THE EVALUATION OF SOIL GENOTOXICITY BY MODIFIED *ALLIUM* TEST

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Abstract. *In Romania, the Middle Jiu River Valley is a well-known coal basin in active exploitation. Nearby the surface coal exploitations there are placed TEPP (Thermo Electric Power Plant) Rovinari and Turceni - surrounded by ash and sterile waste dumps of different ages. Plant bioassays, which are most sensitive in detecting genotoxicity of environmental agents, can serve as the first alert for the presence of environmental hazards in water, air and soil. The aim of this paper was to study the impact of the pollution with radionuclides from ash and sterile waste dumps on vegetation in nearby villages Rosia de Jiu and Turceni, and to find correlations between the frequency of chromosomal aberrations and soil radioactivity, in Allium cepa, exposed to pollution in situ.*
For genotoxicity evaluation, the Allium test (FISKESJÖ, 1995) was modified, because the onion bulbs with roots were harvested from 10 different gardens near the TEEP (area considered to be contaminated). The amount of radionuclides from TEEP nearby village soil induced chromosomal aberrations in A. cepa. Very significant correlations between the percentage of aberrant cells in Allium cepa mitosis and the total content of radionuclides were found, the highest values for the correlation coefficient being recorded in Pb-210, Pb-214, Bi-214, Ra-226, Th-234.

Keywords: *radionuclides, power industry, genotoxicity, modified Allium test.*

Rezumat. Evaluarea genotoxicitatii solului prin testul *Allium* modificat. *Valea Jiului Mijlociu este un binecunoscut bazin de exploatare minieră din România. În vecinătatea exploatărilor miniere se află amplasate două Centrale Electrotermice (CET) - Rovinari şi Turceni, înconjurate de halde de cenuşă şi de steril de vârste diferite. Biotestele la plante, care sunt foarte sensibile în detectarea genotoxicităţii agenţilor de mediu, pot servi ca o primă alertă, pentru prezenţa hazardelor de mediu în apă, aer şi sol. Scopul acestei lucrări este studiul impactului poluării solului şi vegetaţiei din satele limitrofe, cu radionuclizi, proveniţi din cenuşă haldată şi/sau cenuşă uşoară eliberată, utilizând testul Allium modificat. Pentru evaluarea genotoxicităţii testul Allium (FISKESJÖ, 1984, 1995) a fost modificat, datorită faptului că bulbii de ceapă cu rădăcini au fost recoltaţi din 10 grădini diferite, din vecinătatea CET (suprafaţa considerată contaminată). Radionuclizii din sol induc aberaţii cromosomiale la A. cepa. Între conţinutul în radionuclizi ai solului şi procentul de aberaţii în mitoza la A. cepa, au fost găsite corelaţii semnificative, cele mai mari valori înregistrându-se pentru Pb-210, Pb-214, Bi-214, Ra-226, Th-234.*

Cuvinte cheie: *radionuclizi, industria energetică, genotoxicitate, testul Allium modificat.*

INTRODUCTION

The extractive and power industries contribute to the dislocation of a large amount of soil, as well as to the artificial modifications of the soil, by forming sterile and ash waste that present a high content of heavy metals and radionuclides, a permanent pollution source. CHALUPNIK et al. (2001) reported researches on solid waste material with increased natural radioactivity, that have been produced in the power and coal industries and studied the Ra concentration in Poland coal mines and settling ponds. MICHALIK et. al. (2005) studying the same area environmental pollutions concluded that the mining of the coal sometimes causes a significant increase in natural radioactivity in the environment, induced mainly by the release of radium-bearing waste water, as well as the storage of solid waste products on the surface. This phenomenon was observed not only in the Upper Silesian Coal Basin (Poland), but also in the Ruhr District (Germany) (SCHMID & WIEGAND, 2003), and other countries where coal is the only natural resource and fossil fuel available in abundance, as in India (MISHRA, 2003).

