
4. At the highest concentrations of metals (4 x 10^4 ppm Cu, Zn and $6,4x10^4$ ppm Pb) it is noticed that the leaves' transpiration intensity decreases as compared to the one recorded in the variants subjected to the action of the metals at minimum and average concentrations but it still remains significantly superior to the control.
5. Under the action of the lead absorbed at leaf's level, the intensity of the maize leaves transpiration increases, but the increase is insignificant as compared to the control.
6. Among the foliar absorbed analyzed metals, copper had the most significant effect on the transpiration intensity, followed by zinc and lead.
7. The dynamics of maize leaves transpiration intensity after the specific action of the pollutants: heavy metals Cu, Zn transferred on the plant by air during growth and development phases for which the determinations were made, shows a gradual decline since the impact of copper and zinc while the lead effect occurs later.
8. Ribera variety is more sensitive to the effect of heavy metals on the intensity of transpiration as compared with Turda 200 hybrid.

C. The influence of the air pollutants -Cu, Zn, Pb- on the dry substance accumulation in the maize leaves

1. Heavy metals Zn, Pb absorbed at foliar level in increasing concentrations determines the stimulation of the dry weight accumulation in the maize leaves.
2. The highest correlation between metals concentration and dry weight in maize leaves was determined for zinc and lead.
3. Zinc causes the most significant increases in the maize leaves dry weight, at all concentrations examined.
4. The effects of zinc on dry weight accumulation in the maize leaves are less dependent on the specific weather conditions as compared to lead and copper.
5. The effect of lead on the intensification of the dry weight accumulation in leaves is manifested with preference starting from the average concentration (3.2 x 10^4 ppm Pb) applied. The further increase in the concentration of this metal results in a lower rate of the intensification of dry weight accumulation in leaves.
6. The correlation between copper concentration and dry weight content in leaves is reduced.
7. The copper absorbed at foliar level at an applied concentration of 2 x 10^4 ppm results in a significant intensification of the dry weight accumulation.
8. At its smallest (0,2 x 10^4 ppm) and highest concentration (4 x 10^4 ppm), copper brings about the reducing of the dry substance accumulation.
9. Ribera Hybrid is more sensitive to the action of the heavy metals on the dry weight accumulation as compared to Turda 200.

D. The influence of the air pollutants -Cu, Zn, Pb on the total nitrogen content in the maize leaves

1. Copper administrated in growing concentrations brings about a proportionally significant decrease of the total nitrogen content in leaves, averagely by 0.17 units (Turda 200) -0.22 units (Ribera) for each unit of the copper concentration applied at foliar level.
2. The increase of the zinc concentration applied at foliar level results in a very significant reduction of the total nitrogen content in leaves, compared with the control, averagely by 0.21 units (Turda 200) -0.25 units (Ribera) for each unit of the zinc concentration applied at foliar level.
3. Lead determines the decrease of the total nitrogen content in leaves, simultaneously with the increasing concentration, with 0.1 (Turda 200) -0.11 (Ribera) units per unit of lead concentration applied at foliar level.
4. The toxic effect of metals on the total nitrogen content in the maize leaves is higher in zinc and copper, in comparison with lead.
5. Under the influence of microelements copper, zinc, lead, the nitrogen dynamics in the maize leaves show a gradual decrease from the 4-6 leaves phase to the 8-10 leaves one, similar to the control and in agreement with the physiological needs of the plant.
6. In all the variants examined the total nitrogen concentration in leaf 18 days from the impact of heavy metals on plants, fall within the low field of supplying with this macreolement.
7. Ribera hybrid is more sensitive than hybrid Turda 200 to the toxic action of heavy metals Cu, Zn, Pb on the total nitrogen content in leaf.

E. The influence of the air pollutants -Cu, Zn, Pb- over the phosphorus content in the maize leaves

1. All metals analyzed copper, zinc, lead – bring about a reduction in the phosphorus content while simultaneously increasing the metal concentration applied.
2. The lowest concentration of copper and zinc applied at foliar level brings about a stimulation of the phosphorus accumulation in leaves, stronger than in the case of copper.
3. Lead has inhibitory effect on the total phosphorus content of leaves at all concentrations applied, causing an average decrease of the phosphorus content in leaves by an average of 0.005 (Turda 200) - 0.008 (Ribera) units per unit of lead concentration applied at foliar level.
4. Zinc shows less toxic effect on the phosphorus content in leaves, as compared to lead and copper.
5. The phosphorus content in leaves decreases as the plant advances with its vegetation: the dynamics of the phosphorus content in the maize leaves show a gradual decrease from the 4-6 leaves phase to the 8-10 leaves one.
6. Examined metal toxicity expressed by the reduction of phosphorus in leaves occurs in the order: Pb> Cu> Zn.
7. As compared to Turda 200 hybrid, Ribera is more sensitive to the effect of heavy metals over the phosphorus content in leaves.
8. The effect of the heavy metals Cu, Zn, Pb applied at foliar level over the phosphorus content in the maize leaves is intensified in extreme environmental conditions, when

the coefficients of correlation with the concentration of the metals applied have lower values.

F. The influence of the air pollutants -Cu, Zn, Pb- on the potassium content in the maize leaves

1. All metals determine a reduction in the potassium concentration in leaf, together with the increasing metal concentration applied.
2. The toxic effect of metals on the content of potassium in the maize leaves is higher in the case of lead as compared to copper and zinc.
3. The effect of copper and lead on the potassium quantity in the maize leaves occurs at the lowest concentration applied, by stimulating the accumulation of potassium in leaves.
4. Zinc shows less toxic effect on the potassium content in leaves, compared with lead and copper.
5. Zinc has inhibitory effect on the total phosphorus content of leaves at all concentrations applied at foliar level.
6. In periods with higher temperatures and lower humidity environment (2007), the environmental factors have a greater influence on the accumulation of potassium in leaves, the correlation between the concentrations of the metals applied and the potassium content in leaves being smaller.
7. The effect of the heavy metals Cu, Zn, Pb applied at foliar level on the potassium content of leaves is attenuated as the plant's vegetation advances.
8. Ribera hybrid is more sensitive than Turda 200 hybrid to the action of the metals Cu, Zn, Pb on the potassium content of leaves.

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