Strategies of Adaptation to Heavy Metal Pollution in *Deschampsia caespitosa* and *Lychnis flos-cuculi*: Analysis Based on Dose–Response Relationship

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Abstract—The response to copper pollution was studied in the vegetative progeny of tufted hair grass (*Deschampsia caespitosa* (L.) Beauv.) and ragged robin (*Lychnis flos-cuculi* L.) plants growing together in chronically polluted areas around the Middle Ural Copper Smelter or in background areas. The root elongation test was used, with copper sulfate (0.006–0.51 mg Cu/L) being added directly to the nutrient medium. Using multimodel inference, dose–response curves were plotted for each of 85 maternal plants, and their parameters (effective Cu concentrations and curve slope in the linear segment) were evaluated. The pattern of transformation in dose dependence of root increment upon transition from background to impact populations proved to be basically different in the two species. The curves for *L. flos-cuculi* showed a parallel shift, with their shape remaining generally unchanged. In *D. caespitosa*, this transition was accompanied by a decrease in sensitivity to Cu, but tolerance to this metal was found to increase only at the highest concentration range. These results provide evidence for different strategies of adaptation to heavy metal pollution, which are discussed by comparing the physiological and ecological properties of the two species.

Keywords: Lychnis, Deschampsia, Silene, root elongation test, population, adaptation, tolerance, sensitivity, dose–response relationship, effective concentrations, industrial pollution, heavy metals, copper, Middle Ural Copper Smelter

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Although the operation period of industrial enterprises is negligibly small on the evolutionary time scale, plants growing in the vicinities of various metal industries are adapted to excess concentrations of heavy metals in the environment. Compared to populations growing in clean habitats, impact populations are characterized by an increased proportion of metal-tolerant individuals and a higher level of individual tolerance (Ernst, 2006).

Metal tolerance of plants has been actively studied from physiological, biochemical, genetic, ecological, and other aspects. To evaluate this parameter, the root elongation test has been widely used since the mid-20th century. It is based on the comparison of linear root growth rates in a clean nutrient medium and in the same medium containing a toxicant (Wilkins, 1957). The results of this test in a number of species (*Agrostis tenuis*, *A. gigantea*, *D. caespitosa*, *Festuca rubra*, *F. ovina*, *Lolium perenne*, *Sedum alfredii*, *Silene paradoxa*, *S. vulgaris*, etc.) show that populations growing in polluted habitats are more tolerant to heavy metals than background populations (Ernst, 2006).

The root elongation test was initially conducted with a single, most indicative metal concentration (Wilkins, 1978) that could markedly inhibit the growth of tolerant plants without completely suppressing control plants. However, advances in ecotoxicology have provided understanding that correct evaluation of metal tolerance is only possible if the metal is tested in a wide concentration range (Kopittke et al., 2010; Dulya and Mikryukov, 2011). This requirement is observed in most of recent studies (Mengoni et al., 2003), but mathematical approaches to analysis of plant metal tolerance remain archaic. In most cases, they are limited to numerous pairwise comparisons of growth rates at different metal concentrations, with the description of the results being cumbersome and difficult to interpret. In some publications, the authors compare parameters of linear regression of root growth rate on toxicant dose (De Vos et al., 1991; Paschke and Redente, 2002), but this approach is even less valid because of distinct nonlinearity of the relationship between these parameters.

It appears more correct and informative to characterize the response to toxic exposure by nonlinear modeling of the dose–response relationship (Ritz, 2010). According to the concepts of classic toxicology, the relationship between the organism's state and the

Table 1. Characteristics of habitats studied

Parameter	Zone (distance from MUCS, km), coordinates		
	Background (30), 56°48′ N, 59°25′ E	Buffer (4), 56°51′ N, 59°49′ E	Impact (1), 56°50′ N, 59°52′ E
Parameters of vegetation			
Type of community	Grass−herb meadow	Herb−grass meadow	Horsetail−grass meadow
Total coverage of grass stand*, %	91.3 ± 4.75	75.0 ± 7.51	33.4 ± 6.96
Grass stand height*, cm	37.4 ± 4.34	24.8 ± 0.87	23.7 ± 7.95
α-Diversity*	15.5 ± 3.78	12.6 ± 3.00	2.4 ± 0.50
Abundance of *D. caespitosa*, ind./m^2**	1.82 ± 0.09	4.13 ± 0.10	0.56 ± 0.06
Abundance of *L. flos-cuculi*, ind./m^2**	2.55 ± 0.18	1.23 ± 0.14	15.90 ± 1.43
Concentrations of different forms of copper in soil, µg/g			
Water-soluble	0.52 ± 0.02	1.24 ± 0.39	1.27 ± 0.68
Exchangeable	0.08 ± 0.02	2.41 ± 1.35	33.31 ± 9.02
Mobile	22.49 ± 0.40	277.29 ± 107.69	530.93 ± 295.31

Note: Data are presented as mean values with standard deviations for three meadows; *parameters recorded in 20−30 test squares (50 × 50 cm) per meadow, with α-diversity being determined as the number of species per square; **according to Trubina and Dulya, 2007.

