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RESEARCH ARTICLE

Development of a sediment-contact test with rice for the assessment of sediment-bound pollutants

Alexandra Brinke[1] · **Sebastian Buchinger**[1] · **Georg Reifferscheid**[1] · **Roland Klein**[2] · **Ute Feiler**[1]

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Abstract Despite the key role of higher plants in aquatic ecosystems as functional and structural elements, sediment-contact tests with macrophytes are still scarce. Moreover, due to large differences in exposure routes for pollutants as well as in life cycles between the diverse taxa of macrophytes, sensitivities to pollutants vary between taxa. Therefore, the development of new test systems with aquatic macrophytes, in general, is favorable. This study proposes a protocol for a sediment-contact test with *Oryza sativa* and addresses the main question whether the rice plant is a suitable test organism for sediment toxicity testing with higher plants. As a first evaluation step, the variability and sensitivity of the test was investigated using spiked artificial sediments. Thus, according to the protocol, rice was exposed to arsenic-, cadmium-, chromium-, and nickel-spiked sediments. Additionally, it was investigated which classical endpoints for plant bioassays, such as root and shoot elongation, are suitable for this bioassay. As a second evaluation step, the test system was used for assessment of natural sediments. Thereupon, a sensitivity profile of the presented test protocol was analyzed in comparison to other plant-based test systems. Inhibition of root and shoot elongation turned out to be the most sensitive endpoints for single-substance testing in spiked artificial sediments. However, regarding testing of natural sediments, rice shoots responded more sensitive than rice roots. In conclusion, the rice plant clearly showed pollutant-induced effects on growth in sediments, and thus, it is likely a promising test organism to complement sediment-contact tests with higher plants.

Keywords *Oryza sativa* · Sediment-contact test · Higher plants · Sediment quality assessment · Heavy metals and arsenic

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✉ Alexandra Brinke
alexandra.brinke@bafg.de

[1] German Federal Institute of Hydrology, Am Mainzer Tor 1, 56068 Koblenz, Germany

[2] Department VI, Trier University, Biogeography, 54286 Trier, Germany

Introduction

Sediments are an integral part of aquatic ecosystems and can act as a sink as well as a secondary source for sediment pollutants (Brils 2002; Förstner 2004). The assessment of sediment quality, for example, with sediment-contact tests, often proves to be difficult, because sediments form complex environmental compartments which are highly diverse in structure and composition. However, sediment-contact tests are important elements in the ecotoxicological risk assessment of sediment-bound pollutants (Höss et al. 2010; Feiler et al. 2013; Tuikka et al. 2011). Because those bioassays reflect interactions between sediment and test organism, they provide a more realistic exposure scenario for the assessment of hazards arising from bioavailable sediment-bound contaminants. Over recent years, various sediment-contact tests have been developed based on invertebrates, such as *Caenorhabditis elegans* (ISO 2010). However, sediment-contact tests with higher plants are so far only available as international standards based on watermilfoils. One bioassay using *Myriophyllum aquaticum* (ISO 2013) and one bioassay using *Myriophyllum spicatum* in a sediment water system (OECD 2014) have been standardized. This recent increase in standard testing procedures with higher plants reflects the increasing relevance of plant tests in sediment quality assessment studies. However, despite the key role of higher plants in aquatic

ecosystems, as functional and structural elements, sediment-contact tests with macrophytes are still scarce (Diepens et al. 2013). Macrophytes are a systematically, and hence physiologically, highly diverse group of photosynthetic organisms that occupies various niches in freshwater ecosystems (Chambers et al. 2008). Moreover, macrophytes and aquatic plants, in general, provide key functions and thus are the basis of biodiversity in freshwater ecosystems (Bornette and Puijalon 2009). Due to large differences in exposure routes for pollutants as well as in life cycles between the diverse taxa of aquatic plants, sensitivities to pollutants vary between taxa. Thus, it is difficult to extrapolate from, for example, floating *Lemna* spp. to sediment rooting *M. aquaticum*. Arts et al. (2008) discovered large differences in sensitivity of five aquatic plant species to different dissolved or sediment-bound contaminants. No species was regarded as generally the most sensitive to all tested contaminants. As shown by Arts et al. (2008), risks to sediment-rooted macrophytes might not be addressed by testing with *Lemna* spp. because the risk can be underestimated. In conclusion, additional sediment-contact tests with aquatic macrophytes should be developed. Those new bioassays should include physiologically more different vascular plants, which show different sensitivities between taxa toward sediment-bound pollutants, such as heavy metals and some herbicides (Arts et al. 2008; Belgers et al. 2007). For several reasons, rice could serve as a relevant test organism in the divers group of macrophytes: (i) rice grows semiaquatic, so that the roots are in direct contact to the sediment. Therefore, it is able to indicate the presence of bioavailable sediment-bound pollutants on the macroscopic scale by changes in growth behavior (Chakrabarty et al. 2009; Feiler and Krebs 1999); (ii) rice plays an important role in the global food industry. This guarantees a big pool of scientific research information on rice. Those are useful for the interpretation of measured effects; (iii) thus far, no aquatic in vivo testing with rice as test organism was standardized (Wang and Keturi 1990; Rivera et al. 2013; Liu et al. 2007b). Currently, ecotoxicological research on rice focuses on contaminated paddy field soils, which are already a thread to rice farmers and consumers (Li et al. 2005). The development of suitable tools for an adequate risk assessment on rice fields is, consequently, highly required; (iv) as alternative endpoints, molecular endpoints, such as stressor-induced transcriptomic changes, could be considered as well. This is possible because the rice genome was sequenced completely (Yu et al. 2002), and thus DNA microarrays for subsequent toxicogenomic analysis are commercially available.

Therefore, the aim of the present study was to develop a test protocol for a sediment-contact test using the rice plant *Oryza sativa* L. spp. *indica* as test organism and to evaluate the performance of the developed test in comparison to other plant-based bioassays.

Material and methods

Test organism

Grains of *Oryza sativa* spp. *indica* were obtained from the Seeds and Grains Research Center at Costa Rica University. The rice grains were unblended seeds of the variety Palmar-18. If not specified otherwise, preliminary experiments for validation of the test parameters were performed with rice grains of unknown provenience obtained by the Botanical Garden in Mainz/Germany.

