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Effects of polystyrene microparticles, gadolinium salts and their mixtures to soil annelid studied in agar exposure medium

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Environmental risk assessment requires to conduct standardized ecotoxicological bioassays that can, at least, partially imitate realistic exposure scenarios. However, the study of the ecotoxic effects of particulate materials is complicated mainly by the fact that particles have different physical-chemical properties resulting in different distribution in the test medium in comparison with soluble pollutants. Therefore, in this study, the agar-based experimental system has been used to examine the toxicity of plastic microparticles to the soil decomposer Enchytraeus crypticus. Furthermore, of interest was the effect of the presence of plastic particles on the toxicity of gadolinium salts whose harmfulness to soil organisms is less known. The agar-based exposure medium enabled the consistent dispersion and agglomeration state of particles during the experiments under stable conditions with the characterization of the particles directly in the exposure medium. Gadolinium was tested in the form of $Gd(NO_3)_3$ *and two contrast agents - Dotarem® (Acidum gadotericum) and MultiHance® (Dimeglumi gadobenas). The polystyrene particles (65–497 µm) were prepared by cryogenic grinding of coffee cup lids. The estimated 96h* LC_{50} *for gadolinium was higher than 1 g* kg^{-1} *of agar, which suggests a relatively low acute toxicity to E. crypticus. The polystyrene particles exhibited no toxicity to E. crypticus even at very high concentrations (1–25 g* kg^{-1} *of agar) after 10 days of exposure. The survival rate, the content of malondialdehyde in the biomass, or biomass production E. crypticus were not affected after 10-day of exposure to three different concentrations of Gd in the presence of particles.*

Keywords: Ecotoxicity; Microparticles; Gadolinium; Contrast agent; Agar

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Introduction

In recent years, the pollution of the environment by plastic material has received increased attention of scientists. The extensive use of plastics results in an alarming amount of plastic waste occurring in the environment. The estimated global production of plastics was about 350 million tons in 2018 [1,2]. Due to the use of plastics in all the areas of human activity, the sources of plastic debris are very diverse and include, for example, domestic sewage, containing fibers from clothing and microplastic beads from personal care products, fertilizers, landfills from urban and industrial centers, illegal waste dumping, vinyl mulch from agricultural activities, tire abrasion, etc. Tons of plastics are produced yearly also by the medical care [3]. Exposure to ultraviolet radiation and mechanical abrasion are the main fragmentation processes. However, plastics are not completely decomposed and stay in the environment in the form of micro- or nanoparticles [2]. The plastic particles with a size of less than 5 mm are generally considered as microplastics [4]. Plastic debris can be found in each part of the environment across the globe. Monitoring of microplastic particles (MPs) reveals that industrial soils can contain 300–67 500 mg kg^{-1} [5]. The surface of the MPs can host bacteria, viruses, and algae. Microorganisms are often present in the form of biofilms and can potentially enter the food chain, causing harm to higher organisms [6]. MPs can also adsorb abiotic contaminants on their surface. The adhesion of pollutants (organic pollutants, heavy metals, etc.) transforms MPs into a carrier, enabling the adsorbed pollutants to migrate long distances, thus affecting the distribution of other pollutants in the environment [7]. There is also a high concern about the potential toxicological interactions that may occur when organisms are simultaneously exposed to MPs and other environmental contaminants. Such kinds of studies with MPs have started to be investigated recently [8–10]. However, reports on the adverse effects of MPs on soil organisms are still limited and therefore, these studies are of great importance.

The lanthanide group, which includes 15 elements with highly similar atomic properties, together with yttrium and scandium, represents the rare earth elements (REEs). REEs have a wide spectrum of applications in agriculture, clean energy, electronics, medicine, etc., which can cause their increased release into the environment and, together with their low mobility, may promote their higher accumulation in the environment [11,12]. The REEs concentration in the surface layer of soils was found to be 100–200 mg kg^{-1}, but anthropogenic activity can increase their levels above 1 000 mg kg^{-1} [13,14]. REEs can be mobilized and transported in various environmental components through the different transfer mechanisms (infiltration, erosion, etc.). The speciation of REEs and geochemical conditions play a principal role in REEs mobility and partitioning. In the available literature, the ecotoxicity of REEs varies. However, the dose-response relationships of REEs often exhibit biphasic or hormesis-related trends, characterized by beneficial effects at low concentrations and, *vice versa*, inhibitory effects at high concentrations. Although REEs are not essential elements, it has been shown that, at low concentrations, REEs promote the growth of both aquatic and terrestrial organisms [15–18]. These elements are also expected to be emerging contaminants due to the assumed increase of anthropogenic emissions. Gadolinium is one of the REEs with the highest potential to be released and accumulated in the environment. Gadolinium chelates are widely used as a contrast medium for imaging by nuclear magnetic resonance (NMR) and like the plastic particles they can be transported into the surface waters or soil by hospital effluents [19,20]. In Europe, Gd has been found in rivers and lakes downstream of cities, indicating an anthropogenic accumulation [21]. Since REEs can be transported to soils, it is necessary to study their effect on soil organisms. Regarding Gd, its effect on soil organisms is not much addressed; namely, because of income of Gd into the environment through waters and thus, the so far available information about its ecotoxicity concerns mainly aquatic organisms. Data about toxicity to soil organisms are scarce and mostly concern plants.

As mentioned above, both plastic particles and gadolinium often occur in hospital effluents and both has been reported in soil environment [5,13,14]. However, no study exists on their possible mixture toxicity to soil organisms. Due to this lack of information, we were interested in whether plastic particles, gadolinium salts or their mixture would have some effect on the soil biota. Specifically, we chose polystyrene which is one of the most common plastic polymers worldwide often found in the environment. In our study, various forms of gadolinium salts were used for testing its toxicity – gadolinium(III) nitrate and two gadolinium-containing contrast agents. In the case of testing particulate insoluble materials, a homogeneous dispersion in the exposure medium is essential. The respective agglomeration influences the resulting size and toxicity of particles and consequently the reproducibility of ecotoxicological experiments. Therefore, in our study, a model soil organism was exposed in agar instead of a model in soil. Agar-based exposure medium should suppress the interference effect of agglomeration and simplify the complexity of soil to focus only on the effect of the studied contaminants [22]. As a model soil organism, the potworm *Enchytraeus crypticus* was chosen. Besides earthworms, enchytraeids represent significant decomposers and often fully replace earthworms in soil. In contrast to the most common kind *E. albidus*, *E. crypticus* can be easily cultured in agar medium, having also a higher reproductive rate and shorter generation time [23]. Due to the shorter life cycle, tests with *E. crypticus* are faster in comparison to bigger soil decomposers. In addition, because the culture of *E. crypticus* can also be kept on agar plates, the adaptation phase is omitted and the time required for the entire testing procedure can further be shortened. Then, the agar-based experimental system with *E. crypticus* can serve as fast screening method allowing one a rapid assessment of further research steps.