dose of toxicant (in a wide concentration range) is described by a sigmoidal curve. This curve is usually described with the effective toxicant concentrations (EC_x) inhibiting growth by 10, 50, and 90%, compared to the initial (control) level: EC_{10} and EC_{90} are close to the inflection points of the nonlinear function, which mark the start and end of rapid changes in the organism's state, and EC_{50} (the most informative point) divides the dose−response curve by half. The effective concentrations characterize *tolerance* to the toxicant: the higher their values, the more tolerant is the organism. Another important parameter of the curve is the slope of its linear segment, which shows how abruptly the growth slows down upon an increase in the dose of toxicant and, hence, characterizes *sensitivity* to this agent (Weltje, 2003). Differentiated analysis of sensitivity and tolerance has proved very informative in radioecological research (Zhuravskaya, Pozolotina, and Kershengol'ts, 1997).

Distinctive features of the dose−response curve (slope angle, the width of the upper or lower plateau, deviations from the sigmoidal shape) depend on specific effects of a given toxicant and mechanisms of organism's metal tolerance (Weltje, 2003; Slikker et al., 2004). It is logical to assume that this curve in adapted organisms is transformed and differs from the initial (control) curve, e.g., in shape or position within the concentration range.

Variants of this transformation depend on different strategies of adaptation to excess concentrations of the toxicant. Therefore, the diagnosis of the type of transformation, combined with analysis of physiological and ecological properties of the test species, can provide an insight into the mechanisms of adaptation and, hence, make it possible to predict the consequences of toxic pollution for populations and ecosystems.

The purpose of this study was to compare transformations of the dose−response relationship in two plant species well adapted to environmental pollution with heavy metals, namely, tufted hair grass (*Deschampsia caespitosa*) and ragged robin (*Lychnis flos-cuculi*). To this end, the root elongation test was used to evaluate the responses to a wide range of copper concentrations in the vegetative progeny of maternal plants from the territory chronically polluted with emissions from a copper smelter and from the control (background) territory. Such a methodological scheme made it possible to plot individual dose−response curves for each maternal plant.

Our choice of either the toxic agent or the test species was not random but based on the following considerations. As a result of chronic atmospheric pollution over the 70-year operation period of the copper smelter, the contents of copper in its surroundings have become an order of magnitude higher than in the background area. This is regarded as one of the main factors responsible for elimination of sensitive species and evolutionary transformations in populations of tolerant species (Table 1). Currently, copper concentrations in the impact zone are many times higher than their threshold values determined for the species studied (Paschke and Redente, 2002).

Deschampsia caespitosa and *L. flos-cuculi* were selected as test species due to their ability to survive and even increase in abundance under conditions where almost all other species deteriorate or disappear (Cox and Hutchinson, 1980). According to our data (Trubina and Dulya, 2007), their abundance in the

impact zone near the smelter is two to six times higher than in the background zone (Table 1).

Deschampsia caespitosa (L.) Beauv. (Poaceae) [ITIS no. 40586, IPNI no. 30074881-2] is a perennial, obligate mycorrhizal meadow grass reproducing mainly by seeds. Its life span averages 40–50 years, sometimes reaching 70–80 years (Zhukova, 1976; Davy, 1980) This widespread hygromesophyte species is acidophilous and tolerant of low soil fertility. *Lychnis flos-cuculi* L. [ITIS no. 20309, IPNI no. 155082-1; =*Silene flos-cuculi* (L.) Clairv., *Coronaria flos-cuculi* (L.) Braun] (Caryophyllaceae) is also a widespread plant characteristic of moist meadows. This predominantly outcrossed species is pollinated by insects but is also capable of vegetative reproduction (Biere, 1995). Species of the families Poaceae and Caryophyllaceae are evolutionarily distant from each other and differ in many ecological and physiological parameters (The Angiosperm..., 2009), but both have proved to be highly tolerant of industrial pollution (Ernst, 2006; Dulya and Mikryukov, 2011). This is why it is relevant to compare them with respect to the strategy of metal tolerance.

A special note should be made on usage of the term "population" in this study: it refers to a group of plants in a certain pollution zone that is separated by forest areas from other groups, with the distance between the groups being no less than 3 km. Although we have not evaluated the rank of such groups in the hierarchy of population categories, they appear to be spatially isolated from each other to some extent and, hence, may be regarded as micropopulations.

For correct data interpretation, it should be taken into account that exposure to toxic emissions from the smelter during its 70-year operation period could result in selection for increased metal tolerance (in a narrow sense, for genetic adaptation to excess environmental copper) in populations of both species growing in polluted areas. Moreover, to exclude the possible effect of acclimation (i.e., reversible changes in individual plants under the effect of environmental factors), test plants were grown under standard conditions for more than a year.

