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BIOMONITORING OF AIRBORN SOILS CONTAMINATION IN DNIPROPETROVSK MEGAPOLIS

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Abstract: The soil airborn pollution influence on functional state of radish seedlings was studied. It has been shown, that the variability of chemical composition of soils from different polluted sites lead to active reorganization in cell protein system. Comparative analysis of obtained data allows to reveal a direct correlation between the degree of contamination and the decrease of mass. Reduced mass in Dnipropetrovsk was 15-21%, for Dniprodzerzhynsk was 25-45%. To a certain extent, changes in protein concentrations in roots and leaves are in inverse proportion to the reduced mass. According to biological indication of airborn polluted soils in the studied area, it was found that adaptive reactions of test plant to heavy metals pollution reflected in the corresponding variability of different exchange cell processes, which includes a decrease of intensity of general protein synthesis, the formation of different in the composition and intensity of pool readily soluble cytoplasm inclusion proteins, the change in activity of the antioxidant protection system. The peroxidase activity determined in roots and leaves of radish sprouts varies considerably. That is caused by the nonuniformity of the composition in investigated sites.
Key words: airborn soil pollution, biological indication, heavy metals, morpho-physiological indexes

INTRODUCTION:

Since the last century , as a consequence of advanced industrialization and remarkable growth of population and its mobility needs, particularly in urban areas, air pollution attributable to anthropogenic activities has accused more acute forms and often irreversible. There was a continuous accumulation of various pollutants in the environment, with increasingly severe consequences on humans, animals and plants, buildings, works of art and landscape in general (Popa, 2004; Mitran, 2011)

The worsening environmental situation in industrially developed areas is a global problem. It's conditioned by the fact that changes in pollution zones predominate over adaptive ability of a human body. Health of population is closely connected with the worsening environmental situation. It is important to follow up the health status of inhabitants living in the polluted areas, paying particular attention to diseases and its prevention. Within the context of atmospheric pollution measuring comes biomonitoring as an important tool (Garrec, 1996; Gottardini *et al.*, 2004; Bosch-Cano, 2012), which generally completes the automatic measuring devices (Dmuchowski *et al.*, 2011; Bosch- Cano, 2012). In the same time, it is very important to have reliable test methods using "test plants" to characterize possible biological impacts of environmental contaminants.

Dnipropetrovsk is Ukraine's fourth largest city, with about one million inhabitants and the center of Dnipropetrovsk region. It is also a major industrial center of Ukraine. It has several facilities devoted to heavy industry that produce cast-iron, rolled metal, pipes, machinery, different chemicals and many others. Atmospheric emissions made by mining and metallurgy industry account for 54.3% of total air pollutions of the Dnipropetrovsk region. As it has been shown previously, the range of pollutants depends on the number and types of the industrial enterprises located within the urban area (Babiy *et al.*, 2003). In Dnipropetrovsk and surrounding cities the dominant emissions come from the waste of metallurgical industries, which is heavily developed in this area (Habashi *et al.* 2011). Those pollutions have substantial impacts on human health and affect a human body on different levels, including gene regulations, epigenetic substances, proteins and different metabolites. Proteins, being the primary products of gene expression, represent information about genetic systems in the most explicit way (Pernas *et al.*, 2000). A genotype could be characterized through protein phenotype analysis, which makes such assays an efficient way to reflect changes in different organisms affected by the urbanized environment.

The aim of our study was to apply genetic-environmental analysis as a tool to investigate impacts of industrial emissions on conditions of "test plants" in the studied region.

MATERIALS AND METHODS

The multipollution exposure assessment was made for several sites in the cities of Dnipropetrovsk and Dniprodzerzhynsk. One site in each city was selected in clean area; it was noted as Plot.1.

Three urban sites noted as Plot.2, 3 and 4 were selected near industrial enterprises (metallurgical plant, heat power station, coke-chemical plant, etc).

Different distances from metallurgical, chemical plants or roads with motorcar emission were taken in account. The coefficient of heavy metals accumulation (Benselhoub *et al.*, 2015, Kharytonov *et al.*, 2014) in soil of industrial sites has been calculated as following:

$$K_{c_i} = \frac{C_i}{C_b} \quad (1)$$

Where C_i – element content in soil, mg/kg;

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C_b – background content, mg/kg.

Index of soil total airborn pollution (TAP) with heavy metals Z_c was calculated with formula:

$$Z_c = \sum_{i=1}^{n} \left(K_{c_i} - (n-1)\right), \quad (2)$$

where n – elements number.

