NATURAL COMMUNITIES OF URANIUM MINING IMPACTED AREA IN THE VICINITY OF THE SENOKOS VILLAGE

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ABSTRACT
Uranium containing wastes accumulated during mine activities have resulted in a multitude of contaminated sites in Bulgaria. The lack of biomonitoring programs limits the environmental impact assessment of uranium impacted areas. The aim of the study was to investigate the health of natural terrestrial (vegetation, soil bacteria and millipedes) and freshwater (macrozoobenthos) communities in the impacted area of former uranium mine Senokos. The mine is one of the forty-nine former uranium mines in Bulgaria, reclaimed in the beginning of the 90’s, but the reclamation was compromised due to surface erosion of the protective layers. The vegetation is typical for the region and uranium pollution has not caused any significant adverse affects on it. Adverse affects on soil bacterial communities are recorded only to their activity, but not to the abundance. Soil millipedes are in low density dominated by Pachyiulus cattarensis (Latzel 1884). The benthic community of Luda River is influenced by both uranium loaded sediments and infiltrate water from the mine.

Keywords: benthic community, millipedes, soil bacterial communities, uranium pollution, vegetation

Introduction
Contamination of large areas by mining activities is widespread environmental problem in Bulgaria. Not all impacts of uranium mining on the environment are related to the radionuclide content alone. Most of the adverse affects recorded on the biota are due to chemical toxicity of uranium, heavy metals, non-metallic minerals and compounds used in uranium processing (sulfuric acid, kerosene), acid mine seepages. In many countries the impacts of non-radiogenic contaminants were investigated in Environmental Impact Studies for uranium mine development proposals.

Uranium mining in Bulgaria started in 1942 at Buhovo site, near Sofia. In the 1980’s twenty three ore deposits were mined by conventional underground mining techniques, seventeen by in situ leaching, and eleven by in situ leaching in combination with conventional underground mining. Since 1990, the mining and processing of uranium ore have undergone a process of liquidation in Bulgaria. Reclamation of mines was undertaken to a different extent and radiological monitoring was introduced as part of the environmental control, but the lack of biomonitoring programs limits the environmental impact assessment. There are not many researches about the impact and the restoration possibilities of the abandoned uranium mines on the terrestrial and freshwater communities in Bulgaria.

The aim of this study is to assess the changes in terrestrial and freshwater communities in the impacted area in the vicinity of uranium mine Senokos.

Materials and Methods
Uranium mine Senokos is located at the watershed between the rivers Luda and Senokoska and at 1 km west-northwest of Senokos Village (municipality Simitli, district of Blagoevgrad). The mining in the region started in 1988 and continued until 1991 as an open mine ore extraction in the upper reaches of the Luda River. The mine was rehabilitated in the beginning of the 90’s, but the lack of maintenance has led to intense surface erosion of the protective layer and washout of radionuclides. The surface runoff from the mine drains toward Luda River, but part of it seeps to the Senokoska River basin.

The changes in terrestrial and freshwater communities in the impacted area in the vicinity of uranium mine Senokos...
were assessed by different methods. All researching were
done during April – October 2009.

Environmental variables
The pH (H₂O) was measured by HANA pH-meter
(soil:liquid, 1:5). The contents of inorganic nitrogen (NH₄-N and
NO₃-N) and phosphorus (PO₄-P) were determined
spectrophotometrically in water extracts of soil samples (1:10
soil:water) (11), (16). Organic matter was measured
following Turin method (10). Radioactivity of U235 and
U238 as well as of some daughter isotopes of their decay
series were analyzed by γ – spectrophotometer CANBERRA
(CANBERRA PACKARD, Belgium).

Bacterial abundance (BA)
Bacteria were counted in 0.9% NaCl extracts of soil samples
preserved with prefiltered formaldehyde (final concentration
2% v/v) (7). Subsamples (25-50 ml) were filtered through 0.2
μm pore size black polycarbonate filters (Nucleopore, 25 mm
diameter) and stained with acridine orange (0.001% final
concentration) according to (9). Samples were counted using an
epifluorescence microscope. Minimum 300 bacteria were
counted on at least 10 fields of the filter and were calculated
according to (7).

