INVESTIGATIONS TO DETECT ECOSYSTEM DISTURBANCES UNDER THE INFLUENCE OF ANTHROPOGENIC FACTORS

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Abstract. The extractive and energetic industries determined the change of the soil surface in the middle Jiu river basin, as well as the setting up of sterile and ash waste dumps, with a higher heavy metal and radionuclide content. A methodology for the investigation of the affected areas was discussed in order to limit the negative effects of these anthropogenic pollutants. This includes: the quantitative and qualitative analysis of the heavy metals and radionuclides in soil and plants; the establishment of the pioneer and indicator species, as well as of the species tolerant and resistant to the stress factor action, species used for the recovery of the affected ecosystems; methods for the detection of species with a role in the phytoremediation process, as well as methods for the analysis of the stress factor effects, which permit their maintenance at a tolerable level. Among the analysis methods, we mention: growth tests (especially in the laboratory experiments), cytogenetic analysis (structural and metabolic modifications of the chromosomes and of the division spindle), structural and ultrastructural investigations for the evidence of the stress factor effect, the induced lesions and the interaction of the eukaryote cell with the stress factors, the entrance pathways and the accumulation place of the pollutant particles in the eukaryote cell (important aspect in the phytoremediation species), a/o. Bioindicators for the evidence of anthropic pollutant factors (heavy metals and radionuclides in this study) are represented by biological species, or certain features of theirs, altered under the action of the two anthropic factors (alteration of the genetic material, of the nucleus ultrastructure, a/o).

Keywords: heavy metals; radionuclides; biological effects; Jiu river basin.

Rezumat. Investigaţii pentru detectarea perturbării ecosistemelor sub influenţa factorilor antropici. În bazinul mijlociu al Jiului, industria extractivă şi energetică, au contribuit la modificarea suprafeţei solului şi la formarea haldelor de steril şi cenuşă, cu un conţinut ridicat în metale grele şi radionuclizi. Pentru a limita efectele negative ale acestor poluanţi antropici, a fost analizată o metodologie de investigaţie a suprafeţelor afectate. Aceasta implică: analiza cantitativă şi calitativă a prezenţei radionuclizilor şi metalelor grele în sol şi plante; stabilirea speciilor pionier şi a speciilor indicator, precum şi a speciilor rezistente şi tolerante la acţiunea factorilor de stres, specii utilizate în refacerea ecosistemelor afectate; metode de depistare a speciilor cu rol în fitoremediere, precum şi metode de analiză a efectelor acestor factori de stres, care permit menţinerea lor la un nivel tolerabil. Dintre metodele de analiză, se remarcă: testele de creştere (în special la experimente din laborator), analize citogenetice (modificări structurale şi metabolice ale cromosomilor şi ale fusului de diviziune), analize structurale şi ultrastructurale pentru a evidenţia efectul factorilor de stres, leziunile induse şi interacţiunea celulei eucariote cu factorii de stres; căile de pătrundere şi locul de stocare a particulelor poluante în celula eucariotă (aspect important în cazul speciilor cu rol în procesul de fitoremediere), ş.a. Bioindicatori pentru evidenţierea efectului factorilor de stres antropic (metale grele şi radionuclizi) pot fi reprezentaţi prin specii biologice, sau prin anumite caracteristici ale acestora, care sunt modificate specific sub acţiunea celor doi factori de stres antropic consideraţi (alterări la nivelul materialului genetic, caracteristici ultrastructurale ale nucleului ş.a.).

Cuvinte cheie: metale grele; radionuclizi; efecte biologice; bazinul Jiului.

INTRODUCTION

The human society development represented both by its numerical increase and the rise in social necessities, leads to intensive exploitation of natural resources, as well as to the release of different matters in the environment, which adulterate the biocoenosis composition. The research performed under natural or experimental conditions proved the existence of numerous species indicating the presence of different toxic substances in the environment, as well as some species with the capacity of catching different toxic substances. They contribute to the 'cleaning' of the environment (CORNEANU *et al*., 2008). The pollution sources can come from different industries (energetic, extractive, chemical, a/o), transportation, agriculture, human agglomerations, a/o.

The extractive and energetic industries from the middle Jiu river basin represent a major pollution source, because large areas are affected by the destruction of the arable level from the soil surface, by bringing and depositing on the soil surface an immense amount of underground matter with a high heavy metal and radionuclide content. The sterile waste dumps resulted from the coal exploitation shape and modify the soil surface, as they have a high heavy metal and radionuclide content, and they are an improper ground for the spontaneous and forest vegetation, for agriculture, for the animal and human health. Moreover, as a result of the working of the two power stations from Rovinari and Turceni, ash sterile waste dumps with a high heavy metal and radionuclide content appear, which also affect the environment and animal or human health.