The threat caused with technogenic pollution is fixed when $K_{c_i} \geq 1$, and $Z_{c_i} \geq 1$, or more detailed as bellow:

- Z_c<16 – permissible level of soil pollution;
- 16<Z_c<32 – moderate threatening;
- 32< Zc<128 – threatening;
- Zc≥128 – extraordinary threatening.

Soil samples were prepared for chemical analyses by heavy metal extraction with ammonium – acetate buffer (pH4.8) and 1NHCl. The content of heavy metals in samples was determined by flame atomic-absorption spectrophotometer (Model S-115, Ukraine).

The multifactorial soil pollution influence on functional state of test radish seedlings was studied. 4-days radish seedlings were treated in the water-soluble soil extracts in the Petri dishes. The contents of readily soluble proteins of coleoptiles in 4-days red radish plantlets withdrawn by the buffer 0.05 M tris - HCl and pH 7.4 were defined according to the method of Bradford (Bradford *et al.,* 1976) the peroxidase activity was determined right after the secretion (Boyarkin, 1951). Protein spectra in the 4-days radish roots (variety "Frenchpop") were determined with SDS-electrophoresis. While analyzing an individual sample set comprising 50-100 seeds, the following parameters were defined: the total number of spectrum types as an inherent characteristic of each level of polymorphism in the seed proteins, a quantitative ratio of spectrum types, and frequencies of occurrence of the most common spectrum types being studied.

RESULTS AND DISCUSSION:

The obtained data during soil observation were confirmed with heavy metals air pollution monitoring. Three years (2010-2011-2012) average data on air pollution in park area of Dnipropetrovsk city and two metallurgical plants are shown in fig 1.

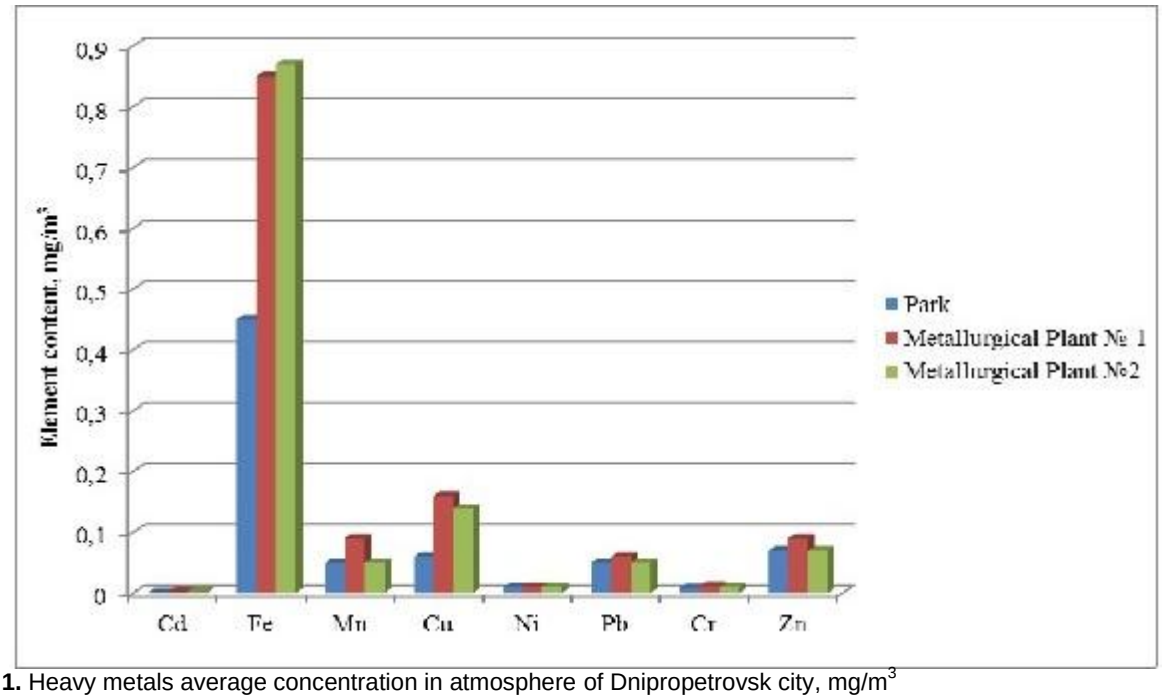

Fig 1. Heavy metals average concentration in atmosphere of Dnipropetrovsk city, mg/m^3

Air pollution near two metallurgical plants with iron, manganese, copper and cadmium exceed 2 times the control. Chemical analysis results of heavy metals in the water-soluble and acid-soluble form of extracts (BSF and ASF) from the soil, which were selected on certain testing sites of two industrial cities, are shown in Table. 1.