Dehydrogenase activity (Dha)
Dehydrogenase activity was assayed under standard
conditions by the method of reduction of 2-(4-iodophenyl)-3-
(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) to INT
formazan according to (4) as modified by (8). Briefly, soil
samples were placed in test tubes (16x100 mm) and 2.5 ml of
INT-Tris buffer (0.1 M Tris-HCl pH 7.9 containing 2% INT)
were added. The tubes were sealed with plastic stoppers and
incubated at 25°C for 3 hrs. The reaction was stopped by
adding 0.2 ml ice acetic acid. The formazan formed was
extracted three times with 10 ml of methanol for 30 min in
the dark. The absorbance was measured at 485 nm and the
amount of INT reduced was calculated from INT formazan
calibration curve.

Invertebrates
The invertebrates (Myriapoda) were collected by digging (0-
20 cm) from four different points of the mine, following by
hand-sorting. Myriapoda was preserved in 96% ethanol and
were identified in laboratory.

Flora and vegetation
The field researches were done using the both method –
veectors and sample plots. Vectors were done in radius 1 km
east, south and north from the former mine and 2 km west,
because of the small river, which probably can expand the
region of influence along the river basin. The main vectors
were in the following directions: 1. main vectors - west and
east; 2. other vector: south and north-west. A checklist of
floristic diversity was done according (5). Phytocoenological
investigations were made in accordance with the
methodology of floristic phytocoenological school (3), (15).
The vegetation types were described in sample plots in
accordance with the microbiological samples.

Macrozoobenthic invertebrates
Macrozoobenthic samples were collected from seven stations
of Luda River located as follow: above the mine (station
one); below the stulm (station two); after the confluen
c of the two major tributaries of the river (station thee); above
Rakitna Village (station four); right tributary of the river
(station five); at Rakitna Village (station six) and downstream
after Rakitna Village (station seven). Samples of Senokoska
River were collected from four station - above Senokos
Village (station one), at Senokos Village (station two), at
Mechkul Village (station three) and below Mechkul village
(station four). The water quality was assessed by the use of
biotic index – Biological Monitoring Working Party
(BMWP) (1).

Results and Discussion

Environment
The soil in the vicinity of the mine is sandy- loam with low
cation exchange capacity and low contents of organic matter
and inorganic nitrogen and phosphorus (see Table1).

Potassium, sodium and magnesium were the most
abundant elements in the soil. The mine activities lead to
additional accumulation of phosphorus (but no bioavailable)
and sulphur in concentrations 1.7 and 9 times higher than the
background, respectively. The concentration of U235, U238,
and their daughter isotopes were also higher than the
background (see Table 2).

Alpha and beta radiation in the mine territory were 4.3
and 2.1 times higher than the background. The concentrations
of heavy metals were under the thresholds (unpublished data)
and thereby the pollution in the territory of mine could be considered mainly as radiological.

**TABLE 1**
Basic characteristic of the soil in the vicinity of mine Senokos

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Mine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil texture</td>
<td>(%)</td>
<td>sand - 66.19±22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>silt - 31.07±23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>clay - 2.80±2.1</td>
</tr>
<tr>
<td>CEC</td>
<td>(meq/100 g)</td>
<td>11.20±7.8</td>
</tr>
<tr>
<td>Organic matter</td>
<td>(%)</td>
<td>3.87±1.8</td>
</tr>
<tr>
<td>pH(H₂O)</td>
<td></td>
<td>7.2±0.57</td>
</tr>
<tr>
<td>pH(KCl)</td>
<td></td>
<td>6.3±1.2</td>
</tr>
<tr>
<td>NH₄-N</td>
<td></td>
<td>0.13±0.3</td>
</tr>
<tr>
<td>NO₃ – N</td>
<td>(mg/kg)</td>
<td>35 547±2 497</td>
</tr>
<tr>
<td>PO₄-P</td>
<td></td>
<td>141±10</td>
</tr>
<tr>
<td>Ca</td>
<td></td>
<td>6.59±4.6</td>
</tr>
<tr>
<td>Mg</td>
<td></td>
<td>8 193.5±572</td>
</tr>
<tr>
<td>Na</td>
<td></td>
<td>1 112.5±77</td>
</tr>
<tr>
<td>K</td>
<td></td>
<td>6.59±4.6</td>
</tr>
<tr>
<td>Na</td>
<td></td>
<td>1 418±100</td>
</tr>
<tr>
<td>Si</td>
<td></td>
<td>16 653±1162</td>
</tr>
</tbody>
</table>

