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THE ECOTOXICOLOGICAL IMPACT OF THE NUCLEAR FACILITIES' EFFLUENT AND Cs-137 ON THE TEST ORGANISM *LEPIDIUM SATIVUM*

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Abstract. *The aim of this study is to assess the impact of nuclear facility effluent during decommissioning process on the seed germination and growth of the test organism Lepidium sativum, as well as to assess the biological effect of Cs-137 on the roots (meristematic cells) and sprouts (cells of parenchyma) of the test organism Lepidium sativum. The investigations were performed on water and the bottom sediment from the monitoring station of Lake Drūkšiai (the cooling-pond of the Ignalina NPP), as well as from the Ignalina NPP's effluent channels IRD-1,2 (industrial rain drainage channel) and TWO (technical water outlet channel) before the INPP shutdown (2007–2009) and after (2010–2015). The ^{137}Cs impact on the test organism using the low activity concentration solutions of 25 and 250 Bq/L was also investigated.*

***Key words*:** Cs-137, ecotoxicological effect, effluent channels, *Lepidium sativum*, the Ignalina NPP

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1. INTRODUCTION

Significant quantities of artificial radionuclides have already entered, and may enter in the future, in the environment. It may happen during operation of NPPs, after their shutdown and during decommissioning process in conjunction with dismantling works, after nuclear accidents (Chernobyl NPP, Fukishima NPP, etc.), and during the implementation of nuclear power development projects. The dispersion of the artificial radionuclides and their distribution in the biotic and abiotic ecosystem components are associated with the ongoing decommissioning of the Ignalina NPP in Lithuania. For the reasons mentioned above, the investigation of the biological effects caused by the artificial radionuclides is always relevant.

It is very important to assess the effects caused by the artificial radionuclides on biota. However, the consequences of the ionizing radiation impact on biota have not been sufficiently investigated yet. So far, the ionising radiation doses are rated only for humans. Therefore, it is necessary to find the assessment ratio of radiation safety criteria for humans and biota. This requires scientific data on ionizing radiation impact on biota at both the organism and cellular level. However, due to the high biodiversity and different environmental conditions the data about biological effects derived by ionising radiation are still limited.

NPPs are the important objects requiring regular and sufficient radioecological assessment. The maximum quantity of some radionuclides intake from the NPP into the cooling pond is a known fact [1, 2]. A variety of the physical, chemical and biological (physiological, genetic, etc.) methods is used for the assessment of radionuclide toxicity. Various biological tests with the so-called test organisms, widely used for the investigations of the toxicity of external factors, can be applied to the studies of the biological effects impacted by radionuclides [3-9].

The aim of this study is to assess the impact of nuclear facility effluent during decommissioning process on seed germination and growth of the test organism *Lepidium sativum*, as well as to assess the biological effect of Cs-137 on the roots (meristematic cells) and sprouts (cells of parenchyma) of the test organism *Lepidium sativum*.

2. OBJECT AND METHODS

In order to assess the radioecological condition of Lake Drūkšiai, the samples of the dominant macrophyte species (*Ceratophyllum demersum, Myriophyllum spicatum, Potamogeton sp.*) were collected with a special hook in the monitoring stations 1, 4, 6 and 7 of Lake Drūkšiai, as well as in the Ignalina NPP effluent channels (the Industrial Rain Drainage channel (IRD–1,2) and the Technical Water Outlet channel (TWO)) in July–August 2007–2015 (Fig. 1). In

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the same time the samples of the bottom sediment were collected using the Ekman bottom grab sampler (the area of sampler 20 x 20 cm).

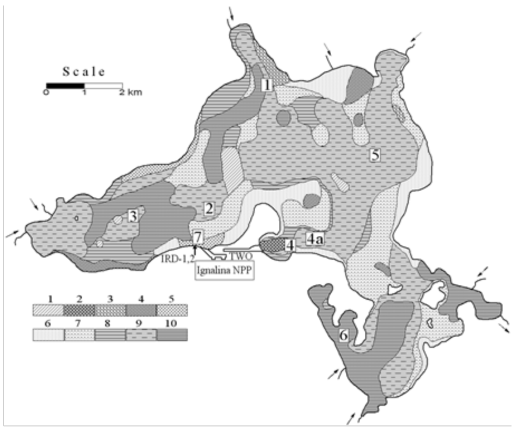

Figure 1. Lake Drūkšiai monitoring stations (used in this work are 1,4,6,7, IRD-1,2 and TWO) and bottom sediment types [10-12]: 1- till (morainic clay, loam and sandy loam), 2 – carbonate sediments, 3 – gravel, 4 – coarse-grained sand, 5 – various-grained sand, 6 – fine-grained sand, 7 – silty sand, 8 – coarse silt mud, 9 – fine silt mud, 10 – silt-pelitic mud.