MATERIAL AND METHODS

The study area is in the surroundings of the Middle Ural Copper Smelter (MUCS) in the city of Revda, Sverdlovsk oblast. The impact of MUCS on the biota has been studied from different aspects (Vorobeichik, Sadykov, and Farafontov, 1994; Nesterkov and Vorobeichik, 2009; Trubina and Vorobeichik, 2012). The test populations grow in meadow communities within three zones differing in the level of pollution. The impact zone extends for 1–1.5 km west of the pollution source; the buffer zone, for 4 km; and the background zone, for 30 km. Descriptions of meadow vegetation have been made, including the assessment of grass stand coverage and height.

To determine copper concentrations in the soil, five 1-m² samples of root soil layer (0–10 cm) were taken in each zone, air dried, and sieved through 2-mm mesh. To determine different forms of the metal, 2-g aliquots of the sample were extracted with 20 mL of deionized water (water-soluble Cu), 5% HNO_3 (mobile Cu), or 0.05 M $CaCl_2 \cdot 6H_2O$ (exchangeable Cu). Measurements were made in a Vario 6 atomic absorption spectrometer (Analytik Jena, Germany).

Plant material. In each zone, the material was collected in three meadows located 0.2–1 km apart and separated by forest massifs. In each meadow, shoots or seeds were sampled from 2–12 plants growing at a distance of at least 15 m from each other. The samples were brought to a greenhouse where the seeds were germinated and the shoots were planted for vegetative propagation. The plants were grown in a garden soil mixture (TerraVita, Ukraine) under standard conditions, at natural illumination, for 15 months. On the whole, the material for toxicological experiments consisted of 1228 vegetative shoots from 57 maternal *D. caespitosa* plants (including 26 plants grown from seeds collected in 2006 and 31 plants sampled in the field in August to September 2008) and 378 vegetative shoots from 28 maternal *L. flos-cuculi* plants collected in August to September 2007.

Assessment of metal tolerance. Root elongation tests were conducted in November to December 2008 with *L. flos-cuculi* and in December 2009 to February 2010 with *D. caespitosa*. Shoots separated from the maternal plant were placed in individual plastic cups with 100 ml of Hoagland's no. 2 medium (Sigma Aldrich, Germany) diluted sixfold and grown for 8 days. Thereafter, the medium was supplemented with different amounts of $CuSO_4 \cdot 5H_2O$, and the shoots were grown for 5 more days. The concentrations of Cu^{2+} in the medium were as follows: 0.006 (control), 0.08, 0.10, 0.13, 0.18, and 0.26 mg/mL (for both species) and, in addition, 0.31, 0.34, 0.41, and 0.51 mg/L for *D. caespitosa*. The maximum root length in each individual was measured before and after this treatment to calculate root increment (growth rate) over the 5-day exposure period. This experiment was performed under illumination with fluorescent lamps (a 16-h photoperiod) at 22°C and 60% relative air humidity. On the whole, 8–42 shoots were taken from each maternal plant (2–4 shoots per Cu^{2+} concentration), which allowed us to obtain a set of data on its responses to different Cu^{2+} concentrations in the nutrient medium. Subsequent analysis was performed with datasets for 57 *D. caespitosa* plants (19 plants per zone) and 28 *L. flos-cuculi* plants (7, 10, and 11 plants from the background, buffer, and impact zones, respectively).

Data analysis. Different models describe the same datasets in different ways, which may result in biased estimates of toxicological parameters. A more correct approach is to use several competitive models and compare their fit to the initial data, e.g., using the
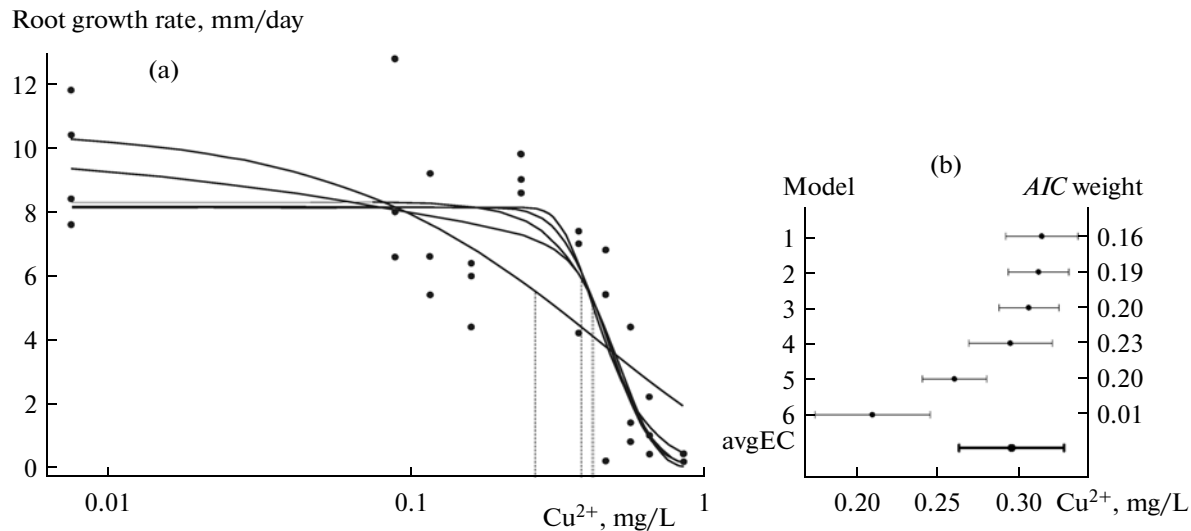
Root growth rate, mm/day