Table 1.

The content of heavy metals in soils of urban territories in Dnipropetrovsk and Dniprodzerzhynsk mg / kg

Element	Dnipropetrovsk							
	Plot.1		Plot.2		Plot.3		Plot.4	
	BSF	ASF	BSF	ASF	BSF	ASF	BSF	ASF
Pb	9,42	42,72	11,70	60,75	7,06	22,41	7,70	24,04
Cd	0,28	1,40	0,37	0,78	0,30	0,45	0,32	0,55
Ni	1,40	9,62	1,51	11,03	1,77	10,99	1,62	9,63
Zn	52,87	254,60	65,77	393,75	41,16	232,06	48,24	267,39
Cu	1,02	25,57	1,24	29,89	1,06	26,32	1,14	38,29
Fe	2,00	1563,64	2,24	1670,82	2,69	1508,29	2,50	1308,34
Mn	11,81	681,91	10,08	591,29	7,48	519,61	6,91	449,63
	Dniprodzerzhynsk							

Pb	6,31	36,09	8,00	36,48	6,33	42,90	3,23	4,61
Cd	0,26	0,39	0,60	1,15	0,44	0,71	1,64	2,70
Ni	1,25	7,96	1,30	6,85	0,89	6,72	0,57	6,89
Zn	15,29	59,90	43,48	258,54	45,59	230,92	15,66	120,24
Cu	0,56	7,43	1,14	27,69	0,63	14,36	0,61	21,92
Fe	1,93	1177,77	9,66	4553,38	2,13	2145,87	2,60	1392,95
Mn	13,24	494,56	23,13	1598,55	22,10	1338,53	13,02	706,64

To summarize the obtained results, polyelement levels of soil contamination were calculated. Determination of Zc was conducted with regard to the content of heavy metals in the form of acid-soluble extract from soils (Fig 2.).

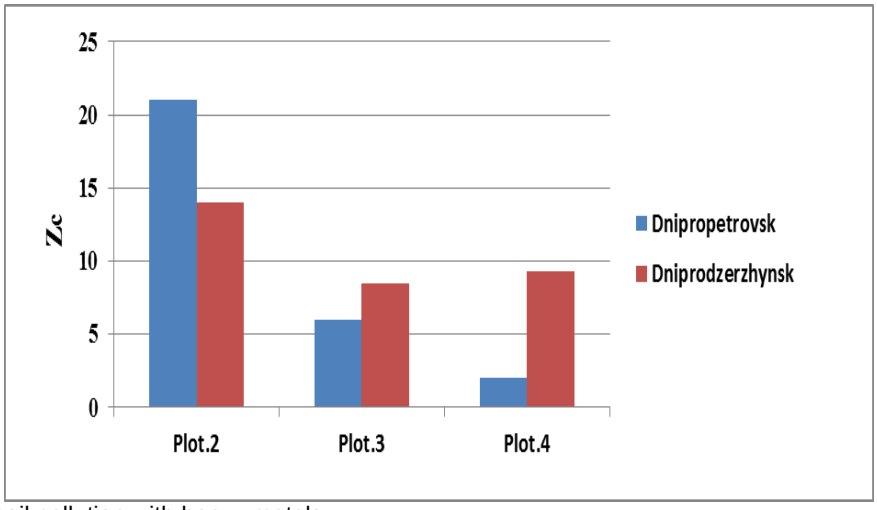

Fig 2. Total index of soil pollution with heavy metals

According to the above gradation of the environmental situation of soil pollution with heavy metals near the metallurgical plants in both cities tends to be judged as moderately hazardous. One of the fastest methods for biotesting environment is the cultivation of test plant in aqueous extracts of soils taken from different levels of man-made pollution zones. In this regard, it was conducted bioassay of soil samples taken near the area of metallurgical coke production and the cities of Dnipropetrovsk and Dniprodzerzhynsk. The evaluation results of morpho-physiological characteristics of 4-days test radish seedlings varieties (French Breakfast) after growing up in the soil extracts from Dnipropetrovsk is given in Table 2.