In Romania, the Middle Jiu River Valley is a well-known coal basin in active exploitation. Nearby the surface coal exploitations there are placed TEPP Rovinari and Turceni - surrounded by ash waste dumps of different ages. Mining activity in the Jiu Valley had an important impact both on environmental and socio-economic terms. In this respect, RĂDULESCU & BUIA (2002) supported the opinion that the main problem is to identify the correct and significant impact of the mining activity, defining also the ways to mitigate and monitor the process associated with coal extraction. Plant bioassays, which are most sensitive in detecting genotoxicity of environmental agents, can serve as the first alert for the presence of environmental hazards in water, air and soil (GOPALAN, 1999). Therefore meristematic and sporogenic tissues of plants generally show patterns of cytotoxic response similar to those of embryogenic and spermatogenic tissues of vertebrates (KRISTEN, 1997). *Allium cepa* (2n=2x =16) is a proper plant bioassay due to the low number of large chromosomes, radiosensitive and cultivated in the most gardens. The aim of this paper was to study the impact of pollution with radionuclides from ash waste dumps on vegetation in nearby villages Rosia de Jiu and Turceni, and to find correlations between the frequency of chromosomal aberrations and soil radioactivity, in *Allium cepa,* exposed to pollution *in situ.*

MATERIAL AND METHODS

The experiments were performed in spring 2007. The stations from which samples were collected – soil and plants - belong to different areas surrounding TEPP Rovinari and Turceni (Table 1). As Control, there were used soil and plants harvested from Stramba Monastery, Stramba Village and as absolute Control from Craiova.

The content of radionuclides from soil was established by the DUGGAN (1988) method, in conformity with IAEA TECDOC 1092 directives, with a gamma spectrometry system, analyzer SPECTRUM-MASTER-ADCAM, model 92X. The work method was elaborated by the National laboratory of Reference for Environment Radioactivity in the framework o fhte National Agency for Environment Protection, being in conformity with the IAEA TECDOC 1092 directives. For the energy and efficiency calibration we used standard gamma punctiform and volume sources with energies of the gamma radiation in the range of interest (5-20.000 keV); Am^{241}, Cs^{137}, Co^{60}, Eu^{152}, Ba^{133}. The collecting time of the natural background amounted to 2000.000 s. Radionuclide activity was expressed in Bq/kg, confidence level 95%.

For genotoxicity evaluation, the *Allium* test (FISKESJÖ G., 1995) was modified, due to the fact that the onion bulbs with roots were harvested in March 2007 from 10 different gardens near the TEEP (area considered to be contaminated) and Stramba village. The 1-2 cm roots harvested from young onion bulbs were fixed in Carnoy II fixing solution and coloured with Carr reactive. The cytogenetic observations were performed to an Olympus optical microscope. Observations were performed on all cells in division in ten tips (belonging to different bulbs) and there were recorded the number of cells in mitosis/tip, the percentage of chromosomal mutations in anaphase and telophase (dicentric, ring chromosomes, fragments), C-mitosis phase, micronuclei and metabolic alterations in prophase and metaphase. 300-400 cells/tip were analysed.

Statistical analyses were performed with STATISTICA 7.0 by Windows.

Table 1. The stations from which samples were collected.
Tabel 1. Stațiunile de unde au fost colectate probele.

Station	Sample type	Specification
Turceni (fresh ash pit)	Fresh ash	3 km West of TEEP Turceni
Ceplea (Ash waste)	Ash waste	Surrounding the ash pit, 5 years old
Cocoreni surface exploitation	Brown coal	Surface exploitation between TEEP Rovinari and TEEP Turceni
Turceni village	Soil *Allim cepa* bulbs	The gardens of the population surround the TEEP Turceni, in the East and South
Unchiasului Pool (fresh ash pit)	Fresh Ash	3 km East of TEEP Rovinari
Rosia de Jiu village	Soil *Allim cepa* bulbs	The village and the gardens of the populations are in the vicinity of TEEP Rovinari, in the South of it
Stramba village – Control	Soil *Allim cepa* bulbs	Considered to be a clean area, situated 25 km far from the experimental area

RESULTS AND DISSCUSIONS

Both TEEP (Turceni and Rovinari) started their activity 30 years ago. TEEP Turceni has 6 working groups x 330 MWatt each (900-1,200 MWatt/day) and there are 30,000 inhabitants in the surrounding area. TEEP Rovinari has 4 working groups x 330 MWatt each (800-900 MWatt/day) and there are 25,000 inhabitants in the surrounding area (Plate 1).