Different papers pointed out the effects of a high amount of heavy metals or an enhanced activity of different radionuclides on the living organisms. LEHMANN & REBELE (2004) evaluated the tolerance towards some heavy metals (Cu, Zn, Pb and Cd) of the *Calamagrostis epigejos* and *Elymus repens* species, from the neighbourhood of some copper smelters in Poland, farm sewage in Berlin and an unpolluted control. These experiments revealed a different tolerance depending on the species and the considered heavy metal and in all cases a constitutional tolerance towards Pb, more pronounced in *C. epigejos*. Other investigations were performed by BANASOVA *et al*., 2007; CONESA *et al*., 2007;

FROUZ *et al.,* 2008; GOPALAN, 1999; KRISTEN, 1997; LEHMANN & REBELE, 2004; LINDENMAYER *et al.*, 2000; RAJAKARUNA et *al.,* 2011; REMON *et al*., 2005, a/o

This study was developed on the basis of the experimental results obtained in the POLMEDJIU grant, regarding the heavy metal and radionuclide effect on the biocoenoses from the middle Jiu river basin. The installation of vegetation on sterile and ash waste dumps with a high heavy metal and radionuclide content was analysed. The investigations performed in the natural biocoenoses, in the crops, or in the experiment with tester species for stress conditions are also discussed, and an investigation method of the anthropogenic affected areas is proposed. In the bioindicator group, some cytogenetic and structural features are included (*bodyguard* and *NAB's*).

INVESTIGATION TECHNIQUES AND BIOINDICATORS OF POLLUTION

The mutagenic action of the environment can be established and studied in different investigations.

A. Studies performed on biocoenoses, or in crops. A particular case is the pioneer species study.

B. Studies performed under experimental conditions, when the effect of some pollutant factors is analysed. These investigations are usually performed on tester species for mutagenesis.

A. INVESTIGATIONS PERFORMED ON BIOCENOSES AND IN CROPS. PIONEER SPECIES.

As a result of the anthropogenic activity (mining industry, oil derrick eruption, waste sterile and ash dump building, domestic waste dumps, geological activity, a/o), or as a result of natural incidents (landslides, earthquakes, volcanic activity, a/o), the amount of mutagenic agents rises (heavy metals and radionuclides, a/o).

Adulterations of the biocoenosis composition, or changes of biocoenoses (desertification process in Oltenia, a/o) take place, or areas without vegetation, named 'terrenum nudum', can result. Moreover, the presence of a mutagenic factor exceeding a certain value is perceived by sensitive species, or by indicator species. Phenotypical mutations, as well as genetic mutations take place and they can be analysed in the laboratory. The vegetal models, which are sensitive for the detection of the environment agent genotoxicity, can be used as primordial indicators for their detection in soil, water and air (GOPALAN, 1999). Thus, the meristematic and sporogenous tissues of plants generally represent models of response to genotoxicity, similar to those of the embryogenic and spermatogenous tissues of the vertebrates (KRISTEN, 1997).

The evaluation of the environment mutagenesis depends on the stress factor, the affected area, climate, degree of contamination with mutagenic factors, a/o. If a catastrophic situation is not present, the following investigation techniques must be considered.

***Investigations on the soil surface**, to establish the pollution degree, the affected area size and the environment features, possible mutations induced by the stress factors, a/o. If possible, some mutants in the plant and animal populations from the affected area will be identified. In this case an *albino* mutant in *Cardaria draba* (L.) DESV. (Fam. Brassicaceae) was identified on a ground of 'terrenum nudum' type (with a great amount of salts, heavy metals and radionuclides), with revegetation after an oil derrick explosion. On an ash waste dump near CET-Turceni (with a great amount of radionuclides and heavy metals) there were antennal mutants (the antennal article number was affected) in three *Thysanoptera* (Insecta) species: *Neohydatothrips gracilicornis*, *Thrips tabaci* and *Fankliniella intonsa* (BĂRBUCEANU & VASILIU-OROMULU, 2011a, 2011b). The mutants recognized in the first two *Thysanoptera* species represent the first results in the scientific literature.

***The heavy metal content and radionuclide activity** were established in soil (the sterile and ash waste dumps near Rovinari and Turceni power stations, as well as in Control, Argineşti, the 5-20 and 20-40 cm levels), as well as in the main crop plants cultivated on them. The heavy metal content was determined by a spectrophotometer of atomic absorption, in the version with solution atomization in air-acetylene. The radionuclide activity was determined by the Duggan method, in the soil and vegetation samples, which were analysed by a gamma-spectrophotometer with an HPGe detector, with a plane crystal and a Be window (DUGGAN, 1988). The time of sample measuring ranged between 20000s – 40000s. The obtained values were related to 1 kg of soil or 1 kg of green mass.

The recorded values indicated a higher amount of radionuclides in the waste dumps from the Rovinari Station (especially on the ash waste dump) as compared to the values recorded in the Control area (Table 1). The mining excavation brings to the ground surface great amounts of soil with heavy metals and radionuclides. The ash waste dumps (formed from the residues resulted from the lignite combustion) have a higher amount of radionuclides. The presence of high values as compared to the average values for the natural radionuclides and the high values for Cs-137 (usually over 10 Bq/Kg soil) points out an artificial radioactive pollution with different contamination degrees.