Materials and methods

Chemicals

The PS-MPs were prepared by cryogenic grinding of polystyrene coffee cup lids. A polycarbonate grinding vial of a cryogenic mill (6970EFM Freezer/Mill, SPEX SamplePrep, Metuchen, NJ, USA) was loaded with 3.5 g of lid. The following operational conditions in the cryogenic mill were applied: precooling time of 15 minutes, grinding time of 2 minutes, recooling time of 2 minutes, three working cycles, impactor frequency of 10 Hz. As a gadolinium salt, gadolinium(III) nitrate ($Gd(NO_3)_3 \cdot 6H_2O$ was used being of analytical reagent grade, Sigma-Aldrich Co., St. Louis, MO, USA) and two contrast agents in the form of injection solutions. First was Dotarem® (Acidum gadotericum, 0.5 mol L^{-1}, lot number: 13GD111B; Geubert, CE, USA), the second MultiHance® (Dimeglumi gadobenas, 0.5 mol L^{-1}, lot number: S3P273A; Bracco Imaging, Konstanz, Germany).

Characterization of PS-MPs

The Raman spectrometry was used for verification of the PS structure preservation during cryogenic grinding (5 frame/20 p, laser frequency: 532 nm, 785 nm). The particle size distribution of PS-MPs dispersed in distilled water was measured with Mastersizer 2000/MU (Malvern Instruments, Worcestershire, UK) by using Fraunhofer approximation. Each sample was dispersed by an ultrasonic finger for 1 minute and then measured three times in the flow cell of the instrument. The size and shape of the fresh ground PS-MPs were analyzed with an optical microscope (Keyence VHX-6000, Osaka, Japan). The dispersion of particles in exposure medium was imaged with optical microscope.

Test species

The culture of *Enchytraeus crypticus* was maintained in plastic Petri dishes with ventilation (Fisherbrand, PS, aseptic, 90 × 14.2 mm, Fisher Scientific, Waltham, MA, USA) filled with agar at a temperature of 21 ± 2 °C. Culture medium was prepared using powder agar (Dr. Hoffmann, Cítov pod Řípem, the Czech Republic), distilled water and salts $NaHCO_3$, KCl, $MgSO_4$ (Lach-Ner, Neratovice, the Czech Republic), and $CaCl_2$ (PENTA, Prague, the Czech Republic) to obtain 1.5% agar with pH 6.5–7.5. Potworms were cultivated in the dark and fed finely ground oatmeal twice a week. Adult worms with a well-developed clitellum were used for all the tests.

Experiments with the gadolinium salts

Two separate experiments were performed to select the Gd concentrations used in the test with PS-MPs. In the first experiment, we examined the acute toxicity of gadolinium nitrate alone. Exposure medium was prepared by the addition of dry powder agar to demineralized water (2% agar). Mixtures were then vigorously stirred with a magnetic stirrer at 95 ± 5 °C for 30 minutes. After other 30 min., the test solutions were added to the stirred agar. The medium was then poured into plastic Petri dishes with ventilation (Fisherbrand, PS, aseptic, 90 × 14.2 mm, Fisher Scientific); of both the bottom and the lid. The tested concentrations of Gd were 0, 100, 250, 500, 750, and 1 000 mg kg^{-1} of agar (wet weight). Twenty adult worms were placed into each Petri dish and maintained in the dark inside a microclimate-controlled box at 21 ± 2 °C for a period of 96 hours. Three independent replicates were performed for each tested concentration level, including the control. The endpoint of the experiment was mortality and from the obtained data were estimated the values of LC_{50} and LC_{10}. Concentrations of Gd in the exposure medium after its preparation and in exposed worms after tests were verified using inductively coupled plasma optical emission spectrometry (ICP-OES; Integra XL, GBC, Regents Park, Australia). The procedures for the sample preparation and analysis conditions are described in the previous study [24].

In the experiment with the contrast agents (CAs), the preparation of exposure medium and working conditions were the same as previously described [24]. Tested concentrations of Dotarem and MultiHance were chosen so that the gadolinium content in the exposure medium corresponded to the Gd LC_{50} and LC_{10} values from experiment with gadolinium nitrate (1 062 and 837 mg Gd kg^{-1} of agar, respectively).

Experiments with the PS-MPs

Prior to the ecotoxicity tests, it was first necessary to optimize the introduction of particles into the agar. The problem with plastic MPs is the low wettability in water and the subsequent formation of agglomerates. This problem can be solved by partial polymerization of the agar, which leads to an increase in its viscosity. The dispersion is carried out by heating the agar first, followed by allowing the agar to cool down to the desired temperature and then pouring MPs into it with intensive stirring with a magnetic bar. The exposure medium was prepared as described in the above chapter and cooled to 90, 60, and 45 °C. The dried PS-MPs (0.1 wt. %) were added to a stirred agar at each temperature and then poured into the test vessels. The degree of agglomeration was verified by the optical microscopy. After evaluating the size of the agglomerates, it has been found that the best dispersion (i.e., least agglomeration) is achieved at the agar temperature of 45 °C and therefore, this temperature has been chosen for the preparation of exposure medium in further tests.