Test compounds

Test compounds for the conducted exposure experiments were chosen based on their relevance as sediment-bound pollutants or as reference substances. From 11 substances, which were tested in a range-finding experiment, the six most toxic compounds with respect to growth inhibition were selected as model substances for the method evaluation. The selected compounds were sodium arsenite [As(III); CAS 7784-46-5; Aldrich], nickel(II)chloride hexahydrate [Ni(II); CAS 7791-20-0; Merck], cadmium chloride [Cd(II); CAS 10108-64-2; Aldrich], chromium(III) chloride hexahydrate [Cr(III); CAS 10060-12-5; Applichem], and potassium dichromate [Cr(VI); CAS 7778-50-9; Aldrich].

Nutrient solution

Steinberg medium was used as nutrient solution. It was prepared according to the guidelines of the sediment-contact test with *M. aquaticum* (ISO 2013) to achieve comparability between both test systems.

Artificial sediment

Artificial sediment was prepared according to the preculturing sediment in the International Standard of the sediment-contact test with *M. aquaticum* (ISO 2013). This artificial sediment was used as the negative control for exposure testing and the spiking experiments with the chosen toxicants.

Sediment spiking

The spiking of the sediment was based on the ISO standard for the sediment-contact test with *M. aquaticum* (ISO 2013). For each test compound, a stock solution was prepared. Heavy metals and arsenic were dissolved in nutrient solution, diluted to the final concentration, and added to the dry artificial sediment. Subsequently, the spiked sediment was kept in a closed vessel for 7 days at 25 °C in darkness for equilibration.

Natural sediments

The natural sediments used in the present study were sampled from selected sampling sites at the watercourses Moselle [Palzem, lock (PZ-R)], Rhine [Schierstein, harbor (SH-R)], and Hunte [harbor (HU-R)] in Germany. These sediments have been previously classified in the joint research project SeKT (Feiler et al. 2013). The sediments were taken with a stainless steel van Veen grab sampler (sampling depth 0–10 cm) and characterized by grain-size distribution, total organic carbon (TOC) content, and sediment contamination. The list of parameters includes anthropogenic contaminants that are typically enriched in sediments, such as heavy metals or persistent organic pollutants. Sediment concentrations of the four metals that were used as model substances in this study are shown in Table 1. The complete physical and chemical investigation program and the methods used are described elsewhere (Feiler et al. 2013).

Test protocol

In order to allow comparability between methods, the test protocol was developed based on the sediment-contact test with *M. aquaticum* (ISO 2013). In order to assure that all test organisms were in the same life stage at the beginning of the test, rice grains were precultured for 48 h (Yoshida 1981). Therefore, rice grains were suspended in nutrient solution in glass trays on filter paper and kept in a climate chamber at (25±1) °C .The grains used for the exposure experiments showed a radical below 0.2 mm in length. Six-well microtiter plates served as test vessel (one well=one replicate). Each experiment consisted of four biological replicates per test concentration. One biological replicate consisted of three seedlings that were grown in one well of a six-well plate. The single seedlings were treated as technical replicates. In each well, 10 g of the prepared artificial sediment or the natural sediment was weighted in. The three pregerminated grains were placed gently on the sediment surface.

To show changes in growth behavior, different concentrations per compound were tested (Table 2). The plates were kept in a climate chamber at (25±1) °C and at a light intensity of (65±5) μmol $m^{-2}s^{-1}$ (neutral white) with a constant light regime for 7 days. According to Yoshida (1981), this experimental conditions result in a linear growth of the plants. To avoid desiccation, the plates were covered with a six-well plate cover for the first 48 h and cultivated in a miniature glasshouse in the climate chamber. After the removal of the six-well plate cover, the watering of the plants was adjusted to the water demand of the plants. The water demand depends on the age of the sprout and the air humidity. The irrigation guaranteed a constant layer of nutrient solution (~1 mm) above the sediment surface. For irrigation, a mixture of one part nutrient solution and one part double-distilled water was used. The cultures were randomized in position every 48 h to compensate deviations in light intensity and temperature, which are possibly present in the climate chamber.

Table 1 Measured concentration of tested compounds in natural sediments[a]

Code	As [mg kg^{-1}_{dw}]	Cd [mg kg^{-1}_{dw}]	Cr [mg kg^{-1}_{dw}]	Ni [mg kg^{-1}_{dw}]
SH-R	10	2.1	70	44
HU-R	25	3.6	80	56
PZ-R	32	1.2	70	44

[a] Measurement based on dry weight in the 2-mm fraction

Evaluation of the test

If not specified otherwise, at the end of the exposures, the individual plants were described (color, shape of shoots and roots, necrosis, and chlorosis). The test endpoints root and shoot elongation were evaluated as follows: each plant was carefully sampled from the sediment, carefully washed in double-distilled water, and dried on tissue paper without damaging it. Length of root and shoot were immediately determined after sampling with an mm grid [mm±0.5]. Shoot length was assessed as the length from the seed coat to the tip of the primary leaf. Root length was assessed as the length from the seed coat to the tip of the primary root. The results of the endpoints root and shoot length are shown as the average of the three technical replicates. If distinct plants from one replicate showed a reduced or enhanced growth of more than 30 % compared to the average growth of the treatment, they were excluded from further analysis. The inhibition of growth for shoot and root elongation (I [%]) was calculated from the shoot and root lengths of control and exposed plants following Eq. 1, which is the calculation of the inhibition of root and shoot elongation I [%]:

$$I\ [\%] = \left(1 - \frac{\text{lenght sample [mm]}}{\text{lenght control [mm]}}\right) \times 100 \quad (1)$$

In terms of the initial development of the test protocol, suitable endpoints were identified. In this context, two more endpoints were measured as follows: fresh weight and dry weight of root, shoot, or total plant. For the determination of the fresh weight, the plant or the respective parts of the plant were immediately weighted after sampling. For the determination of the dry weight, rice plants were dried to constant weight in a drier at 50 °C for 24 h. Subsequently, the plant or the respective parts of the plant were weighted.