Radionuclides activity in solid waste and soil

Artificial radionuclides, appeared as a result of nuclear energy production (Cs-137, Cs-134, Sr-90, I-131 a.o.), as well as due to the natural ones from by-products or wastes (Ra-226, Ra-228, U-238, Th-232, Ac-228, Pb-214, Bi-214, Bi-212) having physicochemical properties similar to some constituent chemical elements of living organisms, are metabolized and arrive finally, through different natural trophic chains, into the human organism (CHIOSILA, 2004). Many of these radionuclides mentioned before were found in the samples collected from our considered area (Table 2). From artificial radionuclides, Cs-137 and U-235 registered a higher activity in villages and in the Ceplea Valley, these ones resulting from the nuclear accident of Cernobil, 1987. In most Romanian regions, the amount of Cs-137 decreased under 1 Bq/kg in the last decade, but there are regions in the Subcarpathians were the values are still high (CHIOSILA, 2004).

During coal combustion, most uranium, thorium and their decay products are released from the coal matrix and distributed in gas phase, as well as in solid combustion products (fly ash and bottom ash). The highest amount of natural radionuclides was found in the bottom ash collected from the ash pit. The highest radioactivity was presented by Th-234, Pb-214, Ra-226, Bi-214 (Table 2). Ra-226 is accumulated in bones and its gaseous decay products are accumulated in soft tissues, inducing lung cancer in time. A relatively small amount of radionuclides was found in the nearby village gardens, in this case significant differences in comparison with the rest of the samples being registered for Cs 137. The towns Turceni, Rovinari and the village Rosia de Jiu are affected by pollution because of the fly ash and bottom ash from the pit, which are dissipated by the wind, as well as by the gases. At the same time, because the ash pit and ash dumps are not restricted areas, the people from the villages use the ash in constructions, under the floor, to keep the rats away, being exposed themselves to a chronic irradiation with Ra-226.

It has to be mentioned that in solid combustion waste the concentration of radionuclides is three times (U-235) to nine times (Pb-214) higher than in the original coal. These findings are in accordance with those of ZIELINSKI & FINKELMAN, (1997), for two coal zones in the USA. According to UNSCEAR (1982) the average concentration in K-40, U-238 and Th-232 in coal is estimated to be 50, 20 and 20 $Bq{\cdot}kg^{-1}$, respectively, based on the analysis of coal samples from 15 countries, with a variation of more than two orders of magnitude (FLUES et al., 2006). The coal that was analyzed, from Cocoreni mine, registered values under the coal world range.

Table 2. The radioactivity ($Bq{\cdot}kg^{-1}$) of the solid combustion waste, brown coal and soil (0-20cm) in the considered area.
Tabel 2. Radioactivitatea ($Bq{\cdot}kg^{-1}$) cenuşii, lignitului şi a solului (0-20cm) în aria considerată.

Site	Radionuclides content (mean ± standard deviation)											
	K-40	Cs-134	Cs-137	Pb-210	Bi-212	Pb-212	Bi-214	Pb-214	Ra-226	Ac-228	Th-234	U-235
Turceni ash (pit)	67.1 ± 14.3	<1.73	<1.88	18.9± 1.5	56.7± 7.67	81.1± 2.55	112.6± 3.35	132.7± 3.61	122.7± 3.5	47.9± 3.44	137.8± 14.9	6.5± 0.2
Ceplea Ash dump -5y	212.8± 17.8	<1.34	0.88± 0.26	9.06± 0.89	32.1± 4.9	40.4± 1.64	35.1± 1.5	42.6± 1.6	38.9± 1.6	28.4± 2.35	44.2± 5.8	7.1± 0.7
Turceni Village	155.4± 15.9	<1.35	46.9± 0.96	7.87± 0.97	26.6± 4.83	37.1± 1.0	19.9± 0.98	22.9± 0.82	21.4± 0.9	26.3± 1.99	<11.0	4.42± 0.28
Balta unchiasului ash (pit)	416.9± 38.8	<1.56	74.9± 4.0	127.5± 7.95	20.0± 3.95	90.8± 8.47	86.8± 4.82	97.3± 5.67	102.3± 33.8	72.7± 7.52	130.1± 15.1	8.46± 1.48
Rosia Village	341.2± 19.9	<1.67	32.1± 1.12	10.8± 1.64	36.1± 6.99	53.4± 1.63	40.0± 1.58	43.9± 1.53	42.0± 1.5	38.8± 2.84	50.3± 6.88	7.53± 0.43
Stramba village	239.8± 18.9	<1.55	51.2± 3.21	6.86± 0.74	27.1± 4.91	37.7± 1.54	20.2± 1.18	24.3± 1.09	22.2± 1.1	26.8± 2.34	<12.7	3.23± 0.39
Brown coal Cocoreni mine	<43.8	-	<1.4	-	-	-	23.5± 2.5	14.8± 1.4	19.1± 2.0	8.7± 0.9	<10.5	2.18± 0.4
Average natural radioactivity for Romania	330-800	*	*	*	*	*	*	*	10-90	13-65	*	*