Since we did not anticipate any high toxicity of plastic particles as in case of gadolinium, other more sensitive endpoints in the ecotoxicity test with PS-MPs have been monitored in addition to mortality; namely, the weight change and oxidative stress. The former was determined as the difference in the wet weight and total protein mass of the exposed organism compared to the control group. Malondialdehyde (MDA) was chosen as a biomarker of oxidative stress. Obtained MDA concentrations were related to mg of total protein. The two assays were performed using the respective set (Lipid Peroxidation Assay Kit and Total Protein Kit, Micro Lowry, Peterson's Modification, Sigma Aldrich). Total protein was determined spectrophotometrically at 680 nm (Epoch, BioTek Instruments, Inc., Winooski, VT, USA, software Gen5 3.02). MDA was determined at a high-performance liquid chromatograph (1260 Infinity II Prime LC System, Agilent, Palo Alto, CA, USA) with a reverse phase C18 column (Nucleosil 120-5 C18; particle size 5 μm; length 250 mm, internal diameter 4 mm). The following operational conditions were applied: isocratic elution with composition of the mobile phase 35 % of 8.3mM phosphate buffer and 65 % of acetonitrile, amount of sample: 15 μL, flow rate: 0.7 mL min^{-1}, temperature: 25 °C, and detection by diode array detector at 532 nm.

Two ecotoxicity tests were performed with PS-MPs. The first one lasted 96 hours. The second test was prolonged to 10 days due to a possible increase in organism response under the prolonged exposure to the stressor. Tested concentrations of PS-MPs were 0, 0.1, 1, and 2.5 wt. % (0, 1, 10 and 25 g kg^{-1} of agar, respectively).

Combined ecotoxicity test with PS-MPs and gadolinium(III) nitrate

According to the results of previously described experiments, the final test was carried out as a prolonged experiment (for 10 days), in which we examined the ecotoxicity of Gd (25, 100 a 500 mg kg^{-1} of agar, concentrations below the detected LC_{10} value) in the presence of the highest concentration of PS-MPs (2.5 wt. %) tested. Gadolinium was applied in the form of nitrate, because in the preliminary experiment, gadolinium in the form of CAs had not affected the mortality of worms.

Statistical analysis

The dose-response curve and calculation of LC_x values were carried out by using the module of nonlinear regression (curve fit) with variable slope (GraphPad Prism 7 software, GraphPad Software, San Diego, CA, USA). The experimental data were fitted with equation $Y = 100/[1+10\text{\^{}}((\log EC_{50} - X) \cdot \text{HillSlope})]$, where Y is normalized response, X log of concentration and *HillSlope* the slope factor. Both nominal and analytical concentrations of Gd (obtained by ICP-OES) were analyzed and the values obtained were compared. The means of replicates and evaluation of data significance were determined by Student's t-test ($n = 3, f = 2, p < 0.05$).

Results and discussion

Characterization of PS-MPs

Raman spectrometry was used for verification of the PS structure preservation during cryogenic grinding. The structures of polystyrene lid and grounded PS-MPs were compared (see Figure 1 a). The basic bands of PS were measured on the spectra: 800, 1 000, 1 600 a 2 900 cm^{-1} (the 520 cm^{-1} band corresponds to the Si substrate). The spectra of the samples did not change with each other, which indicated that the structure remains unchanged during grinding. As PS is moderately thermally stable, nearly no degradation of pure PS occurs at temperatures below 200 °C. But temperatures over 330 °C already result in almost complete degradation, with styrene monomer as the main product [25].

The particle size distribution was determined by laser diffraction and the respective distribution curve can be seen in Figure 1 b. Median size $d(0.5)$ of ground particles was 190 μm, the lower decile $d(0.1)$ 65 μm and the upper decile $d(0.9)$ then 497 μm. The photos imaged with an optical microscope can be seen in Figure 1 c, revealing that the particles ranged in size from tens to hundreds of micrometers, which is consistent with the previous measurement. The particles had different shapes and very often sharp edges. These sharp edges of fresh ground particles can cause the damage of exposed organisms and may thus increase, for example, the oxidative stress. In the environment, MPs have often smooth edges due to abrasion and other degradation processes [26].