Fig. 1. An example of multimodel analysis for copper tolerance: (a) experimental results of testing plants from family no. 7 and their approximation by different models, with projections on the X axis showing EC_{50} values; (b) mean EC_{50} values and their standard errors calculated by (*1*) Weibull I, (*2*) lognormal, (*3*) Weibull II, (*4*) Cedergreen−Ritz−Streibig, (*5*) linear, and (*6*) exponential decay models; avgEC is the weighted average EC_{50} value.

Akaike information criterion (*AIC*), which takes into account both fit and parsimony of the model (with respect to the number of estimated parameters). For practical applications, absolute *AIC* are converted into *AIC* weights showing the normalized relative likelihood of each model (Burnham and Anderson, 2002), which is interpreted as the probability that a given model has the best fit among all models tested.

Toxicological parameters estimated by different models usually differ from each other even when the models fit the data equally well. Therefore, it is necessary to reduce the model-dependent uncertainty of estimates, e.g., by averaging the results obtained with different models. The procedure of model averaging consists in calculating the *AIC*-weighted mean value of the relevant toxicological parameter for all models, together with its standard error and confidence interval (Buckland, Burnham, and Augustin, 1997).

In this study, the dependence of root growth on Cu^{2+} concentration for each family (i.e., the progeny of each maternal plant) was approximated by functions of four classes: log-logistic models, lognormal models, and two classes of Weibull models (a total of 24 models; for details, see Ritz, 2010). The lower limit of root increment in nonlinear models was taken as the zero point. For each model, *AIC* weights were calculated, and a visual test for fit to empirical data was conducted. The *tolerance* of each family to Cu^{2+} was estimated from the weighted mean effective concentrations of the metal (EC_{10}, EC_{50}, and EC_{90}). Data were analyzed using the R software version 2.15.0 (The R Development Core Team, 2012) and drc package version 2.2-1 (Ritz and Streibig, 2005) for dose−response curves fitting.

The sequence of data analysis is illustrated in Fig. 1. Points in Fig. 1a show the growth rates of vegetative shoots from the same family at different Cu^{2+} concentrations in the medium, and lines show approximations of their dependence on metal concentration by different models. Figure 1b illustrates the procedure of averaging the values of effective Cu^{2+} concentrations (the example of EC_{50}).

Parameter *b* characterizing the slope of the linear curve segment in the Weibull class I model was used as an index of *sensitivity* to Cu^{2+}: the higher the *b* value, the steeper the curve slope and, hence, the higher the sensitivity to the toxicant.

Subsequent data analysis involved comparisons of EC_x and *b* values for different samples. Differences were considered significant if the corresponding confidence interval did not pass through zero. The intrapopulation variability of these parameters was evaluated using the estimator S_n, which is computed as the median of distances between all data points in all their combinations in the sample. Compared to the standard or median deviation, this estimator is more robust and independent of symmetry in the distribution pattern (i.e., is valid at asymmetric distributions) (Rousseeuw and Croux, 1993). All comparisons were made using a stratified bootstrap technique with 10000 iterations in the boot R package version 1.3-4 (Canty and Ripley, 2012).

