

The Dynamics of Anthropogenic Polluted Areas in Kabardino-Balkaria and Chechen Republic

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Abstract—The dynamics of the territories exposed to significant anthropogenic pollution by heavy metals (Kabardino-Balkar Republic) and oil products (Chechen Republic) were studied. The level of soil pollution and its genotoxic effect were determined during the intensive activity of the enterprises - sources of pollution, and 10-17 years after their liquidation. The level of soil contamination with heavy metals was determined using the method of X-ray fluorescence analysis, petroleum products by infrared spectroscopy and benz(a)pyrene by spectrofluorometric analysis at low temperatures. To determine the genotoxic effect of pollution we used plants naturally growing in the study areas (*Taraxacum officinale* Wigg. s.l.). We applied anaphase/telophase method in root meristem cells to identify the frequency of chromosomal aberrations. The level of mutations in plants in contaminated areas increased by 4.5 – 6.7 times during the work of enterprises. After the liquidation of enterprises, the level of pollution decreased. Mutation rate also declined but remained higher than in the clean zones by 3.3 – 3.5 times. Thus, it was found that, despite the decrease in soil contamination, the genotoxic effect persists.

Keywords: *benz(a)pyrene, oil products, heavy metals, soils, dandelion, chromosomal aberrations*

I. INTRODUCTION

The deterioration of the environment due to the receipt of significant amounts of pollutants is one of the main to-date problems. Contaminated habitats have both toxic and genetic effects on living organisms. The toxic effect is usually easily detectable. The genotoxic effect is very difficult to identify, and its consequences may be remote. Therefore, the choice and development of methods for determining the genotoxic influence of environmental pollutants are of particular importance. Numerous studies are devoted to the exploration of the environmental pollution effects on plants. Of particular interest are the *in situ* studies carried out on plants, naturally living in contaminated areas, and plant test-systems. The influence of

radiation accidents [1, 2], heavy metal pollution [3, 4], pesticides and oil products [5-7], industrial enterprises [8], as well as the conditions of urban areas [9-11] was studied using these methods.

The aim of this work was to study the changes in the pollution levels and its genotoxic effects in the territories highly contaminated by heavy metals and products of domestic oil refining. The studies were conducted during the period of intensive activity of enterprises and after its termination.

II. MATERIALS AND METHODS (MODEL)

The investigation of the polluted territory dynamics was carried out in two republics of the North Caucasus – Kabardino-Balkar and Chechen. In the Kabardino-Balkar Republic (KBR), the main pollutants are heavy metals (HM), and in the Chechen Republic (Ch.R) – oil products.

A. Characterization of the studied area and sampling

To study the influence of heavy metal pollution we collected the soil sample and seeds in the disposal areas of the wolfram-molybdenum factory (Kabardino-Balkaria, Russia). The factory was working on wolfram-molybdenum field exploitation and ore concentration from 1940 until 2000 when it was closed. The tailing dump studied here was put into operation in 1967 and it was located at the altitude 1000-1200 meters above the sea level. Areas, which were adequate to the studied areas by geo-climatic conditions, were chosen as the “clean zone” (background landscape). These regions were determined according to slope exposure and plants-identifiers. It is located 20-25 km up the gorge of the Baksan river.

In the case of pollution by the products of oil burning and refining, the soil sample and seeds were collected in the Chechen Republic in the surrounding areas of villages, in

which mini-factories for domestic oil refining were located. These mini-factories are usually built in the marginal regions of settlements or in woodland belts; their number can vary from several tens to several hundred around one village.

Two villages in which primary oil refining was practised for over 13 years were chosen as the objects for investigation. They were Dolinsk (Grozneskiy region) and Mesker-Yurt (Shalinskiy region). These territories were selected for analysis because they are located in the same natural-climatic region, are characterized by similar landscape features and similar vegetation. The villages listed above were taken as contaminated area. Village Goity (Urus-Martanovskiy region) that is known to devoid of this industry and belongs to the same natural-climatic region has been chosen as "relatively clean" zone. It was named "relatively clean" because benz(a)pyrene content in soil was 0.03 mg/kg, which is higher than the legal limit (0.02 mg/kg).