**TABLE 2**
Radionuclide analysis of soil samples from the region of former uranium mine Senokos

<table>
<thead>
<tr>
<th>Element (Bqkg⁻¹)</th>
<th>Background</th>
<th>Mine</th>
</tr>
</thead>
<tbody>
<tr>
<td>α - radiation</td>
<td>817±50</td>
<td>3 487±25</td>
</tr>
<tr>
<td>β - radiation</td>
<td>2 671±134</td>
<td>5 133±65</td>
</tr>
<tr>
<td>U235</td>
<td>1.8±0.2</td>
<td>11.6±1.2</td>
</tr>
<tr>
<td>U238</td>
<td>38±4</td>
<td>265±25</td>
</tr>
<tr>
<td>Tr230</td>
<td>340±37</td>
<td>765±71</td>
</tr>
<tr>
<td>Tr232</td>
<td>101±5</td>
<td>81±4</td>
</tr>
<tr>
<td>Ra223</td>
<td>3.8±0.4</td>
<td>32±3</td>
</tr>
<tr>
<td>Ra226</td>
<td>96±10</td>
<td>449±44</td>
</tr>
<tr>
<td>Bi214</td>
<td>50±5</td>
<td>218±19</td>
</tr>
<tr>
<td>Pb214</td>
<td>52±4</td>
<td>307±27</td>
</tr>
<tr>
<td>Pb210</td>
<td>76±7</td>
<td>408±37</td>
</tr>
</tbody>
</table>

Based on radiological and chemical data, components of terrestrial and freshwater biota were studied. Soil biota study was focused over abundance and activity of bacterial communities, as well as the species composition and dynamics of millipedes.

**Terrestrial investigations**

**Bacterial abundance and activity**

The higher radiation and the uranium chemical toxicity caused changes in number and activity of soil bacterial communities (Fig. 1).

![Fig. 1](image)

Fig. 1. Average bacterial abundance (ABA, x 10⁸ cell g⁻¹) and average dehydrogenase activity (ADha, µg F g⁻¹ h⁻¹) of soil bacterial communities in the vicinity of uranium mine Senokos

BA from the mine was on average (8.1±6.4) x 10⁸ cell g⁻¹ varying from 2.9 x 10⁸ cell g⁻¹ to 15.2 x 10⁸ cell g⁻¹ and it did not differ significantly from the control plot (on average (7.4±2.4) x 10⁸ cell g⁻¹) (p=0.05). The wide variability of BA and the lack of linear correlation with the level of radioactivity made it unuseful for assessing the impact of pollution on bacterial communities. More sensitive indicator was the bacterial dehydrogenase activity (Fig. 1). The average dehydrogenase activity of control bacterial communities was two times higher (27.5±3.5 µg F g⁻¹ h⁻¹) than those of the mine soil bacterial communities (13.5±5.9 µg F g⁻¹ h⁻¹). According to many authors (12), (14), (19) dehydrogenase activity is a sensitive indicator for assessment of adverse effects of heavy metals pollution. The study proves that the bacterial dehydrogenase activity can be used also as a sensitive bioindicator of radioactive pollution.

**Invertebrates**

Thee species of Myriapoda (Pachyiulus cattarensis (Latzel 1884),
**Megaphyllum hercules** (Verhoeff 1900) and *Lithobius* (*Lithobius*) aff. *forficatus* (Linnaeus 1758) belonging to two families (*Julidae* and *Lithobiidae*) were found during the study. Relative density and abundance are presented in Fig. 2.

![Graph A](image1.png)  
![Graph B](image2.png)

**Fig. 2.** Relative density and abundance of species (1. *P. cattarensis*; 2. *M. hercules*; 3. *L. aff. forficatus*) collected during April-May (A) and September-October (B) 2009

The determinate density was relatively low, but with similar values during the both phases of the investigation. The populations’ dynamics can be considered as a result of radionuclide rather than the heavy metals pollution which was under the thresholds as it was noted above. No data were found about the impact of radionuclide pollution on *Myriapoda*’s dynamics but some authors specified relationship between their abundance and the levels of heavy metals in the soil (18).