RESULTS

Meadow vegetation changes consistently as the MUCS is approached: its diversity, density, and plant height decrease, and its total coverage in the buffer and impact zones is 1.2−3 times lower than in the back-
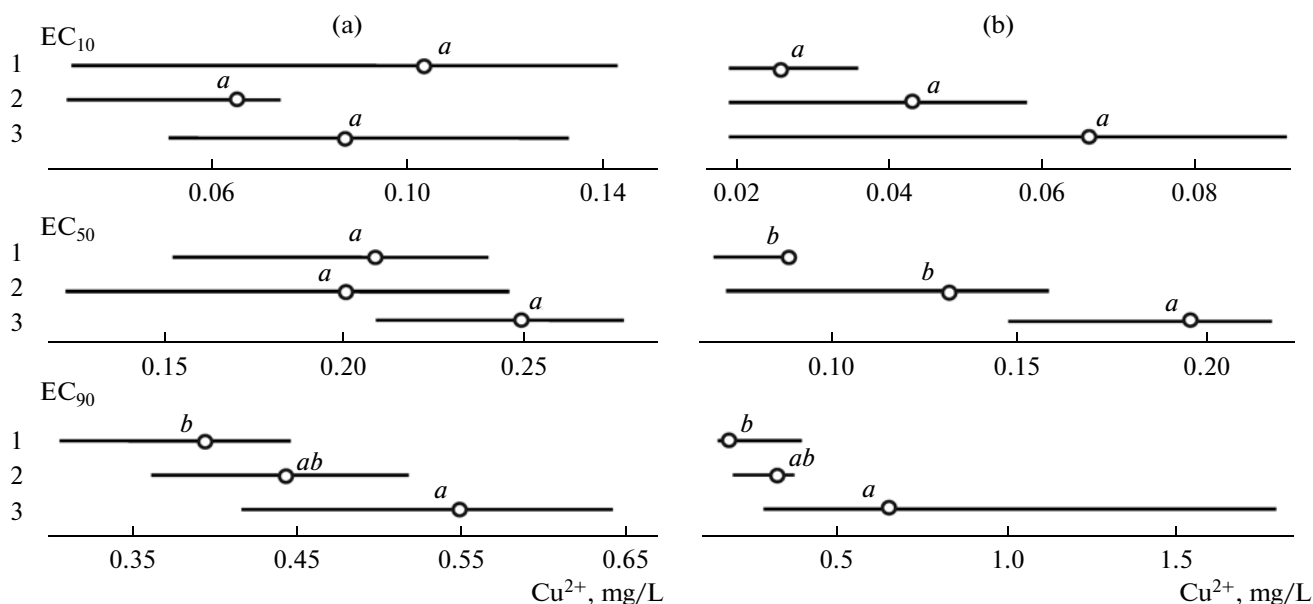
Fig. 2. EC_{10}, EC_{50}, and EC_{90} (median values and 95% confidence intervals) in populations of (a) *D. caespitosa* and (b) *L. flos-cuculi* from (1) background, (2) buffer, and (3) impact zones. Similar letters indicate the absence of differences between median values for conspecific samples from different populations.

ground zone (Table 1). The content of the most bio-available, exchangeable form of copper in the soil exceeds the background level by a factor of 400 in the impact zone and by a factor of 30 in the buffer zone (Table 1).

The results of dose−response modeling show that the group of best fit models includes special cases of the Cedergreen−Ritz−Streibig model, the exponential decay model, linear function, and Weibull models displaying different types of asymmetry (Ritz, 2010).

For *L. flos-cuculi*, all three EC_x increase along the pollution gradient, with EC_{50} in the impact zone being 1.5−2 times higher than in the buffer and background zones and EC_{90} in the impact zone being 3.6 times higher than in the background zone (data statistically significant). In the case of *D. caespitosa*, only EC_{90} increases significantly, being 1.4 times higher in the impact than in the background zone (Fig. 2).

The average value of parameter b (the index of sensitivity) in *D. caespitosa* is lower in plants from the impact than from the background zone, while its values in *L. flos-cuculi* populations do not differ between the pollution zones (Fig. 3).

The variability of all three EC_x does not change significantly along the pollution gradient in *D. caespitosa* but increases in *L. flos-cuculi*: S_n for EC_{10} and EC_{90} is significantly higher than in the buffer and background zones (Table 2). The variability of plant sensitivity in *D. caespitosa* is markedly lower in the impact than in the background population, whereas that in *L. flos-cuculi* remains unchanged along the pollution gradient (Table 2).

In both species, frequency distributions of toxicological parameters plotted for different populations overlap with each other (Fig. 4), which is evidence that all populations contain individuals with similar levels of tolerance. Frequency distributions of EC_x in all *D. caespitosa* populations are right-tailed, indicating the presence of individuals whose tolerance markedly exceeds the population average level. In the case of *L. flos-cuculi*, such individuals occur only in the impact population.

DISCUSSION

If hormesis is not taken into account, then four basic variants of transformation of the dose−response relationship are hypothetically possible (Fig. 5): (I) a "parallel shift," or an equal increase in all EC_x values, with sensitivity remaining unchanged (Fig. 5a); (II) a decrease in sensitivity, with tolerance to the toxicant increasing only at its high concentrations (Fig. 5b); (III) a decrease in sensitivity and an increase in tolerance to the whole range of toxicant concentrations (Fig. 5c); and (IV) an increase in tolerance to moderate toxicant concentrations and simultaneous increase of sensitivity (Fig. 5d).

Transformations of dose−response relationship in populations adapted to growing in industrially polluted areas drastically differs between the two species. The impact population of *D. caespitosa* shows increased tolerance to copper concentrations producing a strong deleterious effect on plants but not to concentrations only slightly inhibiting their growth. Plants from this population also have a decreased sen-
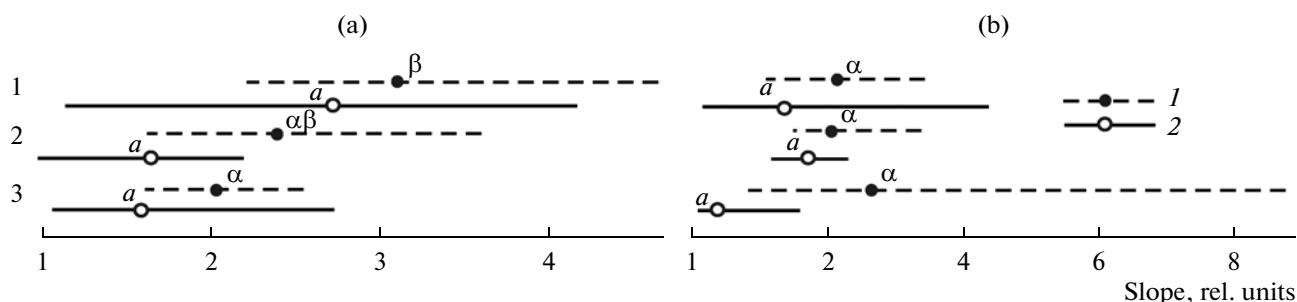
Fig. 3. Parameters of dose−response curve slope in populations of (a) *D. caespitosa* and (b) *L. flos-cuculi* from (1) background, (2) buffer, and (3) impact zones, (*1*) median and (*2*) mean values with 95% confidence intervals. Similar letters (Latin for median and Greek for mean values) indicate the absence of differences between parameters of conspecific samples from different populations.