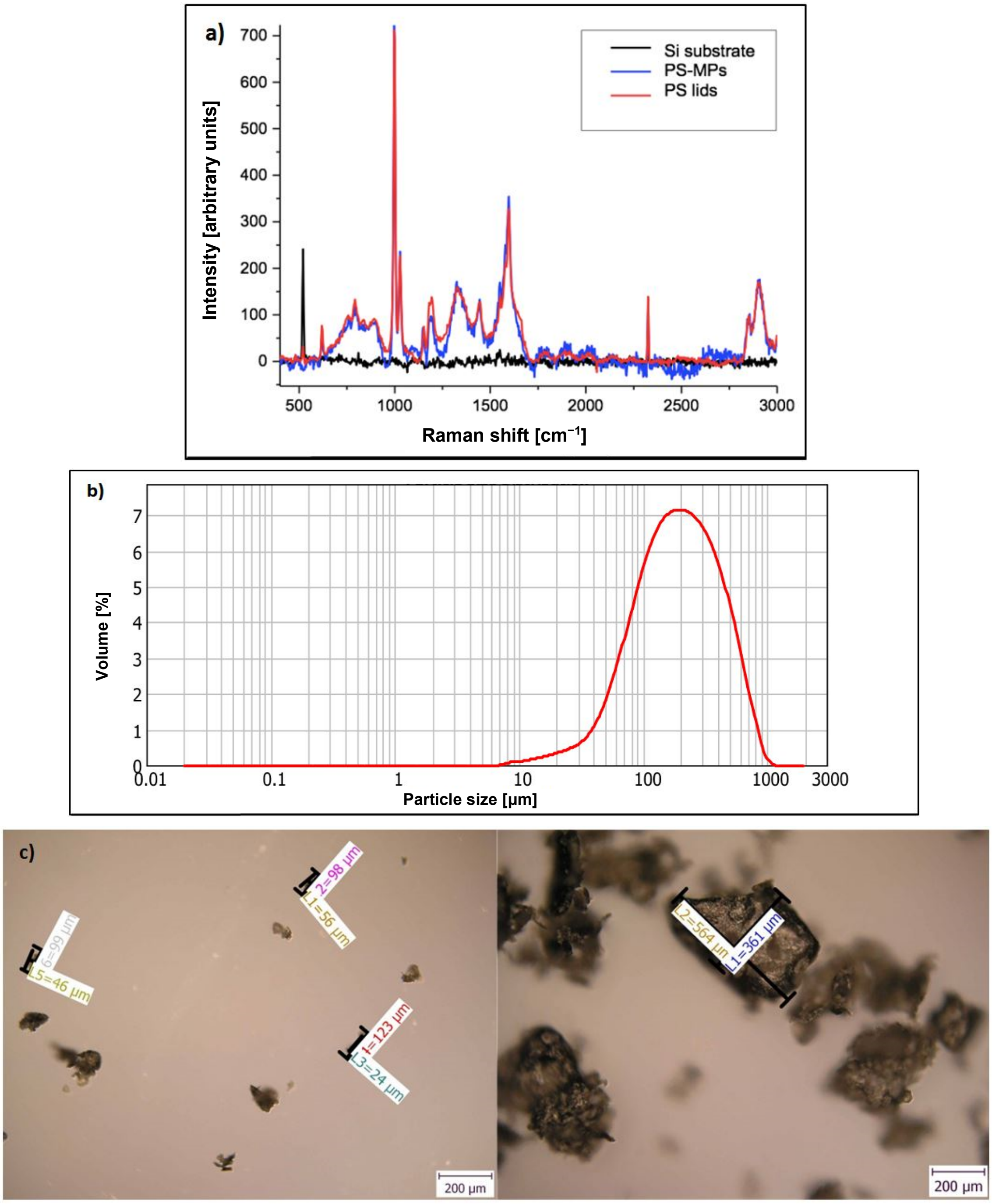

Fig. 1 Raman spectra of PS coffee lids and ground PS-MPs (a), particle size distribution of ground PS-MPs (b) and images of ground PS-MPs from optical microscopy (c)

In order to select the optimal agar temperature for the proper particle dispersion (with the least agglomerates) in the exposure medium, the PS-MPs were observed directly in the exposure medium; again, with the optical microscopy. It was found that the dispersion had been the most homogeneous at a temperature of 45 °C (see Figure 2) and, at this temperature, the smallest agglomerates were evidenced with an average size of around 250 µm. Therefore, we have considered to use this setting to prepare our experimental medium.

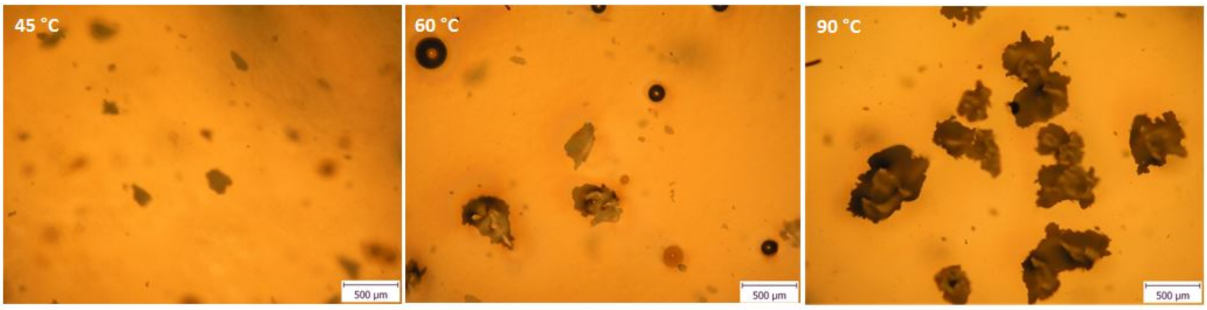

Fig. 2 Images from optical microscopy - comparison of MPs (0.1 wt. %) dispersion at different agar temperatures

Toxicity of gadolinium salts

After 96 hours of exposure to gadolinium(III) nitrate, the worm mortality increased with higher concentration of Gd in the exposure medium (see Table 1). The estimated dose-response curve depicting the acute toxicity of gadolinium originating from $Gd(NO_3)_3{\cdot}6H_2O$ is shown in Figure 3. When nominal concentrations were used for statistical analysis, the estimated value of 96h LC_{50} was 1 062 (1 029–1 123) mg Gd kg^{-1} of agar and LC_{10} being 837 mg Gd kg^{-1} of agar. When analytical concentrations were statistically analysed, the estimated 96h LC_{50} was 1 059 (1 038–1 095) mg Gd kg^{-1} of agar and LC_{10} then 891 mg Gd kg^{-1} of agar. The highest concentration of Gd tested, 1 000 mg kg^{-1} of agar, corresponding to the concentrations of REEs found in polluted areas (locations with anthropogenic activity [13]) in the agar caused a 37% mortality. The toxicity of gadolinium observed for the potworm *E. crypticus* is very low, considering that the experiment took place in a simplified exposure medium. In soils, pollutants interact with soil components, and these interactions often lead to the reduced bioavailability or reactivity of the pollutant. The toxicity in soil is often up to an order of magnitude lower than toxicity observed in agar or other simplified medium, such as humic acids-water environment, etc. [22,27].

Table 1 Mortalities of *E. crypticus* exposed to $Gd(NO_3)_3$ in agar after 96 h

c [mg kg^{-1}]	log nominal *c* [mg kg^{-1}]	log analytical *c* [mg kg^{-1}]	Replicates 1	2	3
control	–	–	0	0	0
100	2.0	1.97	0	0	0
250	2.4	2.40	0	5	0
500	2.7	2.73	0	5	0
750	2.9	2.94	10	10	0
1000	3.0	3.01	45	30	35

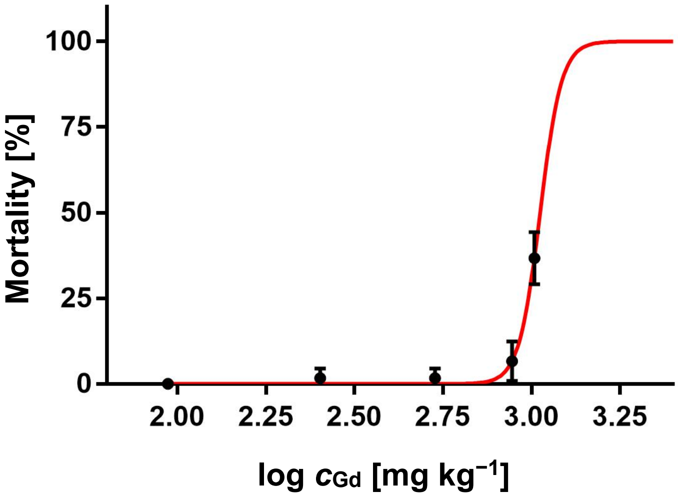

Fig. 3 Estimation of the dose-response curve describing the acute toxicity (96h) of gadolinium originating from $Gd(NO_3)_3$ to *E. crypticus*