Table 2 Numbers of exposures (*n*) with three to four replicates for each concentration and tested substance

Arsenic [mg kg^{-1}_{dw}]	*n*	Cadmium [mg kg^{-1}_{dw}]	*n*	Chromium (III) [mg kg^{-1}_{dw}]	*n*	Chromium (VI) [mg kg^{-1}_{dw}]	*n*	Nickel [mg kg^{-1}_{dw}]	*n*
10	5	1	4	56.3	3	12.5	4	25	4
11	7	2.5	5	113	3	25	2	50	4
12	6	5	5	225	4	56.3	2	100	4
13	9	10	4	300	3	112,5	7	200	4
14	5	15	5	450	2	225	2	300	4
15	3	20	7	900	4	300	3	400	4
20	3	25	4	1,200	4	450	3	500	4
30	3	30	7	1,800	3	900	4	700	4
40	3	65	4						
50	3	95	4						
100	3	130	3						
		180	3						
		230	3						
		280	3						
		330	3						
		380	3						
		400	3						

Statistical analysis

If not specified otherwise, the inhibition of growth [%], standard deviation (SD), coefficients of variance (CV), and the Gaussian error propagation (Gep) based on the standard error (SE) were calculated over all exposure experiments (with four biological replicates each). Dose–response curves were generated in SigmaPlot 12.0 and fitted with a three-parametric sigmoidal curve fit ($f = a / \{1 + \exp(-[x - x_0] / b)\}$) with $a = 100$. To test for statistical differences between the response in natural sediments and the control sediment, one-way analysis of variance tests were performed, and treatments were compared with a post hoc Dunnett's test ($\alpha = 0.05$, two-sided). If the test for normal distribution (Shapiro–Wilk test) and homogeneity of variance (Levene's test) failed, the Kruskal–Wallis one-way analysis of variance on ranks was used ($\alpha = 0.05$).

Results and discussion

Development of the test protocol

Identification of suitable endpoints

To guarantee a good practicability of the test system, endpoints were chosen according to the following criteria: they should be simple, efficiently manageable, and technically feasible, must show a low variability, and must be sufficiently sensitive to the test substances. With respect to published test systems with higher plants (reviewed in Diepens et al. 2013), the well-established endpoints dry and fresh weight (root, shoot, and total) as well as elongation of root and shoot were chosen and evaluated with regard to the abovementioned criteria within the present study.

First, the parameters fresh weight of the whole plant, the shoot and the root were tested as described previously. The small weight of the plants and their constant loss of water made it impossible to reliably assess the weight of the root, the shoot, or the whole plant. This parameter was therefore excluded from further analysis.

Second, the endpoints elongation of root and shoot, as well as the dry weight of the root, the shoot, and the total plant, were studied. A comparison of the investigated endpoints is shown in Fig. 1. The parameters dry weight of the root and the shoot showed slightly higher intratest variability than root and shoot elongation (Fig. 1). Because of the higher variability of the results and the time-consuming procedure, this parameter was judged to be ineligible for the testing of sediments, and hence, it was not further used in the present study. In contrast, the parameters root and shoot elongation of rice plants proved to be suitable and easily assessable. The intertest variability for root and shoot elongation on control sediments from 23 exposure experiments is shown in Fig. 2. The plants showed good and uniform growth for both parameters. Although the observed average root length of rice seedlings grown under control conditions showed a higher intertest variability compared to average shoot length, this variability was acceptable compared to other plant bioassays (Feiler et al. 2014). Therefore, root and shoot elongations were chosen as endpoints for the assessment of possible adverse effects caused by contaminated sediments on the growth of rice plants.

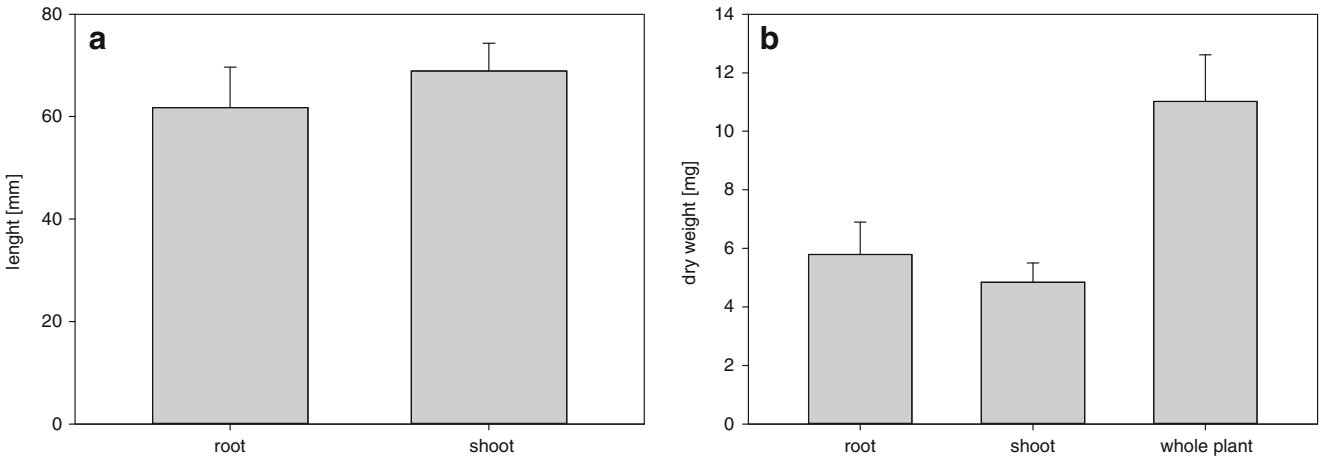

Fig. 1 **a** Length of root and shoot [mm] as well as **b** dry weight [mg] of $n=18$ control plants. The *error bars* indicate the standard deviation. CVs were calculated as 12.7 % (root [mm]), 7.9 % (shoot [mm]), 13.6 % (root [mg]), 19.2 % (shoot [mg]), and 14.5 % (whole plant [mg])

Pretreatments of rice seeds

In order to reduce test variability caused by possible differences in the germination of the rice grains, three different procedures for pretreatment of the seedlings were comparatively investigated: (i) grains were chosen by morphological characteristics; (ii) grains were incubated for 96 h at 50 °C to interrupt the dormancy (Waheed et al. 2012; Yoshida 1981); and (iii) grains were pregerminated in nutrient solution for 48 h in a climate chamber at a temperature of (25±1) °C and a light intensity of (65±5) μmol m^{-2} s^{-1} (Liu et al. 2007a; Yoshida 1981).

For comparison of the pretreatment procedures, the parameters elongation of root and shoot were evaluated following the test protocol. Results are shown in Table 3. After germination, grains with the lowest coefficient of variation concerning lengths of root and shoot were those of the group (iii) with a pregermination period of 48 h. Furthermore, the shoots and roots from pregerminated grains showed a higher growth due to their earlier activation. The 48 h pregermination procedure was already described by Liu et al. (2007a) and Yoshida (1981). Moreover, the pregermination procedure allowed identifying nongerminable grains. In addition, due to the use of constant growth conditions (e.g., temperature and light regime), all grains were in the same life stage at the beginning of the exposure experiment, with a radical of a length of about 0.2 mm. Although not studied systematically, it was observed in the present study that pregerminated seeds with a radical longer than 0.2 mm showed a complete inhibition of growth in both the exposure and the control samples.