Table 2.
Indicators of soil bioassay of Dnipropetrovsk

Plant Part	Soil sampling plots			
	Plot.1	Plot.2	Plot.3	Plot.4
Weight seedling in mg				
Seedling	105,3 ± 2,03	91,3 ± 5,04	87,3 ± 3,01	100,0 ± 7,01
Protein concentration in mg / ml				
Root	2,60 ± 0,03	1,41 ± 0,05	2,32 ± 0,07	2,54 ± 0,02
Leaves	1,65 ± 0,04	1,86 ± 0,11	1,42 ± 0,05	2,22 ± 0,08

The evaluation results of morphological and physiological characteristics of 4-days seedling of radish sprouts (French Breakfast) varieties, after growing in soil extracts from Dniprodzerzhynsk are shown in Table 3.

Table 3 .
Indicators of soil bioassay of Dniprodzerzhynsk

Plant part	Soil sampling plots			
	Plot. 1	Plot. 2	Plot. 3	Plot. 4
Seedling	**Weight seedling in mg**			
	81,2 ± 9,05	61,3 ± 4,11	65,6 ± 3,21	56,0 ± 2,03
	Protein concentration in mg / ml			
Root	2,32 ± 0,06	2,41 ± 0,10	2,44 ± 0,04	2,44 ± 0,03
Leaves	1,20 ± 0,01	1,75 ± 0,05	2,58 ± 0,07	1,82 ± 0,13

Comparative analysis of the data presented in Tables 2 and 3 allows to reveal a direct correlation between the degree of contamination and the decrease of mass. Reduced mass in Dnipropetrovsk was 15-21%, for Dniprodzerzhynsk was 25-45%. To a certain extent, changes in protein concentrations in the roots and leaves are in inverse proportion to reduced mass.

One of the most important features of change in metal-induced metabolism of cells is the change of activities of enzymes antioxidant protection.

The multipollution exposure influence on functional state of 4-days test radish seedlings was studied for 09 sites in both cities (table 4). EF-spectra comparative assessment shows that variability of soil pollution from different sites lead to active reorganization in cell protein system. In our study, the peroxidase activity determined in roots and leaves of radish sprouts varies considerably. That is caused by the nonuniformity of composition in the investigated sites.

Table 4

Components composition and peroxidase activity of the easily soluble proteins of 4-days radish sprouts grown in water soil solutions of samples taken in different sites

pI	sites								
	1	2	3	4	5	6	7	8	9
Roots									
4,50	-	-	-	trace	-	+	+	-	+
4,53	+	+	+	+	++	+++	++	++	+
4,55	-	-	-	-	trace	-	trace	trace	trace
4,60	-	-	-	-	-	+	trace	trace	trace
4,70	-	trace	trace	trace	+++	-	++	+	++
4,80	+	+	++	++	+++	+++	++++	++++	+++
4,95	-	-	-	-	+	trace	+	+	+
5,15	-	+	+	+	+	+	+	+	+
5,30	+	+	+	+	+	++	+	+	+
Sprouts									
4,49	++	+	trace	+	+	-	trace	++	+
4,54	++ ++	+++	++	++++	++++	+	+	++	+++
4,56	+	-	+	+	+	+	-	-	-
4,60	+	+	-	+	+	+	-	+	+
4,65	+	trace	+	+	+	+	-	+	+
4,80	+++	+++	++	+++	+++	+++	+++	+++	+++
4,85	-	trace	trace	+	+	-	-	-	-
5,00	-	-	++	trace	+	+	-	-	-
5,05	-	trace	trace	+	+	+	-	-	-

Note. site 1 and 2 – control, sites 3-9 urban sites in Dnipropetrovsk and Dniprodzerzhynsk
Peroxidase activity: "-" – is absent; "+" – very weak; "++" – weak; "+++" – strong; "++++" – very strong.

CONCLUSION:

From the analysis and interpretation of obtained results in the present study, the concluding remarks should be highlighted:

1. The soil airborn pollution influence on functional state of radish seedlings was studied. It has been shown, that the variability of chemical composition of soils from different polluted sites lead to active reorganization in cell protein system.

2. Comparative analysis of the data shown in Tables 2 and 3 allows to reveal a direct correlation between the degree of contamination and the mass decrease. Reduced mass in Dnipropetrovsk was 15-21%, for Dniprodzerzhynsk was 25-45%. To a certain extent, changes in protein concentrations in the roots and leaves are in inverse proportion to the reduced mass.

3. Adaptive reactions of test plant to heavy metals pollution includes a decreasing of intensity of general protein synthesis, the formation of different in the composition and intensity of pool readily soluble cytoplasm inclusion proteins, the changing in activity of the antioxidant protection system.

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