The diversity of invertebrates studied was low and unevenly distributed across the sites surveyed. According to some authors (6), (13), (17) invertebrate richness, abundance, biomass, species distribution and communities’ composition depend on factors such as geographical region, climate, environment and soil depth. The low species diversity of the *Myriapoda*, in the area of the mine, probably due to the soil texture (sandy loam) and the low soil depth. The sex structure of millipedes was dominated by males over females in the both phases of investigation.

**Flora and vegetation**

The investigated territory is on the western slopes of Northern Pirin Mt. According to (2), the regional vegetation falls in Upper Struma District, Macedono-Tracian Province, European Deciduous Forests Area. The natural vegetation is mostly deciduous termophilous open oak and oriental hornbeam forests. The secondary communities are the shrubs of *Juniperus oxycedrus* and xerophyllous grasslands.

During the field researches 163 species of vascular plants were found on the investigated territory. They belong to 37 families. The probable number of species will be more than 200. The families with a big number of species are *Asteraceae* (28); *Poaceae* (26); *Fabaceae* (17); *Lamiaceae* (9) etc. These families have more species on light, warm terrains in the plains and in the low parts of the mountains. The geographical influence on the flora is mostly transitional Mediterranean one. There is a medial level of ruderalization. The ruderals are mostly annual species which invade the area of abandoned mine, and penetrate in the natural grasslands after the grazing impact.

The primary vegetation of the region was deciduous forests dominated from *Quercus pubescens*, *Q. frainetto*, *Q. daleuschampii*, *Carpinus orientalis*, in the valleys and along the small rivers – riverine forests of *Alnus glutinosa* and *Salix alba*, and mesophilous forests from *Carpinus betulus*. Some fragments from these forests were survived south from the abandoned uranium mine. On the investigated territory, this vegetation was strongly destroyed from three main impacts:

a) Deforestation and the development of the villages and the agriculture activities. The active grazing changed the natural floristic and ecological peculiarities of the natural and semi-natural vegetation. After the active grazing, some non typical species penetrated into the floristic structure of the grasslands in the region.

b) The new forest plantations from coniferous species – mostly *Pinus nigra*. These artificial forests replaced
the natural deciduous and caused some important transformation into the floristic structures, soil acidity and soil structure.

c) The excavation activities during the period of using of the uranium mine. These activities transformed the terrain and created a big negative form – the mine.

The territory of investigation was divided into three main areas according their degree of negatives impact: 1. Quarry of the abandoned mine; 2: Strongly influenced area below the mine; 3: Weakly influenced territory over the mine – so named „control” area.

The vegetation of quarry (mine) is strongly influenced negatively. There is an attempt for recultivation with forest plantation from Robinia pseudoacacia. This plantation is not a good condition. The walls (slopes) of the quarry are bare ones excluding one pioneer community of Epilobium dodonei. The species is an indicator for acidic screes with mobile gravels. There is a possibility for a natural tolerance of the species to different concentration of heavy metals in these pioneer substrates. The primary stages of recolonization of the stable substrates of the abandoned quarry include mostly annual plant species: Taeniatherum caput-medusae, Vulpia myurus, Trifolium arvense, Erodium cicutarium, Medicago minima, Medicago lupulina. This primary phase is in a process of transformation, which is demonstrated form the penetration of single tufts from perennial grasses – Festuca rupicola, Botriochloa ischaemum, Chrysopogon gryllus and some shrubs – Rosa canina gr., Prunus spinosa.

The above mentioned species are common ones in the sample plots from the microbiological samples. The communities are open ones – the coverage varies between 30 do 90%.

The biggest part of the area below the mine was planted with a forest plantation of Pinus nigra. The surrounding fields are open grasslands dominated from Festuca rupicola, Koeleria splendens, Agrostis castellana, Chrysopogon gryllus and with the participation of Juniperus oxycedrus. The area of the coniferous forest plantation has a big degree of human change (including the decrease of pH of soils) because of foliages of Pinus. The soils are sandy ones. The flora of the plantations is a very poor one. But on the surroundings, there is a primary phases of succession on bare sandy substrates. The vegetation of the sample plots, is mostly dominated from some grasses (single tufts) – Koeleria splendens, Festuca rupicola, Agrostis castellana. Some mosses (Bryum argenteum) and Lichenes (Cladonia foliaceae) are dominants in the plots too. The influence of the territory was caused mostly from the forestry human activities and grazing. Any plant indicators for the influence of uranium in the mine were not established because of big degradation of the natural and semi-natural communities.