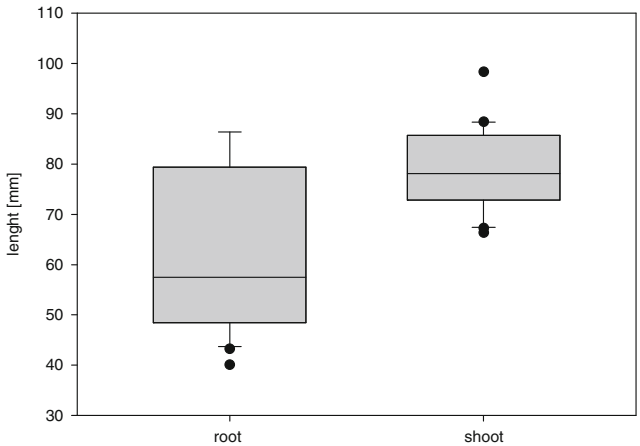

Fig. 2 Average length of rice roots and shoots from control plants in 23 independent exposure experiments with four parallel treatments each ($n=92$). CVs were calculated as 20 % (shoot [mm]) and 26 % (root [mm]). The *boundary of the box closest to zero* indicates the 25th percentile, a *line within the box* marks the median, and the *boundary of the box farthest from zero* indicates the 75th percentile. *Whiskers (error bars) above and below the box* indicate the 90th and 10th percentiles. *Points* mark outliers

Growth behavior of *Oryza sativa* on spiked artificial sediments

In order to assess the sensitivity and repeatability of the selected endpoints for the developed test protocol with *O. sativa*, dose–response curves for four sediment-relevant pollutants were analyzed and EC_{20} and EC_{50} values were derived. For each pollutant, at least three independent exposure experiments were performed at different days with four biologically independent replicates for each concentration step. As shown

Table 3 Influence of different pretreatments on root and shoot elongation

	Shoot [mm]			Root [mm]		
Pretreatment	Mean	SD	CV	Mean	SD	CV
Morphology (i)	12.9	2.4	18.3	8.7	1.4	15.7
96 h at 50 °C (ii)	12.4	3.7	30.2	8.9	1.8	20
Pregermination 48 h (iii)	21.8	3.2	14.9	12.9	0.7	5.3

SD standard deviation, *CV* coefficient of variance

in the following section, clear trends for dose-dependent growth inhibition were detected by decreased elongation of roots and shoots. The root elongation proved to be more sensitive than shoot elongation, with the exception of arsenic, where EC_{20} values of roots and shoots were similar (Table 4). Similar results were observed for rice plants grown in triclosan-spiked natural soil (Liu et al. 2009).

In the following section, the results of the present study will be highlighted in detail, discussed against the background of their ecological relevance, and be compared to toxicological data obtained by other plant bioassays. Additionally, in order to assess the sensitivity, and thus ecological relevance, of the test system, we compared our derived effect concentrations with natural sediment concentrations. Average metal and arsenic concentrations were mainly obtained from the study of Heininger et al. (2003) that investigated natural sediments at five sites of the river Elbe (Germany) during the years 1991–2001.

Arsenite [As(III)]

The most sensitive responses of the rice plants were induced by arsenite (Fig. 3). As(III) induced an inhibition of growth of more than 90 % at concentrations of 30 mg kg^{-1}_{dw} for roots and 50 mg kg^{-1}_{dw} for shoots (Fig. 3). These findings are in accordance with a publication by Azizur Rahman et al. (2007). They reported a complete inhibition of growth in exposure experiments with rice on arsenic-added natural soils at arsenite concentrations above 60 mg kg^{-1}_{dw}. The higher sensitivity of roots compared to shoots toward total arsenic might be explained by a higher concentration of total arsenic in roots. In rice samples from different regions in Bangladesh, total arsenic concentrations of 2.4, 0.73, and 0.14 mg kg^{-1} were found in roots, shoot, and grains, respectively, which imply a limited transport of arsenic in rice plants from root to shoot (Das et al. 2004). Arsenite instead of arsenate was tested in the present study due to its reported higher mobility in paddy soils (Meharg and Jardine 2003) and its higher toxicity. Arsenite in comparison to arsenate was described as more toxic to rice plants grown for 10 days in nutrient solution (Chakrabarty et al. 2009). Furthermore, arsenite has a higher uptake rate due to a phosphate and arsenate competitive uptake (Meharg and Jardine 2003). The uptake of arsenite occurs via the aquaporins of the roots, but varietal differences in arsenite uptake are expected (Meharg and Jardine 2003). This is important against the background of the interest in finding or breeding new rice varieties with limited arsenite uptake in order to decrease the human arsenic uptake and affiliated health risks. Our study indicates that the variety Palmar-18 is limited in arsenite uptake due to the high sensitivity for arsenite, which might limit growth on arsenite-contaminated rice fields.

The determined values for EC_{20} (root=7 mg kg^{-1}_{dw}, shoot=5 mg kg^{-1}_{dw}; see Table 4) and EC_{50} (root=13 mg kg^{-1}_{dw}, shoot=24 mg kg^{-1}_{dw}; see Table 4) of rice on arsenite-spiked sediment were compared to average arsenic concentrations reported by Heininger et al. (2003) from the river Elbe. The measured arsenic concentrations in that study ranged from 33–70 mg kg^{-1}_{dw}. This implies a sufficient sensitivity of the test system to detect growth inhibition by arsenic in environmental relevant concentrations.