***Pioneer species of superior plants**. **Pioneer species** are species which colonize previously uncolonized land, usually leading to ecological succession. They are the first organisms to start the chain of events leading to a liveable biosphere or ecosystem. The ash and sterile waste dumps are primary ecosystems, 'terrenum nudum' respectively. Since uncolonized land may have thin, poor quality soils with few nutrients, pioneer species are often vigorous plants with adaptations such as long roots, root nodes containing nitrogen-fixing bacteria, and leaves that employ transpiration. Pioneer species will die creating plant litter, and break down as 'leaf mould' after some time, making new soil for secondary succession, and nutrients for small fish and aquatic plants in adjacent bodies of water.

After a period of time, through natural processes, there are changes in the biotope. At the same time, the ash and sterile waste dumps are a permanent source of pollution for the nearby places, because they have a high heavy metal and radionuclide a/o content. The pioneer species developed on waste dumps present a natural resistance to radioactivity and to the high amount of heavy metals. In Table 3, some pioneer species from the ash waste dump from Ceplea, near CET-Turceni, with some genetic features, are presented. The degree of polyploidy is a diversification and adaptation to the environment, a reaction to stress factors including. It is natural that all polyploidy species do not have resistance to stress factors. According to RAMSEY *et al.* (1998) "polyploidy is widely acknowledged as a major mechanism of adaptation and speciation in plants". In a recent synthesis, TE BEEST *et al.* (2012) also point out that: "polyploidy has been proposed as an important determinant of invasiveness in plants" (a characteristic of the pioneer species).

Table 1. The soil radionuclide activity (in Bq/kg sol; mean ± SE), in some sites from the middle Jiu river basin (Control and ash or sterile waste dumps from CET-Rovinari, 5-20 cm level). / Tabel 1. Activitatea radionuclizilor din sol (în Bq/kg sol; media ± SE) în unele locuri din bazinul mijlociu al Jiului (Control şi halde de steril şi cenuşă de la Rovinari, orizontul 5-20 cm).

Radionuclide	Control (Argineşti)	Sterile waste dump, Rovinari Station	Ash waste dump, Rovinari station
U-238 (Th-234)	32.09 ± 3.58	30.86 ± 3.53	97.7 ± 10.6
Ra-226	15.9 ± 1.0	22.1 ± 1.31	92.0 ± 4.0
Pb-210	55.8 ± 3.83	36.6 ± 3.29	88.5 ± 6.0
Bi-214	13.0 ± 0.92	29.2 ± 1.42	66.2 ± 4.0
Pb-214	18.9 ± 1.27	25.1 ± 1.31	77.7 ± 3.75
U-235	3.00 ± 0.29	2.15 ± 0.47	5.78 ± 1.67
Ac-228 (Th-232)	25.1 ± 4.21	29.9 ± 2.02	53.48 ± 3.82
Pb-212	31.1 ± 1.13	34.4 ± 1.26	81.67 ± 3.16
K-40	455.8 ± 21.9	401.5 ± 27.6	299.5 ± 3.59
Be-7	< 13.1	< 11.5	< 22.8
Cs-137	19.7 ± 2.93	13.6 ± 1.27	75.8 ± 3.44

For most heavy metals, the recorded values in ash waste dumps were generally higher as compared to similar values from Control and sterile waste dumps (Table 2).

Table 2. The heavy metals content (limits of variability) in some cultivated areas situated on sterile and ash waste dumps and in Control (mg/kg, 5-20 cm level). Tabel 2. Conţinutul în metale grele (limite de variabilitate) în terenuri agricole situate pe halde de steril şi cenuşă şi la Control (mg/kg sol, orizontul 5-20 cm).

Situs	Zn	Cu	Fe	Mn	Pb	Ni	Cr	Co	Cd
Control Argineşti	20.5-61.2	16.4-35.2	25334-36108	578-996	24.7-36.9	42.6-85.8	22.1-46.0	12.4-16.2	Udl
Rovinari-sterile dump	54.8-95.3	16.1-35.4	21912-30207	374-436	19.0-22.0	21.2-55.0	22.0-32.6	9.6-11.5	Udl
Rovinari-ash dump	66.6	52.8	26705	215	38.3	42.6	39.4	11.4	Udl
Turceni-sterile dump	66.0-284	16.6-36.1	12854-30188	550-797	5.1-30.2	17.5-54.6	25.9-47.0	5.2-11.5	0.6-1.8
Turceni-ash dump	45.0-54.7	31.9-36.1	39507-41876	269-321	26.2-36.2	92.1-133.2	10.8-15.5	12.3-16.8	0.2-0.3

Udl – under detection limit.

The analysis of these pioneer species developed on the ash and sterile waste dumps from the middle Jiu river basin (CORNEANU *et al.,* 2010a) points out the predominance of the polyploidy forms and/or polyploidy series (*Convolvulus arvensis, Erophila verna, Stelaria media, Thlaspi perfoliatus*, a/o), species with specific genetic features (diffuse centromere in *Juncus inflexus*), as well as species with a natural resistance determined by the presence of some genes involved in the phytoremediation process, through synthesis of bioactive substances, a/o (*Plantago lanceolata*, *Taraxacum officinalis, Thlaspi perfoliatum*), a/o. These waste dumps are subsequently colonized with shrubs and trees (*Salix* sp., *Populus* sp., a/o), the cuttings being brought by the wind or birds.