The control territory above the mine is close to the Senokos Village. The terrain is covered from open grass-shrub communities with the dominant shrub Juniperus oxycedrus. The vegetation covers open sandy substrates with big single bare rocks. The grasslands are open too – the coverage varies between 50 and 70%. In the vegetation of sample plots, the dominants are Festuca rupicola, Koeleria splendens, Plantago carinata. The bigger level of naturalness is demonstrated from the participation of some Balkan endemics: Silene frivaldskyana, Dianthus gracilis, Hypericum olympicum. In spite of their origin, these species are flexible ones and pioneers too, in some cases. They are typical for sandy, acidic soils and stones, especially on sand-stones. Their participation not only noted the naturalness of the area, as well as the primary geological peculiarities of the area. The control territory is closer to the semi-natural vegetation in the low parts of mountains in South Bulgaria.

Any negative visible effect was not established on the vegetation and flora of the investigated territory from the uranium mine. It probably depends from the long term existing of the dominant types of vegetation – most of them (for example – the plantations of Pinus nigra is 50 years old) exist on the territory before the mine’s exploitation.

The lack of established impact on the vegetation and plants-indicators level is the reason that we made investigation on the pigment content on the leaves of the dominant tree species – Robinia pseudoacacia and Pinus nigra (Fig. 3).

Chlorophyll a (Chl a) and chlorophyll b (Chl b) in Pinus nigra wet mass did not differ significantly between control and mine areas (p=0.05). In a contrast to Pinus nigra, Chl a and Chl b in Robinia pseudoacacia were 6 and 11 times lower in the territory of mine than in the control area. The lower amounts of Chls were probably due to the disturbance of water balance and soil texture rather than the toxicity of uranium. Evidence for this suggestion are the data received...
from the sampling procedure in April 2009 when the contents of Chl a and Chl b in *Robinia pseudoacacia* were higher (1.5±0.35 mg g⁻¹ wet mass and 0.45±0.25 mg g⁻¹ wet mass, respectively).

The low values of the BMWP at station two marks the direct inflow of seepage waters from the stulm to the river. The highest average value of BMWP for Luda River is 104 at station five and the lowest one at station seven (44 with standard deviation of 12.7).

**Freshwater investigations**

**Macrozoobenthic invertebrates**

A total of 73 taxons belonging to 14 benthic groups were found in Luda River during the study. The most abundant benthic groups are: order **Ephemeroptera** (family **Baetidae**, genus *Baetis*) and order **Plecoptera** (family **Nemouridae**, genus *Protonemura*) followed by order **Trichoptera** (family **Rhyacophilidae**, genus *Rhyacophila*; family **Hydropsychidae**, genus *Hydropsyche*) and order **Diptera** (families **Chironomidae** and **Simulidae**). In Senokoska River were found 62 taxons belonging to 12 benthic groups. The most abundant benthic groups are order **Ephemeroptera** (family **Baetidae**, genus *Baetis*; family **Heptagenidae**, genus *Ecdyonurus*) and order **Plecoptera** (family **Leuctridae**, genus *Leuctra*) followed by order **Trichoptera** (family **Rhyacophilidae**, genus *Rhyacophila*; family **Hydropsychidae**, genus *Hydropsyche*) and order **Diptera** (families **Chironomidae** and **Simulidae**). Despite of the close proximity of the rivers in Senokoska River we have not found **Lepidoptera** and **Bivalvia**.

The confluenve to the main river of a number of small unimpacted tributaries determines the quick recovery of the benthic communities below the mine (from station two to station five) (Fig. 4).
The highest values of BMWP for Senokoska River (Fig. 5) are at station one (137 with standard deviation of 21.9) and the lowest – at station three (100 with standard deviation of 19.8). The influence of Senokos and Mechkel villages determine the lower BMWP values at stations two and three. The rapid improvement of the water quality below the mine area have none or little adverse background. The higher radiation and chemical pollution of the river territories is evident of the high rates of self-purification and assimilation capacity of the river.

Conclusions
The radioactivity in the mine territory is increased than the background. The higher radiation and chemical pollution of the mine area have none or little adverse affects on terrestrial and freshwater communities. More impacted were soil bacterial dehydrogenase enzyme complexes and benthic communities. Based on these data we can recommended to use them as sensitive indicators assessing the adverse affects of uranium pollution on natural communities.

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REFERENCES