Chromium

Chromium was assessed as trivalent [Cr(III)] and hexavalent [Cr(VI)] chromium. In contrast, in other sediment quality assessments, chromium speciation is often not taken into account. This is mainly due to methodological complexities in analytical chemistry. However, Cr(III) and Cr(VI) differ widely in terms of toxicity and bioavailability, and thus, it is necessary to assess both metal speciations separately. The more toxic speciation, Cr(VI), is highly mobile in sediments and soils as compared to Cr(III) and thus leaches more easily to groundwater or other water bodies (Lytle et al. 1998; Zayed and Terry 2003). Cr(VI) adsorption to clay is in inverse proportion to the soil pH. The pH of the test sediment was at about pH 6–7, where more than 95 % of Cr(VI) are described to be dissolved and hence became bioavailable (Adriano

Table 4 Toxicological endpoints for the tested elements [mg kg^{-1}_{dw}] referring to the inhibition of root and shoot elongation [%]

	EC_{20}				EC_{50}			
	Root [mg kg^{-1}_{dw}]	95 % CI	Shoot [mg kg^{-1}_{dw}]	95 % CI	Root [mg kg^{-1}_{dw}]	95 % CI	Shoot [mg kg^{-1}_{dw}]	95 % CI
Arsenic	7	±28	5	±19	13	±19	24	±16
Cadmium	56	±9	181	±7	408	±19	477	±21
Nickel	130	±15	157	±12	218	±16	600	±17
Chromium (III)	335	±15	1,117	±21	1,288	±12	2,032	±29
Chromium (VI)	266	±40	299	±43	334	±32	373	±30

The number of treatments (*n*) included in the calculation are shown in Table 2

dw sediment dry weight, *95 % CI* 95 % confidence interval

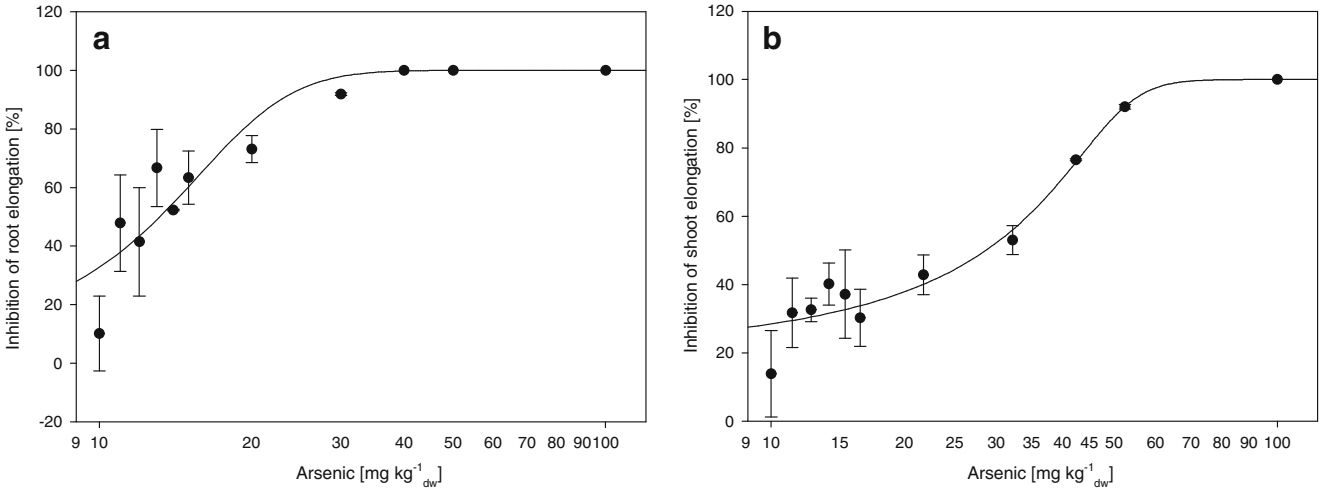

Fig. 3 Concentration–response curve for *Oryza sativa* on arsenic-spiked sediments, expressed as the inhibition of **a** root or **b** shoot elongation with SD

1986). In plants, both speciations lead to oxidative stress (Panda and Choudhury 2005). In addition, Cr(III) affects growth, water balance, and pigment content and initiates lipid peroxidation causing oxidative damage to plants (Panda and Choudhury 2005), and Cr(VI) is described as growth retarding (Han et al. 2004). Our results showed a higher sensitivity of rice to Cr(VI) than to Cr(III) (Figs. 4 and 5), confirming the higher toxicity of Cr(VI) and stressing the need to differentiate between chromium speciations.

In the present study, root elongation was found to be only slightly more sensitive than shoot growth for Cr(III), and in the case of Cr(VI), no differences in the inhibition of root or shoot elongation were detected. In contrast, Hou et al. (2014) conducted exposure treatments with four plant species on natural sediments spiked with Cr(VI) and assessed root elongation as the most sensitive macroscopic endpoint. Test species in that study were, in the order of their sensitivity according to the lowest observed adverse effect concentration (LOAEC), lettuce (LOAEC=20 mg kg^{-1}), wheat, cucumber, cabbage, and corn (LOAEC=50 mg kg^{-1}). This study has been reviewed in Shanker et al. (2005), and the sensitive response of the root was explained with detoxification mechanisms that limit the Cr transport from root to shoot. Moreover, the plant species tested in the study of Hou et al. (2014) seem to be more sensitive than rice toward Cr(VI), as indicated by the determined EC_{20} values (root=266 mg kg^{-1}_{dw}, shoot=299 mg kg^{-1}_{dw}; Table 4) for the rice test. Furthermore, the concentrations of chromium in natural Elbe sediments range from 128 to 226 mg kg^{-1}_{dw} (Heininger et al. 2003). This implies that only weak effects of Cr on rice can be expected in Elbe sediments because the Cr concentrations in those sediments are in the range of the EC_{10} for root elongation on Cr(VI)-spiked sediments. However, according to Heininger et al. (2003), the concentrations of chromium in Elbe sediments are close to local background levels, and thus, if anthropogenic chromium addition occurs, it will likely be detected by the rice test. Anthropogenic chromium additions can be considerable, for example, Zayed and Terry (2003)