* No available data

Genotoxicity evaluation

The analysis of mitotic normal and aberrant stages, as well as of the metabolic modifications, offers interesting data for genotoxicity evaluation in *A. cepa.* The amount of radionuclides from TEEP nearby village soil induced chromosomal aberrations in *A. cepa*, such as: acentric fragments, minutes, simple or double bridges (dicentric chromosomes), centromere and/or kinetocor inactivation, inactivation or destruction of the mitotic spindle, having as a result characteristic aspects of C-mitosis, as well as metabolic modifications of the chromosomes, such as: alteration of the condensation degree of the chromatine fibers (premature chromosome condensation - PCC or delay in chromosome condensation - DCC), parallel disposal of the chromatine fibers with obvious euchromatine and heterochromatine bands, suprachromosomal organisation of the genetic material in prophase, due to the links between telomeres, chromatine fiber depolymerisation, a.o. (Table 3, Plate 2). CARRUYO et al. (2008) evidenced the induction of stickiness, as the effect of different lead concentration (0.25-1.00 ppm) on apical root tips of *A. cepa,* more than 12 hours. The phenomenon was described as chromosome agglutination displaying the sticky appearance and has been reported as an indicator for high toxicity (MARCANO, 1999).

A comparison of data obtained two years ago (2005) in a preliminary study of the village Rosia de Jiu area showed that the percent of metabolic disorder increased, as well as the inhibition of the mitotic spindle formation, phenomenon that was absent in the past experiment (Table 3).

Table 3. Chromosome aberrations (%) in *Allium cepa* L. harvested from villages near the TEPP.
Tabel 3. Aberaţii cromosomiale (%) înregistrate la *Allium cepa* L. recoltată din satele din vecinătatea CET.

Site	Normal mitosis %	Prophase MM %	Metaphase %			Anaphase %		Telophase %		MN %
			MM	CA	C-M	CA	C-A	CA	C-T	
Control 2007	98.36	0.5	1.7	-	-	1.3	-	2.1	-	0.2
Turceni-2007	89.6	8.4	24.1	-	3.6	22.6	-	2.9	2.3	0.0
Rosia de Jiu -2005	94.9	3.7	2.0	2.0	-	9.0	-	4.3	-	2.7
Rosia de Jiu -2007	93.8	6.0	4.8	-	1.8	8.6	1.6	1.4	2.1	0.2
Stramba 2007	95.1	0.4	1.9	3.8	-	4.1	-	0.8	-	0.4

LEGEND: **MM** = metabolic modifications, **CA** = chromosomal aberrations, **C-M** = C-metaphase, **C-A** = C-anaphase
C-T = C-telophase, **MN** = Micronuclei

A decrease in normal mitosis percentage was observed in the case of populations that registered a high percentage of metabolic disorders in metaphase, as well as structural modifications of the chromosomes in anaphase and telophase (Turceni 2007) (Table 4). CHAKRABORTY et al. (2009) reported that the proportion of cells in metaphases, anaphases and telophases taken together decreased dramatically with the content of the ash fly in heavy metals. In the case of *A. cepa* population collected from Stramba (2007), a village considered to be in a clean area, 95.1% normal mitosis was registered, a relatively high percentage of structural modifications in metaphase, mainly due to the metabolic disorders in prophase, which affected the packing degree of the chromatin fibers and chromosome structure (parallel disposal of the chromatin fibers, PCC, DCC). All these resulted in a relatively high number of acentric fragments in anaphase, the value of BR index being 50.99 (number of fragments in anaphase/ number of bridges in anaphase). The BR index was introduced by LAZÁNYI (1966) to characterize the efficiency of radioprotective substances, being utilised in cytogenetics and radiobiology (CORNEANU, 1979; IMREH, 1989). The BR value can offer information on the evolution of the mutational process at chromosome level, in the case of radiosensitivity analysis of some populations belonging to the same species.