The samples were dried in the laboratory at 20–22 °C. Macrophytes samples were incinerated in a muffle furnace (430 °C). The bottom sediment samples were homogenized using a ceramic pestle and were passed through sieves with holes of 0.8 mm in a diameter [1, 2].

The measurements of the radionuclides in the samples were accomplished on the gamma-ray spectrometers with Ge(Li) and HPGe detectors at the Centre for Physical Sciences and Technology, Vilnius, and at the Nature Research Centre, Vilnius, as described in [13].

The *Lepidium sativum* toxicity method was used as modified in [14] methodology. The 9 mL water solution (investigated water from monitoring points or used Cs-137 aquatic solutions and distilled water as control) plus 2 pieces of filter paper in case of the bottom sediment, a 5–6 mm layer of the bottom sediment plus 1 piece of filter paper. On this base, 25 seeds of *Lepidium sativum* (density 1 x 1 cm) were put in 5 repeats. The Petri plates were kept in darkness for 48 hours at a constant 24±1 °C temperature. The sample toxicity was assessed according to the seed germination and roots growth. The toxicity level was assessed according to the modified [15] scale. In cases with Cs-137 solutions (25 and 250 Bq/L activity concentration) after the experiment performance, *Lepidium sativum* plants were washed and divided with the scissors into stems and roots. In these parts, the accumulated Cs-137 activity concentration was measured.

For statistical calculations programme STATISTICA six sigma, © StatSoft (2007) was used.

3. Results and discussion

The Cs-137 activity concentrations in macrophytes of Lake Drūkšiai monitoring stations 1, 4, 6 and 7 have little changed (from 5 to 13 Bq/kg d. w.) (Table 1) before the shutdown of the Ignalina NPP (period 2007–2009). After the Ignalina NPP shutdown (2010–2015), Cs-137 activity concentrations in macrophytes of Lake Drūkšiai monitoring stations 1, 4 and 7 were similar to those before the shutdown. Only at the monitoring station 6, the macrophyte Cs-137 activity concentration was <mda after the Ignalina NPP shutdown.

In the Ignalina NPP's effluent IRD-1,2 channel, Cs-137 activity concentration in macrophytes in 2008 was much higher than in 2007 or 2009 (Table 1). In 2008, even Cs-134 was easy detectable in IRD-1,2 channel macrophytes with level of 183 Bq/kg d. w. Meantime Cs-137 activity concentration in IRD-1,2 channel's bottom sediment was much less than in macrophytes. During the investigated period (2007–2015), Cs-137 activity concentration in IRD-1,2 macrophytes differed slightly (Table 1). However, in the bottom sediment Cs-137 activity concentration was higher after the Ignalina NPP shutdown (from 14 to 27 Bq/kg d. w.) than before the Ignalina NPP shut down (from <0,3 to 11 Bq/kg d. w.).

It was found that water of all monitoring stations and effluent channels of the Ignalina NPP had no impact on the seed germination of the test organism before (2007–2009) and after (2010–2015) the Ignalina NPP shutdown. However, during the 2007–2009 period, water of the effluent channels was found to have the impact upon the root growth of this test organism. After the Ignalina NPP shutdown, only in 2010 the water of the effluent channels (IRD-1,2, TWO) was found to be moderately toxic to the root growth of *Lepidium sativum*. In all other cases, after the Ignalina NPP shutdown, the water was slightly toxic to *Lepidium sativum* root growth.

In 2008, 2009 and 2010, only the bottom sediments from the channels IRD-1,2 and at the monitoring station 7 showed a strong and moderate toxicity to *Lepidium sativum* seed germination. In all other cases, the bottom sediment was not toxic to *Lepidium sativum* seed germination. Very strong toxicity of the bottom sediments to the root growth of the test organism was recorded only in IRD-1,2 channels and at monitoring station 7 (the IRD-1,2 impact zone) in 2008, 2009 and 2010 (Table 2). In all other cases, the bottom sediments had moderate and weak toxicity impact on the root growth of the test organism *Lepidium sativum*. The data suggest that the bottom sediments are more toxic to the test organism *Lepidium sativum* than water.

The data show that in 2008, 2009 and 2010 the bottom sediments from IRD-1,2 and at the monitoring station 7 had a strong toxicity impact on *Lepidium sativum*. As already mentioned, during the same period relatively higher activity concentrations of the artificial radionuclides were determined in macrophytes from IRD-1,2 and 7 monitoring stations.

It was found that over the study period the toxic effect of water at Lake Drūkšiai monitoring stations on the test organism *Lepidium sativum* decreased from the moderate (before the shutdown of the Ignalina NPP) to weak (after the shutdown). However, the toxicity of the bottom sediments to the test organism *Lepidium sativum* remained essentially the same throughout the whole study period.