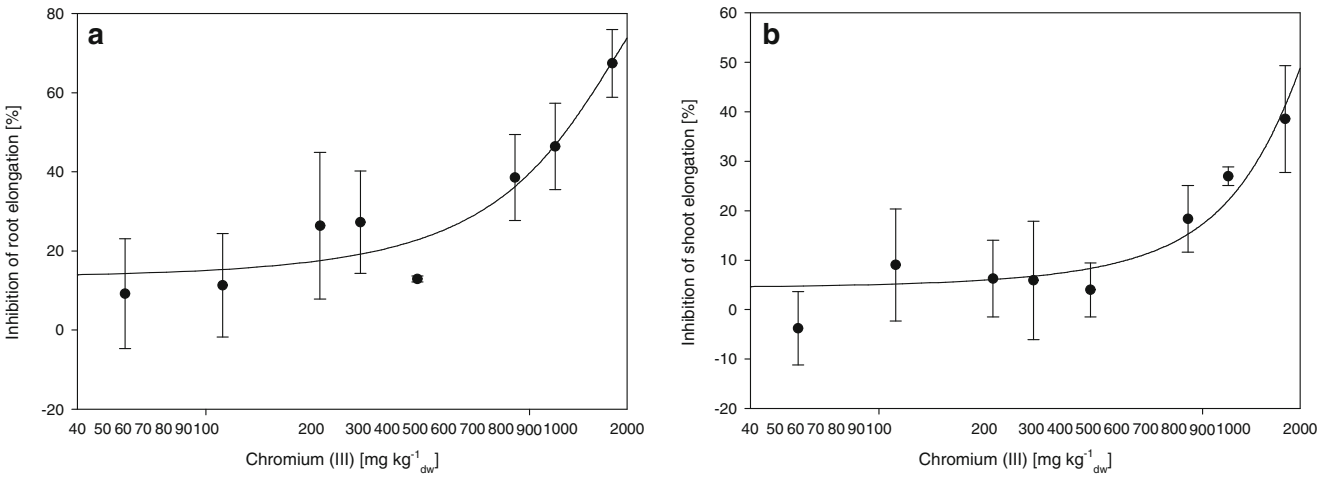

Fig. 4 Concentration–response curve for *Oryza sativa* on chromium(III)-spiked sediments, expressed as the inhibition of **a** root or **b** shoot elongation with SD

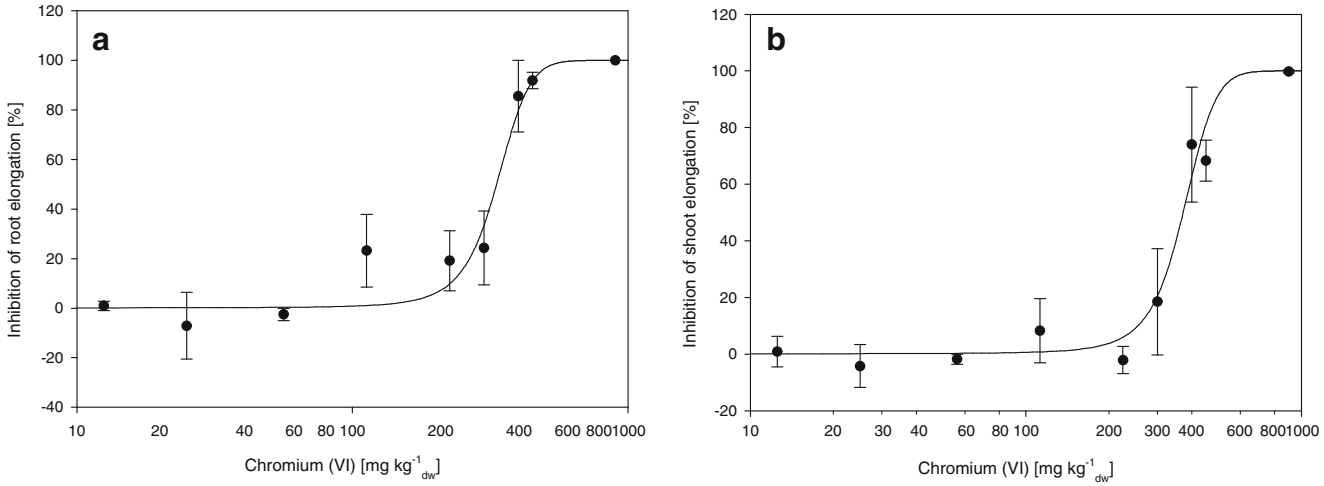

Fig. 5 Concentration–response curve for *Oryza sativa* on chromium(VI)-spiked sediments, expressed as the inhibition of **a** root or **b** shoot elongation with SD

reported up to 3,000 mg kg^{-1} Cr in some natural soils and even more than 25,000 mg kg^{-1} Cr in soils at chromium industry brownfields in Oregon (USA).

Nickel

The EC_{20} values (root=130 mg kg^{-1}_{dw}, shoot=157 mg kg^{-1}_{dw}; Table 4, Fig. 6) and the EC_{50} values (root=218 mg kg^{-1}_{dw}, shoot=600 mg kg^{-1}_{dw}; Table 4, Fig. 6) that were determined with the rice test for nickel are in the range of those obtained by the sediment-contact test with *M. aquaticum* (e.g., EC_{50}= 436 mg kg^{-1}_{dw}; Feiler et al. 2006). However, no complete inhibition of growth was observed in the rice test up to the highest nickel concentration, and thus, it has to be considered that higher uncertainties may be associated with the derived effect concentrations. Nonetheless, it can be also deduced from the effect concentrations that root elongation was more sensitive to nickel exposure than shoot elongation. Moreover, if the effect concentrations are compared to nickel concentrations in field samples, no significant growth inhibition can be expected on sediments of Elbe River with concentrations ranging from 64 to 94 mg kg^{-1} (Heininger et al. 2003). However, for contamination hot spots with nickel concentrations between 200 and 26,000 mg kg^{-1} (Nagajyoti et al. 2010), the rice test will likely show significant inhibitory effects.

Cadmium

Cadmium is less known for its phytotoxic potential, but for causing severe human health risk. In this context, rice consumption is described to be one of the major indirect vectors for cadmium toxicity in humans (Kirkham 2006; Simmons et al. 2005). This is due to the ability of some plant species, such as many rice varieties, to accumulate high concentrations of cadmium (Kirkham 2006). Described phytotoxic effects caused by cadmium exposure are physiological effects, such as oxidative stress, affected transpiration, interference with nutrient and water uptake, altered RNA synthesis, inhibited