The same species were also met on another nude terrene of about 80 ha (resulted from oil derrick mud and other petroliferous products), caused by an oil derrick explosion at Moşneni-Almăj (Dolj district; POPESCU *et al.,* 1998), as well as around the oil derrick from the Moreni Oil Extraction Site (Dâmboviţa district; DUMITRU *et al.,* 2000).

The pioneer species present some biological features which ensure their resistance on *soils* with a high amount of heavy metals and radionuclides (as well as to other stress factors), such as: small size chromosomes, sometimes with a diffuse centromere; the presence of a polyploidy series, or other ploidy forms (aneuploidy, a/o); the presence of protective substances (traps for free radicals, substances involved in the annihilation of some chemical mutagens, a/o); specific genes involved in the synthesis of some substances with a chelating role which bind with the pollutant factor (*phytochellatine*, *metalthioneine*), a/o. The research performed by WOŹNIAK *et al*. (2005) in the Upper Silesia, Poland, regarding the vegetation development on coal mine heaps, points out pioneer species of similar plants. LEI and DUAN (2008) studied the restoration potential of the pioneer plants developed on lead-zinc mine tailings in China.

The main investigations performed in the pioneer species analysis are similar to the investigations performed in the experiments with genetic tester species (presented in section B). They are of a different nature: phenotypic and biometrical (growth tests), cytogenetic, biochemical, flow-cytometry, structural and ultrastructural investigations, a/o. The vegetation succession on soils contaminated with heavy metals was performed by REMON *et al.* (2005) on a former metallurgical landfill in France; CONESA *et al.* (2007) in mine tailings from Cartagena, Spain; FROUZ *et al*. (2008) in a coal exploitation from the Czech Republic, a/o.

Table 3. Pioneer species of plants, met on ash and sterile waste dumps, resistant to a high heavy metal and radionuclide content. / Tabel 3. Specii pionier de plante, întâlnite pe haldele de cenuşă şi steril, rezistente la un conţinut înalt de metale grele şi radionuclizi.

Genotype	Family	Basic chromosome number (x)	Somatic chromosome number (2n)	Observations
Arenaria serpyllifolia	*Caryophyllaceae*	8	2n=4x=32	-
Atriplex patula	*Chenopodiaceae*	9	2n=4x=36	-
Berteroa incana	*Brassicaceae*	8	2n=2x=16	Bioactive stuff
Calamagrostis epigeios	*Chenopodiaceae*	7	2n=4x=28	Risomes
Carduus acanthoides	*Asteraceae*	11	2n=2x=22	Flavonoids
*Convolvulus arvensis***	*Convolvulaceae*	5	2n=10x=50	-
Cynodon dactylon	*Poaceae*	9	2n=4x=36	-
Capsella bursa-pastoris	*Brassicaceae*	8	2n=4x=32	-
Descurainia Sophia	*Brassicaceae*	7	2n=4x – 8x	-
Erophila verna	*Brassicaceae*	6, 7, 8	2n=2x - 10x	Cytotypes
*Juncus inflexus**	*Juncaceae*	10	2x, 4x, 8x	Aneuploids
*Lepidium ruderale***	*Brassicaceae*	8	2x, 4x	Aneuploids
*Medicago lupulina***	*Fabaceae*	7, 8	2n=4x=28-32	-
*Mentha pulgerina***	*Lamiaceae*	5	2n=4x=20	-
Phytolacca americana	*Phytolaccaceae*	18	2n=2x=36	Bioactive stuff
*Polygonum aviculare***	*Polygonaceae*	10	2n=6x=60	-
*Pimpinella saxifraga***	*Apiaceae*	9, 10	2n=4x=36-40	-
Solanum nigrum	*Solanaceae*	12	2n=6x=72	
Stelaria media	*Caryophyllaceae*	10,11,12,13,14	2n=40,42,44	Aneuploids
Taraxacum officinalis	*Asteraceae*	8	2n=3x=24	Bioactive stuff
Thlaspi perfoliatum	*Brassicaceae*	7	2x, 6x, 10x	Specific genes

Legend: *- diffuse centromere; ** - facultative halophyte

***Indicator species, tolerant and resistant to pollution**.

An '**indicator species** is any biological species that defines a trait or characteristic of the environment' (FARR, 2002). Regarding the modification of the abiotic conditions and/or changes in ecological processes, an indicator species is an indicator of environmental changes, such as modification of the climate, the presence of a toxic element, a/o (modified after LINDENMAYER *et al.,* 2000). According to MCGEOCH (1998), 'a **bioindicator** can be loosely defined as a species or a species group, that reflect the abiotic or biotic state of the environment, represent the impact of environment change on a habitat, community or ecosystem, or indicate the diversity of other species' (reproduced after AVGIN & LUFF, 2010). Thus, in present (2012 year) is considered that, **bioindicator** is a species whose death or unusual behaviour may indicate the presence of toxic substances which were not detected by test instruments. The investigations performed by different authors pointed out that every individual manifested a certain response to stress factors and in some cases induced characteristic adulterations. For these reasons, the 'indicator' term can be re-defined. Thus, **bioindicator** represents a species or its different biological features (structural or ultrastructural, cytogenetic, biochemical, a/o), which offer information about the presence of some stress factors in the environment, as well as the active response (defence, survival, a/o) of the considered organism.