Gadolinium ecotoxicity to soil organisms has been studied only very seldom. Most available information is known for aquatic organisms or plants. In the literature, we can find a report where the authors have investigated the toxicity of Gd toward maize plants in hydroponic solution [21]. It was found that Gd concentration of 1 mg L^{-1} had suppressed the biomass production by 37 % for the shoot and 27 % for the roots and 10 mg L^{-1} reduced a shoot growth by 67 % and root biomass by 35 % compared to the control group. The results indicated higher Gd toxicity, but similarly to our work, the experiments had not been performed in a soil environment. In other study [12], the toxicity of Gd was evaluated with using a battery of aquatic tests. In the case of the planktonic crustacean *Daphnia magna*, benthic ostracod *Heterocypris incongruens,* and bacteria *Vibrio fischeri,* the EC_{50} values are anticipated to be greater than 6 400 mg Gd L^{-1} (the highest nominal concentration tested in this study). In case of rotifer *Brachionus calyciflorus,* the estimated EC_{50} was 1 120 mg Gd L^{-1}, for fresh-water polyp *Hydra attenuata* then 2 549 mg Gd L^{-1} and for algae *Pseudokirchneriella subcapitata* attaining 3 111 mg Gd L^{-1}. These values are also quite high and, in a conclusion, the authors have stated that the presence of lanthanides in the environment apparently does not represent a high environmental risk except at some hotspots.

Since the behavior of the elements in the lanthanide group is quite similar, our results can be compared with those from the studies dealing with other REEs; lanthanum being a very often studied representative of them. Similarly to gadolinium, lanthanum affects the organism at the biochemical level mainly due to the exchange with calcium, thus blocking the calcium channels, and interfere with calcium metabolism due to the close resemblance of the ionic ratio of La and Ca [28]. Lanthanum toxicity to soil invertebrates in natural soil LUFA 2.2 was studied by Li et al. [29], reporting on affected survival of *E. crypticus,* springtail *Folsomia candida,* and earthworm *Eisenia Andrei* with the corresponding LC_{50} of 1 650 (1 500–1 820), 1 690 (1 600–1 790), and 1 850 (>1 088, <3 133) mg La kg^{-1} dry soil, respectively. In this study, an isopod *Porcellio scaber* was the most sensitive organism with LC_{50} 960 (441–2 090) mg La kg^{-1} dry soil, whereas the lowest toxicity was observed in experiments with a mite *Oppia nitens* where LC_{50} is expected to be higher than 5 820 mg La kg^{-1} dry soil. The lanthanide chemical homogeneity has been commonly considered as a basis to predict toxicity across the whole group. The decrease in toxicity of REEs in relation with the increasing atomic number has been associated with higher stability constants of heavy lanthanides [28]. Thus, differences within the lanthanide group and chemical composition of the exposure medium make a definite comparison with our results quite difficult; however, our results and the available data suggest a low toxicity of lanthanides to soil organisms.

During the experiments with CAs, no mortality of exposed organisms was observed for both concentrations of Gd tested (see Table 2). To the best of our knowledge, there are no data on the toxicity of contrast agents to soil organisms in the literature, only some information on the toxicity to human can be traced up. As far as chemistry is concerned, the chemical bonds in CAs are made of a gadolinium ion and a carrier molecule that represents a chelating agent modifying the distribution of gadolinium within the body to overcome its toxicity while maintaining its contrast properties. Toxicity of CAs has primarily been attributed to the dissociation of Gd^{3+} from the complexes with the chelate structure. Such dissociation is related to differences in stability of the complexes among the various types of CAs [11]. Dotarem® represents the macrocyclic form of gadolinium based Cas, whereas MultiHance® is a linear form. Macrocyclic CAs form cage-like structures with Gd^{3+} enclosed in the complex. The macrocyclic CAs have lower dissociation constants and therefore, they are more stable than the linear CAs [30]. In the environment, under normal circumstances, lanthanides are present in trivalent oxidation states. With lower temperature and higher pH, the solubility of lanthanides decreases due to a possible precipitation of lanthanides as hydroxides or carbonates. Heavy lanthanides (Tb–Lu), such as Gd, give rise to stronger complexes with carbonate ions than those formed by light lanthanides due to the stability constants increasing with atomic number [28]. In the human body, the CAs are being removed renally without metabolization. In addition, CAs are not degraded in wastewater treatment and thus, they are released into the aquatic and, subsequently, soil environment in the original form [31].

If we consider the above mentioned, we can assume that gadolinium in the chelate form was, compared to gadolinium nitrate, far less available for potworms and therefore, no mortality was observed for the concentrations given.

Table 2 Mortalities of *E. crypticus* exposed to different form of gadolinium salts

Nominal c_{Gd} [mg kg^{-1} of agar]	Mortality [%]		
	$Gd(NO_3)_3 \cdot 6H_2O$	Dotarem®	MultiHance®
837	10	0	0
1 062	50	0	0

Toxicity of PS-MPs

After 96 hours of exposure to PS-MPs, the worm mortality did not increase with the rising PS-MPs concentration in the exposure medium. None of the tested concentrations led to a statistically significant change in the mortality, weight, or MDA concentration in *E. crypticus* biomass compared to the control group (see Figure 4, first row). Nor even prolonged exposure to 10 days did affect any of the observed endpoints (see Figure 4, second row). Research on the toxic effects of MPs on soil organisms is limited because water pollution by MPs has been regarded as one of the most important and serious global concerns [3]. In general, when compared to other common synthetic packing materials, PS and the products of its decomposition seem to be of low toxicity. Amounts of degradation products, including monomer styrene that can be released from PS package materials exposed to high temperatures (over 200 °C) are associated with a relatively low toxicity of this polymer [25]. It has also been found that *Enchytraeus albidus* is able to prevent lipid peroxidation with its antioxidant defense mechanisms during short exposures (in a few days), but not after longer exposures (more than 3 weeks) [32]. In most studies, MPs affect rather growth or reproduction than the mortality of soil organisms [33,34]. Jiang et al. [33] studied the toxicity of PS particles (100 and 1 000 μg kg^{-1} of soil) to earthworms. According to the mortality, growth measurements, histological changes, the results of DNA damage and oxidative stress analyses, the acute toxicity of 100 μg kg^{-1} of soil and 1 300 nm sized particles to earthworms was found to be extremely low. However, exposure to PS with particle size 1 300 nm at a concentration of 1 000 μg kg^{-1} of soil caused DNA damage, oxidative stress, and histopathological changes in earthworm intestines compared to the lower tested concentration or smaller particle size. On the other hand, in a study by Lahive et al. [35] it was assessed that there had been the effect of nylon particles (20, 50, 90, and 120 g kg^{-1} of soil ~ 2–12 wt. %) on *E. crypticus* survival and reproduction. Regarding survival, it was not affected, but reproduction was reduced at high exposure concentrations (>90 g kg^{-1}). Compared to the previous study, the particles with smaller size (13–18 μm) had a greater effect compared to that of larger sizes (>63 μm), with a calculated EC_{50} (13–18 μm) of 108 ± 8.5 g kg^{-1} of soil. In another study [36] the toxicity of polystyrene MPs (58 μm) was investigated on the earthworm