The comparative analysis of the metabolic disorder percentage (especially PCC and DCC type) and the anaphase aberration percentage reveals the existence of a causal relation between these two types of modifications. Thus *A. cepa*, Turceni 2007, registered PCC in 30.48% prophase cells and a high number of fragments (41.51/100 anaphases) and bridges (26.46 bridges/100 anaphases).

Table 4. The causal relation between metabolic modifications (in prophase, prometaphase and metaphase) and structural modifications of the chromosomes (in anaphase and telophase) in *Allium cepa.*
Tabel 4. Relațiile cauzale între modificările metabolice (în profaza, pro-metafaza şi metafaza) şi modificările structurale ale cromosomilor la *Allium cepa.*

Variant	% Normal Mitosis	Metabolic modifications						Structural modifications			
		Prophase %		Prometaphase %		Metaphase %		Anaphase %		Telophase %	
		Cond.	Oth.	Cond.	Oth.	Cond.	Oth.	CF	CB	CF	CB
Control 2007	98.4	0.28	0.00	0.00	0.00	0.00	0.00	1.30	0.00	0.80	0.00
Turceni 2007	89.6	5.37	2.58	0.00	0.00	3.61	20.48	41.51	26.42	7.83	2.61
Rosia de Jiu 2005	94.9	0.82	3.27	0.00	0.00	3.85	7.70	12.23	5.77	9.78	1.09
Rosia de Jiu 2007	93.8	0.35	4.12	3.85	3.85	4.13	1.20	12.60	10.24	1.73	1.30
Stramba 2007	95.1	0.40	0.00	0.00	0.00	2.73	3.18	4.11	1.20	0.80	0.40

LEGEND: **Cond.** = DNA condensation process alterations, **Oth.** = other metabolic modifications
CF = chromosomal fragments , **CB** = chromosomal bridges

Table 5. The correlations (r) between the radionuclides content in soil and the percent of metabolic modifications and chromosomal aberrations in mitosis in *Allium cepa.*
Tabelul 5. Corelațiile dintre conținutul de radionuclizi în sol şi procentul de modificări metabolice şi aberații cromosomiale înregistrate în mitoza la *Allium cepa.*

Mitotic phase	Prophase	Metaphase		Anaphase				Telophase				MN
RN	MM	CA	C-M	CA	C-A	CF	CB	CA	C-T	CF	CB	
K40	**0.3455**	0.2297	0.0613	0.1187	**0.6401**	0.0297	0.0812	0.0458	0.2775	0.2275	0.2641	**0.4140**
	p=0.029	p=0.154	p=0.707	p=0.466	**p=0.000**	p=0.856	p=0.618	p=0.779	p=0.083	p=0.158	p=0.100	**p=0.008**
Cs137	**0.5286**	0.1098	**0.4621**	**0.3671**	**0.6866**	**0.3118**	**0.3911**	**-0.3446**	**0.6355**	-0.0189	**0.4857**	-0.0488
	p=0.000	p=0.500	**p=0.003**	**p=0.020**	**p=0.000**	**p=0.050**	**p=0.013**	**p=0.029**	**p=0.000**	p=0.908	**p=0.001**	p=0.765
Pb210	**0.3863**	**-0.3496**	0.2645	0.0020	**0.9974**	-0.0295	0.1053	**-0.3331**	**0.5765**	-0.2688	0.1636	-0.1970
	p=0.014	**p=0.027**	p=0.099	p=0.990	**p=0.000**	p=0.857	p=0.518	**p=0.036**	**p=0.000**	p=0.093	p=0.313	p=0.223
Bi212	**0.3738**	**0.5160**	0.1503	**0.4679**	-0.0820	**0.3844**	**0.3291**	**0.3997**	0.0992	**0.6799**	**0.5009**	**0.7181**
	p=0.018	**p=0.001**	p=0.355	**p=0.002**	p=0.615	**p=0.014**	**p=0.038**	**p=0.011**	p=0.543	**p=0.000**	**p=0.001**	**p=0.000**
Pb212	**0.5187**	-0.0183	0.2862	0.2389	**0.8017**	0.1663	0.2502	-0.0516	**0.5260**	0.1448	**0.3951**	0.2361
	p=0.001	p=0.911	p=0.073	p=0.138	**p=0.000**	p=0.305	p=0.119	p=0.752	**p=0.000**	p=0.373	**p=0.012**	p=0.142
Bi214	**0.4557**	-0.1635	0.2235	0.1074	**0.9041**	0.0454	0.1495	-0.0915	**0.5087**	0.0229	0.2753	0.1421
	p=0.003	p=0.313	p=0.166	p=0.510	**p=0.000**	p=0.781	p=0.357	p=0.575	**p=0.001**	p=0.889	p=0.086	p=0.382
Pb214	**0.4610**	-0.1182	0.2215	0.1271	**0.8792**	0.0610	0.1594	-0.0734	**0.4982**	0.0570	0.2934	0.1790
	p=0.003	p=0.468	p=0.170	p=0.435	**p=0.000**	p=0.708	p=0.326	p=0.653	**p=0.001**	p=0.727	p=0.066	p=0.269
Ra226	**0.4474**	-0.1858	0.2310	0.0922	**0.9253**	0.0339	0.1434	-0.1337	**0.5225**	-0.0232	0.2605	0.0904
	p=0.004	p=0.251	p=0.152	p=0.572	**p=0.000**	p=0.835	p=0.377	p=0.411	**p=0.001**	p=0.887	p=0.104	p=0.579
Ac228	**0.5061**	-0.0644	0.2810	0.2048	**0.8438**	0.1361	0.2284	-0.0853	**0.5364**	0.0930	**0.3651**	0.1871
	p=0.001	p=0.693	p=0.079	p=0.205	**p=0.000**	p=0.402	p=0.156	p=0.601	**p=0.000**	p=0.568	**p=0.021**	p=0.248
Th234	**0.3879**	-0.2409	0.1549	0.0057	**0.9347**	-0.0489	0.0631	-0.0993	**0.4610**	-0.0474	0.1764	0.0964
	p=0.013	p=0.134	p=0.340	p=0.972	**p=0.000**	p=0.765	p=0.699	p=0.542	**p=0.003**	p=0.772	p=0.276	p=0.554
U235	**0.5702**	-0.0098	0.2513	0.3335	**0.6189**	0.2507	0.2990	0.2804	**0.4320**	**0.4458**	**0.4736**	**0.5437**
	p=0.000	p=0.952	p=0.118	p=0.035	**p=0.000**	p=0.119	p=0.061	p=0.080	**p=0.005**	**p=0.004**	**p=0.002**	**p=0.000**