Table 1. Cs-137 activity concentrations (Bq/kg d. w.) in macrophytes and bottom sediment in Lake Drūkšiai monitoring stations and the Ignalina NPP effluent channels

Year	Lake Drūkšiai monitoring stations								Ignalina NPP effluent channels			
	1		4		6		7		TWO		IRD-1,2	
	macro phytes	bottom sediment	macro phytes	bottom sediment	macro phytes	bottom sediment	macro phytes	bottom sediment	macro phytes	bottom sediment	macro phytes	bottom sediment
	Before the Ignalina NPP shutdown											
2007	13±1	184±3	7±1	145±9	6±1	64±2	11±1	6.0±0.4	4±1	1.0±0.1	20±2	<0,3
2008	5±2	133±7	–	76±4	–	–	11±3	19±2	8±2	1.0±0.1	**309±39**	**11±2**
2009	6±1	128±8	7±1	78±4	10±1	184±9	–	31±3	4±1	2.0±0.2	18±1	1.1±0.4
	After the Ignalina NPP shutdown											
2010	8±2	–	15±4	37±4	–	–	–	42±2	10±4	–	19±3	14±2
2011	9±3	115±5	4±1	28±2	<mda	151±14	8±4	12±2	4±2	–	23±5	–
2012	13±4	111±7	<mda	46±6	–	107±10	–	15±3	<mda	–	37±8	19±3
2015	15±4	124±7	–	–	<mda	93±6	–	–	13±5	–	25±4	27±3

Table 2. Toxicity impact of bottom sediments from Lake Drūkšiai monitoring stations and the Ignalina NPP effluent channels on root growth of the test organism *Lepidium sativum*

Monito-ring stations and effluent channels	**Before the Ignalina NPP shutdown**						**After the Ignalina NPP shutdown**							
	2007		**2008**		**2009**		**2010**		2011		2012		2015	
	Root growth, %	Toxi-city	Root growth, %	Toxi-city	Root growth, %	Toxi-city	Root growth, %	Toxi-city	Root growth, %	Toxi-city	Root growth, %	Toxi-city	Root growth, %	Toxi-city
	Lake Drūkšiai													
1	76±3	interme diate	85±3	weak	86±2	weak	–	–	55±2	potent	71±2	interme diate	105±8	Stimu-lant
4	61±4	interme diate	78±3	interme diate	77±1	interme diate	67±1	interme diate	82±2	weak	88±8	weak	–	–
6	68±3	interme diate	–	–	80±3	interme diate	–	–	70±2	interme-diate	75±1	interme diate	96±3	not
7	71±4	interme diate	0	**very toxic**	15±1	**very toxic**	0	**very toxic**	68±3	interme-diate	82±2	weak	–	–
	The Ignalina NPP effluent channels													
TWO	52±6	potent	64±2	interme diate	–	–	–	–	–	–	–	–	–	–
IRD-1,2	80±5	interme diate	38±2	**toxic**	–	–	0	**very toxic**	–	–	65±3	interme diate	82±4	weak

Macrophytes are perennial organisms, however their biomass (stems and leaves) grows and accumulates admixtures only during vegetation period (from spring to ~September). After vegetation period the disintegrating biomass drops to the bottom and forms fraction of bottom sediment composition. It is known, Cs-137 is a hydrophobic element. Entered into aquatic ecosystem it rapidly distributes between bottom sediment and ecosystem biotic components (plants, fishes, insect larvae's, phyto- and zooplankton, etc.) [16]. In their live cycle the macrophytes compete with water and are a barrier retaining Cs-137 (in this case) along water flow in IRD-1,2 channel [17]. In all investigated stations, it is significant differences between Cs-137 activity concentration and toxicity impact of water on *Lepidium sativum* root growth ($p=0.038$).

As discussed above, the increase of Cs-137 activity concentration in IRD-1,2 macrophytes in 2008 evidenced the higher annual discharge rate of this radionuclide. Despite the Cs-137 activity concentration in IRD-1,2 channel bottom sediment was very low (only about 11 Bq/kg d.w., see Table 1), a strong toxic response of *Lepidium sativum* roots to this material was detected in 2008 and two years later (Table 2).

According to macrophytes live cycle for all studied stations was found significant moderately correlation

(R=0.608231, $p<0.05$) between Cs-137 activity concentrations in macrophytes and bottom sediment and the toxicity of bottom sediment to *Lepidium sativum* root growth in next year (the toxicity effect delay - one year). For the IRD-1,2 channel and 7 monitoring station of Lake Drūkšiai it was not found correlation of Cs-137 activity concentration in macrophytes and water or bottom sediment toxicity with delay of one or two years. The correlation coefficients between Cs-137 activity concentration in bottom sediment and toxicity of bottom sediment on *Lepidium sativum* roots grows were following: R=0.48 with delay of one year, R=0.54 with delay of two years. Compared to previous case the correlation coefficients between Cs-137 activity concentration in bottom sediment and toxicity of water on *Lepidium sativum* roots grows were higher: R=0.79 with delay one year, R=0.89 with delay of two years.