Eisenia foetida exposed in agricultural soil at concentrations of 0, 0.25, 0.5, 1 and 2 wt. % for 30 days. The results showed no dose-response effect. Also, the MPs had had insignificant effect on the fitness of earthworms under low exposure concentrations (≤ 0.5 wt. %), while exposure to 1 a 2 wt. % inhibited the growth and exposure to 2 wt. % having increased mortality of earthworms to 40 %.

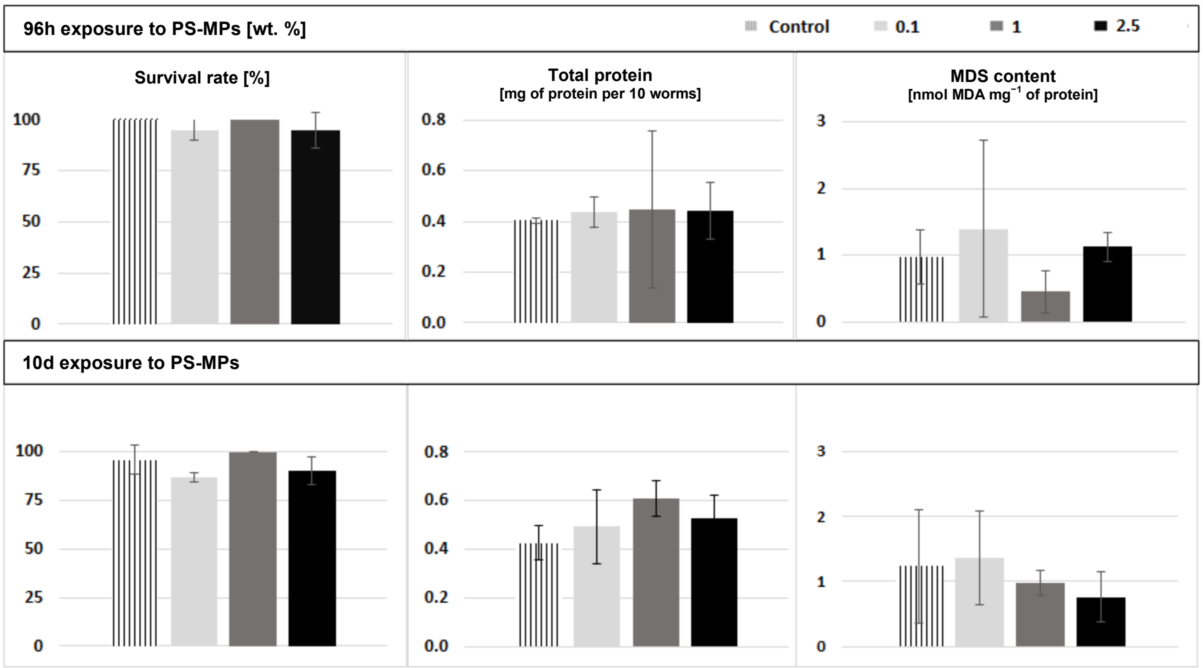

Fig. 4 *E. crypticus* survival rate, total protein and MDA content in the biomass after 96h exposure to three different concentrations of PS-MPs (first row) and after 10d exposure (second row)

In general, MPs with a particle size less than 1 μm can be easily ingested by soil animals and further transported from the intestines to other tissues via the intestinal wall [37]. In the case of *E. crypticus,* the mouth part, drawing food particles into the fore gut, is approximately 72 μm across and 90 μm in length [38]. Based on these physiological limitations, the particles at or below these dimensions could potentially be ingested. No spherical larger particles could have been smaller in one dimension (see Figure 1 c, left image) and so might be ingested, too. However, in our study, the average size of PS-MPs in the exposure medium was around 250 μm and such large particles were not ingested by the test organisms, whereas smaller particles were apparently subsequently eliminated without further effect. Dermal exposure to polystyrene particles clearly does not pose a significant risk to *E. crypticus*.

Combined ecotoxicity test with PS-MPs and gadolinium nitrate

The 10-days exposure of *E. crypticus* to different concentrations of gadolinium (25, 100 a 500 mg Gd kg^{-1} of agar) in the presence of 2.5 wt. % of PS-MPs did not influence the mortality, total protein or MDA content in the *E. crypticus* biomass in the dose-response manner (see Figure 5). Concentrations of Gd were chosen below the detected LC_{10} value, where it was expected that pollutants affect biological processes in the organism, such as the biomass production or oxidative stress. Our tested concentrations of Gd were higher than the average worldwide natural background, which ranges from units up to tens mg of Gd per kg of soil [39]. No mortality, no MDA increase, and the slight increase in biomass of worms (calculated as a total protein per wet weight of 10 worms) exposed to concentrations under the found Gd LC_{10} value could somewhat correspond to the hormesis effect found also in other studies. This effect on the growth was found in the roots of rice *Oryza sativa* exposed to the increasing concentrations of La^{3+} (0.05, 0.1, 0.5, 1.0, and 1.5 mmol L^{-1}). The results indicated that the presence of La^{3+} had promoted the growth of rice roots at 0.05 mmol L^{-1}, but inhibited the growth at 1.0 and 1.5 mmol L^{-1} La^{3+} after 13 days of exposure [40].