sitivity to copper. Therefore, exposure to pollution in this case leads to type II transformation of the dose−response relationship (Fig. 5b). In contrast, the impact population of *L. flos-cuculi* shows an increased tolerance to copper in the whole concentration range, with sensitivity to this toxicant being equal in plants from all pollution zones. Therefore, the dose−response relationship is transformed according to type I (Fig. 5a).

Transformations of the dose−response relationship may be interpreted from different standpoints. In physiological terms, adaptation to excess copper in *D. caespitosa* may be based on accessory mechanisms of tolerance that are activated only by extreme toxicant level. In particular, these can be the mechanisms of intracellular repair (Hall, 2002). Characteristic of *L. flos-cuculi* plants from polluted habitats are protective mechanisms that operate more actively (compared to the baseline level) in the whole range of toxicant concentrations. They include mechanisms maintaining Cu homeostasis in the cell (e.g., reduced inflow and increased outflow of these ions and their compartmentalization in vacuoles), which have been revealed in copper-tolerant lines of the related species *Silene vulgaris* (Verkleij et al., 2001). It is noteworthy that serial root elongation tests for resistance to copper (with Cu^{2+} concentrations of 0.01 to 20 mg/L) in *S. vulgaris* populations from background, buffer, and impact habitats with different soil copper contents (De Vos et al., 1991) produced results similar to those obtained in our study: adaptation of *S. vulgaris* to this metal was accompanied by a similar increase in all EC_x values, and, consequently, the dose−response curve retained the same shape in the pollution gradient but only changed its position along this gradient.

From the standpoint of evolutionary genetics, tolerance characterizes the phenotypic plasticity of the genotype: the higher the tolerance, the lower the plasticity level (Weltje, 2003). In these terms, adaptation in *D. caespitosa* is achieved due to an increase in the plasticity of mechanisms providing for metal tolerance, whereas *L. flos-cuculi* shows a different adaptation strategy: its phenotypic plasticity remains unchanged, but the average level of metal tolerance increases.

Table 2. Variation in parameters of copper tolerance and sensitivity in *D. caespitosa* and *L. flos-cuculi* populations (average S_n values and, in parentheses, 95% confidence intervals). Similar letters indicate that corresponding values do not differ between populations

Parameter	Pollution zone		
	background	buffer	impact
D. caespitosa			
EC_{10}	0.090 (0.069−0.15)*a*	0.053 (0.013−0.128)*a*	0.063 (0.026−0.143)*a*
EC_{50}	0.084 (0.046−0.133)*a*	0.093 (0.051−0.146)*a*	0.063 (0.044−0.136)*a*
EC_{90}	0.130 (0.044−0.202)*a*	0.143 (0.063−0.412)*a*	0.225 (0.115−0.523)*a*
Slope	2.412 (1.667−5.168)*a*	1.412 (0.806−3.249)*ab*	0.971 (0.547−1.685)*b*
L. flos-cuculi			
EC_{10}	0.010 (0−0.024)*a*	0.024 (0.008−0.044)*a*	0.058 (0.036−0.09)*b*
EC_{50}	0.029 (0−0.043)*a*	0.043 (0.014−0.089)*a*	0.079 (0.029−0.239)*a*
EC_{90}	0.040 (0.003−0.357)*a*	0.099 (0.036−0.202)*a*	0.561 (0.127−1.217)*b*
Slope	1.705 (0.013−2.321)*a*	0.873 (0.352−2.098)*a*	0.430 (0.075−4.15)*a*

Fig. 4. Frequency distributions of EC_{10}, EC_{50}, EC_{90}, and parameters of dose−response curve slope in populations of (a) *D. cae-spitosa* and (b) *L. flos-cuculi* from (1) background, (2) buffer, and (3) impact zones.