Tolerance is the property of an organism to identically react to the action of some mechanical, physical, chemical, medical, a/o factors in increased doses. The medical tolerance is the capacity of an organism to support increased doses of drugs, or of other exogenous factors, obtaining the same effect.

Shade-tolerant species are species that are able to thrive in the shade and in the presence of natural competition by other plants. **Shade-intolerant species** require full sunlight and little or no competition. **Intermediate shade-tolerant** trees fall somewhere in between the two.

Resistance is the degree in which an organism is unaffected by different stress factors from internal or external environment. Resistance is dependent on the genetic constitution of the organism, as well as on other factors, such as biochemical factors, the degree of adaptation to the environmental conditions, a/o. The plant resistance to different stress factors can be a breeding factor. As a result of selection of the most resistant individuals from the crops on the sterile waste damps, for 30 years, landraces were obtained in: *Phaseolus vulgarius*, *Pisum sativum*, *Lycopersicon esculentum*, *Capsicum annuum*, *Allium sativum*, *Cucurbita pepo*, *Cucurbita pepo oblonga*, *Cucumis sativus*, *Zea mays*, *Avena sativa*, *Ocimum basilicum*, a/o (CORNEANU *et al.,* 2011). They present a high resistance to heavy metals and radionuclides.

***Mushrooms,** especially in their mycorrhizal habitat, are involved in long-term accumulation of radionuclides and their transfer from the alimentary chain, regulating their accumulation in host plants (DIGHTON et al., 2008). The radioresistance of some mushroom species is linked to the melanin presence, which can absorb all types of electromagnetic radiations (MEREDITH & SARNA, 2006). The melanised mushroom species around Chernobyl reactor responded to the ionizing radiation presence through accelerated growth (DADACHOWA & CASADEWALL (2008). Their resistance in an environment with a high heavy metal and/or radionuclide content, as well as their involvement in the phytoremediation process, are a result of the presence of genes from the phytochelatin and metallothionein group. The research performed by COLLIN-HANSEN *et al.* (2007) pointed out the presence of phytochelatines in the *Boletus edulis* mushroom. On the sterile and ash waste dumps around Rovinari and Turceni power stations, the *Agaricus arvensis, Boletus edulis, Coprinus comatus, Macrolepiota procera, Pleurotus eryngii, Suillus luteus*, a/o species of macromycetes were met. They have the capacity of accumulating heavy metals and/or radionuclides (DOĞAN *et al*., 2006; BUSUIOC *et al*., 2008; FALANDYSZ *et al*., 2008; MICHELOT *et al*., 1999, a/o).

Lichens vegetating on a substrate extract different substances from it and from the air, pollutant elements including (NIEBOER *et al*., 1972; PUCKET *et al*., 1973; BANASOVA *et al*., 2007, a/o). Thus, some lichen species adapted on media with a high heavy metal, radionuclide and other abiotic pollutant content, becoming indicator species for their presence in the environment (ASLAN *et al.,* 2011; RAJAKARUNA *et al*., 2011; STATE *et al*., 2010; ZSIGMOND & URAK, 2011, a/o).

Insecta, Thysanoptera. Investigations performed in this polluted area led to the establishment of some *Thysanoptera* (Insecta) species with an indicator, tolerant or resistant capacity (BĂRBUCEANU *et al.,* 2011b). Some differences regarding the Thysanoptera species with a specific role in the two polluted areas were also remarked. Thus, on the sterile waste dumps from the power station Turceni, the indicator species were *Frankliniella intonsa* and *Neohydatothrips gracilicornis*, whereas on the waste dumps from the power station Rovinari, there was a single indicator species, *Haplothrips leuchanthemi*. The resistant species were *Chirothrips manicatus* and *Thryps physapus* on the waste dumps from the power station Rovinari, whereas on the waste dumps from the power station Turceni, there were three resistant species: *Chirothrips manicatus*, *Thryps physapus* and *Thrips validus* (BĂRBUCEANU & VASILIU-OROMULU, 2011a).

Investigations of some stress factors affecting the vegetal communities must be completed by the publication of a **Catalogue with indicator, tolerant and resistant superior plant species** to these stress factors. This catalogue must be put at the disposal of the authorized bodies from the affected area (prefect's office, town halls, cadastre offices, environment protection agencies, ROMSILVA, a/o). On the basis of this Catalogue, the plant and animal communities in the affected areas will be restored, using new species as well. Interspecific relations of the news components of the biocoenosis must be established. The features of "tolerant" and "resistant" species in the new environmental conditions (under the action of stress factors) must also be analysed.

B. INVESTIGATIONS PERFORMED UNDER EXPERIMENTAL CONDITIONS.

These investigations and techniques can be applied both to organisms harvested from the affected area and in *ex situ* experiments. These experiments can be performed with ordinary species, or with radiobiological tester species, in experimental culture (on soil surface), or in the mutagenesis laboratory.