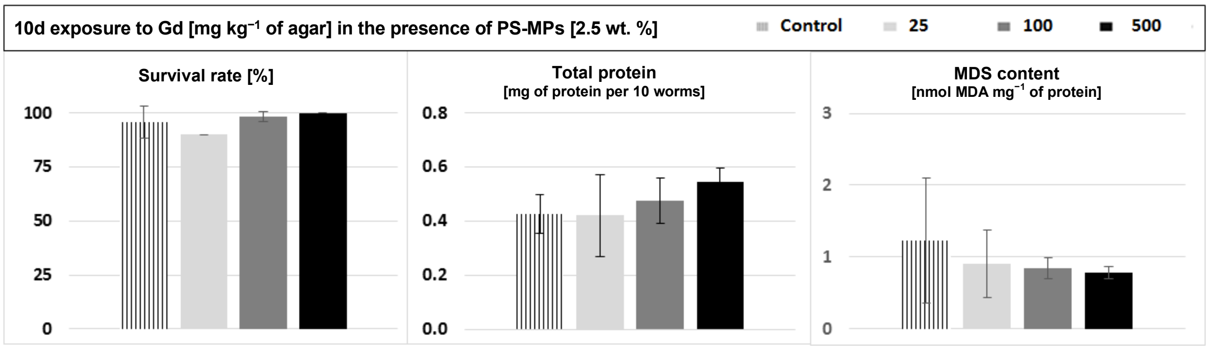

Fig. 5 *E. crypticus* survival rate, total protein and MDA content in the biomass after 10d exposure to three different concentrations of Gd in the presence of PS-MPs

Our highest tested concentration of MPs was 25 g kg^{-1} of agar, being close to the upper limit of concentrations found in industrial soils 0,3–67.5 g kg^{-1} [5]. And even such a high concentration was not toxic to our representative of soil decomposer *E. crypticus* and did not affect the Gd toxicity in agar exposure medium.

Conclusions

The agar-based experimental system has been used to study the toxicity of polystyrene particles, gadolinium salts, and their mixtures toward *Enchytraeus crypticus* as a representative of the soil decomposer. The agar-based exposure medium enabled the characterization of the tested particles directly in the test medium and stable agglomeration status of particles during the whole test under static and well-adjustable conditions. In the agar, we have easily demonstrated that gadolinium shows a relatively low acute toxicity to *E. crypticus* with estimated LC_{50} higher than 1 g kg^{-1} of agar, which agrees with the literature data. Furthermore, the toxicity in the environment could be expected to be even lower compared to result obtained in the agar, because the interactions of pollutant with the soil matrix almost always reduce the bioavailability or reactivity. The gadolinium in the form of contrast agents showed reduced toxicity when compared to Gd nitrate. This could be attributed to the chelated form, in which gadolinium is less bioavailable. The polystyrene particles with size from tens to hundreds µm exhibited no toxicity to *E. crypticus* even at very high concentrations (1–25 g kg^{-1} of agar). In the mixtures of PS-MPs and gadolinium, no toxicity has been reported. In our study, however, the effect of particle size on the resulting toxicity has been observed. Despite the fact that agar enabled to disperse the particles better in the medium keeping them dispersed, most particles had not been probably ingested and dermal exposure did not elicit a significant response even at high concentrations of PS-MPs.

Despite the above mentioned, the present study represents a first step in the investigation as there have been assessed only the effects of short-term exposure. Because the amounts of microplastic particles and REEs are likely to increase with time and because they will affect the soil biota in the long term, understanding how could this pose a risk for the soil environment is important and thus, such kinds of studies are necessary.

References

[1] Guo J.J., Huang X.P., Xiang L., Wang Y.Z., Li Y.W., Li H., Cai Q.Y., Mo C.H., Wong M.H.: Source, migration and toxicology of microplastics in soil. *Environment International* **137** (2020) 105263.

[2] Wang W., Ge J., Yu X., Li H.: Environmental fate and impacts of microplastics in soil ecosystems: Progress and perspective. *Science of the Total Environment* **708** (2020) 134841.

[3] Chae Y., An Y.J.: Current research trends on plastic pollution and ecological impacts on the soil ecosystem: A review. *Environmental Pollution* **240** (2018) 387–395.

[4] Law K.L., Thompson R.C.: Microplastics in the seas. *Science* **345** (2014) 144–145.

[5] Fuller S., Gautam A.: A procedure for measuring microplastics using pressurized fluid extraction. *Environmental Science and Technology* **50** (2016) 5774–5780.

[6] Bejgarn S., Macleod M., Bogdal C., Breitholtz M.: Toxicity of leachate from weathering plastics: an exploratory screening study with *Nitocra spinipes*. *Chemosphere* **132** (2015) 114–119.

[7] Gong J., Xie P.: Research progress in sources, analytical methods, eco-environmental effects, and control measures of microplastics. *Chemosphere* **254** (2020) 126790.

[8] Ferreira P., Fonte E., Soares E.M., Carvalho F., Guilhermino L.: Effects of multi-stressors on juveniles of the marine fish *Pomatoschistus microps*: Gold nanoparticles, microplastics and temperature. *Aquatic Toxicology* **170** (2016) 89–103.

[9] Khan F.R., Syberg K., Shashoua Y., Bury N.R.: Influence of polyethylene microplastic beads on the uptake and localization of silver in zebrafish (*Danio rerio*). *Environmental Pollution* **206** (2015) 73–79.

[10] Luis L.G., Ferreira P., Fonte E., Oliveira M., Guilhermino L.: Does the presence of microplastics influence the acute toxicity of chromium (VI) to early juveniles of the common goby (*Pomatoschistus microps*)? A study with juveniles from two wild estuarine populations. *Aquatic Toxicology* **164** (2015) 163–174.

[11] Rogosnitzky M., Branch S.: Gadolinium-based contrast agent toxicity: A review of known and proposed mechanisms. *BioMetals* **29** (2016) 365–376.

[12] Gonzalez V., Vignati D.A.L., Pons M.N., Montarges-Pelletier E., Bojic C., Giamberini L.: Lanthanide ecotoxicity: First attempt to measure environmental risk for aquatic organisms. *Environmental Pollution* **199** (2015) 139–147.