It is known, that concentration of stable Cs-133 about 200 μM can be toxic to plants [18]. The data presented in [19] shows that the concentration of Cs-137 of 0.2 nM can have an impact on the electrophysiological parameters of algae *Nitellopsis obtuse*. On other hand, the experiments showed that Cs-137 activity concentration of 400 Bq/L and higher causes a weak stimulated response to *Lepidium sativum* stems and roots [19,20]. In this study, we carried out the experiment with the lower Cs-137 concentration. This was supported by two findings. Firstly, the toxicological response of *Lepidium sativum* to bottom sediment from Lake Drūkšiai was strongly negative during period of 2008-2010. Secondly, Cs-137 activity concentration in the IRD-1,2 channel was 11±2 Bq/kg d.w. in bottom sediment and 309±39 Bq/kg d.w. in macrophytes in 2008 (Table 1).

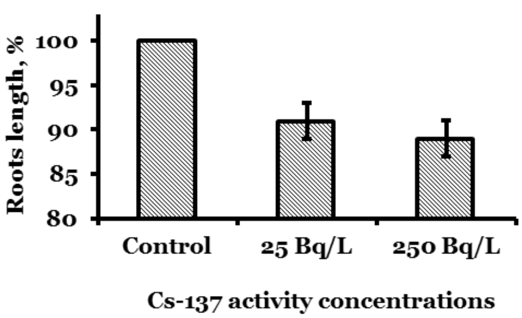

Figure 2. The effect of Cs-137 (activity concentrations 25 and 250 Bq/L) on the *Lepidium sativum* root length

Figures 2 and 3 show the effect of Cs-137 on the morphological parameters of *Lepidium sativum*– root length and biomass of roots and stems. In case of Cs-137, the activity concentration increased 10–fold (from 25 to 250 Bq/L), the root length decreased by 9 and 11 % respectively, as compared to the control. Increasing the Cs-137 activity concentration, stem and root biomass decreased very slightly. In both cases the impact of the increased Cs-137 activity concentration was weak toxicity compare to control and cannot give the reaction observed in natural condition 2008-2010 period in IRD-1,2 channel.

Despite the increased Cs-137 accumulation in roots of the test organism *Lepidium sativum*, at 250 Bq/L activity concentrations, the toxic effects on the root lengths were equal in both cases of 25 and 250 Bq/L activity concentrations. It can be inferred that *Lepidium sativum* is not very sensitive to the Cs-137 impact.

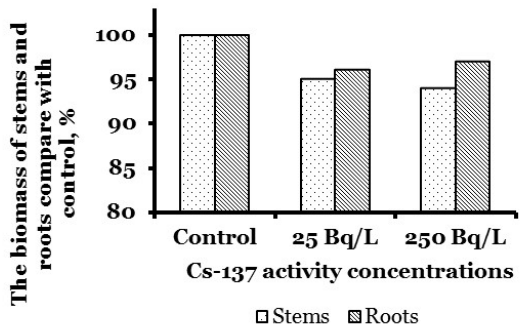

Figure 3. The effect of Cs-137 (activity concentrations 25 and 250 Bq/L) on biomass of roots and stems of test organism *Lepidium sativum*

4. CONCLUSIONS

After the shutdown of the Ignalina NPP Unit One, the activity concentrations of Cs-137 in the bottom sediments and macrophytes of Lake Drūkšiai decreased, changing slightly in the subsequent decommissioning period. The highest Cs-137 activity concentrations in macrophytes were found in the effluent channels IRD-1,2 earlier (especially in 2008).

The assessment of water and the bottom sediment toxicity to the test organism (plant) *Lepidium sativum* in Lake Drūkšiai and effluent channels of the Ignalina NPP shows that the ecotoxicological condition of the lake has changed a little (2007-2015). The exception is IRD-1,2 effluent channel and 7 monitoring stations (impact zone of IRD-1,2) where toxic response existed in 2008-2010.

However, it exists statistically significant moderate correlation between in Cs-137 in macrophytes and bottom sediment and toxicity of bottom sediment to *Lepidium sativum* roots growth with one year delay. Investigated Cs-137 activity concentrations were not toxic to the plant test organism *Lepidium sativum*, i.e. they had no toxic impact on the growth of both roots and stems.

It can be assumed that the elevated toxicity of water and the bottom sediments in IRD-1,2 and 7 at monitoring stations could have been induced not only by the presence of Cs-137, but also by the total sum of the chemicals getting into the coastal area of Lake Drūkšiai from the Ignalina NPP industrial site. Synergetic toxic effects may be caused by the exposure to the whole variety of toxicants.

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