In the laboratory, the species are exposed to an abiotic stress factor, or they are cultivated under some conditions from the environment, being cultivated on soil and wetted with water from the affected area, or which reflect a stress factor (a concentration of heavy metals or radionuclides, a/o).

As radiobiological tester species, annual species are recommended, which are sensitive to stress factor action ('a species, sensitive to a stress factor, also manifests sensitivity to other stress factors'), and have a small number of chromosomes in the somatic cells (preferably up to 2n=20), the division cycle quite synchronous, such as: *Vicia faba* (2n=12), *Hordeum vulgare* (2n=14), *Nigella damascena* (2n=12), *Tradescantia* sp. (2n=24), *Allium cepa* (2n=16) or *Allium sativum* (2n=16), **the *Allium t*est** (FISKEJÖ, 1985) being used in the environmental mutagenesis analysis. The tester species, *Allium test* particularly, present some advantages as compared to microbial and animal cells, as being more sensitive to pollutants from the environment (FISKEJÖ, 1985), heavy metals (PANDA *et al*., 1996; PALACIO *et al*., 2005), radionuclides including (CORNEANU *et al*., 2005; CORNEANU *et al.,* 2008, 2009), and being also used for the monitoring of the synergic effects of several pollutants, hydrophilic or lipophilic chemical substances including.

Investigations performed under experimental conditions are of different types.

1. Growth tests, conducted according to the recommendation of authorized bodies (IAEA-VIENNA), 14 days after the germination beginning (for monocotyledonous plants), 21 days for *Allium test*, and 28 days (for dicotyledonous species). In all cases, real leaf length must exceed cotyledonous leaf length. Phenotypical mutants will be also remarked. The individual observations are statistically interpreted.

2. Analysis of cell cycle and of DNA amount (flow-cytometry) performed in the laboratory enables the analysis of the genetic stability of some genetic material resulting from field or laboratory experiments. By this method, it can be established if a potential mutagenic agent affects the genetic stability of a genotype, if different genetic mutations are induced (at chromosomal and genomic level), respectively.

3. Analysis of cytogenetic modifications (chromosomal aberrations, metabolic chromosome modifications, abnormalities of the division spindle, micronucleus presence in the next interphase) is usually conducted in the first mitotic divisions in the radicular meristematic tissue. Investigations are performed in the meristematic tissue of the radicel top, with squash preparations, CARR stain, analysed by an optical microscope.

3.a. Chromosomal aberrations are analysed in all the cell cycle stages, and usually in anaphase and telophase. The main chromosomal aberrations are: **acentric fragments**, **minutes** (very small acentric fragments, up to 1 μm in diameter), **acentric rings**, **bridges** (single, double, parallel or crossed), **arches**, **centric rings**, a/o. In the next interphase, all the acentric fragments and other chromosomal matter without centromere are amalgamated, and micronuclei result. Their numeric analysis offers information about the mutagen process, the efficiency of an anticarcinogen treatment a/o. 'Retarded chromosomes' can result from mistakes during performed of the chromosomal preparations. "Gaps" must be also analysed with much caution: there can be real modifications, uncoloured regions on the chromosome length, or secondary chromosomal constrictions.

3.b. Metabolic modifications of the genetic matter, usually visible in prophase, prometaphase and metaphase, but also in anaphase and telophase (CORNEANU *et al.*, 2010b). They are especially determined by the adulteration of the condensation degree of the chromatin fibres, the modification of the division spindle orientation, the centromere and/or kinetochore inactivation, a/o. They are represented through: **PCC** (**premature chromatin condensation**), **DCC** (**delay in chromatin condensation**), **PCD** (**premature centromeric division**), the presence of some chromosomes or the whole **chromosome set with banding aspect**, **multipolar division spindle**, or its **wrong orientation** (the division spindle in the same cell half, as a result of the mitotic division, sometimes tetraploid cells a/o can result. (CORNEANU *et al.*, 2010b).

4. Biochemical analysis of the main substance groups involved in the active response to modified environment conditions, having as a result the organism survival.

5. Analysis of structural and ultrastructural modifications of different cell organelles (chloroplast, mitochondria, Golgi complex, peroxisome presence, a/o) conducted with a transmission electron microscope (TEM). It can offer information regarding the induced adulterations, the pollutant particles interaction with cell organelles, their spread in the organism, and/or their accumulation place, a/o (CORNEANU *et al.*, 1999). The main structural adulterations of the nucleus induced by stress factors are represented by: invaginations of the nuclear envelope in which cytoplasm can be present, with or without cellular organelles; lysis areas in nucleus or nucleolus, adulteration of the ultrastructural shape of nucleolus, a/o.

