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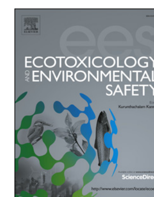
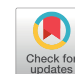


Toxicity assay of lanthanum and cerium in solutions and soil



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ABSTRACT

Lanthanum (La) and cerium (Ce) are one of the most abundant rare earth elements (REEs). In spite of quite extensive studying of the effects of these lanthanides on biota, some contradictions remain in the results. Also little is known about the effect of lanthanum and cerium on plant cells and their mitotic cycle, especially in soils. In this study, the effects of La and Ce in solutions and soil samples on root growth, mitotic index (MI) and frequency of aberrant cells (FAC) were assayed using one of the most convenient objects for testing of cytotoxicity – onion *Allium cepa* L. Bulbs were germinated on media containing La and Ce in concentrations 0–200 mg/l and 0–50 mg/l respectively for solutions and 0–200 mg/kg for soil samples. After 5 days of germination in solutions, a significant decrease in root elongation and MI in apical meristem cells are shown. We have also observed an increase in the number of cells with aberrations at 50 mg/l La and Ce concentration. The number of observed stickiness and disturbed metaphase has increased significantly. Soil samples turned out to be less toxic compared to the solutions probably due to the decreased availability of REEs. In spite of this, significant cytotoxicity of soil samples containing the highest concentration of La and Ce (200 mg/kg) is observed. The latter may indicate the importance of considering the cytotoxicity of soils containing high lanthanides concentrations – in extraction and production areas and actively fertilized fields.

1. Introduction

The active use of rare earth elements (REEs) in many sectors of the economy in recent decades has led to a rapid increase of interest in studying their behavior in the environment and their impact on living organisms. In soil lanthanum and cerium – the most abundant REEs in earth's crust – originate from the parent material, in the process of mining, with improperly utilized wastes and the use of organic and inorganic, especially phosphate, fertilizers (Hu et al., 2006; Sadeghi et al., 2013; Todorovsky et al., 1997; von Tucher and Schmidhalter, 2005). The last mentioned source of possible contamination became especially significant with the beginning of their use as microfertilizers in last decades (Liang et al., 2005). The content of RREs in the surface layer of the soil, where they can be absorbed by plants and interact most actively with other biota, varies considerably and reaches 100–200 mg/kg (Liang et al., 2005). These values can increase up to 1000 mg/kg as a result of human activity (Li et al., 2013).

Despite a large number of various works devoted to the problem of the effect of REEs on biota, there is still no consensus on this issue (Gonzalez et al., 2014). Depending on the concentration of the studied elements and the selected testing objects, the researchers note both positive and negative effects of lanthanides. It is especially important to note here a small number of works devoted to the effects of REEs on plants directly in the soil (Ramos et al., 2016).

The studies of RREs influence on the processes of cell division are rare. There are few studies devoted to the effect of REEs on the process of cell division in plant, and the results do not allow making clear conclusions. In the experiment with *Vicia faba* L. seedlings damage induced by lanthanum in the DNA structure was shown (Wang et al., 2011). This effect could lead to a decrease in the rate of root growth in combination with nutritional imbalances in plants (Wang et al., 2012). Previously, with the same test object, the clastogenic effect of praseodymium and neodymium was detected (Jha and Singh, 1994). As a result, further cell cycle disorders can occur, including the formation of micronuclei. A reliable mitotoxic effect (decrease in cell division activity) was shown for two species of plants – wheat (*Triticum durum*

Abbreviations: RREs, rare earth elements; MI, mitotic index; FAC, frequency of aberrant cells

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Desf.) and garlic (*Allium sativum* L.) (d'Aquino et al., 2009; Xu et al., 2016). A significant increase in micronuclei formation was discovered at the maize roots tips treated with REEs nitrates (Huang et al., 2007).

For soybean plants, on the other hand, an increase in cell proliferative activity of the root tips was found for La (de Oliveira et al., 2015). A positive effect on proliferative activity of *Vicia faba* root tips was also shown for the concentration of holmium in solution below 4 mg/l (Qu et al., 2004). But the authors found certain cytotoxic and genotoxic effects at increased concentration. This effect is called hormesis and it was previously observed at low test concentrations of lanthanides (Pagano et al., 2015). A positive effect on the root growth activity is most often associated with the intensity of cell division in root tips. A similar effect of low concentrations of lanthanides on plants was reported previously (Fashui et al., 2003; Li et al., 2007; Song et al., 2002; Wang et al., 2011; Xu et al., 2007).

All mentioned studies were carried out using solutions containing various concentrations of lanthanides. Some researchers assume that toxicity of soil can be higher than toxicity of extracts from the same soil. Moreover, biotests allow to detect soil cytotoxicity even if pollution indicators, for example, concentration of heavy metals, do not indicate an environmental risk (Koleva et al., 2018). Apparently, the studies of cytotoxicity of soil samples or their extracts containing lanthanides were not performed. For many chemical elements including lanthanides the level of bioavailability was shown to be dependent on chemical and physical properties of the soil (Liang et al., 2005; Zhang et al., 2017). Consequently, further research should include the study of different soil types, containing lanthanides in various concentrations.

Plant test systems have several advantages over the use of animals as test objects. Showing a good correlation with the results of determining the cytotoxicity of various substances on mammalian cells, plants can be recommended as a convenient tool for determining possible genetic disorders (Grant, 1978). Among the plant test objects used to assess the cytotoxicity of various substances, one of the most common is onion *Allium cepa* L. (Grant, 1982; Leme and Marin-Morales, 2009). It has 16 large chromosomes (2n = 16), rapidly growing bulbs, large cells, which makes it a convenient object for biotesting. The Allium-test allows assessing both the phytotoxicity of the studied factor for inhibiting root growth, and genotoxicity. The latter includes mitotoxicity, assessed with changes in the cells proliferative activity – the mitotic index (MI), as well as mutagenicity, assessed by measuring the frequency of aberrant cells (FAC) (Tkalec et al., 2009).