Indirect evidence for this conclusion is provided by the results of studies on morphological variation of the two species in a wide range of ecological conditions. They show that *D. caespitosa* is a highly plastic species

(Davy, 1980; Seliskar, 1985) and that the level of physiological plasticity may differ between individuals from different habitats (Tieszen and Helgager, 1968), whereas the phenotypic plasticity of *L. flos-cuculi* in
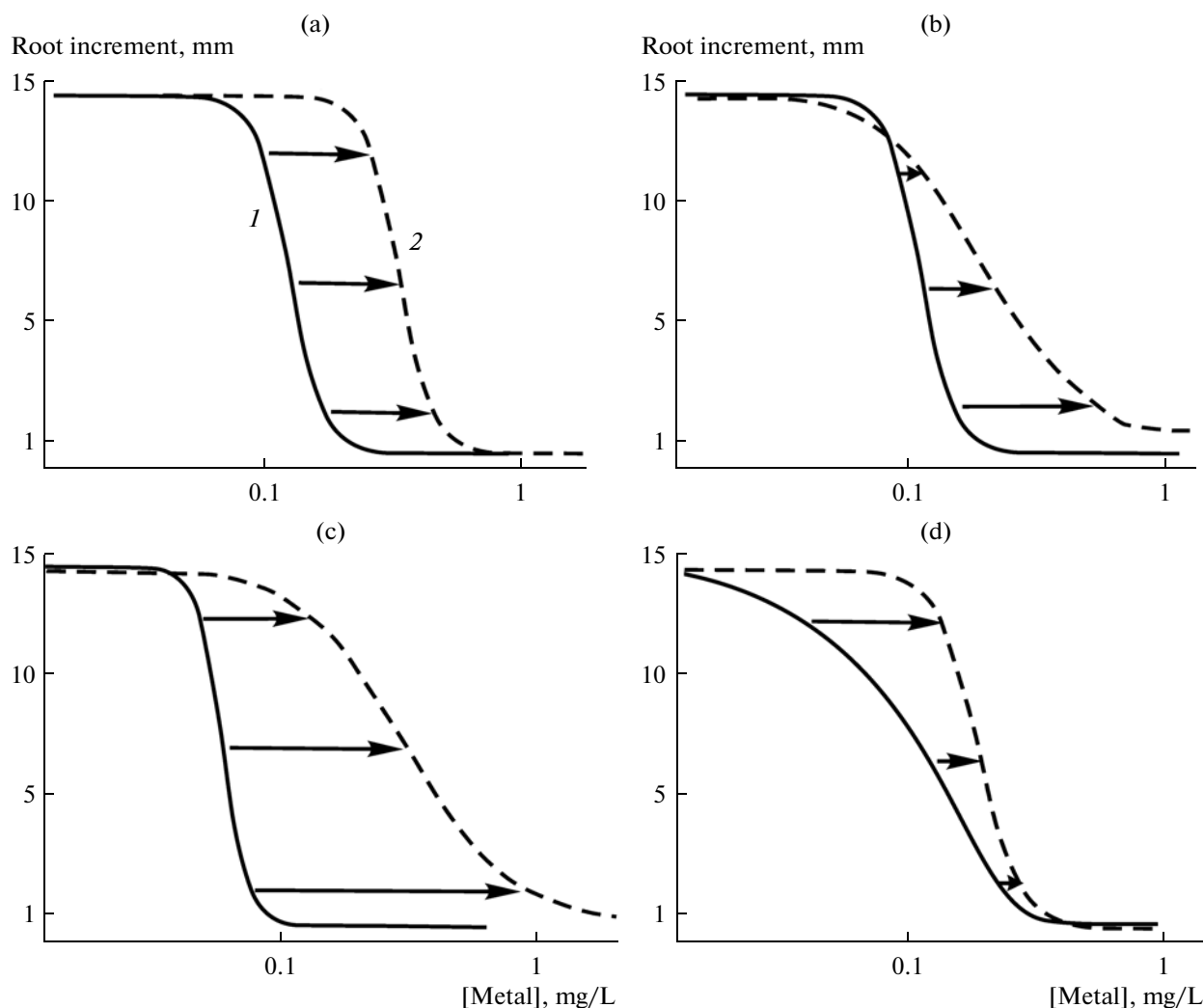
Fig. 5. Hypothetical types of transformation of the dose–response relationship upon plant adaptation to excess heavy metal contents: (*1*) dose–response curve for nontolerant plants, (*2*) dose–response curve for adapted plants.

response to a variety of factors is low, with genotypes differing in the ecological optimum for growth and reproduction showing similar levels of plasticity (Biere, 1995).

From the ecological standpoint, transformation of the dose–response relationship in *D. caespitosa* may be interpreted as follows: in polluted habitats, the decrease in the rate of linear root growth caused by excess soil copper is to a certain extent acceptable for plants as long as it does not endanger their survival. In the case of *L. flos-cuculi*, in contrast, adaptation is aimed at avoiding any degree of root growth inhibition. These features may be due to differences in root system structure between the species. The root system of *D. caespitosa* consists of numerous adventitious roots whose length usually varies between 15 and 30 cm but may reach 60–70 cm (Davy, 1980); therefore, the harmful effect of copper (manifested mainly in the inhibition of apical root meristem) can be partly lev-

eled off due to the compensatory growth of new adventitious roots. In *L. flos-cuculi*, the root system consists of a short (5–8 cm) primary root and weakly developed lateral roots (Chaloupecká and Lepš, 2004), and the impairment of the primary root growth can result in a critical reduction of the absorptive surface area and consequent decline in plant competitiveness.