Marked correlations are significant at 0.010< p < 0.050; distinct significant at 0.001< p < 0.010; very significant p < 0.0010;

LEGEND: RN = radionuclide, **MM** = metabolic modifications, **CA** = chromosomal aberrations, **C-M** = C-metaphase, **C-A** = C-anaphase, **CF** = chromosomal fragments, **CB** = chromosomal bridges, **C-T** = C-telophase, **MN** = Micronuclei

The values of BR index in the case of *Allium* populations can be explained by the relationship between metabolic disorders in prophase and metaphase and the percentage of fragments and bridges in anaphase and telophase. The analysis of table 4 reveals a causal relation between the percentage of metabolic disorders and structural modifications in *Allium cepa*.

Correlations

There were found very significant correlations between the percent of aberrant cells in *Allium cepa* mitosis and the content in radionuclides in soil. All analyzed radionuclides induced metabolic disorders, especially concerning the condensation degree of DNA, in prophase, the highest values for the correlation coefficient being recorded for Cs-137, Pb-212, Ac-228 and U-235 (Table 5). Data analysis showed significant correlations between the contamination of the soil with radionuclides and the inhibition of mitotic spindle formation (C-metaphase, C-anaphase, C-telophase). This phenomenon is similar with the one observed by CHAKRABORTY et al. (2009), who reported that fly ash seems to have an effect on fragmoplast that is a complex assembly of microtubules, microfilaments and endoplasmic reticulum. DOVGALIK et al. (2001) reported an increase of binuclear cells in *A. cepa* apical meristems, after treatment by salts of Cd, Al, Pb, Cu and Zn, where cytoskeleton could be a cell target for salts of some metals.

The analysis of the correlations between the metabolic modifications, represented by the different degree of DNA fibers compaction and mitotic spindle alteration, on one hand and the structural modification of the chromosomes (chromosomal aberrations), on the other hand pointed out that our first hypothesis was right, that there exist causal relations between the phenomena. The percent of chromosomal fragments and bridges counted in anaphase and telophase are very significantly correlated with the percent of cells in prophase and metaphase, which present metabolic disorders (Table 6). Very significant correlations were found also, between the percent of chromosomal fragments in anaphase and telophase and the percent of cells with micronuclei.