6. Analysis of the metabolic and ultrastructural modifications of the nucleus, conducted with a TEM. The metabolic modifications represented by **bodyguard** and **NAB's** are involved in the resistance to the action of some strong stress factors. **Bodyguard** is represented by large heterochromatin blocks, disposed on the inner surface of the nuclear envelope (CORNEANU *et al.*, 1999). Just some genes involved in the cell survival remain active in the rest of euchromatin. Bodyguard is the cell response to the action of a strong stress factor. **NAB's** (**nucleolus associated bodies**) are spherical corpuscles, with a diameter of about 0.5 μm, situated near the nucleolus or in intimate association with it. They are made of chromatin fibres with a diameter of about 24-26 nm (CRĂCIUN *et al.*, 1996). Their presence indicates an intense metabolic activity, normal (meristem or secretor cells, a/o), or pathological (stress factor action). In Fig. 1, there is a nucleus with **NAB's** in *Typha latifolia*, developed on a sterile waste dump from Olari.

7. Environmental pollution and human infertility. In the studied area from the middle Jiu river basin, with a high amount of heavy metals and radionuclides, there were numerous cases of human infertility (female). The cytogenetic analysis of some young women proved some cytogenetic adulterations involved in human infertility: **chromatidal and chromosomal breaks,** anomalous elongation of the secondary constriction from the 1q chromosome (+1q), probably a premature expulsion of supplementary heterochromatin in metaphase, a/o. Some abnormalities in ovogenesis also determined the presence of an ectopic pregnancy, a/o.

8. Soil surface decontamination can be performed by the use of plant species involved in the phytoremediation process. These genotypes extract the excess of heavy metals and radionuclides from the environment. In the POLMEDJIU grant, investigations in herbaceous plants, shrubs and trees were conducted. These investigations identified some new species with a phytoremediation role, not mentioned before in the scientific literature, such as *Taraxacum officinale*, *Quercus rubra*, *Robinia pseudoacacia* var. *oltenica*, a/o. Moreover, these investigations discovered the entrance pathways of pollutant particles in the plant (leaf or root), their spread (mainly through the plant conducting system and through the aeriferous conducting system of the leaf) or penetration of the cell wall (Fig. 2), as well as the interaction of the pollutant particles with the cell organelles. In some species, the pollutant particles are accumulated in the intercellular spaces or are complexed with some chelating substances (synthesized in plants, by some specific genes; Fig. 3) and are neutralized (CORNEANU *et al.*, 2012). Syntheses on the field crops involved in the phytoremediation of contaminated metals were elaborated by many researchers (CORNEANU *et al.*, 2011; VAMERALI *et al.*, 2010; a/o). Sometimes exogenous particles are present inside some organelles, in nucleus including (Fig. 4). SEREGIN & KOZHEVNIKOVA (2008) also conducted investigations in some plants with a natural resistance to a great amount of heavy metals (*Zea mays*, *Lupinus* sp., *Thlaspi perfoliatum*, *T. caerulescens* and *Amaranthus retroflexus*). They analysed the role of root and offshoot tissues in the transport and accumulation of some heavy metals (Cd, Pb, Ni and Sr). The specific features of transport and distribution of metals in the roots of different plant species are essential determinants for the ability of plants to accumulate metals in their organs.

CONCLUSIONS

The coal extractive and energetic industries are the main sources for the environment pollution with heavy metals and radionuclides. They affect the environment quality, human and livestock health. The research performed in the middle Jiu river basin contributed to establish some indicators for pollution with heavy metals and radionuclides. The analysis of the environment pollution must include several investigations.

1. The establishment of heavy metal and radionuclide content in the environment (soil, vegetation, water, air).
2. The vegetation analysis to establish the pioneer species installed on the affected areas, as well as the tolerant, resistant and sensitive plant species.
3. The use of tolerant and resistant species, together with pioneer species for the revegetation (or reforestration) of the affected areas.
4. The establishment of indicator, resistant and tolerant species from other organism groups: mushrooms, lichens, some animals (Insecta, Thysanoptera, in this paper).
5. The use of different methods of analysis of the pollutant effects on the natural biocoenosis, crops or experimental investigations.
6. The main work methods for the analysis of the stress factor effect in the affected areas, are: the analysis of the morphological and structural adulterations of the organisms; growth tests; cytogenetic investigations (numeric, structural and metabolic modifications of the chromosomes, as well as the spindle division activity, a/o), structural, ultrastructural and metabolic adulterations of the nucleus or different cell organelles; analysis of the interaction of the eukaryote cell with pollutant particles; modifications in the human population (the fertility state of women), a/o.
7. The results of these researches must be put at the disposal of the authorized bodies in the affected area.
8. The experimental investigations led to redefining the bioindicator term. Bioindicator is represented both by individuals, and by some of their features, which indicate the presence of a stress factor in the external or internal environment, even without discovering it.