The plants were collected as follows: a square territory with the dimensions $10\ 000 \pm 200\ m^2$ was virtually marked off, and then the whole territory was divided along diagonal on areas of approximately $50\ m^2$. In each area, we selected 10 plants of approximately the same habits at the stages of flowering and seed ripening and collected seeds from these plants. Altogether, in each object, we collected seeds from at least 50 plants and then mix them.

B. Chemical analysis

We determined the level of soil contamination. Soil samples were taken on terraces of dump area (KBR) or in the territories located approximately $100 \pm 20\ m$ apart of the oil-refinery plants (Ch.R) in dry summer days, the same when the seeds of wild plants were collected.

The level of heavy metals in the soil we studied was determined using x-ray fluorescence analysis. To determine oil products, the method of accelerated determination of oil content in various natural environments (waters, soils) by infrared spectroscopy was used. The method of spectrofluorometric analysis at low temperatures was used to determine the content of benz(a) pyrene.

C. Tests procedure

To determine the genotoxicity of soils we used dandelion (*T.officinale*) naturally growing in the study areas. The collected seeds were air-dried and stored in marked paper bags. For cytogenetic studies, the shelf life of seeds was the same for all items and was one month. It is important that the seeds collected in "clean" and "contaminated" zone should have the same time of storage.

Seeds were germinated in glass Petri dishes on filter paper soaked with tap water in a thermostat at $+26^{\circ}C$. Germination time varies from 7 to 10 days. When necessary, the filter paper was additionally moisturized. The roots 10-15 mm long were fixed in a mix of ethanol and glacial acetic acid (3:1) for at least 2-3 h. For long-term storage, the fixed material was thoroughly washed in distilled water and transferred to 70° alcohol. It can be stored up to 1-2 months in the refrigerator at $4^{\circ}C$. The fixed seedlings were stained in acetocarmine (2% solution of carmine in 45% acetic acid) in a water bath during 10-12 min. Temporary squashed slides were prepared from root meristem according to the standard method [6, 12]. At least 1000 anaphases have been scored. Chromosome

fragments, single and double "bridges" were registered. The percentage of abnormal anaphases to the total number of scored anaphases was calculated.

D. Statistical processing

To determine the reliability of the observed differences we used Fisher's conversion for the match against shares [13]

III. RESULTS AND DISCUSSION

A. Heavy metals

The main source of heavy metal pollution in the Kabardino-Balkar Republic is the Tyrnyauz tungsten-molybdenum factory. During the activity millions of tons of waste were accumulated in the dumps and tailings of the factory, containing a range of HM 1st and 2nd class of danger. The first studies of the pollution level and genotoxic effects of tailings were carried out in 1997 when the remediation was not completed [4]. During this period the factory operated. The upper terraces and the open part of the tailing dump gave a huge amount of toxic dust that spread over long distances and settled on the plants. Table I presents data on the content of heavy metals in soils and chemozem of the tailing dump. HM were determined both in the pulp discharged from the factory (chemozem) and in the thin (5-20 cm) layer of soil. This soil was poured on chemozem during the remediation.

As can be seen from Table I, the concentrations of Fe, Mn, Zn, As and especially Mo and W are increased in chemozem and soil from the tailing dump. In these areas, dandelion seeds were collected. In the seedlings of these seeds we determined the level of chromosomal aberration (Table II).

Remediation at the tailings dump was completed in 2005. The same studies of the mutation level were conducted at the same areas in 2014 (17 years after the first studies) (Table II). As it is known, HMs in soils have extremely low mobility and are not removed from biogeocenoses. So, we did not re-determine the content of heavy metals in soils.

TABLE I. THE CONTENT OF HEAVY METALS IN TAILING DUMP OF THE FACTORY AND CLEAN ZONE OF THE KBR (G/KG).