[13] Li X., Chen Z., Chen Z., Zhang Y.: A human health risk assessment of rare earth elements in soil and vegetables from a mining area in Fujian Province, Southeast China. *Chemosphere* **93** (2013) 1240–1246.

[14] Liang T., Zang S., Wang L., Kung H.T., Wang Y., Hu A., Ding S.: Environmental biogeochemical behaviors of rare earth elements in soil plant systems. *Environmental Geochemistry and Health* **27** (2005) 301–311.

[15] Gwenzi W., Mangori L., Danha C., Chaukura N., Dunjana N., Sanganyado E.: Sources, behaviour, and environmental and human health risks of high-technology rare earth elements as emerging contaminants. *Science of the Total Environment* **636** (2018) 299–313.

[16] Migaszewski Z.M., Gałuszka A.: The characteristics, occurrence, and geochemical behavior of rare earth elements in the environment: A review. *Critical Reviews in Environmental Science and Technology* **455** (2015) 429–471.

[17] Pagano G., Aliberti F., Gida M., Oral R., Siciliano A., Trifuoggi M., Tommasi F.: Rare earth elements in human and animal health: State of art and research priorities. *Environmental Research* **142** (2015) 215–220.

[18] Tyler G.: Rare earth elements in soil and plant systems - a review. *Plant and Soil* **267** (2004) 191–206.

[19] Pedreira R.M.A, Pahnke K., Böning P., Hatje V.: Tracking hospital effluent-derived gadolinium in Atlantic coastal waters off Brazil. *Water Research* **145**: (2018) 62–72.

[20] Kümmerer K., Helmers E.: Hospital effluents as a source of gadolinium in the aquatic environment. *Environmental Science and Technology* **34** (2000) 573–577.

[21] Saatz J., Vetterlein D., Mattusch J., Otto M., Daus B.: The influence of gadolinium and yttrium on biomass production and nutrient balance of maize plants. *Environmental Pollution* **204** (2015) 32–38.

[22] Hrda K., Pouzar M., Knotek P.: Study of zinc oxide nanoparticles and zinc chloride toxicity to annelid *Enchytraeus crypticus* in modified agar-based media. *Environmental Science and Pollution Research* **25** (2018) 22702–22709.

[23] Castro-Ferreira M.P., Roelofs D., van Gestel C.A.M., Verweij R.A., Soares A.M.V.M., Amorim M.J.B.: *Enchytraeus crypticus* as model species in soil ecotoxicology. *Chemosphere* **87** (2012) 1222–1227.

[24] Patocka J., Krejcova A., Stojarova K., Hrda K., Pouzar M.: The ICP-OES method for determination of zinc in *Enchytraeus crypticus* and agarose gel from ecotoxicological tests. *Chemical Papers* **73** (2019) 159–164.

[25] Kik K., Bukowska B., Sicinska P.: Polystyrene nanoparticles: Sources, occurrence in the environment, distribution in tissues, accumulation and toxicity to various organisms. *Environmental Pollution* **262** (2020) 114297.

[26] Gray A.D., Weinstein J.E.: Size- and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (*Palaemonetes pugio*). *Environmental Toxicology and Chemistry* **36** (2017) 3074–3080.

[27] Li L.Z., Zhou D.M., Peijnenburg W.J.G.M., van Gestel C.A.M., Jin S.Y., Wang Y.J., Wang P.: Toxicity of zinc oxide nanoparticles in the earthworm *Eisenia fetida* and subcellular fractionation of Zn. *Environment International* **37**: (2011) 1098–1104.

[28] Gonzalez V., Vignati D.A.L., Leyval C., Giamberini L.: Environmental fate and ecotoxicity of lanthanides: are they a uniform group beyond chemistry? *Environment International* **71** (2014) 148–157.

[29] Li J., Verweij R.A., van Gestl C.A.M.: Lanthanum toxicity to five different species of soil invertebrates in relation to availability in soil. *Chemosphere* **193** (2018) 412–420.

[30] Port M., Idee J.M., Medina C., Robic C., Sabatou M., Corot C.: Efficiency, thermodynamic and kinetic stability of marketed gadolinium chelates and their possible clinical consequences: A critical review. *BioMetals* **21** (2008) 469–490.

[31] Rogowska J., Olkowska E., Ratajczyk W., Wolska L.: Gadolinium as a new emerging contaminant of aquatic environments. *Environmental Toxicology and Chemistry* **37** (2018) 1523–1534.

[32] Howcroft C.F., Amorim M.J.B., Gravato C., Guilhermino L., Soares A.M.V.M.: Effects of natural and chemical stressors on *Enchytraeus albidus*: Can oxidative stress parameters be used as fast screening tools for the assessment of different stress impacts on soils? *Environment International* **35** (2009) 318–324.

[33] Jiang X., Chang Y., Zhang T., Qiao Y., Klobucar G., Li M.: Toxicological effects of polystyrene microplastics on earthworm (*Eisenia fetida*). *Environmental Pollution* **259** (2020) 113896.

[34] Rillig M.C., Ziersch L., Hempel S.: Microplastic transport in soil by earthworms. *Scientific Reports* **7** (2017) 1362–1368.

[35] Lahive E., Walton A., Horton A.A., Spurgeon D.J., Svendsen C.: Microplastic particles reduce reproduction in the terrestrial worm *Enchytraeus crypticus* in a soil exposure. *Environmental Pollution* **255**: (2019) 113174.

[36] Cao D., Wang X., Luo X., Liu G., Zheng H.: Effects of polystyrene microplastics on the fitness of earthworms in an agricultural soil. *IOP Conference Series: Earth and Environmental Science* **61** (2017) 012148.

[37] Farrell P., Nelson K.: Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). *Environmental Pollution* **177** (2013) 1–3.

[38] Westheide W., Graefe U.: Two new terrestrial *Enchytraeus* species (Oligochaeta, Annelida). *Journal of Natural History* **26** (1992) 479–488.

[39] Ramos S.J., Dinali G.S., Oliveira C., Martins G.C., Moreira C.G., Siqueira J.O., Guilherme L.R.G.: Rare Earth elements in the soil environment. *Current Pollution Reports* **2** (2016) 28–50.

[40] Liu D., Wang X., Zhang X., Gao Z.: Effects of lanthanum on growth and accumulation in roots of rice seedlings. *Plant, Soil and Environment* **59** (2013) 196–200.