Table 6. The correlations (r) between metabolic modifications (in prophase and metaphase) and structural modifications of the chromosomes (in anaphase and telophase) in *Allium cepa*.
Tabel 6. Corelaţiile între modificările metabolice (în profază şi metafază) şi modificările structurale ale cromosomilor la *Allium cepa*.

Mitotic phase	Metaphase	Anaphase				Telophase				MN
	C-M	CA	C-A	CF	CB	CA	C-T	CF	CB	
MM Prophase	**0.9155**	**0.9098**	**0.3533**	**0.8958**	**0.9367**	**0.3334**	**0.9045**	**0.5569**	**0.9603**	0.2410
	p=0.000	**p=0.000**	**p=0.025**	**p=0.000**	**p=0.000**	**p=0.035**	**p=0.000**	**p=0.000**	**p=0.000**	p=0.134
MM Metaphase	**0.9308**	**0.9419**	-0.1210	**0.9689**	**0.9615**	0.2067	**0.7506**	**0.4469**	**0.8932**	-0.0030
	p=0.000	**p=0.000**	p=0.457	**p=0.000**	**p=0.000**	p=0.201	**p=0.000**	**p=0.004**	**p=0.000**	p=0.985
CA Anaphase	**0.9023**	1.0000	-0.0355	**0.9951**	**0.9831**	0.3891	**0.7564**	**0.6600**	**0.9853**	0.2800
	p=0.000	p= ---	p=0.828	**p=0.000**	**p=0.000**	p=0.013	**p=0.000**	**p=0.000**	**p=0.000**	p=0.080
CA Telophase	0.0614	**0.3891**	**-0.3684**	**0.3617**	0.2822	1.0000	-0.0773	**0.9123**	**0.3648**	**0.8844**
	p=0.707	**p=0.013**	**p=0.019**	**p=0.022**	p=0.078	p= ---	p=0.635	**p=0.000**	**p=0.021**	**p=0.000**
Marked correlations are significant at 0.010< p < 0.050; distinct significant at 0.001< p < 0.010; very significant p < 0.0010;										
LEGEND: MM = metabolic modifications, **CA** = chromosomal aberrations, **C-M** = C-metaphase, **C-A** = C-anaphase, **CF** = chromosomal fragments, **CB** = chromosomal bridges, **C-T** = C-telophase, **MN** = Micronuclei										

CONCLUSIONS

The highest radioactivity was presented by Th-234, Pb-214, Ra-226, Bi-214 (Table 2). Ra-226 is accumulated in bones and its gaseous decay products are accumulated in soft tissues, inducing lung cancer in time. A relatively small amount of radionuclides was found in nearby village gardens, in this case significant differences in comparison with the rest of the samples being registered for Cs 137.

In the solid combustion waste, the concentration of radionuclides is three times (U-235) to nine times (Pb-214) higher than in the original coal.

The amount of radionuclides from TEEP nearby village soil induced chromosomal aberrations in *A. cepa*, such as: acentric fragments, minutes, simple or double bridges (dicentric chromosomes), centromere and/or kinetocor inactivation, inactivation or destruction of the mitotic spindle, as well as metabolic modifications of the chromosomes, such as: alteration of the condensation degree of the chromatine fibers (premature chromosome condensation - PCC or delay in chromosome condensation - DCC), parallel disposal of the chromatine fibers with obvious euchromatine and heterochromatine bands, suprachromosomal organisation of the genetic material in prophase, due to the links between telomeres, chromatine fiber depolimerisation, a.o.

A decrease in normal mitosis percentage was observed in the case of populations that registered a high percentage of metabolic disorders in metaphase, as well as structural modifications of the chromosomes in anaphase and telophase.

There were found very significant correlations between the percent of aberrant cells in *Allium cepa* mitosis and the content in radionuclides in soil. All analyzed radionuclides induced metabolic disorders, especially concerning the condensation degree of DNA, in prophase, the highest values for the correlation coefficient being recorded for Cs-137, Pb-212, Ac-228 and U-235.

Using the biological material, harvested from the polluted area, *Allium* test offers a more accurate image on the presence of environmental hazards.

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PLATE 1 / PLANŞA 1

Photo 1.

Photo 2.

Photo 3.

Photo 4.

Photo 1. TEPP Turceni; Photo 2. TEPP Rovinari: Ash pit Ceplea (Turceni); Photo 4. Ash pit Balta Unchiasului (Rovinari) in a windy day.