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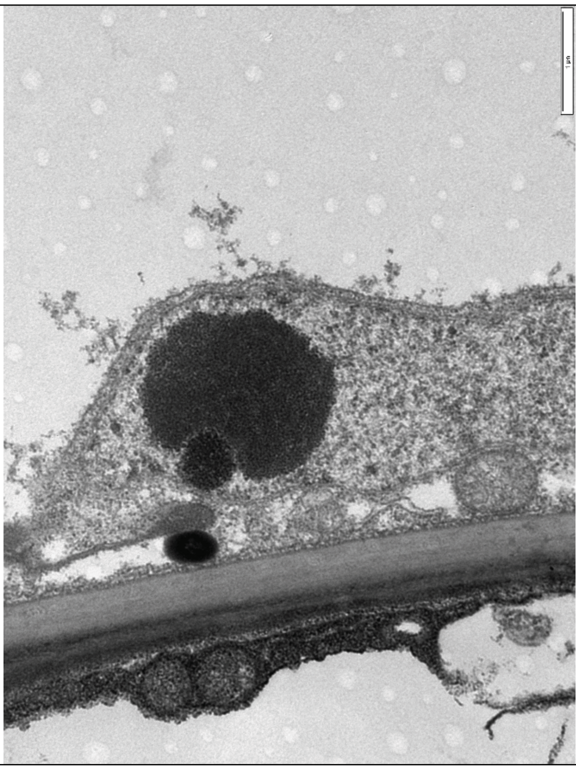

Figure 1. *Thypha latifolia*, sterile waste dump, Olari. Nucleus with NAB's and granular exogenous particles in vacuole Bar = 1 µm. / Figura 1. *Thypha latifolia*, halda steril Olari. Nucleu cu NAB's; particule exogene granulare în vacuolă. Bara = 1 µm.

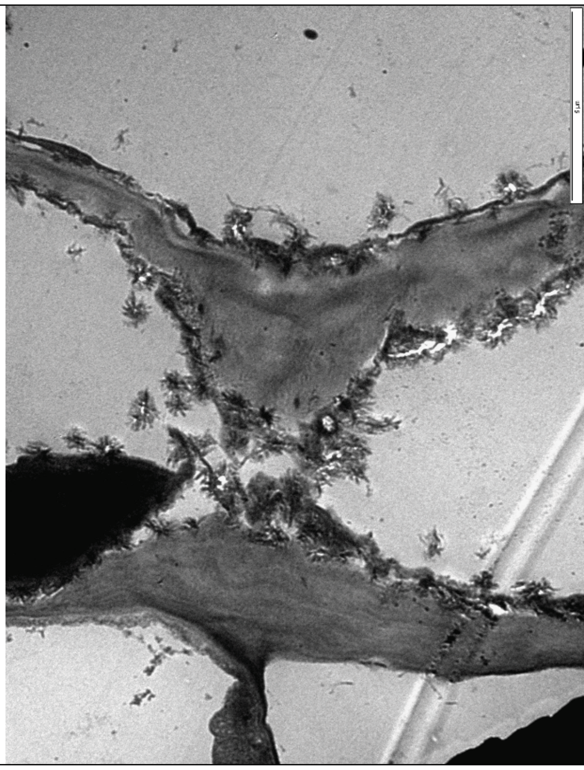

Figure 2. *Helianthus annuus*, sterile waste dump, Olari. Exogenous acicular particles in cell. Bar = 5 µm. / Figura 2. *Helianthus annuus*, halda de steril, Olari. Particule aciculare exogene în celulă. Bara = 5µm.

Figure 3. *Quercus rubra*, sterile waste dump, Rovinari. Vacuole with acicular matter and synthesized chelating substance. / Figura 3. *Quercus rubra*, halda steril Rovinari. Vacuolă cu material aciculare acicular şi substanţa chelatoare sintetizată. Bara = 2 µm.

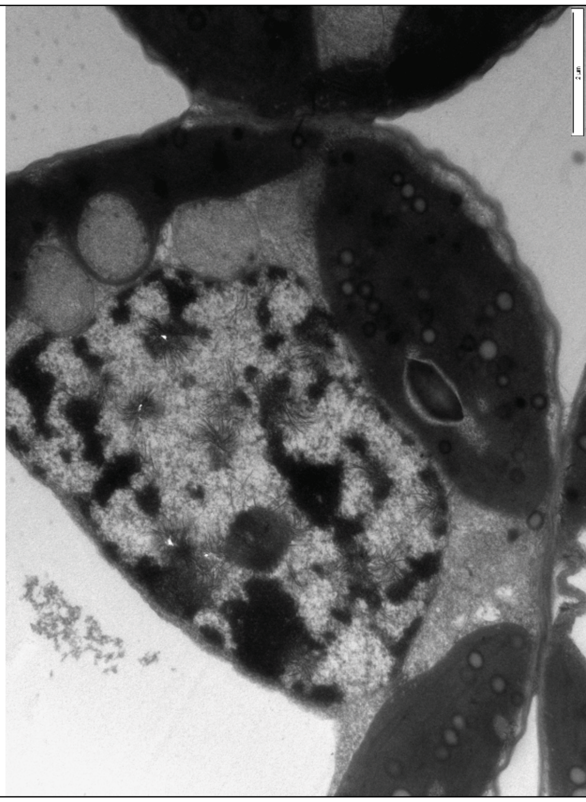

Figure 4. *Helianthus annuus*, sterile waste dump, Olari. Acicular particles among euchromatin fibers in nucleus. Bar = 2 µm. / Figura 4. *Helianthus annuus* halda steril Olari. Particule între fibrele de eucromatină din nucleu. Bara = 2 µm.