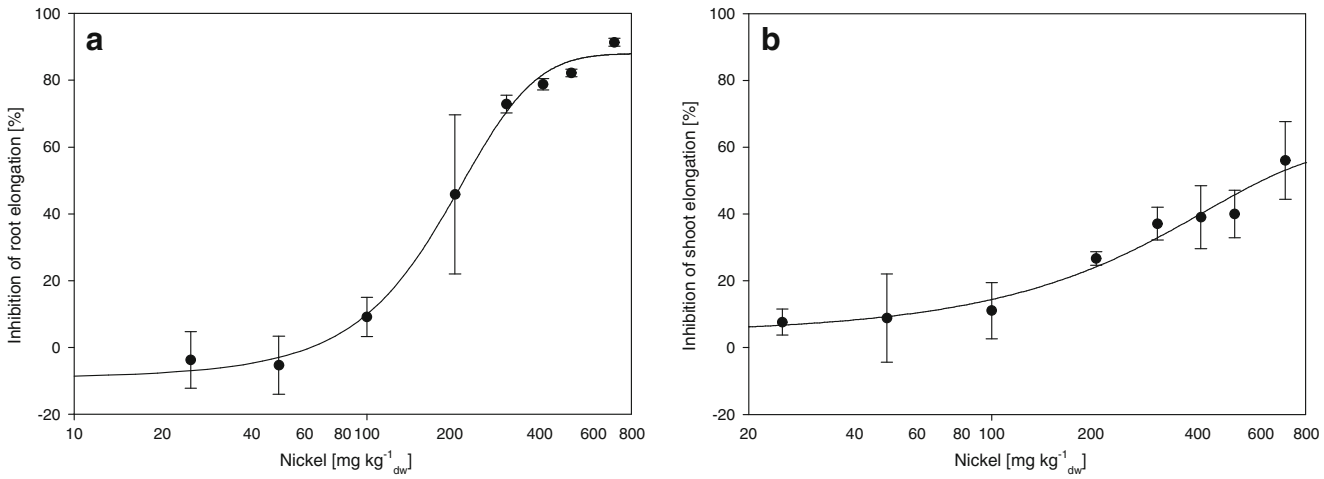

Fig. 6 Concentration–response curve for *Oryza sativa* on nickel-spiked sediments, expressed as the inhibition of **a** root or **b** shoot elongation with SD

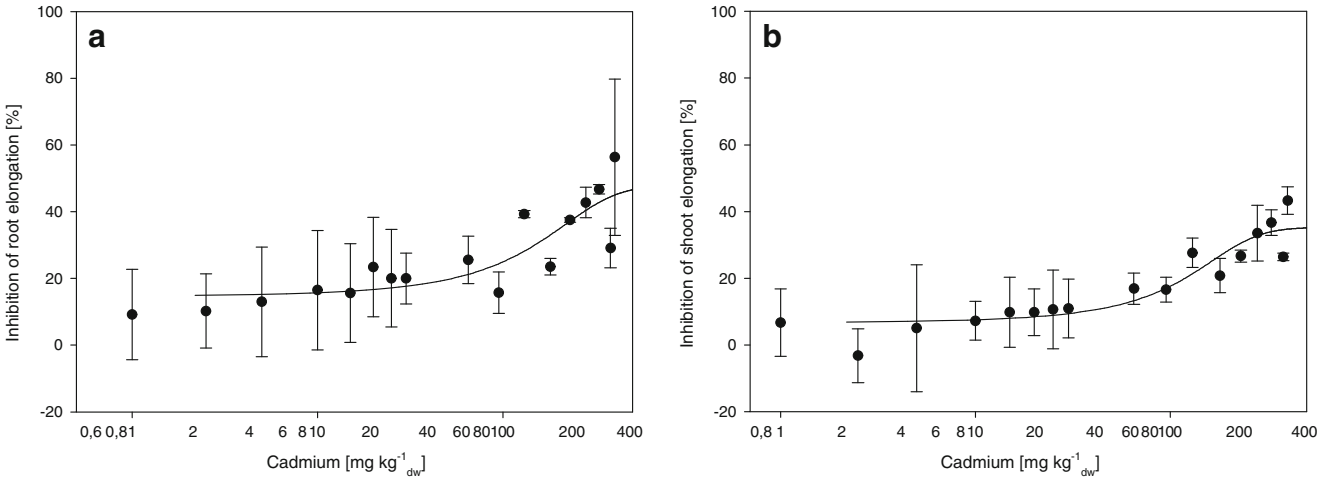

Fig. 7 Concentration–response curve for *Oryza sativa* on cadmium-spiked sediments, expressed as the inhibition of **a** root or **b** shoot elongation with SD

ribonuclease activity, and damage of the photosynthetic apparatus. These physiological effects are resulting in macroscopic effects, such as clorosis, growth inhibition, changes in root morphology, browning of root tips, and finally death (An 2004; Benavides et al. 2005; Chen et al. 2003; Ci et al. 2009; Clemens 2006; Hattab et al. 2009; Nagajyoti et al. 2010; Rodríguez-Serrano et al. 2006; Sanitá di Toppi and Gabbrielli 1999; Xiong et al. 2009).

Some of these authors investigated effects of cadmium on root elongation. Rodríguez-Serrano et al. (2006) reported cadmium-induced growth inhibition of roots from pea plants grown for 15 days in hydroponic medium (50 μM $CdCl_2$), and Xiong et al. (2009) detected a decreased elongation of primary and mesocotyl roots in rice plants cultivated in cadmium-spiked nutrient solution (100 μM).

However, although both studies demonstrated adverse effects of cadmium on roots, the comparability of these results to the present study is limited because cadmium shows a higher bioavailability from nutrient solution than from soil (Sanitá di Toppi and Gabrielli 1999), where cadmium binds to organic matter (Prokop et al. 2003). Hattab et al. (2009) investigated pea plants grown on cadmium-spiked soil, which is a more comparable exposure scenario to the present study. Just like in the studies of Rodríguez-Serrano et al. (2006) and Xiong et al. (2009), EC values were not reported, but an EC_{50} of 7 mg kg^{-1}_{dw} for root length could roughly be estimated. EC values assessed in the present study (Table 4, Fig. 7) were much higher indicating a higher cadmium sensitivity of pea plants than rice. This observed insensitivity of rice toward cadmium might be due to its ability to accumulate high concentrations of cadmium. Rubio et al. (1994) reported a 30-fold increase in Cd-treated rice plants compared to controls. In contrast to effects on root elongation, changes in root morphology were observable already at lower concentrations in the present study. On cadmium-spiked sediments, rice developed a primary root that was growing thicker close to the seed, with a long, very fine tip. Lateral roots and especially mesocotyl roots grew shorter at higher concentrations (Fig. 8) compared to control roots (Fig. 9). Similar effects on root morphology were also shown in the study of Xiong et al. (2009). The changed ratio of root length to root diameter implies changes in the root volume, and thus, changes in nutrient uptake can be expected, because the surface was relatively diminished. Although these morphology changes cannot be measured accurately by conventional methods, such as measurement of root elongation or root dry weight, they might have a high impact on the general fitness of the plant and hence should not be ignored. Especially in case of cadmium, the fine root tip likely impacts the function of root uptake, although this functional damage is not displayed by the inhibition of root length, which is equal to an unaffected root in control sediment. Moreover, the changed morphology of the primary root after cadmium exposure is not necessarily reflected in the dry weight of the root as well. Consequently, the cadmium-exposed root would not be distinguishable from an unaffected and therefore longer root. These findings