Element	Clean zone	Soil of the tailing dump	Chemozem of the tailing dump
Fe	104.2	127.16	124.28
Al	58.55	55.67	26.99
Ti	12.01	10.73	6.20
Mn	2.16	8.28	9.43
Sr	1.17	2.21	1.33
V	0.48	0.41	0.25
Zn	0.40	0.915	2.05
Cu	0.39	0.39	0.38
Pb	0.12	0.09	0.285
Cr	0	0.14	0
Mo	0	0.27	0.71
As	0	0.18	0.58
W	0	0	4.144

After the termination of factory activity and the completion of remediation, the frequency of mutations in plants living in tailings decreased. In 1997 the frequency of chromosomal aberration (CA) in plants of the contaminated zone exceeded that in the clean zone by 5.0 times, and after 17 years - by 2.1 times. The differences between the

frequency of mutations in the clean zone in 1997 and 2014 are not statistically significant.

TABLE II. THE LEVEL OF CHROMOSOMAL ABERRATION IN ROOT CELLS OF PLANT SEEDLINGS *T. OFFICINALE*.

Variant	No of cells (ana/telo)	Cells with aberrations	Aberrations (%)	P
1997				
Clean zone	1033	41	3.97	
Tailing dump	1007	194	19.72	≤0.001
2014				
Clean zone	707	20	2.83	
Tailing dump	745	45	6.04	≤0.01

TABLE III. TYPES OF CHROMOSOMAL ABERRATIONS IN ROOT CELLS OF PLANT SEEDLINGS *T. OFFICINALE*

Variant	No of cells (ana/telo)	Cells with fragments	%	Cells with «bridges»	%
1997					
Clean zone	1033	34	3.23	7	0.61
Tailing dump	1007	152	15.03	42	4.11
2014					
Clean zone	707	16	2.26	4	0.56
Tailing dump	745	31	4.16	14	1.88

Since the types of chromosomal aberrations recorded in ana/telophase are quite diverse and of different origin, they are shown in Table III. Abnormal anaphases possess acentric fragments and “bridges”. Fragments of variable sizes emerge as a result of deletions and chromosome lagging during their movement to poles. Joining of two centromere-containing fragments leads to the formation of the dicentric chromosome, which is affected by two mitotic centres and, being stretched between two daughter groups of anaphase or telophase chromosomes form a “bridge”. Depending on the type of chromosome damage, different types of “bridges” may occur. Re-joining of two broken sister chromatids led to the formation of a chromatid (usually single) “bridge”, whereas lateral re-joining of two broken chromosomes led to the formation of a chromosomal (usually double) “bridge”.

Among all types of the induced alterations the highest increase was observed in the frequencies of fragments. The decrease in both types of aberrations in 2014 was approximately proportional.

After the remediation in the tailing dumps, the dust pollution of the territory stopped. The frequency of mutations in the seed offspring of plants growing in the contaminated area also decreased. But, nevertheless, in the territories exposed to significant pollution of HM a hidden genetic impact on plants remained, despite the improvement of the ecological situation.

B. Oil products

In the Chechen Republic the main pollutants of the environment are the products of domestic oil refining in mini-factories. The deterioration of the environment in the Ch. R is due to the widespread practice of uncontrolled domestic oil production in the region and its processing in mini-factories. The total number of such factories reached 15 000 in 1994-2005. It is known that the products of low-temperature oil refining and combustion are polycyclic aromatic hydrocarbons (PAHs) - known mutagens and carcinogens [14-16]. They are very slowly metabolized by microorganisms and plants [17, 18]. On the territory of the Ch. R the similar work was carried out in the most polluted settlements, where dozens of mini-plants were located. The level of soil contamination by oil products and genotoxicity of contaminated soils were studied using the same species *T. officinale* [5]. Table IV shows the concentrations of oil products and benz(a)pyrene in soils during the existence of mini factories and after their liquidation.