Comparing dose–response curves for *L. flos-cuculi* from the background and buffer zones, type IV transformation of this relationship could be expected as a theoretically possible intermediate variant upon transition from the background to the impact population. Transformation of this type would have been evidence for the existence of additional mechanisms providing for tolerance of plants from the buffer zone to moderately harmful concentrations of the toxicant. The distinctive feature of such mechanisms is their low "detoxification capacity," as is the case with metallothioneins, though these proteins play only a second-

ary role in detoxification of metals (Mengoni et al., 2003). However, we have found that, in either species, the dose–response curves for plants from the buffer and impact populations have similar shape; therefore, irrespective of the toxicant level in the environment, the type of transformation of the dose–response relationship is a species-specific attribute. The same follows from the results of other studies (De Vos et al., 1991; Macnair, Smith, and Cumbes, 1993; Mengoni et al., 2003).

Type III transformation (also not revealed in our study) implies that new mechanisms of tolerance are induced at low copper concentrations but, unlike in types IV and I, their activity increases nonlinearly with an increase in the concentration of the toxicant. Such an adaptation strategy is more probable in species exposed to seasonal fluctuations of environmental toxicity, e.g., in aquatic plants.

Variability in parameters of metal tolerance in the background population of *D. caespitosa* is high, indicating that this population contains metal-tolerant genotypes. The increased tolerance to copper in part of plants from this population (Fig. 4) may be accounted for by a naturally high level of plasticity in physiological characters. A similar phenomenon has been described for the response of this species to temperature (Tieszen and Helgager, 1968). In contrast, intrapopulation variability in the metal tolerance of *L. flos-cuculi* in the background zone is lower than in the buffer and impact zones, which is indicative of a low phenotypic plasticity with respect to the toxic factor.

In addition to metal-tolerant genotypes, samples from populations of both species growing in polluted habitats have proved to contain genotypes with a low level of metal tolerance, which explains the observed high level of intrapopulation variability. This finding is unexpected, since it is generally considered that exposure to toxic pollution leads to elimination of low-tolerant genotypes (Meerts and Van Isacker, 1997). Meanwhile, soil toxicity in polluted habitats is accounted for not only by heavy metal concentrations but also by many other parameters, including soil pH and the contents of phosphorus compounds and organic matter, and often displays high spatial heterogeneity, even on a microscale (Vorobeichik and Pozolotina, 2003). The microscale spatial heterogeneity of substrate toxicity may be responsible for major differences in individual metal tolerance between plants growing close to each other (Bradshaw, 1965) and, consequently, for a high intrapopulation variation in this character. It should also be taken into account that populations of impact areas are formed under the effect of not only excess heavy metals but also of other extreme environmental factors (specific temperature and moisture conditions, deficit or excess of particular nutrients, deficit of pollinators, reduced competition, etc.). For this reason, it appears somewhat incorrect to characterize plant populations from polluted habitats simply as metal-tolerant, even though this is accepted in studies on evolutionary genetics (Ducousso et al., 1990; Mengoni et al., 2001).

CONCLUSIONS

Analysis of dose–response relationships in two species growing together in areas with different levels of industrial pollution have shown that the transformation of the dose –response curve upon plant adaptation to excess environmental copper differs between the species: the curves for *L. flos-cuculi* show a parallel shift, with their shape remaining generally unchanged, while those for *D. caespitosa* are indicative of decrease in sensitivity to copper, with tolerance to this metal increasing only at the highest concentration range. Different types of transformation of the dose–response relationship are evidence for interspecific difference in the strategy of adaptation to toxic impact. Thus, species successfully growing together in the same polluted habitats during the same period of time demonstrate different mechanisms of adaptation to pollution.

Since the area around the MUCS is polluted not only with Cu but also with considerable amounts of Pb, Zn, Cd, and As (Vorobeichik, Sadykov, and Farafontov, 1994), it may well be that plants from the studied populations are also tolerant to these elements. However, the transformation pattern of the dose–response relationship revealed for Cu cannot be directly extrapolated to other elements, since there is evidence that detoxifying mechanisms are specific for individual heavy metals and rarely provide for co-tolerance to more than one of them. In particular, co-tolerance to Cu in plant lines tolerant of other metals is a very rare phenomenon. Assumptions concerning multiple tolerance, i.e., simultaneous functioning of several metal-specific detoxifying mechanisms, appear to be more valid (Hall, 2002).

The results of this study show that the mathematical apparatus developed in classical toxicology for analyzing dose–response relationships can be successfully used in research on metal tolerance in plants. An advantage of this approach is that it prevents the loss of information on plant tolerance to a given metal in different segments of the total range of its concentrations and also on sensitivity to it and on intrapopulation variation in toxicological parameters along the pollution gradient.

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