Foto 1. CET Turceni; Foto 2. CET Rovinari; Foto 3. Depozitul de cenusă Ceplea (Turceni); Foto 4. Depozitul de cenuşă Balta Unchiaşului (Rovinari) într-o zi cu intensificări ale vântului.

PLATE 2 / PLANŞA 2

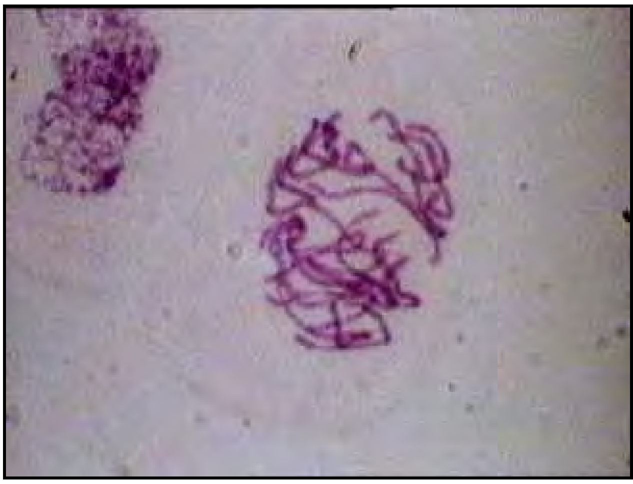

Photo 5.

Photo 6.

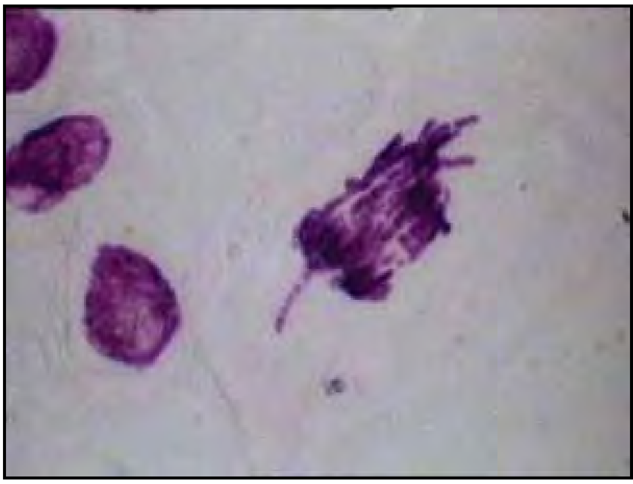

Photo 7.

Photo 8.

Photo 9.

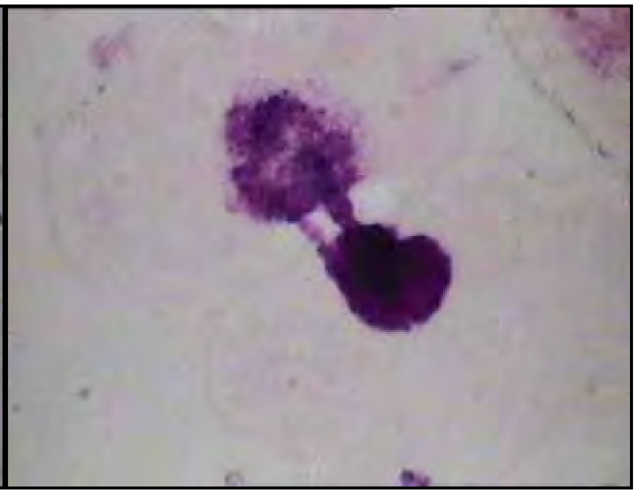

Photo 10.

Photo 5. Prometaphase – deley in chromosome condensation (DCC); Photo 6. C-Metaphase; Photo 7. Anaphase with bridges and aspect of banded chromosomes; Photo 8. Anaphase with double bridges; Photo 9. Polyploid anaphase; Photo 10. Son nuclei linked by chromatine double bridge and asincrony in chromosome decondensation.

Foto 5. Prometafaza – întârziere în condensarea cromosomilor; Foto 6. C-metafaza; Foto 7. Anafaza cu punți şi aspectul de cromosomi bandati; Foto 8. Anafaza cu punți duble; Foto 9. Anafaza poliploida; Foto 10. Nuclei fii legați prin punte dublă de cromatina şi asincronie în decondensarea cromosomilor.