During the period from 2002 to 2012 mini-factories were liquidated almost the entire territory of the Chechen Republic. Nevertheless, the soils of the contaminated areas are characterized by an increased level of oil products and benz(a)pyrene compared to the soils of the relatively clean area. During this period, there was a decrease in the level of pollution, however, the content of benz(a)pyrene exceeds the maximum allowable concentration (MAC) in Dolinsk by 3 times and in Mesker-Yurt by 4.8 times.

The results of the study of the genotoxic effect of soils using *T. officinale* are given in Tables V and VI. In 2002, the frequency of mutations in the contaminated zone exceeded that in the clean zone by 4.5-5.3 times. 10 years after the liquidation of pollution sources, this index decreased to 3.5 times.

TABLE IV. THE CONTENT OF OIL PRODUCTS AND BENZ(A)PYRENE IN THE SOILS OF THE STUDIED SETTLEMENTS OF THE CHECHEN REPUBLIC

Site of soil sampling	Oil products (%)		Benz(a)pyrene (mg/kg)	
	2002	2012	2002	2012
Goity	0.02	0.04	0.03	<0.001
Mesker-Yurt	1.10	0.69	1.83	0.37
Dolynsk	1.56	0.86	0.15	0.05
Maximum allowable concentration	0.80±0.20		0.02	

TABLE V. CHROMOSOMAL ABERRATIONS IN ROOT CELLS OF PLANT SEEDLINGS IN *T. OFFICINALE* COLLECTED IN 2002 AND 2012.

Site of soil sampling	Year	No of cells (ana/telo)	Cells with aberrations		P
			No of cells	%	
Goity	2002	1056	24	2.27	
	2012	1141	24	2.10	
Mesker-Yurt	2002	1012	204	10.28	<0.001
	2012	1376	101	7.37	<0.001
Dolynsk	2002	1044	126	12.07	<0.001
	2012	1241	88	7.09	<0.001

Reduction of all types of CA occurred uniformly (Table. VI). The content of oil products in 2012 was below the MAC value. It can be assumed that the main factors causing mutations are polycyclic aromatic hydrocarbons, and in particular, benz(a)pyrene.

Thus, in the Chechen Republic the level of soil contamination with oil and PAH decreased after the elimination of mini-factories, although it still exceeds the MAC value. Also, the frequency of mutations in plants growing in the contaminated zone decreased, but the genotoxic effect remained.

TABLE VI. TYPES OF CHROMOSOMAL ABERRATIONS OF THE DANDELION (*T. OFFICINALE*), GROWING IN THE OIL-CONTAMINATED SOILS (2002 AND 2012)

Site of soil sampling	Year	No of cells (ana/telo)	Cells with fragments		Cells with «bridges»		Cells with «duble bridges»	
			No of cells	%	No of cells	%	No of cells	%
Goity	2002	1056	19	1.78	4	0.38	1	0.09
	2012	1141	20	1.75	4	0.35	0	0
Mesker-Yurt	2002	1012	79	7.81	21	2.07	4	0.39
	2012	1376	77	5.52	18	1.31	6	0.44
Dolynsk	2002	1044	91	8.72	27	2.59	8	0.77
	2012	1241	73	5.88	12	0.97	3	0.24

IV. CONCLUSION

A. Heavy metals

After the termination of the work of the wolfram-molybdenum factory and the completion of remediation in the tailing dump, dust contamination of the territory stopped. The level of soil contamination has not changed. The frequency of mutations in plants of wild flora growing in the contaminated area decreased, but the genotoxic effect remained.

B. Oil products

As a result of the liquidation of mini-factories for domestic oil refining, the level of soil pollution with oil products and PAH decreased in Chechen Republic. The frequency of CA in plants growing in contaminated areas also decreased, but the genotoxic effect remained.

Thus, the hidden genetic influence on plants has been preserved in the territories that have undergone significant pollution of both HM and PAH despite the improvement of the ecological situation.

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