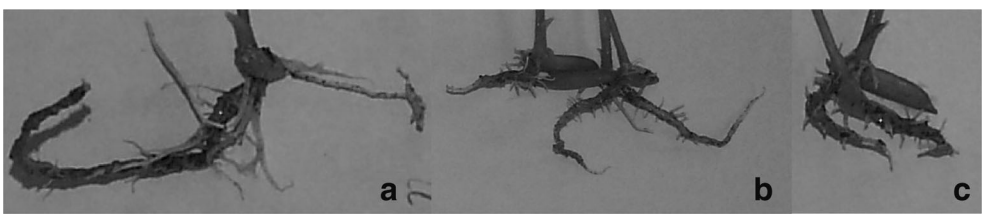

Fig. 8 Rice root exposed to sediments spiked with the following concentrations of cadmium: **a** 30, **b** 180, and **c** 400 mg kg^{-1}_{dw}

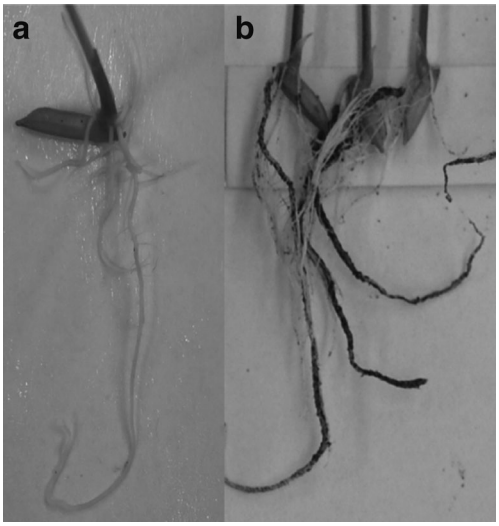

Fig. 9 Morphology of control plants. **a** Rice root grown in nutrient solution after 9 days. **b** Rice roots after 2-day pregermination and a 7-day growth on control sediment

underpin that further research on these sensitive endpoints, and their accurate determination, is desirable. Other macroscopic effects as described in the literature, such as clorosis, were not detected in this study. This might be due to a constant supply of nutrients and water by irrigation with nutrient solution, which is why severe effects might have been masked (Liu et al. 2003).

Concentrations of cadmium in Elbe sediments ranged between 4.9 and 14 mg kg^{-1}_{dw} (Heininger et al. 2003). Disregarding potential synergistic effects of other sediment contaminants, these concentrations of cadmium would not be detectable with the presented test system using only the macroscopic endpoints root and shoot elongation. However, concentrations ranging from 5 to 284 mg kg^{-1} were reported from paddy fields in western Thailand (Simmons et al. 2005) that are in the range of the effect concentrations of both endpoints (root EC_{20}=56 mg kg^{-1}_{dw}, shoot EC_{20}= 181 mg kg^{-1}_{dw}; see Table 4). Furthermore, in these areas, an accumulation of cadmium in rice grains with a concentration of 0.05–7.7 mg kg^{-1} was detected (Simmons et al. 2005), and thus, a low sensitivity of rice toward cadmium is indicated, which is in accordance with the results of the present study.

Growth behavior of *Oryza sativa* on natural sediments

The tested sediments induced an inhibition of root and shoot growth for all sediments and endpoints, with the exception of HU-R that induced growth-promoting effects for roots. Differences in sensitivity of the test system in comparison to the results for the same sediments obtained with the *M. aquaticum* sediment-contact test (Feiler et al. 2013) were detected. The inhibition of shoot elongation of rice was higher than the inhibition of growth rate of *M. aquaticum* (*I* [%]: SH-R=19.4 %, HU-R=9.7 %, and PZ-R=3.2 %). While *M. aquaticum* was significantly inhibited by the sediments SH-R and HU-R, shoot growth of rice plants was also significantly inhibited on HU-R and even more on PZ-R sediments. In contrast to exposures of rice on spiked artificial sediments, the endpoint inhibition of root elongation did not prove sensitive for the tested natural sediments. In all cases, observed inhibition of root elongation was insignificant (Table 5). This might be due to the measured concentrations in the natural sediments that were below the EC_{20} values for Cd, Cr, and Ni. Only the arsenic concentrations measured in the three tested sediments, ranging from 10 to 32 mg kg^{-1}_{dw}, were in the same order of magnitude as the EC_{50} values of arsenite for root and shoot elongation. However, it has to be kept in mind that the data from chemical analysis are total arsenic concentrations, which do not differentiate between element speciations. Therefore, the actual share of arsenite of the measured arsenic is unknown.

Conclusions and perspectives

In summary, based on the presented findings, the rice plant can be regarded as a possible new test organism for sediment-contact tests with higher plants, as it clearly showed an inhibition of shoot growth on natural sediments and in single-

Table 5 Response of the endpoints root and shoot elongation to natural sediment samples

Code[a]	N	Mean [mm]	SD	Root			Mean [mm]	SD	Shoot		
				CV	*I* [%]	Gep			CV	*I* [%]	Gep
C	11	61.4	10.7	17.5	0.0	5.8	76.2	21.9	28.7	0.0	10.7
SH-R	11	57.2	19.5	34.2	7.0	9.1	54.8	13.2	24.0	28.0	10.9
HU-R	11	62.1	25.4	40.9	−1.0	10.5	48.7	30.3	62.3	36.1[b]	18.9
PZ-R	7	52.5	27.1	51.7	14.6	15.9	43.5	19.1	43.8	43.0[b]	17.6

[a] For code abbreviations of sediment samples, see "Natural sediments"

[b] Significantly reduced with regard to the respective control ($p<0,05$, one-way analysis of variance)

C test-specific control, *SD* standard deviation, *CV* coefficient of variance, *I [%]* Inhibition of root/shoot growth (Eq. 1), *Gep* Gaussian error propagation

substance tests on artificial sediments. Effects on rice caused by natural sediments differed to those obtained by the sediment-contact test with *M. aquaticum*. Thus, the proposed new sediment-contact test with rice should be considered to complement ecotoxicological test batteries for aquatic ecosystems, because it provides additional information about the impact of pollutants on macrophytes. Shoot growth is the recommended endpoint for the assessment of toxicity of natural sediments. In addition, because the rice genome is completely sequenced, rice offers the possibility to amend macroscopic endpoints, as used in this study, with molecular endpoints. The latter can be assessed by means of gene expression analysis, which is planned as a subsequent step in this study. It is likely that such an approach will lead to a more comprehensive understanding of pollutant impacts on higher plants.

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