Currently, Allium-test is adapted to work with chemical (Mesi and Kopliku, 2013; Türkoğlu, 2012), biological (Laughinghouse et al., 2012; Tedesco, Laughinghouse, 2012) and physical factors (Pesnya and Romanovsky, 2013) in aqueous solutions. Some study shows the perspective of using the method for soil biotesting (Kovalchuk et al., 1998; Saghirzadeh et al., 2008), but most of them are devoted to testing of soil extracts (Cabrera and Rodriguez, 1999; Cotelle et al., 1999) and suspensions (Koleva et al., 2018). In addition to the availability of insoluble substances for plants, it is worthwhile to take into account the influence of various properties of the tested soils (pH, organic carbon content, particle size distribution, etc.). They can increase the availability of toxic factors or mask their effect on living organisms (Alexander, 1995).

In this study, the toxicity of the two most abundant lanthanides (La and Ce) is assayed using the Allium-test. To evaluate the effect of soil properties on the possible toxicity of lanthanum and cerium, aqueous solutions of La and Ce chlorides and soil samples with various concentrations of REEs were tested.

2. Materials and methods

2.1. Experimental setup

To study the effects of REE contained in water solutions and soil samples on root elongation and process of cell division chloride hydrate forms of lanthanum and cerium were used. The solutions were prepared from $LaCl_3 \times 6H_2O$ and $CeCl_3 \times 6H_2O$ (chemically pure, Novosibirsk rare metals factory, Russia). The solutions with following concentrations of La^{3+} were studied: 0 (control, distilled water), 10, 20, 50, 100, and 200 mg/l. Preliminary experiments showed higher toxicity of Ce^{3+} at these concentrations compared to La^{3+}, which does not allow obtaining material for cytological study. Therefore, Ce^{3+} solutions were studied in lower concentrations: 0 (control, distilled water), 2, 5, 10, 20 and 50 mg/l. Concentrations of the prepared solutions were controlled with an ICP–AES spectrometer ICP–AES 720ES (Agilent Technologies, USA).

Samples of agro soddy-podzolic soil (Eutric Albic Retisol (Loamic, Aric, Cutanic, Ochric)) collected on the experimental field located in the Moscow region (56°7.09' N, 37°49.10' W) (IUSS Working Group, 2014). Samples were collected from the upper soil layer (0–20 cm). Soil samples were prepared in accordance with ISO 11464:2006 "Soil quality – Pretreatment of samples for physico-chemical analysis" (ISO 11464, 2006). Before the experiment the following basic soil parameters were measured in four replicates: pH of aqueous and KCl extracts, humus content, the mobile P content and the exchange K, content of NO_3. The soil is characterized by an acid reaction ($pH_{aqueous}$ = 6.4, pH_{KCl} = 4.7), a low content of humus (1.05%), and a weak supply of nitrogen (NO_3 content – 17.7 mg/kg), phosphorus (P_2O_5 content – 74.5 mg/kg), and potassium (K_2O content – 90.3 mg/kg).

To ensure the homogeneous incorporation of $LaCl_3$ and $CeCl_3$ in soil samples the following procedure was used. For each concentration 8 kg of soil was irrigated with a 250 ml of solution containing an appropriate amount of La^{3+} or Ce^{3+}. After that, the soil samples were dried. For both RREs the following concentrations introduced into soil samples were studied: 0 (control), 10, 20, 50, 100, and 200 mg/kg air-dry soil. Samples were prepared six months before the experiment.

2.2. Test procedure

Locally available onion bulbs (*Allium cepa* L., "Stuttgarter Riesen" cv.) of the same size (d = 1.5–2 cm) were used. Dormant bulbs without grown roots were placed in test-tubes containing 15 ml of solutions or 5 g of soil samples with different concentrations of La^{3+} and Ce^{3+}. Bulbs were grown in 5 replicates for each concentration for 5 days in the dark at 20–25 °C. Equal volumes of the solutions were added when necessary, soil is brought to full moisture capacity and constant soil moisture was maintained (moisture 50%).

After 5 days of treatment the roots were washed from solutions and soil samples in distilled water. The length of 10 roots from each bulb was measured. The root tips (10 mm) were fixed in acetic alcohol (ethanol: glacial acetic acid, 3:1 v/v) for 24 h according to the existing method (Mesi and Kopliku, 2013; Tedesco, Laughinghouse, 2012). Afterwards, the root tips were washed from acetic alcohol in 70% ethanol and transferred to 70% ethanol for long-term storage in a refrigerator (at 4 °C).

Before preparing slides, rootlets were washed from ethanol in distilled water and stained with 2% aceto-orceine for 30 min, while being heated on the burner. The root tips were then washed from aceto-orceine in 45% acetic acid and slides were prepared from the meristematic region.

Root slides were analyzed using light microscope Mikmed-6 (LOMO, Russia) and images were captured with a camera Canon EOS 1100 D (Canon Inc., Japan) attached to a microscope using opto-mechanical adapter (Zenit, Russia). Image analysis software EOS Utility version 2.10.00 (Canon Inc., Japan) was employed (Stolbova et al., 2016).

About 3000 cells were analyzed per replication, thus 15,000 cells per each treatment concentration. The slides were analyzed with 40× magnification. The cells were counted in various stages of mitosis i.e., interphase and prophase (P), metaphase (M), anaphase (A) and telophase (T), different types of aberrant cells (chromosome stickiness, fragments, lagging chromosomes, anaphase bridges, c-mitosis etc.)

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